Haemostatic factors associated with ischaemic heart disease in men aged 45 to 64 years
The Speedwell study

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SUMMARY In the Speedwell study set up to examine primarily the relation of plasma lipids and ischaemic heart disease in men aged 45 to 64 years drawn randomly from the practices of 16 general practitioners, various haemostatic factors which may contribute both to thrombogenesis and atherogenesis were measured. Fibrinogen measured nephelometrically and plasma viscosity were positively associated with the prevalence of ischaemic heart disease. Antithrombin III was negatively associated with the prevalence of ischaemic heart disease. These associations were statistically significant at the 1% level of probability. Fibrinogen measured by a clotting method and the heparin neutralising activity of platelet poor plasma had negative and positive associations with ischaemic heart disease, respectively, but neither association achieved statistical significance. Because of the interrelation of these variables and also age, a multiple logistic regression analysis was undertaken separately for the two measures of fibrinogen. The negative association of "clottable" fibrinogen and the positive association of plasma viscosity with the prevalence of ischaemic heart disease were confirmed and both were statistically significant at the 5% level of probability. Apart from age the independent association of the other variables with ischaemic heart disease did not achieve statistical significance. Fibrinogen measured by both methods had positive and statistically significant associations with serum total cholesterol, but no associations with serum total triglycerides, smoking, or alcohol consumption. "Clottable" fibrinogen had an inverse and statistically insignificant association with serum high density lipoprotein cholesterol. The observed associations support the concept of the involvement of some haemostatic factors in the aetiology of ischaemic heart disease, and these associations are now being examined more critically in a longitudinal study.

In considering the aetiology of ischaemic heart disease it is necessary to study the elements of thrombogenesis as well as atherogenesis. Patients dying from myocardial infarction show evidence of coronary artery thrombosis, which antedates the infarction, as well as atheroma.[1][2] Other evidence indicates that substances in the blood which are thrombogenic may also be involved in the process of atherogenesis and arterial occlusion and thereby other clinical manifestations of ischaemic heart disease. Fibrin and platelets are present in mural thrombi and atherosclerotic plaques.[3–6] In developing plaques in the human aorta, Smith et al.[7] found increased concentrations of free

low density lipoprotein and fibrinogen and in more advanced plaques high concentrations of the same lipoprotein bound to fibrin. Platelets may also aggregate and embolise causing myocardial damage and irritability[8][9]; or by the release of active substances alter arterial tone[10]; or contribute to atheroma formation.[11] At the same time, a number of elements exist, like antithrombin III, which can neutralise activated clotting factors and which may influence the extent of the above thrombotic and atherogenic processes. Finally, fibrinogen is also important in determining plasma viscosity and thereby influencing blood flow through occluding arteries.

Within the design of the Speedwell study[12] which is to examine longitudinally the relation between serum lipoproteins and ischaemic heart disease the feasibility

of measuring some of these haemostatic factors was considered. Selection of the factors measured was influenced by the need to collect large numbers of blood specimens at a distance from laboratories as well as intra- and inter-subject variability of the individual factors. The results of the pilot study and the associations between the factors measured and prevalent ischaemic heart disease and other risk indications for ischaemic heart disease are reported.

Patients and methods

The design of the study has been described in the paper giving results of lipid and related measurements and ischaemic heart disease in the same population.[12] Venous blood samples were collected from men aged 45 to 64 years who were in a fasting state in the early morning. For subjects reporting minor illness, or major illness or surgery, blood samples were collected, respectively, two weeks or three months later. Fibrinogen was estimated by two different methods. "Clottable" fibrinogen (C) was measured in citrated plasma by the method of Clauss.[13] In the second method using EDTA plasma, fibrinogen (N) was measured by a heat precipitation nephelometric method.[14] Plasma viscosity was measured within a few hours of blood collection in the EDTA specimen using the method of Harkness.[15] Platelet count and haematocrit were determined by the Coulter S counter. Antithrombin III was measured biologically in serum by the method of von Kaulla[16] and immunologically by single radial immunodiffusion according to the method of Mancini *et al*.[17] The heparin neutralising activity of platelet poor plasma was measured using the heparin thrombin clotting time test.[18] The plasma response to thrombin was also monitored by a dilute thrombin clotting time test.[18] Estimations of platelet aggregation response to dilutions of adenosine diphosphate were carried out turbidometrically on citrated platelet rich plasma within two hours of collection. Within the four dilutions of adenosine diphosphate used we were unable to obtain consistent patterns of aggregation with which to quantify the responses of subjects and relate them to the prevalence of ischaemic heart disease. These estimations were discarded. Determination of the prevalence of ischaemic heart disease and other "risk factors" has been described in the paper giving results for the lipid analysis.

Results

Reproducibility of measurements was estimated from split specimens of blood taken from a subsample of subjects. Expressed as coefficients of variation, the estimations of reproducibility are shown in Table 1.

Table 1 *Reproducibility—duplicate pair analysis*

Test	No. of pairs	Overall mean	Coefficient of variation (%)
Fibrinogen			
Clottable (g/l)	46	2·97	7·5
Nephelometric (g/l)	46	4·20	6·0
Viscosity (centipoises)	47	1·65	1·8
Thrombin clotting time (s)	46	17·4	6·1
Heparin thrombin clotting			
time (s)	46	31·1	14·1
Antithrombin III			
Biological (s)	44	37·5	27·8
Immunological (g/l)	44	0·22	11·6
Platelet count (10^9/l)	47	184 000	8·2
Haematocrit (%)	47	0·45	2·5

Reproducibility of all measurements was acceptable except for the "biological assay" of antithrombin III by the von Kaulla method.

Mean values for the various factors measured for the population divided into those with "any" evidence of ischaemic heart disease by history and electrocardiogram and those without any such evidence are shown in Table 2. The variables for which the difference between those with and without ischaemic heart disease reached statistical significance were fibrinogen (N), plasma viscosity, and antithrombin III by immunological assay. While fibrinogen (N) levels were higher in men with ischaemic heart disease, fibrinogen (C) was higher in men without ischaemic heart disease. Antithrombin III activity estimated by the von Kaulla test gave differences for men with and without ischaemic heart disease in the same direction as that measured immunologically, but the differences were not statistically significant. The thrombin clotting times were similar in men with and without evidence of ischaemic heart disease. The heparin thrombin clotting times were shorter in men with ischaemic heart disease, indicating increased heparin neutralising activity (Table 3).

Interrelations existed between age, fibrinogen measured by both methods, heparin thrombin clotting times, viscosity, and antithrombin III as shown in the correlation matrix in Table 4. As fibrinogen has heparin neutralising activity and contributes significantly to plasma viscosity, a multiple logistic regression analysis was performed to see which if any of these variates (excluding antithrombin III) were independently associated with ischaemic heart disease. The regression was performed twice—one model included fibrinogen (N) and the second model included fibrinogen (C). Table 5 shows the regression coefficients for both models expressed in standard units. These units show the change in the logit of risk for one standard deviation change in the variable and allow for easier comparison of the relative importance of the independent variables. Age was an important predictor of ischaemic heart disease in both models and its positive associations were statistically

Table 2   *Association of ischaemic heart disease with haematological variables*

Variable	No ischaemic heart disease		Any ischaemic heart disease	
	No. of subjects	Mean (SD)	No. of subjects	Mean (SD)
Fibrinogen				
Clottable (g/l)	226	2·97 (0·63)	55	2·87 (0·61)
Nephelometric (g/l)	223	4·02 (0·93)	53	4·39 (0·85)*
Viscosity (centipoises)	223	1·64 (0·09)	53	1·69 (0·10)*
Platelet count (10⁹/l)	221	184 (54)	52	188 (51)
Haematocrit	225	0·455 (0·03)	54	0·448 (0·04)
Antithrombin III				
Biological (s)	217	37·3 (17·1)	52	34·8 (16·8)
Immunological (g/l)	217	0·23 (0·03)	54	0·21 (0·03)*

*$p<0.01$.

Table 3   *Association of ischaemic heart disease and heparin thrombin clotting test*

Variable	No ischaemic heart disease		Any ischaemic heart disease	
	No. of subjects	Mean (SD)	No. of subjects	Mean (SD)
Thrombin clotting test time (s)	223	17·4 (1·2)	54	17·6 (1·2)
Heparin thrombin				
clotting test time (s)	223	32·2 (10·0)	54	30·0 (8·8)

Table 4   *Haematological variables—correlation matrix*

	Age	Fibrinogen (N)	Fibrinogen (C)	Viscosity	Heparin thrombin clotting time	Antithrombin III (i)
Age	1					
Fibrinogen (N)	0·24***	1				
Fibrinogen (C)	0·09	0·40***	1			
Viscosity	0·20**	0·60***	0·35***	1		
Heparin thrombin						
clotting time	−0·12*	−0·40***	−0·46***	−0·35***	1	
Antithrombin III (i)	−0·19**	−0·14*	0·14*	−0·13*	0·09	1

Fibrinogen (N), nephelometric; Fibrinogen (C), clottable; Antithrombin III (i), immunological.
No. of subjects varies between 265 and 281.
*$p<0.05$, **$p<0.01$, ***$p<0.001$.

Table 5   *Multiple logistic regression coefficients for two models with different estimations of fibrinogen*

Variable	Standardised regression coefficient (SE)	Variable	Standardised regression coefficient (SE)
Age	0·365 (0·164)	Age	0·388 (0·166)
Fibrinogen (N)	0·068 (0·214)	Fibrinogen (C)	−0·513 (0·200)
Viscosity	0·336 (0·198)	Viscosity	0·497 (0·180)
Heparin thrombin		Heparin thrombin	
clotting time	−0·045 (0·180)	clotting time	−0·262 (0·196)

Fibrinogen (N), nephelometric; Fibrinogen (C), clottable.

significant ($p<0.05$). In the model containing the variate of fibrinogen (C), both fibrinogen and viscosity showed moderate associations in opposite directions with ischaemic heart disease, and both associations were statistically significant ($p<0.01$). A moderate positive association of viscosity with ischaemic heart disease was shown also in the other model, but this association did not reach statistical significance at the 5% level of probability. The associations of fibrinogen measured by the two methods with ischaemic heart disease independent of the effect of age, viscosity, and heparin thrombin clotting times were in opposite directions. The heparin thrombin clotting times are negatively associated with ischaemic heart disease (that is positive associations for the heparin neutralising activity measured), but the associations in both models are not statistically significant at the 5% level.

Fibrinogen measured by both methods was positively associated with age (Table 4). Positive but weak associations with serum total cholesterol existed with

both measures of fibrinogen (fibrinogen (N) $r=0.15$, $p<0.01$; fibrinogen (C) $r=0.18$, $p<0.01$). A negative but statistically insignificant association ($r=-0.11$) existed between serum high density lipoprotein cholesterol and fibrinogen (C). No association was shown with fibrinogen (N) and high density lipoprotein cholesterol, or between either of the two measures of fibrinogen and serum total triglycerides. Fibrinogen (N) had a weak positive association ($r=0.12$, $p<0.05$) with the obesity index, weight-height^2. No associations with smoking habit or alcohol consumption were apparent with either measure of fibrinogen.

Plasma viscosity showed positive associations with serum total cholesterol ($r=0.23$, $p<0.001$) and serum total triglycerides ($r=0.22$, $p<0.001$). No further associations were shown for smoking, alcohol consumption, or obesity index with plasma viscosity, heparin thrombin clotting times, or antithrombin III. Antithrombin III measured immunologically had an inverse association with age (Table 4).

Discussion

The study was satisfactory in that 86% of the random samples of men gave fasting blood specimens on which the haemostatic factors were measured. The reproducibility of the measurements, except for the von Kaulla biological assay for antithrombin III, was acceptable. Estimations of platelet aggregation response to dilutions of adenosine diphosphate turbidometrically were inconsistent over the period of the study. Because of the difficulties of interpretation and quantification, this form of assessment of platelet aggregation seems unsuitable for large epidemiological studies.

The two different methods of measuring fibrinogen gave associations with the selected manifestations of ischaemic heart disease. It is not uncommon for measurements of different properties of proteins to have dissimilar associations with other variables. The correlation coefficient of the two measurements of fibrinogen was 0·40. Hampton *et al.*,[19] using a chemical estimation of fibrinogen, found higher mean levels and greater variability in levels on repeated samples in subjects with ischaemic heart disease than in subjects of similar age without a history or electrocardiographic evidence of ischaemic heart disease.[19] Pilgeram[20] using a method of estimation of fibrinogen which depends on its conversion to fibrin and spectrophotometric quantification of fibrin yield by ninhydrin found fibrinogen levels 20% higher in patients six months after myocardial infarction than in age matched controls. Meade *et al.*[21] have shown positive associations between prevalent ischaemic heart disease and clottable fibrinogen levels in men aged 40

to 64 and also increased fibrinogen levels in the same population of men who die from cardiovascular disease compared with those who survive over a mean 33 month follow up period. Further studies by Pilgeram[22] have indicated that raised fibrinogen in patients with coronary thrombosis is associated with an increased rate of biosynthesis and turnover of the molecule. There may also be defects in those patients' capacity to lyse fibrin.[22]

Fibrinogen measured by both methods was associated with age and serum total cholesterol, but not with smoking or alcohol consumption as in the studies by Meade *et al.*[23] and Korsan-Bengtsen *et al.*[24] The differences in association may relate to selective factors acting upon the populations studied as well as to differences in measurements. Fibrinogen in this study determined some of the variation in plasma viscosity. But plasma viscosity itself was shown to be independently and positively associated with ischaemic heart disease. Plasma viscosity was also positively associated with serum total cholesterol and total triglycerides. Plasma viscosity determined by fibrinogen, other proteins, lipids, and glucose will influence blood viscosity which is determined further by erythrocyte flexibility and mass.

The heparin neutralising activity of platelet poor plasma may be derived from a variety of sources. Heparin neutralising activity is raised after acute myocardial infarction and in the post-infarction state[25][26] and this rise relates, at least in part, to raised fibrinogen levels. When fibrinogen and other heat labile proteins are removed by heating platelet poor plasma, however, the heparin neutralising activity of the plasma remains raised in a proportion of cases of acute myocardial infarction and other thrombotic conditions.[27] Thus the heparin thrombin clotting time test must be considered as a non-specific measure of the heparin neutralising activity of a number of proteins in platelet poor plasma which have association with ischaemic heart disease.

Antithrombin III was measured in serum and represents residual levels after clotting. The lower levels of antithrombin III observed in subjects with ischaemic heart disease may represent lower pre-existing plasma levels contributing to a prothrombotic state or increased usage during in vitro clotting of blood containing higher concentrations of thrombogenic substances.

In considering haemostatic factors in the aetiology of ischaemic heart disease it is realised that there are a large number of such factors which could be measured. Histological studies indicate the involvement of various factors like fibrinogen, fibrin, and platelets. But other work now indicates the importance of endothelial damage,[28] intraendothelial and intraplatelet prostaglandins,[10] other clotting factors,[21] and

mechanisms that neutralise or lyse thrombotic factors[23] in the processes of thrombogenesis and atherogenesis. Generating and testing hypotheses with these large numbers of factors and mechanisms in epidemiological studies concerned with the aetiology of ischaemic heart disease will be exhausting of resources and public co-operation. The problem is being tackled by separate studies determining initially associations of ischaemic heart disease and its risk indicators with different factors and by the use of more global, non-specific tests like the heparin thrombin clotting times.[18]

The association of blood levels of the above factors with ischaemic heart disease does not necessarily imply causation, and their interpretation must be viewed with caution in cross-sectional studies in particular when only those subjects surviving ischaemic heart disease and other diseases are available. This study has shown both the feasibility of measuring some haemostatic factors in a random population and interesting associations with ischaemic heart disease and some of the established risk indicators. These factors, and others having associations with cardiovascular deaths in the Northwick Park heart study,[21] are now being measured in a large random sample of men aged 45 to 59 to determine their associations with the incidence of ischaemic heart disease.

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