Plasma and blood viscosity in the prediction of cardiovascular disease and mortality in the

Scottish Heart Health Extended Cohort Study

Sanne A.E. Peters (1), Mark Woodward(1-4), Ann Rumley(2), Hugh D. Tunstall-Pedoe(3), Gordon D.O.

Lowe(2)

(1) The George Institute for Global Health, University of Oxford, UK

(2) Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK

(3) Cardiovascular Epidemiology Unit, Institute of Cardiovascular Research, University of Dundee,

UK

(4) The George Institute for Global Health, University of Sydney, Australia

Correspondence : Professor G D O Lowe, University of Glasgow, New Lister Building, Royal Infirmary,

10 Alexandra Parade, Glasgow G31 2ER, UK.   E-mail: Gordon.Lowe@glasgow.ac.uk

SUMMARY

Background: There is increasing evidence that blood viscosity and its major determinants (haematocrit and plasma viscosity) are associated with increased risks of cardiovascular disease (CVD) and premature mortality; however, their predictive value for CVD and mortality is not clear.

Methods: We prospectively assessed the added predictive value of plasma viscosity and whole-blood viscosity and haematocrit in 3386 men and women aged 30-74 years participating in the Scottish Heart Health Extended Cohort study.

Results: Over a median follow-up of 17 years, 819 CVD events and 778 deaths were recorded. Hazard ratios (95% confidence intervals) for a 1SD increase in plasma viscosity, adjusted for major CVD risk factors were 1.12 (1.04, 1.20) for CVD, and 1.20 (1.12, 1.29) for mortality. These remained significant after further adjustment for plasma fibrinogen: 1.09 (1.01, 1.18) and 1.13 (1.04, 1.22). Corresponding results for blood viscosity were 0.99 (0.90, 1.09) for CVD, and 1.11 (1.01, 1.22) for total mortality; and 0.97 (0.88, 1.08) and 1.06 (0.96, 1.18) after further adjustment for fibrinogen. Haematocrit showed similar associations to blood viscosity. When added to classical CVD risk factors, plasma viscosity improved discrimination of CVD and mortality by 2.4% (0.7, 4.4) and 4.1% (2.0, 6.5).

Conclusion: While plasma and blood viscosity may play a role in pathogenesis of CVD and mortality, much of their associations with CVD and mortality are due to mutual effects of major CVD risk factors. However, plasma viscosity adds to discrimination of CVD and mortality and might be considered for inclusion in multivariable risk scores.

Keywords : viscosity, haematocrit, fibrinogen, mortality, cardiovascular disease

Key messages

What is already known about this subject?

- Blood viscosity and its major determinants (haematocrit and plasma viscosity) are associated with increased risks of cardiovascular disease and premature mortality.

What does this study add?
- Plasma and blood viscosity are may play a role in pathogenesis of CVD and mortality, much of their associations with CVD and mortality are due to mutual effects of major CVD risk factors.
- Plasma viscosity adds significantly to discrimination of CVD and mortality

How might this impact on clinical practice?
- Plasma viscocity might be considered for inclusion in multivariable risk scores

Introduction

Plasma viscosity and whole-blood viscosity are important determinants of blood flow rheology; and hence may play roles in atherosclerosis, thrombosis and ischaemia.[1, 2] There is increasing evidence that levels of plasma and whole-blood viscosity are associated with risks of cardiovascular disease (CVD; comprising coronary heart disease and stroke) and mortality in samples of the general population.[3-6] However, viscosity levels are also associated with most major CVD risk factors,[7-9] so it is important to establish whether or not viscosity levels are associated with risks of CVD or mortality, independently of such risk factors. It is also important to establish the additional predictive value of viscosity levels to established risk scores for CVD and mortality, which are based on major risk factors. To date, no such study has been performed.

The aim of this study was to establish the associations of plasma viscosity and blood viscosity with incident CVD and mortality in the Scottish Heart Health Extended Cohort (SHHEC) Study. We adjusted these associations for major CVD risk factors; and additionally for plasma fibrinogen, which is a major determinant of plasma and blood viscosity,[1] and an established CVD risk factor.[10, 11] Finally, we determined the additional predictive value of plasma and blood viscosity to variables in the ASsessing cardiovascular risk, using SIGN guidelines (ASSIGN) risk score,[12] which is currently recommended for estimation of risks of CVD and total mortality in general practice in National Health Service Scotland.[13]

Methods

Study participants

SHHEC was derived from the Scottish Heart Health Study,[14] and the MONICA (Multinational MONItoring of trends and determinants in CArdiovascular disease) Project,[15] which recruited random population samples of adult men and women in 1984-7 (across Scotland) and (in north Glasgow only) 1989, 1992 and 1995. All participants completed a questionnaire, which solicited information on demographics, past medical history and lifestyle, including tobacco use. They were invited to attend clinics where blood pressure, weight and height were measured, a 12-lead electrocardiogram (ECG) was applied, and a blood sample taken. This study was restricted to those aged 30-74 years, free of clinical evidence of CVD at baseline,[12, 16] and having data on viscosity or haematocrit. CVD was diagnosed if they reported having received a previous doctor diagnosis of angina, heart attack or stroke or previously undergoing CABG or PTCA on the baseline questionnaire; if the ECG was suggestive of myocardial infarction using Minnesota codes;[17] or if the national records obtained showed that they had been hospitalized for coronary heart disease or stroke or had undergone any coronary surgical procedures prior to recruitment (and post-1980).

Follow-up and endpoint definition

Individuals who gave written permission were followed up for cause-specific mortality through linkage with two national systems: death registrations and the record linkage database.[12] The latter was also used to follow-up, and trace back, hospitalizations. Records were obtained from the period from 1981 to 2005. CVD endpoints were: deaths attributed to a cardiovascular cause (International Classification of Disease (ICD) 9 codes 390-459, ICD 10 codes 100-199); any hospital discharge diagnosis post-recruitment of coronary heart disease (ICD 9 410-414, ICD 10 120-125) or cerebrovascular disease (ICD 9 430-438, ICD 10 G45, 160-169); or surgical codes for coronary artery bypass graft (CABG) or percutaneous coronary angioplasty (PTCA).

Assessment of rheology

Rheological methods have been described previously.[7, 8] Venous blood was anticoagulated with dry dipotassium EDTA (1.5 mg/ml). Whole-blood viscosity and plasma viscosity were measured at high shear rates (>300 s^{-1}) in a Coulter-Harkness capillary viscometer at 37^C. Haematocrit was measured using a Hawksley microcentrifuge (13,000 g for 5 minutes) and reader. To examine the contribution of determinants other than haematocrit, whole-blood viscosity was corrected to a standard haematocrit of 45% (corrected blood viscosity).[7] Relative blood viscosity (corrected blood viscosity / plasma viscosity) was calculated as a measure of red cell deformability.[7] Fibrinogen was assayed in stored, thawed citrated plasma (0.11 M, 9:1 v:v) using the von Clauss method in an automated coagulometer (Organon Teknika, Cambridge, UK) and standardized across SHHEC samples using a general linear model.[11, 18]

Plasma viscosity and fibrinogen were measured in three phases of SHHEC recruitment: the first Glasgow MONICA survey (MONICA-1) in north Glasgow and the west Scotland portion of the Scottish Heart Health Study;[6, 7] the third north Glasgow MONICA survey (MONICA-3);[8] and the fourth north Glasgow MONICA survey (MONICA-4).[9] Whole-blood viscosity and haematocrit were not measured in MONICA-4. We have previous reported correlations of rheological variables with CVD risk factors, and with each other, in these studies.[6-9] Intra-and inter-coefficients of variation were; haematocrit, both <1%; plasma viscosity, both <1%; whole-blood viscosity, 1.4% and 1.7%; fibrinogen, 2.6 and 3.7%.

Statistical analyses

Cox proportional hazards models were used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for first-time cardiovascular disease and total mortality for each rheological variable. We predefined three models; model 1 was adjusted for age and sex only, model 2 was also adjusted for the CVD risk factors in the ASSIGN score (systolic blood pressure (SBP), serum total and high density lipoprotein (HDL) cholesterol, cigarettes smoked per day, diabetes status, family history of coronary heart disease, and the Scottish Index of Multiple Deprivation (SIMD) score), and model 3 was additionally adjusted for standardized fibrinogen. Analyses were conducted for one standard deviation increase in each rheological variable, and, for plasma viscosity only, in thirds of the range of values in the study sample using floating absolute risks.[19, 20] Survival data-based c-statistics[20] for the ASSIGN score variables with and without plasma viscosity, and their difference, were computed to assess discrimination. The ability of plasma viscosity to correctly reclassify those with and without CVD events or total mortality, compared to using the ASSIGN score variables alone, was quantified using the categorical net reclassification improvement (NRI) for time-to-event data,[20, 21] both overall and separately for individuals who would, or would not, experience an event during the first 10-years of follow-up. The risk categories used for the NRI were <10%, 10 to 20% and >20%. We also calculated the integrated discrimination improvement (IDI) with addition of plasma viscosity to the risk prediction model.[20, 22] The relative IDI was used to measure the percentage improvement in reclassification, averaged over all potential thresholds. 95% confidence intervals for the c-statistic, its change, and the reclassification measures were generated through bootstrapping (n=500). All statistical analyses were performed using R, version 2.15.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Table 1 shows the baseline characteristics of the 3386 study participants contributing to the baseline surveys according to calendar time and overall. The mean age was 49 years (standard deviation [SD]: 11), 49% were male, mean systolic blood pressure was 131mm/Hg, and 39% were current smokers. Mean (SD) values in mPa.s for rheological variables were 1.31 (0.08) for plasma viscosity, 3.27 (0.52) for whole blood viscosity, 3.32 (0.39) for corrected blood viscosity, and 2.52 for relative blood viscosity. Over a median (interquartile range) follow-up of 17.3 (14.4, 18.0) years, 819 CVD events and 778 deaths were recorded.

Table 2 shows the hazard ratios and 95% CIs for CVD and total mortality for a one standard deviation increase in plasma viscosity, blood viscosity, corrected blood viscosity, relative blood viscosity and haematocrit. After adjustment for age and sex, the HRs (95% CI) for plasma viscosity were 1.21 (1.13, 1.29) for CVD, and 1.23 (1.15, 1.31) for total mortality. Following further adjustment for CVD risk factors, HRs were attenuated to 1.12 (1.04, 1.20) for CVD and to 1.20 (1.12, 1.29) for total mortality. HRs were 1.09 (1.01, 1.18) for CVD and 1.13 (1.04, 1.22) for mortality after further adjustment for plasma fibrinogen. The age- and sex-adjusted HRs (95% CI) for whole-blood viscosity were 1.16 (1.06, 1.27) for CVD, and 1.23 (1.13, 1.35) for total mortality. Following further adjustment for CVD risk factors, HRs were attenuated, to non-significance for CVD: 0.99 (0.90, 1.09) and to marginal significance for mortality: 1.11 (1.01, 1.22). HRs were 0.97 (0.88, 1.08) for CVD and 1.06 (0.96, 1.18) for total mortality following further adjustment for plasma fibrinogen. Corrected blood viscosity was significantly associated with CVD and total mortality in the age- and sex-adjusted models, but not following further adjustment. Age-and sex adjusted HRs (95% CI) were 1.15 (1.06, 1.26) for CVD and 1.15 (1.05, 1.25) for total mortality, and were 1.04 (0.95, 1.14) for CVD and 1.10 (1.00, 1.20) for total mortality in the multiple-adjusted models. Relative blood viscosity did not show significant associations with either CVD risk or mortality in any of the models. For haematocrit, the HRs were very similar to those for whole-blood viscosity. After adjustment for age and sex, HR was 1.14 (1.04, 1.25) for CVD, and 1.22 (1.11, 1.33) for mortality. Further adjustment for CVD risk factors attenuated both HRs to non-significance (Table 2).

Figure 1 shows the graded association between plasma viscosity and CVD and total mortality in thirds of plasma viscosity. Compared to those with plasma viscosity levels in the lowest third (<1.27 mPa.s), individuals with levels in the highest third (≥1.34 mPa.s) had a 1.60 (1.43, 1.78) times increased risk of CVD, and a 1.58 (1.41, 1.79) higher risk of total mortality in the models adjusted for age and sex. HRs attenuated to 1.28 (1.13, 1.45) for CVD, and 1.45 (1.25, 1.68) for total mortality following multiple adjustment.

Table 3 shows the reclassification of 10-year predicted risk and changes in risk discrimination for cardiovascular disease and total mortality after addition of plasma viscosity to a model including ASSIGN risk score variables only. The c-statistic (95% CI) increased by 0.002 (0.0000, 0.005) for CVD and by 0.006 (0.001, 0.008) for total mortality after plasma viscosity was added to the risk model. Classification of individuals in risk categories did not improve after addition of plasma viscosity; the categorical NRI (95% CI) was 0.0156 (-0.0196, 0.0560) for CVD and was 0.0024 (-0.0324, 0.0581) total mortality. However, plasma viscosity added significantly to discrimination: the IDIs were small, but statistically significant for both cardiovascular disease and total mortality (Table 3); the relative IDIs were 2.4% and 4.1%, respectively.

Discussion

In this largest prospective study yet reported of plasma viscosity and whole-blood viscosity in prediction of cardiovascular disease and total mortality in men and women, we have shown that plasma viscosity is associated with both CVD risk and total mortality. While plasma viscosity is associated with age, sex, and major cardiovascular risk factors in the SHHEC study,[6-9] we have shown that these associations persist following adjustment for these variables. Our findings are consistent with those of a previous meta-analysis of plasma viscosity and coronary heart disease risk in four prospective studies of healthy populations;[3] and extend it significantly as (a) the largest single study of CVD (coronary heart disease and stroke) and also of total mortality; and (b) the first study to show that plasma viscosity adds significantly to risk discrimination for both CVD and total mortality, improving the ASSIGN risk score, which was developed from the SHHEC, and which is currently used for routine CVD risk prediction in NHS Scotland.

Plasma fibrinogen is also a determinant of plasma viscosity; however the associations of plasma viscosity remained significant after further adjustment for fibrinogen, presumably due to the contributions of other large, asymmetrical proteins including lipoproteins and immunoglobulins.[4] We have recently shown that plasma fibrinogen, measured by four different methods, did not add substantially to risk prediction by the ASSIGN risk score.[11, 23] These findings are similar to those from a recent Danish study which found that, although fibrinogen and 14 other risk markers were independently associated with CVD, none of these added significantly to the performance of the SCORE risk algorithm.[24]

Whole-blood viscosity is determined not only by plasma viscosity, but also by haematocrit and red cell deformability.[1] While a previous meta-analysis of prospective studies in healthy populations has established that haematocrit is a risk factor for coronary heart disease, there is little data for blood viscosity.[3] We have shown that while blood viscosity is associated with both CVD risk and total mortality, adjustment for major cardiovascular risk factors, which are associated with blood viscosity,[6-8] attenuates these associations to null. This contrasts with the independent associations of plasma viscosity, which may be more sensitive than blood viscosity to changes in plasma proteins which are associated with CVD risk and mortality, such as fibrinogen. The associations between blood viscosity and CVD risk and mortality appeared largely due to associations with haematocrit: correction of blood viscosity to a standard haematocrit of 45% abolished its associations with CVD risk and mortality. Relative blood viscosity, a measure of red cell deformability, showed no association with CVD risk or mortality.

Strengths of this study include: a large prospective study of men and women across the age range 30-74 years, with high rate of follow-up over 10-21 years; a high number of incident cases of CVD and mortality; and a wide range of rheological variables assayed. Limitations include that the participants were largely Caucasian; hence the results cannot be extrapolated to other ethnic groups.

We conclude that, while plasma and blood viscosity may play a role in pathogenesis of cardiovascular disease and total mortality,[2] their associations with these events are largely due to the effects of major CVD risk factors. However, plasma viscosity adds to discrimination of both CVD and mortality and might be considered for inclusion in future multivariable risk scores.

Conflicts of interest

None

Author contributions: MW, HTP, and GL were involved in the concept and design of the study. SP and MW conducted the statistical analyses. All authors were involved in the acquisition and/or interpretation of the data. SP and GL prepared the first draft of the manuscript. All authors made critical revisions, provided final approval, and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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Caption for figure

Figure 1: Age- and sex (squares) and multivariable (circles) adjusted hazard ratios and 95% confidence intervals (CIs) for cardiovascular disease (left) and total mortality (right) in thirds of plasma viscosity

Table 1: Baseline characteristics of study participants

	MONICA 1 & West Scotland, 1984-7 (n=1070)	MONICA 3, 1992 (n=1114)	MONICA 4, 1995 (n=1202)	Total (n=3386)
Age, years	49 (7)	51 (13)	46 (10)	49 (11)
Male sex, %	50	46	48	49
Systolic blood pressure, mmHg	133 (19)	133 (23)	128 (19)	131 (21)
Total cholesterol, mmol/L	6.4 (1.3)	6.1 (1.1)	6.0 (1.1)	6.1 (1.2)
HDL cholesterol, mmol/L	1.6 (0.4)	1.5 (0.4)	1.3 (0.4)	1.5 (0.4)
Body Mass Index, kg/m^2	26 (4)	26 (5)	26 (5)	26 (5)
Current smokers, %	35	42	40	39
Diabetes, %	1	1	2	2
Family history of CVD, %	29	28	33	30
Scottish Index of Multiple Deprivation	25 (23)	46 (24)	45 (24)	39 (26)
Rheology, mPa.s				
Plasma viscosity	1.32 (0.09)	1.31 (0.08)	1.31 (0.08)	1.31 (0.08)
Whole blood viscosity	3.35 (0.53)	3.18 (0.50)	-	3.27 (0.52)
Corrected blood viscosity	3.39 (0.37)	3.24 (0.39)	-	3.32 (0.39)
Relative blood viscosity	2.58 (0.25)	2.47 (0.28)	-	2.52 (0.28)
Haematocrit, %	43.96 (4.10)	43.64 (3.77)	-	43.81 (3.94)
Outcomes				
Cardiovascular disease, n (%)	310 (29)	327 (30)	182 (16)	819 (25)
Total mortality, n (%)	286 (27)	340 (31)	152 (13)	778 (23)

Values shown are mean (standard deviation) for continuous variables and percentages for dichotomous variables.

Table 2: Hazard ratios and 95% confidence intervals for cardiovascular disease and total mortality for a standard deviation increase in rheology measures and haematocrit

			Model 1		Model 2		Model 3	
Cardiovascular disease								
	Mean (SD), mPa.s							
	No event	Event	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Plasma viscosity	1.31 (0.08)	1.34 (0.09)	1.21 (1.13; 1.29)	<0.001	1.12 (1.04; 1.20)	0.002	1.09 (1.01; 1.18)	0.03
Whole blood viscosity	3.22 (0.50)	3.37 (0.55)	1.16 (1.06; 1.27)	<0.001	0.99 (0.90; 1.09)	0.84	0.97 (0.88; 1.08)	0.61
Corrected blood viscosity	3.29 (0.37)	3.41 (0.42)	1.15 (1.06; 1.26)	0.001	1.04 (0.95; 1.14)	0.38	1.03 (0.93; 1.13)	0.60
Relative blood viscosity	2.52 (0.27)	2.54 (0.28)	1.02 (0.93; 1.12)	0.71	0.98 (0.89; 1.07)	0.64	0.99 (0.90; 1.08)	0.76
Haematocrit	43.56 (3.94)	44.41 (3.90)	1.14 (1.04; 1.25)	0.004	0.98 (0.90; 1.08)	0.73	0.97 (0.88; 1.07)	0.50
Total mortality								
Plasma viscosity	1.30 (0.08)	1.35 (0.09)	1.23 (1.15; 1.31)	<0.001	1.20 (1.12; 1.29)	<0.001	1.13 (1.04; 1.22)	0.003
Whole blood viscosity	3.21 (0.49)	3.40 (0.57)	1.23 (1.13; 1.35)	<0.001	1.11 (1.01; 1.22)	0.03	1.06 (0.96; 1.18)	0.24
Corrected blood viscosity	3.29 (0.38)	3.41 (0.41)	1.15 (1.05; 1.25)	0.002	1.10 (1.00; 1.20)	0.05	1.05 (0.95; 1.15)	0.36
Relative blood viscosity	2.52 (0.27)	2.53 (0.27)	1.00 (0.91; 1.09)	0.91	0.98 (0.89; 1.07)	0.60	0.98 (0.89; 1.08)	0.68
Haematocrit	43.49 (3.83)	44.62 (4.12)	1.22 (1.11; 1.33)	<0.001	1.07 (0.97; 1.18)	0.16	1.05 (0.95; 1.15)	0.38

Model 1 is adjusted for age and sex, model 2 is additionally adjusted for systolic blood pressure, total cholesterol, HDL cholesterol, diabetes mellitus, number of cigarettes smoked per day, family history of cardiovascular diseases, and the Scottish Index of Multiple Deprivation; model 3 is additionally adjusted for standardized fibrinogen. HR, hazard ratio; CI, confidence interval; SD, standard deviation.

Table 3: Reclassification of 10-year predicted risk and changes in risk discrimination for cardiovascular disease and total mortality after addition of plasma viscosity to a model including ASSIGN risk score variables*

	Cardiovascular disease	Total mortality
Change in c-statistic†	0.002 (0.000, 0.005)	0.006 (0.001, 0.008)
Net Reclassification Improvement, categorical±		
Overall	0.0156 (-0.0196, 0.0560)	0.0024 (-0.0324, 0.0581)
With event	0.0174 (-0.0201, 0.0508)	-0.0033 (-0.0347, 0.0520)
Without event	-0.0018 (-0.0079, 0.0140)	0.0057 (-0.0081, 0.0134)
Integrated discrimination improvement	0.0022 (0.0005, 0.0038)	0.0051 (0.0022, 0.0081)
Relative integrated discrimination improvement, %	2.40 (0.67, 4.39)	4.14 (1.97, 6.46)

Values shown are point estimates (95% confidence intervals).

*ASSIGN risk score variables are age and sex, systolic blood pressure, total cholesterol, HDL cholesterol, diabetes mellitus, number of cigarettes smoked per day, family history of cardiovascular diseases, and the Scottish Index of Multiple Deprivation.
†Reference C-indexes (95% confidence interval) for the model including ASSIGN risk score variables only were 0.7484 (0.7299; 0.7668) for cardiovascular disease and 0.8134 (0.7965; 0.8304) for total mortality.
±Risk categories were <10%, 10-20%, ≥20%.

Figure 1