Haemostatic factors and ischaemic heart disease

The Caerphilly study

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SUMMARY In a preliminary report from the Caerphilly study four haemostatic factors showed univariate associations with prevalent ischaemic heart disease after adjusting for age. These factors were fibrinogen concentration, plasma viscosity, white cell count, and the heparin-thrombin clotting time. Age and these haemostatic variables were entered into a stepwise multiple logistic regression analysis; after age the white cell count and heparin-thrombin clotting time remained significantly associated with ischaemic heart disease. Further regression analyses indicated that diastolic blood pressure contributed additionally to this association with ischaemic heart disease but that smoking habit did not.

Epidemiological studies have indicated that existing risk factors such as plasma cholesterol concentration, smoking habit, and hypertension predict only a modest proportion of new cases of ischaemic heart disease.¹ Data on possible haemostatic mechanisms in the pathogenesis of ischaemic heart disease are scarce, but two prospective studies have indicated that haemostatic factors may be independently predictive of ischaemic heart disease and stroke respectively.^{2,3} A recent postmortem case series of subjects with ischaemic heart disease who had died within six hours after the onset of chest pain found coronary thrombi present in 74% of cases.⁴ No intraluminal thrombi were found in age matched controls.

These reports provide evidence that haemostatic factors may be implicated in the pathogenesis of ischaemic heart disease. Several such factors are being investigated in the Caerphilly prospective study. Preliminary data are available for cases of ischaemic heart disease defined cross sectionally by the use of a standardised questionnaire⁵ and a 12 lead electrocardiogram. These data form the basis of the present report.

Subjects and methods

STUDY POPULATION

A random sample of men was drawn from the electoral registers for the town of Caerphilly, Mid Glamorgan, and two outlying villages (population 38 000). All men were sent an initial letter but were considered to be eligible only if born between, or in, 1911 and 1949—that is, were aged between 30 and 69 at the time of the survey. A total of 797 men came into this category and were invited to attend a local clinic. They were seen from September 1979 to September 1980.

SURVEY METHODS

Detailed medical questionnaires, which included the London School of Hygiene questionnaire on ischaemic heart disease,⁵ were administered at an evening clinic; anthropometry⁶ and 12 lead electrocardiography were carried out. Casual blood pressures were measured by a single observer using a Hawksley random zero sphygmomanometer.⁷ Electrocardiographic tracings were coded independently by two experienced observers according to the Minnesota convention.⁵ Subjects were classified as having ischaemic heart disease if any of the following criteria were satisfied: a definite history of myocardial infarction (chest pain defined by the London School of Hygiene questionnaire with hospital admission in excess of five days plus doctor's diagnosis); angina

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Accepted for publication 8 January 1984

Table 1 Number (%) of men with or without ischaemic heart disease (IHD), according to age

	Age (years)			Total
	30-44	45-54	55-69	
No IHD	297 (90)	141 (78)	143 (71)	581 (82)
Any IHD	33 (10)	39 (22)	58 (29)	130 (18)

(defined by the same questionnaire); Q, ST, or T wave changes or left bundle branch block (Minnesota codes 1.1-1.3, 4.1-4.4, 5.1-5.3, or 7.1) on the electrocardiogram.

Each man was invited to attend an early morning clinic, where a single venous blood sample was taken with minimum stasis after a minimum fast of eight (mean 12) hours. Aliquots were placed in two tubes containing sodium citrate (3.8%) and one containing edetic acid. Plasma was separated, usually within one hour after collection.

LABORATORY METHODS

Fibrinogen concentration was estimated by two methods; a nephelometric determination after heat precipitation in buffered saline (Fibrinogen N)⁸ and a clotting assay (Fibrinogen C).⁹ Plasma viscosity was estimated on plasma in edetic acid by the method of Harkness.¹⁰ The heparin neutralising activity of platelet poor plasma was measured using the heparin-thrombin clotting time.¹¹ A test of dilute thrombin clotting time¹¹ was also carried out and was used in part to screen out samples that may have been affected by partial clotting. A value of ≥ 25 s for the dilute thrombin clotting time was used. Subjects with values above or equal to this had a mean heparin-thrombin

clotting time of 82 s, whereas subjects with a thrombin time of <25 s had a mean heparin-thrombin clotting time of 31 s. Samples from six subjects, two of whom had some evidence of ischaemic heart disease and four of whom did not, were excluded on these grounds. Antithrombin III concentrations were estimated using a chromogenic assay (antithrombin III C)¹² and also immunologically (antithrombin III I).¹³

A full blood count, which included a white cell and platelet count, was done using a Coulter model S-plus cell counter in whole blood anticoagulated with edetic acid. Cell counts were carried out within four to 10 (mean six) hours after venepuncture and the nephelometric fibrinogen and plasma viscosity usually within 12 hours after collection. The assays on platelet poor plasma (the heparin-thrombin clotting time, thrombin time, and clottable fibrinogen) were carried out after a standard delay overnight of 28 to 32 hours. In hot or warm weather conditions a frozen cooler pack was placed inside the container. Antithrombin III assays were carried out in large batches on platelet poor citrated plasma frozen at -20°C and stored for up to three months.

A 5% sample of duplicate samples was presented "blind" to each laboratory during the course of the study. The coefficients of variation of the duplicate pairs for fibrinogen N and fibrinogen C concentrations, viscosity, white cell count, heparin-thrombin clotting time, platelet count, and antithrombin III C and antithrombin III I concentrations were 7%, 11%, 2%, 3%, 24%, 7%, 11%, and 10%, respectively. A second blood sample was taken from a small group of men ($n=8-12$) some four to 12 weeks after the initial sample. Coefficients of variation for these pairs of results for fibrinogen N and fibrinogen C concentrations, viscosity, white cell count, and heparin-

Table 2 Mean (SD) values of haemostatic variables in subjects with or without any evidence of ischaemic heart disease (IHD)

Variable	Age (years)	No IHD		Any IHD	
		No of subjects	Mean (SD)	No of subjects	Mean (SD)
Fibrinogen N (g/l)	<45	286	3.2 (0.7)	33	3.6 (0.8)
	45-54	134	3.6 (0.8)	37	3.9 (0.8)
	55-69	141	3.8 (1.0)	57	4.1 (0.8)
Fibrinogen C (100/s)	<45	279	7.1 (1.4)	29	7.7 (1.5)
	45-54	130	7.5 (1.5)	33	7.8 (1.8)
	55-69	138	8.0 (1.5)	56	8.5 (1.7)
Viscosity (cP)	<45	286	1.68 (0.09)	33	1.70 (0.10)
	45-54	132	1.71 (0.09)	38	1.76 (0.14)
	55-69	142	1.74 (0.13)	57	1.75 (0.13)
Heparin-thrombin clotting time* (log s)	<45	275	29.7 (14-62)	29	25.6 (12-55)
	45-54	128	30.0 (14-64)	33	27.2 (11-69)
	55-69	138	28.9 (14-61)	55	24.7 (12-49)
White cell count* (log $10^9/\text{l}$)	<45	287	7.0 (4.1-12.0)	33	7.6 (4.2-13.8)
	45-54	135	7.1 (4.1-12.1)	38	7.5 (4.2-13.6)
	55-69	142	7.0 (4.4-11.3)	57	8.1 (4.6-14.3)

Fibrinogen N, nephelometric; fibrinogen C, clottable.

*For log transformed variables the mean is the antilog of the mean value on the log scale. Range values given are the antilogs of mean -2SD and mean $+2\text{SD}$ on the log scale—that is, the range within which 95% of the values lie.

Table 3 Correlation matrix for haemostatic variables and age

	Fibrinogen N	Fibrinogen C*	Viscosity	Heparin-thrombin clotting time†	White cell count†
Age	0.34	0.30	0.24	-0.08	-0.06
Fibrinogen N		0.62	0.60	-0.43	0.41
Fibrinogen C*			0.47	-0.66	0.31
Viscosity				-0.33	0.25
Heparin-thrombin clotting time†					-0.34

Fibrinogen: N, nephelometric; C, clottable.

* Measured as 100/s.

† Transformed to logarithms.

Numbers of pairs of values on which correlation coefficients are based range from 646 to 692. All coefficients are significantly different from zero at $p < 0.001$ except those between heparin-thrombin clotting time and age when $p < 0.05$ and between white cell count and age, which is not significant.

thrombin clotting time were 10%, 18%, 2%, 11%, and 21% respectively.

Results

Of 797 men eligible for inclusion in the study, 711 (89%) were examined and 700 (88%) provided a blood sample. Where tables include fewer than 700 observations a test may not have been carried out owing to insufficient blood or some other technical reason.

Subjects were classified as having ischaemic heart disease if one or more of the criteria described above were satisfied. Table 1 shows that 33 subjects (10%) aged 30 to 44 and 58 (29%) aged 55-69 had some evidence of ischaemic heart disease classified by the use of epidemiological criteria.

The relation between ischaemic heart disease and the haemostatic variables was examined in a series of detailed analyses. Several of the haemostatic variables were dependent on age, and after age adjustment five of the haemostatic variables showed significant associations with ischaemic heart disease: fibrinogen N concentration ($p < 0.001$), fibrinogen C concentration ($p < 0.01$), viscosity ($p < 0.05$), white cell count ($p < 0.001$), and heparin-thrombin clotting time ($p < 0.001$). Two variables (heparin-thrombin clotting time and white cell count) had distributions that were appreciably skewed, and in all analyses these data were transformed to logarithms. Platelet count and the two assays for antithrombin III did not show significant associations with ischaemic heart disease, and most subsequent analyses do not include these variables.

Table 2 shows the mean values of the five haemostatic variables that were significantly associated with ischaemic heart disease by age and ischaemic heart disease category.

Table 3 shows the correlation matrix for the five haemostatic variables and age. There were highly significant ($p < 0.001$) associations between the haemostatic variables; those between the fibrinogen concentrations and viscosity and between the

fibrinogen concentrations and heparin-thrombin clotting time were particularly strong. The associations between age and the haemostatic variables were much less pronounced. There were significant positive associations between fibrinogen concentrations, viscosity, and age. The correlation coefficients between heparin-thrombin clotting time and age and between white cell count and age were both very small.

Age and the five haemostatic variables were then entered into a stepwise multiple logistic regression analysis with prevalent ischaemic heart disease as the dependent variable. Table 4 shows a summary of the results. Age entered the regression model first followed by white cell count and heparin-thrombin clotting time. Neither the fibrinogens nor viscosity added significantly to this basic model. Regression coefficients were standardised by transforming each independent variable to zero mean and unit standard deviation. The regression coefficients for the different variables are thus directly comparable.

Smoking habit was strongly related to many of the haemostatic variables. Table 5 shows that cigarette smokers had a much higher mean white cell count and much lower mean heparin-thrombin clotting time than non-smokers, while exsmokers and cigar and pipe smokers held an intermediate position.

The addition of smoking habit to the basic regression model did not add significantly to the model and did not affect the coefficients for white cell count and heparin-thrombin clotting time in other than a trivial

Table 4 Standardised regression coefficients (SE) from stepwise multiple logistic regression

Variable	Standardised coefficient† (SE)	t
Age	0.596 (0.112)	5.3***
White cell count‡	0.387 (0.116)	3.3***
Heparin-thrombin clotting time‡	-0.239 (0.118)	-2.0*

$p < *0.005; ***0.001$.

† Regression coefficient standardised by transforming independent variable to zero mean and unit standard deviation.

‡ Transformed to logarithms.

Table 5 Effect of smoking habit on white cell count and heparin-thrombin clotting time

Smoking habit	White cell count		Heparin-thrombin clotting time	
	No of subjects	Mean (95% CI)*	No of subjects	Mean (95% CI)*
Non-smoker	129	6.0 (5.8-6.2)	120	33.9 (31.8-36.1)
Ex-smoker	173	6.4 (6.2-6.6)	164	31.1 (29.2-33.2)
Cigar/pipe smoker	74	7.2 (6.8-7.7)	73	29.4 (26.9-32.1)
Cigarette smokers:				
1-14/day	92	8.1 (7.7-8.5)	87	25.9 (24.1-27.9)
15-24/day	113	8.4 (8.0-8.8)	112	25.7 (24.1-27.4)
≥25/day	107	8.2 (7.8-8.6)	98	25.7 (24.1-27.5)

*Mean values and 95% confidence interval (CI) for the mean were calculated on logarithmic scale and transformed back to natural units.

manner. For the purpose of this analysis smoking habit was treated as a factor at six levels (Table 5). A similar lack of effect was found when alcohol consumption and obesity (Quetelet's index) were added to the basic model. The addition of diastolic blood pressure significantly improved ($\chi^2 = 8.6$; $p < 0.01$) the fit of the model but did not alter the association between prevalent ischaemic heart disease and either white cell count or heparin-thrombin clotting time. Table 6 shows the values of the standardised regression coefficients obtained. The addition of systolic instead of diastolic blood pressure yielded a similar but slightly weaker effect ($\chi^2 = 6.6$; 1 df; $p < 0.01$). Values of χ^2 for the non-significantly associated variables were as follows: smoking habit (2.0, 5 df); nephelometric fibrinogen concentration (1.4, 1 df); amount of clottable fibrinogen (0.1, 1 df); log alcohol (0.7, 1 df); Quetelet's index (0.1, 1 df); viscosity (0.1, 1 df).

Discussion

This report indicates that certain haemostatic factors have a close association with prevalent ischaemic heart disease. This largely confirms the findings of a pilot study among the population of Speedwell in Bristol, in which identical laboratory techniques were used in the case of fibrinogen, heparin-thrombin clotting time, and viscosity.¹⁴ It is fully compatible with the concept of a hypercoagulable state, shown to be associated with incident ischaemic heart disease in an early report.² Many haemostatic factors are

rapidly modified by stressful stimuli or as a response to infection in the "acute phase response"^{15 16}; among these factors are fibrinogen and white cell count, which both show strong univariate (but age adjusted) associations with prevalent ischaemic heart disease in the present data. Smoking habit is also associated with higher than average concentrations of fibrinogen^{17 18} and white cell count; and also with increased heparin neutralising activity of plasma reflected in the decrease of heparin-thrombin clotting time. The present data, however, fail to show an association between prevalent ischaemic heart disease and smoking habit. This is true of both largely symptomatic subjects and asymptomatic subjects. This is contrary to expectations generated by prospective studies^{19 20} and may be due to selection, through survival, of prevalent ischaemic heart disease compared with the complete identification in incident studies and also stopping smoking in symptomatic cases. All variables associated with the acute phase response show significant intercorrelations (Table 3) (white cell count, nephelometric fibrinogen, heparin-thrombin clotting time, and viscosity). Further regression analyses, however, indicate that: any association between viscosity and ischaemic heart disease disappears when nephelometric fibrinogen concentration is included in the model; the inclusion of heparin-thrombin clotting time largely removes the association of ischaemic heart disease with amount of clottable fibrinogen (the inclusion of nephelometric fibrinogen concentration removes the remainder of this association); and the addition of white cell count largely removes the contribution of nephelometric fibrinogen concentration to the basic model. As noted elsewhere, these techniques are not infallible,²¹ but on the present evidence only the white cell count and heparin-thrombin clotting time show a significant association with prevalent ischaemic heart disease.

The association between white cell count and ischaemic heart disease has been reported previously for incident ischaemic heart disease in studies in the USA,²² France,²³ and Japan.²⁴ White cell count also shows an association with incident cerebrovascular disease.²⁵ These associations do not appear to be

Table 6 Standardised regression coefficients (SE) from multiple logistic regression analysis with diastolic blood pressure added to basic model

Variable	Standardised coefficient (SE)	t
Age	0.540 (0.115)	4.7***
White cell count†	0.360 (0.117)	3.1**
Heparin-thrombin clotting time†	-0.267 (0.119)	-2.2*
Diastolic blood pressure	0.317 (0.110)	2.9**

p < *0.05; **0.01; ***0.001.

†Transformed to logarithms.

explained by smoking habit and encourage further prospective studies among disease free populations. A mechanism that is mediated by complement activation (C5a) and that causes clumping of granulocytes and monocytes (but not lymphocytes) and subsequent leucoembolism of small coronary vessels has been postulated.²⁶ Other possible mechanisms have been discussed elsewhere,²⁷ but larger numbers are required to test these hypotheses adequately in both prevalence and prospective studies.

This work was supported by a grant from the British Heart Foundation.

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