Plasma fibrinogen — a major coronary risk factor

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SUMMARY. The role of plasma fibrinogen as a potential indicator of susceptibility to heart attacks was studied in a sample of 297 men aged 40–69 years at entry who were initially free from overt coronary heart disease. During a mean observation period of 7.3 years (range 0.1–16.1) new heart attacks occurred in 40 men. There was a significant positive correlation between initial plasma fibrinogen levels and the subsequent incidence of heart attacks. In men with high cholesterol or high systolic blood pressure levels the incidence of heart attacks was respectively six times and 12 times greater in those with high plasma fibrinogen levels than in those with low fibrinogen levels. In multivariate models plasma fibrinogen was a highly significant and independent explanatory variable, at least as important as serum cholesterol, blood pressure or cigarette smoking. These results suggest that high plasma fibrinogen levels are an important coronary risk factor and should be included in profiles used to identify those at high risk of heart attacks.

Introduction

THE evidence that the plasma fibrinogen level may be a coronary risk factor in the same sense as plasma cholesterol, blood pressure and cigarette smoking, came initially from a number of case control studies[1-3] in which men with coronary heart disease were found to have higher mean fibrinogen levels than healthy controls, and from a prospective study[4] which reported that men dying from coronary heart disease had higher mean fibrinogen levels at initial examination than those who remained healthy. It has been shown in an angiographic study[5] that raised plasma fibrinogen levels were positively correlated with the extent of coronary artery disease and fibrinogen has also been implicated[6] in the development of atherosclerotic lesions.

We report here the results of a study begun in 1965 in which the role of plasma fibrinogen as a coronary risk factor has been examined using a technically simple and relatively specific fibrinogen assay. The predictive value of the plasma fibrinogen level has been evaluated by both univariate and multivariate statistical analyses. A brief account of some of these results has been published previously.[7]

Method

In a general practice of 2800 patients, men aged 40–69 years were invited to attend for examination with a view to studying the incidence of heart attacks. There were 505 men in this age range of whom 384 (76%) completed all stages of the examination procedure. Of the remaining 121 men 92 either did not attend at all or did not complete the examination procedure. A further 29 had left the area or had died before they could be examined or had developed a major disease which would have excluded them from the study.

We excluded from the 384 men who completed the examination procedure 46 with known coronary heart disease and 24 suffering from diabetes mellitus or from severe hyperlipidaemia or hypertension for which they were already being treated by diet or drugs. Men who were found on examination to have hypertension or hyperlipidaemia discovered for the first time and for which they were not receiving treatment were not excluded. Seventeen men left the area before the end of the study and were lost to follow up. The remaining 297 (59% of all the men in this age range) on whom this report is based remained under the care of one of us (M.C.S.) throughout the study.

Most of the examination procedures and laboratory methods used in this study have been described in an earlier publication;[8] they included a baseline electrocardiogram (ECG) at rest, analysis of plasma lipoproteins by micronephelometry[9] and a colorimetric estimation of serum total cholesterol.[10] The latter method was subsequently changed to an enzymatic assay (CHOD-PAP Boehringer-Mannheim). Plasma fibrinogen was estimated nephelometrically by measuring the light scattering intensity in diluted plasma by heat aggregation, with strict control of pH and temperature.[11,12]

End points were limited to acute heart attacks. Myocardial infarction was diagnosed on the basis of clinical presentation and serial ECG changes which when compared with the baseline ECG (recorded at entry into the study) showed Q wave, ST segment or T wave changes characteristic of Minnesota codes 1.1, IV.1 or V.1. Less severe Q wave, ST segment or T wave changes were accepted when accompanied by characteristic serial enzyme changes — aspartate aminotransferase (AST) and lactic dehydrogenase (LDH) in the early years of the study, or creatine kinase and hydroxybutyrate dehydrogenase (HBD) in later years. Sudden death was judged to have been due to coronary heart disease after autopsy carried out by a pathologist.

Statistical analyses were carried out using the computer package SAS.[13] Correction of event rates to allow for age differences in sub-groups was effected by reference to the linear relationship between the logarithm of the event rate and age, derived from three equal sized age sub-groups in the study itself. Rates were corrected to a mid-observation age of 56 years (that is, average at entry plus half the mean observation period). Because of the skewed distribution of values for plasma fibrinogen and serum very low-density lipoprotein (VLDL) a logarithmic transformation of these variables was used for the majority of the analyses reported.

Results

The 297 men in this study were observed for a total of 2165 man-years, 57% being under observation for at least five years. During the period of observation (mean 7.3 years, range 0.1–16.1 years) new heart attacks occurred in 40 men: 16 died in their first heart attack, 12 more died subsequently and 12 were still alive when the study closed. A further 48 men suffered other major disease events; angina pectoris (7), stroke (2), cardiac failure other than that attributable to coronary heart disease (6), new diabetes mellitus (4), malignant disease (19) and other conditions (10).

Table 1. Comparison between mean values of baseline variables for the 297 men at initial examination.

Variable		New heart attack group ($n=40$)	Heart attack-free group ($n=257$)	'Healthy' group ($n=209$)	New malignant disease group ($n=19$)
Age (years)	Mean	58.4	50.6 ***	49.2 ***	54.6 *
	(CV)[a]	*(12.7)*	*(16.6)*	*(15.9)*	*(14.0)*
Obesity index (kg m^{-2})	Mean	27.1	26.0 *	26.1	25.2 *
	(CV)	*(12.2)*	*(14.7)*	*(14.6)*	*(14.8)*
Cigarettes (no. smoked per day)	Mean	7.9	10.4	9.9	17.9 **
	(CV)	*(122.7)*	*(108.0)*	*(119.2)*	*(66.1)*
Systolic blood pressure (mmHg)	Mean	153.2	131.8 ***	130.2 ***	134.1 *
	(CV)	*(17.1)*	*(19.2)*	*(15.1)*	*(20.5)*
Serum cholesterol (mM)	Mean	6.4	5.6 ***	5.7 **	5.2 ***
	(CV)	*(18.9)*	*(20.8)*	*(21.0)*	*(24.0)*
Plasma fibrinogen[b] (g l^{-1})	Mean	3.92	3.13***	3.00***	3.58
	(CV)	*(3.5)*	*(4.0)*	*(3.6)*	*(5.5)*
Serum VLDL[b,c](g l^{-1})	Mean	1.88	1.44*	1.47*	1.23*
	(CV)	*(11.2)*	*(13.5)*	*(13.7)*	*(14.2)*
Time in study (years)	Mean	6.6	7.4	7.4	7.1
	(CV)	*(68.5)*	*(65.8)*	*(67.7)*	*(43.4)*

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus new heart attack group, assuming that the variances are unequal (except in the few cases where they do not differ significantly between the sub-groups).

[a] CV = coefficient of variation (%) = $\frac{\text{standard deviation}}{\text{mean value}} \times 100$. [b] After log tranformation. [c] VLDL = very low-density lipoproteins. Can be converted into triglyceride levels using the equation: TG (mM) = $\frac{(0.83 \times \text{VLDL}) - 23}{88.6}$.

Baseline variables

Table 1 shows the mean values of the baseline variables in the 'heart attack' group, in the whole of the 'heart attack-free' group, and in the 209 men who remained 'healthy' (that is, free from major disease events throughout the study). Table 1 also shows the mean values of the variables in the 19 men who suffered from new malignant disease. The remaining sub-groups were considered to be too small for meaningful statistical analyses.

The heart attack group had significantly higher mean levels of plasma fibrinogen, systolic blood pressure, serum cholesterol, serum VLDL and obesity index than the heart attack-free and healthy groups, but the mean number of cigarettes smoked was not significantly different between the heart attack and attack-free groups. The malignant disease sub-group had higher fibrinogen levels than the healthy subjects but lower than the heart attack group, but the latter difference was not statistically significant at the 5% level. However, the malignant disease group differed from the heart attack group in a number of other variables, for example, serum cholesterol levels were lower and cigarette smoking was higher in the malignant disease group.

Age-related differences

It can be seen from Table 1 that the heart attack group was significantly older than the attack-free group; therefore, in order to show that the higher mean fibrinogen level in the heart attack patients was not attributable to this age difference, the mean

Table 2. Comparison between mean values of baseline variables at initial examination for men in the heart attack and attack-free groups in two age groups.

Variable		Age 40–54 years		Age 55–69 years	
		Heart attack-free group ($n=180$)	Heart attack group ($n=11$)	Heart attack-free group ($n=77$)	Heart attack group ($n=29$)
Age (years)	Mean	45.9	48.5	61.6	62.1
	(CV)[a]	*(9.5)*	*(8.6)*	*(7.0)*	*(6.6)*
Obesity index (kg m^{-2})	Mean	26.2	28.2	25.6	26.7
	(CV)	*(14.1)*	*(12.6)*	*(16.3)*	*(12.0)*
Systolic blood pressure (mmHg)	Mean	126.8	153.2 *	142.5 †††	153.2 *
	(CV)	*(14.7)*	*(19.2)*	*(15.5)*	*(16.6)*
Serum cholesterol (mM)	Mean	5.7	6.5 *	5.5	6.4 ***
	(CV)	*(21.4)*	*(20.5)*	*(18.9)*	*(18.6)*
Plasma fibrinogen[b] (g l^{-1})	Mean	2.99	4.03***	3.38†††	3.87**
	(CV)	*(3.8)*	*(4.2)*	*(4.5)*	*(3.2)*
Serum VLDL[b](g l^{-1})	Mean	1.52	2.22*	1.30	1.77***
	(CV)	*(14.2)*	*(15.3)*	*(11.5)*	*(9.1)*
Time in study (years)	Mean	7.6	5.4	6.9	7.0
	(CV)	*(64.7)*	*(81.7)*	*(68.4)*	*(64.6)*
Cigarette smokers[c]	%	*54.0*	*82.0*	*55.0*	*38.0*

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus attack-free group. ††† $P<0.001$ versus 40–54 years age group.

[a] CV = Coefficient of variation (%). [b] After log tranformation. [c] Chi-square test.

baseline variables in the heart attack and attack-free groups were compared in the age ranges 40–54 and 55–69 years at entry into the study (Table 2). It can be seen that in the heart attack-free subjects the mean systolic blood pressure and plasma fibrinogen levels were significantly higher in the older than in the younger subjects. However these age-related differences could not account for the highly significant differences in these variables between the heart attack and attack-free groups. These results demonstrate clearly that the difference in fibrinogen levels between the men who had heart attacks and those who had not was independent of age.

Nine of the 11 men in the 40–54 year age group who suffered heart attacks were cigarette smokers compared with only 11 of the 29 older men who had heart attacks. This difference between the age groups was statistically significant (Table 2). However, the difference in the proportion of cigarette smokers between the heart attack and attack-free groups did not reach statistical significance at the 5% level even in the younger age range.

The heart attack incidence rate in this study was 1.85% per annum and the mean age at the event was 64.9 years. In men aged 40–54 years at entry the incidence rate was 0.77% per annum and the mean age of this group at the end of the study was 53.5 years (range 41–69 years). In men aged 55–69 years at entry the rate was 3.96% per annum and the mean age at the end of the study was 68.6 years (range 58–82 years).

Plasma fibrinogen

In these 297 men the plasma fibrinogen level correlated weakly with age ($r = 0.29$), systolic blood pressure ($r = 0.21$), serum cholesterol levels ($r = 0.13$) and VLDL levels ($r = 0.13$), but its multiple correlation with all six baseline variables showed that only 13.5% of its variance was explained by such correlation. Thirty of the 40 new heart attacks occurred in the 98 men who had plasma fibrinogen levels greater than 3.50 g l^{-1}, an incidence of 31%. In men with fibrinogen levels less than 3.51 g l^{-1} the incidence was 5%.

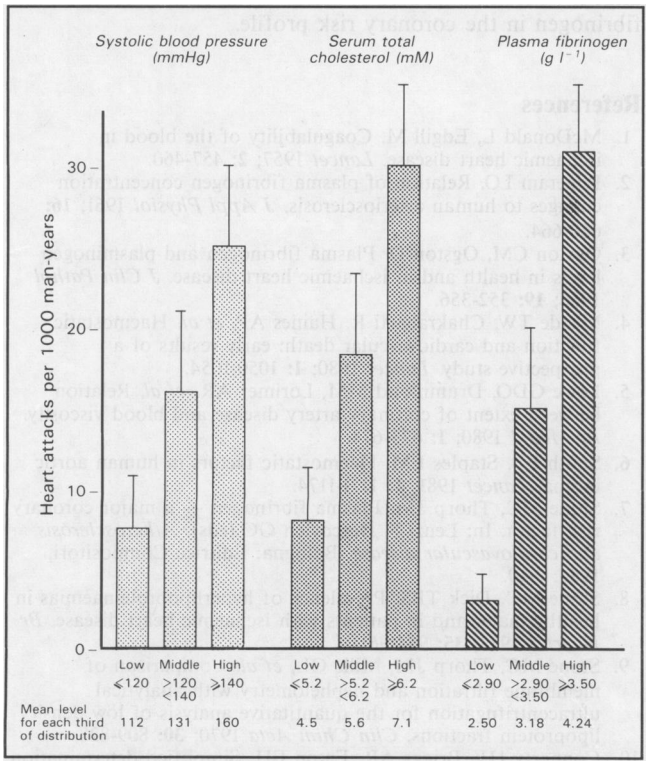

Figure 1. *Heart attack incidence rates (+ standard error of mean) in relation to increasing levels of each variable.*

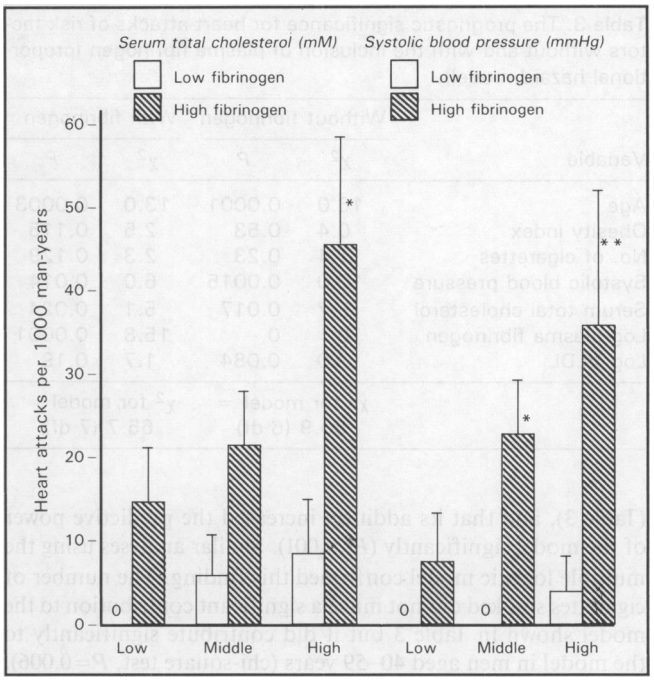

Figure 2. *Heart attack incidence rates (+ standard error of mean) at low and high thirds of fibrinogen for each third of serum cholesterol and systolic blood pressure.* *P<0.05, **P<0.01.

There were 28 men who died from heart attacks before the end of the study. In these men the mean plasma fibrinogen level was significantly higher than in the heart attack-free subjects (4.02 versus 3.13 g l^{-1}, $P<0.001$). Furthermore the 16 men who died in their first heart attack had significantly higher mean fibrinogen levels than the 12 men who survived their first heart attack and lived to the end of the study (4.29 versus 3.69 g l^{-1}, $P=0.025$), but these two sub-groups did not differ with respect to any other variable.

The association between the incidence of heart attacks and three of the baseline variables — systolic blood pressure, serum total cholesterol levels and plasma fibrinogen levels — was compared by calculating the incidence rate in each third of the distribution of each variable and correcting for age (Figure 1). All three univariate relationships were statistically significant: the Spearman rank correlation coefficients were 0.30 for systolic blood pressure, 0.22 for serum cholesterol and 0.34 for log plasma fibrinogen.

A number of bivariate relationships were also examined, for example, men in each third of the serum cholesterol or systolic blood pressure distribution were also ranked according to their plasma fibrinogen level. Men whose serum cholesterol was in the top third and who also had plasma fibrinogen levels in the top third had a six times greater incidence of heart attacks than those with plasma fibrinogen in the bottom third (Figure 2). Men with systolic blood pressure in the top third who also had plasma fibrinogen levels in the top third had a 12 times higher heart attack rate than those with plasma fibrinogen in the bottom third (Figure 2).

Multivariate analyses (by the proportional hazard method)[14] showed that the contribution of log fibrinogen in predicting heart attacks was high compared with most of the other variables

Table 3. The prognostic significance for heart attacks of risk factors without and with the inclusion of plasma fibrinogen (proportional hazard model).

	Without fibrinogen		With fibrinogen	
Variable	χ^2	P	χ^2	P
Age	16.0	0.0001	13.0	0.0003
Obesity index	0.4	0.53	2.5	0.115
No. of cigarettes	1.4	0.23	2.3	0.129
Systolic blood pressure	10.0	0.0015	6.0	0.014
Serum total cholesterol	5.7	0.017	5.1	0.024
Log plasma fibrinogen	0	0	15.8	0.0001
Log VLDL	3.0	0.084	1.7	0.19
	χ^2 for model = 50.9 (6 df)		χ^2 for model = 65.7 (7 df)	

(Table 3), and that its addition increased the predictive power of the model significantly ($P<0.001$). Similar analyses using the multiple logistic model confirmed this finding. The number of cigarettes smoked did not make a significant contribution to the model shown in Table 3 but it did contribute significantly to the model in men aged 40–59 years (chi-square test, $P=0.006$).

Discussion

The predictive power of plasma fibrinogen levels for the incidence of heart attacks reported here is consistent with earlier reports.[4,15] However, the results differ from these studies in certain respects, for example, in the Northwick Park Study[4] a higher proportion of coronary heart disease events (7 out of 25) occurred in the lowest third of fibrinogen than in the present study (1 out of 40), and in the Gothenberg subjects,[15] fibrinogen was associated with an increased risk of myocardial infarction only by univariate analysis. A possible explanation of part of these differences may lie in the methods used for estimating fibrinogen. Our nephelometric fibrinogen assay differs from methods used in earlier studies which employed techniques involving thrombin clotting of fibrinogen. A comparison of the two methods has demonstrated a high correlation between them[11] except in conditions associated with high levels of fibrinogen degradation products, when thrombin clotting methods tend to give lower values for fibrinogen than those found with the nephelometric technique used in this study.

Our results are also consistent with findings in the Stockholm prospective study[16] in which erythrocyte sedimentation rate (ESR) was positively correlated with heart attack incidence. A significant correlation between ESR and fibrinogen has been reported[17] as has a strong correlation between fibrinogen level and reduction in ESR after defibrination.[18] However ESR (which is significantly affected by the haematocrit levels) explains only about 50% of the variance in fibrinogen. Fibrinogen therefore provides a more direct assessment of acute phase protein change than is provided by the ESR.

Like other measured variables associated with coronary heart disease plasma fibrinogen varies from time to time even in healthy individuals. It responds to physiological changes (for example, pregnancy and varying thyroid activity) and also to injury, inflammation, malignant disease and other stresses (the acute phase reaction). It can be seen from Table 1 that in the present study the mean plasma fibrinogen levels were elevated in both the new heart attack group and also in the men who developed malignant disease. The heart attack group had higher fibrinogen levels than the malignant disease group but this difference was not statistically significant at the 5% level. However, these two disease sub-groups differed markedly in some of the other variables, for example, serum cholesterol levels were significantly lower and the number of cigarettes smoked significantly higher in the malignant disease group.

A pathway by which tissue reactions such as inflammation, malignant disease or atherosclerosis may influence circulating plasma fibrinogen levels has been reported.[19] This pathway involves the local production of fibrinogen degradation products (particularly fragment D) which stimulate macrophages to release a regulator protein (hepatocyte stimulating factor) which increases hepatic synthesis of fibrinogen and other acute phase proteins.

It is possible therefore that atherosclerosis and its complications provoke this acute phase response as a chronic phenomenon before clinical manifestations become apparent, and our data are consistent with this hypothesis. However, raised levels of plasma fibrinogen may be more than merely a marker for vascular system damage but may also be involved in the development of atherosclerosis. Smith and colleagues found high concentrations of fibrinogen in gelatinous intimal lesions and were able to show an association between fibrinogen and the binding of low-density lipoproteins within the intimal gel, and Copley[20] has suggested that an early event in atherosclerosis is the adsorption of low-density lipoproteins to the endothelial fibrin lining. In addition to its possible implication in the development of atherosclerosis fibrinogen is also a major factor in thrombosis, platelet[21] and red cell aggregation, and in blood viscosity particularly in small vessels.

This small prospective study, carried out entirely within a single general practice, has demonstrated that the plasma fibrinogen level is a significant, independent risk factor for coronary heart disease, at least as important as blood pressure, lipid levels or cigarette smoking. Our results suggest therefore that the identification of men at high risk of sustaining heart attacks would be improved by including an estimation of plasma fibrinogen in the coronary risk profile.

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Acknowledgements

We thank Mr J.A. Lewis of Imperial Chemical Industries Pharmaceuticals Division for helpful statistical advice and Dr T.W. Meade and Dr K.G. Green for their valuable criticism. We gratefully acknowledge research grants from the Medical Research Council (during the early days of this study) and from the North Western Regional Health Authority and Imperial Chemical Industries Pharmaceuticals Division.

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