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Original article

Evaluation of D-Dimer and CRP in cases of Atherosclerosis (CAD)

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ABSTRACT

Abstract— Elevated levels of C-reactive protein (CRP) and D-dimer (DD) have been associated with the presence and progression of various forms of atherosclerotic disease, particularly coronary heart disease. It is hypothesized that there is a relationship between elevated levels of baseline CRP and DD and progression of coronary arterial disease (CAD) in patients with symptomatic CAD. C-reactive protein (CRP), a marker of the reactant plasma protein component of the inflammatory response, has been associated with the risk of future ischemic heart disease (IHD), not only among patients with stable and unstable angina and high-risk subjects The current study is a prospective evaluation of this hypothesis. Plasma levels of C-reactive protein (CRP, a marker of the reactant plasma protein component of the inflammatory response) and of fibrin D-dimer (a marker of cross-linked fibrin turnover) has each been associated in recent studies with the risk of future ischemic heart disease (IHD). Previous experimental studies have shown that fibrin degradation products, including D-dimer, have effects on inflammatory processes and acute-phase protein responses. We therefore measured CRP and D-dimer levels in stored plasma samples from 100 men aged 49 to 77 years who were followed-up for incident IHD for an average of 45±4 months (mean±SD) and studied their associations with each other, with baseline and incident IHD, and with IHD risk factors. CRP and D-dimer levels were each associated with age, plasma fibrinogen, smoking habit, and baseline evidence of IHD. CRP was associated with D-dimer (r=0.21, P<0.00001). On univariate analyses, both CRP and D-dimer were associated with incident IHD. The incidence of IHD increased with CRP independently of the level of D-dimer (P=0.0002) and also increased with D-dimer independently of the level of CRP (P=0.048). In multivariate analyses, inclusion of D-dimer and conventional risk factors reduced the strength of the association between CRP and incident IHD; likewise, inclusion of CRP and conventional risk factors reduced the strength of the association between D-dimer and incident IHD.It is concluded that although these respective markers of inflammation and fibrin turnover show modest association with each other in middle-aged men, they may have additive associations with risk of incident IHD. Further larger studies are required to test this hypothesis

Introduction

Fibrin D-dimer, a marker of cross-linked fibrin turnover, has also been shown in recent studies to be associated with the risk of future IHD in persons with and without baseline evidence of vascular disease. Local fibrin formation and lysis are part of the inflammatory response, and fibrin degradation products, including D-dimer, have been shown to have diverse effects on inflammatory processes and acute-phase responses, including neutrophil and monocyte activation; secretion of cytokines, including interleukin-6 and interleukin-1; and hepatic synthesis of acute-phase proteins, including fibrinogen and CRP. Interpreting D-dimer results

The association between elevated D-dimer levels and thrombotic disease continues to grow. When interpreting a D-dimer result there are some clinical aspects that should be considered. The precise level of cross-linked fibrin derivatives (D-dimer) circulating in the blood at a given time will depend on a number of parameters:

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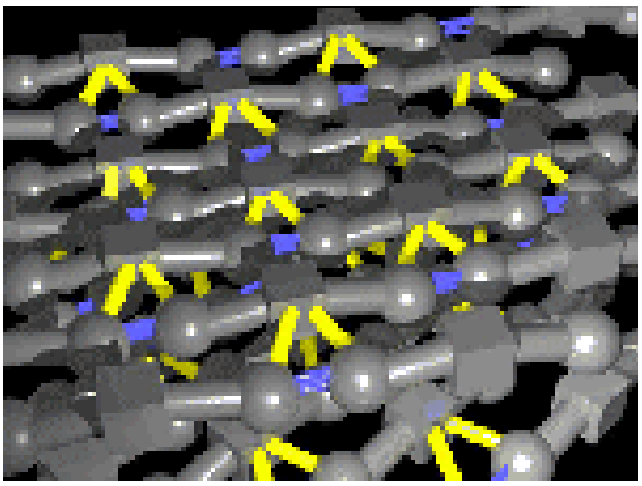
Time elapsed since the thrombotic event	D-dimer has a half life of approximately 6 hours in the circulation of individuals with normal renal function. Patients with stabilised clots and not undergoing active fibrin deposition and plasmin activation, may not give detectable D-dimer elevations.
The initial size of the clot.	The larger the clot size, the higher the expected level of circulating D-dimer. Obviously the converse is also true
The rate of fibrinolysis.	Blood fibrinolysis is a highly regulated process and in delicate dynamic balance. Should any of the components be compromised (hereditary or acquired deficiency or dysfunction) then the rate of fibrinolysis will be altered.
Alternative fibrin sites.	Fibrin may be present at alternative sites other than that suspected. For example, atherosclerotic lesions or extravascular fibrin deposits. Some tumours can be encapsulated in a fibrin sheath
Differing antibody specificity.	All D-dimer assays are not alike - Depending on the commercial source, different antibodies used in a test have differing specificities for fibrinogen and fibrin and their derivatives. There are still today many FDP assays calling themselves D-dimer specific.

It is therefore hypothesized that (1) plasma levels of CRP and D-dimer and their associations with incident IHD in the general population might be linked; (2) linkage might result from focal, vessel wall–related fibrin formation and lysis and an inflammatory response associated with unstable atherosclerotic plaque activity; and (3) CRP and D-dimer might be related to IHD risk factors associated with thrombogenesis and inflammation, particularly cigarette smoking. We tested these hypotheses by studying the mutual relationships of CRP, D-dimer, incident IHD, and risk factors (especially smoking habit) in the middle-aged. D-dimer is a global indicator of coagulation activation and fibrinolysis and, therefore, an indirect marker of thrombotic activity. The utility of D-dimer measurement has been evaluated in several clinical situations including the exclusion of venous thromboembolism (VTE), prediction of future risk of VTE, and the diagnosis and monitoring of disseminated intravascular coagulation (DIC). This article will review the available evidence for the utilization of D-dimer antigen measurement in the management of thrombotic and bleeding disorders.

D-dimer is a reliable and sensitive index of fibrin deposition and stabilization. As such, its presence in plasma should be indicative of thrombus formation. There are many conditions unrelated to thrombosis in which D-dimer concentrations are high, however, making its positive predictive value rather poor.



C-reactive protein (CRP) is a phylogenetically highly conserved plasma protein, with homologs in vertebrates and many invertebrates, that participates in the systemic response to inflammation. Its plasma concentration increases during inflammatory states, a characteristic that has long been employed for clinical purposes. CRP is a pattern recognition molecule, binding to specific molecular configurations that are typically exposed during cell death or found on the surfaces of pathogens. Its rapid increase in synthesis within hours after tissue injury or infection suggests that it contributes to host defense and that it is part of the innate immune response. Recently, an association between minor CRP elevation and future major cardiovascular events has been recognized, leading to the recommendation by clinicians. The Centers for Disease Control and intermediate risk of coronary heart disease might benefit from measurement of CRP. This review will largely focus on our current understanding of the structure of CRP, its ligands, the effecter molecules with which it interacts, and its apparent functions. C-reactive protein (CRP) is a phylogenetically highly conserved plasma protein, with homologs in vertebrates and many invertebrates, that participates in the systemic response to inflammation. Its plasma concentration increases during inflammatory states, a characteristic that has long been employed for clinical purposes. CRP is a pattern recognition molecule, binding to specific molecular configurations that are typically exposed during cell death or found on the surfaces of pathogens. Its rapid increase in synthesis within hours after tissue injury or infection suggests that it contributes to host defense and that it is part of the innate immune response. Recently, an association between minor CRP elevation and future major cardiovascular events has been recognized, leading to the recommendation by clinicians. The Centers for Disease Control and intermediate risk of coronary

heart disease might benefit from measurement of CRP. This review will largely focus on our current understanding of the structure of CRP, its ligands, the effecter molecules with which it interacts, and its apparent functions.

Modified" CRP

CRP is an ancient protein whose initial role as a pattern recognition molecule may have been to defend against bacterial infections, but whose present biological role appears quite complex. It is protective against a variety of bacterial infections and inflammatory stimuli in mice. It is likely that the activity of CRP in humans, either pro- or anti-inflammatory is dependent on the context in which it is acting. Recent data have raised the possibility that it may participate in the pathogenesis of disease.

Denatured and aggregated forms of CRP (neo-CRP or modified CRP) have been reported to be powerfully pro-inflammatory in a number of experimental systems, although the existence of this material in vivo has not been unequivocally established. It is conceivable that at local sites of deposition, small amounts of modified CRP may be generated with a set of properties distinct from those of the native protein. It has recently been reported that modified CRP increased the release of the inflammatory mediators monocyte chemoattractant protein-1 and IL-8 and up-regulated the expression of ICAM-1 in endothelial cells. In this model, modified CRP was shown to be a much more potent inducer than native CRP .

CRP and Atherosclerosis

Evidence in support of the possibility that CRP itself plays a role in the pathogenesis of atherosclerosis has been summarized in a recent review. Examples include the finding that CRP binds the phosphocholine of oxidized low density lipoprotein up-regulates the expression of adhesion molecules in endothelial cells, increases low density lipoprotein uptake into macrophages, inhibits endothelial nitric-oxide synthase expression in aortic endothelial cells,and increases plasminogen activator inhibitor-1 expression and activity. A recent study utilizing a mouse strain expressing transgenic CRP and deficient in apolipoprotein E reported a modest acceleration in aortic atherosclerosis in male animals expressing high levels of CRP. A second report demonstrated increased arterial occlusion in transgenic mice expressing CRP in a model of vascular injury. Despite these suggestive findings, a role for CRP in the pathogenesis of atherosclerosis is far from established.

Material and Methods

A total of 100 men were eligible for inclusion, and 92 (92%) attended the first examination. At that time their ages ranged from 45 to 68 years. The current study uses as its baseline the second examination conducted between 2013 and 2014, at which time men were aged 49 to 67 years.

Survey Methods

The general design and procedures have been carried out. In brief, at each examination the men were invited to attend OPD of GGS Medical College & Hospital where a detailed medical and lifestyle history was obtained; chest pain questionnaire was administered; a full 12-lead ECG was recorded; and height, weight, and blood pressure were measured. Current or last occupation was recorded, and from this information, social class was coded according to the Re

At the first examination, the men returned after an overnight fast to an early morning OPD to give a blood sample. Standard methods were used for the estimation of lipids. At the second examination, a non-fasting blood sample was taken from 20 (98%) of the men seen. Fibrinogen was measured by heat precipitation . Plasma samples from dipotassium edetate–anticoagulated blood at the nonfasting second examination were stored at −20°C. CRP and D-dimer levels were measured on these; CRP was measured by a sensitive Ichroma assay and D-dimer, with chemiluminescence's method. CRP was measured for 100 men, who were a 100% whom D-dimer was measured first.

Observation

Levels of CRP and D-dimer were both significantly higher ($P<0.001$). Fibrinogen, measured on the fresh, prestorage samples, and even fibrinogen and total cholesterol, as measured at the first examination, were all higher among the subjects whose stored, second-examination sample thawed. This suggests that the subjects whose samples had been thawed were, by chance, not a representative sample of the whole group. Further support for this explanation comes from the Caerphilly study. When D-dimer was measured there, 1 batch of samples had also previously been thawed, and D-dimer levels were slightly but not significantly lower among the thawed samples. It was thus decided not to exclude the results from the thawed samples but to include them in all analyses with an adjustment for the effect of thawing.

Incidence IHD

Incidence of IHD was measured between the second examination (the baseline for this report) and the fourth examination, which took place between 2006 to 2008. At that fourth examination, the men were seen in the same order as far as possible, and the average follow-up period was 75 months (mean±SD, 75±4). Death coded as 44 to 54 International Classification of Diseases (ICD) was used as the definition of fatal IHD. Questions about admission to hospital with severe chest pain and lists from hospital activity analysis of all men admitted with a diagnosis of ICD 40 to 54 were used as the basis for a search of hospital notes for events meeting standard World Health Organization criteriafor acute myocardial infarction (MI). Finally, ECG of major or selected moderate Q waves was recorded.

Under these definitions, there were 76 major IHD events of which 34 were fatal. The average annual incidence rate was 1.5%. Among the 69 men with a measurement for CRP, there were 53 IHD events, whereas among the 100 for whom a D-dimer measurement was available, there were 59 such events.

Statistical Methods

The distributions of CRP and D-dimer both had a marked, positive skewness. In all analyses where they were used as continuous variables, they were transformed to (natural) logarithms. The transformations produced distributions that were close to gaussian, with back-transformed geometric means of 1.57 mg/L for CRP and 42.0 ng/mL for D-dimer.

Adjusted mean differences in CRP and D-dimer between men who developed IHD and those who did not were obtained by ANCOVA by using standard multiple regression techniques. The remainder of the analysis was performed by using multiple logistic regression with the occurrence or not of any of the 3 types of incident IHD as the dependent variable. Logistic regression takes no account of the duration of follow-up, but this factor is likely to be

immaterial because follow-up was at a nearly constant interval of 75 months, with an SD of only 4 months. Furthermore, any model such as the Cox proportional-hazards model that involves the time to the event would face the problem that no time to event is available for the ECG-defined MIs. These would either have to be excluded or allocated an arbitrary time to event.

In the logistic regression analyses, CRP and D-dimer were first divided into 5 equally sized groups by using 4 cutpoints: 0.6, 1.1, 2.0, and 4.2 mg/L for CRP and 26, 37, 47, and 63 ng/mL for D-dimer. Results were then presented as the odds of IHD in each group relative to the odds in the 20% of men with the lowest levels. Tests for trend were obtained by entering CRP or D-dimer as logarithmically transformed continuous variables, and the trends were summarized by standardized relative odds (SROs): the odds associated with a 1-SD increase in the logarithm of CRP or D-dimer.

Evidence of ischemia at baseline was assessed from the chest pain questionnaire and the ECG. Three categories, namely angina; history of at least 1 episode of prolonged, severe chest pain; and ECG ischemia were defined in a standard manner. Among the 100 men, 24 (24%) had some evidence of ischemia at baseline. This prevalence is slightly lower than that found by the British Regional Heart Study for men of similar age. These men were not excluded from the analysis. Exclusion of such a large group, among whom 42% of the incident events occurred, does not seem satisfactory. Neither does the usual practice of excluding just a very small percentage (<5%) of men for whom there is good evidence of a previous MI. Instead, we have chosen to include all men and to adjust for the presence of ischemia at baseline by including the 3 standard measures of confounders in the logistic regression analyses.

Results

Baseline Characteristics

The baseline characteristics of the 191 men who developed major IHD are compared in Table 1 with those of the 1864 men who did not. Those who developed IHD were slightly older (P<0.001) and had higher total cholesterol (P=0.001), body mass index (P=0.029), and systolic (P<0.001) and diastolic (P=0.002) blood pressures. They were more likely to be smokers (P=0.001), to be diabetic (P=0.003), and to have a family history of MI (P=0.024). The proportion from the manual social classes was similar in the 2 groups.

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Table 1: Baseline Characteristics of Men With and Without Incident IHD

	No Incident IHD (n=1864)	Incident IHD (n=191)
Age, y	57.1 (4.4)	58.6 (4.3)
Smoking status		
Never	16%	8%
Past	45%	41%
Current	39%	50%
Body mass index, kg/m^2		
Blood pressure, mm Hg	25.9 (3.3)	26.4 (3.9)
Systolic	151 (23)	158 (25)
Diastolic	93 (12)	96 (13)
Total cholesterol, mmol/L	5.85 (1.21)	6.15 (1.25)
Diabetes	2.2%	5.8%
Family history of MI	25%	33%
Manual social class	64%	62%

Univariate Analyses of CRP, D-Dimer, and Incident IHD

In Table 2, the data show that mean CRP and D-dimer levels were each higher among the men who developed IHD. The age- and thawing-adjusted mean difference between the 2 groups was highly significant for CRP (P=0.00008) and significant for D-dimer (P=0.017).

Table 2: Baseline Levels of CRP and D-Dimer Among Men Who Developed IHD Compared With Those Who Did Not

	No IHD		IHD		Age6Adjusted[†] Mean Difference (95% CI) and *P*
	n	Mean (SD)	n	Mean (SD)	
Log$_e$ CRP	1528	0.408 (1.141)	162	0.858 (1.166)	0.376 (0.192 to 0.559);*P*=0.00008
Geometric mean CRP, mg/L		1.50		2.36	
Log$_e$ D6dimer	1554	3.724 (0.637)	165	3.887 (0.638)	0.124 (0.022 to 0.225);*P*=0.017
Geometric mean D6 dimer, ng/mL		41.4		48.7	

In Table-3, the data show that the incidence of IHD increased steadily from 5.7% in the 20% of men with the lowest levels of CRP to 15.5% among the 20% with the highest levels. Unadjusted, the corresponding relative odds increased steadily to 3.07 (95% CI, 1.80 to 5.22) in the top 20% of the distribution. This trend was highly statistically significant (P<0.00001), and the SROs, the relative odds associated with a 1-SD increase in CRP, were 1.48. Adjusting for age slightly reduced the relative odds in the top 20% to 2.73 (95% CI, 1.60 to 4.67) and the SRO to 1.41, but the trend was still highly significant (P=0.00005). Further adjustment for whether or not the sample had been thawed during the freezer failure had no material effect on these relative odds Table-3

	Quintile of CRP or D6Dimer					SROs and *P*for Trend
	1	2	3	4	5	
CRP						
Range, mg/L	≤0.6	0.7–1.1	1.2–2.0	2.1–4.2	≥4.3	
Number of men	100	93	92	87	66	
Number (%) with incident IHD	21 (5.7%)	26 (7.9%)	28 (8.5%)	36 (10.9%)	51 (15.5%)	
Relative odds of IHD for model						
CRP alone	1.0	1.43	1.54	2.03	3.07 (95% CI, 1.80–5.22)	1.48;*P*<0.00001
CRP, age, sample thawed/unthawed	1.0	1.40	1.45	1.87	2.67 (95% CI, 1.56–4.58)	1.39;*P*=0.00010
D6dimer						
Range, ng/mL	≤26	27–37	38–47	48–63	≥64	
Number of men	100	98	93	90	78	
Number (%) with incident IHD	25	28	32	41	39 (11.2%)	

	Quintile of CRP or D6Dimer					SROs and *P*for Trend
	1	2	3	4	5	
	(7.0%)	(8.1%)	(9.8%)	(12.0%)		
Relative odds of IHD for model						
D6dimer alone	1.0	1.18	1.43	1.81	1.68 (95% CI, 1.00–2.82)	1.27;*P*=0.0018
D6dimer, age, sample thawed/unthawed	1.0	1.11	1.33	1.67	1.40 (95% CI, 0.82–2.38)	1.21;*P*=0.017

Table 3. Incidence and Relative Odds of IHD by Quintiles of CRP and D-Dimer

The incidence of IHD increased from 7.0% in the 20% of men with the lowest levels of D-dimer to 12.0% and 11.2% in the fourth and fifth highest quintile groups, respectively (Table 3⇑). Unadjusted, the corresponding relative odds increased steadily to 1.68 (95% CI, 1.00 to 2.82) in the top 20% of the distribution. This trend was highly statistically significant (P=0.0018), and the SROs were 1.27. Adjusting for age slightly reduced the relative odds in the top 20% to 1.44 (95% CI, 0.85 to 2.45) and the SRO to 1.22, but the trend was still significant (P=0.012). Adjustment for thawing had only a minor effect (Table 3).

Associations With Cardiovascular Risk Factors

The data in Table 4⇓ show how CRP and D-dimer varied with cigarette smoking habit and with evidence of IHD at baseline. Current smokers had geometric mean levels of CRP that were nearly double those among men who had never smoked. Among those current smokers, the lowest geometric mean level (1.87 mg/L) was found among the lightest smokers, and even this lowest level was significantly (P<0.001) higher than that found among the men who had never smoked. There was no clear dose response among current smokers, but among ex-smokers there was a clear trend with the length of time since quitting. Even those who gave up more than 10 years ago had a geometric mean CRP of 1.36 mg/L, which was still higher (P=0.037) than the geometric mean of 1.13 mg/L among the men who had never smoked. The average length of time since quitting among these men was nearly 23 years, with a range from 10 to 48 years. Current smokers also had higher D-dimer levels than men who had never smoked (P=0.046), with no dose response. Again, there was a clear trend with the length of time since quitting; 5 to 9 years after quitting, their D-dimer levels returned to the levels observed in nonsmokers.

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	n	Geometric Mean CRP, mg/L	n	Geometric Mean D6Dimer, ng/mL
Cigarette smoking habit				
Never	265	1.13	272	41.0
Ex6smokers, time since quitting				
≥10 y	477	1.36	485	39.5
5–9 y	113	1.34	116	39.2
1–4 y	115	1.66	113	48.6
<1 y	29	2.10	30	58.1
Current cigarette smokers				
1–14/d	189	1.87	193	46.9
15–24/d	210	2.32	214	43.7
≥25/d	128	2.05	129	44.6
Evidence of ischemia at baseline				
Angina				
No	1549	1.49	1579	41.8
Yes	141	2.70	140	45.7
History of severe chest pain				
No	1558	1.53	1590	41.6
Yes	132	2.20	129	48.1
ECG ischemia				
None	1434	1.46	1460	41.2
Possible[1]	184	2.23	186	46.0
Probable[2]	72	2.59	73	52.3

All manifestations of IHD at baseline were associated with higher geometric mean levels of CRP. Men with angina from the Rose chest pain questionnaire had levels of CRP nearly double those of men without angina. CRP was raised by ≈50% among men with a history of prolonged, severe chest pain or with evidence of ischemia on ECG. All of these differences were highly statistically significant (P<0.001). D-dimer levels compared with CRP showed less elevation in men with evidence of ischemia, but these elevations were still statistically significant for men with a history of severe chest pain (P=0.014) or with evidence of ischemia on ECG (P<0.001). CRP was also raised by 50% among the small number (2.8%) of diabetics. Among the much larger proportion (26%) of men with a first-degree relative with a history of MI, CRP was raised by 17% (P=0.011). There was no association between CRP and social class. D-dimer was not significantly associated with diabetes, family history of MI, or social class.

CRP increased with age (r=0.15) and showed positive associations with other conventional cardiovascular risk factors such as total cholesterol (r=0.08), diastolic blood pressure (r=0.07), and body mass index (r=0.14). All of these were statistically significant (P<0.01) but modest in size. There was a much stronger association with fibrinogen (r=0.42). D-dimer also increased with age (r=0.15,P<0.01) and fibrinogen (r=0.16, P<0.01) but not with total cholesterol (r=0.01), diastolic blood pressure (r=0.03), or body mass index (r=0.00).

There was a positive correlation between CRP and D-dimer (r=0.21, P<0.00001). This association did not arise simply because both were positively associated with age, smoking habit, baseline evidence of IHD, and fibrinogen. On adjusting for all of these factors, the partial correlation declined to only 0.17 and remained statistically significant (t=5.29, P<0.00001).

Incidence of IHD with Increasing CRP and D-Dimer

The data in Table 5⇓ show that the incidence of IHD increased with CRP at each level of D-dimer and that it also increased with D-dimer at every level of CRP. The trend for incidence of IHD to increase with CRP independently of the level of D-dimer was statistically significant (P=0.00015), as also was the trend for incidence to rise with D-dimer independently of the level of CRP (P=0.048). There was no evidence that the association between IHD and CRP was different at different levels or D-dimer or that the association between IHD and D-dimer differed with level of CRP (test for interaction, χ^2 (2 df) =3.54, P=0.17).

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Tertile of D6Dimer, Range in ng/mL	Tertile of CRP, Range in mg/L			All
	1 (≤0.9)	2 (1.0–2.5)	3 (≥2.6)	
1 (≤33)	15/228 (6.6%)	11/189 (5.8%)	13/141 (9.2%)	39/558 (7.0%)
2 (34–51)	9/189 (4.8%)	19/192 (9.9%)	25/171 (14.6%)	53/552 (9.6%)
3 (≥52)	13/155 (8.4%)	16/171 (9.4%)	41/234 (17.5%)	70/560 (12.5%)
All	37/572 (6.5%)	46/552 (8.3%)	79/546 (14.5%)	162/1670 (9.7%)

Tertile of D6Dimer, Range in ng/mL	Tertile of CRP, Range in mg/L			
	1 (≤0.9)	2 (1.0–2.5)	3 (≥2.6)	All
1 (≤33)	15/228 (6.6%)	11/189 (5.8%)	13/141 (9.2%)	39/558 (7.0%)
2 (34–51)	9/189 (4.8%)	19/192 (9.9%)	25/171 (14.6%)	53/552 (9.6%)
3 (≥52)	13/155 (8.4%)	16/171 (9.4%)	41/234 (17.5%)	70/560 (12.5%)
All	37/572 (6.5%)	46/552 (8.3%)	79/546 (14.5%)	162/1670 (9.7%)

Table 5. Incidence of IHD Jointly by Tertiles of CRP and D-Dimer

Multivariate Analysis for CRP

In Table 6 are shown the results of a series of multiple logistic regression analyses that examined the change in association between CRP and incident IHD as groups of cardiovascular risk factors were successively added to the regression model. In the first model, which adjusted only for age and the thawing (or not) of the sample, the relative odds of IHD rose to 2.49 (95% CI, 1.44 to 4.30) among men in the top 20% of the distribution of CRP, and the SROs were 1.37 (P=0.0002). These figures differ from those in Table 3 only because those in Table 3 were based on all 1690 men with a measurement of CRP, whereas the figures in Table 6 were based on the 1595 men who had a complete set of data for all of the variables included in the models of

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Table 6. Effect on the Association between CRP or D-Dimer and Incident IHD after Adjusting for Other Cardiovascular Risk Factors

	Relative Odds (95% CI) Among the 20% of Men With the Highest CRP*/D6Dimer†	SROs	*P* for Trend
Variables in model for CRP			
CRP, age, sample thawed/unthawed	2.49 (1.44 to 4.30)*	1.37	0.0002
+Smoking, body mass index, diastolic BP, total cholesterol, evidence of ischemia at baseline	1.60 (0.90 to 2.83)*	1.20	0.049
+Fibrinogen	1.51 (0.83 to 2.77)*	1.18	0.103
+D6dimer	1.45 (0.79 to 2.66)*	1.15	0.16
Variables in model for D6dimer			
D6dimer, age, sample thawed/unthawed	1.32 (0.76 to 2.30)†	1.21	0.023
+Smoking, body mass index, diastolic BP, total cholesterol, evidence of ischemia at baseline	1.15 (0.65 to 2.05)†	1.17	0.072
+Fibrinogen	1.09 (0.61 to 1.96)†	1.15	0.11
	Relative Odds (95% CI) Among the 20% of Men With the Highest CRP*/D6Dimer†	SROs	*P* for Trend
+CRP	1.04 (0.58 to 1.87)†	1.13	0.17

Addition of a set of conventional cardiovascular risk factors (smoking habit, body mass index, diastolic blood pressure, total cholesterol, and evidence of ischemia at baseline) reduced the relative odds in the top 20% of the distribution of CRP to 1.60 (95% CI, 0.90 to 2.83) and the SROs to 1.20. The test for trend was still just significant at the conventional 5% level. The further addition of fibrinogen and then D-dimer to the model reduced the relative odds in the top 20% of the CRP distribution to 1.45 (95% CI, 0.79 to 2.66) and the SROs to 1.15. The test for trend (P=0.16) was no longer statistically significant.

A stepwise multiple logistic regression analysis was then performed for CRP (Table 7). The variables considered for inclusion in the stepwise regression were all those cardiovascular risk factors appearing in the models of Table 6. The base model again consisted of age and whether or not the sample had been thawed, as well as CRP. At each stage of the stepwise procedure, the cardiovascular risk factor that produced the largest reduction in the SROs of IHD was added to the model. First to be so added was evidence of ischemia at baseline, which caused the SROs to decline from 1.37 to 1.27, but the test for trend remained significant (P=0.008). At the next stage the addition of smoking habit caused the largest further reduction to 1.21 (P=0.033). Thereafter the addition of fibrinogen and then D-dimer reduced the SROs first to 1.19 (P=0.084) and then to 1.16 (P=0.13). No individual risk factor then caused any further substantial reduction in SROs. The joint addition of body mass index, diastolic blood pressure and total cholesterol only reduced the SROs to 1.15 (P=0.16) as shown in Table 6⇑. When family history of MI instead of social class and whether or not the subject was a diabetic were added jointly to the model, the SROs declined only from 1.16 (P=0.13) to 1.14 (P=0.17).

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	SROs	*P* for Trend
Variables in model for CRP		
CRP, age, sample thawed/unthawed	1.37	0.0002
+Evidence of ischemia at baseline	1.27	0.008
+Smoking	1.21	0.033
+Fibrinogen	1.19	0.084
+D6dimer	1.16	0.13
Variables in model for D6dimer		
D6dimer, age, sample thawed/unthawed	1.21	0.023
	SROs	*P* for Trend
+CRP	1.15	0.11
+Evidence of ischemia at baseline	1.13	0.16
+Fibrinogen	1.12	0.19

Table 7. Confounding Factors That Successively Cause the Largest Reduction in the Relative Odds of IHD Associated With A 1-SD Increase in CRP or D-Dimer

	SROs	*P* for Trend
Variables in model for CRP		
CRP, age, sample thawed/unthawed	1.37	0.0002
+Evidence of ischemia at baseline	1.27	0.008
+Smoking	1.21	0.033
+Fibrinogen	1.19	0.084
+D6dimer	1.16	0.13
Variables in model for D6dimer		
D6dimer, age, sample thawed/unthawed	1.21	0.023
+CRP	1.15	0.11
+Evidence of ischemia at baseline	1.13	0.16
+Fibrinogen	1.12	0.19

Multivariate Analysis for D-Dimer

The data in Table 6 show that addition of the set of cardiovascular risk factors reduced the relative odds in the top 20% of the distribution of D-dimer to 1.15 (95% CI, 0.65 to 2.05) and the SROs to 1.17. The test for trend after this adjustment was nonsignificant and was reduced further by adjustment for fibrinogen and CRP. Stepwise multiple logistic regressions showed that the confounding factor that most reduced the association of D-dimer with IHD was CRP, followed by evidence of ischemia at baseline and fibrinogen (Table 7).

Discussion

To our knowledge, this is the first report to compare CRP (a marker of the reactant plasma protein component of the inflammatory response) with D-dimer (a marker of fibrin turnover) in the prediction of incident IHD in a population cohort. The inflammatory and the thrombotic components of coronary atherosclerosis and IHD are of current interest in pathophysiology, and there is some experimental evidence that they may be linked. Because both CRP and D-dimer are easily measured in stored plasma (or serum) samples, their potential use in risk stratification for IHD merits a comparison.

We observed that in this population men aged 49 to 67 years, plasma CRP and D-dimer levels showed a moderate correlation ($r=0.21$, $P<0.00001$). On adjusting for potential confounders (age, smoking, baseline evidence of IHD, and fibrinogen), the association was reduced but remained highly statistically significant ($r=0.17$, $P<0.00001$). This correlation supports our hypothesis that there may be a link between these respective markers of inflammation and fibrin turnover in middle-aged men, which may be due in part to their associations with asymptomatic and symptomatic arterial lesions. However, the correlation is not strong, which suggests that other factors have different effects on plasma levels of these 2 variables.

As expected, we found that cigarette smoking habit had important, reversible effects on both CRP and D-dimer.The elevations of CRP and D-dimer in current cigarette smokers were not dose dependent but were reversible after quitting (Table 4). Mean plasma CRP was approximately doubled in current smokers compared with never-smokers and may partly reflect elevations in smokers of interleukin-6, which is a major regulator of the reactant plasma protein component of the inflammatory response. Although plasma CRP levels fell progressively with time since quitting smoking, they remained significantly elevated 10 years after quitting compared with those in never-smokers (Table 4). In contrast, the elevation in mean plasma D-dimer in current smokers was only ≈10% and fell to levels seen in nonsmokers, 5 to 9 years after quitting (Table 4). These data suggest that the "inflammatory" effect of cigarette smoking is both larger in magnitude (10-fold) and longer-lasting than its effect on cross-linked fibrin turnover. The relationships of these observations to underlying smoking-related pathology in the arteries, respiratory tract, and other organs and to the time course of reduction of IHD risk in smokers who quit merit future study.

As with smoking, the relationships of CRP to both baseline IHD (Table 4) and incident IHD (Tables 2and 3) were stronger than those of D-dimer to baseline and incident IHD. These relationships to IHD are consistent with the literature for CRP7and D-dimer. We are not aware of previous studies directly comparing the predictive value of CRP and D-dimer for IHD. The present study suggests that measurement of both variables may be useful in risk stratification (Table 5). The incidence of IHD increased with CRP independently of the level of D-dimer and vice versa. The risk of IHD over ≈6 years follow-up was ≈6% (ie, 1% per year) in middle-aged men with levels in the lower third of both CRP and D-dimer compared with almost 18% (ie, almost 3% per year) in those in the upper third of both CRP and D-dimer. These data, combined with the practical issue that both CRP and D-dimer are easily measured in stored plasma (or serum) samples, suggest the need for further evaluation of both variables in risk stratification for IHD. This suggestion has a plausible pathophysiological basis: both inflammation and thrombosis are important in the pathogenesis of IHD.

In the present study, we observed that inclusion of conventional risk factors as well as CRP or D-dimer in multiple logistic regression analyses of the relationships of the other variable to incident IHD reduced the strength of the association (Tables 5, 6, and 7). With regard to the relationship between CRP and incident IHD, the inclusion of fibrinogen in the model reduced the relationship to below the conventional level of statistical significance (reduction of SROs from 1.21, $P=0.033$ to 1.19, $P=0.084$); however, because CRP and fibrinogen are both measures of the reactant plasma protein component of inflammation, the validity of this adjustment is debatable. The addition of D-dimer to the model further reduced the relationship (SROs of 1.16, $P=0.13$), which suggests that the relationship between CRP and incident IHD is partly confounded by their mutual relationships to D-dimer. Conversely, with regard to the relationship between D-dimer and incident IHD, the inclusion of CRP in the model reduced the relationship from an SRO of 1.21 ($P=0.023$) to 1.15 ($P=0.11$). However, the limited number of major IHD events in this study (191) results in wide confidence intervals for estimates of the mutual relationships between CRP, D-dimer, conventional risk factors, and incident IHD. Hence, further prospective cohort studies and collaborative meta-analyses are required to define these with greater precision.

The results of the present study are very similar to the overall results in meta-analyses of CRP and D-dimer (J. Danesh, personal communication, 2000).

In conclusion, our data suggest that in a population cohort of middle-aged men, markers of inflammation (CRP) and of fibrin turnover (D-dimer) are related to each other, smoking, age, plasma fibrinogen, and baseline (as well as incident) IHD. These findings may be related to the association of inflammation and fibrin turnover in arterial lesions and at other body sites. However, measurement of both CRP and D-dimer may be a logical and practical enhancement of current risk stratification for IHD. Further studies are required to test these hypotheses.

Conclusions

In subjects with symptomatic CAD, elevated baseline DD, a marker of thrombotic activity, was significantly associated with the occurrence of myocardial infarction. This study did not confirm a relationship between progression of CAD and baseline DD or CRP during the first 3 years. Baseline DD and CRP do not provide useful risk stratification in patients at high risk for progression of symptomatic CAD. Future studies should evaluate serial levels of these markers to assess their utility in predicting progression of symptomatic PAD.

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