Blood Viscosity, Fibrinogen, and Activation of Coagulation and Leukocytes in Peripheral Arterial Disease and the Normal Population in the Edinburgh Artery Study

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Background. Increased blood and plasma viscosity, hematocrit, fibrinogen, and activation of coagulation and leukocytes have been reported in patients with claudication; however, their associations with symptomatic and asymptomatic peripheral arterial disease have not been reported in an epidemiological study.

Methods and Results. Blood and plasma viscosity, hematocrit, fibrinogen, urinary fibrinopeptide A, plasma leukocyte elastase, and uric acid were measured in a random sample of 1,581 men and women aged 55–74 years in Edinburgh, Scotland, and related to peripheral arterial stenosis (ankle–brachial systolic pressure index, ABPI) and to lower limb ischemia (intermittent claudication and reactive hyperemia test). Each variable (except fibrinopeptide A) was significantly related to prevalent symptomatic and asymptomatic peripheral arterial disease. On multivariate analysis, blood viscosity (p<0.05) and fibrinogen (p<0.01) were independently associated with peripheral arterial narrowing (ABPI); a positive interaction was found between fibrinogen and smoking in the association with ABPI. Plasma viscosity was associated with claudication in the presence of a given degree of arterial narrowing (odds ratio of claudication in top quintile compared with bottom quintile of plasma viscosity, 3.35; 95% CI, 1.32, 8.51). Leukocyte elastase and uric acid were each associated with reactive hyperemia independently of arterial narrowing (p<0.01).

Conclusions. Blood rheological factors and leukocyte activation as well as arterial narrowing are associated with lower limb ischemia in the general population and may be implicated in its pathogenesis.

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Key Words • plasma viscosity • fibrinopeptide A • atherosclerosis • claudication

Chronic ischemia of the lower limbs may result from not only atherosclerotic stenoses but also from thrombotic occlusions and rheological abnormalities such as increased blood viscosity and impaction of activated leukocytes in the nutritive microcirculation.2,3 Previous studies have suggested that patients with intermittent claudication may have activation of blood coagulation,4,5 increased blood viscosity caused by elevation of hematocrit and plasma fibrinogen,6–9 and increased leukocyte activation and rigidity.10,11 However, these factors have not been related to peripheral arterial disease in epidemiological studies in the general population, nor have relations with other cardiovascular risk factors (e.g., smoking) been taken into account.

The Edinburgh Artery Study is a cross-sectional survey of 1,592 men and women aged 55–74 years residing in Edinburgh, Scotland. A high prevalence of symptomatic and asymptomatic peripheral arterial disease has been identified in this population as well as associations with conventional cardiovascular risk factors.12 We now report the relations of whole blood viscosity and its major determinants (hematocrit, plasma viscosity, fibrinogen) as well as measurements of activation of blood coagulation (urinary fibrinopeptide A) and of blood leukocytes (plasma leukocyte elastase) to symptomatic and asymptomatic peripheral arterial disease in this population. We also tested the hypothesis that, for a given degree of atherosclerotic arterial narrowing (measured by the ankle–brachial pressure index, ABPI), leg ischemia (measured by the World Health Organization [WHO] intermittent claudication questionnaire and by a reactive hyperemia test) was related to viscosity and to leukocyte activation.10

Methods

In this cross-sectional survey,12 1,592 men and women aged 55–74 years were selected from the age/sex regis-
ters of 10 general practices with catchment area populations spread geographically and socioeconomically throughout the city. The sample was selected randomly within sex-specific 5-year age groups to produce equal numbers in each group and an adequate sample size to conduct a future cohort study. Subjects attended a university clinic to complete a questionnaire and have a comprehensive medical examination. The response rate was 65%, and follow-up of a sample of nonresponders did not show any significant bias. Details of the study population, recruitment, and prevalence of peripheral arterial disease are described elsewhere.\textsuperscript{12} The questionnaire included validated questions on cardiovascular history, intermittent claudication and angina (WHO questionnaire\textsuperscript{16}), and smoking history. A 12-lead ECG was taken and coded independently by two observers using the Minnesota code.\textsuperscript{17} Arm blood pressure was taken supine after 10 minutes of rest using a random zero sphygmomanometer. Peripheral pulses were palpated, and ankle systolic pressures were then measured using a Sonicaid Doppler probe and random zero sphygmomanometer with the patient supine. The ABPI was calculated as a measure of arterial narrowing in the lower limb.\textsuperscript{12}

A reactive hyperemia test was then carried out in which ankle systolic pressures were measured 15 seconds after the release of a cuff occluding arterial flow for 4 minutes above the knee at 50 mm Hg above systolic pressure.\textsuperscript{12} Reactive hyperemia tests have been shown to have adequate validity in detecting angiogram-positive disease in hospital patients.\textsuperscript{18,19} In preliminary studies, we found that our technique detected the greatest hyperemic response and had adequate reproducibility.\textsuperscript{20} The main purpose of this test was to detect those with substantial peripheral atherosclerosis who might have had a normal ABPI, as may occur in diabetics. Details of the results of the reactive hyperemia test in this population are published elsewhere.\textsuperscript{21}

From a fasting blood sample taken on each patient at about 9:00 AM, serum uric acid was estimated on a Cobas Bio analyzer, using a standard kit. Fibrinogen was measured in citrated plasma by a thrombin-clotting turbidimetric method in a centrifugal analyzer.\textsuperscript{22} Blood and plasma viscosity were measured from a blood sample anticoagulated with dry dipotassium edetate (EDTA, 1.5 mg/mL) at high shear rates (over 300 sec\textsuperscript{-1}) in a Coulter-Harkness viscometer at 37°C.\textsuperscript{23} Hematocrit was measured using a Hawksley microcentrifuge and reader. Blood viscosity was corrected to a standard hematocrit of 45% using the formula of Matrai et al.\textsuperscript{24} Relative blood viscosity (corrected blood viscosity/plasma viscosity) was calculated as a measure of red cell deformability.\textsuperscript{3,25} Urinary fibrinopeptide A was measured by radioimmunoassay as previously described,\textsuperscript{14} using reagents from IMCO (Stockholm). Plasma leukocyte elastase was also measured by radioimmunoassay as previously described.\textsuperscript{15} Quality control was monitored by means of blind duplicate samples taken intermittently throughout the study.

Data were analyzed on the Edinburgh University mainframe computer using SPSSX and BMDP statistical packages. In the univariate analysis, the population was divided for descriptive purposes into four categories of peripheral arterial disease:\textsuperscript{12} intermittent claudication (WHO questionnaire positive and ABPI ≤0.9 or reactive hyperemia >20%), major asymptomatic disease (ABPI ≤0.9 and reactive hyperemia >20% or ABPI ≤0.7 or reactive hyperemia >35%), minor asymptomatic disease (ABPI ≤0.9 or reactive hyperemia >20%), and normal (none of the above). Because these categories have not been used in other studies, the validities were unknown, but results of studies comparing the ABPI and reactive hyperemia separately with arteriography\textsuperscript{26} suggest that the classification has adequate face validity. The main analysis, however, concentrated on the ABPI because it was almost completely recorded and is a continuous measure, thus giving more power to detect associations. The minimum ABPI in the two legs was used because disease often occurs unilaterally. The minimum of two measurements induced slight negative skewness caused by random variation between legs, but this was not sufficient to justify transformation.

Multiple linear regression was used to investigate the relations between the ABPI and age, sex, blood and plasma viscosity, hematocrit, fibrinogen, leukocyte elastase, urinary fibrinopeptide A, uric acid, and smoking. Logarithmic transformations\textsuperscript{27} were carried out on the values for leukocyte elastase and urinary fibrinopeptide A because of positive skewness in their distributions. Height was also included in the regressions because a positive association with the ABPI was demonstrated.\textsuperscript{12} This may have been due to the widening of pulse pressure, as blood flows through arteries leading to a relatively high systolic pressure at the ankle in taller individuals.\textsuperscript{28} Exclusion of height would have affected the association with sex, tending to increase the ABPI in male subjects. The multivariate analysis was carried out with all of the above factors estimated simultaneously, with the subsequent insertion of total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, and diabetic status. (The prevalence of diabetes was 10% in claudicants and 6% in those without evidence of peripheral arterial disease.\textsuperscript{13})

The association with smoking was modeled using number of “pack-years” with additional variables for current smokers, recent ex-smokers (stopped within the last 5 years), and smokers of pipe/cigars only. The additional variable for current smokers, for example, measures the difference between ABPI in a current smoker and an ex-smoker who gave up more than 5 years ago but who had smoked the same amount of pack-years. The distribution of pack-years was highly skewed with a few very heavy smokers, therefore the square root of the pack-years was used to reduce the influence of these few individuals. The smoking histories were considered reasonably valid because the stated amount was related to mean thiocyanate levels, another measure (albeit imperfect) of cigarette consumption. Only smoking history is reported in this article.

Multiple regressions of age, sex, height, smoking, and the rheological factors were also carried out on intermittent claudication, the reactive hyperemia test, and three separate measures of heart disease: 1) subject recall of physician's diagnosis of angina or heart attack, 2) WHO questionnaire positive for angina or previous myocardial infarction, and 3) ECG evidence of ischemia.

Results

The mean levels of the rheological factors in the total population of 1,581 men and women from whom blood
was obtained are shown in Table 1. Analysis by age and sex showed that blood viscosity, hematocrit, urinary fibrinopeptide A, and leukocyte elastase were significantly higher in men (p<0.001), whereas fibrinogen was higher in women (p<0.001). Fibrinogen increased markedly with age from 2.52 g/L (SEM, 0.03) in those aged 55–59 years to 2.90 g/L (SEM 0.04) in those aged 70–74 years (p<0.001). A significant increase with age also occurred with plasma viscosity (p<0.001) and leukocyte elastase (p<0.05). Substantial positive correlations were present between many of these factors and also with cigarette consumption (measured by pack-years). The highest correlations were between hematocrit and blood viscosity (r=0.69), fibrinogen and plasma viscosity (r=0.46), and blood viscosity and plasma viscosity (r=0.45).

Table 1 also shows the mean levels of the rheological factors according to categories of peripheral arterial disease. Blood viscosity, hematocrit, hematocrit-corrected blood viscosity, plasma viscosity, fibrinogen,
leukocyte elastase, and uric acid were each related significantly to the severity of the disease. Urinary fibrinopeptide A levels were higher in claudicants than in the other groups, but there was no significant trend across all categories of peripheral arterial disease ($p=0.62$). Relative blood viscosity (a measure of red cell deformability) was unrelated to peripheral arterial disease ($p=0.26$).

Multiple logistic regression on claudication and multiple linear regression on the ABPI are shown in Table 2. Blood viscosity and fibrinogen remained significantly independently related to the ABPI but not to claudication (although this may have been related to the small number of claudicants). Plasma viscosity was related to both claudication ($p<0.01$) and the ABPI ($p<0.001$) on univariate analysis, but this disappeared on multivariate analysis including blood viscosity and fibrinogen. Blood viscosity and fibrinogen remained significantly associated with the ABPI on the inclusion of other vascular risk factors (diabetes mellitus, cholesterol, HDL cholesterol, and triglycerides). Conversely, these vascular risk factors did not lose their independent relations with the ABPI on the inclusion of fibrinogen and viscosity.

Analysis of possible interactions among age, sex, cigarette smoking, fibrinogen, and blood viscosity with the ABPI showed that the relations between both fibrinogen and blood viscosity and the ABPI occurred predominantly in male subjects ($p<0.01$) and were less marked and nonsignificant in female subjects. Figure 1 shows that the association between fibrinogen and the ABPI was strongly related to the amount of cigarette smoking, with the slope of the graphs increasing at higher levels of smoking. No interactions were found among blood viscosity, smoking, and the ABPI.

Multiple logistic regressions of age, sex, height, smoking, and the rheological factors on separate measures of ischemic heart disease showed that fibrinogen was associated only with a history of heart disease on the WHO questionnaire ($p<0.01$). Blood viscosity was not related to any measure of heart disease, although plasma viscosity was associated with recall of a physician's diagnosis of heart disease ($p<0.1$). A major difference from the findings in peripheral arterial disease was that uric acid remained independently related to all measures of heart disease, namely ECG evidence of ischemia ($p<0.001$), WHO questionnaire positive for heart disease ($p<0.01$), and recall of physician's diagnosis of heart disease ($p<0.1$).

To study the possible impact of rheological factors on ischemic symptoms and on reaction to vascular stress in the presence of a given degree of arterial narrowing, multiple logistic regressions were carried out separately on intermittent claudication and the reactive hyperemia test, with the inclusion of the ABPI as a measure of arterial narrowing. Plasma viscosity was the only factor that was independently related to claudication after adjustment for the ABPI: Figure 2 shows that the odds of having claudication in the top quintile of plasma viscosity were 3.35 times that in the bottom quintile (95% CI, 1.32, 8.51). Leukocyte elastase and uric acid were the only factors associated with the results of the reactive hyperemia test independently of the ABPI and other factors ($p<0.01$).

**Discussion**

Blood rheological factors (viscosity, hematocrit, fibrinogen, activated leukocytes) may contribute to ischemia by...
promoting atherosclerosis, thrombosis, or obstruction to microcirculatory flow distal to atherosclerotic stenoses.\textsuperscript{2,3} The results of our study support the hypothesis that rheological factors are related in an older population aged 55–74 years to both atherosclerotic peripheral arterial disease (measured by the ABPI) and to leg ischemia (measured by the presence of intermittent claudication on questionnaire or an abnormal reactive hyperemia test).

Blood viscosity and its major determinants (hematocrit, plasma viscosity, and fibrinogen) as well as leukocyte activation (plasma leukocyte elastase) were found to be significantly related to increasing severity of peripheral arterial disease within the population (Tables 1 and 2). No such relation was evident for activation of blood coagulation (urinary fibrinopeptide A), but this may have been related to insensitivity of the assay because 38% of the population had levels <1 ng/mL. Indeed, plasma fibrinopeptide A levels have been related to several major cardiovascular risk factors\textsuperscript{22} as well as to angiographic coronary artery disease\textsuperscript{29} and peripheral arterial disease.\textsuperscript{4}

In the Edinburgh Artery Study, peripheral arterial disease has been related to conventional risk factors such as age, cigarette smoking, systolic blood pressure, and HDL and non-HDL cholesterol.\textsuperscript{13} Several rheological factors were also associated with these risk factors, particularly age and cigarette smoking, as previously reported.\textsuperscript{2,3,23} On multivariate analyses including age, sex, and cigarette smoking, the associations between the rheological factors and intermittent claudication became nonsignificant (Table 2). However, this finding may be related to the small number of claudicants and does not exclude the possibility that increases in viscosity, fibrinogen, and leukocyte activation may be mechanisms whereby age and smoking promote development of peripheral arterial disease.

Are rheological factors related to the extent of atherosclerotic narrowing in the arteries to the lower limbs? The latter was assessed using the ABPI, which at a level of 0.9 has been shown to be up to 95% sensitive in detecting angiogram positive peripheral arterial disease\textsuperscript{30} and in the Edinburgh Artery Study has been shown to be related to the severity of disease on duplex scanning.\textsuperscript{31} The ABPI was significantly associated with blood viscosity, plasma viscosity, and fibrinogen, and the relations with blood viscosity and fibrinogen persisted after multivariate analysis including conventional risk factors (Table 2). These findings suggest that blood viscosity and fibrinogen each may have an independent role in atherogenesis. Several biologically plausible mechanisms have been suggested, including an effect of blood viscosity on the localization of atherosclerotic lesions.\textsuperscript{22} Fibrinogen levels may influence infiltration of fibrinogen into the arterial wall, platelet aggregation, and fibrin formation as well as increasing plasma and blood viscosity.\textsuperscript{21} Interestingly, the association between fibrinogen and the ABPI was strongly related to the amount of cigarette smoking (Figure 1). Possible explanations include a synergistic effect of smoking (which disturbs endothelial cells and activates platelets) and fibrinogen (which infiltrates the arterial wall through damaged endothelium and promotes platelet aggregation).\textsuperscript{2,3} An interaction between cigarette smoking and plasma fibrinogen has also been reported in the prediction of occlusion of femoropopliteal grafts in peripheral arterial disease.\textsuperscript{32}

In the presence of a given degree of atherosclerotic narrowing (ABPI), do rheological factors predispose to leg ischemia? If blood viscosity is reduced by lowering the hematocrit, blood flow in the leg increases,\textsuperscript{33} but this may reflect vasodilation caused by changes in oxygen carriage and blood volume rather than blood viscosity.\textsuperscript{34} However, lowering plasma fibrinogen also reduces plasma and blood viscosity and increases leg blood flow, including nutritive skin flow,\textsuperscript{35} and this cannot be ascribed to changes in oxygen carriage or blood volume. The importance of plasma viscosity has been shown in the present study, in which higher levels significantly increased the likelihood of symptomatic intermittent claudication at a given level of arterial narrowing (Figure 2). It is likely that this association reflects a direct effect of plasma viscosity on leg muscle blood flow distal to arterial stenosis, not only from theoretical considerations\textsuperscript{36} but also because reductions in plasma viscosity after exercise training\textsuperscript{37} cessation of cigarette smoking,\textsuperscript{22,38} or treatment with some pharmacological agents\textsuperscript{39} are accompanied by improvements in claudication that are quite consistent with the relation shown in Figure 2. Indeed, the relation shown in Figure 2 between plasma viscosity in the population and prevalent symptomatic leg ischemia is very similar to the recently described relation between plasma viscosity in the male population and incident coronary heart disease.\textsuperscript{40} It is therefore possible that plasma viscosity may also promote myocardial ischemia distal to coronary arterial stenoses, although measurement of the latter in population studies is more problematic than measurement of lower limb arterial stenoses using the ABPI.

We have also shown that leukocyte elastase was related to leg ischemia as measured by the reactive hyperemia test. Again, it is possible that this is a direct effect: Elastase release is a measure of leukocyte activation, which is also associated with production of oxygen metabolites that cause skeletal muscle vasodilation.\textsuperscript{41} Uric acid was also related to leg ischemia as measured by the reactive hyperemia test as well as ischemic heart disease. Because of the importance of the uric acid/xanthine oxidase pathway in reactive oxygen metabolism, these associations may also be relevant to oxygen metabolite–related ischemia. Leukocyte activation may play a role in atherosclerosis and thrombosis as well as in ischemia.\textsuperscript{3,42} However, an overall interpretation of the findings of the present study is that, in the presence of a given degree of arterial narrowing, determinants of microcirculatory blood flow (plasma viscosity, leukocyte activation) may be important determinants of leg ischemia.\textsuperscript{2,3} Conversely, reduction of plasma viscosity (for example, by reduction of plasma fibrinogen or lipoproteins) and inhibition of leukocyte activation may be rational approaches to prevention and treatment of ischemia.\textsuperscript{39}

Conclusions

Our findings suggest that rheological factors are associated with both the severity of atherosclerosis in the older population and with the presence of leg ischemia for a given degree of arterial occlusion. We are currently assessing the predictive value of rheological factors for arterial events in the limbs, heart, and brain.
in both this and other cohorts as well as the effects of interventions on blood rheology and prognosis in peripheral arterial disease.

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