ROLE OF FIBRINOGEN AND FIBRIN D-DIMER IN PERIPHERAL ARTERIAL DISEASE

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DECLARATION

THESIS: Role of Fibrinogen and Fibrin D-Dimer in Peripheral Arterial Disease.

I declare that I am the sole author of this thesis. It has not been submitted for any other degree, and all sources of information have been acknowledged. I conducted all aspects of the research except for the baseline examination in the Prognostic Study, the laboratory assays and the more complex statistical analyses.

Signed: .....

Date: 6th April 1998

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SUMMARY

Haemostasis is the physiological response to blood loss following vascular injury. The process produces a fibrin-platelet clot which seals the damaged blood vessel. Fibrinogen is polymerised to fibrin by thrombin which is produced by the coagulation pathways. Plasma fibrinogen has been related in several studies to the development of ischaemic heart disease and stroke, but the relationship with peripheral arterial disease is less well established. Another haemostatic factor, fibrin D-dimer, is the terminal breakdown product of cross-linked fibrin, and is thought to reflect the degree of active fibrin formation and subsequent activation of fibrinolysis. Fibrin D-dimer has not been widely investigated in terms of cardiovascular risk, and little information is available on its relationship to peripheral arterial disease.

This thesis is composed of two studies. The principal aim of the first study, the Sites of Atheroma Study, was to determine whether plasma fibrinogen, fibrin D-dimer and other haemostatic factors (von Willebrand Factor and plasminogen activator inhibitor - type 1) were related to the angiographic site and severity of atherosclerosis in the arteries of the lower limb. The principal aim of the second study, the Prognostic Study of Intermittent Claudication, was to determine whether plasma fibrinogen, fibrin D-dimer and other haemostatic factors (von Willebrand Factor and tissue plasminogen activator), were related to the future incidence of atherothrombotic events, and deterioration of peripheral arterial disease in subjects with intermittent claudication.

The study samples in both studies consisted of men and women with ischaemic symptoms in the lower limb referred to the Peripheral Vascular Clinic, Royal Infirmary of Edinburgh. In

the Sites of Atheroma Study, 192 patients referred for angiography were categorised by site and severity of peripheral atherosclerosis using the Bollinger angiographic scoring system. A clinical examination was conducted on each patient including the administration of a questionnaire and taking of a blood sample for the measurement of haemostatic factors. In the Prognostic Study, 607 patients with intermittent claudication who had had a comprehensive examination at baseline, including measurement of haemostatic factors, were followed up over six years to determine the incidence of fatal and non-fatal ischaemic heart disease and stroke and deterioration of peripheral arterial disease. Follow-up data were obtained from hospital records, general practitioners, self-administered questionnaires, the Information and Statistics division of the Common Services Agency and the Scottish National Health Service Central Registry.

Results from the Sites of Atheroma Study indicated that 34 (17.7%) patients had predominantly aorto-iliac disease, 85 (44.3%) had femoro-popliteal disease and 73 (38.0%) had dual-site disease. There were no significant differences in the mean levels of the haemostatic factors between patients with disease affecting different sites. An independent relationship was found between nephelometric fibrinogen and between fibrin D-dimer and disease severity only in the femoro-popliteal arteries. On multiple regression, fibrinogen remained independently associated with disease severity in the femoro-popliteal arteries, when life-time smoking or current smoking were taken into account. There was no influence of current smoking on the association between fibrin D-dimer and disease severity but, on inclusion of life-time smoking, the association became non-significant.

In the Prognostic Study of Intermittent Claudication, a total of 210 (34.6%) patients died

during the six year follow-up period. Of these 90 (42.9%) died from ischaemic heart disease, 29 (13.8%) from stroke and 27 (12.9%) from other vascular causes, including cardiac arrhythmias and ruptured aneurysm. Ninety three (15.3%) patients had a non-fatal myocardial infarction and 79 (13.0%) had a fatal or non-fatal stroke. Forty five (7.4%) patients underwent investigations for peripheral arterial disease and 64 (10.5%) patients progressed to severe chronic leg ischaemia. A total of 203 (33.4%) patients did not have a vascular event or show any deterioration of limb ischaemia.

Baseline median levels of plasma fibrinogen, fibrin D-dimer and von Willebrand Factor were significantly higher in patients who died from ischaemic heart disease compared to those who had no vascular events. Tissue plasminogen activator antigen levels were significantly elevated in patients who suffered a stroke. All the relationships between the haemostatic factors and vascular events became weaker and statistically non-significant in analysis adjusting for cardiovascular risk factors and baseline ischaemic heart disease.

von Willebrand Factor levels were significantly raised in claudicants who developed severe chronic leg ischaemia (rest pain, ulceration and gangrene). In multivariate analyses adjusting for life-time smoking, fibrinogen became significantly associated with the risk of vascular intervention, and von Willebrand Factor was associated with the risk of severe chronic leg ischaemia.

In conclusion, these results indicate that there may be a stronger relationship between chronic smoking and increased fibrin turnover than coagulation in symptomatic peripheral arterial disease. Increased coagulation and fibrinolytic activity may also contribute to thrombosis or

progression of atherosclerosis in the coronary and cerebral arteries in claudicants. The effect that fibrinogen, fibrin D-dimer and other haemostatic factors may have on the progression of peripheral arterial disease was mostly independent of cigarette smoking.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Peripheral Arterial Disease

1.1.1 Histopathology of lesions

Atherosclerosis in the lower limb may cause stenosis or occlusion of the distal aorta, iliac, femoral, popliteal or tibio-peroneal arteries. Lesions in these arteries tend to be segmental although, overall, the distribution of disease may be highly diffuse and affect multiple sites within the lower limb arteries. The histopathology of peripheral atherosclerosis is, however, similar to atherosclerosis occurring at other sites within the arterial tree. There are four main types of lesions : early lesions, fatty streaks, advanced and complicated lesions (Badimon et al 1993).

Atherogenesis is thought to be initiated by the disruption of endothelial cell function caused by factors such as cigarette smoking, hypercholesterolaemia, immuno-complexes and infection (Ross 1993). Early lesions form within the intima, the innermost layer of the arterial wall, and are characterised by accumulation of lipid-laden macrophages (foam cells). Progression of the disease to a fatty streak is dependent on further accumulation of lipid and smooth muscle cells within the intima. Fatty streaks can be either flat or raised. Most children will have these lesions occurring in the aorta between the ages of two and 15 years of age.

When the structure of the intima becomes disrupted and a lipid core has developed, the lesion is classified as advanced and is common in young adults from about the age of 20 years onwards. Although it may not cause a reduction in blood flow, this type of lesion can be clinically significant because it is prone to rupture or fissuring due to thinning of the fibrous cap overlying the lipid core. Progression of disease is also related to increases in fibrous tissue within the intimal, medial and adventitial layers of the artery. This can result in projection of the lesion into the lumen and blood flow may be reduced. Mural thrombi also can become incorporated within the vessel wall and contribute to narrowing of the lumen.

Finally, when the lesion undergoes degenerative changes, such as ulceration, fissuring or haemorrhage, it is classified as a complicated lesion. Such changes can cause thrombosis and occlusion of the artery so that symptoms of ischaemia may occur. Inflammation, the cytotoxic products released from macrophages, shear stress and vasospasm have been implicated as causal factors in intimal disruption. Levels of systemic risk factors and the degree of disruption may also determine the size and persistence of the thrombus and the severity of the resulting clinical event (Badimon et al 1993).

1.1.2 Definition of peripheral arterial disease

The term 'peripheral arterial disease', which is synonymous with 'peripheral vascular disease', is used primarily to describe atherosclerotic disease of the arteries of the upper limb and of the lower limb distal to the aortic bifurcation. Disease in the upper limb arteries is comparatively rare, however, and therefore the term is generally used to denote atherosclerotic disease in the arteries supplying the lower limb.

An individual cannot be classified as simply 'diseased' or 'non-diseased' because the pathological process begins early in life and by the age of 40 years, most adults will have lesions in the peripheral arteries (Mitchell and Schwartz 1965). However, ischaemic symptoms do not appear until the disease is relatively advanced. For the purpose of initial clinical evaluation and for use in epidemiological surveys, peripheral arterial disease is usually diagnosed by the presence of symptoms, such as intermittent claudication, and by non-invasive tests to detect the degree or effects of arterial narrowing. Traditionally, angiography is considered the best objective method of quantifying atherosclerotic disease and is used as the reference point against which non-invasive techniques are validated.

1.1.3 Measurement of peripheral arterial disease

The WHO Intermittent Claudication Questionnaire, which was developed in the early 1960's (Rose 1962), has been until recently the most widely used method of detecting symptomatic peripheral arterial disease in the general population. However, new questionnaires have recently been developed in an attempt to improve the validity of the WHO questionnaire, which has a particularly poor sensitivity in comparison to diagnosis made by a doctor. For example, the Edinburgh Claudication Questionnaire demonstrated a sensitivity of 85% compared to 65% for the WHO questionnaire when evaluated in community and hospital surveys (Leng and Fowkes 1992), although the specificity was over 95% for both questionnaires. Consequently, the Edinburgh Claudication Questionnaire is now being used in an increasing number of epidemiological studies investigating the prevalence of peripheral arterial disease.

A variety of non-invasive techniques have been used to assess both symptomatic and asymptomatic disease in epidemiological studies. These include palpation of peripheral pulses, segmental pressure measurements, pulse waveforms detected by oscillography, the ankle brachial pressure index (ABPI), exercise and reactive hyperaemia tests.

Measurement of the ankle brachial pressure index (ABPI) is perhaps the most common non-invasive method of assessing the severity of lower limb atherosclerosis and is used extensively both in clinical practice and in population surveys. In selected hospital patients, an ABPI of ≤ 0.9 has been shown to be approximately 95% sensitive and 100% specific in identifying angiogram positive disease in the lower limb (Bernstein and Fronek 1982; Laing and Greenhalgh 1983). However, this cut-off point may be less valid in confirming the presence of asymptomatic disease in individual cases within the community. In the Edinburgh Artery Study, for example, the distribution of the ABPI was normal, but with a slight negative skew, and with no obvious level differentiating abnormally low levels. About 18% of the population had an ABPI ≤ 0.9 (Fowkes et al 1991).

In subjects who have a raised central aortic pressure, or a condition such as diabetes mellitus, where arteries can be relatively rigid due to medial calcification, the ABPI can be normal at rest in spite of considerable atherosclerotic disease. A stress test, such as an exercise or reactive hyperaemia test, can identify the presence of haemodynamically significant lesions and hence improve diagnostic sensitivity in such cases. In subjects with peripheral arterial disease, systolic ankle pressures are reduced and pressure recovery time is increased after exercise, or after occluding blood flow in the leg during the reactive hyperaemia test (Laing and Greenhalgh 1983). However, the exercise test may not be appropriate for claudicants or

those with concomitant coronary disease, who may be unable to complete the test.

The reactive hyperaemia test, although uncomfortable, is probably more suitable for use in epidemiological surveys than a treadmill test, since it is conducted at rest and uses simple equipment. A 20% change in ankle systolic pressure from the resting to the hyperaemic state is usually considered valid in identifying cases of peripheral arterial disease. The accuracy of this test has recently been assessed. The cut-off point of 20% was shown to have a sensitivity of only 52% in identifying true cases of peripheral arterial disease (Leng et al 1993). Overall, the findings suggest that a combination of non-invasive tests should be used to investigate the wide spectrum of disease which may occur in the general population.

Duplex ultrasonography is a more sophisticated non-invasive technique which is being increasingly used in vascular units and has recently been employed in two large scale epidemiological surveys, the Atherosclerosis Risk in Communities (ARIC) Study in the United States and the Edinburgh Artery Study (Howard et al 1993; Mowbray et al 1997). Duplex ultrasound detects arterial disease by a combination of high resolution ultrasound imaging and assessment of blood flow within the vessel using the Doppler effect, which describes the effect on a waveform when there are differences between transmitter and receiver frequencies, due to movement between them.

Arteries produce characteristic waveforms on Doppler which are altered by the presence of atherosclerotic disease. Criteria have been developed which relate the changes in waveform to the extent and severity of disease. The criteria for the peripheral arteries are based on alterations in peak systolic velocity and spectral broadening which occur at sites of disease.

One of the major advantages of duplex ultrasound is its ability to directly visualise the severity and location of atherosclerosis within arteries. On the other hand, it is also recognised as being relatively expensive, complex and time consuming (Allan 1991). Studies comparing ultrasound to angiography in the diagnosis of stenoses > 50% diameter in the aorta, iliac, femoral and popliteal vessels in symptomatic patients have reported good sensitivity (82%-87%) and specificity (92%-96%) (Kohler et al 1987; Cossman et al 1989). The validity and reproducibility of results using this technique have yet to be established in epidemiological surveys in the community, however.

Angiography (or arteriography) is considered the best objective method of quantifying atherosclerotic disease in living subjects and is used as the reference against which non-invasive measurement techniques of existing arterial disease are validated. The visualisation of the lower limb arterial system was first reported by Brooks in 1924 and the technique was improved by the development of organic contrast media in 1929 (Swick 1929). The use of a percutaneous catheter technique to cannulate the peripheral arteries has further refined the technique. Modern radiological practices have also greatly improved diagnostic accuracy, and have reduced the risk of patients developing significant complications and also the level of discomfort which can occur during the procedure.

Angiography was developed to directly visualise arterial segments since the disease process changes the geometry of the affected artery by narrowing the vessel lumen. Therefore, it is primarily used to illustrate the location and extent of atherosclerosis. It is less accurate, however, at predicting the haemodynamic effects of a stenosis, or progression of disease and is unable to detect the morphological changes of a complicated plaque which can lead to

thrombosis, such as haemorrhage, ulceration and necrosis.

Disease severity is expressed as 'percentage stenosis', or the percentage reduction in the lumen. In the lower limb arteries, developing lesions tend to develop eccentrically, affecting the posterior wall more severely, and atheroma occurs more commonly at bifurcations and origin of vessels. Therefore, the extent of atherosclerosis can be under-estimated unless projections in more than one plane are assessed.

1.1.4 Prevalence of peripheral arterial disease

The first large scale survey to determine the prevalence of intermittent claudication in the general population was conducted in Framingham, USA, in 1949 (Kannel and Shurtleff 1973). Since then, major epidemiological studies assessing the prevalence of both symptomatic and asymptomatic peripheral arterial disease have been carried out in many other countries, including Switzerland, Finland, Israel and Scotland (Leng and Fowkes 1993).

Most of these surveys have used the WHO questionnaire (Rose 1962) to determine the prevalence of intermittent claudication. The prevalence has varied widely, ranging from 0.3% in one survey in the East coast of America to 7.7% in a Finnish survey (Leng and Fowkes 1993). These differences reflect not only possible population differences, but also the different age and sex structures of the population samples, possible healthy worker effects and varying quality of measurement. Also, discrepancies in translation of the questionnaire may have contributed to differences in the reported prevalences internationally. Overall, however, it can be concluded that the prevalence of intermittent claudication increases with age and occurs

more frequently in men than in women (Leng and Fowkes 1993).

The prevalence of asymptomatic disease in the general population has been less widely studied, but appears to be much higher than that of claudication. In surveys which have used only an ABPI ≤ 0.9 as an indicator of disease, prevalence has varied from 4% in two studies from Belguim and Israel (De Backer et al 1979; Gofin et al 1987) to as high as 17% in the Edinburgh Artery Study (Fowkes et al 1991). However, the study population in the latter survey was considerably older (between 55 and 74 years) which may have partly accounted for the difference in disease prevalence. In another study, which used a combination of non-invasive tests, including a reactive hyperaemia test, the prevalence of asymptomatic disease in large peripheral arteries increased from 3% in subjects less than 60 years of age to over 20% in subjects of 75 years and older, although the inclusion of hyperlipidaemic subjects may have affected the prevalence rates (Criqui et al 1985).

1.1.5 Incidence of peripheral arterial disease

The incidence of peripheral arterial disease has been reported in only four large scale epidemiological studies at present. After 26 years follow-up in the Framingham Study, the incidence of claudication in men aged 40-49 years was estimated to be 0.2% a year and showed a progressive increase to 1.2% in men aged 60-69 years (Kannel and McGee 1985). Among chemical workers in the Basle Study, five year incidence rates, although not directly comparable, were similar, and ranged between 1% in men aged 35-44 years to 6% in those aged 65 years and above (Widmer et al 1985). The estimates from the Speedwell prospective Study were also comparable. Symptoms of claudication developed in 0.3% of men aged 45-49

years and 0.5% of men aged 60-63 years (Bainton et al 1994). The most recent survey, the Edinburgh Artery Study reported the highest incidence of 9% overall, but included 'probable' claudicants, a term defined by Criqui et al (1985) to improve the sensitivity of the WHO questionnaire (Leng et al 1996). All four studies were in agreement that the trend for incidence increased with age, and was higher in men than women, although the gap between men and women closed with increasing age and particularly after the menopause.

1.1.6 Aetiology of peripheral arterial disease

Risk factors associated with the development of peripheral arterial disease have been identified in an increasing number of epidemiological surveys, although most are of cross-sectional or case-control design (Leng and Fowkes 1993). The strength of the observed relationships has varied greatly from study to study; this probably reflects not only differing methods of diagnosis, but also the age and sex of the population studied and methods used to determine levels of risk factors. Although risk factors are thought to act synergistically, the role of the main risk factors in peripheral arterial disease is described individually below.

Cigarette smoking

Cigarette smoking is a major risk factor for peripheral arterial disease, both in the onset and progression of disease (Murabito et al 1997). It also appears to be a comparatively more important risk factor for this disease than for ischaemic heart disease (Fowkes et al 1992). Cross-sectional surveys have shown that the relative risk of developing peripheral arterial disease in smokers ranges between 1.4 to 10.0 (Leng and Fowkes 1993). It is probable,

however, that self-reported smoking in population-based surveys is inaccurate, and this may explain the wide range of risk estimates.

In one prospective study, the Framingham Study, 78% of incident cases of intermittent claudication were attributed to smoking (Kannel and Shurleff 1973). Furthermore, a strong dose-dependent effect of smoking on the risk and severity of peripheral arterial disease has been observed (Criqui et al 1985; Fowkes et al 1992). In more severe disease, approximately 90% of patients attending vascular clinics are cigarette smokers (Hughson et al 1978a). Moreover, the clinical prognosis in patients is worse in those who continue to smoke. For example, the rate of amputation and re-occlusion of grafts is far higher in smokers than those who stop smoking (Juergens et al 1960).

Blood pressure

Elevated blood pressure is considered a relatively weak risk factor for intermittent claudication. Systolic blood pressure is on the whole, however, thought to be more closely related to peripheral arterial disease than diastolic pressure (Schroll and Munck 1981; Gofin et al 1987). After 26 years of follow-up, the Framingham Study reported a three times higher risk of claudication for men with hypertension, systolic pressure being more strongly associated with the level of risk than diastolic pressure (Kannel and McGhee 1985). In contrast, the Basle Study found that there was no effect of either diastolic or systolic pressure on development of disease (Da Silva et al 1979). However, since blood pressure may rise as a consequence of peripheral arterial disease through increases in peripheral resistance or because of a decrease in aortic compliance (Levenson et al 1982), it may be difficult to

establish whether blood pressure has a truly independent effect on risk of disease.

Diabetes mellitus

Peripheral arterial disease is a major complication of diabetes mellitus. Diabetes has long been recognised as associated with vascular disease in the distal arteries, and with a poor prognosis in terms of development of severe chronic leg ischaemia, graft failure and amputation, although peripheral neuropathy may also contribute (Jarrett 1991). It is not surprising therefore, that population studies have consistently found the incidence of intermittent claudication to be significantly higher in diabetics compared to non-diabetics, although most of those affected are non-insulin dependent (Herman et al 1977; Slitonen et al 1986; Kannel and McGhee 1985).

The relationship between non-diabetic glucose intolerance and peripheral arterial disease is not so well defined, however. Community surveys have shown inconsistent associations between prevalent or incident claudication and either elevated fasting glucose or blood glucose after an 'oral glucose load' (Leng and Fowkes 1993). This lack of effect could be related to small numbers, particularly when cases are further subdivided by gender. Only the Framingham Study has reported an unequivocal independent relationship between impaired glucose tolerance and risk of disease (Kannel and McGhee 1985). On balance, therefore, glucose intolerance cannot be confirmed as a risk factor for peripheral arterial disease.

Blood lipids

Serum total cholesterol is an important risk factor for ischaemic heart disease, but evidence suggests that, overall, it may be a weaker risk factor for peripheral arterial disease. Patients with peripheral arterial disease have demonstrated raised levels of serum cholesterol and also lower levels of HDL cholesterol in most hospital-based case-control studies (Jacobsen et al 1984; Rühling et al 1989; Cardia et al 1990), but not all (Dormandy et al 1973a; Bradby et al 1990). Conflicting results have also been found cross-sectionally (Fowkes 1988).

In longitudinal studies, the association between total cholesterol and development of intermittent claudication, or disease estimated by the ABPI is weak, but statistically significant on multivariate analysis (Schroll and Munck 1981; Kannel and McGhee 1985). These estimates could have under-estimated the true risk, however; firstly because they are based only on one measurement of cholesterol which tends to fluctuate over time and secondly, because levels are associated with LDL cholesterol, which demonstrates an imperfect correlation with total cholesterol levels. Correction of these types of bias led to a substantial increase in the degree of risk associated with total cholesterol in one prospective study investigating ischaemic heart disease (Law et al 1994), and this effect may also be applicable to lower limb disease.

The relationship between triglycerides and peripheral arterial disease is inconclusive. Triglyceride levels are often raised in hospital patients with claudication, but in population studies, an independent effect is usually not maintained after adjusting for other lipids. In the Edinburgh Artery Study, however, there appeared to be a significant independent relationship

between triglycerides and more severe lower limb disease, but further confirmation is required (Fowkes et al 1992). It has been suggested that lipoprotein subfractions may, in fact, be better discriminators of peripheral arterial disease than lipid levels (Pilger et al 1988).

Hypercoagulability

Fibrinogen has been investigated more than any other haemostatic factor in studies relating hypercoagulability to the occurrence of peripheral arterial disease. In case-control studies of subjects with established and also asymptomatic disease, plasma fibrinogen levels are consistently elevated compared to controls (Dormandy et al 1973a; Christe et al 1984; Smith et al 1993) and have also been associated with severity of angiographic lower limb atherosclerosis (Lassila et al 1993). From the limited prospective data available, it appears that fibrinogen may also be related to the future clinical onset of peripheral arterial disease (Kannel et al 1992). The presence of both symptomatic and asymptomatic peripheral arterial disease has been associated with elevated plasma levels of other haemostatic factors, including tissue plasminogen activator (Smith et al 1994), von Willebrand Factor (Blann and McCollum 1992; Folsom et al 1993), plasminogen activator inhibitor and fibrin D-dimer (Cortellaro et al 1993; Lassila et al 1993). In particular, the importance of fibrin D-dimer as a risk factor for peripheral arterial disease, both in primary and secondary prediction is becoming increasingly apparent (Al-Zahrani et al 1992; Fowkes et al 1993; Woodburn et al 1995).

1.2 Overview of Haemostasis

This section provides an overview of haemostasis as background to a more detailed literature

review of the roles of fibrinogen and fibrin D-dimer in this process.

Haemostasis is the physiological response to blood loss following vascular injury. The process produces a fibrin-platelet clot which effectively seals the damaged blood vessel. Generation of the clot and subsequent clot lysis involves complex interactions between vascular endothelium, blood platelets, coagulation factors and components of the fibrinolytic system which are finely regulated to localise the clot to the area of damage.

1.2.1 Primary haemostasis

Following injury to the vessel, vasoconstriction mediated by serotonin and thromboxane A_2 precedes platelet adhesion, aggregation and formation of a platelet plug. This process is termed primary haemostasis and in small blood vessels, such as arterioles and capillaries, is usually adequate to control bleeding. Platelet adhesion is stimulated by vessel wall damage and exposure of blood to collagen from the subendothelial matrix. Platelet-collagen binding is mediated by a specific platelet membrane receptor, glycoprotein Ia/IIa. However, binding to von Willebrand Factor (vWF), a multimeric protein expressed by endothelial cells, is essential for normal platelet adhesion via the platelet receptor, glycoprotein Ib (Sixma 1987).

These interactions result in a monolayer of platelets spreading across the vessel endothelium. Further recruitment of platelets occurs only after platelet activation which is stimulated by agonists, such as thrombin, adenosine diphosphate (ADP) and thromboxane A_2. This autocatalytic process involves morphological changes in the platelet shape, the expression of the glycoprotein receptor IIb/IIIa for binding of fibrinogen and exposure of negatively charged

phospholipid molecules on the platelet surface for the binding of coagulation factors. In addition, platelet micro-particles are formed which have strong pro-coagulant activity.

During activation, platelets release the contents of four types of intra-cellular granules: α-granules, dense bodies, lysosomes and peroxisomes. High concentrations of thrombin or collagen stimulate α-granule release of proteins which mediate coagulation, including fibrinogen, vWF, factor V, platelet-derived growth factor, platelet factor 4, beta-thromboglobulin (β-TG), plasminogen, and plasminogen activator inhibitor (PAI). Dense bodies secrete serotonin, adenosine, nucleotides and inorganic phosphates, in response to stimulation by ADP, adrenaline and relatively lower concentrations of thrombin and collagen. Lysosomes contain proteolytic enzymes and peroxisomes contain catalases and other proteins.

Expression of the platelet glycoprotein receptor IIb/IIIa for fibrinogen leads to platelet aggregation by calcium-dependent inter-platelet bridging. Under high shear conditions, e.g. at arterial stenoses, binding of vWF to this receptor is necessary for platelet aggregation.

1.2.2 Coagulation pathways

The function of the blood coagulation system is to produce fibrin in order to stabilise and strengthen the platelet plug formed as a result of endothelial damage. Blood coagulation is described as a cascade of reactions which involves the formation of enzyme-cofactor complexes and substrates (Mann 1988). Inactive factors (zymogens) are converted sequentially to the active protease enzymes which bind to regulatory factors (co-factors) and calcium ions to generate thrombin which polymerises the substrate, fibrinogen to fibrin. In

vivo assembly of these complexes takes place on negatively charged phospholipid surfaces of activated platelets or endothelial cells which amplifies the activation rate of the coagulation factors and also localises the clot to a particular site. It is generally accepted that there are two distinct systems in fibrin generation, the intrinsic and extrinsic pathways. However, this division is arbitrary since the pathways are integrated physiologically. These pathways are shown in Figure 1.

Intrinsic coagulation pathway

This pathway is described as 'intrinsic' because all the components are present in blood in an inactive precursor form. It can be initiated experimentally by the absorption of factor XII and a complex of three proteins, high molecular weight kininogen, prekallikrein and factor XI, onto a negatively charged surface, and is termed the 'contact phase'. The conversion of factor XII to activated factor XII (XII_a) is followed sequentially by activation of factors XI, IX, X and prothrombin. A co-factor, factor VIII, is required for factor IX_a to convert factor X to X_a in conjunction with ionised calcium, phospholipid and factor IX_a on cell surfaces. The function of factor VIII is to orientate factor IX_a and its co-factor $VIII_a$ on the phospholipid surface for optimal catalysis. The phospholipid surfaces of platelets can further promote coagulation pathways by the expression of binding sites for factors V_a and $VIII_a$.

Although the activation of factor XII is involved in other physiological processes, e.g. fibrinolysis, inflammation and the complement system (Colman 1993), the relevance of factor XII to coagulation in vivo is not clear. Patients with a deficiency of factor XII do not exhibit abnormal haemostasis, whereas a lack of factor XI can cause a severe bleeding disorder. Thus,

an alternative mechanism may exist to produce factor XI_a which does not require factor XII. It has been proposed that thrombin produced by the extrinsic pathway directly activates factor XI, which in turn generates additional factor XI_a through autoactivation, although this has not been demonstrated in vivo (Gailani and Broze 1991).

Extrinsic coagulation pathway

It is generally accepted that in vivo coagulation is initiated when tissue factor (TF), the high affinity glycoprotein receptor for factor VII, is expressed on activated cells. Although TF is present on many extravascular cells such as subendothelial collagen and adventitial cells, intravascular expression of TF on endothelial cells and monocytes occurs through stimulation by thrombin and the cytokines, interleukin-1, endotoxin and tumour necrosis factor, agonists which are also involved in the inflammatory response and infection (Gladal 1984).

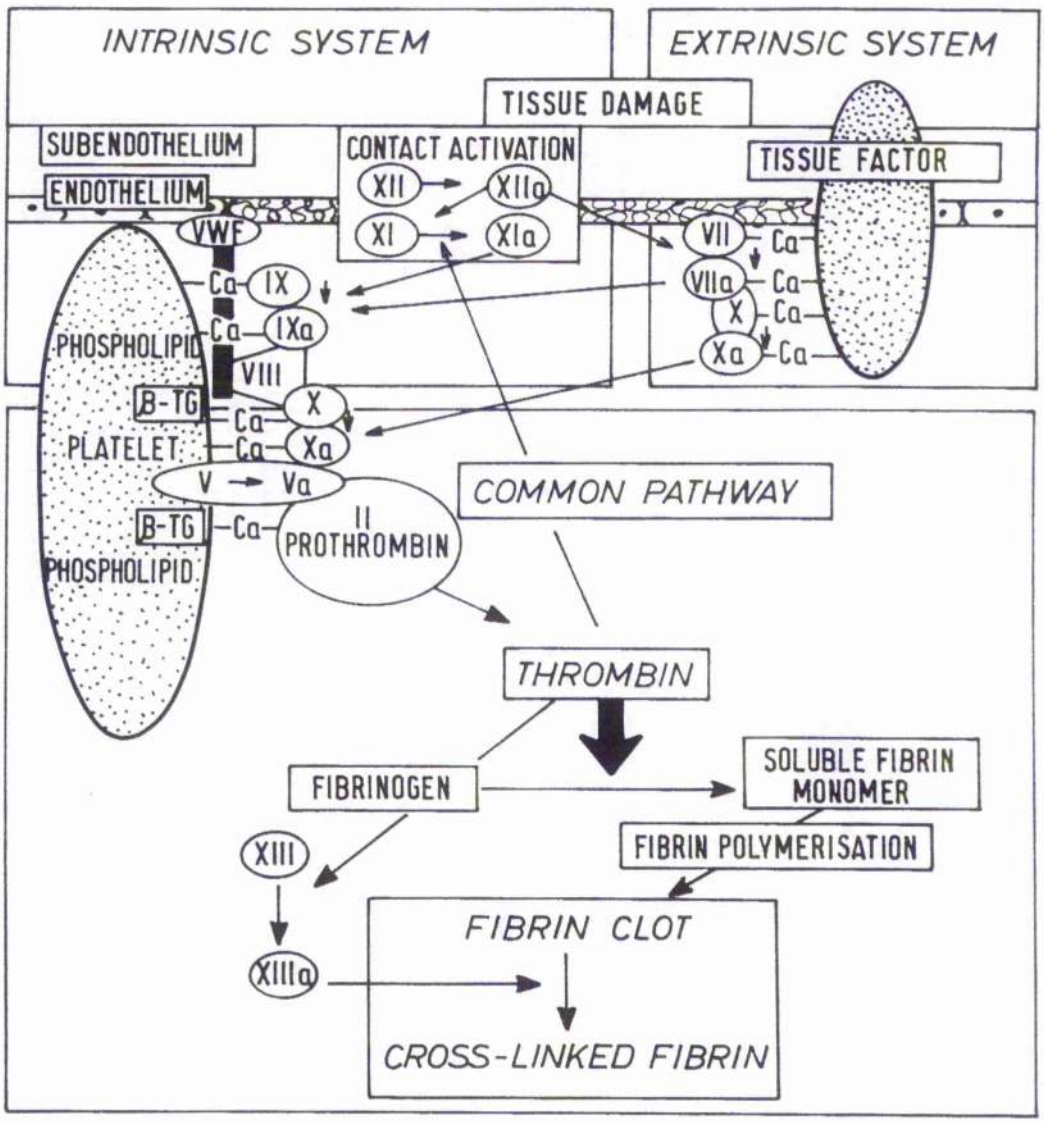
Subsequent exposure of TF to blood due to injury results in the rapid formation of a 1:1 complex with factor VII and activation of factor IX to IX_a. Approximately 1% of factor VII circulates in plasma in the active form. Factor X_a can also be directly generated by the TF-factor VII_a complex (although this is rapidly inactivated by tissue factor pathway inhibitor when a trace of factor X_a is produced) and by the factor IX_a-factor $VIII_a$ complex. The rate of factor X activation by the factor IX_a-$VIII_a$ complex is much greater than the rate of generation catalysed by the TF-factor VII_a complex (Mann et al 1990), and may be the main mechanism of factor X_a formation in vivo. Finally, a third complex, the prothrombinase complex, is formed between factor X_a (generated by either of the two coagulation pathways) and its co-factor V_a on the phospholipid surface of activated platelets or endothelial cells.

The production of thrombin from prothrombin is described as the final common pathway of coagulation. Factors X_a and prothrombin from the prothrombinase complex bind to the cell membranes by gamma carboxyglutamic residues and calcium ion bridging. Prothrombin undergoes three catalytic cleavages regulated by factor V_a to produce a two-chain thrombin molecule linked by disulphide bonds which is released from the cell surface (Krishnaswamy et al 1987).

Formation of the fibrin clot

Thrombin catalyses the polymerisation of fibrinogen by the initial release of fibrinopeptide A by peptide bond cleavage at the amino terminal end of the Aα chain, and then release of fibrinopeptide B from the Bβ chain. This allows the resulting fibrin monomers to interact in a half-overlap and side-to-side manner to form protofibrils which grow into linear networks, called fibrin gel. The structure of the gel is influenced by the rate of fibrinogen activation by thrombin, the formation of which is dependent on the fibrinogen concentration (Blombäck 1994). At high fibrinogen concentrations, the gel becomes rigid and tight and occupies a greater volume than gel formed at lower fibrinogen concentrations (Blombäck et al 1994). The stability of the fibrin clot or gel is increased by cross-linkage of fibrin polymers catalysed by factor XIII, activated by thrombin in the presence of ionised calcium. Gamma chains of adjacent protofibrils are rapidly linked to form dimers by covalent isopeptide bonding, followed by slower Aα chain linkage. This cross-linking increases the elasticity, tensile strength and resistance to plasmin degradation of the fibrin clot. Factor XIII also interacts with the adhesive platelet protein, fibronectin to promote binding of platelets and the fibrin clot to the vessel wall, thus effectively sealing the damaged endothelium.

FIGURE 1 THE COAGULATION SYSTEM



Regulation of blood coagulation

There are a number of mechanisms which prevent coagulation from becoming generalised. Central to this control is the ability of the endothelium to modify its function in response to pro-coagulant stimuli by expressing anti-coagulant activity. Antithrombin-III (AT-III) can inactivate circulating thrombin. In addition, AT-III is able to neutralise the activity of factors XII_a, XI_a, IX_a, and X_a (Clause and Comp 1986) and the tissue factor-VII_a complex (Lawson et al 1993). The formation of these complexes is accelerated by exogenous heparin and by the endogenous AT-III co-factor, heparin sulphate, which is synthesised and expressed on endothelial cells and binds to AT-III on the cell surface.

A second anti-coagulant complex is formed between thrombin and thrombomodulin, a specific thrombin-binding receptor expressed in high concentrations on resting vascular cells (Esmon et al 1993). Subsequently, thrombin becomes conformationally altered so that it is unable to either recognise fibrinogen or activate platelets. The thrombin-thrombomodulin complex also catalyses the activation of protein C, which with its co-factor protein inhibits the coagulation factors V_a and $VIII_a$ (Stern et al 1986) and increases plasminogen activator inhibitor (PAI) formation.

Thrombin can itself directly stimulate the secretion of two vasodilators from the endothelial cells, prostacyclin and nitric oxide. These mediators act synergistically to inhibit local platelet aggregation (Radomski et al 1988) and also nitric oxide can inhibit platelet adhesion, thus limiting the extent of platelet deposition intravascularly.

1.2.3 Fibrinolysis

The fibrin clot is removed from the blood vessel when normal vascular structure and function is restored. The fibrinolytic system controls the degradation of fibrin and comprises an inactive proenzyme, plasminogen which is converted by plasminogen activators to the active serine protease, plasmin. The system is illustrated in Figure 2. Astrup (1956) suggested that balanced interactions between fibrin formation and degradation occurs even in healthy vasculature. Interaction with intrinsic coagulation is demonstrated by the generation of plasmin by activated factor XII (Francis and Marder 1986).

Two extrinsic plasminogen activators (PA) have been identified: tissue type PA (t-PA) and urokinase type PA (u-PA). u-PA may participate mainly in tissue remodelling and repair, macrophage activity and tumour invasion, through matrix degradation or growth factor activation. In contrast, t-PA is believed to be mainly responsible for the degradation of fibrin at the endothelial surface. Vascular endothelial cells synthesise and secrete t-PA into plasma (Van Hinsbergh et al 1994) but platelets also contain a form of t-PA. Other cells, such as monocytes can be stimulated to produce t-PA by the cytokine, interleukin-4 (Hart et al 1989). Certain stimuli, such as venous occlusion, catecholamines, exercise and bradykinin cause a rapid release of t-PA from cellular storage pools in endothelial cells.

Plasminogen is converted to plasmin by cleavage of a peptide bond which creates a heavy and a light amino acid chain. The activation rate of plasminogen by t-PA is enhanced markedly in the presence of fibrin and is due to three processes: fibrinopeptide A release, fibrin polymerisation and release of fragment X, an early fibrin degradation product (Suenson et al

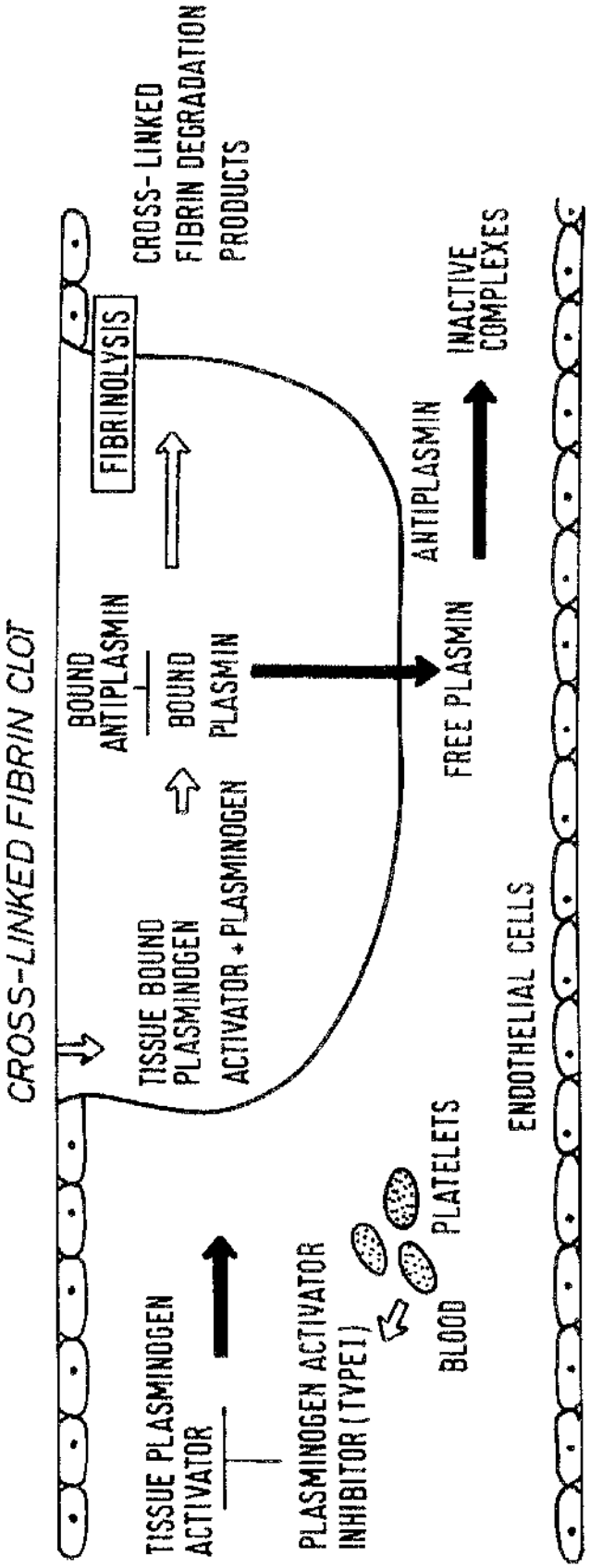
1984). A complex is subsequently formed between t-PA, fibrin and plasminogen by absorption onto the fibrin surface. Optimal orientation of plasminogen by the binding of t-PA to fibrin by kringle domains also produces enhanced catalysis of plasminogen by t-PA (Horrevoets et al 1994). The enzymatic activity of plasmin via binding to plasminogen receptors is also increased on cell surfaces (Gonzalez-Gronow et al 1991).

Regulation of fibrinolysis

Regulation of this system occurs through plasmin inhibitors, t-PA binding to fibrin and cellular receptors, or via the activity of the specific t-PA inhibitors, plasminogen activator inhibitors (PAI). This effectively limits fibrinolysis to the site of injury and prevents systemic fibrin breakdown.

The main inhibitor of plasmin is alpha $(\alpha)_2$-antiplasmin, which belongs to the α_1-proteinase inhibitor class of serine protease inhibitors (serpins). It forms a irreversible complex with plasmin in a two-stage reaction, although the speed of this reaction is influenced by the availability of fibrin binding sites on plasminogen (Wiman and Collen 1978). Plasmin, when its lysine binding sites are occupied by fibrin, is inhibited at a rate two to three times slower than free plasmin (Collen and Lijnen 1991). Cross-linking of α_2-antiplasmin to fibrin, catalysed by factor $XIII_a$, may enhance fibrin resistance to degradation (Aoki and Harpel 1984). Other proteolytic inhibitors, such as α_2-macroglobulin, α_1-antitrypsin and complement 1-inhibitor may play a secondary role in plasmin inhibition, but only when the functional concentration of α_2-antiplasmin is lower than that of plasmin.

FIGURE 2 THE FIBRINOLYTIC SYSTEM



Regulation of fibrin breakdown is also controlled by specific t-PA cellular receptors. These receptors control rates of elimination of t-PA by the liver, or localise its activity to specific cell surfaces. Uptake of t-PA in the liver is mediated by carbohydrate-specific receptors or low density lipoprotein (LDL)-receptor related protein receptors. Lack of the carbohydrate, mannose on t-PA side chains, for example, decreases the rate of t-PA clearance time by hepatic endothelial cells (normally about five minutes) and hence fibrinolytic activity is effectively increased (Otter et al 1991).

The interaction of t-PA with cellular receptors expressed on endothelium, platelets and monocytes can mediate t-PA binding at these sites and increase pro-fibrinolytic activity. For example, t-PA binding to annexin II on endothelial cells, in conjunction with plasminogen, enhances plasminogen activation by supporting the formation of a ternary complex, analogous to that occurring on the surface of fibrin (Lijnen et al 1990).

Fibrinolysis is also regulated by specific t-PA inhibitors, (PAI). PAI are synthesised by a wide variety of cells and are found in plasma, platelets, placenta and in the extracellular matrix. There are at least four forms. PAI-1 (released from endothelial cells, hepatocytes and platelets), PAI-2 (found in the placenta, pregnancy, plasma and also in monocytes), PAI-3 (found in urine) and protease-nexin 1, which is synthesised by smooth muscle cells and fibroblasts (Rosenblatt et al 1987). Synthesis and secretion of PAI-1 can be altered by agonists such as hormones, growth factors and cytokines. It is synthesised in an active form, which spontaneously converts to a latent form that may be reactivated on the phospholipid surfaces of endothelial cells when required (Emeis and Kooistra 1986)). Most PAI-1 in platelets (and hence in blood) occurs in the latent form.

Active PAI-1 bound to the co-factor, vitronectin, complexes rapidly with t-PA and thus inhibits further plasmin formation. The binding to vitronectin is thought to stabilise PAI-1 activity and protect it from inactivation (Seiffert et al 1990). In addition, PAI-1 strongly binds to fibrin and may protect it from plasmin degradation and premature clot lysis.

Fibrin degradation

The removal of the fibrin clot is controlled by the interaction between plasminogen, plasmin, and their specific activators and inhibitors. Cross-linked fibrin has a greater resistance to plasmin degradation than either fibrinogen or non cross-linked fibrin due to the stabilising effect of factor $XIII_a$-induced isopeptide bonds. These bonds are also responsible for the unique degradation products produced in the breakdown of cross-linked fibrin. Initial degradation of the two-stranded protofibril is produced by cleavage of the Aα chain, which is followed by cleavage of all three polypeptide chains. A series of non-covalently bound complexes are formed, the smallest of which consists of two cross-linked fragment D or terminal domains of fibrinogen, named D-dimer (Gaffney and Brasher 1973).

1.3 Fibrinogen

Fibrinogen was first described in 1836 by a Glasgow surgeon, Buchanan, who noted that blood appeared to coagulate because it contained 'at once fibrin and substances capable of reacting upon it, and so occasioning coagulation' (Buchanan 1836). A potential role for fibrinogen and fibrin in the pathogenesis of arterial disease was first suggested only a few years later when the German pathologist, von Rokitansky, concluded that atheroma in the

arterial wall was formed by fibrin deposits from the blood (von Rokitansky 1852). In 1946, another pathologist, Duguid, in Aberdeen, confirmed that fibrin was incorporated both within and on the surface of atherosclerotic plaques and contributed to mural thrombosis (Duguid 1946).

Although it was recognised that there was an association between thrombosis and the occurrence of myocardial infarction as long ago as 1952 (Gilchrist and Tulloch 1952), the study of haemostatic factors in the pathogenesis of arterial disease was largely ignored in favour of blood lipids. Only in the last twenty years has enough evidence accumulated from clinical, experimental and epidemiological studies to support the contribution of fibrinogen and other haemostatic factors to the aetiology of arterial disease.

1.3.1 Structure of fibrinogen

Fibrinogen is an elongated molecule which is highly asymmetrical. It is a dimeric glycoprotein with a molecular weight of approximately 340,000 Daltons. It is composed of three pairs of polypeptide chains, two Aα, two Bβ and two γ, bound together by disulphide bonds (Doolittle 1981). The three dimensional structure of fibrinogen has been determined from electron microscopy and biochemical data and is illustrated in Figure 3. The diagram shows a central nodule (A) or domain connected to two identical terminal domains (D) by coiled chains comprised of interlocked Aα, Bβ, and γ chains. The smaller central domain is made up of the amino-terminal ends of all six polypeptide chains, whereas the terminal domains consist of carboxy-terminal regions of the β and γ chains. The fibrinopeptides A and B, which are released when fibrinogen is converted to fibrin, are situated at the amino-terminal

ends of the Aα and Bβ chains.

The composition of fibrinogen varies due to differences in amino acid residues in the three polypeptide chains. Heterogeneity is predominantly due to changes in amino acids at the carboxy and amino terminal ends of the Aα chains. Three forms of fibrinogen have been identified due to Aα chain heterogeneicity - high molecular weight fibrinogen and two low molecular weight fibrinogen variants (Mosesson 1983). High molecular weight fibrinogen clots more readily than the lower weight variants because of a faster polymerisation rate (Holm et al 1985). Variation occurs less frequently in the γ chain at the carboxy terminal regions but results in differences in charge and molecular weight. Changes also occur in the Bβ chain producing differences in sialic acid residues (Töpfer-Peterson et al 1976).

1.3.2 Regulation of fibrinogen synthesis

Plasma fibrinogen is synthesised in the liver by hepatic parenchymal cells. Each individual polypeptide chain is synthesised separately and assembled into the dimer before secretion by hepatocytes. It circulates in plasma at a concentration of between 1.5 g/L and 4.5 g/L, which is well above the concentration (0.5g/L) required for normal haemostasis. About 3% of circulating fibrinogen is contained in platelet alpha-granules, but appears to lack the γ chain. The biological half life of fibrinogen is approximately 100 hours, but the main catalytic pathway responsible for its breakdown has not, as yet, been determined (Green and Humphries 1989).

Fibrinogen shows a high degree of biological variation within an individual and between

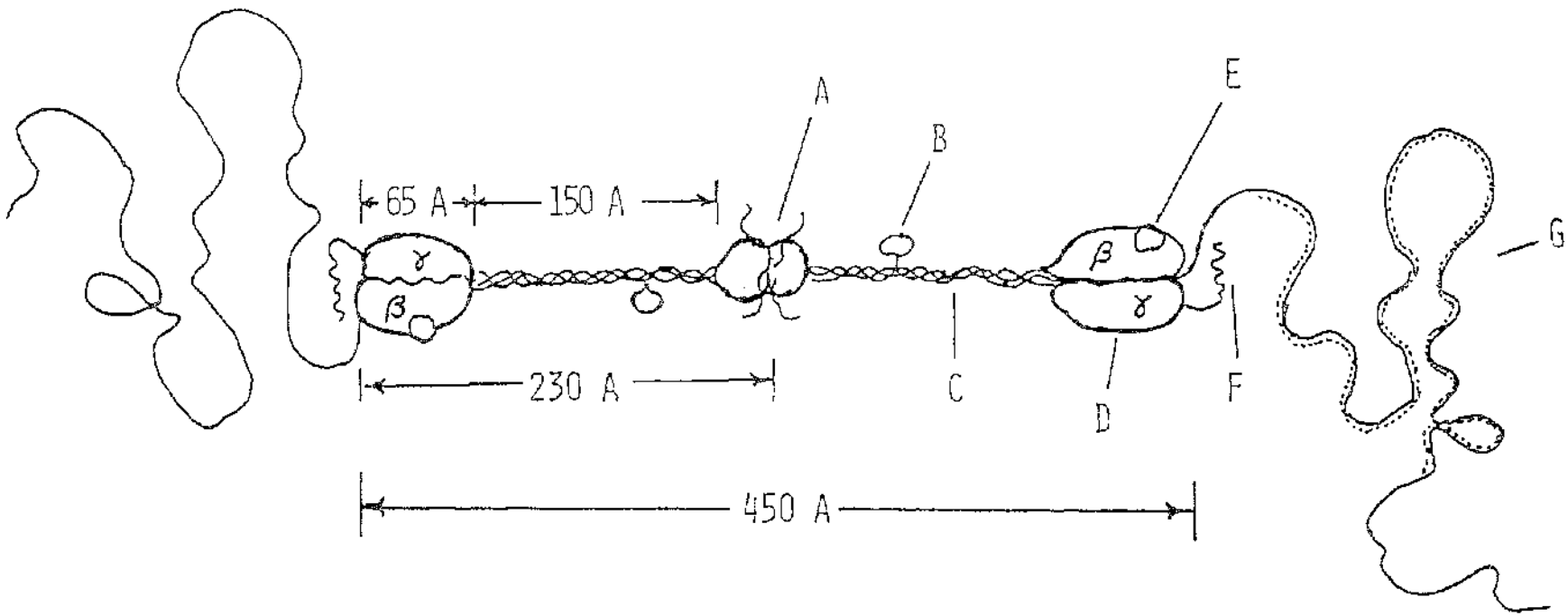


FIGURE 3 Schematic depiction of vertebrate fibrinogen molecule. A, central domain including fibrinopeptides; B, γ chain carbohydrate cluster; C, "coiled-coils" interdomainal connector; D, terminal domain consisting of homologous β and γ chain segments; E, β chain carbohydrate cluster; F, γ chain cross-linking site; and G, α chain carboxy-terminal extension.

individuals in response to many stimuli. It is one of a number of proteins whose plasma levels rise in conditions such as inflammation, infection, neoplasia and pregnancy. This systemic response is called the acute phase reaction. Fibrinogen levels can rise up to 20 times during this reaction and is due to increases in mRNA levels. Hepatic synthesis of fibrinogen is believed to be controlled by at least three inflammatory cytokines, interleukin-6 (IL-6), leukaemia inhibitory factor and oncostatin M, which are secreted by activated monocytes or damaged endothelial cells. In vitro experiments have shown that the addition of these factors to culture medium can produce up to a six fold rise in fibrinogen synthesis by the hepatoma cell line, HepG2 (Vasse et al 1994). There is also some evidence that the production of IL-6 by monocytes is dependent on prior stimulation by fibrin degradation products and therefore may indirectly control fibrinogen levels through a positive feed-back mechanism. Glucocorticoid hormones (including insulin) also directly affect fibrinogen synthesis in the liver and may be ultimately responsible for controlling the duration of the acute phase response (Green and Humphries 1989).

1.3.3 Function of fibrinogen in haemostasis, atherogenesis and thrombogenesis

One of the key roles of fibrinogen in haemostasis is its polymerisation to fibrin by the action of thrombin. Fibrinogen is also involved in a number of other cellular interactions which are important in haemostasis and potentially implicated in atherogenesis and thrombogenesis.

During haemostasis, the rate of clot formation may be controlled by fibrinogen binding to thrombin (Liu et al 1979), but conversely, the binding of fibrinogen to fibrin can inhibit further fibrin formation. The structure of the fibrin clot also appears be influenced by the level of

plasma fibrinogen, with tight, rigid clots being formed at a higher fibrinogen concentration which may be potentially more thrombogenic (Blombäck 1994). Fibrinogen also appears to be involved in the control of fibrinolysis in which interactions with the fibrinolytic proteins, plasminogen and t-PA are thought to increase the rate of clot lysis.

Fibrinogen is an essential co-factor in optimum platelet aggregation, a process which initiates normal haemostasis. By binding to the platelet glycoprotein receptor, IIb/IIIa, fibrinogen can bridge adjacent platelets previously activated by ADP, thrombin, collagen and adrenaline (Marguerie and Plow 1983). Data from the Northwick Park Heart Study has demonstrated a direct correlation between increasing fibrinogen levels and ADP-stimulated platelet aggregation under laboratory conditions (Meade et al 1985). When high levels of fibrinogen occur in vivo, the size and speed of platelet-rich clot formation may be increased in potentially thrombotic events, such as plaque rupture.

Fibrinogen is a major determinant of plasma viscosity and red cell aggregation, two factors which strongly influence the flow properties of blood. A two-fold increase in fibrinogen raises plasma viscosity by 20% and also blood viscosity by the same amount. A rise in viscosity may also increase blood pressure and shear stress at the vessel wall, which could promote platelet activation and subsequent thrombus formation in areas where there is restricted vasomotor activity. Where conditions of low shear predominate, such as at arterial bends and bifurcations, high levels of fibrinogen (and hence increased local blood viscosity) could contribute to atherogenesis through reduced blood flow and accumulation of atherogenic factors (Lowe 1986). Furthermore, under these low shear conditions, fibrinogen can contribute to the formation of erythrocyte aggregates which may be important in disturbed microvascular

blood flow and may further promote ischaemia.

In vitro studies using cultured endothelial cells have also found that fibrinogen is implicated in arterial wall damage, a process which may initiate atherogenesis. Both fibrinogen and fibrin can alter endothelial permeability by disrupting endothelial cell organisation and cause their migration into the intima (Dejana et al 1985). Other fibrinogen or fibrin-induced mechanisms related to the onset of atherosclerosis, include the migration and stimulation of smooth muscle cell proliferation and a reduction in prostacylin synthesis (Watanabe et al 1984; Naito et al 1992). Fibrinogen also binds to lipids, particularly Lp(a) and LDL cholesterol and provides a substrate for their accumulation within the lesion (Thompson and Smith 1989). The process of intimal thickening observed after induced vascular injury is also influenced by fibringen levels in vitro (van Pelt-Verkuil et al 1989).

There is also evidence that fibrinogen is implicated in the progression of arterial disease. Firstly, the amount of fibrin present in atherosclerotic lesions is directly correlated with plasma fibrinogen levels (Gurewich et al 1976). Using immunological techniques, Bini et al (1987) have further shown that the proportion of fibrin II (lacking fibrinopeptides A and B) rises with increasing severity of atherosclerotic lesions and concluded that the particular distribution of fibrinogen and fibrin within these lesions indicated dynamic interactions between fibrinogen, macrophages and smooth muscle cells within the evolving plaque.

Fibrinogen may be involved in other cell interactions which are central to the progression of atherosclerosis. Fibrinogen may facilitate cholesterol ester production and cholesterol transfer from platelets to macrophages. The process is thought to occur through the binding of

fibrinogen to two receptors, Mac-1 on activated macrophages and the platelet receptor IIb/IIIa, with subsequent uptake of fibrinogen into the macrophage. It was hypothesised that this may be a mechanism for the transport of cholesterol from platelets to macrophages by thrombophagocytosis, resulting in foam cell formation which is indicative of progression of atherosclerosis (Loscalzo 1992).

1.3.4 Measurement of fibrinogen levels

Four main types of assays are used to measure fibrinogen in clinical and epidemiological studies: clotting rate assays, clottable protein assays, precipitation assays and immunological assays.

Clotting rate assays

The Clauss assay measures the time between the addition of thrombin to diluted plasma and polymerisation of clottable fibrinogen. The clotting time is calibrated against the clotting times of standard fibrinogen plasmas and the fibrinogen concentration of the test plasma is then calculated (Clauss 1957). The advantage of this assay is that it is easy to perform, but there may be a degree of variability associated with heterogeneity in fibrinogen structure, and also because other haemostatic factors, such as fibrin degradation products which can become attached to the clot may alter the clotting time.

Clottable protein assays

In this type of assay, fibrinogen is quantitated directly. Thrombin is added to a sample of plasma and allowed to set until a clot is formed. The amount of fibrinogen in the clot mass is then estimated e.g. by spectrophotometry (optical density at 279nm or 315nm) or by gravimetry (dry weight). Although fibrinogen heterogeneity seems to have a minimal effect on measurement, this type of assay has a number of disadvantages. Like the Clauss assay, it requires blood dilution of approximately 10% by citrate anti-coagulants and sample processing within hours of blood sampling. It is also time-consuming and laborious.

Precipitation assays

Precipitation assays measure total circulating fibrinogen and hence estimated levels tend to be higher in this type of assay compared to clotting assays. In this method, fibrinogen is precipitated either by heating plasma at 56^{0}C diluted in saline buffered at pH 6.3 (Stone and Thorp 1985) or by using salts, such as sodium sulphite. Fibrinogen is then measured either by nephelometry (change in light scatter) or by turbidometry. One limitation of this technique is that other plasma proteins, such as fibrin degradation products can also be precipitated and hence the true level of fibrinogen may be over-estimated.

Immunological assays

This technique was originally based on polyclonal antibodies which were non-specific and bound to fragments of fibrinogen and fibrin, in addition to the intact molecule. An ELISA

(enzyme linked immunosorbent assay) has been developed which uses monoclonal antibodies specific to the amino and carboxy terminal ends of the Aα fibrinogen chain but does not recognise other fibrinogen derivatives. One advantage of this particular assay is that it only takes one hour to complete.

1.3.5 Standardisation of fibrinogen

Epidemiological studies have employed a variety of different assays to measure fibrinogen levels using a number of commercial or in-house standards. As a result, plasma fibrinogen levels have varied widely between the studies and there has been considerable discussion about which is the best assay method to employ in assessing the risk of arterial disease. For example, whereas the majority of studies have used clotting or clotting rate assays (which are thought to represent the functionality of fibrinogen), recent evidence has suggested that the assay which measures total circulating fibrinogen by nephelometry may be a better predictor of arterial disease (Sweetnam et al 1998).

Although there has not, as yet, been agreement on the type of assay which should be routinely used in epidemiological studies, a WHO International Standard for fibrinogen measurement has been established at 2.4 g/L of clottable fibrinogen (Gaffney and Wong 1992). This should ensure better inter-laboratory comparability and also help to establish reference ranges which are important in defining what level might constitute an increase in the risk of arterial disease.

1.3.6 Determinants of fibrinogen levels

Many intrinsic and extrinsic factors can affect fibrinogen levels both in the general population and in patients with atherosclerotic diseases. Identification of these factors is important in establishing the reference range within different populations and also in the interpretation of the relationship between fibrinogen and arterial disease. In the following section, the major determinants of fibrinogen are discussed based on results from epidemiological studies.

Geographical variation

Fibrinogen levels vary considerably among different populations. In general, levels increase with the population risk of ischaemic heart disease. Fibrinogen levels are highest among the Irish, Scots and Finns, intermediate among English and Caucasian Americans, and lowest among Asians, such as the Japanese, which is consistent with their respective degree of risk of ischaemic heart disease. However, there are exceptions. For example, rural Gambians and Greenland Eskimoes have high levels of fibrinogen and low risk of ischaemic heart disease. In the case of the Africans, this elevation may be attributable to parasitic infection (Folsom 1995), whereas a high dietary intake of polyunsaturated omega-3 fatty acids may account for these associations in Eskimos (Bjerregaard and Dyerberg 1988).

Age

Plasma fibrinogen levels rise progressively with age in both sexes. This trend is particularly evident in men from the fourth decade, and in post-menopausal women from the fifth decade

onwards. It has been estimated that in adults, fibrinogen increases at a rate of approximately 0.2g/L every ten years (Meade et al 1979; Lee et al 1990; Folsom et al 1991). This is consistent with the higher incidence of vascular disease among older age groups. On the other hand, elevations in fibrinogen recorded in healthy centenarians in a recent case-control study suggest that high levels are not always deleterious (Mari et al 1994).

Sex

Clotting fibrinogen levels are consistently higher in women compared to men, despite a lower risk of vascular disease in pre-menopausal women (Kannel et al 1987). Levels are reported to be similar in men and women, however, if fibrinogen is measured by a heat precipitation assay (GDO Lowe, personal communication). This suggests that there may be variation in the molecular structure of fibrinogen between the sexes or there is a relatively higher proportion of clotting fibrinogen in total circulating fibrinogen in women.

Cigarette smoking

Cigarette smoking is considered one of the most important environmental determinants of fibrinogen levels. Current smokers have approximately 10% higher fibrinogen levels compared to non-smokers (Ernst and Resch 1993). Most of the major epidemiological studies have found that the levels of fibrinogen are highest in current smokers and lowest in non-smokers, with intermediate levels found in ex-smokers (Wilhelmsen et al 1984; Balleisen et al 1985; Meade et al 1986; Kannel et al 1987). The effect is also dose-dependent, in that fibrinogen rises linearly with the number of cigarettes smoked per day (Ernst et al 1987).

Although fibrinogen levels start to fall within two weeks of stopping smoking, it may take up to 10 years for levels to return to that of a never-smoker (Meade et al 1987).

It is not clear which components of cigarette smoke are responsible for stimulating fibrinogen synthesis. The effect may not be mediated by nicotine because levels of fibrinogen in oral snuff takers have been found to be similar to non-smokers, even though the snuff takers had higher levels of cotinine (the main metabolite of nicotine) than current cigarette smokers (Eliasson et al 1995). The mechanism by which smoking increases fibrinogen is not fully understood, but it may be through an inflammatory response to injury to pulmonary or arterial endothelium and involves the production of fibrin degradation products which activate leucocytes to release cytokines, such as interleukin-6. These in turn may stimulate liver hepatocytes to up-regulate fibrinogen synthesis.

Blood pressure

Most epidemiological studies have reported significant univariate associations of fibrinogen with systolic and diastolic blood pressure which were not maintained on multivariate analysis (Wilhelmsen et al 1984; Kannel et al 1987; Rosengren et al 1990). One exception is the Münster Study which reported an independent relationship between systolic pressure and fibrinogen in women (Balleisen et al 1985). In the Framingham Study, fibrinogen levels rose linearly with the severity of hypertension in both men and women (Kannel et al 1992). There is a direct correlation between fibrinogen and levels of systolic and diastolic blood pressure in hypertensive patients.

Blood lipids

Reports on the correlation between plasma fibrinogen and blood lipid levels indicate weak effects within the major epidemiological studies. Increases in fibrinogen have been related to a threshold level of serum cholesterol of 232mg/dL in the Framingham Study, which may account for the low overall correlations (Kannel et al 1992). The PROCAM Study found small positive correlations between fibrinogen and serum cholesterol and between fibrinogen and triglycerides, which tended to be stronger in women (Heinrich et al 1990). In contrast, the ARIC Study reported an inverse correlation between triglycerides and fibrinogen which was stronger in Japanese men (r=-0.29) than in other racial groups (Iso et al 1993). However, this negative effect may reflect the associations of triglycerides with other factors,such as HDL cholesterol. Other epidemiological studies have reported inverse associations between fibrinogen and HDL cholesterol (Iso et al 1993).

Obesity

Obesity is frequently associated with increases in blood pressure, total cholesterol and triglycerides and decreases in HDL cholesterol and physical activity. It is therefore difficult to determine any truly independent association between fibrinogen and excess body weight. Obese subjects have demonstrated higher levels of fibrinogen compared to lean individuals in some cross-sectional studies, regardless of whether obesity is measured as the body mass index (Lee et al 1990), the Broca index (Balleisen et al 1985), or skin thickness (Meade 1981). In another study, the significant univariate association between fibrinogen and abdominal fat (represented by the waist-to-hip ratio) was not maintained in multivariate analysis which

included physical fitness (Møller and Kristensen 1991), whereas another survey among working men showed no univariate relationship between fibrinogen and these variables (De Bocver et al 1995). In post-menopausal women, fibrinogen was correlated with body mass index, but not with the waist-to-hip ratio (Meilahn et al 1996).

Menopause

Fibrinogen levels rise more rapidly after the onset of menopause as does the incidence of ischaemic heart disease in women. This suggests that sex hormones, such as oestrogen may have a protective effect against ischaemic heart disease, possibly through haemostatic mechanisms. Meade et al (1983) estimated that fibrinogen levels were between 6% and 10% higher in postmenopausal women compared to premenopausal women of similar age. This has been confirmed in other studies (Balleisen et al 1985; Meilahn et al 1992; Iso et al 1993; Brunner et al 1996).

Oral contraceptives and hormone replacement therapy

Fibrinogen levels are consistently raised in women who use oral contraceptives containing oestrogen (Balleisen et al 1985; Lee et al 1993). In spite of these elevations, current or past use of low-dose contraceptives does not appear to increase the risk of ischaemic heart disease in healthy women (Brezinka and Padmos 1994). There is, however, a significant increase in the risk of myocardial infarction and stroke in women over 35 years who both smoke and use contraceptives. In contrast, oestrogen replacement therapy taken by post-menopausal women is associated with a decrease in fibrinogen of 0.1-0.15g/L compared to non-users (Iso et al

1993; Lee et al 1993; Meilahn et al 1995). An interaction between fibrinogen, distribution of body fat and oestrogen use was also noted by Meilahn et al (1995). Women using hormone therapy had less abdominal fat than non-users and this was significantly associated with their lower levels of fibrinogen in multivariate analysis.

Early development and psychosocial factors

There is increasing evidence that certain factors which operate in early life may partly determine fibrinogen levels in adulthood. Barker et al (1992) found that men who had low rates of infant growth or foetal growth showed higher levels of fibrinogen in adulthood. Other factors associated with childhood environment (short stature, father's social class and a subject's level of education) were inversely related to fibrinogen levels in both men and women and these associations were only partly mediated by current lifestyle factors (Brunner et al 1996).

The association between the risk of cardiovascular disease and low social class and occupational stress is well documented (Marmot et al 1978; Hein et al 1992) and this relationship may be partly mediated by fibrinogen (Markowe et al 1985). Emotional stress can directly increase fibrinogen under controlled conditions (Jern et al 1989). However, the influence of psychosocial factors on fibrinogen is more difficult to determine in the general population. While low social class and occupational stress has been found to be independently associated with increases in fibrinogen (Markowe et al 1985), other studies have suggested that this relationship was due to the confounding effects of smoking, body weight and physical activity (Baker et al 1988; Rosengren 1990). Similarly, the association between elevated

fibrinogen and low work control was attenuated by adjustment for these variables and also health status (Brunner et al 1996).

Genetic regulation of fibrinogen

Measurable environmental and personal factors account for only 20% of the differences in plasma fibrinogen levels observed between individuals. The extent to which genetic variation affects fibrinogen levels has been investigated in several recent studies. The genes that encode each pair of fibrinogen polypeptide chains (Aα, Bβ and γ chains) occur within a cluster of 50 kb of DNA on chromosome 4 (Kant et al 1985) and in cell culture experiments, synthesis of the β chain has been shown to control the formation of fibrinogen (Yu et al 1983).

Using path analysis, Hamsten et al (1987) estimated that 51% of the variance of plasma fibrinogen among families with early myocardial infarction was due to genetic heritability. Humphries et al (1987) found that three different genotypes, defined as restriction fragment length polymorphisms (RFLPs) at the α and β gene loci, contributed 15% of the variance of fibrinogen. The authors concluded that this was probably an underestimation, since this was based on only one fibrinogen measurement and other RFLPs associated with fibrinogen genotype were not included in the analysis. In contrast, a Norwegian study found no association between levels of fibrinogen and the α and β genotype (Berg and Kierulf 1989).

Exercise

Regular physical activity is considered a protective factor against the development of

cardiovascular disease. The protective effect may be mediated metabolically, through reductions in weight, heart rate, blood pressure, insulin and triglyceride levels and increases in HDL cholesterol. In relation to haemostatic function, cross-sectional studies have consistently found that plasma fibrinogen is lower in subjects who exercise regularly (Morris et al 1990; Rosengren et al 1990; Folsom et al 1991; Elwood et al 1993). Strenuous, acrobic and long-term exercise appear to have the greatest effect on fibrinogen reduction (Connelly et al 1992; Wosornu et al 1992). In studies of individuals who have undergone exercise programmes lasting between 2-6 months, a reduction in fibrinogen appeared to be dependent on a number of factors, such as the age of the individual, period and level of activity and presence of other risk factors (Mannucci 1995).

Alcohol and diet

Light regular drinkers have a lower risk of cardiovascular disease than non-drinkers or heavy drinkers, as well as lower rates of cigarette smoking, blood pressure and body mass index. Moderate alcohol consumption is also associated with a decrease in levels of fibrinogen (possibly due to reduced hepatic fibrinogen synthesis) and may contribute to the lower overall risk of arterial disease (Meade et al 1979; Yarnell et al 1983; Lee et al 1990; Folsom et al 1991; Iso et al 1993).

The influence of diet on fibrinogen levels has been more controversial. Some studies have found that fish oil intake reduces fibrinogen levels (Lee et al 1990; Iso et al 1993), but there are exceptions (Marckmann et al 1991). Dietary fat intake appears to have no significant effect on fibrinogen (Miller et al 1986). In a diet supplemented with soluble dietary fibre, however,

fibrinogen levels were significantly raised, but a decrease in the density of the fibrin clot was observed. This suggests that the functional properties of fibrinogen may be influenced by diet (Veldman et al 1994).

1.3.7 Associations with angiographic disease

Several studies have shown elevations in fibrinogen in patients with symptomatic ischaemic heart disease. A causal link between fibrinogen levels and the extent of arterial disease defined by angiography has also been suggested. In one of the earliest studies, Lowe et al (1980) observed an increase in fibrinogen in men with stenoses in two or three coronary vessels compared to men with none or single vessel disease, which was independent of smoking history. Further studies have confirmed that fibrinogen is significantly related to the extent (the number of affected vessels) and severity (degree of stenosis) of angiographic ischaemic heart disease in both men and women (Hamsten et al 1986; Leschke et al 1987; Handa et al 1989; ECAT Angina Pectoris Study Group 1993; Heinrich et al 1995). In only one study (Schmitz-Huber et al 1988) was the fibrinogen level found not to correlate with the severity of coronary atherosclerosis. The results from the largest of these studies, the European Concerted Action on Thrombosis and Disabilities (ECAT) Angina Pectoris Study, further indicated that fibrinogen levels were more strongly associated with vessel occlusion rather than stenoses, suggesting that fibrinogen may not only play a role in the development of atherosclerosis, but also in thrombogenesis (ECAT Angina Pectoris Study Group 1993).

Although clinical case-control studies of patients with symptomatic peripheral arterial disease have consistently shown raised fibrinogen levels (Dormandy et al 1973a; Stormer et al 1974;

Lipinska et al 1979; Christe et al 1984), there have been few studies relating fibrinogen to the angiographic severity of peripheral arterial disease. Overall, results have been inconclusive. Lassila et al (1993) reported a strong correlation between fibrinogen (both clotting and nephelometric) and the severity of peripheral atherosclerosis, assessed jointly by angiography, ABPI and duplex ultrasonagraphy. In contrast, however, Woodburn et al (1995) reported that fibrinogen levels were not independently related to the extent of peripheral atherosclerosis in patients with intermittent claudication or severe chronic leg ischaemia.

1.4 Fibrin D-dimer

The potential role of other components of the haemostatic system in thrombogenesis is now being evaluated in both clinical and epidemiological studies. One factor which has attracted considerable interest as a possible marker of intravascular clot formation is fibrin D-dimer. Fibrin D-dimer is the terminal degradation product of cross-linked fibrin and measurement of levels of this factor in plasma is thought to reflect the degree of active fibrin formation and subsequent activation of fibrinolysis during thrombus formation. Elevated fibrin D-dimer levels have been found in other conditions associated with overt thrombosis, such as disseminated intravascular coagulation, pulmonary thromboembolism and deep venous thrombosis. Fibrin D-dimer is also derived extravascularly, and is raised in patients with haematoma, inflammation, tumours, liver and renal disease, in pregnancy and after surgery (Lip and Lowe 1995).

1.4.1 Structure of fibrin D-dimer

The fibrin clot is broken down into a series of degradation products, and removed from the site of injury by the action of plasmin, after wound healing has taken place. Some of these end products are structurally different from the degradation products derived from fibrinogen and non cross-linked fibrin, because the factor $XIII_a$-catalysed isopeptide bonds (cross-links) formed between adjacent γ-chains (and also α-chains) in stabilised fibrin are resistant to plasmin and are degraded less extensively during fibrinolysis. Under physiological conditions, low concentrations of soluble fibrinogen degradation and non cross-linked fibrin degradation products circulate in plasma, and are also found within healthy arterial intima (Smith 1994), suggesting that some degree of fibrin formation and degradation occurs normally, either intravascularly or extravascularly. These products have been identified in vitro using a variety of techniques, including gel electrophoresis, affinity and gel filtration chromatography. Based on structural studies, the sequence of fibrinogen and fibrin degradation was described approximately 25 years ago (Gaffney 1973).

The lysis of fibrinogen and non cross-linked fibrin by plasmin initially cleaves the Aα chain and removes the Aα-chain polar appendages. Another peptide fragment, Bβ(1-42) is released from the Bβ chain. This forms fragment X. Progressive cleavage by plasmin produces fragment Y, composed of linked D (terminal) and E (central) domains, and a single D domain or fragment D. The coiled chains linking the E and D domains are then broken, forming the terminal 'core' fragments D and E.

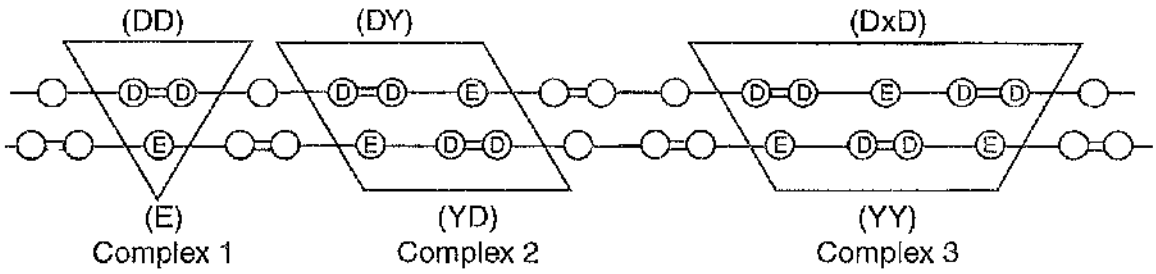
Plasmic degradation of cross-linked fibrin proceeds more slowly, but also commences with

cleavage of the Aα chain and removal of the Aα-chain polar appendages from a fibrin polymer, whilst the γγ cross-links remain intact. The remaining product is termed fragment XX, but progressively larger soluble complexes (X-oligomers) can be formed depending on the length and number of protofibrils within the fibrin clot or gel. A series of these complexes unique to cross-linked fibrin has been identified and their molecular weights have been predicted to be over 1,000,000 Daltons (Francis and Marder 1986). However, clot reduction only occurs when X-oligomers containing fragment X are released spontaneously (Gaffney 1973). The rigidity of the fibrin gel then gradually decreases and disintegrates into soluble fragments. When these high molecular soluble complexes are released into plasma, they continue to be degraded into a series of smaller fragments by cleavages at sites near the γγ chain, or at the D and E domains between the coiled chains (Figure 4). Fibrin D-dimer (fragment DD) is the smallest terminal fragment derived from cross-linked fibrin after prolonged exposure to plasmin, and consists of the D domains of two adjacent fibrin monomers, cross-linked through the γγ chains. However, in vitro studies indicate that fibrin D-dimer primarily circulates in plasma in a non-covalent association with fragment E, derived from the second fibrin strand of a protofibril (Gaffney 1973).

1.4.2 Function of fibrin D-dimer in haemostasis, atherogenesis and thrombogenesis

In normal haemostasis, interactions between fibrin and cross-linked fibrin degradation products may inhibit fibrin gel formation. The formation of complexes has been demonstrated in vitro between fragments X, Y and D with fibrin monomers, which can block the extension of the protofibrils and also inhibit their lateral growth. The rate of clotting and clot stability may therefore be reduced, since the length of the protofibrils are too short to promote adequate

FIGURE 4 CROSS-LINKED FIBRIN DEGRADATION



Two linear fibrin polymers are shown in a half-staggered overlap array with covalent crosslinks between chains indicated as double lines between adjacent D domains. Complex 1 shows D-dimer and DD apposed to Fragment E of the other fibrin strand. The complexes can be expressed as DD/E (complex 1), DY/YD (complex 2), and DXD/YY (complex 3).

fibre formation (Hermans and McDonagh 1982). In addition, a role in fibrinolysis is indicated because these fragments can increase the rate of activation of plasminogen to plasmin, the main fibrinolytic enzyme (Suenson et al 1984). Conversely, D-dimer can compete with plasminogen for surface binding sites on fibrin, thus effectively prolonging clotting time.

There is also evidence that fibrin degradation products may contribute to both plaque growth and thrombus formation. In immunoassay experiments, high concentrations of fibrin D-dimer have been found in atherosclerotic lesions, whereas in normal arterial intima, only small quantities of intact fibrinogen are detected. Moreover, as the lesions become more advanced, the proportion of fibrin D-dimer increases and are distributed around foam cells, cholesterol and macrophages (Bini et al 1989). Both fibrin and fibrin D-dimer are also concentrated in large amounts in the centre of mural thrombi, surrounding platelets and leucocytes. The source of fibrin and fibrin D-dimer appears to originate from within the thrombi through thrombin and plasmin activity, rather than originating from transport of fibrinogen into the arterial wall (Smith 1993).

These findings may be significant because fibrin D-dimer appears to affect a number of cell functions which may be relevant to atherothrombosis. Firstly, it can increase endothelial permeability through disorganisation and retraction of the cell monolayer (Rabbani and Loscalzo 1994). Other in vitro effects on the endothelium include a reduction of prostacyclin synthesis (Ishida and Tanaka 1982). As prostacyclin is a potent inhibitor of platelet aggregation occurring in response to injury at the vessel wall, this suggests fibrin D-dimer may be involved in the earliest stages of endothelial dysfunction. Furthermore, fibrin D-dimer can stimulate migration and proliferation of vascular smooth muscle cells, collagen and growth

factors, processes which characterise the development of the fibrous plaque. Fibrin D-dimer is also chemotactic for monocytes, another of the major cell types found in atherosclerotic lesions and implicated in lipid accumulation within these lesions (Thompson and Smith 1989). Lastly, high levels of fibrin D-dimer may increase hepatic synthesis of fibrinogen through interactions with monocytes. This reaction is thought to be controlled in part by the cytokine, interleukin-6, released by the monocyte in response to changing levels of D-dimer. This relationship may partly explain the strong correlation found between fibrin D-dimer and fibrinogen in most epidemiological studies.

1.4.3 Measurement of fibrin D-dimer levels

Early assays for the detection of fibrin degradation products, such as the red cell haemoagglutination inhibition immunoassay or the Thrombo-Wellco test were performed on serum rather than plasma samples and lacked specificity for the individual fibrin fragments. It was also suggested that the levels of serum fibrin degradation products do not accurately represent levels in plasma since serum concentrations usually tend to be lower (Gaffney and Perry 1985).

Current methods used to measure fibrin D-dimer are based on detecting the specific structure of cross-linked fibrin degradation products which differ from those of fibrinogen and non cross-linked fibrin because of the plasmin-resistant $\gamma\gamma$ chain cross-links. The four most common techniques used at present to determine D-dimer are gel electrophoresis, immunoelectrophoresis, the semi-quantitative latex agglutination test and the enzyme linked immunosorbent assay (ELISA). However, the ELISA is now the preferred quantitative

method employed in clinical and epidemiological surveys to assess the role of D-dimer in cardiovascular disorders.

Gel electrophoresis assay

In this type of assay, the proportion of D-dimer formation compared to other degradation products can be determined by separating the fibrin fragment from plasma samples, diluted with sodium dodecyl sulphate-polyacrylamide (SDS-PAGE) gel, at a concentration of between 2-10%. Purified fibrinogen, labelled with radioactive ^{125}iodine is first exposed to thrombin to increase the amount of cross-linked fibrin protofibrils and then the fibrinogen and plasma samples undergo electrophoresis after addition of t-PA. After staining the gel with Coomassie blue dye, a procedure known as Western blotting transfers the gel pattern of the migrating protein onto nitrocellulose sheets. A band corresponding to the migration of fibrin D-dimer through the gel can be detected by autoradiography (Gaffney et al 1975). The proportion of fibrin D-dimer is then determined by densitometry.

Immunoelectrophoresis assay

In immunoelectrophoresis, fibrin D-dimer is detected using a combination of immunoprecipitation and Western blotting. Plasma samples are incubated with thrombin and the resultant serum is treated with an anti-fibrinogen antibody to remove any fibrin-related antigens from the sera. Analysis by SDS-PAGE gel electrophoresis and blotting with a monoclonal antibody specific to fibrin D-dimer detects a precipitation band where the D-dimer antigen and antibody have reacted (Connaghan et al 1985).

Latex agglutination test

This technique employs a monoclonal antibody (DD-3B6) coated onto latex beads which are specific for fibrin cross-linked between the D-domains. These beads are mixed with plasma which has been centrifuged with bentonite to remove fibrinogen and left at room temperature for three minutes. The slide is then read against a black background for signs of agglutination, which indicates that the antibody has recognised and complexed with fibrin D-dimer (Greenberg et al 1987). The concentration of fibrin D-dimer levels are calculated semi-quantitatively by multiplying the highest doubling dilution of plasma showing bead agglutination by 200ng/mL, which is considered the sensitivity of the beads for fibrin D-dimer.

Enzyme linked immunosorbent assay

This is a simple and precise enzyme immunoassay which recognises the fibrin derivatives, D-dimer, D-dimer/E and high molecular weight complexes containing the D-dimer fragment. The reactions are carried out on microlitre plates, which have been coated with the D-dimer specific monoclonal antibody (the capture antibody). This binds with the D-dimer antigen in the plasma sample. A second antibody, (the tag antibody) conjugated with horse-radish peroxidase enzyme is added and complexes with the bound antigen and any residual untagged antigen. A colour reaction is produced by adding hydrogen peroxide and a substrate, 2,2'-azino-di-3-ethylbenzthiazoline sulphinate. The absorbance of each microtitre well is then determined at 405-420nm and values calculated with reference to a standard curve.

1.4.4 Determinants of fibrin D-dimer levels

There is relatively little published data on the factors which can influence fibrin D-dimer levels in the general population and therefore normal values within the community are not clearly known. At present, only three population surveys have examined associations between fibrin D-dimer and its possible determinants (Giansante et al 1994; Lee et al 1995; GDO Lowe, personal communication). There is also evidence of associations between fibrin D-dimer and cardiovascular risk factors based on patients with atherosclerotic disease, but it is probable that interactions between fibrin D-dimer and other factors may be different in these patients compared to healthier subjects.

Geographical variation

There are no specific reports at present on variation in levels of fibrin D-dimer among different populations or by race, in the community. However, the ARIC Study reported that there was an increase in the risk of early carotid atherosclerosis across quartiles of D-dimer in whites, but not in blacks, although the differences in fibrin D-dimer levels were non-significant (Salomaa et al 1995). Conversely, Gaines et al (1992) found that raised fibrin D-dimer levels were more common in black patients who suffered a stroke than in white patients.

Age

Age appears to be a major determinant of plasma fibrin D-dimer levels in both men and women in the general population. Fibrin D-dimer levels rise linearly with increasing age,

although the trend is slightly stronger in men (GDO Lowe, personal communication; Giansante et al 1994; Lee et al 1995). Strong correlations have also been reported between D-dimer concentrations and increasing age in men with ischaemic heart disease (Heinrich et al 1995) and in patients with more extensive atherosclerosis (Panchenko et al 1995).

Sex and the menopause

Overall, women tend to have higher levels of fibrin D-dimer than men (Lee et al 1995). Higher fibrin D-dimer levels have also been reported in pre-menopausal women compared to men of similar age (GDO Lowe, personal communication). This sex difference is not maintained after the menopause. This may be due to an increase in extravascular fibrin turnover during menstruation. Also, fibrin D-dimer levels rise in women after the onset of the menopause. This suggests that sex hormones, such as oestrogen may influence fibrin D-dimer levels.

Oral contraceptives and pregnancy

The use of oral contraceptives containing oestrogen has been associated with an increase in plasma fibrin D-dimer levels (Lip and Lowe 1995). This has also been observed in healthy pregnancy and also in women who develop pre-eclampsia. Furthermore, pre-eclamptic pregnant women who have significantly raised fibrin D-dimer levels had higher blood pressures, more abnormal liver function, higher protein levels in the urine and a greater risk of early caesarean section and premature delivery than those with lower fibrin D-dimer levels.

Cigarette smoking

Two studies in the general population have observed that fibrin D-dimer levels are higher in male current and ex-cigarette smokers compared to never smokers (Giansante et al 1994; GDO Lowe, personal communication). No consistent relationship was shown between fibrin D-dimer levels and smoking habit in women. In the Trieste Study, however, there was an interaction between age and smoking with fibrin D-dimer in women. A progressive significant increase in fibrin D-dimer levels in women smokers was demonstrated with each successive decade between 25 and 64 years (Giansante et al 1994). The association between long-term smoking and fibrin D-dimer levels appeared to be stronger than the relationship between D-dimer levels and current smoking, both in population surveys (Salomaa et al 1995), and in subjects (particularly men) with peripheral arterial disease (Smith et al 1993; Lassila et al 1993; Lee et al 1995). Other studies have found no relationship between fibrin D-dimer levels and cigarette smoking in claudicants or patients with severe chronic leg ischaemia (Al-Zahrani et al 1992; Lee et al 1996).

Blood pressure

Relatively low correlations have been reported between fibrin D-dimer and blood pressure in two population surveys. In the larger Edinburgh Artery Study, age adjusted correlations between fibrin D-dimer and both systolic and diastolic blood pressure were stronger in women than in men (Lee et al 1995). In contrast, the WHO MONICA survey found that fibrin D-dimer correlated more highly with blood pressure in younger men (GDO Lowe, personal communication). A significant association was also demonstrated with systolic blood pressure

in a pooled case-control analysis investigating early carotid atherosclerosis from the ARIC Study (Salomaa et al 1995), but conversely, no relationship was found in men with prevalent ischaemic heart disease (Heinrich et al 1995). Elevations in plasma levels of fibrin D-dimer have also been shown in hypertensive patients (Varizi et al 1993; Giansante et al 1994; Lip and Beevers 1994) and this has been suggested as a contributory factor in the increased risk of stroke and thrombotic episodes observed in hypertensive individuals.

Blood lipids

Evidence is also conflicting regarding the relationship between fibrin D-dimer and serum lipids in the general population. Whereas Giansante et al (1994) found no significant correlations between fibrin D-dimer levels and serum lipids in analysis in which the sexes were combined, only a significant inverse correlation between D-dimer and triglycerides in men, and a positive association with fibrin D-dimer and total cholesterol among women were reported (GDO Lowe, personal communication). The Edinburgh Artery Study, on the other hand, found that the strongest correlations were between fibrin D-dimer and low HDL cholesterol in both sexes, but significant associations were also observed with other lipids, particularly in women (Lee et al 1995).

Ridker et al (1994a) suggested that the relationship between fibrin D-dimer and serum lipids may be non-linear, in that a significant increase in fibrin D-dimer only occurs at high threshold levels of cholesterol and triglycerides, and at a low level of HDL cholesterol. It is also possible that a sex differential effect exists in associations between fibrin D-dimer and lipids.

1.4.5 Associations with angiographic disease

Evidence that fibrin D-dimer may have pathological significance in peripheral arterial disease has been provided by recent prospective, epidemiological and hospital-based studies. Elevated fibrin D-dimer levels have consistently been demonstrated in subjects with asymptomatic or symptomatic peripheral arterial disease, both in clinical practice and in the general population (Al-Zahrani et al 1992; Cortellaro et al 1993; Smith et al 1993; Herren et al 1994; Heinrich et al 1995; Panchenko et al 1995). The Edinburgh Artery Study has further shown that fibrin D-dimer was independently related to the severity of peripheral arterial disease (as determined by the ABPI), after adjustment for a range of cardiovascular risk factors, including smoking consumption (Lee et al 1995).

These findings have been confirmed in two clinical studies of patients in whom the extent of peripheral arterial disease was measured directly by angiography. The first of such studies described a linear relationship between fibrin D-dimer levels and severity of disease, although the presence of peripheral atherosclerosis was assessed by angiography in only 38% of patients, with the remainder assessed by non-invasive techniques (ABPI and duplex scanning) (Lassila et al 1993). However, no account was taken of the effect of cigarette smoking on this association. In a more recent angiographic study of patients diagnosed as having intermittent claudication or severe chronic leg ischaemia, fibrin D-dimer showed the strongest association of any haemostatic or risk factor with increasing severity of peripheral arterial disease, which was independent of smoking habit (Woodburn et al 1995). Overall, these findings are indicative of a role of intravascular fibrin turnover in the development of peripheral arterial disease, which may be related to increased formation of cross-linked fibrin thrombi.

1.4.6 Prediction of thrombotic events

The role of fibrin D-dimer in the prediction of thrombotic vascular events has not been widely studied, either in the general population or in patients with prevalent peripheral arterial disease. From data derived from the the Edinburgh Artery Study, fibrin D-dimer was independently related to the risk of stroke after five years of follow-up, in analysis which adjusted for cigarette smoking, LDL cholesterol, systolic blood pressure and pre-existing ischaemic heart disease (Smith et al 1997). Fibrin D-dimer was also predictive of ischaemic heart disease (especially myocardial infarction), although the relationship was weaker and not maintained on multivariate analysis. These findings were in contrast to findings from the Caerphilly Study (Lowe et al 1998), and the Physicians Heart Study (Ridker et al 1994a). Comparison of these studies suggests that there are differences in the age structures, end points and the covariates entered into the analysis which may account for the conflicting results.

At present, few studies have investigated the associations between fibrin D-dimer and adverse outcome in symptomatic peripheral arterial disease. In the PLAT Study, baseline levels of fibrin D-dimer were associated with the incidence of thrombotic events, such as myocardial infarction and stroke in patients with peripheral atherosclerosis, after one year of follow-up (Cortellaro et al 1994). Levels of fibrin D-dimer, measured pre-operatively, have also been shown to have predictive value in patients who suffered graft occlusion or death following reconstructive surgery for severe peripheral arterial disease (Woodburn et al 1996).

1.5 Background to Research

Thrombosis is an important pathogenic factor in the causation of cerebral and myocardial infarction (Davies and Thomas 1984) and thrombus formation has been related to high systemic levels of haemostatic factors, particularly fibrinogen. The relationship between haemostatic function and peripheral arterial disease is attracting interest, partly because the pathogenesis of disease in the lower limb is less well understood, but also because it is recognised as a disease associated with a high risk of mortality from thrombotic ischaemic heart disease and stroke events.

In the research described in this thesis, the author decided to investigate two haemostatic factors, fibrinogen and fibrin D-dimer for the following reasons. Firstly, plasma fibrinogen has been related to the development of ischaemic heart disease and stroke in several studies, but the relationship with peripheral arterial disease is less well established. Secondly, fibrin D-dimer is a relatively new factor to be investigated in terms of cardiovascular risk and little evidence is available on peripheral arterial disease. Measurement of fibrin D-dimer levels may indicate the level of cross-linked fibrin turnover formed in arterial thrombi and hence may have prognostic value as a marker of intravascular thrombosis.

Previously, the author conducted a case-control study on men and women, aged 55-74 years, who were selected from the Edinburgh Artery Study. This study began as a cross-sectional survey investigating the prevalence of symptomatic and asymptomatic peripheral arterial disease in the general population in 1988. The purpose of the nested case-control study was to determine the relationship between the haemostatic factors, fibrinogen, fibrin D-dimer, von

Willebrand Factor and plasminogen activator inhibitor and peripheral arterial disease. The influence of cigarette smoking on these associations was also considered, for two reasons. Firstly, smoking is probably the most important risk factor in the development and progression of peripheral arterial disease. Secondly, it may affect plasma levels of haemostatic factors, such as fibrinogen (Leng and Fowkes 1993), and hence might influence the strength of the relationships between haemostatic function and disease.

Interpretation of the results from the case-control study indicated that neither life-time or current smoking had any real impact on the increased risk of peripheral arterial disease associated with raised fibrinogen levels. Rather, the main effect of smoking on peripheral atherosclerosis appeared to be more through fibrin formation than through fibrinogen levels (Smith 1993). In view of these findings, which suggested differing associations of haemostatic function in peripheral arterial disease compared to that of ischaemic heart disease, two further studies, the subject of this thesis, were proposed to examine the inter-relationships between cigarette smoking, haemostatic factors (in particular fibrinogen and fibrin D-dimer) and peripheral arterial disease.

The first study, the Sites of Atheroma Study, was conducted by the author between January 1992 and January 1993. This study was designed to investigate the relationship between haemostatic factors and the site and severity of atherosclerosis of the lower limb to confirm whether the above relationships were sustained in patients with more severe disease, as defined by angiography. The effect of smoking was again considered, since risks due to smoking appear to differ by site. For example, in addition to being a stronger risk factor for peripheral arterial disease compared with ischaemic heart disease (Fowkes et al 1992), smoking is more

strongly associated with aorto-iliac disease than with disease in the femoral vessels (Sackett et al 1968; Lawton 1973; Strong and Richards 1976).

The second study, the Prognostic Study of Intermittent Claudication, was established in 1989 to determine whether haemostatic factors were associated with the future development of acute vascular events in individuals with intermittent claudication. The author was employed in 1995 to conduct a follow up study of events occurring within the six years since baseline.

1.6 Sites of Atheroma Study: Aim and Objectives

Aim

To determine whether plasma fibrinogen , fibrin D-dimer and other haemostatic factors (von Willebrand Factor and plasminogen activator inhibitor-type 1) are related to the angiographic site and severity of atherosclerosis in the arteries of the lower limb, in order to enhance understanding of aetiological mechanisms.

Objectives

I To determine mean differences in levels of plasma fibrinogen, fibrin D-dimer and other haemostatic factors between subjects with the following sites of atherosclerosis predominantly in:

1. Aorto-iliac arteries
2. Femoro-popliteal arteries

3. Dual-site arteries

II To determine associations between the above haemostatic factors and the severity of disease separately within the aorto-iliac and femoro-popliteal arteries.

III To determine differences in cigarette smoking and other vascular risk factors between subjects with atherosclerosis predominantly in:

1. Aorto-iliac arteries
2. Femoro-popliteal arteries
3. Dual-site arteries

IV To determine the influence of cigarette smoking on the associations between plasma fibrinogen, fibrin D-dimer and other haemostatic factors, and severity of disease within the aorto-iliac and femoro-popliteal arteries.

1.7 Prognostic Study of Intermittent Claudication: Aim and Objectives

Aim

To determine whether plasma fibrinogen, fibrin D-dimer and other haemostatic factors, and cardiovascular risk factors are related to the incidence of atherothrombotic events in subjects with intermittent claudication, in order to identify possible mechanisms involved in the aetiology and progression of disease.

Objectives

I To determine in patients with intermittent claudication, univariate associations between the following haemostatic factors:

1. Plasma fibrinogen
2. Fibrin D-dimer
3. von Willebrand Factor
4. Tissue plasminogen activator

and the development of vascular events during six years, in terms of :

1. Ischaemic heart disease events (fatal and non-fatal)
2. Stroke (fatal and non-fatal)

II To determine in patients with intermittent claudication, univariate associations between the above haemostatic factors and the progression of peripheral arterial disease, in terms of:

1. Vascular intervention
2. Severe chronic leg ischaemia

III To determine multivariate associations between the above haemostatic factors and the incidence of ischaemic heart disease and stroke events in those patients with intermittent claudication, taking into account the possible effects of life-time cigarette smoking and other cardiovascular risk factors.

IV To determine multivariate associations between the above haemostatic factors and progression of peripheral arterial disease in patients with intermittent claudication, taking into account the possible effects of life-time smoking and other cardiovascular risk factors.

CHAPTER TWO

SITES OF ATHEROMA STUDY: METHODS

2.1 Study Design

The Sites of Atheroma Study comprised a consecutive case series of patients in the form of a cross-sectional study, which is an observational epidemiological study in which the risk factors and disease status of individuals in a defined population are assessed simultaneously. The defined population of consecutive cases of intermittent claudication or rest pain consisted of both new and existing cases, and their current health status was examined in respect to the risk factors of interest. The data collected were used to determine the distribution of physiological and biochemical measurements and the characteristics of disease within this defined population at a specific time.

2.2 Sample Size

In a recent study designed to classify 300 patients by retrospective examinations of angiograms into groups with predominantly disease in either the aorto-iliac or femoro-popliteal segments, one-third of patients were categorised as dual-site because they did not have disease predominantly in one site or the other (M Whyman, unpublished observations). Of the remaining 200 subjects, more than 100 had disease in the femoro-popliteal arteries and less than 100 had disease in the aorto-iliac arteries. The angiograms were coded by site and severity of disease described by Bollinger et al (1981). In the present study, it was therefore

assumed that three approximately equal sized groups defined according to site of disease would be identified.

The sample size had to be adequate to detect significant differences between the groups in the main variables of interest, plasma fibrinogen and fibrin D-dimer. From data derived from the Edinburgh Artery Study, mean levels of plasma fibrinogen were as follows:

	Mean	**Standard Deviation**
Fibrinogen (g/L)	2.72	0.80

Based on the following formula:

Number of patients in each group

$$n = \frac{2\sigma^2}{(\mu_2 - \mu_1)^2} \times f(\alpha, \beta)$$

where $(\mu_2 - \mu_1)^2$ is the difference in mean fibrinogen which is important to detect.

σ is the standard deviation of fibrinogen

α is the type I error, normally 0.05

β is the type II error, or power, 0.1 in this case.

It was estimated that a sample size of 200 would have 90% power to detect a mean difference of plasma fibrinogen 0.26g/L between groups at 5% level of significance.

2.3 Study Population

The study population comprised men and women with ischaemic symptoms in the lower limb.

The study sample was selected prospectively from consecutive patients referred for angiography to the Royal Infirmary of Edinburgh. These patients were experiencing symptoms of chronic ischaemia, that is, intermittent claudication in the calf, buttock or hip, or more severe symptoms, such as pain at rest in the foot or toes. Referral of these patients for angiography had been made either from the Peripheral Vascular Clinic (out-patients), or the Vascular Surgery Unit (in-patients). The clinical symptoms of these patients were assessed by a vascular consultant over a period of several months to determine the probable site and severity of disease. If it was considered that their condition had worsened significantly or severely affected their life-style, they were then referred for angiographic investigation.

2.4 Study Exclusions

Patients with ischaemic symptoms of ulceration and gangrene were excluded from the study because infection and inflammation induce a systemic rise of acute-phase reactant levels. Patients who had undergone amputation or reconstructive surgery were also excluded because the removal of a severely diseased limb, or the replacement of occluded arterial segments, would not give a true reflection of the severity or site of arterial disease when assessed by angiography.

2.5 Patient Selection

Before the study commenced, permission to recruit patients from the vascular wards was obtained from the senior consultant, Professor CV Ruckley. The author was introduced to the ward nursing and other staff who were informed about the study. The Radiology department

was also visited and permission was obtained from Dr I Gillespie, consultant radiologist, to remove angiographic X-rays from the film store when required for coding purposes.

The patients were selected prospectively from a list of patients undergoing radiological investigations during the forthcoming week. This list was issued at approximately 4 pm on Friday afternoon in the Radiology department. The day and date of procedure, name of patient, ward number, and type and time of procedure were given. Any patient who was listed as undergoing aortography, femoral or iliac angiography or femoral or iliac angioplasty was noted and considered a potential recruit for the study. This list was also checked on each weekday because appointment cancellations frequently occurred and new patients were then listed.

In addition to this list, patients with symptoms of severe chronic leg ischaemia were occasionally admitted without prior notification to the vascular wards. Therefore, regular assessment of all admissions to these wards was required, and hence the numbers potentially suitable for inclusion to the study varied from week to week. Once a list of potential subjects had been drawn up, the patients' notes were examined within the vascular wards to ensure that they had none of the exclusion criteria.

2.6 Sample Recruitment

Patients undergoing angiography or angioplasty were normally admitted on the day before, or the day of the procedure, and discharged 24 hours later. Men or women with more acute symptoms of limb ischaemia who were scheduled for vascular surgery were in-patients on the

vascular wards. Patients with less severe symptoms of peripheral arterial disease were admitted for a short stay to a Programmed Investigation Unit.

There were only certain times available for recruitment of subjects. This could only take place after the patients had been admitted and interviewed by the nursing staff and after they had been 'clerked in' by the ward House Officer, who gave them a full medical examination, including palpation of peripheral pulses, blood pressure measurements, electrocardiogram, and a venepuncture. Also, no recruitment was possible during consultant ward rounds between 9am and 10 am, at lunch-time between 12.15 pm and 1.30 pm, tea-time between 5 pm and 6 pm and visiting times between 3 pm and 4 pm.

It was also important that the patients had sufficient time to recover from the initial examination by the ward doctor, because the examination required by the present study involved a second venepuncture and blood pressure recordings. However, it was essential to recruit patients before angiography because the contrast media could activate platelets, leucocytes and other blood factors.

2.7 Examination Procedures

The ward sister was approached to ascertain whether the patient scheduled for angiography had arrived and had been examined by nursing staff and the house officer. If so, and a reasonable amount of time had elapsed since their last examination, the patient was approached prior to their angiography. The purpose of the study and the examination procedure was explained to the patient. If they were willing to take part in the study, they

were then asked to complete a consent form which emphasised the anonymity and the confidentiality of the results. This form is shown in Appendix I. The subjects were informed that the study had been given ethical approval by a Medical Ethics sub-committee of Lothian Health Board and the examination then took place.

Patients were recruited and examined over a period of 13 months, between January 1992 and January 1993. Equipment and patient forms were kept at the Vascular Studies Unit which was situated in close proximity to the vascular wards. When a patient was recruited from any ward, the equipment required for the examination was taken by a small portable trolley to the ward. The patient was examined in the ward at the bed-side. Each examination took approximately 45 minutes and consisted of venepuncture, blood pressure measurements and administration of a questionnaire.

A blood sample was taken following ten minutes rest in the supine position. A total of 25 mLs of blood was withdrawn from the ante-cubital vein using a 21g butterfly infusion set and without tourniquet wherever possible to prevent platelet lysis. The needle was held in position using micropore tape. Bruising was prevented after withdrawal of the needle by pressure on the vein, with the arm held in a vertical position for 20 seconds. Details of the venepuncture procedure were recorded on a form which identified the subject by name and study number (Appendix II). Time of venepuncture was also recorded because certain haemostatic factors e.g. plasminogen activator inhibitor are subject to diurnal variation. The form was designed to record whether venepuncture was successful, because slow venepuncture can lead to spuriously high levels of some haemostatic factors. If venepuncture was not possible, the subject was excluded from the study.

Blood pressure measurements were taken after venepuncture, when the subject had rested in the supine position for a further 10 minutes. The systolic and diastolic (phase V) blood pressures were taken in the right arm using an adult size latex inflation cuff and a Hawksley random zero sphygmomanometer. The ankle systolic blood pressure was recorded on each leg by a cuff inflated proximally to the ankle, and the return of blood flow detected using a Sonicaid doppler probe placed over the posterior tibial or dorsalis pedis artery. The ankle brachial pressure index (ABPI), which is the ratio of ankle to brachial systolic pressure was calculated as a measure of severity of peripheral atherosclerosis (Appendix III).

Following blood pressure measurements, a self-administered questionnaire was completed by the subject. The questionnaire was then checked and assistance given if there was difficulty in answering any of the questions. The questionnaire is shown in Appendix IV. The subject was asked to provide their name and date of birth and the questionnaire was then divided into five sections. Firstly, details of past medical history were sought, including questions on previous myocardial infarction, angina, stroke and other cardiovascular disease.

The second section was to ascertain whether the subjects were prescribed regular medication from their general practitioner. It was important to quantify those who were receiving aspirin or anti-coagulant medication because these can affect haemostatic function. Thirdly, the subject was asked to provide details on family history of heart attack, angina and intermittent claudication. This section was included because it is recognised that cardiovascular disease in first-degree relatives is a risk factor, especially for ischaemic heart disease, although this information was not subsequently used in the study analysis. The smoking section was designed to elicit detailed information on current smoking status and to distinguish clearly

between current, ex- and never cigarette smokers. Questions were listed sequentially to quantify the amount of tobacco consumption, duration of smoking in current users and those who had quit smoking. For ex-smokers, the length of time since cessation of smoking was requested.

The Edinburgh Claudication Questionnaire was also administered (Leng and Fowkes 1992). Finally, details on previous arterial surgery was obtained. This was to ensure that subjects had not undergone previous reconstructive surgery or angioplasty which would have excluded them from the study.

2.8 Blood Assays

2.8.1 Preparation of anti-coagulants

Tri-sodium citrate

This anti-coagulant was prepared by the author in the Cardiovascular Research Unit, Department of Biochemistry, University of Edinburgh. The absolute concentration required was 0.32 g/mL. Two hundred and fifty white topped Z_5 plastic tubes with 0.5 mL tri-sodium citrate solution were prepared. Using a Sauter balance, 8.0 g tri-sodium citrate crystals were weighed out into a 250 mL conical flask. The crystals were dissolved by shaking gently in distilled water and more distilled water was added until the 250 mL mark was reached.

Using an Oxford pipette fitted with a plastic disposable tip, 0.5 mL of tri-citrate solution was

measured into each Z_5 tube. The tubes were then placed in racks and immediately frozen in a -40^{0}C freezer. If not used immediately, liquid coagulants must be frozen to prevent deterioration and resulting changes in anti-coagulant activity.

Tri-sodium citrate trasylol

Two hundred and fifty Z_{10} tubes were filled with 1.0 mL solution of 3.8% tri-citrate and 100,000 units of trasylol per 100 mL of tri-sodium citrate solution. To formulate this solution, 3.8 g tri-sodium citrate was measured into a 100 mL conical flask. Distilled water was added to dissolve the crystals and water added to the 100 mL mark. One ampoule of trasylol, equivalent to 100,000 units was then added using a 5 mL syringe and 21 g needle, and mixed into the solution.

A further 250 mL of the solution was prepared. 9.5 mL of tri-sodium citrate were measured into a 250mL conical flask again using a Sauter balance, and 12.5 mL trasylol were added. One mL of the 250 mL tri-sodium citrate trasylol solution was added to the Z_{10} 10 mL tubes using an Oxford pipette and the tubes were placed in the -40^{0}C freezer.

2.8.2 Blood processing

The procedure for handling and carrying out the initial processing of blood specimens is detailed in Appendix V. For estimation of levels of plasma fibrinogen, von Willebrand Factor and fibrin D-dimer, 9 mL of blood from the 30 mL syringe were added to a Z_{10} 10 mL tube containing 1 mL tri-sodium citrate trasylol anti-coagulant. The tube was inverted five times

to ensure adequate mixing of blood and anti-coagulant and placed on a roller for 10 minutes. The sample was then centrifuged at 2800 RPM for 10 minutes at 4^0C. Five 0.5mL aliquots of plasma were removed from the supernatant plasma and pipetted into red microtubes using a plastic disposable syringe. The tubes were then labelled with the patients' study number and immediately placed onto dry ice and stored in the -40^0C freezer.

For plasminogen activator inhibitor, 4.5 mL of blood were added to a Z_5 plastic tube, which contained 0.5 mL of the anti-coagulant tri-sodium citrate (9:1 V:V, 0.11M). This tube was also inverted five times and centrifuged at 2800 RPM for 10 minutes at 4^0C. Two 0.5 mL aliquots of plasma were removed from the supernatant plasma and pipetted into two green microtubes. These were placed onto dry ice and then placed into the -40^0C freezer.

Serum for assay of total cholesterol, HDL cholesterol, serum thiocyanate and serum cotinine was prepared by adding 10mL of blood to a Z_{10} glass tube without anti-coagulant, and this was left to stand for approximately one hour until clotting had taken place. The sample was then centrifuged at 4^0C and 2800 RPM. After centrifugation, 3 mLs of serum were removed, pipetted into a clear plastic Z_5 tube and placed in the freezer for subsequent determination of lipid levels. In addition, two 0.5 mL aliquots of serum were placed in blue 0.5 mL microtubes for estimation of serum thiocyanate and serum cotinine levels.

2.8.3 Laboratory assays

The assays of the haemostatic factors were carried out in the Haemostasis, Thrombosis and Vascular Medicine Unit, Glasgow Royal Infirmary, University of Glasgow, under the direction

of Professor GDO Lowe.

Fibrinogen

Fibrinogen levels were measured in this study by two different types of assay. The Clauss clotting method (Clauss 1957) was performed on a Coag-U-Mate analyser using appropriate reagents and standards (Organon Technika). It was based on a modified Clauss method in which the clotting time of fibrinogen was not measured directly, but increase in turbidity was measured after addition of thrombin. The coefficient of variation was 6%.

In the nephelometric heat precipitation assay (Stone and Thorp 1985), fibrinogen was aggregated by heating plasma diluted in saline buffered at pH 6.3 at a temperature of 56^{0}C. The concentration of the suspension was estimated by nephelometry which measures the light scattering intensity of fibrin in diluted plasma. The coefficient of variation was 6%.

Fibrin D-dimer

Fibrin D-dimer was assayed using a commercial enzyme-linked immunosorbent assay (ELISA) supplied by AGEN (Parsippany, New Jersey) based on a monoclonal antibody. The immunological reactions were carried out in microlitre plates. Two antibodies, one tagged with an enzyme (horse radish peroxidase) specific to the target antigen were added to the plate. The untagged antibody bound any antigen present in the plasma sample, and the second tagged antibody was then added to the plate which complexes with the bound antigen and residual untagged antigen. A substrate, hydrogen peroxide, and a colour detector, phenylene diamine,

was then added. The colour generated was proportional to the concentration of fibrin D-dimer present. The coefficient of variation was 5%.

Plasminogen activator inhibitor

Plasminogen activator inhibitor activity levels were measured by an amidolytic assay (Kabi, Stockholm). A fixed amount of tissue plasminogen activator was added in excess to undiluted plasma where it formed an inactive complex with PAI. Plasminogen was activated to plasmin by the residual t-PA in the presence of a stimulator (3.3 mg human fibrinogen fragments). The amount of plasmin was directly proportional to the residual t-PA activity and therefore conversely proportional to the PAI activity within the plasma sample. The amount of plasmin was determined by measuring the amidolytic activity with the chromogenic substrate S-2251. The release of p-nitro-aniline was determined at 405 nm in a photometer.

von Willebrand Factor

von Willebrand Factor antigen levels were also assayed using an ELISA assay based on a monoclonal antibody (DAKO, Copenhagen, Denmark). The coefficient of variation was 5%.

Serum lipids

Serum total cholesterol and HDL cholesterol were assayed in the University of Pathological Biochemistry Laboratory, Department of Medicine, Glasgow Royal Infirmary under the direction of Professor CJ Packard.

Total cholesterol was measured on the BM Hitachi 704 analyser by the CHOD-PAP method using reagents supplied by Boehringer-Mannheim. HDL cholesterol was also measured on the BM analyser after precipitation with heparin manganese.

Serum thiocyanate and serum cotinine

To validate self-reporting levels of smoking consumption, two biochemical markers of tobacco inhalation were assayed, serum cotinine and serum thiocyanate. These were carried out in the Cardiovascular Epidemiology Unit, University of Dundee under the direction of Dr R Tavendale. Serum thiocyanate was estimated on a COBAS BIO centrifugal analyser, by precipitation with trichloracetic acid. The supernatant was reacted with ferric chloride and absorbance measured at 450 nm. Serum cotinine was measured by gas-liquid chromatography based on the method of Feyerabend and Russell (1980), except that lidocaine was used as the internal standard.

2.8.4 Quality control

To assess laboratory reproducibility, a small number of patients at recruitment were asked to donate twice the usual quantity of blood. The venepuncture technique was similar to the routine method, except that after withdrawal of the first 25 mL of blood, the 30 mL syringe was replaced by a second 30 mL syringe in the 21 g butterfly infusion set and a second 25 mL of blood withdrawn. Each sample was placed into a duplicate set of tubes and identified by different patient study numbers. The tubes were centrifuged at 2800 rpm at a temperature of 4^0C for 10 minutes. The separated plasma was pipetted into two sets of 0.5 mL micro-tubes

using a different plastic disposable pipette for each tube. The tubes were then placed into a -40^{0}C freezer and sent to the University of Glasgow, Haemostasis, Thrombosis and Vascular Medicine Unit for analysis. The laboratory was unaware as to the source of these split plasma samples. A total of 11 patients donated a double quantity of blood.

2.9 Angiography

2.9.1 Angiographic technique and procedure

The preferred method used for investigating peripheral atherosclerosis at the Royal Infirmary of Edinburgh Radiology Department is percutaneous transfemoral catheterisation. This technique permits serial examination of the aorta for inflow disease, and multiple views of the pelvic, thigh and distal calf vessels.

Patient preparation

Patients referred for angiography were examined by the resident house officer on the vascular wards to evaluate their clinical need for angiography, and to ascertain their physical status by a number of tests including palpation of peripheral pulses and measurement of blood pressure. They were also required to sign a consent form after being informed of the procedure and risks of the technique. If the subject was receiving anti-coagulant medication, such as warfarin, this was discontinued to prevent excess bleeding and haematoma formation. In addition, the patient was required to fast for 4-6 hours prior to angiography, since ingestion of contrast media can induce nausea and vomiting.

Procedure

The arterial puncture site for catheterisation was determined by presence or absence of femoral pulses. The femoral artery approach was used if a femoral pulse was palpable in either leg. However, if not, entry into the brachial artery was used. The groin was then shaved and cleaned with an antiseptic solution of betadine and then draped with sterile towels. Following injection of 10 mLs 1% solution of local anaesthetic, lidocaine, the femoral artery was punctured using a 18 g needle below the inguinal ligament. A cannula was inserted after incision by a scalpel blade after first checking that the artery had been penetrated. A guide wire with a flexible tip was then manoeuvred through the femoral and iliac arteries and into the abdominal aorta.

A catheter was introduced through a teflon sheath, once the guide wire was in place in the abdominal aorta. Either a size 4 or 5 French Lodis pigtail catheter was used (preferably the smaller outer diameter of catheter whenever possible). The function of the catheter was to divert radio-opaque contrast medium through its side-holes into the aortic side branches at the T12-L1 interspace opposite the renal branches. Once the catheter was in place, the guide-wire was withdrawn through the sheath at the puncture site.

Injection of 90 mL contrast media, niapam 300 delivered at a rate of 10 mL per second triggered the filming mechanism which involved an automatic serial film change and a programmable moving table-top with synchronised kilovoltage regulation. Exposure of the peripheral arteries was carried out by a stepping mechanism in which the exposure was

decreased by half, as the arteries were filmed sequentially down the lower limb. At 80 KV, the pelvic vessels were filmed at 40 mAs, the femoral arteries at 20mAs, the calf arteries at 10mAs and the ankle arteries at 5mAs. The film exposure rate at the pelvis was one per second for 4 seconds, one per second for 3 seconds at the thigh, one per second at the knee and one per second for 4 seconds below the knee. Factors such as filming rates, contrast quality and clearance rate, pattern of disease and patient size were also assessed for optimal visualisation of the peripheral arteries.

The procedure was concluded by withdrawal of the catheter and by applying pressure at the puncture site for approximately ten minutes to minimalise bleeding and the formation of haematoma. A pressure dressing was applied to the site and the patient was kept at bed rest in the recumbent position for 24 hours. The procedure lasted approximately one hour.

2.9.2 Collection of angiograms

All angiograms were stored initially in the X-Ray film store situated in the Radiology Department of the Royal Infirmary of Edinburgh. The X-rays were filed by the day and month of date of birth initially, and then each section was arranged sequentially in ascending year of birth. However, if patients were retained on the vascular wards for further treatment after angiography, their angiograms were filed there for consultation. In addition, the angiograms of patients attending the Surgical Consultation Department for follow-up examination were removed from the film store a week before their appointment.

2.9.3 Arterial segment definition

The arterial segments evaluated by the scoring system are shown in Figure 5. A total of 13 segments were coded in each patient. These were the abdominal aorta (standardised to the distal 5cm before bifurcation), and both the left and right sides of each of the following: common iliac artery (beginning at the aortic bifurcation and ending at the origin of the internal iliac artery); external iliac artery; internal iliac artery (origin to the first branching); profunda femoris (the first 15 cm of its main descending branch); superficial femoral and the popliteal artery.

2.9.4 Grading of angiograms

At the end of patient recruitment, a final list of all patients in the study was drawn up and identified by patient number, name and date of birth. Their angiograms were collected in groups of six from the radiology film store, or vascular wards. These were taken to the University Medical School and coded by the author on a portable viewing box using the Bollinger scoring system. The data were recorded on a standard form which is shown in Appendix VI. The time taken to code each angiogram varied between 30 minutes and one hour, depending on the quality, complexity and number of previous angiograms. If a patient had had prior angioplasty, the treated arterial segments were identified. Coding of these segments was taken from X-rays prior to angioplasty since this was considered more representative of the true disease status of the artery. At the end of each day, the angiograms were returned to the radiology film store. The names of the patients who had their angiograms coded were listed on the computer and any difficulties noted.

2.9.5 The Bollinger scoring system

The site and severity of atherosclerotic disease was assessed by uniplanar angiographic images obtained from each patient, using a scoring system developed by Bollinger et al (1981). Biplanar views, although requested, could not be provided by the Radiology Department of the Royal Infirmary of Edinburgh.

This system was developed primarily for evaluation of disease progression and regression occurring in the peripheral arteries. However, it is also suitable for the assessment of the severity and location of disease because it codes both narrowing of the lumen and the pattern of disease numerically at one point of time without requiring a comparison of successive angiograms.

The system consists of an additive score for each defined arterial segment. This score is the summation of code numbers each corresponding to the degree of percentage reduction of the lumen for lesions within the segment. Four categories of lesions were defined: i) complete occlusion of the segment; ii) stenoses narrowing more than half of the diameter of the lumen (stenoses > 50%); iii) stenoses narrowing the lumen by more than 25% but less than or equal to 50% (stenoses ≤ 50%) and iv) plaques narrowing the diameter by a maximum of 25% (plaque ≤ 25%).

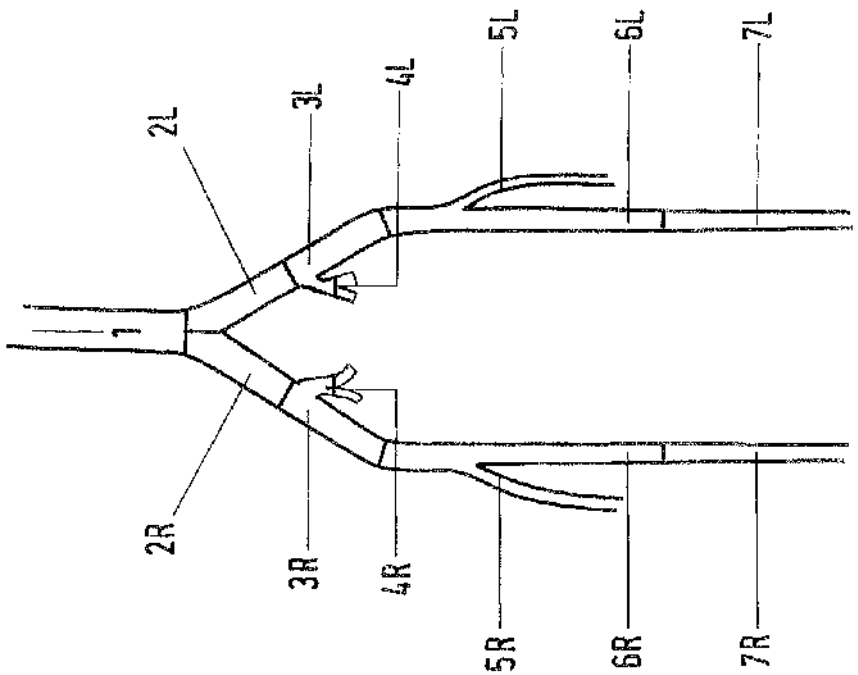
The system was designed to differentiate between perceived clinical severity of stenoses because the overall additive score for plaques and stenoses in a segment is never as high as a single occlusion score. Several rules are implemented during scoring. Firstly, when

occlusions are present, plaques and stenoses are not scored. The additive score of an arterial segment with an occlusion along half or less the length of the segment will be 13, even with stenoses in the remainder of the segment. Secondly, if there are stenoses of > 50% or ≤ 50% present in the same segment, plaques are not coded. Thirdly, only one occlusion is coded in each segment.

2.10 Data analysis

Univariate analysis was carried out by the author using the SPSS-X statistical package on the mainframe computer at the University of Edinburgh. Chi-square analysis was used to test for differences in categorical variables within each class of disease. Analysis of covariance was carried out with age and sex as covariables to test for differences in mean levels of cardiovascular and haemostatic risk factors across the three sites of disease. Partial correlation coefficients adjusted for age and sex reflected the linear association between each haemostatic factor and the additive score within the femoro-popliteal and aorto-iliac segments for all patients in the study. Multiple linear regression was used to examine the independence of each haemostatic factor on additive score at each site. All factors were entered simultaneously with the additional variables age, sex and time of venepuncture having forced entry. The magnitude of the unstandardised regression coefficients indicated the average increase or decrease in additive score for every relevant unit change in the haemostatic factors. The model for the femoro-popliteal additive score was repeated with the forced entry of packyears and then serum thiocyanate (to assess the effect of adjusting for life-time and current cigarette smoking, respectively). The residuals of the multiple regression model were approximately normally distributed.

FIGURE 5 ARTERIAL SEGMENT DEFINITION



Definition of arterial segments evaluated by the Bollinger scoring system. 1) Abdominal aorta; 2) common iliac; 3) external iliac; 4) internal iliac; 5) profunda femoris; 6) superficial femoral; 7) popliteal. L=Left, R=Right.

CHAPTER THREE

PROGNOSTIC STUDY OF INTERMITTENT CLAUDICATION: METHODS

The Prognostic Study of Intermittent Claudication was established in 1989 and consisted of two phases. Subjects were initially followed-up over a one year period and an interim analysis was conducted (Fowkes et al 1993). An overall analysis was then conducted after the total follow-up period of six years from baseline was completed, and the results are described in the present thesis. A research nursing sister conducted the baseline and one year clinical examination, and collected information on incident vascular events on the subjects during the initial one year period. The author was responsible for identifying and confirming any vascular events occurring within the study population during the six year follow-up period, including events which occurred during the first year of follow-up.

3.1 Study Design

The Prognostic Study of Intermittent Claudication is an example of a prospective cohort study, which is sometimes referred to as a follow-up, longitudinal or prospective study. In this type of study, a sample from a defined population is identified and the subjects are then followed over a specified period of time to determine the incidence of a particular outcome of interest. Prognostic factors which are considered to influence the development of the specified outcome are determined and measured at baseline. Differences in the baseline characteristics of those who develop the outcome and those who do not, can then be analysed to establish whether the factors are related to the outcome under investigation.

3.2 Sample Size

The sample size had to be sufficiently large so that clinically significant differences in fibrinogen levels and other variables at baseline could be detected between those who would or would not have a vascular event at one year (and six years) of follow-up. Using information based on the variability of fibrinogen in a previous case-control study of intermittent claudication, it was calculated that 600 subjects would be required to have 90% power at 5% level of significance to detect a 9% difference in mean levels of plasma fibrinogen (0.32g/L) between those who would and would not have a vascular event. The formula is as follows:

Number of patients in each group

$$n = \frac{2\sigma^2}{(\mu_2 - \mu_1)^2} \times f(\alpha, \beta)$$

where $(\mu_2 - \mu_1)^2$ is the difference in mean fibrinogen which is important to detect.

σ is the standard deviation of fibrinogen

α is the type I error, normally 0.05

β is the type II error, or power, 0.1 in this case.

3.3 Study Population

The study population comprised men and women with symptoms of intermittent claudication in the calf, thighs or buttocks. The study sample was a consecutive series of patients who had been diagnosed as suffering from intermittent claudication by a vascular consultant at the Peripheral Vascular Clinic, Royal Infirmary of Edinburgh. As this hospital provides the only

vascular service for the Lothian Health Board area in Scotland, these patients were probably representative of those with moderate to severe claudication, referred from this defined population. Subjects with minor symptoms often do not seek medical help, or if they do, are less likely to be referred by their general practitioner and were therefore not represented in the study sample.

3.4 Exclusion Criteria

Patients were excluded from entry into the study if they had symptoms of severe chronic leg ischaemia, defined as rest pain, ulcer or gangrene. Ulcer and gangrene are necrotic conditions associated with high levels of acute phase reactants, such as fibrinogen. Since these conditions may therefore affect the levels of haemostatic factors, they were considered to be potentially confounding factors, and therefore only patients with symptoms of uncomplicated peripheral arterial disease (intermittent claudication) were selected. Previous or impending arterial surgery (including angioplasty) and amputation also precluded patients from selection, because these procedures may alter the natural history of peripheral arterial disease and hence affect the progression of vascular disease. Those with a serious disability that would make attending follow-up difficult were also considered unsuitable to participate.

3.5 Patient Selection

The study sample was selected from patients attending the Peripheral Vascular Clinic between January 1989 and December 1990. These included newly diagnosed claudicants referred by letter by their general practitioner, and also patients who had been diagnosed with claudication

at the clinic during the previous two years. The patients were examined at their first visit by a consultant vascular surgeon or physician to establish whether intermittent claudication was present based on their medical history, peripheral pulse palpation and WHO diagnostic criteria. If confirmed, the probable site of disease was then allocated a diagnostic code, according to criteria used in the Lothian Surgical Audit.

This code was recorded on a form by the attending consultant and identified the patient by name, address, date of birth and general practitioner. These forms were subsequently sent to the Vascular Surgery Office within the hospital and the data was entered onto a vascular register using a micro-computer. A second form was completed by the consultant which recorded the date of the first visit to the clinic and also indicated whether the patient was suitable for inclusion in the study. These latter forms were then forwarded to the research nursing sister at the University of Edinburgh Medical School.

The Vascular Surgery Office provided a monthly computer print-out from the vascular register of new patients attending the Peripheral Vascular Clinic with the specified diagnostic codes indicating either claudication in the aorto-iliac or femoro-popliteal arteries. This was used by the research sister, in conjunction with hospital records and the out-patient diagnosis forms to ensure that all possible patients were identified. A total of 742 patients diagnosed with intermittent claudication were identified as initially suitable to participate in the study. As each patient was selected, their name, address, date of birth, name and address of their general practitioner and date of first appointment at the Peripheral Vascular Clinic and date of appointment for the baseline examination was entered onto a Dbase IV database.

3.6 Sample Recruitment

Following selection of patients from the Peripheral Vascular Clinic, patients were recruited retrospectively between January 1989 and December 1990. Each patient was identified by a computerised subject number. Patients were then sent a letter of invitation (Appendix VII), signed jointly by the consultant vascular physician (Dr E Housley) and the research sister, asking them if they would be willing to return to the Peripheral Vascular Clinic to take part in the study. The letter explained the purpose of the research and the nature of the clinical examination. In order to maximise response, the appointment date, day, time and place of examination were printed on the letter for each patient. Travel expenses were offered.

The patient was required to indicate on the enclosed reply slip whether they would attend the clinic at the appointment time suggested, if the appointment was not suitable or if they would require an alternative appointment. If necessary, the patient was transported to the clinic by ambulance. Non-responders to the first appointment letter were sent a second letter inviting them to attend the clinic at a later date. Those who had refused to participate or did not reply to this second invitation were not contacted again. Patients who had accepted the invitation but did not subsequently attend the clinic were contacted by telephone and offered another appointment.

3.7 Baseline Clinical Examination

Examinations conducted by the research sister were held on Wednesdays and Fridays, between 9am and 2pm. Two physiological measurement technicians assisted in these clinical

examinations. A maximum of ten patients was invited to each clinic; two appointments spaced at one hourly intervals and commencing at 9 am in the morning. Occasional home visits were organised if requested.

On arrival at the clinic, the subjects were informed of the procedures, and given a consent form to sign (see Appendix I) which explained the purpose of the research and the examination they would undergo. It also emphasised the anonymity and confidentiality of the results. The form indicated that the study had been given ethical approval by a Medical Ethics Sub-Committee of Lothian Health Board.

In an examination room within the clinic, the patient underwent measurement of the brachial and diastolic (phase V) blood pressures in the right arm, after 10 minutes rest in the supine position, using a Hawksley random zero sphygmomanometer and stethoscope. The femoral, posterior tibial and dorsalis pedis pulses were palpated, but the results were subsequently considered to be too variable to be of any practical use. Ankle systolic pressures were recorded on each leg at the posterior tibial artery whenever possible, using a Sonicaid doppler probe and the same sphygmomanometer. The results of the measurements were recorded on a form which also enabled the observer to insert the value of the ABPI on each leg (see Appendix III).

A 12.5 mL specimen of venous blood was then obtained from the ante-cubital vein, using a 20 mL syringe and 23 g needle following a further ten minutes rest for estimation of the haemostatic factors. Details of the venepuncture were recorded on a form (see Appendix II), which identified the patient by name and study number. The name of the technician was

recorded, since it was not always possible for the research sister to take blood on every occasion. The form was designed to record the success of venepuncture and the quantity of blood removed from the patient and time of venepuncture. A 12-lead electrocardiogram was also obtained from the patient, if a recent copy was not available from the patients' case notes. A blood sample for measurement of glucose and total cholesterol levels had previously been obtained by the vascular consultant at the Peripheral Vascular Clinic on the patients' first attendance.

Following the ECG recording, patients were requested to complete a questionnaire, with the assistance of the research sister. This is shown in Appendix VIII. The questionnaire was divided into six sections. Firstly, the sex and date of birth were recorded. The patients were then asked whether they were receiving any regular medication prescribed by their doctor and details of past medical history were sought, including questions on heart disease, stroke, diabetes and thrombosis and embolism. To assess the presence and current severity of peripheral arterial disease, and the presence or absence of angina, the WHO questionnaires on intermittent claudication and angina were included. Lastly, the smoking section was designed to elicit detailed information on current smoking status and to distinguish clearly between current, past and never cigarette smokers. The amount of tobacco consumption and duration of smoking in current and in ex-smokers was quantified. The length of time since cessation of smoking in ex-smokers was also noted.

3.8 Exclusions

Several patients who had been diagnosed with intermittent claudication in the preceding two

years were found to have symptoms of severe chronic leg ischaemia (rest pain, ulceration or gangrene), or had other exclusion criteria discovered at the baseline examination. In addition, several patients refused to take part or did not arrive for their appointment. Subsequently, one hundred and twenty five patients were excluded from the baseline cohort of 742 patients. A total of 617 patients from the baseline cohort of 742 was thus followed-up prospectively.

3.9 Blood Processing

For estimation of plasma fibrinogen, von Willebrand Factor, tissue plasminogen activator and fibrin D-dimer, 5mL from the 20 mL syringe were added to a Z_5 white plastic tube containing 3.8% tri-sodium citrate trasylol anti-coagulant and inverted five times. The tube was placed on a roller for two minutes. The sample was then centrifuged at 2800 rpm for 10 minutes, at 4°C in a Denley BR 401 refrigerated centrifuge. Five 0.5mL aliquots were pipetted into five microtubes labelled by the identifying study number, and placed on dry ice and stored in a -40° C freezer. The tubes were sent to Professor GDO Lowe at the University of Glasgow every four weeks by Red Star delivery for laboratory assay.

3.9.1 Laboratory assays

The assays for the haemostatic factors were carried out in the Haemostasis, Thrombosis and Vascular Medicine Unit, Glasgow Royal infirmary, under the direction of Professor GDO Lowe. The assays for clotting fibrinogen, von Willebrand Factor and fibrin D-dimer have previously been described in Chapter Two.

Tissue plasminogen activator

Plasma levels of tissue plasminogen activator (t-PA) antigen were measured by a commercial enzyme linked immunosorbent assay (ELISA) obtained from Biopool AB, Umea, Sweden. Both human single chain and two chain t-PA antigen were quantified. Each plasma samples were added to two wells of a microtitre plate, one containing the normal goat antibody IgG and the other containing goat anti-human t-PA antigen IgG. After initial binding to the pre-coated well, the second antibody, conjugated to the enzyme horseradish peroxidase, was applied, which bound to the target t-PA antigen. After a period of incubation, a specific peroxidase substrate, orthophenylenediamine dihydrochloride was added. The colour generated was proportional to the concentration of t-PA present.

Total cholesterol and glucose

Total cholesterol and glucose were assayed in the Department of Clinical Biochemistry, Royal Infirmary of Edinburgh, under the direction of Dr J Roulston. Total cholesterol was measured on the BM/Hitachi 737 analyser by the CHOD-PAP method. This is an enzymatic colorimetric test in which free cholesterol was produced by the action of the enzyme, cholesterol esterase on cholesterol ester from the serum sample. Oxidation of cholesterol produced a substance which reacts with a chromogenic receptor. The optical density of the resulting solution was proportional to the total cholesterol concentration of the sample measured at a wavelength of 505 nm.

Glucose was measured by the GOD-PAP assay, supplied by Randox Laboratories. Glucose

concentrations were determined after enzymatic oxidation in the presence of glucose oxidase. Formed hydrogen peroxide reacts with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator. The absorbance of the sample was measured at a wavelength of 500nm.

3.10 One Year Follow-Up Examination

After one year, all surviving patients were invited by letter to attend a second clinical examination at the Peripheral Vascular Clinic. The procedure was similar to the baseline examination, except that a blood specimen was not taken. A second questionnaire was administered which enquired about any new illnesses or conditions occurring during the year since the baseline examination, and also current medication status. Any new treatment for claudication, including vascular surgery was documented. Changes in the severity of their leg pain or chest pain or new occurrence of chest pain were ascertained. Finally, the patient was asked about any changes in smoking habit since their last visit to the study.

3.11 Follow-up of Vascular Events

Subjects were followed over a total period of six years to determine the incidence of the following fatal and non-fatal vascular events: angina pectoris, myocardial infarction, stroke, and coronary vascular procedures, including angiography, angioplasty and coronary by-pass grafting. Progression of peripheral arterial disease was determined by the development of severe chronic leg ischaemic events (rest pain, ulceration, gangrene and amputation) and the performance of peripheral arterial procedures, such as angiography, angioplasty, lumbar

sympathectomy and by-pass grafting. Data were collected from five main sources:

i Scottish National Health Service Central Registry

ii Information and Statistics Division of the Common Services Agency

iii Self-administered questionnaires

iv General practitioners

v Hospital records

Multiple sources of information were used to ensure that as many events as possible were identified and subsequently verified.

3.11.1 Identification of fatal events: Scottish National Health Service Central Registry

To identify all fatal events occurring in the study sample, each patient's record was flagged at the Scottish National Health Service Central Registry at baseline. This ensured that all death certicates were provided automatically. The name, address, date of birth, sex and study number of each patient was typed onto an index card and forwarded to the Central Registry. When notification of death was received by the Central Registry, the death certificate was sent to the author (after a period of approximately two months) at the University of Edinburgh Medical School. The primary and secondary causes of death were recorded on the database in a file restricted to deceased patients. All causes of death had been allocated a diagnostic code according to International Classification of Disease, version 9 (ICD-9). In addition, the cause of death was allocated a code indicating whether the patient had died of myocardial infarction (code 1), stroke (code 2), other cardiovascular disease (code 3), or non-cardiovascular causes (code 4).

3.11.2 Fatal events: autopsy reports

When the cause of death is unknown, or there are suspicious circumstances, or the patient has been an in-patient at a hospital for less than 24 hours prior to death, an autopsy (post-mortem) may be requested by the Procurator Fiscal. Also, post-mortems may be carried out at the request of the clinician in charge if the relatives give their consent. Post-mortem reports were used to verify the underlying cause of death in six patients participating in this study, because confirmation of cause of death could not be confirmed from hospital case notes, or through notification from Central Registry.

3.11.3 Fatal and non-fatal events: Information and Statistics Division of the Common Services Agency

The Information and Statistics Division of the Common Services Agency (ISD) of the Scottish Office in Edinburgh holds patient data derived from summaries created when patients are discharged from general, maternity, geriatric and psychiatric hospitals. An application was submitted in November 1995 to ISD to obtain access to Scottish Morbidity Records (SMR) on all patients in the study, dating from the time of recruitment. The procedure entailed record linkage to computerised ISD data with our own patient data. The merged file was then released in the form of a computer print-out, with the cases listed in a specified order.

To complete the application, a summary of the study aim, background and study methods was requested in addition to identifying the range of data to be supplied for each individual case requiring linkage. The study number, surname, initials, sex, date of birth and postcode was

provided for each patient within the study.

Criteria for selection of cases from the SMR were defined as:

1. New outpatients and inpatients attending hospitals between 1989 and 1996.
2. The age range of the study participants (born between 1st January 1902 and 31st December 1941).
3. Area and post-code of residence.
4. Diagnosis (both fatal and non-fatal) according to specified vascular ICD codes.
5. Any hospital attended for treatment of a vascular event and the dates of admission and discharge.

Following acceptance of the application by the Privacy Advisory Committee of the ISD, data identifying patients were transferred to an ASCII file onto a diskette from our database and sent to ISD. The Information and Statistics Division subsequently provided a computer printout in January 1996, identifying all study subjects (listed alphabetically) who had attended a hospital for a vascular event according to the specified ICD codes, in addition to the relevant hospital and dates of admission and discharge.

3.11.4 Fatal and non-fatal events: general practitioners

To ensure that as many events as possible were identified, each surviving patient's general practitioner was also contacted by letter. The Primary Care Division of the Lothian Health Board was first contacted and asked to supply a list of all general practioners and practice

addresses in the Lothian Health Board area in Scotland. This allowed the names and existing addresses of general practitioners on the database files to be updated from their initial entry in 1989. A letter was sent to each listed doctor from the database file explaining the purpose of the study which was signed by a clinical research fellow and the author, as study co-ordinator. A separate form for each named patient with their date of birth was enclosed, requesting whether the patient was still attending the practice and also requesting information concerning any new cardiovascular events, hospital admissions (including date of attendance), and changes in medication occurring within the last six years (Appendix IX and X). A pre-stamped envelope was also included for the reply. Non-responders were not contacted again. One medical practice had previously intimated that they did not want to be contacted on matters concerning research, and were therefore not contacted.

3.11.5 Non-fatal events: self-administered questionnaires

In February 1996, each surviving patient was sent a questionnaire to complete, with a covering letter reminding them of their participation in the study between 1989 and 1990 and asking them for information about their health since that time (Appendix XI). The first section inquired about any new vascular-related illnesses or medical conditions which they may have had within the last six years. Details of hospital attendances and current medication were sought. The Edinburgh Claudication questionnaire was included, and a self-assessment made by the subjects to ascertain whether their symptoms of peripheral arterial disease had changed. They were also asked whether they had had any new treatment for their leg pain, and for information on any hospital attendances relating to this treatment. A modified WHO questionnaire on angina was used to determine whether they had developed angina within the

six year follow-up period and self-assessment was made of chest pain since recruitment. The last section was designed to determine changes in smoking history since baseline.

3.12 Retrieval of Hospital Records

Patients who had a vascular event identified during follow-up, from either the ISD computer print-out, the general practitioner event form, or the self-administered questionnaire were listed, so that their medical records could be investigated. It became apparent, however, from preliminary examination of records, that there were omissions in the reported number, or errors in diagnoses of events in the three sources used for event identification. It was therefore decided that hospital records of all study patients who had at least one event reported from any of the three sources should be examined. Hospital records were not examined if patients had no events reported in the ISD data, GP forms and patient questionnaires. In these instances, patients were contacted by telephone (where possible) to confirm that they had had no events during the six years since recruitment.

The records were first sought at the Royal Infirmary of Edinburgh, because the patients had initially attended the Peripheral Vascular Clinic within the hospital for diagnosis of intermittent claudication. In the Royal Infirmary records department, hospital records were grouped by patients with the same day of date of birth, and within these groupings, alphabetically by surname. The records of deceased patients were stored separately and were allocated a special number. A patient management information system, termed RHOMER, was used to access the deceased number, by micro-computer. The records of patients who had not attended the Royal Infirmary for at least three years had been sent to the Princess Margaret

Rose (PMR) hospital in Edinburgh for storage.

To retrieve these records, a separate tracer card was completed for each patient and the relevant records were collected and delivered to the Royal Infirmary on Fridays for checking. However, case notes of patients who had not attended hospital for ten years or more had been systematically destroyed. Study patients whose records had been destroyed or whose case notes could not be traced or had been destroyed at other hospitals were excluded from the study, since the reported events could not be confirmed.

If patients had attended an out-patient clinic, or were admitted for in-patient treatment within the Royal Infirmary (or at other relevant hospitals) during the previous month, or were due for treatment in the forthcoming week, their records were removed from the Records Department for consultation at the relevant department. Tracer cards were completed in the records department indicating the whereabouts of the records. In these cases, patient records were traced and examined within the particular ward, or ward office after the hospital attendance had taken place.

If patient records were incomplete or could not be located at the Royal Infirmary, other hospitals cited in the ISD print-out, general practitioner forms or patient questionnaire were contacted between May and June 1996. Permission to visit hospitals was requested by letter at eight other hospitals within the Lothian and Border regions. One particular hospital, St Johns in Livingston, required a further application to the Director of Vascular Surgery for permission to examine case notes. Over a period of six months, the following hospitals were visited and the case notes examined: Western General Hospital, Eastern General Hospital, City

Hospital, Royal Victoria Hospital, Liberton Hospital, Royal Edinburgh Hospital, Borders General Hospital, St. Johns Hospital (Livingston).

Also, requests were made to several other hospitals for records to be sent by recorded delivery when only a small number of patient records was lodged and the hospital was outwith the District of Edinburgh. The hospital was reimbursed for recorded delivery costs.

3.13 Criteria for Diagnosis of Vascular Disease and Death

Criteria used to diagnose fatal and non-fatal myocardial infarction and fatal and non-fatal stroke were adapted from those proposed by the American Heart Association (Gillum et al 1984) and for angina from the World Health Organisation (Rose 1962) and were as follows:

3.13.1 Myocardial infarction

Non-fatal, definite

Two of the following criteria:

a) Prolonged cardiac pain anywhere in the anterior chest, left arm, or jaw (possibly also affecting the back, shoulder, right arm, or abdomen) and lasting at least 20 minutes.

b) Diagnostic electrocardiographic codes, including Minnesota codes: 1.1.1-1.2.5, 1.2.7, or 9.2 plus 5.1 or 5.2;

(c) Raised enzyme concentrations (creatine phosphokinase greater than twice the upper limits of normal, and one of the following also greater than twice the upper limits of

normal: lactate dehydrogenase, aspartate aminotransferase, or the MB isoenzyme of creatine phosphokinase).

The enzymes must have been measured within 72 hours of the acute event.

Non-fatal, possible

(a) One of the above definite criteria plus either equivocal electrocardiographic codes (1.2.8-1.3.6, 4.1-4.3, 5.1-5.3, or 9.2) or equivocal enzyme levels (above normal but not twice normal, or one above twice normal but could be attributed to another cause; or

(b) Equivocal electrocardiographic codes and enzyme concentrations.

Fatal, definite

(a) Postmortem evidence of acute myocardial infarction; or

(b) Definite criteria for myocardial infarction within the four weeks before death; or

(c) ICD-9 codes for cause of death 410-414 plus history of a definite or possible myocardial infarction or 410-414 plus definite or possible criteria for myocardial infarction immediately preceding death or 410-414 plus post-mortem evidence of severe coronary atherosclerosis or previous myocardial infarction.

Fatal, possible

Death certificate codes 410-414 but no other evidence.

3.13.2 Stroke

Non-fatal, definite

(a) History of onset of symptoms of less than 48 hours, plus clinical confirmation of a focal or global disturbance of cerebral function lasting more than 24 hours; or

(b) Computed tomography showing evidence of cerebral infarction or haemorrhage.

Non-fatal, possible

(a) Primary or secondary discharge diagnosis including ICD-9 codes, 431, 432, 434, 436, or 437.

Fatal, definite

One of the following criteria:

(a) Post-mortem evidence of cerebral infarction or haemorrhage;

(b) Criteria for definite stroke met within six weeks before death.

Fatal, possible

(a) Death certificate codes of underlying or immediate cause of death were ICD-9 431-437, but no other evidence.

3.13.3 Other vascular events

Other fatal vascular events (such as ruptured aortic aneurysm or thromboembolism) were recorded if the diagnosis was confirmed by laboratory, radiological, surgical or postmortem evidence.

Transient ischaemic attack

History of rapid onset of clinical signs of focal (or global) disturbance of cerebral function lasting less than 24 hours.

Angina pectoris

This was defined as pain or discomfort in the centre of the chest, or left anterior chest and left arm when walking up hill or hurrying requiring the person to stop or slow down for ten minutes or less, whereupon the pain is relieved.

Thrombosis and embolism

Clinical diagnosis confirmed by laboratory, radiological or surgical evidence.

Amputation

Amputation of any part of the lower limb due to only diabetes, or vascular causes.

3.14 Recording of Vascular Events

All possible cardiovascular events occurring since the patient was recruited into the study were followed up by direct examination of the relevant hospital records. Events were included in the data analysis if they met the predetermined diagnostic criteria.

Two separate forms were designed for the recording of the fatal or non-fatal events (Appendices XII and XIII). In both cases, a patient identification section was included, with the name and address of the patient's general practitioner. The information source for the reported event and the provisional diagnosis was required. The final diagnosis with the relevant ICD-9 code was recorded and was entered into the database. Vascular surgical procedures were coded using the Office of Population Censuses and Surveys' Classification of Surgical Operations 4th revision manual. For fatal events, the source of confirmation was noted.

Prior to examination of the hospital records, a separate form for each new non-fatal event reported in either the ISD printout, the general practitioner event form, or the self-administered questionnaire was prepared for every listed patient. The name, study number and provisional diagnosis was inserted. Diagnosis was then finalised from examination of the hospital records. If a patient's hospital record or a reported event could not be traced, it was coded as missing (code 99).

Nine non-fatal event files and one fatal event file were subsequently created when the data was entered onto computer. Each confirmed non-fatal event per patient was entered onto a separate

data file. The record structure for each non-fatal event file consisted of the study number of the patient, date of birth, date of event, ICD code and final diagnostic code. A file restricted to deceased patients recorded study number, date of birth, date of death, primary and secondary ICD death codes and final diagnostic code. Another file listed patients who did not have a vascular event or deterioration of limb ischaemia during follow-up. Patients who were excluded from the study because of missing data were also identified.

3.15 Definition of Ischaemic Heart Disease and Stroke Categories

Four categories of ischaemic heart disease and stroke were defined which were used in the data analysis: combined fatal and non-fatal stroke, non-fatal myocardial infarction, coronary death (fatal myocardial infarction and deaths from ischaemic heart disease), and total coronary events which included fatal and non-fatal myocardial infarction and deaths from ischaemic heart disease. Cases of fatal and non-fatal stroke were combined because of the low numbers in each group. Multiple events of the same type in a subject were recorded only once.

3.16 Definition of Progression of Peripheral Arterial Disease Categories

In this part of the analysis, patients were divided into two groups: those whose symptoms had deteriorated sufficiently to require vascular intervention, but did not develop symptoms of severe chronic leg ischaemia (defined as rest pain, ulceration or gangrene) during follow-up, and secondly, those who developed definite symptoms of severe chronic leg ischaemia during the six years follow-up period.

3.17 Data Analysis

Univariate analysis was carried out by the author on the University of Edinburgh mainframe computer using the SPSS-X statistical package. Means and percentages for baseline risk factor characteristics were calculated for the whole study population. Differences in medians of each haemostatic factor were tested across the four categories of ischaemic heart disease and stroke and categories of progression of peripheral arterial disease, relative to a group who had no coronary or cerebrovascular disease and no deterioration of limb ischaemia during follow-up.

Multiple logistic regression was carried out by Dr AJ Lee, the study statistician, using the statistical package SAS on the University mainframe computer. Associations between prognostic factors and incidence of vascular events and progression of peripheral arterial disease were expressed as an increase in risk of outcome for a given change in the prognostic factor. Relative risks were estimated which adjusted for age and sex and then further adjusted for other risk factors.

CHAPTER FOUR

SITES OF ATHEROMA: RESULTS

This chapter presents the results of the Sites of Atheroma Study. The results are divided into four main sections describing (i) the study sample, (ii) the distribution of angiographic disease, (iii) the relationship between cardiovascular risk factors and site of peripheral arterial disease, and (iv) the relationship between haemostatic factors and site and severity of peripheral arterial disease. The tables and figures are presented at the end of each of these four main sections.

4.1 Description of Study Sample

One hundred and fifty men and fifty women were recruited to the study. Six patients were subsequently excluded from the study sample because their angiograms could not be located within the hospital. Two further patients were excluded because they showed no evidence of significant atherosclerotic disease in any of the 13 arterial segments under consideration. The overall number of subjects participating in the study was therefore 192, which was composed of 144 men and 48 women.

4.1.1 Mean age and sex distribution of study sample

Table 4.1 shows the age and sex distribution of the study sample. The proportion of males was exactly three times higher than that of females (75% to 25% respectively). The age range of the study sample was between 37 and 81 years. The mean overall age was 63.7 years. The

mean age of females was approximately three years greater than that of males (66.2 v 62.9 years).

4.1.2 Clinical measures of severity of peripheral arterial disease in study sample

Symptoms of peripheral arterial disease in the 192 subjects were assessed by responses to the Edinburgh Claudication Questionnaire, a modified form of the WHO Intermittent Claudication Questionnaire, and are shown in Table 4.2. Overall, females reported significantly more severe symptoms of peripheral arterial disease than the males ($p \leq 0.05$). Although a similar proportion of males and females had intermittent claudication grade II, i.e. symptoms occurring while walking 'at an ordinary pace on the level', 34.8% of women had symptoms of more severe limb ischaemia (rest pain) compared to 28.2% of men. Comparison of the mean ABPI, as a more objective measure of peripheral arterial disease, showed only a slightly lower level in women (0.54) than in men (0.56, $p > 0.5$) which suggested that the severity of disease may be similar in women and men in the study sample.

4.2 Distribution of Angiographic Disease

The angiograms of the patients were examined to determine the distribution of lower limb disease. A total of 2496 arterial segments (13 segments per patient x 192 patients) from the study sample was evaluated. The number of segments which was coded as missing values was relatively small. Forty two segments (1.7%) could not be graded because of poor visualisation on angiogram. The percentage of segments which were coded was therefore 98.3%.

Table 4.1

AGE AND SEX DISTRIBUTIONS OF STUDY SAMPLE

	Males		Females	
	Number	% Study Sample	Number	% Study Sample
Age				
Mean Years (SE)	62.9 (9.7)		66.2 (10.0)	
≤ 49 years	16	11.1	5	10.4
50 - 59 years	37	25.7	2	4.2
60 - 69 years	54	37.5	22	45.8
≥ 70 years	37	25.7	19	39.4
Sex				
% Male	144	75		
% Female	48	25		

SE - standard error

Table 4.2

CLINICAL MEASURES OF PERIPHERAL ARTERIAL DISEASE IN STUDY SAMPLE BY SEX

Peripheral Arterial Disease	Males Number	%	Females Number	%	P-value
Intermittent Claudication: WHO Grade I	18	12.7	3	6.5	≤0.05
WHO Grade II	84	59.2	27	58.7	
Rest Pain	40	28.2	16	34.8	
Ankle Brachial Pressure Index Mean (SE)	0.56 (0.02)		0.54 (0.03)		NS

Intermittent claudication Grade I : Pain on hills only
Grade II : Pain on hills and flat
SE : Standard error

Table 4.3 shows the frequency of arterial occlusions in the study sample. Only 57 (29.7%) of the patients did not show occlusions within the 13 coded arterial segments. One hundred and thirty five (70.3%) patients had at least one occlusion. One patient had as many as nine segments occluded.

4.2.1 Site and severity of occlusions

A total of 335 arterial occlusions was recorded (Table 4.4). There was no real difference in the prevalence of occlusion in the right leg compared to the left leg. The right superficial femoral artery was the most common occlusion site, with 20.7% of the total number of occlusions recorded in the study sample within this segment. The left profunda femoris artery was least often occluded (apart from the abdominal aorta which was never completely occluded) with 2.7% of the total occlusions.

The severity of occlusions in the study sample is shown in Table 4.5. An occlusion less than or equal to half the length of a segment was coded by an additive score of 13. A score of 15 was recorded if the occlusion was longer than half the length of the segment. Only one occlusion (the more severe) was coded in each segment. Out of the total of 335 occlusions recorded, 192 (57.3%) were longer than half the length of the arterial segment. When shorter occlusions occurred, there were more occlusions in the right leg (n=84) than the left (n=59). In contrast, the left leg was the preferred site (or where disease progressed more rapidly) of longer occlusions, with the exception of the profunda femoris and superficial femoral arteries. The distribution of disease within the arteries also varied, depending on the severity of occlusion. Although the superficial femoral artery was the most common site of occlusions

of both lengths, longer occlusions were present more often in the common, external and internal iliac arteries.

4.2.2 Site and severity of stenoses

Table 4.6 shows sites of arterial stenoses graded by an additive score > 3, indicating that the patient had at least one lesion narrowing the lumen of an artery by > 25%. There were 853 (34.2% of the total graded segments) moderate to severe stenoses recorded. The left internal iliac was the most commonly affected vessel with 10.6% of total stenoses > 3, whereas the abdominal aortic segment showed the least number of stenoses (2.0%). The sites of arterial stenoses graded by a score ≤ 3 are also shown (Table 4.7). This score indicates that there is, at most, one stenosis narrowing the vessel lumen by between 25-50%. As expected, the prevalence of these less severe stenoses was higher than that of both more severe stenoses > 25%, and occlusions. The abdominal aortic segment (13.6%) and the right common iliac (10.7%) had the most number of mild stenoses. The superficial femoral segments had the fewest stenoses which reflected the high overall severity of disease within these vessels.

4.3 Classification of Patients by Site

The patients were classified into three groups, aorto-iliac, femoro-popliteal or dual-site by assessing the additive scores of the abdominal aorta, combined left and right leg iliac segments and left and right leg femoro-popliteal segments separately. The worst additive score for each of the six iliac (common, external and internal) segments, six femoro-popliteal (femoral, profunda and popliteal) segments and the total score for the six segments in each

group were compared. The aorta was compared separately so as to give an equal weighting of six segments in each of the two groups. If any segment was occluded in one of the two groups of arteries or abdominal aorta, or had a moderate to severe stenosis (additive score > 3) in these segments, the patient was assigned to that group. If there were occlusions or stenoses with an additive score > 3 present in both the aorto-iliac and femoro-popliteal segments, the patient was classified as having dual-site disease.

The classification of patients by site of disease is shown in Table 4.8. Seventy three patients (38%) were classified as having multi-segment disease at more than one site (dual-site disease). Eighty five (44%) of patients were identified as having substantially more disease in the femoral, profunda, or popliteal arteries compared to the aorta and the common, external and internal iliac arteries. The third group comprised 34 (18%) subjects who were considered to have predominately more atherosclerotic disease in the aorta and iliac segments. Table 4.8 also shows that 28 patients (82.4%) were classified into the aorta-iliac and 75 patients (88.2%) into the femoro-popliteal groups on the sole criterion of an occlusion (an additive score of 13 or 15). Only 32 subjects (43.8%) of those with dual-site disease were so classified due to the presence of occlusions at both sites. Several patients were grouped according to the criterion of stenoses only (lesions with an additive score > 3). Ten patients from the femoro-popliteal and six from the aorto-iliac group were categorised in this way. In contrast, 41 (56.2%) of the subjects with dual-site disease were classified by stenoses only, rather than occlusion.

4.3.1 Mean additive scores by site

In Table 4.9, the mean total additive score for the aorta-iliac and femoro-popliteal segments

are shown in relation to subjects in the three sites of classification. Some overlap occurred between the three groups because of the diffuse distribution of peripheral atherosclerosis in the study sample. The aorto-iliac patient group had significantly higher additive scores in those segments than in their femoro-popliteal segments. Likewise, the femoro-popliteal group had relatively higher scores in the femoro-popliteal segments. The dual-site group had a slightly higher mean additive score in the femoro-popliteal segments than in the other group of segments but this difference was not statistically significant. Seventeen patients classified into the dual-site disease group had far more severe and extensive disease in the femoro-popliteal segments than in the iliac segments, suggesting some bias in the dual-site group towards femoro-popliteal disease.

4.4 Cardiovascular Risk Factors

In this section, age, sex, the presence of clinical cardiovascular disease, and risk factors (cigarette smoking, serum lipids and blood pressure) are described in the three groups of patients with different sites of peripheral atherosclerosis.

4.4.1 Age and sex

Table 4.10 at the end of this section (4.4) shows the distribution of age and sex in patients with predominately aorto-iliac, femoro-popliteal and dual-site disease. Chi-square analysis was used to test for differences in categorical variables within each class of disease. The distribution of age was significantly different between the three groups. Those with aorto-iliac disease were approximately eight years younger than patients from the other two groups ($p \leq$

0.001). The mean age of the femoro-popliteal group was only marginally higher than that of the dual-site group (65.55 years compared to 64.98 years). There was a greater proportion of women in the aorto-iliac group compared to either the femoro-popliteal or dual-site group. Differences in sex distribution across the three classes were not statistically significant.

4.4.2 Vascular diseases

Clinical findings of peripheral arterial disease in the three groups of patients are shown in Table 4.11. All three groups reported a far higher prevalence of grade II symptoms of intermittent claudication (pain walking on the flat and uphill) than the less severe grade I (pain walking uphill only). The aorto-iliac group had relatively more rest pain and a slightly higher ABPI than the other two groups. The femoro-popliteal group showed the least percentage of patients with rest pain, but the lowest ABPI. However, none of the observed differences were statistically significant ($p > 0.05$). No patient reported symptoms of severe chronic leg ischaemia, such as ulceration or gangrene or had a previous amputation.

The prevalence of other cardiovascular diseases is reported in Table 4.12. The dual-site group showed more evidence of a history of angina pectoris and previous myocardial infarction (both $p \leq 0.05$), stroke and diabetes mellitus than the other groups which probably indicates a higher degree of generalised atherosclerosis. Angina pectoris and myocardial infarction occurred less frequently in the aorto-iliac group than in the femoro-popliteal group. There were no cases of stroke in the aorto-iliac group. Likewise, diabetes mellitus was less common in the aorto-iliac group than in either of the two other classes of disease. However, the differences for stroke and diabetes mellitus were not statistically significant ($p > 0.05$).

Table 4.3

FREQUENCY OF ARTERIAL OCCLUSIONS IN STUDY SAMPLE

Number of Occlusions	Number of Patients	% of Study Sample
0	57	29.7
1	51	26.6
2	34	17.7
3	22	11.5
4	9	4.7
5	9	4.7
6	4	2.1
7	4	2.1
8	1	0.5
9	1	0.5
Total	192	100

Table 4.4

SITE OF ARTERIAL OCCLUSIONS IN STUDY SAMPLE

Arterial Segment		Occlusions Number	% of Total
Abdominal Aorta		0	0
Common Iliac	Left	15	4.5
	Right	10	3.0
External Iliac	Left	19	5.7
	Right	22	6.6
Internal Iliac	Left	22	6.6
	Right	20	6.0
Profunda Femoris	Left	9	2.7
	Right	12	3.6
Superficial Femoral	Left	63	18.9
	Right	70	20.7
Popliteal	Left	37	11.0
	Right	36	10.7
All Segments		335	100.0

Table 4.5

SEVERITY OF ARTERIAL OCCLUSIONS IN STUDY SAMPLE

Arterial Segment		Occlusions ≤ Half Length of Segment Number	% of Total	Occlusions > Half Length of Segment Number	% of Total
Abdominal Aorta		0	0	0	0
Common Iliac	Left	0	0	15	4.5
	Right	2	0.6	8	2.4
External Iliac	Left	6	1.8	13	3.9
	Right	10	2.9	12	3.6
Internal Iliac	Left	0	0	22	6.6
	Right	4	1.2	16	4.8
Profunda Femoris	Left	4	1.2	5	1.5
	Right	6	1.8	6	1.8
Superficial Femoral	Left	27	8.1	36	10.7
	Right	32	9.6	38	11.3
Popliteal	Left	22	6.6	15	4.5
	Right	30	8.9	6	1.8
All Segments		143	42.7	192	57.3

Table 4.6

SITE OF STENOSES WITH ADDITIVE SCORE >3 IN STUDY SAMPLE

Arterial Segment		Stenoses with Additive Score >3 Number	% of Total
Abdominal Aorta		17	2.0
Common Iliac	Left	54	6.3
	Right	69	8.1
External Iliac	Left	81	9.5
	Right	56	6.6
Internal Iliac	Left	90	10.6
	Right	75	8.8
Profunda Femoris	Left	47	5.5
	Right	65	7.6
Superficial Femoral	Left	69	8.1
	Right	71	8.3
Popliteal	Left	85	10.0
	Right	74	8.7
All Segments		853	100.0

Table 4.7

SITE OF STENOSES WITH ADDITIVE SCORE ≤3 IN STUDY SAMPLE

Arterial Segment		Stenoses with Additive Score ≤3 Number	% of Total
Abdominal Aorta		172	13.6
Common Iliac	Left	123	9.7
	Right	135	10.7
External Iliac	Left	102	8.1
	Right	103	8.1
Internal Iliac	Left	78	6.2
	Right	96	7.6
Profunda Femoris	Left	124	9.8
	Right	106	8.4
Superficial Femoral	Left	37	2.9
	Right	35	2.8
Popliteal	Left	76	6.0
	Right	79	6.2
All Segments		1266	100.0

Table 4.8

PATIENTS BY SITE OF DISEASE : PERCENTAGE IDENTIFIED BY OCCLUSIONS OR STENOSES

Site of Disease	Number of Patients by Site	% Patients by Site Identified by Occlusion	Stenoses
Aorto-Iliac	34	82.4	17.6
Femoro-Popliteal	85	88.2	11.8
Dual-Site	73	43.8	56.2

Table 4.9

MEAN TOTAL ADDITIVE SCORES BY SITE OF DISEASE

	Total Additive Scores		
Site of Disease	Aorto-Iliac Mean ± SD	Femoro-Poplitcal Mean ± SD	P-valuc†
Aorto-Iliac	39.7 (±13.4)	20.7 (±11.8)	≤0.001
Femoro-Popliteal	24.6 (±9.1)	42.8 (±13.2)	≤0.001
Dual-Site	36.9 (±17.3)	40.5 (±17.5)	NS

† - Significance of differences between additive scores
SD - Standard deviation

4.4.3 Cigarette smoking

In the study sample, serum thiocyanate and serum cotinine were highly positively skewed and were transformed by taking the natural logarithm to produce a more normal distribution. This allowed for valid statistical assumptions of significance to be made from the sample data. The mean levels of the transformed factors were presented as geometric means by taking the anti-log values. The confidence intervals were also transformed back to geometric units.

Life-time smoking was modelled using pack-years (the average number of packs of 20 cigarettes smoked per day multiplied by the number of years as a smoker). The distribution of packyears was slightly skewed and so the square root of this variable was used to reduce the influence of a few heavy smokers. The definition of a smoking 'deceiver' was any individual with a level of serum thiocyanate above 63.7 μmol/L and above 17.5 ng/ml for serum cotinine who had declared themself to be a non-smoker. The distributions of total cholesterol, systolic and diastolic blood pressure were approximately normal and levels were therefore presented as the arithmetic means. Analysis of covariance was carried out with age and sex as covariates to test for differences in levels of the risk factors within each class of disease. The mean values were adjusted for age and sex to control for any confounding effects on the levels of the cardiovascular risk factors and on peripheral arterial disease.

Self-reported smoking status, mean levels of biochemical smoking measures and packyears, adjusted for age and sex by site of disease are shown in Table 4.13. There were no significant differences in cigarette smoking between the three classes. As expected with patients with clinically confirmed peripheral arterial disease, few individuals had never smoked. The high

percentage of ex-smokers occurring in all three classes of disease possibly indicated cessation of smoking because of the onset of intermittent claudication or symptomatic ischaemic heart disease or cerebrovascular disease. The aorto-iliac group had more current smokers (44.1%) and fewer ex-smokers (41.2%) than the other groups. The percentage of smoking 'deceivers' was approximately the same in each group of patients and ranged from 9.6% in dual-site patients to 11.8% in the aorto-iliac patients. Serum cotinine and serum thiocyanate levels were also higher in the aorto-iliac patients, reflecting the greater percentage of current smokers in this group. Packyear levels, as a measure of life-time smoking consumption were slightly higher for patients with femoro-popliteal disease than for aorto-iliac disease, suggesting that the femoro-popliteal patients may have smoked more heavily and/or had begun smoking at an earlier age.

4.4.4 Lipids and blood pressure

Mean serum lipid and blood pressure levels adjusted for age and sex were also compared (Table 4.14). As with cigarette smoking, the differences in levels of these risk factors between the three groups were not statistically significant. Of the three groups of patients, those with aorto-iliac disease had the lowest mean levels of total cholesterol, but the highest levels of HDL cholesterol (5.75 mmol/L and 1.27 mmol/L respectively). The levels of total cholesterol in the femoro-popliteal and dual-site groups were approximately the same at 6.08 mmol/L and 6.04 mmol/L. The levels of total cholesterol in all groups was only marginally higher than the clinically accepted 'normal' level of 5.2 mmol/L. Examination of hospital records showed that only two individuals took lipid-lowering drugs, although it is possible that other patients with hyperlipidaemia may have been advised to modify their diet.

Table 4.10

MEAN AGE AND SEX DISTRIBUTION IN PATIENTS BY ANGIOGRAPHIC SITE OF LOWER LIMB ATHEROSCLEROSIS

Factor	Site of Atherosclerosis			P-Value†
	Aorto-Iliac (n=34)	Femoro-Popliteal (n=85)	Dual-Site (n=73)	
Age				
Mean Years (SE)	56.60 (1.82)	65.55 (1.03)	64.98 (1.00)	≤0.001
Sex				
% Male	67.6	74.1	79.5	NS
% Female	32.4	25.9	20.5	

† - P-Value for differences between classes based on chi-square analysis
SE - Standard error
NS - Non-significant

Table 4.11

CLINICAL FINDINGS IN PATIENTS BY ANGIOGRAPHIC SITE OF LOWER LIMB ATHEROSCLEROSIS

Peripheral Arterial Disease	Site of Atherosclerosis			
	Aorto-Iliac (n=34)	Femoro-Popliteal (n=85)	Dual-Site (n=73)	P-Value†
Intermittent Claudication :				
WHO Grade I (%)	2.9	12.0	14.1	
WHO Grade II (%)	61.8	62.7	53.5	NS
Rest Pain (%)	35.3	25.3	32.4	
Ankle Brachial Pressure Index Mean (SE)	0.60 (0.04)	0.56 (0.03)	0.56 (0.03)	NS

† - P-Value for differences between classes based on chi-square analysis
Intermittent claudication Grade I : Pain on hills only
Grade 2 : Pain on hills and flat
NS - Non-significant
SE - Standard error

Table 4.12

OTHER VASCULAR DISEASES IN PATIENTS BY ANGIOGRAPHIC SITE OF LOWER LIMB ATHEROSCLEROSIS

Vascular Diseases	Site of Atherosclerosis			
	Aorto-Iliac (n=34)	Femoro-Popliteal (n=85)	Dual-Site (n=73)	P-Value†
Angina Pectoris (%)	15.2	24.7	40.3	≤0.05
Myocardial Infarction (%)	12.1	23.8	33.8	≤0.05
Stroke (%)	0	8.2	14.1	NS
Diabetes Mellitus (%)	5.9	10.6	12.3	NS

† - P-Value for differences between classes based on chi-square analysis
NS - Non-significant

Table 4.13

CIGARETTE SMOKING BY ANGIOGRAPHIC SITE OF LOWER LIMB ATHEROSCLEROSIS

Cigarette Smoking	Site of Atherosclerosis		
	Aorto-Iliac (n=34)	Femoro-Popliteal (n=85)	Dual-Site (n=73)
Current Smoker (%)	44.1	25.9	31.5
Ex Smoker (%)	41.2	58.8	57.5
Never Smoker (%)	2.9	4.7	1.4
Deceiver‡ (%)	11.8	10.6	9.6
Serum Thiocyanate (μmoL)†	64.06 (51.45,79.76)	54.36 (47.77,61.85)	54.47 (46.91,63.11)
Serum Cotinine (ng/mL)†	34.01 (13.01,88.93)	13.24 (7.58,23.12)	16.40 (8.61,31.24)
Packyears ($\sqrt{}$)*	2.07 (0.12)	2.30 (0.07)	2.25 (0.08)

All analyses adjusted for age and sex
All P-values for differences between classes were non-significant at the 5% level
‡ - Defined by levels above 63.7 μmol/l for serum thiocyanate and 17.5 ng/ml for serum cotinine
† - Geometric mean (transformed confidence intervals)
* - Mean (standard error)

Table 4.14

SERUM LIPIDS AND BLOOD PRESSURE BY ANGIOGRAPHIC SITE OF LOWER LIMB ATHEROSCLEROSIS

	Site of Atherosclerosis		
	Aorto-Iliac (n=34)	Femoro-Popliteal (n=85)	Dual-Site (n=73)
Serum Lipids (mmol/L)			
Total Cholesterol*	5.75 (0.25)	6.08 (0.14)	6.04 (0.15)
HDL Cholesterol†	1.27 (1.13,1.43)	1.23 (1.15,1.32)	1.14 (1.06,1.22)
Blood Pressure (mmHg)			
Systolic Pressure*	148.65 (4.78)	148.08 (2.90)	152.39 (3.12)
Diastolic Pressure*	79.83 (2.76)	75.99 (1.68)	76.77 (1.82)

All analyses adjusted for age and sex
All P-values for differences between classes were non-significant at the 5% level
* - Mean (standard error)
† - Geometric mean (transformed confidence intervals)

Systolic blood pressure was highest in those with dual-site disease and diastolic pressure was highest in the aorto-iliac group (Table 4.14). It should be noted, however, that the intake of anti-hypertensive medication was highest in the dual-site group, with 20.5% of patients taking these drugs, compared to 11.8% in the femoro-popliteal and 8.8% in the aorto-iliac groups.

4.5 Haemostatic Factors

The distributions of von Willebrand Factor, fibrin D-dimer, plasminogen activator inhibitor were highly positively skewed and logarithmic transformations were used in the analyses. Both clotting fibrinogen and nephelometric fibrinogen were normally distributed and levels were therefore presented as the arithmetic means (Figures 6-10).

4.5.1 Univariate analysis of haemostatic factors and site of disease

Mean levels of the haemostatic factors across the site of disease groups were examined using analysis of covariance with age and sex as covariates. There were no significant differences in mean levels of haemostatic factors between patients in the three groups (Table 4.15). This lack of statistically significant differences could have occurred because of the small numbers, particularly in the aorto-iliac group. However, with the exception of fibrin D-dimer, levels of the factors tended to be highest in the dual-site group and lowest in patients with predominately aorto-iliac disease. In contrast, fibrin D-dimer showed the highest levels in the aorto-iliac group. In all three groups, the levels of heat precipitated fibrinogen measured by nephelometry were higher than clotting fibrinogen estimated by the modified Clauss assay.

4.5.2 Univariate analysis of haemostatic factors and severity of disease

Partial correlation coefficients adjusted for age and sex were calculated to examine associations between the haemostatic factors and severity of disease within the femoro-popliteal and aorto-iliac segments. Disease severity was estimated by a quantitative variable (the additive score) in all 192 patients which enabled examination of linear relationships between the variables and hence permitted more power to detect significant associations.

The range of additive scores for both groups of segments was identical (5 to 86), but the mean additive score for the femoro-popliteal segments was significantly higher than the mean score for the aorto-iliac segments (38.09 compared to 31.95, $p \leq 0.001$). Table 4.16 shows that increasing severity of disease within the aorto-iliac segments was significantly associated with nephelometric fibrinogen ($r=0.12$, $p < 0.05$). There were no significant relationships between the other haemostatic factors and the additive score in the aorto-iliac segments. Both nephelometric fibrinogen and fibrin D-dimer were significantly correlated with increasing disease severity in the femoro-popliteal segments ($r=0.20$, $p \leq 0.01$ and $r=0.22$, $p \leq 0.001$ respectively). A weaker positive association was found with von Willebrand Factor ($r=0.14$, $p \leq 0.05$). Plasminogen activator inhibitor (PAI) showed a weak non-significant negative correlation with increasing additive scores in both groups of segments.

4.5.3 Multivariate analysis of haemostatic factors and severity of disease

Multiple regression analyses were carried out to identify any statistically independent associations of the haemostatic factors with disease severity within each of the two groups of

segments. The regression coefficients were obtained from the statistical package BMDP and represented changes in the dependent variable (the additive score) when a haemostatic factor (the independent variable) increased in value by approximately one standard deviation.

Since PAI activity showed a significant linear trend with time of venepuncture ($p \leq 0.001$), this was used as a covariate together with age and sex. Aspirin consumption or other anti-platelet medication showed no relationship with any of the haemostatic factors and these were therefore not included in the model. Table 4.17 shows that none of the haemostatic factors had a statistically independent effect on disease severity within the aorto-iliac segments. Therefore no further analysis was carried out on this group of segments.

Table 4.17 also shows that, for the femoro-popliteal segments, both nephelometric fibrinogen and fibrin D-dimer were independently related to disease severity in the femoro-popliteal segments (both $p \leq 0.05$). In the case of PAI, the regression coefficient was negative, suggesting that higher PAI activity was associated with a lower severity of disease. The association between PAI and disease severity was only just non-significant ($p = 0.08$).

In Table 4.18, the relationships between nephelometric fibrinogen, between fibrin D-dimer and between PAI and the severity of disease within the femoro-popliteal segments were estimated after further adjustment for life-time smoking (packyears) and for current smoking (serum thiocyanate) in the multiple regression model. On adjusting for packyears, nephelometric fibrinogen remained independently associated with disease severity. Adjustment for serum thiocyanate also had little effect on the association of fibrinogen with disease. Adjustment for packyears had a greater effect on the relationship between PAI and disease severity than

current smoking (thiocyanate), although the association remained non-significant (p= 0.06). The negative association between PAI activity and additive score in the femoro-popliteal segments was examined further and found to be due to a strong inverse correlation between men, whereas women showed a weak positive correlation (Figure 11). There was no significant difference in additive scores within the femoro-popliteal segments between the sexes. However, packyears were significantly higher in men (mean = 2.31, SE = 0.06) compared to women (mean = 2.05, SE = 0.10, p=0.02).

The magnitude of the relationship between fibrin D-dimer and disease severity (Table 4.18) was reduced when life-time smoking (packyears) was taken into account, and the association became non-significant (p= 0.06). On the other hand, there was no influence of current smoking on the relationship between fibrin D-dimer and severity of disease in the femoro-popliteal segments and the value of the regression coefficient was unchanged (3.95) on adjustment for serum thiocyanate.

Figure 6: Frequency Distribution of Clotting Fibrinogen

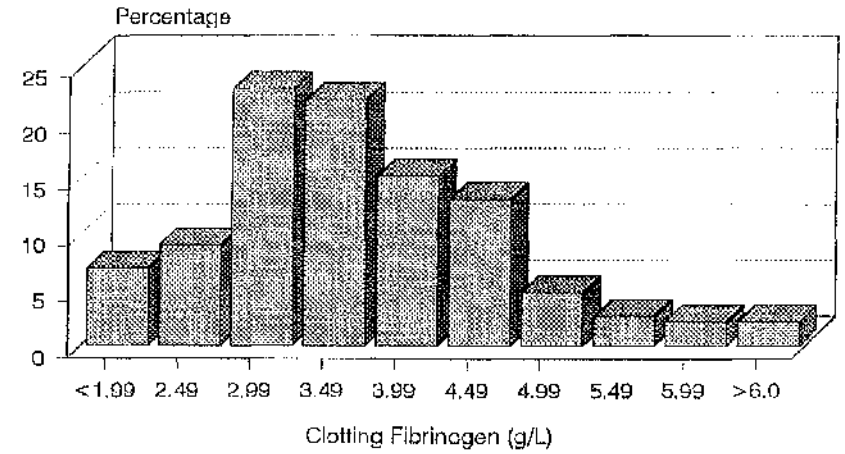


Figure 7: Frequency Distribution of Nephelometric Fibrinogen

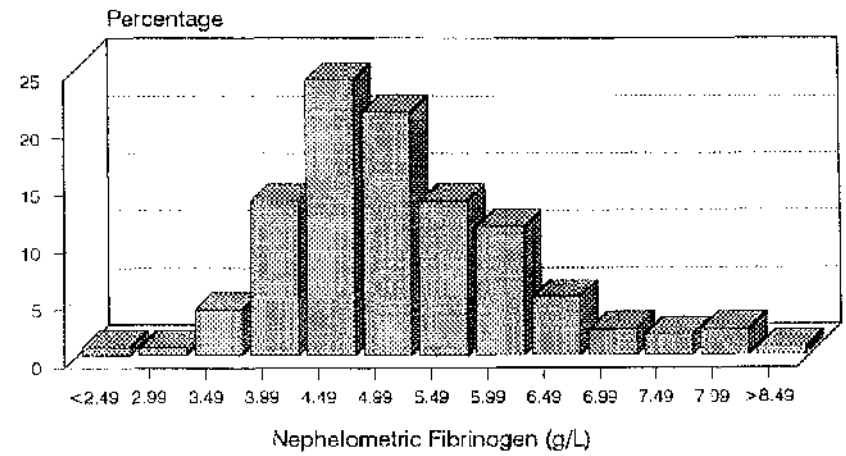


Figure 8: Frequency Distribution of von Willebrand Factor

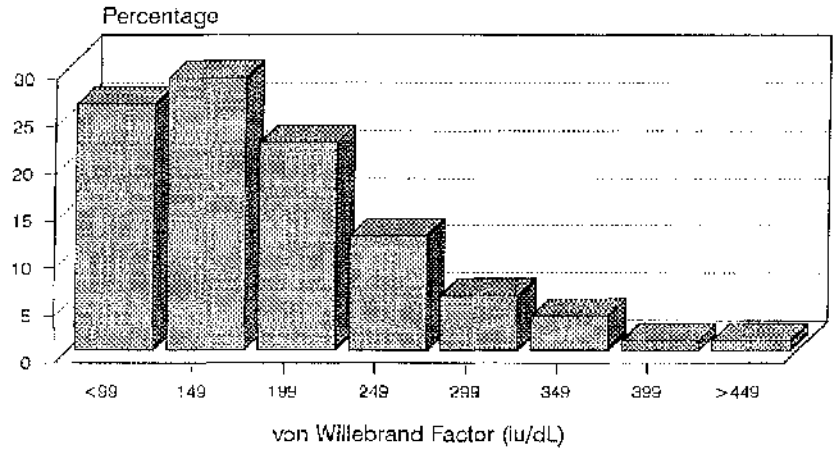


Figure 9: Frequency Distribution of Fibrin D-dimer

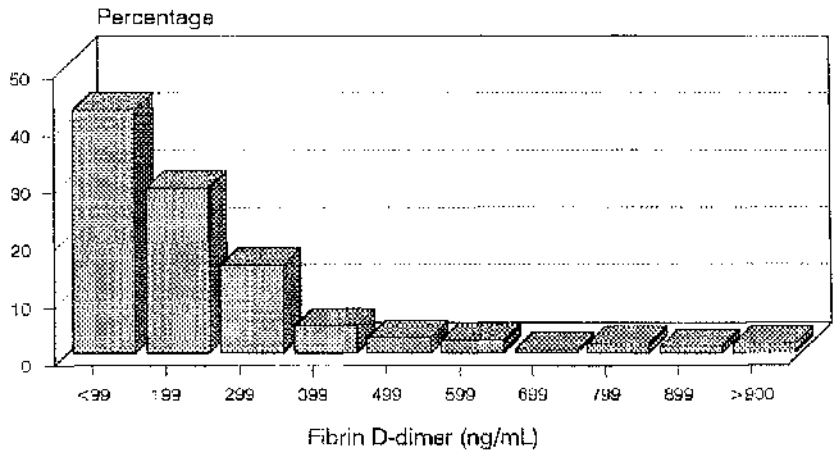


Figure 10: Frequency Distribution of Plasminogen Activator Inhibitor-1

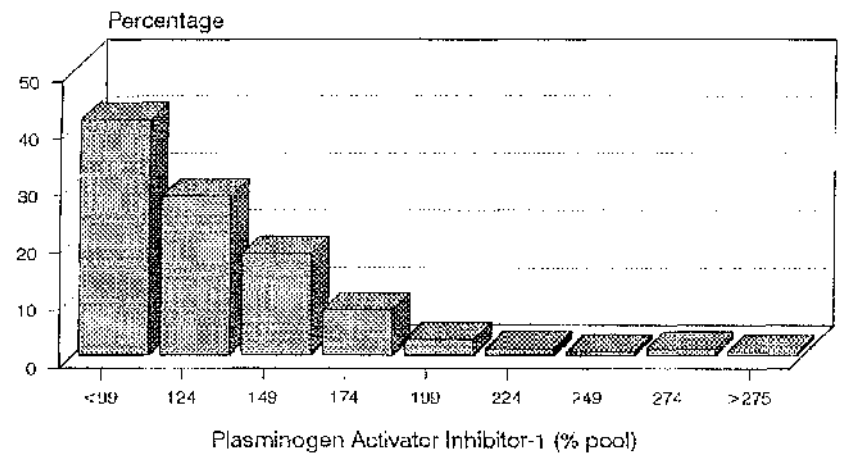


Table 4.15

MEAN LEVELS OF HAEMOSTATIC FACTORS BY ANGIOGRAPHIC SITE OF LOWER LIMB ATHEROSCLEROSIS

Haemostatic Factor	Site of Atherosclerosis		
	Aorto-Iliac (n=34)	Femoro-Popliteal (n=85)	Dual-Site (n=73)
Fibrinogen : Clotting (g/L)[a]	3.38 (0.18)	3.47 (0.11)	3.52 (0.11)
Nephelometric (g/L)[a]	4.47 (0.23)	4.70 (0.14)	4.87 (0.15)
von Willebrand Factor (iu/dL)[b]	130.73 (110.73,154.34)	146.86 (133.03,162.12)	149.35 (134.24,166.16)
Fibrin D-dimer (ng/mL)[b]	134.12 (98.80,182.07)	121.74 (101.58,145.90)	133.26 (109.77,161.78)
Plasminogen Activator Inhibitor (% Pool)[b]	110.74 (99.69,123.00)	111.28 (104.47,118.54)	113.11 (105.56,121.20)

All analyses adjusted for age and sex
All P-values for differences between classes were non-significant at the 5% level
[a] - Mean (standard error)
[b] - Geometric Mean (transformed confidence intervals)

Table 4.16

AGE AND SEX ADJUSTED CORRELATION COEFFICIENTS BETWEEN HAEMOSTATIC FACTORS AND ADDITIVE SCORE WITHIN THE AORTO-ILIAC AND FEMORO-POPLITEAL SEGMENTS

Haemostatic Factor		Additive Score	
		Aorto-Iliac	Femoro-Popliteal
Fibrinogen :	clotting (g/L)	0.08	0.06
	nephelometric (g/L)	0.12*	0.20**
von Willebrand Factor (iu/dL)†		0.08	0.14*
Fibrin D-dimer (ng/mL)†		0.10	0.22***
Plasminogen Activator Inhibitor (% Pool)†		-0.05	-0.07

† - Logged values used
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Table 4.17

MULTIPLE REGRESSION MODELS OF HAEMOSTATIC FACTORS ON ADDITIVE SCORE WITHIN THE AORTO-ILIAC AND FEMORO-POPLITEAL SEGMENTS

Factor		Aorto-Iliac Additive Score	Femoro-Popliteal Additive Score
Fibrinogen :	clotting (+1.21 g/L)	0.29 (1.56)	-1.64 (1.45)
	nephelometric (+0.93 g/L)	1.34 (1.20)	2.42 (1.11)*
von Willebrand Factor (+1 Log iu/dL)		1.50 (2.78)	3.59 (2.58)
Fibrin D-dimer (+1 Log ng/mL)		0.82 (1.55)	3.36 (1.48)*
Plasminogen Activator Inhibitor (+ Log % Pool)		-0.19 (4.81)	-7.82 (4.47)

All values are unstandardised regression co-efficients and standard errors
All factors entered simultaneously with age, sex and time of venepuncture
* $p \leq 0.05$

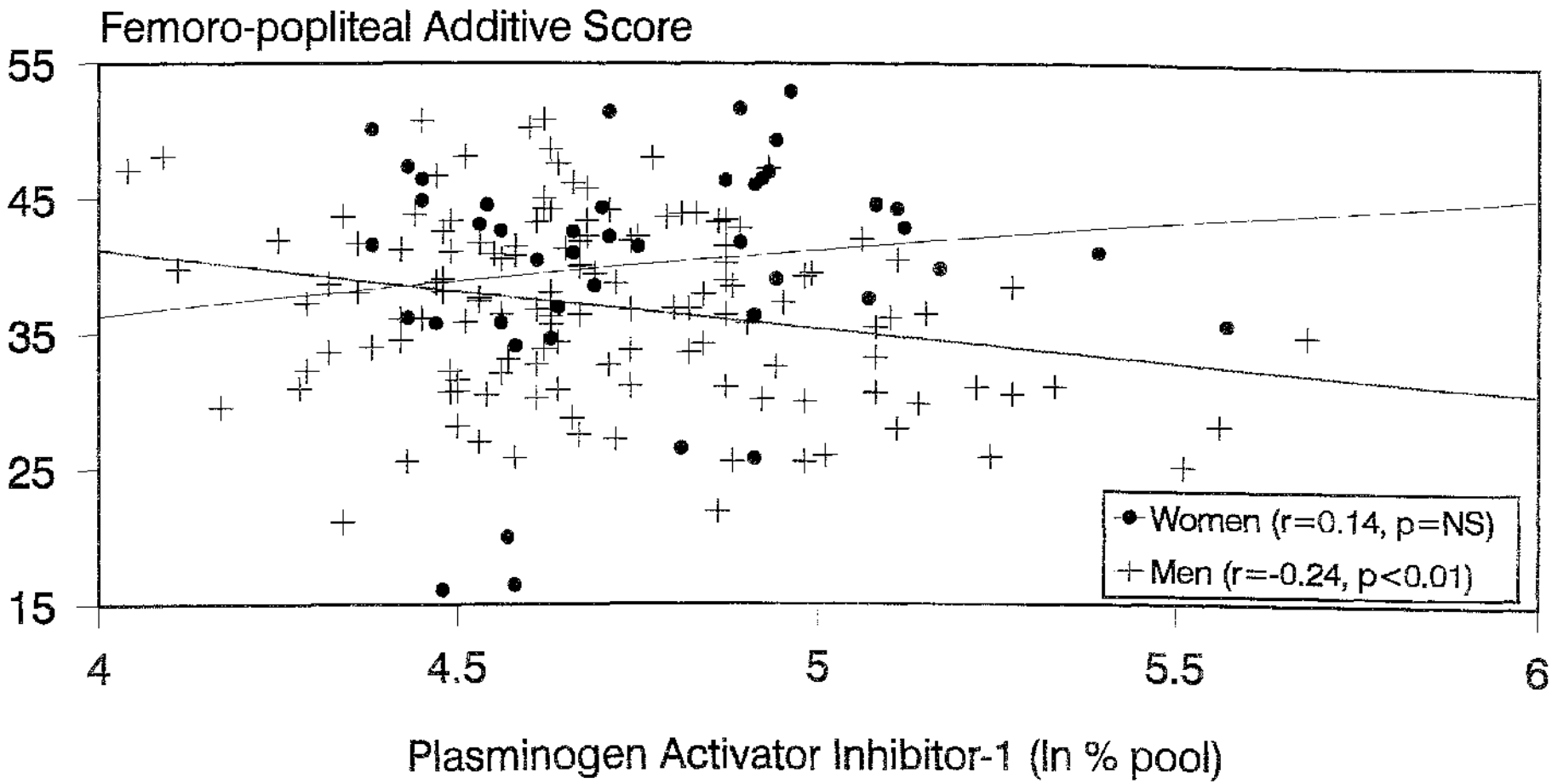
Table 4.18

MULTIPLE REGRESSION MODELS OF FIBRINOGEN, FIBRIN D-DIMER AND PLASMINOGEN ACTIVATOR INHIBITOR ON ADDITIVE SCORE WITHIN THE FEMORO-POPLITEAL SEGMENTS

Forced Entry	Nephelometric Fibrinogen (+0.93 g/L)	PAI (+1 Log % Pool)	Fibrin D-dimer (+1 Log ng/mL)
Age, sex, time of venepuncture	2.32 (0.93)*	-7.69 (4.27)	3.95 (1.37)**
Age, sex, time of venepuncture, packyears	2.43 (0.97)*	-3.57 (4.80)	2.91 (1.52)
Age, sex, time of venepuncture, serum thiocyanate	2.28 (0.96)*	-8.58 (4.53)	3.95 (1.46)**

All values are unstandardised regression co-efficients and standard errors
All haemostatic factors are entered simultaneously in each model
* $p \leq 0.05$; ** $p \leq 0.01$

Figure 11: Age adjusted Regression Lines of Plasminogen Activator Inhibitor-1 on Femoro-popliteal Additive Score in Men and Women



4.6 Summary of Results

1. One hundred and ninety two patients (144 men and 48 women) with either intermittent claudication or rest pain were recruited into the study.

2. Thirty four (18%) patients were identified using the Bollinger angiographic scoring system as having predominantly aorto-iliac disease, 85 (44%) femoro-popliteal disease and 73 (38%) patients were classified as having dual-site disease.

3. Levels of cardiovascular risk factors (blood pressure, lipids and cigarette smoking) differed between patients with different sites of peripheral atherosclerosis, although the differences were statistically non-significant.

4. With the exception of fibrin D-dimer levels (which were highest in aorto-iliac disease), haemostatic factor levels were highest in patients with dual-site disease, although the differences were statistically non-significant.

5. Plasma fibrinogen, as measured by nephelometry, fibrin D-dimer and von Willebrand Factor were significantly correlated with increasing disease severity (additive score) in the femoro-popliteal arteries. There were no significant associations between any haemostatic factors and additive score in the aorto-iliac arteries, after adjusting for age and sex.

6. On multiple regression, fibrinogen remained independently associated with disease

severity in the femoro-popliteal arteries, when life-time smoking, or current smoking were taken into account. There was no influence of current smoking on the association between fibrin D-dimer and disease severity, but on inclusion of life-time smoking, the association became non-significant.

CHAPTER FIVE

PROGNOSTIC STUDY OF INTERMITTENT CLAUDICATION: RESULTS

This chapter describes the results from the Prognostic Study of Intermittent Claudication. The results are divided into four main sections describing (i) the baseline characteristics of the study sample, (ii) the incidence of vascular events, (iii) the relationships between severity of peripheral arterial disease, cardiovascular risk factors, and haemostatic factors to incident ischaemic heart disease and stroke events and (iv) the relationships between cardiovascular risk factors and haemostatic factors to progression of peripheral arterial disease.

5.1 The Study Sample

Ten patients were excluded from the baseline cohort of 617 patients because on examination of hospital records by the author, six were found to have evidence of previous or impending arterial surgery and four had symptoms of severe chronic leg ischaemia (rest pain, gangrene or ulceration) at the time of recruitment to the study. A total of 607 patients was therefore followed up prospectively over the six year period.

5.1.1 Age, sex and clinical characteristics

The mean age and sex distribution and clinical characteristics of the study sample at baseline are shown in Table 5.1. The mean age of the 607 patients in the study was 65.5 years. There were proportionally more males (n=389, 64.1%) than females (n=218, 35.9%). Symptoms of

intermittent claudication were assessed by responses to the WHO Claudication Questionnaire (Rose 1962) which was completed at the time of the baseline clinical examination. A total of 47.1% of the patients had moderate intermittent claudication (grade I), whereas 52.5% had symptoms of more severe claudication (grade II). The mean ABPI of the sample was 0.57.

5.1.2 Cardiovascular risk factors by sex

Baseline risk factor characteristics of the study sample are shown in Table 5.2. Most of the patients had a history of cigarette smoking (91.3%), and at recruitment, current cigarette smokers comprised 38.2%, ex-smokers 48.9% and never smokers 8.7% of the study sample. The high percentage of ex-smokers possibly indicated cessation of smoking when intermittent claudication was first diagnosed. Mean levels of systolic and diastolic blood pressure were 152.4 mmHg and 83.9 mmHg respectively. The mean level of total cholesterol in the study sample was relatively high at 6.7 mmol/L.

Comparison of the mean age between males and females (Table 5.3) showed that females were approximately two years older and the sex difference was statistically significant ($p \leq 0.05$). The males in the study sample had a higher prevalence of more severe grade II symptoms of intermittent claudication than females (54.3% compared to 50.0% respectively, $p > 0.05$). The ABPI was almost identical in the sexes, 0.57 in males and 0.56 in females.

Cigarette smoking status and mean levels of cardiovascular risk factors were compared in males and females (Table 5.4). Smoking status was very different between the sexes. Although the proportion of current smoking was similar, there were more ex-smokers in

males, so that only 4.9% had never smoked compared to 16.3% in females. The square root of packyears was used to reduce the effect from 'outliers' (occasional results with extremely high or low values). Mean levels of packyears, as a measure of life-time smoking consumption were also significantly higher in males than females ($p< 0.001$), and reflected the higher percentage of current and ex-smokers in males. In contrast, females showed higher mean levels of systolic blood pressure (155.2 mmHg compared to 150.8 mmHg in males, $p\leq 0.05$), and a slightly lower diastolic pressure, which was not statistically significant. Also, mean levels of total cholesterol were far higher in females, 7.1 mmol/L compared to 6.4 mmol/L ($p\leq 0.001$), which was as expected in these mainly post-menopausal women. Random glucose levels were also higher in females, but the difference was not significant.

5.1.3 Associations between cardiovascular risk factors and severity of disease

The relationships between cigarette smoking and other cardiovascular risk factors to severity of peripheral arterial disease in the study sample at baseline were examined. Partial correlation coefficients were calculated to examine associations between the risk factors and the ABPI, which was used as a measure of the severity of disease. Table 5.5 shows that only systolic blood pressure was significantly related to the ABPI. The strength of the correlation was moderate ($r=-0.21$) but highly statistically significant ($p\leq 0.001$), probably because of the relatively large numbers in the study sample. The direction of the correlation was negative, indicating that as systolic blood pressure increased, the ABPI decreased (or severity of disease increased). However, an association was not unexpected because ABPI and systolic blood pressure are not independent variables. Estimation of the ABPI involves using systolic pressure and changes in systolic pressure would therefore affect both the numerator and

denominator of ABPI.

5.1.4 Haemostatic factors

The distributions of the haemostatic factors are illustrated in Figures 12-15. Clotting fibrinogen and von Willebrand Factor were positively skewed and were square root transformed. Tissue plasminogen activator and fibrin D-dimer required logarithmic transformations to normalise their distributions.

The medians and inter-quartile ranges of the haemostatic factors for the study sample are shown in Table 5.6. In Table 5.7, the extent to which baseline levels of the haemostatic factors might be related to cardiovascular risk factors was examined univariately by calculating partial correlation coefficients. These were adjusted for age and sex because of possible confounding. Transformed variables were used where appropriate. There were no significant correlations between fibrinogen and any of the cardiovascular risk factors, including cigarette smoking. von Willebrand Factor was inversely related to levels of total cholesterol ($r=-0.09$, $p< 0.05$). Significant associations were found between t-PA and packyears, systolic blood pressure, diastolic blood pressure and total cholesterol, but not random glucose levels. Fibrin D-dimer was significantly correlated with both measures of blood pressure (systolic, $r=0.10$, $p\leq 0.05$; diastolic, $r=0.11$, $p\leq 0.01$).

In Table 5.8, univariate relationships between haemostatic factors and ABPI at baseline adjusted for age and sex are presented. Calculation of partial correlation coefficients showed that all the factors were significantly related to increasing severity of disease. The strongest

Table 5.1

AGE, SEX AND CLINICAL CHARACTERISTICS OF STUDY SAMPLE AT BASELINE

		Study Sample (n=607)
Age mean years (SE)		65.5 (0.4)
Sex % Male		64.1
% Female		35.9
Intermittent Claudication :	Grade I %	47.1
	Grade II %	52.5
Ankle Brachial Pressure Index mean (SE)		0.57 (0.01)

SE - Standard error

Table 5.2

MEAN CARDIOVASCULAR RISK FACTOR LEVELS OF STUDY SAMPLE AT BASELINE

Factor	Study Sample (n=607)
Smoking Status	
Current %	38.2
Ex %	48.9
Never %	8.7
Packyears (√) mean (SE)	4.8 (0.12)
Blood Pressure (mmHg)	
Systolic Pressure mean mmHg (SE)	152.4 (1.0)
Diastolic Pressure mean mmHg (SE)	83.9 (0.5)
Total Cholesterol mean mmol/L (SE)	6.7 (0.1)
Random Glucose mean mmol/L (SE)	6.0 (0.1)

SE - Standard error

Table 5.3

AGE AND CLINICAL CHARACTERISTICS OF STUDY SAMPLE BY SEX AT BASELINE

Factor	Males (n=389)	Females (n=218)	P-Value
Age mean years (SE)	64.8 (0.45)	66.7 (0.73)	<0.05
Peripheral Arterial Disease			
Intermittent Claudication : Grade I (%)	45.8	50.0	
Grade II (%)	54.3	50.0	NS
Ankle Brachial Pressure Index mean (SE)	0.57 (0.01)	0.56 (0.01)	NS

NS - Non-significant

Table 5.4

MEAN CARDIOVASCULAR RISK FACTOR LEVELS BY SEX IN STUDY SAMPLE AT BASELINE

Factor	Males (n=389)	Females (n=218)	P-Value
Smoking Status			
Current %	40.7	38.6	
Ex %	54.4	45.1	≤0.001
Never %	4.9	16.3	
Packyears (√) mean (SE)	5.2 (0.2)	4.1(0.2)	≤0.001
Blood Pressure			
Systolic Pressure mean mmHg (SE)	150.8 (1.2)	155.2 (1.83)	≤0.05
Diastolic Pressure mean mmHg (SE)	84.5 (0.6)	82.8 (0.81)	NS
Total Cholesterol mean mmol/L (SE)	6.4 (0.07)	7.1 (0.09)	≤0.001
Random Glucose mean mmol/L (SE)	5.9 (0.10)	6.2 (0.18)	NS

NS - Non-significant

Table 5.5

PARTIAL CORRELATION COEFFICIENTS ADJUSTED FOR AGE AND SEX BETWEEN CARDIOVASCULAR RISK FACTORS AND ANKLE BRACHIAL PRESSURE INDEX AT BASELINE

Factor	ABPI
Packyears (√)	-0.07
Systolic Blood Pressure	-0.21***
Diastolic Blood Pressure	0.02
Total Cholesterol	0.02
Random Glucose	-0.04

*** $p<0.001$

Figure 12: Frequency Distribution of Fibrinogen

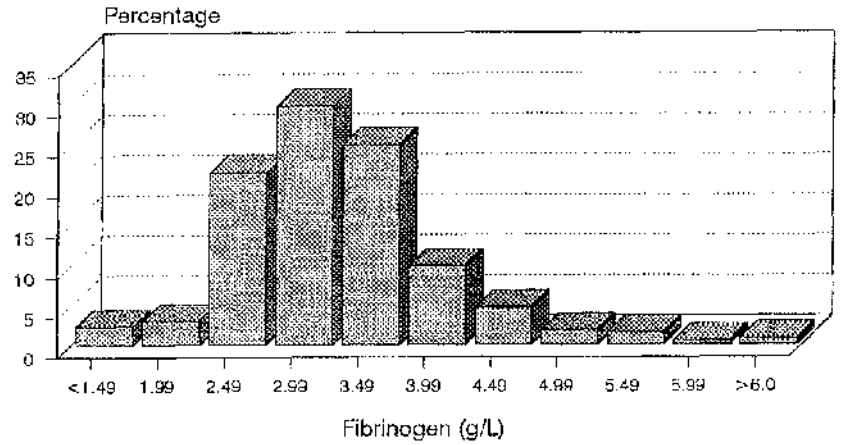


Figure 13: Frequency Distribution of von Willebrand Factor

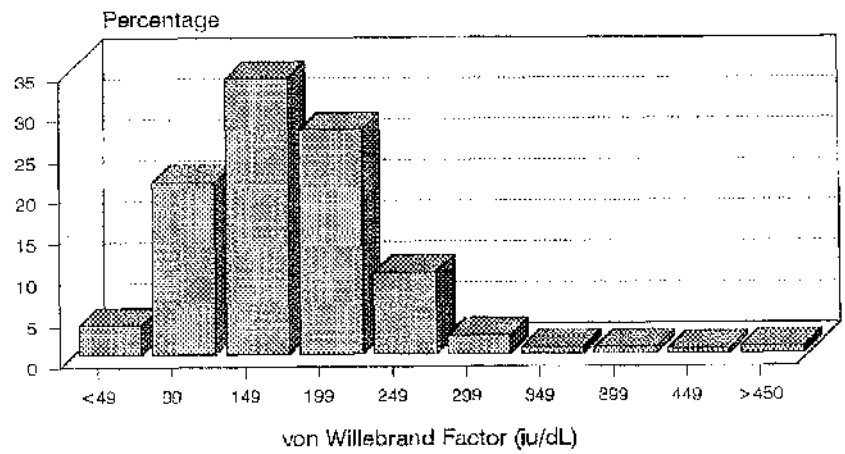


Figure 14: Frequency Distribution of Tissue Plasminogen Activator

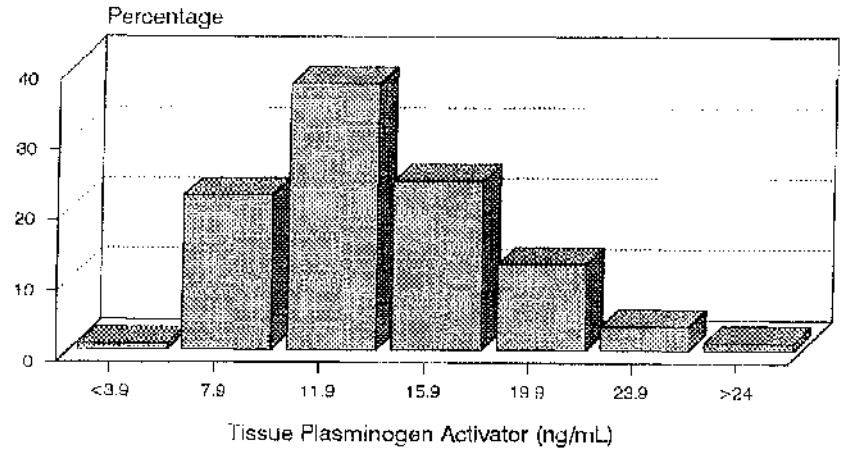


Figure 15: Frequency Distribution of Fibrin D-dimer

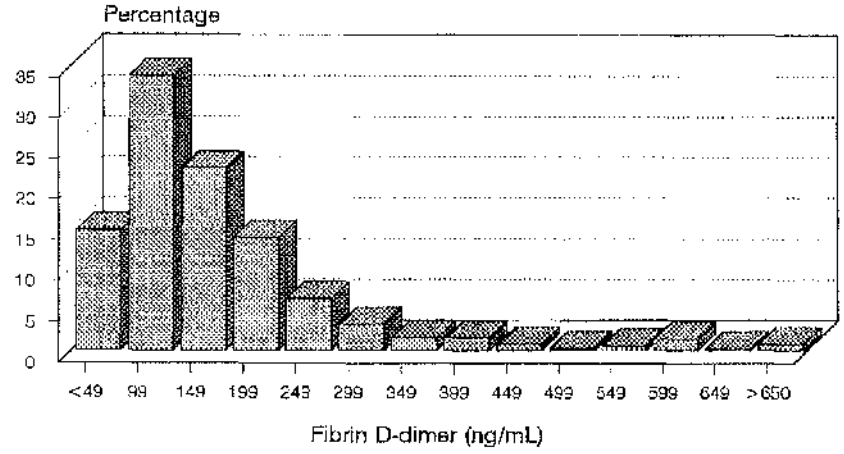


Table 5.6

MEDIANS (INTERQUARTILE RANGES) OF HAEMOSTATIC FACTORS IN STUDY SAMPLE AT BASELINE

Haemostatic Factor	Study Sample (n=607)
Fibrinogen (g/L)	2.89 (2.49,3.37)
von Willebrand Factor (iu/dL)	138.0 (105.0,179.0)
Tissue Plasminogen Activator (ng/mL)	11.0 (8.2,13.6)
Fibrin D-dimer (ng/mL)	104.0 (71.0,167.0)

Table 5.7

PARTIAL CORRELATION COEFFICIENTS ADJUSTED FOR AGE AND SEX BETWEEN HAEMOSTATIC FACTORS AND CARDIOVASCULAR RISK FACTORS AT BASELINE

Haemostatic Factor	Cardiovascular Risk Factor				
	Packyears (√)	Systolic BP	Diastolic BP	Total Cholesterol	Random Glucose
Fibrinogen (√)	0.05	0.04	0.02	-0.01	0.02
von Willebrand Factor (√)	-0.03	-0.01	0.00	-0.09*	0.06
Tissue Plasminogen Activator (log)	0.10*	0.11*	0.12**	0.10*	0.02
Fibrin D-dimer (log)	0.01	0.10*	0.11**	-0.04	-0.06

* $p \leq 0.05$; ** $p \leq 0.01$
BP - Blood Pressure; log - logarithm

Table 5.8

PARTIAL CORRELATION COEFFICIENTS ADJUSTED FOR AGE AND SEX BETWEEN HAEMOSTATIC FACTORS AND ANKLE BRACHIAL PRESSURE INDEX AT BASELINE

Haemostatic Factor	ABPI
Fibrinogen (√)	-0.08*
von Willebrand Factor (√)	-0.09*
Tissue Plasminogen Activator (log)	-0.18***
Fibrin D-dimer (log)	-0.14***

* $p \leq 0.05$; *** $p \leq 0.001$
log - logarithm

inverse correlations with ABPI were found with factors associated with fibrin breakdown, t-PA ($r=-0.18$, $p \leq 0.001$) and fibrin D-dimer ($r=-0.14$, $p \leq 0.001$). Weak correlations were also shown between fibrinogen and ABPI ($r=-0.08$) and between vWF and ABPI ($r=-0.09$). Both associations were significant at the 5% level.

5.2 Incidence of Vascular Events

5.2.1 Fatal events by sex

A total of 210 patients (34.6% of the study sample) died during the six year follow-up period. The cause of death could not be confirmed in two patients and these patients were therefore not included in any subsequent analysis. The mortality rate in males for all causes of death was higher than in females, with the exception of stroke. In total, 39.8% of males in the study sample died compared to 24.2% of the females. Figure 16 shows the survival curves for men and women separately. A comparison of the survival experience between the sexes was highly statistically significant (Wilcoxon statistic 17.47 with one degree of freedom, $p \leq 0.0001$), with the men dying at a faster rate than the women.

Causes of death in males and females during the six years of follow-up are shown in Table 5.9. Results are expressed as number of events and as the percentage of total number of males and females in the study sample. Mortality was primarily due to vascular disease in both sexes. The most common cause of death was due to ischaemic heart disease and a higher mortality rate was found overall in males compared to females. A total of 64 subjects died from a myocardial infarction. Of these, 48 (12.3%) were male and 16 (7.3%) were female. There

were a further 26 deaths due to ischaemic heart disease other than myocardial infarction (n=19, 4.9% males and n=7, 3.2% females). These included deaths due to coronary atherosclerosis. In contrast, the mortality rate from stroke was almost identical in males (n=19, 4.9%), compared to females (n=10, 4.6%). It should be noted that stroke cases were not differentiated by ischaemic or haemorrhagic origin because of the small number on whom computed tomography (CT) scans or necropsy were performed. An additional 27 patients died from vascular causes other than ischaemic heart disease or stroke, for example, ruptured aortic aneurysm. There were 62 deaths due to non-cardiovascular causes and 31 of these were from cancer. There was also a predominence of male deaths in this category, 48 (12.3%) compared to 14 (6.4%) which were female.

5.2.2 Non-fatal events by sex

The number of non-fatal vascular events occurring during the six year follow-up period for each subject is shown in Table 5.10 and is illustrated in Figure 17. Two hundred and three subjects (33.4% of the total sample) did not have a new vascular event and their limb ischaemia did not deteriorate during follow-up. Almost half of the subjects in the study (47.6%) had at least one event during the six years. The maximum number of confirmed events was nine, which was recorded by one patient.

The number of incident non-fatal events due to ischaemic heart disease, and cerebrovascular disease in males and females is shown in Table 5.11. During the six years follow-up period, a total of 56 subjects (9.2%) developed new symptoms of angina pectoris. This comprised 38 males (equivalent to 9.8% of the total number of males in the study sample) and 18, 8.3%

of the study sample of females. Ninety three subjects had a non-fatal myocardial infarction; 65 (16.7%) men and 28 (12.8%) women.

The incidence of self-reported or clinically diagnosed transient ischaemic attacks was higher than the incidence of hospital-diagnosed strokes in men. Overall, men developed slightly more cerebrovascular disease events than women. Thirty four men (8.7%) reported that they had had a transient ischaemic attack compared to 13 (6.0%) women during the six years of follow-up. In contrast, out of a total of 61 non-fatal strokes occurring during follow-up, 40 (10.3%) were male and 21 (9.6%) were female.

5.2.3 Progression of peripheral arterial disease by sex

The study subjects were also followed up over the six year period to determine the incidence of severe chronic leg ischaemia (rest pain, ulceration, gangrene and amputation), and peripheral vascular procedures, including angiography, angioplasty, lumbar sympathectomy and by-pass grafting. Events related to the progression of peripheral arterial disease by gender are shown in Table 5.12.

A total of 45 subjects (7.4%) underwent hospital investigations for treatment for peripheral arterial disease; 19 of these had femoral angiography; 9 femoral angioplasty; 1 iliac angiography; 6 iliac angioplasty; 1 iliac embolectomy; 4 aortic angiography and 1 subject underwent a lumbar sympathectomy. Three patients had femoral by-pass grafting and one patient aortic by-pass grafting. Although information was not collected on the particular leg requiring intervention, it is probable that vascular investigations were mostly performed on

the leg which had the lower ABPI at baseline. None of these patients progressed to severe chronic leg ischaemia or required leg amputation during the follow-up period. Eight of these patients died during follow-up, five from vascular causes. Thirty five men (9.0% of the study sample) required vascular intervention compared to ten women (4.6%) during the six years.

The worst outcome in patients who developed severe chronic leg ischaemia during the six years was also recorded. Sixty four patients (10.5%) progressed to symptoms of severe chronic leg ischaemia; 20 developed rest pain only; 30 further progressed to leg ulceration, and 13 to gangrene. One patient had a below knee amputation because of severe peripheral arterial disease. This event was reported separately because there was no evidence in the hospital case notes of any prior symptoms of severe chronic leg ischaemia. A further 18 patients within this disease category subsequently required leg amputation and 32 died, 27 from vascular causes (data not shown).

Table 5.12 shows that, in contrast to the vascular intervention group, in which the ratio of males to females undergoing treatment was approximately 2:1, slightly more women than men (other than the group who developed leg ulceration) showed deterioration to severe chronic leg ischaemia in the study sample. The percentage of men who developed ulceration of the lower limb was approximately the same in men (5.4%) and women (4.8%). Given that baseline levels of ABPI were similar between the sexes, these results suggest that the rate of deterioration of symptomatic peripheral arterial disease may be slightly faster in women than in men, although these results are based on a relatively low number of events.

Figure 16 : Survival Curves for Men and Women

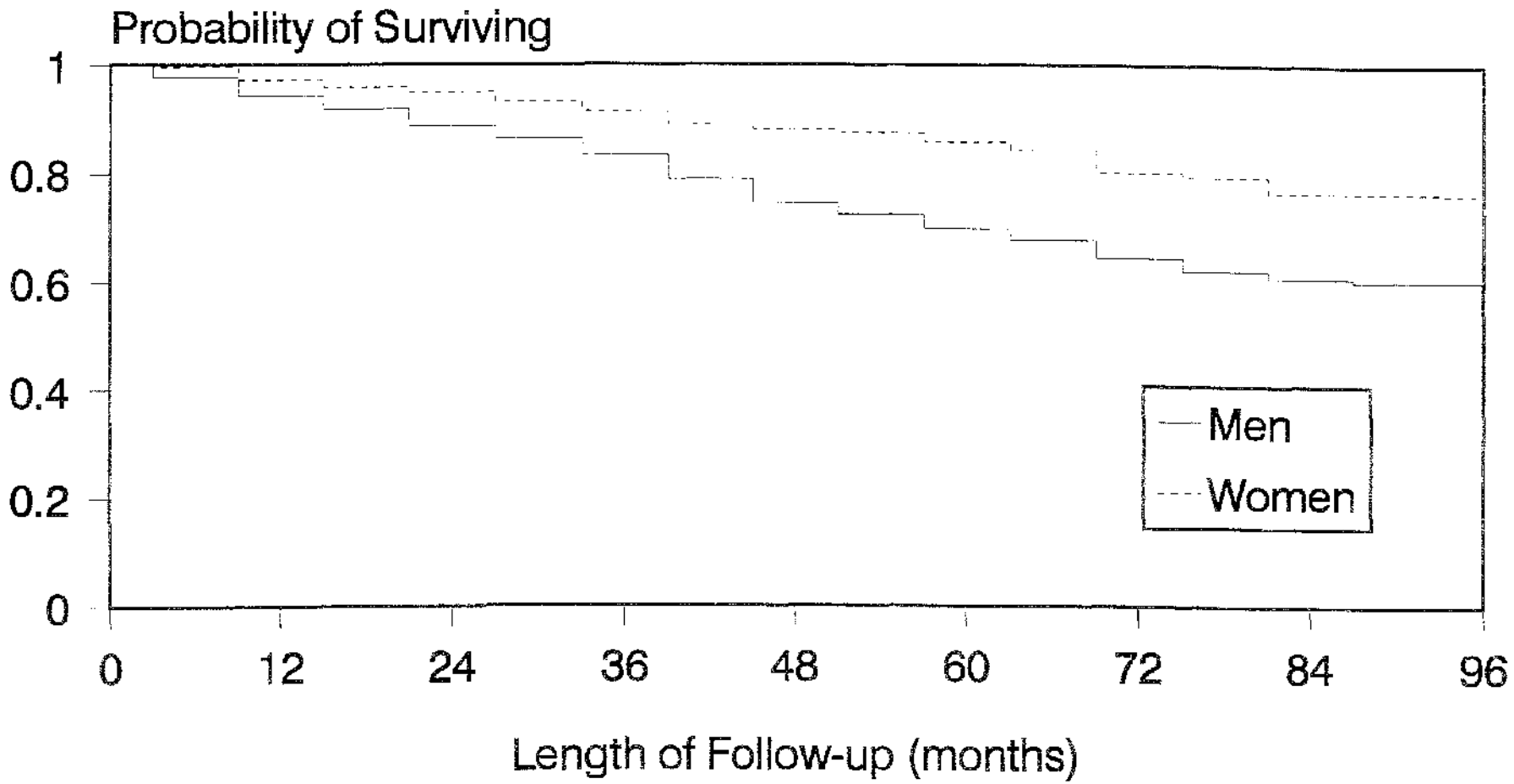


Figure 17: Distribution of Incident Non-fatal Vascular Events in Study Sample

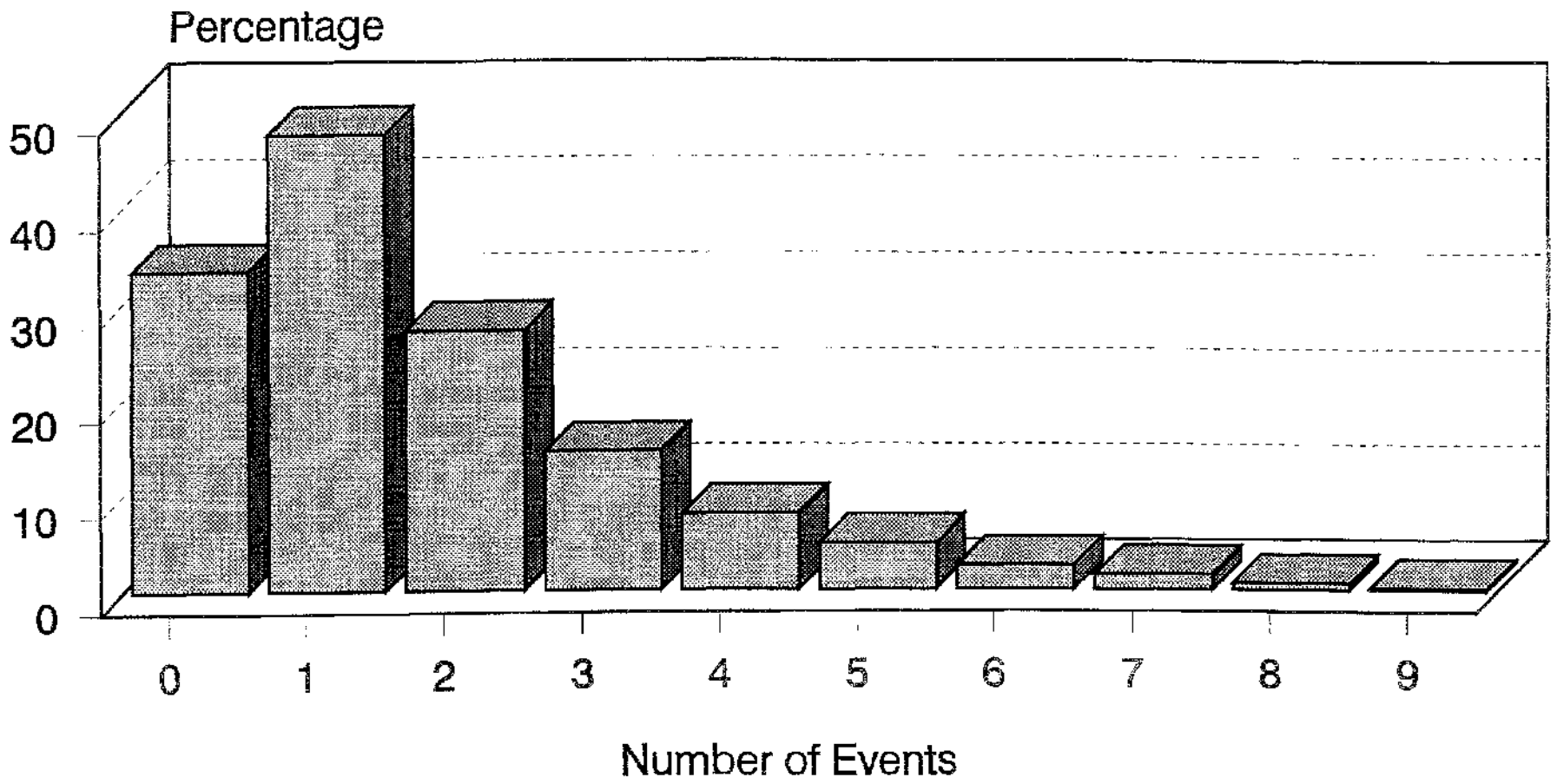


Table 5.9

CAUSES OF MORTALITY IN STUDY SAMPLE BY SEX

	Males		Females	
Cause of Mortality	Number	% Study Sample	Number	% Study Sample
Myocardial Infarction	48	12.3	16	7.3
Other Ischaemic Heart Disease	19	4.9	7	3.2
Stroke	19	4.9	10	4.6
Other Vascular Causes	21	5.4	6	2.8
Non Vascular Causes	48	12.3	14	6.4

Table 5.10

DISTRIBUTION OF INCIDENT NON-FATAL VASCULAR EVENTS IN STUDY SAMPLE

Number of Events	Number of Patients	% Study Sample
0	203	33.4
1	289	47.6
2	165	27.2
3	88	14.5
4	48	7.9
5	29	4.8
6	15	2.5
7	10	1.6
8	4	0.7
9	1	0.2

Table 5.11

INCIDENCE OF NON-FATAL ISCHAEMIC HEART DISEASE, TRANSIENT ISCHAEMIC ATTACKS AND STROKE EVENTS IN STUDY SAMPLE BY SEX

	Males		Females	
Vascular Event	Number	% Study Sample	Number	% Study Sample
Angina Pectoris	38	12.3	18	8.3
Myocardial Infarction	65	16.7	28	12.8
Transient Ischaemic Attack	34	8.7	13	6.0
Stroke	40	10.3	21	9.6

Table 5.12

INCIDENCE OF VASCULAR INTERVENTION AND SEVERE CHRONIC LEG ISCHAEMIA IN STUDY SAMPLE BY SEX

	Males		Females	
PAD Progression	Number	% Study Sample	Number	% Study Sample
Vascular Intervention	35	9.0	10	4.6
Rest Pain	10	2.6	10	4.6
Ulcer	21	5.4	9	4.8
Gangrene	6	1.5	7	3.2
Amputation	1	0.3	0	0

PAD - Peripheral Arterial Disease

5.2.4 Univariate analysis of baseline severity of peripheral arterial disease and incident ischaemic heart disease and stroke events

The univariate relationship between the severity of baseline peripheral arterial disease (ABPI), and the relationship between severity of symptoms of intermittent claudication to ischaemic heart disease and stroke events are shown in Table 5.13. Four categories of vascular events were defined: combined fatal and non-fatal stroke (n=79), non-fatal myocardial infarction (n=93), fatal ischaemic heart disease (fatal myocardial infarction and deaths due to other ischaemic heart disease) (n=90) and total ischaemic heart disease events, which included fatal and non-fatal myocardial infarction and deaths due to ischaemic heart disease (n=160). Student's t-test, or chi-square analysis were used to test for differences across the categories of disease relative to a group who had no new vascular event or deterioration of limb ischaemia during follow-up (n=203).

The mean baseline level of ABPI in claudicants who had no vascular event during follow-up was 0.59 (Table 5.13). In comparison with the no event group, the mean ABPI was significantly lower across all categories of disease with the exception of the non-fatal myocardial infarction group. The lowest ABPI (0.51) was recorded in those who subsequently suffered a stroke ($p \leq 0.01$) or who died from ischaemic heart disease ($p \leq 0.001$).

The relationship of severity of self-reported symptoms of intermittent claudication to incident ischaemic heart disease and stroke events is also presented in Table 5.13. In the no event group, the proportion of those who reported moderate grade I symptoms (49.8%) was similar to those who had more severe grade II symptoms of intermittent claudication (50.2%).

Although the stroke group had recorded the lowest mean ABPI, these subjects reported significantly less severe symptoms of claudication than the no event group (55.7% grade I, 44.3% grade II; $p \leq 0.05$). In contrast, all categories of patients who developed ischaemic heart disease had more severe symptoms of intermittent claudication at baseline than the comparison group, although the differences were non-significant.

5.2.5 Univariate analysis of age, sex, cardiovascular risk factors and incident ischaemic heart disease and stroke events

Table 5.14 shows mean age, percentages of males and mean levels of cardiovascular risk factors across the categories of events. Clustering of elevated risk factors occurred particularly in the stroke and fatal ischaemic heart disease categories. For example, in those who subsequently had a fatal or non-fatal stroke, baseline levels of both systolic (163.45 mmHg, $p \leq 0.001$) and diastolic pressure (87.24 mmHg, $p \leq 0.01$) were significantly higher than in the no event group and were also the highest recorded in any event category. Life-time cigarette consumption (packyears) and total cholesterol were also highest in this category of disease, but the elevations in the levels were not statistically significant in relation to those who experienced no events. The mean age (70.82 years, $p \leq 0.001$), the percentage of males (74.4%, $p \leq 0.001$) and random glucose level (6.86 mmol/L, $p \leq 0.001$) in the group who died of ischaemic heart disease during follow-up were significantly elevated compared to levels in the no event group, and were also higher than in the other event groups. Conversely, baseline levels of total cholesterol and packyears in the fatal ischaemic heart disease group were lowest compared to other categories of disease and the no event group, but this may have reflected more active intervention in this group which had more evidence of baseline ischaemic heart

disease than the other disease categories (data not shown).

5.2.6 Multivariate analysis of cardiovascular risk factors, ABPI and incident ischaemic heart disease and stroke events

Table 5.15 shows the multivariate relationship between baseline cardiovascular risk factors and between ABPI and subsequent risk of ischaemic heart disease and stroke throughout the six year follow-up period. As expected, given the strength of the univariate association, the risk of stroke associated with a standard deviation increase in systolic blood pressure was statistically significant, after adjusting for age and sex (RR 1.57, 95 % CI 1.23, 2.01 $p \leq 0.001$). The relative risk of stroke associated with a unit increase in random glucose levels was also substantially raised (RR 2.65, 95% CI 1.35, 5.18 $p < 0.01$). For a standard deviation increase in ABPI (or decreased severity of lower limb disease), the relative risk related to stroke was significantly less than one, suggesting that increasing severity of peripheral arterial disease may increase the risk of stroke.

With the exception of random glucose, there were no significant relationships between any of the cardiovascular risk factors and the risk of all ischaemic heart disease events, after adjustment for age and sex. Random glucose was significantly related to the risk of ischaemic heart disease, across all categories of events. The strongest association was between glucose levels and risk of myocardial infarction (RR 2.22, 95% CI 1.24, 3.95 $p < 0.01$). A higher ABPI was associated with a significant decrease in the risk of total ischaemic heart disease events (RR 0.83, 95% CI 0.69, 0.99 $p \leq 0.05$).

5.3 Haemostatic Factors

5.3.1 Univariate analysis of haemostatic factors and incident ischaemic heart disease and stroke events

Median levels and inter-quartile ranges for each haemostatic factor across the four categories of ischaemic heart disease and stroke are presented in Table 5.16. Differences in medians across the categories of disease relative to the group who had no coronary or cerebrovascular events and no deterioration of limb ischaemia during follow-up were tested by the Kolmogorov-Smirnov test.

The results show that in those who subsequently died of ischaemic heart disease, baseline levels of fibrinogen ($p \leq 0.01$), von Willebrand Factor ($p \leq 0.05$) and fibrin D-dimer ($p \leq 0.001$) were significantly raised compared to levels in the no event group. The levels of these haemostatic factors were also the highest recorded in any disease category. Fibrin D-dimer levels were also markedly elevated in the other ischaemic heart disease groups. In those who suffered a stroke during the follow-up period, only t-PA antigen levels showed any significant elevation at baseline (12.0 ng/mL, $p \leq 0.01$) compared to those who experienced no events (10.3 ng/mL).

5.3.2 Multivariate analysis of haemostatic factors and incident ischaemic heart disease and stroke events

Relative risks of each event group for a unit increase in each haemostatic factor level were

estimated by multiple logistic regression models that adjusted for age and sex, and then further adjusted for life-time cigarette smoking (packyears), systolic blood pressure, glucose and baseline evidence of ischaemic heart disease (angina and/or myocardial infarction). Baseline angina and myocardial infarction were defined according to a positive response to the WHO questionnaire and recall of a doctor's diagnosis. Relative risks were calculated using the statistical package, SAS.

Table 5.17 shows that fibrinogen was significantly related to total ischaemic heart disease events, after adjusting for age and sex ($p \leq 0.05$). There were no significant associations between vWF and the risk of any vascular event, after adjusting for age and sex. The relative risk of stroke, associated with a unit increase in t-PA antigen was statistically significant after adjusting for age and sex, (RR 1.87, 95% CI 1.04, 3.34 $p \leq 0.05$), but not after other cardiovascular risk factors and baseline evidence of ischaemic heart disease were taken into account. The risk, however, on multivariate analysis remained substantially greater than one (RR 1.60, 95% CI 0.88, 2.92 $p > 0.05$).

The relationship between fibrin D-dimer and non-fatal myocardial infarction, and between this factor and total ischaemic heart disease events also appeared to be partly attributable to the confounding effects of the risk factors and baseline ischaemic heart disease. The magnitude of the relative risk of myocardial infarction was reduced on multiple adjustment from 1.50, (95% CI 1.09, 2.06 $p \leq 0.01$) to 1.37 (95% CI 0.97, 1.92 $p > 0.05$). Similarly, the relative risk of total ischaemic heart disease events associated with fibrin D-dimer dropped from 1.37 to 1.26, $p > 0.05$, when all factors were taken into account.

Figure 18 shows the relative risk for combined fatal and non-fatal stroke across tertiles of t-PA antigen after adjusting for all risk factors. Subjects with values of t-PA antigen in the 2nd and 3rd tertiles had increased relative risks of 1.22 (95% CI 0.63, 2.35) and 1.79 (95% CI 0.99, 3.26) respectively when compared with those in the lowest tertile. A similar pattern for the relative risks of total ischaemic heart disease events according to tertile of fibrin D-dimer level is shown in Figure 19. The highest relative risk was found in the top tertile of fibrin D-dimer (RR 1.46, 95% CI 0.93, 2.29). The relative risk of subjects in the 2nd tertile of fibrin D-dimer, however, was similar to that in the lowest tertile (RR 1.03, 95% CI 0.65, 1.63).

The extent to which the associations between each factor and ischaemic heart disease and stroke events could be explained by interactions with other components of the haemostatic system was also estimated (Table 5.18). The relative risks of each disease category were recalculated in the multiple logistic regression model for each haemostatic factor, adjusting for all the haemostatic factors simultaneously, as well as age and sex. After further adjustment for the effects of the other haemostatic factors, the relative risk of every category of ischaemic heart disease and also stroke for each haemostatic factor was reduced and most became non-significant. However, fibrin D-dimer remained independently associated at the 5% level of significance to the risk of non-fatal myocardial infarction.

5.4 Progression of Peripheral Arterial Disease

For this part of the analyses, two groups of patients were identified: those whose symptoms had deteriorated sufficiently to require vascular intervention, but did not develop severe chronic leg ischaemia during follow-up (n=45), and secondly, a group comprising those who

developed definite symptoms of severe chronic leg ischaemia, defined as rest pain, ulceration or gangrene during the six years (n=64).

5.4.1 Univariate analysis of baseline severity of peripheral arterial disease and progression of disease

Mean levels of the ABPI and the percentages of grade I and grade II symptoms of intermittent claudication at baseline were calculated for each of the two follow-up categories of peripheral arterial disease, and compared with the group who had no cerebrovascular or coronary events, or deterioration of limb ischaemia during follow-up. Table 5.19 shows that the mean ABPI of those who underwent vascular intervention was slightly higher than the no event group, but the difference was not statistically significant (0.62 compared to 0.59, $p > 0.05$). As expected, the group who developed more severe symptoms of limb ischaemia had a significantly lower ABPI at baseline ($p \leq 0.001$) than those who did not deteriorate, or than those who had required vascular intervention. Conversely, the vascular intervention group reported a far higher percentage of more severe grade II symptoms of intermittent claudication at baseline, according to responses to the WHO claudication questionnaire than the no event group (70.5% compared to 50.2%, $p \leq 0.05$) and also than the group which developed severe chronic leg ischaemia (53.1%).

5.4.2 Univariate analysis of cardiovascular risk factors and progression of disease

Baseline characteristics of the vascular intervention group and those patients who developed severe chronic leg ischaemia compared to those who experienced no vascular events are shown

Table 5.13

RELATIONSHIP OF BASELINE MEASURES OF SEVERITY OF PERIPHERAL ARTERIAL DISEASE TO INCIDENT ISCHAEMIC HEART DISEASE AND STROKE EVENTS IN STUDY SAMPLE

			Ischaemic Heart Disease		
Measure of Peripheral Arterial Disease	No Vascular Event (n=203)	Stroke (n=79)	Non-Fatal MI (n=93)	Fatal IHD (n=90)	Total IHD Events (n=160)
Ankle Brachial Pressure Index mean (SE)	0.59 (0.01)	0.51 (0.02)**	0.55 (0.02)	0.51 (0.02)***	0.53 (0.01)***
Intermittent Claudication :					
Grade I (%)	49.8	55.7 } *	45.2	44.9	42.8
Grade II (%)	50.2	44.3 }	54.8	55.1	57.2

MI - Myocardial Infarction; IHD - Ischaemic Heart Disease
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Table 5.14

AGE, SEX AND MEAN LEVELS OF CARDIOVASCULAR RISK FACTORS AT BASELINE BY CATEGORY OF ISCHAEMIC HEART DISEASE AND STROKE EVENTS

Risk Factor	No Vascular Event (n=203)	Stroke (n=79)	Ischaemic Heart Disease: Non-Fatal MI (n=93)	Ischaemic Heart Disease: Fatal IHD (n=90)	Ischaemic Heart Disease: Total IHD Events (n=160)
Age (years)	65.08 (0.65)	66.53 (0.90)	64.56 (1.22)	70.82 (0.74)***	67.04 (0.83)
Sex (% male)	49.5	65.0*	69.9**	74.4***	71.9***
Total Cholesterol (mmol/L)	6.82 (0.92)	6.92 (0.18)	6.72 (0.14)	6.44 (0.13)*	6.57 (0.10)
Systolic Blood Pressure (mmHg)	150.41 (1.75)	163.45 (2.74)***	153.14 (2.73)	159.61 (2.86)**	156.04 (2.03)*
Diastolic Blood Pressure (mmHg)	82.91 (0.85)	87.24 (1.40)**	84.23 (1.25)	85.34 (1.42)	84.91 (0.96)
Random Glucose (mmol/L)	5.62 (0.10)	6.57 (0.33)**	6.27 (0.26)*	6.86 (0.40)***	6.66 (0.26)***
Packyears ($\sqrt{}$)	4.66 (0.20)	5.38 (0.34)	4.79 (0.31)	4.15 (0.34)	4.57 (0.25)

All values other than sex (%) are mean (standard error)
MI - Myocardial Infarction: IHD - Ischaemic Heart Disease
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p < 0.001$

Table 5.15

RELATIVE RISKS (95% CI) OF VASCULAR EVENTS FOR UNIT INCREASE IN CARDIOVASCULAR RISK FACTORS ADJUSTED FOR AGE AND SEX

Unit Increase in Risk Factor†	Stroke (n=79)	Ischaemic Heart Disease		
		Non-Fatal MI (n=93)	Fatal IHD (n=90)	Total IHD Events (n=160)
Total Cholesterol (+1.3 mmol/L)	1.15 (0.93,1.44)	1.03 (0.83,1.28)	0.99 (0.78,1.26)	0.98 (0.83,1.17)
Systolic Blood Pressure (+25.43 mmHg)	1.57 (1.23,2.01)***	1.14 (0.93,1.40)	1.18 (0.96,1.45)	1.15 (0.98,1.35)
Random Glucose (+1 ln mmol/L)	2.65 (1.35,5.18)**	2.17 (1.07,4.41)*	2.22 (1.24,3.95)**	1.91 (1.22,3.00)**
Packyears ($\sqrt{2.95}$)	1.18 (0.93,1.49)	0.97 (0.78,1.21)	0.85 (0.69,1.08)	0.95 (0.81,1.11)
Ankle Brachial Pressure Index (+0.2)	0.75 (0.59,0.95)*	0.84 (0.67,1.05)	0.81 (0.64,1.03)	0.83 (0.69,0.99)*

† - Unit increase in cardiovascular risk factors equates approximately with one standard deviation or one unit on a logarithmic scale
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Table 5.16

MEDIANS (INTERQUARTILE RANGES) OF HAEMOSTATIC FACTORS AT BASELINE BY SUBSEQUENT VASCULAR EVENT

Haemostatic Factor	No Vascular Event (n=203)	Stroke (n=79)	Ischaemic Heart Disease		
			Non-Fatal MI (n=93)	Fatal IHD (n=90)	Total IHD Events (n=160)
Fibrinogen (g/L)	2.74 (2.42,3.15)	2.89 (2.51,3.36)	2.92 (2.45,3.47)	3.08** (2.56,3.58)	3.02** (2.55,3.51)
von Willebrand Factor (iu/dL)	131.0 (106.0,165.0)	126.0 (99.0,184.0)	134.0 (95.5,187.5)	151.0* (112.0,209.0)	143.0 (99.0,189.5)
Tissue Plasminogen Activator (ng/mL)	10.3 (7.9,13.2)	12.0** (9.7,15.4)	11.1 (8.1,13.5)	11.1 (8.3,13.7)	11.1 (8.2,13.7)
Fibrin D-dimer (ng/mL)	96.0 (64.5,135.8)	104.0 (75.8,174.3)	115.0* (75.8,188.5)	142.5*** (88.8,212.3)	120.5*** (81.0,195.5)

MI - Myocardial Infarction; IHD - Ischaemic Heart Disease
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Table 5.17

RELATIVE RISKS (95% CI) OF VASCULAR EVENTS FOR UNIT INCREASE IN HAEMOSTATIC FACTORS ADJUSTING FOR CARDIOVASCULAR RISK FACTORS AND BASELINE IHD

Unit Increase in Haemostatic Factor†	Adjusted	Stroke (n=79)	Ischaemic Heart Disease: Non-Fatal MI (n=93)	Ischaemic Heart Disease: Fatal IHD (n=90)	Ischaemic Heart Disease: Total IHD Events (n=160)
Fibrinogen (+0.21 √g/L)	*a*	1.15 (0.91,1.47)	1.20 (0.99,1.46)	1.18 (0.98,1.43)	1.16 (1.01,1.34)*
	b	1.14 (0.89,1.45)	1.17 (0.95,1.43)	1.14 (0.94,1.39)	1.13 (0.97,1.31)
von Willebrand Factor (+2.59 √iu/dL)	*a*	0.98 (0.78,1.24)	1.09 (0.90,1.32)	1.05 (0.88,1.25)	1.07 (0.93,1.25)
	b	0.97 (0.78,1.21)	1.06 (0.88,1.29)	1.02 (0.85,1.22)	1.04 (0.90,1.19)
Tissue Plasminogen Activator (+1 ln ng/mL)	*a*	1.87 (1.04,3.34)*	1.01 (0.58,1.74)	1.03 (0.60,1.77)	1.06 (0.70,1.63)
	b	1.60 (0.88,2.92)	1.02 (0.58,1.78)	0.97 (0.55,1.71)	1.04 (0.67,1.60)
Fibrin D-dimer (+1 ln ng/mL)	*a*	1.21 (0.84,1.74)	1.50 (1.09,2.06)**	1.38 (0.97,1.97)	1.37 (1.06,1.76)*
	b	1.27 (0.86,1.85)	1.37 (0.97,1.92)	1.29 (0.89,1.86)	1.26 (0.97,1.65)

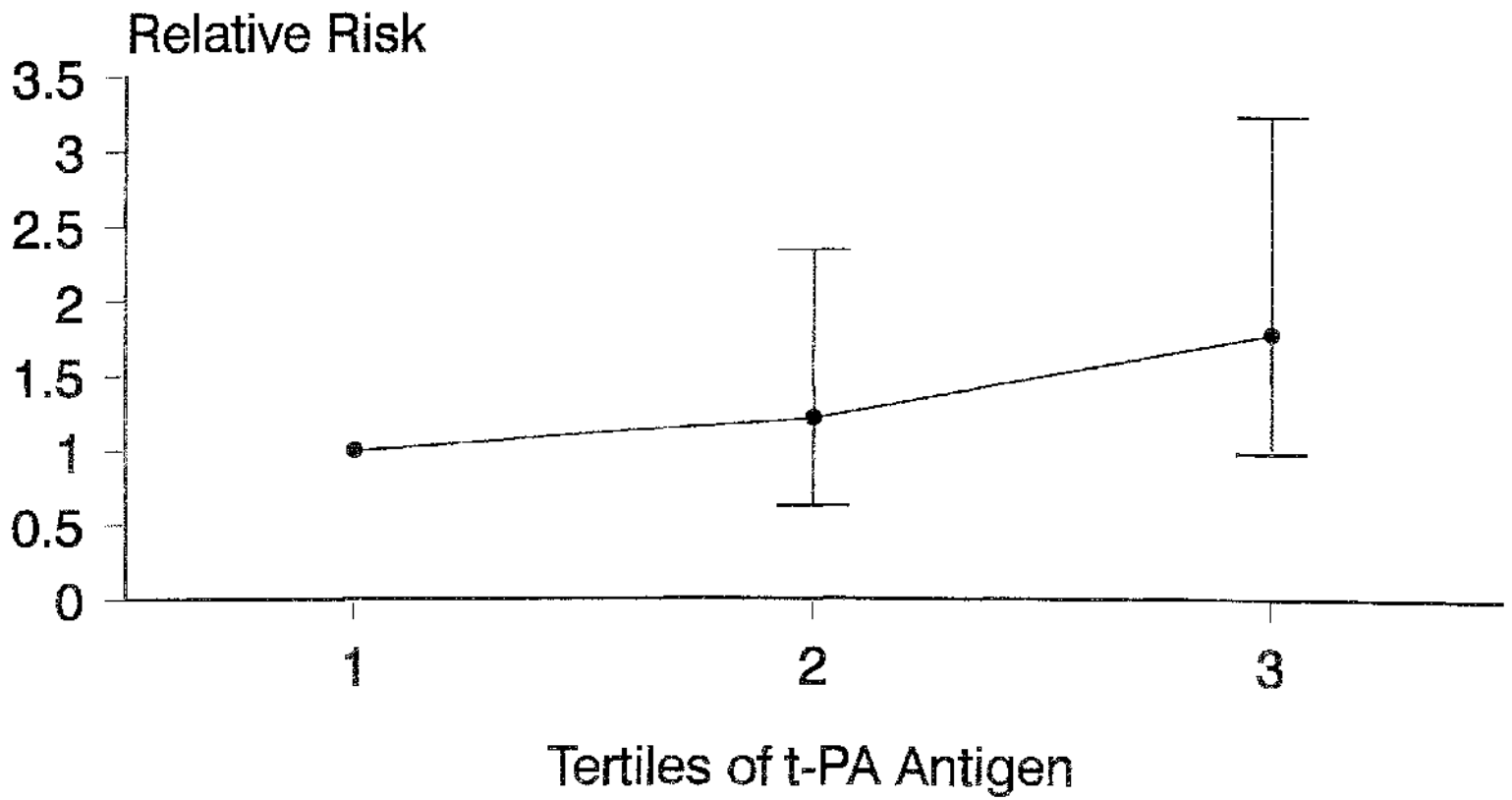
IHD - Ischaemic Heart Disease; MI - Myocardial Infarction

† - Unit increase in haemostatic factors equates approximately with one standard deviation or one unit on a logarithmic scale

a - Analyses adjusted for age and sex. *b* - Analyses adjusted for age, sex, cigarette smoking, systolic blood pressure, glucose, baseline IHD

* $p \leq 0.05$; ** $p \leq 0.001$

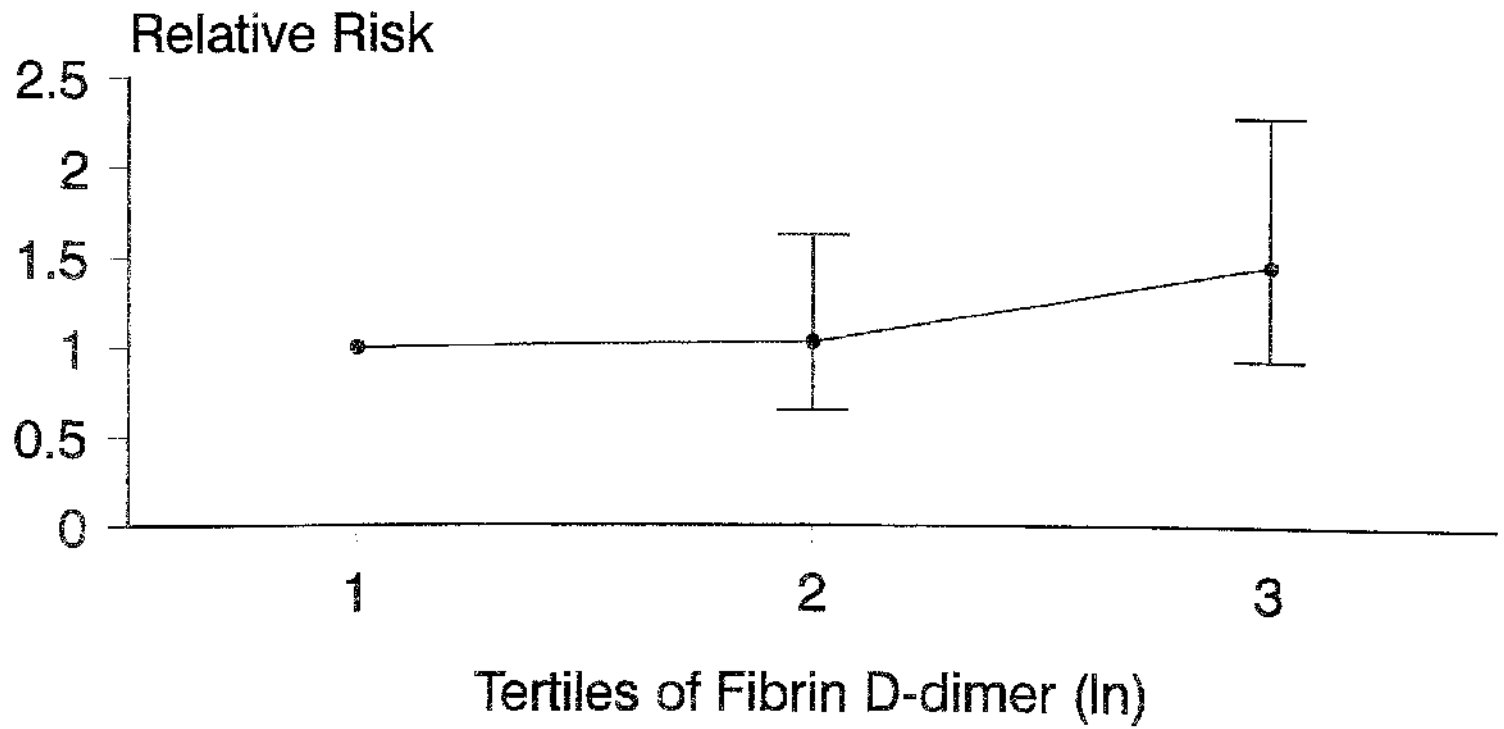
Figure 18: Relative Risks (95% CI) for Fatal and Non-fatal Stroke across Tertiles of t-PA Antigen



Figures adjusted for age, sex, cigarette smoking, systolic blood pressure, glucose and baseline IHD

Tertile cutpoints were <9.2ng/mL; 9.21 to 12.71ng/mL and >12.72ng/mL

Figure 19: Relative Risks (95% CI) for Total IHD Events across Tertiles of Fibrin D-dimer



Figures adjusted for age, sex, cigarette smoking, systolic blood pressure, glucose and baseline IHD

Tertile cutpoints were <4.39ng/mL; 4.40 to 4.92ng/mL and >4.93ng/mL

Table 5.18

RELATIVE RISKS (95% CI) OF VASCULAR EVENTS FOR UNIT INCREASE IN HAEMOSTATIC FACTORS ADJUSTING FOR AGE, SEX AND OTHER HAEMOSTATIC FACTORS

Unit Increase in Haemostatic Factor†	Adjusted	Stroke (n=79)	Ischaemic Heart Disease: Non-Fatal MI (n=93)	Ischaemic Heart Disease: Fatal IHD (n=90)	Ischaemic Heart Disease: Total IHD Events (n=160)
Fibrinogen	*a*	1.15 (0.91,1.47)	1.20 (0.99,1.46)	1.18 (0.98,1.43)	1.16 (1.01,1.34)*
(+0.21 √g/L)	*b*	1.12 (0.86,1.45)	1.10 (0.89,1.36)	1.13 (0.92,1.40)	1.10 (0.94,1.28)
von Willebrand Factor	*a*	0.98 (0.78,1.24)	1.09 (0.90,1.32)	1.05 (0.88,1.25)	1.07 (0.93,1.25)
(+2.59 √iu/dL)	*b*	0.92 (0.72,1.19)	1.08 (0.88,1.32)	1.01 (0.83,1.22)	1.04 (0.90,1.21)
Tissue Plasminogen	*a*	1.87 (1.04,3.34)*	1.01 (0.58,1.74)	1.03 (0.60,1.77)	1.06 (0.70,1.63)
Activator (+1 ln ng/mL)	*b*	1.77 (0.97,3.23)	0.93 (0.54,1.63)	0.94 (0.54,1.63)	0.99 (0.65,1.52)
Fibrin D-dimer	*a*	1.21 (0.84,1.74)	1.50 (1.09,2.06)**	1.38 (0.97,1.97)	1.37 (1.06,1.76)*
(+1 ln ng/mL)	*b*	1.03 (0.69,1.54)	1.43 (1.01,2.02)*	1.26 (0.86,1.85)	1.28 (0.98,1.69)

MI - Myocardial Infarction; IHD - Ischaemic Heart Disease

† - Unit increase in haemostatic factors equates approximately with one standard deviation or one unit on a logarithmic scale

a - Analyses adjusted for age and sex. *b* - Analyses adjusted for age, sex, and other haemostatic factors

* $p \leq 0.05$; ** $p \leq 0.01$

in Table 5.20. The vascular intervention group was significantly younger (61.9 years compared to 65.1 years, $p \leq 0.05$) and had a far higher proportion of males (77.8% compared to 49.5%, $p \leq 0.001$) than the no progression group. The severe chronic leg ischaemics had a lower mean level of total cholesterol at baseline than the group which had not deteriorated (6.3 mmol/L compared to 6.8mmol/L, $p \leq 0.001$), even although none were receiving lipid-lowering medication. There were no significant differences in life-time smoking consumption (packyears), random glucose or blood pressure levels between either of the two event groups compared with those who showed no progression, although the mean levels of these factors were slightly higher in the severe chronic leg ischaemia group. There was also a higher percentage of current smokers in this group. A history of smoking was particularly common among those proceeding to vascular intervention, in that only 2.2% were never smokers, although a relatively high percentage had given up (71% of the vascular intervention group).

5.4.3 Multivariate analysis of cardiovascular risk factors, ABPI and progression of disease

Relative risks of the two disease groups for a unit increase in each of the cardiovascular risk factors and ABPI were estimated by multiple logistic regression models which adjusted for age and sex. Table 5.21 shows that the risk of vascular intervention associated with a 1.3mmol/L increase in total cholesterol was substantially less than one (RR 0.70, 95% CI 0.52, 0.95 $p \leq 0.05$), suggesting that an elevated cholesterol level may *reduce* the risk of requiring vascular intervention. Random glucose was associated with an increased risk of vascular intervention ($p \leq 0.01$) but not severe chronic leg ischaemia. Neither cigarette smoking, systolic blood pressure nor the ABPI were independently associated with the risk of

intervention, after adjusting for age and sex. In contrast, only a standard deviation increase in the ABPI was significantly and inversely related to the risk of developing severe chronic leg ischaemia (RR 0.57, 95% CI 0.43, 0.76 p≤ 0.001).

5.5 Haemostatic Factors

5.5.1 Univariate analysis of haemostatic factors and progression of peripheral arterial disease

The median levels and interquartile ranges of each haemostatic factor across the two groups whose peripheral arterial disease had worsened, relative to those who showed no progression are shown in Table 5.22. Differences in medians of the haemostatic factors were tested by the Kolmogorov-Smirnov test across the two categories, relative to the group which showed no deterioration.

The results show that there were no significant differences in baseline levels of fibrinogen, vWF, fibrin D-dimer or t-PA antigen between the vascular intervention group and the comparison group. Levels of fibrinogen were slightly lower in the severe chronic leg ischaemia group than in the no progression group but not significantly so. vWF levels were significantly higher in patients who developed severe chronic leg ischaemia compared with the no event group (154.0 iu/dL compared to 131.0 iu/dL, p≤ 0.01) and were also higher than in the vascular intervention group (138.0 iu/dL).

5.5.2 Multivariate analysis of haemostatic factors and progression of peripheral arterial disease

Relative risks of the two disease groups for a unit increase in each of the haemostatic factors were estimated by multiple logistic regression models which adjusted for age and sex, and then further adjusted for life-time smoking consumption (packycars). The confounding effect of cigarette smoking on the risk of disease progression was examined separately from the other cardiovascular risk factors because smoking may strongly influence the relationship between certain haemostatic factors and peripheral arterial disease.

Table 5.23 shows that after adjustment for age and sex, there were no significant relationships between any of the haemostatic factors and the risk of vascular intervention. However, after further adjustment for cigarette smoking, the magnitude of risk of vascular intervention increased slightly for fibrinogen and became statistically significant (RR 1.33, 95% CI 1.00, 1.77 $p \leq 0.05$). Similarly, no haemostatic factor was independently related to the risk of severe chronic leg ischaemia on adjustment for age and sex, but when smoking history was taken into account, the relative risk of severe chronic leg ischaemia associated with a unit increase in vWF levels rose from 1.24 ($p > 0.05$) to 1.27 and became significant at the 5% level. However, the associations between the haemostatic factors and progression of peripheral arterial disease after adjustment for smoking were only marginally altered.

Further adjustment for the other cardiovascular risk factors on the risk of vascular intervention and severe chronic leg ischaemia associated with the haemostatic factors in the multiple logistic regression models is shown in Table 5.24. In comparison with adjustment for smoking

only, the magnitude of each of the risk estimates in the vascular intervention group, with the exception of fibrinogen increased slightly. None reached statistical significance, however.

In those with severe chronic leg ischaemia, adjusting for all cardiovascular risk factors increased the magnitude of risk associated with fibrinogen and this became statistically significant (RR 1.35, 95% CI 1.02, 1.79 $p \leq 0.05$). Further analysis showed that this effect probably reflected the negative age and sex adjusted correlations between fibrinogen and cholesterol (r=-0.1) and between fibrinogen and glucose (r=-0.05) (data not shown).

The influence of the other haemostatic factors on the relationships between each haemostatic factor and peripheral arterial disease progression was also estimated in the logistic regression models. No real effect was noted on the risk of vascular intervention on inclusion of all the factors, although the relative risk of intervention for each haemostatic factor was reduced marginally (Table 5.25). Similarly, inclusion of the haemostatic factors into the model had little impact on the magnitude of risk of severe chronic leg ischaemia. This resulted in a reduction of relative risk for all factors other than vWF. The relative risk for vWF rose slightly reflecting the negative correlation with fibrin D-dimer (r=-0.1) and the significance level also increased (RR 1.28, 95% CI 1.01, 1.64 $p \leq 0.05$).

Table 5.19

BASELINE MEASURES OF SEVERITY OF PERIPHERAL ARTERIAL DISEASE BY CATEGORY OF PERIPHERAL ARTERIAL DISEASE PROGRESSION

Measure of Peripheral Arterial Disease	No PAD Progression (n=203)	Vascular Intervention (n=45)	Severe Chronic Leg Ischaemia (n=64)
Ankle Brachial Pressure Index mean (SE)	0.59 (0.01)	0.62 (0.03)	0.48 (0.002)***
Intermittent Claudication :			
Grade I (%)	49.8	29.5 } *	46.9
Grade II (%)	50.2	70.5 } *	53.1

PAD - Peripheral Arterial Disease
* $p \leq 0.05$; *** $p \leq 0.001$

Table 5.20

AGE, SEX AND CARDIOVASCULAR RISK CHARACTERISTICS OF PATIENTS BY CATEGORY OF PERIPHERAL ARTERIAL DISEASE PROGRESSION

Factor	No PAD Progression (n=203)	Vascular Intervention (n=45)	Severe Chronic Leg Ischaemia (n=64)
Age (years)	65.1 (0.6)	61.9 (1.3)*	65.3 (1.0)
Sex (% Male)	49.5	77.8***	60.9
Total Cholesterol (mmol/L)	6.8 (0.1)	6.7 (0.2)	6.3 (0.2)***
Systolic Blood Pressure (mmHg)	150.4 (1.8)	147.4 (3.1)	149.2 (3.5)
Diastolic Blood Pressure (mmHg)	82.9 (0.8)	84.3 (1.7)	80.5 (1.5)
Random Glucose (mmol/L)	5.6 (0.1)	6.1 (0.2)	6.8 (0.4)
Packyears (√)	4.7 (0.2)	4.7 (0.4)	5.0 (0.3)
Smoking Status (%)			
Current	36.5	24.4	43.8
Ex	49.8	71.1	45.3
Never	10.8	2.2	6.3

Values are mean (SE) or %
PAD - Peripheral Arterial Disease
* $p \leq 0.05$; *** $p \leq 0.001$

Table 5.21

RELATIVE RISKS (95% CI) OF PERIPHERAL ARTERIAL DISEASE PROGRESSION FOR UNIT INCREASE IN CARDIOVASCULAR RISK FACTORS ADJUSTING FOR AGE AND SEX

Unit Increase in Risk Factor†	Vascular Intervention (n=45)	Severe Chronic Leg Ischaemia (n=64)
Total Cholesterol (+1.3 mmol/L)	0.70 (0.52,0.95)*	0.98 (0.72,1.34)
Systolic Blood Pressure (+25.43 mmHg)	0.99 (0.76,1.28)	1.10 (0.78,1.55)
Random Glucose (+1 ln mmol/L)	2.72 (1.35,5.47)**	2.47 (0,79,7.65)
Packyears (+√2.95)	1.08 (0.81,1.43)	0.91 (0.66,1.25)
Ankle Brachial Pressure Index (+0.2)	1.09 (0.79,1.50)	0.57 (0.43,0.76)***

† - Unit increase in risk factor equates approximately with one standard deviation increase or one unit on a logarithmic scale
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p < 0.001$

Table 5.22

MEDIANS (INTERQUARTILE RANGES) OF HAEMOSTATIC FACTORS AT BASELINE BY CATEGORY OF PERIPHERAL ARTERIAL DISEASE PROGRESSION

Haemostatic Factor	No PAD Progression (n=203)	Vascular Intervention (n=45)	Severe Chronic Leg Ischaemia (n=64)
Fibrinogen (g/L)	2.74 (2.42-3.15)	2.73 (2.46-3.42)	2.71 (2.36-3.26)
von Willebrand Factor (iu/dL)	131.0 (106.0-165.0)	138.0 (100.8-181.3)	154.0** (122.0-187.0)
Tissue Plasminogen Activator (ng/mL)	10.3 (7.9-13.2)	10.4 (8.4-13.2)	11.9 (9.2-13.6)
Fibrin D-dimer (ng/mL)	96.0 (64.5-135.8)	94.5 (57.0-156.8)	97.0 (69.0-168.0)

PAD - Peripheral Arterial Disease
** $p \leq 0.01$

Table 5.23

RELATIVE RISKS (95% CI) OF PERIPHERAL ARTERIAL DISEASE PROGRESSION FOR UNIT INCREASE IN HAEMOSTATIC FACTORS ADJUSTING FOR AGE, SEX AND CIGARETTE SMOKING

Unit Increase in Haemostatic Factor†	Adjusted	Vascular Intervention (n=45)	Severe Chronic Leg Ischaemia (n=64)
Fibrinogen (+0.21√g/L)	*a*	1.30 (0.98,1.74)	1.04 (0.80,1.34)
	b	1.33 (1.00,1.77)*	1.04 (0.80,1.35)
von Willebrand Factor (+2.59√iu/dL)	*a*	1.13 (0.86,1.48)	1.24 (0.98,1.57)
	b	1.14 (0.87,1.50)	1.27 (1.00,1.61)*
Tissue Plasminogen Activator (+1 ln ng/mL)	*a*	1.13 (0.50,2.58)	1.61 (0.82,3.15)
	b	1.20 (0.52,2.79)	1.56 (0.78,3.08)
Fibrin D-dimer (+1 ln ng/mL)	*a*	1.22 (0.74,2.02)	1.35 (0.92,1.98)
	b	1.24 (0.75,2.06)	1.37 (0.93,2.01)

† - Unit increase in haemostatic factors equates approximately with one standard deviation or one unit on a logarithmic scale

a - Analyses adjusted for age and sex; *b* - Analyses adjusted for age, sex and packyears

* - $p \leq 0.05$

Table 5.24

RELATIVE RISKS (95% CI) OF PERIPHERAL ARTERIAL DISEASE PROGRESSION FOR UNIT INCREASE IN HAEMOSTATIC FACTORS ADJUSTING FOR AGE, SEX, CARDIOVASCULAR RISK FACTORS AND BASELINE IHD

Unit Increase in Haemostatic Factor†	Adjusted	Vascular Intervention (n=45)	Severe Chronic Leg Ischaemia (n=64)
Fibrinogen	*a*	1.30 (0.98,1.74)	1.04 (0.80,1.34)
(+0.21√g/L)	*b*	1.05 (0.80,1.37)	1.35 (1.02,1.79)*
von Willebrand Factor	*a*	1.13 (0.86,1.48)	1.24 (0.98,1.57)
(+2.59√iu/dL)	*b*	1.21 (0.95,1.53)	1.10 (0.85,1.43)
Tissue Plasminogen	*a*	1.13 (0.50,2.58)	1.61 (0.82,3.15)
Activator (+1 ln ng/mL)	*b*	1.55 (0.75,3.77)	1.21 (0.50,2.91)
Fibrin D-dimer	*a*	1.22 (0.74,2.02)	1.35 (0.92,1.98)
(+1 ln ng/mL)	*b*	1.46 (0.99,2.16)	1.39 (0.82,2.37)

IHD - Ischaemic Heart Disease

† - Unit increase in haemostatic factors equates approximately with one standard deviation or one unit on a logarithmic scale

a - Analyses adjusted for age and sex; *b* - Analyses adjusted for age, sex, cigarette smoking, systolic blood pressure, glucose and baseline IHD

* - $p \leq 0.05$

Table 5.25

RELATIVE RISKS (95% CI) OF PERIPHERAL ARTERIAL DISEASE PROGRESSION FOR UNIT INCREASE IN HAEMOSTATIC FACTORS ADJUSTING FOR AGE, SEX AND OTHER HAEMOSTATIC FACTORS

Unit Increase in Haemostatic Factor†	Adjusted	Vascular Intervention (n=45)	Severe Chronic Leg Ischaemia (n=64)
Fibrinogen	*a*	1.30 (0.98,1.74)	1.04 (0.80,1.34)
(+0.21√g/L)	*b*	1.22 (0.86,1.74)	0.92 (0.69,1.23)
von Willebrand Factor	*a*	1.13 (0.86,1.48)	1.24 (0.98,1.57)
(+2.59√iu/dL)	*b*	1.12 (0.85,1.49)	1.28 (1.01,1.64)*
Tissue Plasminogen	*a*	1.13 (0.50,2.58)	1.61 (0.82,3.15)
Activator (+1 ln ng/mL)	*b*	1.07 (0.47,2.46)	1.34 (0.67,2.71)
Fibrin D-dimer	*a*	1.22 (0.74,2.02)	1.35 (0.92,1.98)
(+1 ln ng/mL)	*b*	1.06 (0.62,1.81)	1.27 (0.83,1.96)

† - Unit increase in haemostatic factors equates approximately with one standard deviation or one unit on a logarithmic scale

a - Analyses adjusted for age and sex; *b* - Analyses adjusted for age, sex and other haemostatic factors

* - $p \leq 0.05$

5.6 Summary of Results

1. Six hundred and seven patients (389 men and 218 women) with intermittent claudication were recruited into the study, and followed up prospectively over a six year period.

2. Two hundred and ten patients died during follow-up, with a higher mortality rate in men than in women. Mortality was primarily due to vascular disease in both sexes.

3. Fifty six patients developed angina pectoris, 93 patients had a non-fatal myocardial infarction, and 61 patients had a non-fatal stroke during follow-up. Forty five patients underwent vascular intervention for possible progression of peripheral arterial disease, and 64 patients developed clinically confirmed symptoms of severe chronic leg ischaemia (rest pain, ulceration and gangrene). Two hundred and three patients had no incident cardiovascular or cerebrovascular events, or deterioration of limb ischaemia.

4. Baseline median levels of plasma fibrinogen, fibrin D-dimer and von Willebrand Factor were significantly higher in patients who subsequently died from ischaemic heart disease. Tssue plasminogen activator was significantly elevated in patients who developed a stroke, compared to those who had no vascular events during follow-up.

5. In multivariate analysis adjusting for age, sex, cigarette smoking, random blood glucose, systolic blood pressure and baseline evidence of ischaemic heart disease, all

the relationships between haemostatic factors and future events became weaker and were no longer statistically significant.

6. There were no significant differences in the baseline levels of haemostatic factors between patients who underwent a vascular intervention due to deterioration of peripheral arterial disease, in comparison to the group of patients who experienced no deterioration of limb ischaemia. von Willbrand Factor levels were significantly raised in patients who subsequently developed severe chronic leg ischaemia.

7. When life-time smoking was taken into account, plasma fibrinogen became significantly associated with the risk of vascular intervention and von Willebrand Factor became predictive of severe chronic leg ischaemia.

CHAPTER SIX

DISCUSSION

In this chapter, the limitations of the methods employed in each study are evaluated separately. The principal results of each study are also discussed separately and the findings from other comparable studies in peripheral arterial disease, ischaemic heart disease and stroke are described. Finally, there is a brief discussion on the potential for using plasma fibrinogen and fibrin D-dimer as routine measures in clinical practice.

6.1 Sites of Atheroma Study: Methods

The Sites of Atheroma Study consisted essentially of a consecutive series of patients with a concomitant cross-sectional design. This study design has several inherent limitations. Its main disadvantage is that, because potential aetiological factors and disease are assessed at one point in time, it cannot usually distinguish between whether the factor preceded the disease in question, or whether levels were altered as a result of development of the disease. Hence, cross-sectional studies can provide information on associations between disease and other factors, but the direction of any causal relationship may be difficult to interpret. Cross-sectional studies are also susceptible to bias, defined in this context as any systematic error in design or analysis that results in incorrect estimates of risk factor associations with disease. In this study, potential major sources of bias might have arisen in the representativeness of the study sample in the measurement of disease using angiography, to score the site and severity of atherosclerosis, in the classification of patients and in the measurement of aetiological

factors. Each of these sources are discussed briefly.

6.1.1 Representativeness of study sample

It is important to ascertain whether the study sample was representative of subjects referred from the defined population of interest for angiography because of symptoms of peripheral arterial disease. If not, the severity and distribution of peripheral arterial disease, and the occurrence of possible aetiological factors in the subjects would not reflect those of the defined population. Since the subjects were all referred to the Royal infirmary of Edinburgh, which provides the only vascular service for the population resident in the Lothian and Borders Health Board areas, it is probable that these subjects were reasonably representative of patients referred from that defined population. Very few, if any patients would have been referred from elsewhere in Scotland.

In addition to referral bias, representativeness might have been affected if the characteristics of those who participated were different from those who were eligible but did not take part in the study. Although the study population was a consecutive series of patients, a few would not have been picked up because of cancellation. Only three subjects refused to participate in the study at the time of recruitment, and a blood sample could not be obtained from two patients who had agreed to participate. A further six subjects were subsequently excluded from the study because their case notes could not be traced and two subjects were also excluded because they showed no evidence of atherosclerotic disease on angiography. Out of a total of 205 subjects who were eligible to take part in the study, 13 subjects (6.3%) were therefore excluded. Although bias was possible, it was unlikely to be strong enough to affect

associations between exposure and disease, given the low percentage of non-participation.

6.1.2 Limitations of angiography

Angiography has traditionally been considered as the 'gold standard' for the measurement of atherosclerosis in the lower limb, but the technique has limitations. A main source of error in angiography is derived from the subjective interpretation of the X-ray images, particularly in the identification of what constitutes a 'normal' reference artery and in estimating a percentage stenosis (Thiele and Strandness 1983). These problems were demonstrated in one study investigating variability between 11 observers in the interpretation of angiographic images of 21 patients with intermittent claudication. Agreement on the degree of atherosclerosis was poor for the aorta, iliac arteries and origin of the profunda femoris artery. Reasonable agreement was found only in the detection of occlusion in the superficial femoral artery (Bruins Slot et al 1981).

In this study, only single plane views were available and this may have led to errors in estimating the true extent of disease. For example, atheroma at the origin of the profunda femoris and in the iliac vessels (where atheroma tends to develop eccentrically) can be under-estimated unless projections in more than one plane are assessed (Strandness and Stahler 1966; Lea Thomas and Andress 1972; McDonald et al 1976). Angiography is also known to over-estimate occlusion length and fail to adequately opacify areas both distal and proximal to occlusion (Cossman et al 1989). An additional problem was the variable length of the abdominal aorta shown for each patient, which made it difficult to standardise disease in this segment. Overall, however, the technical quality of the angiograms produced by the hospital

was typical and adequate for the purposes of the research. Nevertheless, 21 patients had incomplete results, which was usually attributable to inadequate film contrast in one or more of their X-rays.

One of the main difficulties in the interpretation of the angiograms was in estimating the percentage stenosis. This is dependent on identifying a normal part of the segment as a reference point to estimate the degree of narrowing but in severely diseased segments, it was difficult to locate any part which was not atherosclerotic. Standardising the width and length of an artery is not possible because of anatomical variation in these parameters. Another difficulty lay in grading the abdominal aorta and the iliac vessels when they showed dilatation. Such changes are associated with atherosclerotic disease, but were difficult to quantify as the lumen diameter is increased relative to normal.

In this study, patients with symptomatic peripheral arterial disease could not be easily classified by a predominant site of disease because of the diffuse distribution of atherosclerosis within their lower limb arteries. Only five patients had a single focal lesion within the arterial segments. The majority had lesions which were multi-segmental and ranged in severity from intimal plaques to total occlusion. Moreover, as angiography can only provide morphological information and not information about pressure and flow changes caused by narrowing of the lumen, the site of the lesion responsible for production of symptoms could not be identified.

6.1.3 The Bollinger scoring system

When reading and coding the angiograms, the author became aware that the Bollinger coding

system underestimated the volume of disease, especially if the surface area was extensively diseased. The system was devised to primarily measure the degree of luminal narrowing (severity of disease) and weights the importance of lesions so that the additive score for plaques and stenoses do not reach that of an occlusion. According to the system's rules, when one occlusion is present within a segment, other occlusions and stenoses are not coded. When stenoses which both narrow the lumen by more than 50% and between 25-50% are present, plaques are not coded. As a result, the volume of atherosclerosis was not fully quantified in each patient. This underestimation may have been consistent across the study sample, but was more likely to have been greater in those with femoro-popliteal disease, given the relatively longer length and hence greater surface area of these segments. Hence, it could have led to inaccuracies in classifying by site.

6.1.4 Classification of patients

The Bollinger scoring system was used to classify patients by site of disease to permit a more objective comparison between both the extent and severity of disease between the aorto-iliac and femoro-popliteal segments. In categorising patients by site, firstly occlusions and secondly at least one stenosis narrowing the diameter of the lumen by more than 25% were used. The latter criterion was considered as indicative of atherosclerotic changes greater than those which would be typically be present in a normal population of comparable age. This method of classification had the advantage of being relatively simple to apply and would appear to have been reasonable, in that the proportion of patients identified by each site was roughly that expected in clinical practice. Also, the distribution of additive scores between the groups of segments accorded with the selection into predominate site of disease.

However, it should be recognised that such criteria were chosen simply because of their face validity; different methods of classification might have led to different results. For example, in another angiographic study of 67 patients with a similar mean age and with rest pain or intermittent claudication, the criteria used were occlusions and a critical stenosis (i.e. greater than 50% narrowing of the lumen diameter). Only 11% of these subjects were classified as having aorto-iliac disease compared to 18% in the present study, although a similar percentage (42%) as in the present study were classified as having femoro-popliteal disease. Only 16% in comparison to 38% in the present study were classified as dual-site, but this difference may be partly explained by the presence of a fourth group which were described as simply having 'generalised disease' (i.e. no occlusions or critical stenoses) and were not grouped by a particular site of disease (Aston et al 1992).

6.1.5 Measurement of aetiological factors

There was potential bias in the collection of data on some aetiological factors reported by the participants, because the information provided by the subjects occurred after the onset of peripheral arterial disease. For example, as the subjects were all claudicants, it is likely that they were advised to stop smoking on diagnosis, and would certainly have been advised to stop smoking just prior to being admitted to hospital for angiography. This could have caused an underestimation in the reporting of current cigarette consumption. However, such biased recall would probably have caused a reduction in the exposure effect and would not detract from any positive findings between smoking and peripheral arterial disease in the study.

Most of the haemostatic factors measured in the study show considerable inter- and intra-

individual variability. A single measurement of each factor may therefore have led to misclassification of the usual haemostatic levels of an individual and could bias the relative risks associated with vascular disease. The components of variability in levels of clotting fibrinogen were determined in one study of healthy volunteers, based on measurements made during one day, over five days and six weeks (Rosenson et al 1994). Laboratory error accounted for 10.7% of the variability when repeat measures were made on the same day. The intra-individual coefficient of variation increased from 14.2% over the five day period to 17.8% at six weeks, which was equivalent to only 68% of the subjects being correctly classified from one fibrinogen measurement. It was concluded that four repeat measurements over several weeks are required to accurately determine the 'true' fibrinogen level in an individual.

6.2 Sites of Atheroma Study: Results

6.2.1 Angiographic disease in study sample

The arterial segments demonstrated marked differences in the prevalence of occlusion. The most common occlusion site was the superficial femoral artery which has also been reported in other angiographic studies of patients with intermittent claudication (Mavor 1956; Haimovici 1967; Walden et al 1985; Bergqvist and Karacagil 1994). Distal to the superficial femoral artery, the popliteal artery also showed a high prevalence of occlusion, whereas occlusive disease was far less common in the more proximal iliac vessels. The severity of occlusion also varied between the arteries. Although the iliac vessels were occluded less frequently than the femoro-popliteal arteries (with the exception of the profunda femoris

arteries), the length of the occlusion in the iliacs was more likely to be greater than half the segment. Given the relatively short length of the iliac segments, it is not clear whether this indicates that the rate of propagation of occlusion is comparable throughout the lower limb arteries or whether the progression of atherosclerosis is perhaps more extensive in the iliac arteries.

The reasons why atherosclerosis only occurs at certain sites in the lower limb arteries are unknown. It is possible that mechanical and haemodynamic factors may contribute to the localisation of disease by affecting endothelial biology (Mavor 1956; Nerem et al 1993). In vitro studies have shown that the endothelium is capable of altering both its structure and function in response to high shear stresses, resulting in the release of vasoactive substances and inflammatory mediators that have been associated with the occurrence of atherosclerosis (Nerem 1993).

6.2.2 Cardiovascular risk factors and site of peripheral arterial disease

In this study, the levels of the three main cardiovascular risk factors (blood pressure, serum lipids and cigarette smoking) varied in patients, depending on the main site of peripheral atherosclerosis. Although the differences were not statistically significant, this may have been due to the small numbers of subjects, particularly in the aorto-iliac group.

The patients with aorto-iliac disease were on average younger, and more likely to be female and to be current smokers compared to those in the other two groups. In contrast, duration of smoking and higher levels of total cholesterol were perhaps more important in those who

developed femoro-popliteal disease. On the other hand, patients with dual-site disease had the highest levels of systolic blood pressure and the lowest levels of HDL cholesterol of any group. In addition, they were more likely to have a history of ischaemic heart disease, stroke and diabetes mellitus, suggesting a higher degree of generalised atherosclerosis.

Although some differences across the three groups could have been due to variation in the overall severity of atherosclerosis, adjusting for history of ischaemic heart disease and for the number of occlusions had little effect on the absolute levels of risk factors (data not shown). It is therefore conceivable that different combinations or single risk factors may be selective in the initiation and/or progression of peripheral atherosclerosis.

Cigarette smoking

In the present study, current cigarette smoking was more strongly associated with aorto-iliac disease and life-time consumption (packyears) with femoro-popliteal disease. Any such differences are difficult to interpret particularly as the amount and presence of current smoking is correlated with life-time consumption. One possible explanation is that patients with femoro-popliteal disease had more severe symptoms and were more likely to give up smoking. Alternatively, differences in smoking variables more strongly related to disease may have occurred by chance. There is, however, good evidence that the site of disease within the cardiovascular system is influenced by smoking. In one population survey, the Edinburgh Artery Study, cigarette smoking was found to be more strongly related to the risk of peripheral arterial disease than to ischaemic heart disease, independently of the severity of underlying atherosclerosis (Fowkes et al 1992).

The association between cigarette smoking and particularly aorto-iliac disease has been well-documented in other clinical and autopsy studies. It has consistently been shown that the severity of aortic atherosclerosis increases both with the number of cigarettes smoked and the duration of smoking. In addition, the angiographic studies have reported that smoking appears more strongly associated with aortic and iliac atherosclerosis than with disease in the femoral vessels (Sackett et al 1968; Weiss 1972; Lawton 1973; Strong and Richards 1976). However, most of this early research had methodological limitations in that it did not allow for the possible confounding effects of other risk factors, nor did it adjust for underlying severity of disease. It also relied solely on self-reported cigarette smoking which is known to under-estimate true levels of consumption. In this present study, for example, about 10% of self-declared non-smokers were found to be probable smokers, based on cut-off points for serum cotinine and thiocyanate (Woodward and Tunstall-Pedoe 1992).

Why the aorta might be particularly vulnerable to the effects of cigarette smoking, or indeed why the peripheral arteries may be more susceptible than the coronary arteries is unknown. Reasons may be related to differences in wall composition or haemodynamics at certain sites, differential responses to the toxic smoke constituents or because of interactions with other risk factors (Allen et al 1988; Fowkes 1989).

Blood Pressure

In the present study, raised systolic blood pressure appeared to be more strongly related to diffuse (but less severe) disease within the lower limb arteries and a raised diastolic pressure was associated more with disease in the aorto-iliac vessels. However, any relationships shown

between blood pressure and site of disease should be interpreted with caution. Blood pressure may rise as a consequence of peripheral arterial disease through increases in peripheral vascular resistance, or because of a decrease in aortic compliance (Levenson et al 1982). Furthermore, the levels of blood pressure in the study are likely to be an underestimate of the true values because approximately 15% of all patients were receiving anti-hypertensive medication. Although considered to be a relatively weak risk factor for the development of intermittent claudication (Schroll 1982; Schurtleff 1983; Davey-Smith et al 1990), elevated systolic pressure is a recognised risk factor for ischaemic heart disease and is one of the primary risk factors for ischaemic stroke (Kannel et al 1970). It is also associated with the development of carotid intimal-medial thickness (Mowbray et al 1997). Blood pressure therefore does appear to have an effect on the site of atherosclerosis. However, there is, at present, no other published data relating either systolic or diastolic blood pressure to a particular site of lower limb atherosclerosis, and thus the results of the present study must be interpreted with caution and cannot be corraborated.

Blood lipids

There is little conclusive evidence on the relationship between serum lipids and site of disease. Previous reports have suggested that patients with coronary and peripheral atherosclerosis differ in the pattern of lipoprotein and apolipoprotein abnormalities (Greenhalgh et al 1971; Rajput-Williams et al 1988; Fowkes et al 1992; Leng and Fowkes 1992). Whereas elevated LDL cholesterol has been particularly strongly associated with disease in the coronary arteries, hypertriglyceridaemia is considered to be relatively more pathogenic for peripheral atherosclerosis (Greenhalgh et al 1971; Leng and Fowkes 1991), although this may only be

applicable for severe disease (Fowkes et al 1992). Also, in most studies, the regression models do not take into account the high degree of correlation between the lipoprotein fractions and triglyceride levels. However, variation in levels of apolipoproteins by site have also been noted, including relatively high levels of apolipoprotein B in ischaemic heart disease and peripheral arterial disease (Rajput-Williams et al 1988; Powell et al 1997), and elevated levels of apolipoprotein C III in patients with early carotid artery disease (Wiseman et al 1991).
In most of these studies investigating cardiovascular risk factors and site of disease, non-invasive techniques have been used to assess the severity of peripheral arterial disease which detect perfusion effects rather than the degree or site of stenoses within individual arteries. The number of studies which have used angiography to evaluate the precise anatomical location of lesions and thus able to make valid comparisons of the risk factors which might be associated with a particular site of disease are limited. Also, the differences in the risk factor profiles in these patients with different sites of peripheral atherosclerosis does not necessarily mean that the levels of the factors precede or are causally related to a site of disease, but may occur as a consequence of disease or from associations with other factors. The possibility of confounding by factors which were not measured in the study, such as serum triglycerides, obesity, alcohol consumption, menopausal status and insulin resistance should also be considered.

6.2.3 Fibrinogen, fibrin D-dimer and disease

The main objective of the study was to determine the relationships between the haemostatic factors, particularly fibrinogen and fibrin D-dimer, with severity of disease within the femoro-popliteal and the aorto-iliac arteries. Disease severity was estimated by a quantitative variable

(the additive score) which enabled examination of linear relationships between the variables and hence permitted more power to detect significant associations.

Plasma fibrinogen (measured by nephelometry), von Willebrand Factor and fibrin D-dimer were more strongly related to the severity of disease in the femoro-popliteal than in the aorto-iliac segments. On multivariate analysis, both nephelometric fibrinogen and fibrin D-dimer remained independently related to disease. However, it is possible that these findings could be related to differing severities of disease between the segments because the mean additive score was significantly higher for the femoro-popliteal than aorto-iliac arteries.

The results imply that elevated fibrinogen, endothelial disturbance and fibrin turnover may contribute to the extent of atherosclerosis at different sites within the lower limb arteries. Alternatively, these findings may partly reflect an inflammatory response to the presence of atheroma (Stuart et al 1981) or to the extent of endothelial damage, rather than contributing to the development of atherosclerosis.

In a previous study, Lassila et al (1993) reported a strong correlation between fibrinogen (both clotting and nephelometric) and functional severity of peripheral atherosclerosis. In accordance with the findings in the present study, Woodburn et al (1995) found that there was no relationship between clotting fibrinogen and the angiographic extent of peripheral arterial disease. However, nephelometric fibrinogen was not measured in that study and nephelometric fibrinogen has been shown to have a stronger relationship with arterial disease than either clotting fibrinogen or fibrin D-dimer (Smith et al 1993; Sweetnam et al 1998). A level of nephelometric fibrinogen above 6.1g/L has recently been implicated in the

development of symptomatic peripheral arterial disease in smokers (Powell et al 1997). Fibrinogen has also been found to be independently associated with mortality, primarily from ischaemic heart disease in claudicants (Banerjee et al 1992). As coronary death is known to be mediated by thrombosis, following the rupture of an atherosclerotic plaque (Fuster et al 1990), it is therefore noteworthy that the femoro-popliteal segments were characterised by a far higher prevalence of occlusion than in the other segments.

This study was the first to examine variation in the levels of several haemostatic factors at different sites of peripheral atheroclerosis in patients with severe limb ischaemia. The results show that there were no significant differences in the mean levels of the haemostatic factors among patients with disease affecting the femoro-popliteal arteries, aorto-iliac arteries or with dual-site disease. However, relatively small numbers in each of these sub-groups may have affected the power to detect significant differences. Furthermore, the three groups were not differentiated exclusively by site because of the diffuse distribution of atherosclerosis in the lower limb. Differences in the levels of factors across the three groups were, with the exception of von Willebrand Factor, quite small and thus probably not of biological significance.

The levels of fibrinogen measured by heat precipitation were more than 1g/L higher than clotting fibrinogen levels across all three groups, which is comparable to findings in other studies of peripheral arterial disease (Baker et al 1982; Smith et al 1993). However, the difference was greatest in those with dual-site disease which was characterised by a lesser prevalence of occlusion. It is therefore conceivable that a relatively higher percentage (or ratio) of nephelometric fibrinogen to clotting fibrinogen may predispose individuals to more

extensive but less severe peripheral atherosclerosis. Alternatively, the characteristics of lesions, such as their composition or compliance may be altered making them less likely to occlude.

6.2.4 Smoking, fibrinogen, fibrin D-dimer and disease

Adjustment for either life-time or current smoking had no real effect on the relationship between fibrinogen and femoro-popliteal disease, whereas life-time smoking appeared to partly explain the association between fibrin D-dimer and severity of disease in these segments. This is in accordance with results from an earlier case-control study in which smoking appeared to influence peripheral arterial disease more through fibrinolytic disturbance and fibrin formation and breakdown than through a direct effect on fibrinogen levels (Smith et al 1993). One reason for the lack of an association between fibrinogen and smoking in severe peripheral arterial disease may be that interactions between the two variables may be stronger at an earlier stage of disease.

Nevertheless, increasing fibrinogen levels have been associated with a greater reduction in the ABPI in smokers than in non-smokers (Lowe et al 1993) and the magnitude of the relative risk of intermittent claudication appears to be related to an interaction between cigarette smoking and fibrinogen levels (Fowkes et al 1996). This effect may be due to smoking-induced endothelial damage causing a greater infiltration of fibrinogen through the arterial wall, or through an interaction between smoking and genotype which may influence the functional and structural characteristics of fibrinogen (Humphries 1995). Such changes may be related to the formation of an abnormal fibrin gel consisting of long, densely packed fibrin strands which

are resistant to lysis and may contribute to thrombogenesis (Curran et al 1995).

There are conflicting reports on the relationship between fibrin D-dimer, smoking and peripheral arterial disease. In one large case-control study of hospital claudicants who also attended the Royal Infirmary of Edinburgh, and with comparable mean ABPI and smoking consumption to this study, life-time smoking appeared to have little effect on the association between fibrin D-dimer and the risk of claudication (Lee et al 1996). The Edinburgh Artery Study also found that fibrin D-dimer was independently related to the risk of intermittent claudication, after adjustment for a range of risk factors, including packyears, although the number of claudicants was low (n=45) (Lee et al 1995).

The association of fibrin D-dimer with severity of peripheral arterial disease, using the continuous variable ABPI was reduced to non-significance in women (but not men) on multiple adjustment in this study. Among men, the relationship between fibrin D-dimer and the ABPI was strongly related to the amount of smoking consumption, especially in those who had a packyear level of 25 or more (Lee et al 1995). Conversely, a previous study found no correlation between cigarette smoking and fibrin D-dimer among claudicants, but no details were given on either smoking status or current levels of smoking consumption (Al-Zahrani et al 1992).

Fibrin D-dimer was the strongest predictor (among several haemostatic factors) of symptomatic peripheral arterial disease in the only other study which has used the Bollinger scoring system to quantify the extent of peripheral atherosclerosis and the inter-relationship with haemostatic factors and cigarette smoking (Woodburn et al 1995). The results were,

however, not directly comparable with the present study because cigarette smoking was included in the regression model only as smoking status (classified as current, ex- or non-smoking), and life-time smoking consumption was not considered. Furthermore, the inclusion of patients with previous revascularisation surgery or leg amputation may not have given a true reflection of the current severity of atherosclerosis within the study sample.

The elevated fibrin D-dimer levels shown in this study may thus reflect increased turnover of fibrin in advanced peripheral arterial disease, which may occur in response to thrombus formation. However why cigarette smoking should be more strongly associated with fibrin formation and breakdown rather than fibrinogen is not clear. It is possible that high levels of thrombin (or suppression of thrombin inhibitors) resulting from chronic smoking-induced endothelial damage could be responsible for extensive generation of fibrin which implies activation of coagulation and generation of thrombin (Lowe et al 1993).

6.2.5 Smoking, other haemostatic factors and disease

The interaction between cigarette smoking and vWF has been investigated in several studies (Blann and McCollum 1993; Conlan et al 1993). von Willebrand Factor levels are used as an indication of endothelial damage and are raised in current smokers compared to non-smokers, and in subjects with peripheral arterial disease (Blann and McCollum 1992; Smith et al 1993). It has been suggested that smoking may be responsible for a major part of endothelial dysfunction in peripheral arterial disease (Smith et al 1993). However, the univariate association of vWF with disease severity found in the present study was not sustained on multivariate analysis and hence may simply reflect the presence of other confounding factors,

such as hypertension or the extent of endothelial dysfunction, rather than the promotion of atherosclerosis.

The inverse relationship found between PAI activity and severity of disease was surprising. Elevated PAI activity has been consistently demonstrated in peripheral arterial disease (Smith et al 1993; Cortellaro et al 1994) and is also predictive of thrombotic cardiovascular events in claudicants (Cortellaro et al 1994). In the present study, PAI activity decreased with disease severity in men, whereas women showed a positive correlation. Since there was no difference in severity of disease between the sexes, this conflicting relationship may have been due to the significantly higher packyears in the men.

Higher thrombin levels in men resulting from a greater degree of endothelial damage may be responsible for the inverse association observed between PAI activity and disease. When fibrin is present, the release of t-PA (which converts plasminogen to plasmin) and PAI (the specific inhibitor of t-PA) are triggered from the endothelium (Wojta et al 1993), or from platelets (Sprengers and Kluft 1987). A high (possibly threshold) level of thrombin may stimulate an additional release of t-PA which complexes rapidly with PAI, thus effectively depleting systemic PAI activity.

Alternatively, PAI activity may be directly inactivated by thrombin through proteolysis or indirectly through thrombin-mediated protein C inhibition (Van Hinsbergh et al 1987). Although the degree of endothelial damage may be greater, a relative increase would result in men, such that the severity of disease would not be appreciably different between the sexes. It is also possible, however, that there may be sex differences in the relationship between PAI

and peripheral arterial disease which are unrelated to smoking, but due to associations with other risk factors, for example, serum triglycerides, obesity and insulin levels (Juhan-Vague and Alessi 1993).

6.3 Prognostic Study of Intermittent Claudication: Methods

In a prospective cohort study, there may often be several sources of bias. These may include selection bias and losses to follow-up leading to a non-representative study sample, and problems in the assessment of the prognostic variables due to measurement variability and changes in exposure during the period of follow-up. In this study, the measurement of outcome was not considered to be a major problem because of the detailed verification from the case records and other sources.

6.3.1 Representativeness of study sample

The patients were similar to those who composed the study sample in the Sites of Atheroma Study, in that they had all been consecutive referrals to the Royal Infirmary of Edinburgh which provides the only vascular service within the Lothian Health Board area. These subjects could be considered representative of the defined population with respect to moderate to severe intermittent claudication, but not sufficiently severe to warrant vascular intervention. Since many of those with minor symptoms of claudication are unlikely to attend a vascular clinic, the study results would not be generalisable to all claudicants in the general population.

There is some evidence that responders participating in epidemiological studies may differ

from non-responders in ways related to health and risk factor status (Hennekens and Buring 1987). In a cohort study, if non-response is related to risk factors or prognostic factors associated with outcome, then it is possible that bias is introduced. In this study, 742 subjects were initially identified as eligible to participate and 49 of these refused to take part. However, since the non-responders were not followed up to determine either incidence of disease or their prognostic factor status, some degree of bias in the association between prognostic factors and outcome cannot be excluded.

Out of the total of 647 eligible study participants, 30 (4.6%) were not included in the final analysis because their medical records, although available at the inception of the cohort at baseline were not available at the time of follow-up. In most of these cases, the medical records had been destroyed because the patient had not attended the particular hospital within a designated period of time for treatment. The reasons for non-attendance were unknown. It could be that these losses to follow-up developed less disease than those who participated, or became too ill to attend, or had migrated out of the study area. The proportion lost to follow-up was so low, however, that any bias would have had a minimal effect on the results of the study.

6.3.2 Measurement of prognostic factors

Another source of potential bias is error in the classification of subjects by prognostic factor and disease status, either at baseline or during the follow-up period. For example, as previously discussed, self-reported cigarette smoking by questionnaire may have under-estimated the true levels of smoking in the study sample, particularly as biochemical markers

for smoking (serum cotinine and thiocyanate) were not measured in the present study to verify current smoking status. By using multiple sources to obtain information on disease during follow-up, however, the possibility of misclassification of disease status during follow-up was minimised.

The extent to which cardiovascular risk factors change during follow-up and influence the relationship with peripheral arterial disease has recently been assessed in one prospective cohort study of males aged 55 years (Ögren et al 1996). Twenty seven per cent of men had stopped smoking; 40% of men developed hypertension; and more than 50% had higher cholesterol levels when re-examined at 68 years of age. Furthermore, increased mortality, and hence loss to follow-up in those at high risk, was thought to bias the associations between the risk factors and peripheral arterial disease at follow-up. In respect to the present study, the change in exposure to smoking was assessed by examining the questionnaires of surviving patients returned during 1996. Out of a total of 225 questionnaires, 30 (13%) of patients had stopped smoking, whereas 16 (7%) patients had restarted smoking during the six years since baseline. However, since only about 50% of the 1996 questionnaires were returned, it was not possible to determine the true extent to which smoking behaviour had changed in the study sample after six years. Furthermore, since only one classification of exposure was made for each subject at baseline, the analysis could not take into account the total length of exposure and changes in exposure which may have occurred.

6.3.3 Variability in haemostatic factors

Another difficulty in the measurement of prognostic factors is the variability in haemostatic

factors, as previously discussed. Bashir et al (1997) examined the effects of correcting for measurement error in several haemostatic factors (t-PA, PAI, vWF and factor VII) on the risk of transient ischaemic attack and minor stroke. This was based on one preliminary measurement and a second measurement was then taken one year later for each factor. Errors related to laboratory measurement were found to be relatively low, ranging between 4% and 7%. However, the temporal variation in the factor levels over one year was substantial, particularly for PAI (41%) and vWF (30%). Similarly, inter-individual variation was very high. When the risk estimates were corrected for misclassification due to the differences in factor levels changing over one year, the odds of stroke for a standard deviation or one unit change in vWF increased from 1.88 to 3.56 and a slight increase occurred in the odds ratios of the other three factors. One large cohort study, the ARIC Study, has also demonstrated increased relative risks for coronary heart disease when haemostatic factor levels were corrected for measurement error (Folsom et al 1997).

The above findings indicate that some degree of misclassification will probably occur in any study relating one estimation of haemostatic factor levels to disease. The associations found in the previous study are unlikely to be erroneous, but since the effect of variability is to mask associations between haemostatic factors and disease, it is possible that there may be real associations which are not revealed. Furthermore, the long term predictive power of these factors may become weaker because regression dilution bias, which tends to underestimate the magnitude of the true association, will be greater over a longer period of time.

6.4 Prognostic Study of Intermittent Claudication: Results

6.4.1 Incidence of cardiovascular and cerebrovascular events in claudicants

Patients with intermittent claudication have a high risk of death, not from deterioration of limb ischaemia, but from premature ischaemic heart disease and stroke (Reunanen et al 1982; Dormandy et al 1989; Criqui et al 1992). In previous studies, the cumulative five year mortality rate in men with intermittent claudication has been estimated at approximately 15% (Reunanen et al 1982), and claudicants at the time of presentation are three times more likely to die than the general population after ten years (Kallero 1981). Between 50%-60% of deaths of those with symptomatic peripheral arterial disease have been reported to be due to ischaemic heart disease, whereas the proportion of cases dying of cerebrovascular disease is approximately 10%-15%. Ten per cent of patients will die from causes related to visceral ischaemia or rupture of an abdominal aortic aneurysm (Dormandy et al 1989). The remainder die from non cardiovascular causes, primarily smoking-related cancer and respiratory disease. Identification of risk factors which can predict subsequent events in this high risk group of patients is therefore potentially important from the perspective of clinical intervention.

In the present study, the overall mortality was 34.6% after six years of follow-up. This finding is in keeping with the majority of studies of hospital patients with claudication with a similar period of follow-up, regardless of differences in patient selection in respect to age, severity of disease and stage of presentation (Dormandy et al 1989). Cause of mortality, primarily from ischaemic heart disease and stroke, was also in agreement with other studies of hospital claudicants where comparisons were made with non-claudicant controls (Kallero 1981;

Dormandy et al 1989). Analysis of cause of death showed that 30.5% of the claudicants died from a myocardial infarction in the present study, which is comparable to results from the recent Prevention of Atherosclerotic Complications by Ketanserin (PACK) study, in which 36% died from a myocardial infarction (Dormandy and Murray 1991). However, this latter study reported twice as many deaths due to stroke than the present study (28% compared to 13.8% respectively).

The all cause mortality rate was significantly higher in males than in females in the present study. More specifically, the death rate from ischaemic heart disease, especially myocardial infarction, was greater in males compared to females, but there was relatively little difference in the incidence of fatal stroke by gender. It is conceivable that the sex differential in mortality rates for vascular disease may reflect differences in baseline severity of co-existing coronary and cerebrovascular disease between men and women. Alternatively, an increased susceptibility to, or higher risk factor prevalence in men, in factors such as cigarette smoking, hypertension and hyperlipidaemia may partly contribute to the sex differential in coronary mortality (Price and Fowkes 1997).

The high mortality from ischaemic heart disease and stroke in claudicants may be due primarily to concomitant disease in the coronary, carotid and cerebral arteries. While between 30% and 50% of hospital claudicants have ischaemic heart disease diagnosed by ECG, or by clinical history, as many as 90% of patients have evidence of heart disease if examined by coronary angiography. The percentage of claudicants with a history of cerebrovascular disease appears to be lower overall, with a prevalence of between 0.5-52%, but angiography or duplex scanning is rarely performed to assess the presence of asymptomatic disease. Thus, the true

extent of concomitant coronary and cerebrovascular disease in patients with claudication may be an underestimation and may account for some of the conflicting results regarding mortality when adjustments are made for co-existing vascular disease.

There is comparatively little data on the incidence of non-fatal ischaemic heart disease and stroke events in patients with intermittent claudication. Most information is derived from population studies in which there are relatively few subjects with claudication. In the Northwick Park Study, which followed up 400 claudicants for over five years, 14% of the subjects had a non-fatal coronary event (Gilliland et al 1986). The Basle study, based on a sample of workers in the pharmaceutical industry, found that 17% of the surviving cases with peripheral arterial disease developed angina pectoris, 15.0%, a non-fatal myocardial infarction and 12.4%, a cerebrovascular event during 11 years of follow-up (Widmer and Da Silva 1991). These results are comparable to the present study, in which 15.3% of subjects suffered a non-fatal myocardial infarction, and 10% developed a stroke but the incidence of angina was considerably less than the Basle study at 9.2%. These differences may be because of survival bias effects in the Basle study or may be related to differences in severity of vascular disease and risk factor prevalence at baseline.

In the present study, a male predominance in the incidence of non-fatal myocardial infarction was not so apparent, as was the case in fatal infarction. Also, a similar proportion of males and females developed angina pectoris, transient ischaemic attack and non-fatal stroke. Since males were more prone to a fatal coronary thrombosis than females, those at higher coronary risk may have died at an earlier stage of follow-up. Hence selective survival may have led to a apparent narrowing of the incidence rate in non-fatal myocardial infarction between the

sexes.

6.4.2 Fibrinogen and prediction of cardiovascular and cerebrovascular events

Increased mortality is only partly explained by the co-existent ischaemic heart disease associated with claudication and adjusting for conventional cardiovascular risk factors has only a small effect on risk estimates (Davey Smith et al 1990; Criqui et al 1992; Bainton et al 1994). This suggests that lifestyle changes and therapeutic interventions to reduce risk factor levels will not eliminate the increased risk and that other therapeutic measures are required. An overview of randomised controlled trials has shown that anti-platelet therapy can reduce the incidence of cardiovascular events in patients with intermittent claudication (Antiplatelet Trialists' Collaboration 1994). This suggests that thrombotic risk factors may be important in determining cardiovascular outcome in the later stages of established peripheral arterial disease.

Several studies have reported that levels of plasma fibrinogen, t-PA antigen and vWF were independent predictors of mortality from ischaemic heart disease in men and women who have had a myocardial infarction or who have angina pectoris (Haines et al 1983; Cooper et al 1991; Jansson et al 1991; Thompson et al 1995; Benderly et al 1996). However, few have investigated the role of fibrinogen and other haemostatic factors in the prediction of ischaemic heart disease and stroke in subjects with peripheral arterial disease.

Results from this prospective cohort of men and women showed that levels of fibrinogen, t-PA antigen, vWF and fibrin D-dimer were higher in claudicants who subsequently developed an

ischaemic heart disease or stroke event. Only t-PA antigen levels were significantly higher in those who subsequently had a stroke. After adjusting for age and sex, t-PA antigen remained significantly associated with increased risk of a future stroke, whereas elevated fibrin D-dimer and fibrinogen levels were more strongly associated with a future ischaemic heart disease event. All of these relationships became weaker and were no longer statistically significant after adjusting for the combined effects of smoking, glucose, systolic blood pressure and baseline evidence of ischaemic heart disease.

These results suggest that the associations between the haemostatic factors and adverse outcome in symptomatic peripheral arterial disease are partly due to inter-relationships with confounding risk factors. However, since the claudicants who experienced ischaemic heart disease and stroke events had higher levels of haemostatic factors at baseline than claudicants who had no events, the elevations in these factors, regardless of their origin, are likely to be of pathological importance and may have contributed to the development of these thrombotic events.

In an interim analysis of this study, which was conducted after one year, plasma fibrinogen was an independent predictor of death from coronary disease (Fowkes et al 1993). The magnitude of this relationship was weaker in the longer term. Possible explanations for this discrepancy could be related to the fact that the early analysis was based on small numbers of events (15 fatal and 21 non-fatal coronary events). In addition, in the one year multivariate analysis, there was no adjustment for baseline ischaemic heart disease, which may have raised the mean level of, for example, fibrinogen among those claudicants who subsequently developed a vascular event.

Only two other studies have so far investigated the relationship between haemostatic function and clinical outcome in claudicants. Banerjee et al (1992) studied the association between mortality rate and fibrinogen levels in stable claudicants. The authors reported that there was a two-fold increase in the odds of dying which was highly significant and associated with an increase in fibrinogen of 1g/L. In the present study, fibrinogen was also significantly associated with all-cause mortality, but the relative risk was substantially lower, based on a standard deviation increase of only 0.21g/L in fibrinogen (data not shown). In contrast, in the A.D.E.P. Study, which was a multi-centre trial of patients with claudication assessing the effects of an anti-platelet drug (picotamide), the follow-up period was only 18 months, and no association was found between fibrinogen and mortality. However, a statistically significant relationship was noted between fibrinogen and the risk of cerebrovascular disease (Violi et al 1994).

Elevated plasma fibrinogen levels have also been shown to be related to incident vascular events in those with established heart disease. Several studies have reported that raised levels of plasma fibrinogen are an independent predictor of mortality from ischaemic heart disease in men and women who have survived a myocardial infarction or have prevalent angina pectoris (Cooper and Douglas 1991; Thompson et al 1995; Benderly et al 1996). One large angiographic study has also provided evidence that fibrinogen may have a stronger association with occlusion than with atherogenic changes in the coronary arteries, which implies that high fibrinogen levels may contribute to a tendency to thrombogenesis in patients with established vascular disease (ECAT Angina Pectoris Study Group 1993).

Chronic inflammation may play an important role in both the initiation and progression of

atherosclerosis (Ross 1993). An important question is whether raised levels of haemostatic factors are due to an inflammatory response to the extent of arterial disease, or are causally related to thrombogenesis. In the ECAT study, the correlation observed between C-reactive protein (an acute phase reactant and marker for systemic inflammation), and increasing risk of myocardial infarction or sudden death, suggests that inflammation may be involved in the clinical outcome of atherosclerosis (Thompson et al 1995). Moreover, as fibrinogen levels were also positively associated with C-reactive protein in that study, it is conceivable that fibrinogen levels may rise, at least in part, because of inflammatory activity within advanced arterial lesions.

All the major prospective studies to date have identified fibrinogen as an independent predictor of future ischaemic heart disease or stroke in initially healthy subjects in the general population (Wilhelmsen et al 1984; Stone and Thorp 1985; Meade et al 1986; Kannel et al 1987; Heinrich et al 1994; Sweetnam et al 1996; Folsom et al 1997; Smith et al 1997; Woodward et al 1998). Three of these prospective studies have follow-up periods of ten years or more, which strengthens the evidence that any reported association between fibrinogen and ischaemic heart disease could be causal. In a recent meta-analysis of six of these studies which compared the highest tertile of fibrinogen to the lowest tertile, the odds ratio for ischaemic heart disease was estimated at 2.3 (95% CI 1.9-2.8) (Ernst and Resch 1993). The relationship between fibrinogen and the risk of ischaemic heart disease appeared to be stronger in younger men (Meade et al 1986) and slightly higher in men compared to women (Folsom et al 1997). The magnitude of fibrinogen-mediated risk of heart disease also seems to diminish with age in women (Kannel et al 1987). However, differences in defining ischaemic heart disease events, race, the age and sex structures of the populations and the numbers of subjects may

account for the varying strength of associations observed between fibrinogen and heart disease in these prospective studies.

In the Gothenborg Study and the Edinburgh Artery Study, both of which recruited comparatively older participants, baseline fibrinogen levels were more strongly associated with the risk of stroke than with myocardial infarction (Wilhelmsen et al 1984; Smith et al 1997). It is possible that these findings may indicate survival bias effects. The results are also based on relatively few stroke events (37 and 45 respectively) compared to the number of ischaemic heart disease events. Data from the Framingham Study have shown that the risk of stroke rises progressively in men with fibrinogen levels between 1.3-7.0 g/L. This effect was only apparent in men aged between 55-79 years, however and did not occur in either younger men or in women of any age (Kannel et al 1987).

Although it is agreed that high fibrinogen levels probably increase the risk of ischaemic stroke, its role in haemorrhagic stroke is unclear, but is generally thought not to be implicated in the pathophysiology. There are clear difficulties in distinguishing between cases of ischaemic and haemorrhagic stroke based on clinical signs, and the use of computed tomography scanning is not uniformly performed. Although ischaemic stroke accounts for approximately 80% of all strokes (Warlow 1987), the true strength of the association between fibrinogen and ischaemic stroke may be greater than observed in these studies, given that cases of haemorrhagic origin may not have been reliably excluded from the stroke categories.

Fibrinogen has also been found to be an independent risk factor for transient ischaemic attack and minor ischaemic stroke (Qizilbash et al 1991). In secondary risk prediction, fibrinogen

predicted a second cardiovascular event within two years in those who survived an ischaemic stroke (Resch et al 1992). The observation that fibrinogen levels were elevated in a small sample of patients who suffered a transient ischaemic attack and did not subsequently rise suggests that fibrinogen may have a causal role in promoting cerebrovascular events (Ernst et al 1988). However, these findings have yet to be confirmed.

6.4.3 Fibrin D-dimer and prediction of cardiovascular and cerebrovascular events

There are few previous reports about the relationship between fibrin D-dimer and prediction of vascular events in claudicants (Cortellaro et al 1992; Fowkes 1993) or the incidence of these events in the general population (Ridker et al 1994a; Lowe et al 1998). Although in the one year interim analysis conducted in the present study, fibrin D-dimer was independently associated with the risk of combined fatal and non-fatal coronary events, this was not maintained over the longer follow-up period of six years. The Edinburgh Artery Study recently demonstrated that fibrin D-dimer was related to the risk of stroke, independently of cigarette smoking, systolic blood pressure, LDL cholesterol and baseline ischaemic heart disease (Smith et al 1997). In one cross-sectional survey, D-dimer was linked to the extent of atherosclerosis within the cerebral arteries (Heinrich et al 1995). Takano et al (1992) further showed that fibrin D-dimer levels were strongly associated with re-embolisation following acute ischaemic stroke. This implies that high fibrin turnover may contribute to a prothrombotic state which may be critical for progression of disease within the cerebral arteries.

Data has recently been published describing the relationship between fibrin D-dimer and future

ischaemic heart disease. In the Physicians' Health Study, there was an increased risk of a first myocardial infarction across quartiles of fibrin D-dimer, the relative risk being two times higher in the top quartile compared to that of men in the bottom quartile (Ridker et al 1994a). Similarly, Lowe et al (1998) observed a substantially greater risk of ischaemic heart disease in middle-aged men with high fibrin D-dimer levels, which was unrelated to the correlation with fibrinogen levels. These results were in contrast to another high-risk population sample, the Edinburgh Artery Study, which found that multi-adjustment for cardiovascular risk factors and baseline ischaemic heart disease reduced the association between fibrin D-dimer and risk of myocardial infarction (but not stroke) to non-significance (Smith et al 1997). This may be a reflection of stronger interactions between fibrin D-dimer, pre-existing disease and the conventional risk factors in subjects who developed a myocardial infarction than for subjects with stroke.

6.4.4 Other haemostatic factors and events and prediction of cardiovascular and cerebrovascular events

Tissue plasminogen activator

To date, only one other prospective study has examined possible associations between t-PA antigen and the risk of stroke, although the study population was limited to a selected group of male physicians (Ridker et al 1994b). In that study, exclusion of haemorrhagic events from the multivariate analysis had no substantial effect on the magnitude of relative risk. Increases in t-PA antigen levels in both the acute and chronic phases of ischaemic stroke have been reported (Lindgren et al 1996) and t-PA antigen has also been found to be a strong

discriminator of subjects with and without a history of cerebrovascular events (Margaglione 1994). These findings suggest that abnormal fibrinolytic activity may identify those at risk of cerebrovascular events.

A strong long-term association between high levels of t-PA and incidence of ischaemic heart disease has been reported in several studies of patients with prevalent coronary disease (Jansson et al 1993; Thompson et al 1995). However, Ridker et al (1993) suggested that high t-PA may represent a secondary response to the progression of atherosclerosis, because adjusting for atherosclerotic risk factors reduced the association of t-PA and myocardial infarction (but not stroke) to non-significance. This finding was also confirmed in the most recent prospective survey of incident ischaemic heart disease (Lowe et al 1998). The stronger relationship observed between t-PA and stroke than between t-PA and myocardial infarction may reflect differences in risk factor associations between the two disease groups. A recent case-control study has also provided evidence of an association between t-PA and the risk of myocardial infarction, which was markedly reduced by inclusion of cardiovascular risk factors in the multivariate analysis. However, a genetic polymorphism of the t-PA was found to be independently associated with increased risk of non-fatal myocardial infarction in this study (van der Bom 1997).

It is not known which component of the fibrinolytic system is the more important in predicting vascular risk. t-PA antigen levels reflect inactive t-PA/PAI complexes rather than free active t-PA (Nicoloson et al 1988; Nilsson 1989), and thus raised levels may indicate elevated PAI activity and impaired fibrinolytic activity, rather than a dysfunctional endothelium. Furthermore, because these two factors are strongly correlated with each other and also with

lipid and metabolic factors, it is difficult to determine their separate effects in epidemiological studies.

von Willebrand Factor

The evidence relating vWF to the development of cardiovascular disease is conflicting at present. The Edinburgh Artery Study found no relationship between vWF and the risk of myocardial infarction or stroke (Smith et al 1997), in contrast to two studies which observed that vWF was independently related to the incidence of ischaemic heart disease (Meade et al 1994; Lowe et al 1998). One large prospective survey, the ARIC Study, reported a strong univariate association between vWF and combined myocardial infarction and ischaemic heart disease mortality, especially in blacks (Folsom et al 1997). This relationship, however, did not persist on multivariate analysis.

Data from clinical studies suggest that there may be a stronger relationship between vWF and poor prognosis among patients with established vascular disease than among initially healthy individuals. For example, vWF has consistently predicted a secondary acute coronary event in patients with either prevalent angina pectoris (Thompson et al 1995) or in those who have already experienced a myocardial infarction (Jansson et al 1991). In the Progetto Lombardo Atero-Trombosi (PLAT) Study, elevated vWF levels also predicted further clinical events in patients with angina pectoris (Cortellaro et al 1992).

Whether the relationship between vWF and co-existing vascular disease represents part of an acute phase reaction to injury to the endothelium or is causally related to progression of arterial

disease is uncertain. vWF is considered to be a marker of endothelial damage and is raised in conditions associated with risk factors for atherosclerosis, such as hypertension, hypercholesterolaemia, cigarette smoking, obesity and diabetes mellitus. It is also an essential co-factor for the development of occlusive thrombi through interactions with platelets at sites of vascular injury and at arterial stenoses. As elevated levels of vWF have been associated with the development of new vascular events in small cohorts of patients with hyperlipidaemia (Blann et al 1997) and with hypertension (Blann and Waite 1996), damage to the endothelium (and resulting thrombus formation) may be mechanisms by which high blood pressure and lipoprotein levels influence haemostatic function and contribute to atherogenesis or thrombogenesis.

In general, the statistical 'independence' observed in population studies, but not found in high risk studies, such as this present study, may be due to the choice of covariates included in multivariate analysis. On the other hand, there may be stronger interactions between the haemostatic factors and cardiovascular risk factors in subjects with peripheral arterial disease than in apparently healthy subjects. In addition, what is termed 'disease-based spectrum bias' may attenuate the risk estimates in high risk studies, such as in the present study (Miller 1994). In contrast to population-based surveys, where the comparison group consists of healthy individuals, the comparison group in this study consisted of claudicants who have substantially more atherosclerosis than the general population. Thus, the absolute severity of disease between the claudicants who develop events and those who do not may be narrower than equivalent groups in population surveys. It follows therefore, that the levels of haemostatic factors in the claudicants who do not develop subsequent events will probably be higher than they would be in an apparently healthy comparison group, and this could effectively lower the

risk estimates.

6.4.5 Clinical progression of peripheral arterial disease

Studies of intermittent claudication have shown that about 25% of patients referred to a peripheral vascular clinic will deteriorate significantly (Dormandy et al 1989). Some will be treated by angioplasty; up to 74% of angioplasties performed in vascular units are conducted on claudicants (Belli et al 1990). Some patients will progress to rest pain, ulceration and gangrene, and of these, more than 90% will undergo major amputation, arterial reconstruction or angioplasty, procedures which involve a high risk to the patient and major cost to hospital services (Wolfe 1986).

Although factors such as cigarette smoking (Jonason and Bergstrom 1987), diabetes mellitus (Bowers et al 1993), systolic blood pressure (Smith et al 1996), hypertriglyceridaemia (Smith et al 1996) and low ABPI (Cronenwett et al 1984) have been independently associated with deterioration of limb ischaemia, the role of haemostatic factors in the clinical progression of peripheral arterial disease is not clear.

In this prospective cohort of claudicants, a total of 109 (18%) patients developed clinical deterioration of limb ischaemia over six years of follow-up. The six year incidence of severe chronic leg ischaemia was approximately 10.5%, which is comparable to some studies (Cronenwett et al 1984; Jelnes et al 1986), but less than reported in earlier studies, in which early intervention techniques, such as angioplasty were not readily available (Imparato et al 1975; Hughson et al 1978b; Kozol et al 1984; Naschitz et al 1988). The high mortality rate

associated with severe chronic leg ischaemia reported in these studies was also confirmed in the present study; 50% of the patients subsequently died, primarily from ischaemic heart disease and stroke.

6.4.6 Fibrinogen, fibrin D-dimer and clinical progression

The results from this study indicated that baseline levels of fibrinogen were significantly associated with the risk of vascular investigations when life-time smoking consumption was taken into account, but not with symptoms of severe chronic leg ischaemia. One possible explanation for these findings is that vascular intervention could have been performed on those who actually had more advanced disease. However, the baseline ABPI was not significantly different in the patients who subsequently underwent vascular intervention compared to those who had no evidence of peripheral arterial disease progression, although the former group had reported more severe symptoms of intermittent claudication at baseline. Another interesting result from this study was that fibrinogen and total cholesterol levels were slightly *lower* in those who subsequently developed severe chronic leg ischaemia. The reasons for this are not known, but this may reflect an on-going negative acute-phase haematological reaction in response to the underlying severity of disease, which was significantly higher at baseline (as indicated by the lowest ABPI of 0.48) in those with severe chronic leg ischaemia. Fibrinogen levels, measured prior to angioplasty, have also been found to be significantly lower in patients who developed restenosis following angioplasty of the iliac and femoro-popliteal arteries for intermittent claudication or rest pain (Price et al 1997).

The mechanisms whereby fibrinogen may influence the apparent worsening of disease is

uncertain. An elevation in fibrinogen may have important effects on reduction of blood flow which could contribute to atherogenesis and to the progression of disease. Fibrinogen is a major determinant of blood and plasma viscosity and a strong association has recently been reported between fibrinogen and plasma viscosity with incident ischaemic heart disease events in the general population (Sweetnam et al 1996). In a previous case-control analysis based on these claudicants, plasma viscosity was associated with the risk of claudication, independently of age, sex and packyears, but not after adjusting for the ABPI (Lee et al 1996). It is increasingly thought that increases in viscosity could be the most important pathway by which fibrinogen promotes vascular disease. The findings in the present study are consistent with the hypothesis that fibrinogen may directly promote symptomatic and progressive worsening of leg muscle ischaemia during exercise due to reductions of microcirculatory blood flow, distal to arterial stenoses, regardless of the extent of peripheral arterial disease (Lowe et al 1993).

To date, only two other studies have examined the prognostic significance of haemostatic factors in the progression of intermittent claudication (Dormandy et al 1973b; Violi et al 1996). The first was based on a series of 62 patients, of which only eight demonstrated definite signs of clinical deterioration. The results were in accordance with the present study in that fibrinogen was significantly correlated with deterioration of peripheral arterial disease, assessed by changes in walking distance and flow patterns recorded by plethysmography (Dormandy et al 1973b). In contrast, the A.D.E.P. Study which was a multi-centre clinical trial of patients with claudication who were followed up for 18 months, found no association between fibrinogen and peripheral arterial deterioration (Violi et al 1996).

The risk of severe chronic leg ischaemia associated with elevated fibrin D-dimer, although marginally non-significant at the 5% level was substantially raised. Adjustment for life-time smoking had little effect on the relative risk, suggesting that any relationship between this factor and progression of peripheral arterial disease in claudicants is not explained by cigarette smoking. There have been no previous studies which have investigated the relationship between fibrin D-dimer and clinical progression of peripheral arterial disease. However, in the interim analysis conducted after one year, fibrin D-dimer was independently associated with a reduction of ABPI (Fowkes et al 1993). This association with deterioration of peripheral arterial disease was weaker after the longer follow-up period, but may have been related to the small numbers in each disease category. The results from the six year follow-up are also not directly comparable to those of the one year follow-up. In the current analysis, since a change in ABPI could not be assessed, deterioration of disease was mainly based on symptoms indicative of clinical progress, assessment of which are subjective and known to lack reliability. In contrast, the ABPI measure used in the preliminary analysis is a more objective measure of underlying atherosclerotic disease and is also a continuous variable allowing more power to detect associations with progression of underlying lower limb atherosclerosis.

6.4.7 Other haemostatic factors and clinical progression

Tissue plasminogen activator

The role of tissue plasminogen activator in the progression of peripheral arterial disease in claudicants has not previously been investigated. Although t-PA antigen levels are elevated

in subjects with prevalent peripheral arterial disease (Smith et al 1995), the concentration of this factor, measured at baseline in the present study was not associated with either the risk of vascular intervention or risk of severe chronic leg ischaemia in claudicants. This suggests that altered fibrinolysis is not involved in the aetiology of deterioration of symptomatic peripheral arterial disease.

von Willebrand Factor

The results from the present study showed that von Willebrand Factor was predictive of the future development of severe chronic leg ischaemia. Adjustment for life-time smoking history increased the relative risk only slightly, although the level of statistical significance was raised to the 5% level. This suggests that the effect of vWF on the progression of peripheral arterial disease was mostly independent of smoking history.

The role of vWF in the outcome of peripheral arterial disease has not been widely investigated. In a clinical study, vWF was predictive of graft occlusion in patients who had undergone infra-inguinal revascularisation (Woodburn et al 1996). The mechanisms by which vWF may promote thrombosis are not certain. vWF is thought to mediate platelet adhesion to damaged subendothelium (Baumgartner 1973), and is a co-factor for platelet aggregation at high shear rates (Sixma 1987). Furthermore, vWF is raised in subjects with peripheral arterial disease (Blann and McCollum 1992, Smith et al 1993), and in smokers compared to non-smokers (Blann and McCollum 1993); it has also been suggested that smoking may be responsible for a major part of endothelial dysfunction in peripheral arterial disease (Smith et al 1993). In the present study, elevated vWF was associated with the development of severe chronic leg

ischaemia, an asssociation which persisted after adjusting for age, sex and smoking habit. This may partly have reflected greater smoking-induced endothelial injury in those who subsequently developed severe chronic leg ischaemia, since their baseline packyear levels tended to be higher. Alternatively, it also could reflect the microcirculatory endothelial disturbance associated with ischaemia, as this is a major source of raised vWF levels (Blann and McCollum 1994). However, it is more likely that the elevated vWF levels contributed to occlusive platelet-fibrin thrombi formation in the severely stenosed arteries of these patients and hence may have promoted the development of severe chronic leg ischaemia.

It should be noted that the categorisation of these patients by symptoms, rather than by an objective assessment of worsening severity of disease may have biased the results of this study. It is not known whether the vascular intervention group had deteriorated over the six year follow-up, or whether they were merely considered more suitable for early intervention, given that they were younger and reported more severe symptoms at baseline. The occurrence of vascular intervention may also have reflected the particular intervention practice of the treating surgeon. It is also plausible that this group represented a pathologically different group with a slower rate of progression of peripheral arterial disease compared to the severe chronic leg ischaemia patients. This hypothesis is supported by the finding that over half of those who had vascular intervention underwent angiography only, and none progressed to severe chronic leg ischaemia.

6.5 Measurement in Clinical Practice

6.5.1 Fibrinogen in clinical practice

At present, measurement of plasma fibrinogen is not routinely included in clinical practice for use in either the primary or secondary prevention of vascular disease. Although current data indicate a clear role for elevated fibrinogen in the prediction of future events, further research in a number of areas is required before this factor would be considered relevant to practising physicians in evaluating patients at risk.

Firstly, an optimal assay must be agreed upon. A variety of assay techniques are used in epidemiological studies at present, based on clotting, precipitation and immunological methods, partly because there is disagreement about which form of fibrinogen should be measured. Whereas the Clauss method which measures only clotting fibrinogen has been widely adopted in many surveys, total circulating fibrinogen (e,g. as measured by heat precipitation) may in fact be a better predictor of thrombotic risk (Sweetnam et al 1998). Also, measurement of fibrinogen has been poorly standardised which has made it difficult to establish normal reference ranges for the distribution of fibrinogen levels in the population. An international standard has now been developed, but this has been based only on the Clauss assay and further work on standardisation using other assays, such as the heat precipitation method is required. Ideally, the routine measurement of fibrinogen should be simple, rapid and inexpensive and be resistant to the variation in sampling and storage occurring in different laboratories and therefore protocols regarding these problems should be established. Another problem is related to the high inter- and intra-individual variability in fibrinogen levels in reponse to many lifestyle and other factors and therefore a single reading may not accurately define the 'normal' level in an individual. The number of fibrinogen measurements and optimal timing of repeat measurements has yet to be defined.

Another area which requires further work is in establishing the predictive value of a fibrinogen level for a single individual, rather than for groups within a population, in relation to cardiovascular events. This will depend on establishing the exact relationship and interaction between fibrinogen, environmental and other cardiovascular risk factors. The extent to which genetic variation determines an individual's response to these factors should also be further elucidated, since there is increasing evidence that fibrinogen genotype is related to inter-individual differences in levels in the general population. However, measurement of fibrinogen in clinical practice will probably only be included if it provides additional information on the degree of risk which factors, such as smoking, cholesterol and blood pressure show at present.

From a clinical perspective, incorporation of fibrinogen into risk models will only be considered worthwhile if reduction of fibrinogen levels lowers the risk of arterial events. Life-style changes, such as exercise, weight loss and particularly smoking cessation may be initially be advised as these changes appear to have some effect in reducing fibrinogen levels. Several oral drugs are now available which have been shown to lower fibrinogen. Among these drugs, fibric acid derivatives, such as bezafibrate and the platelet inhibitor, ticlopidine are proving the most effective, but it is not clear exactly how these drugs lower fibrinogen and the mechanism may not be specific to fibrinogen. At present, there are a number of randomised controlled trials assessing bezafibrate in progress which are investigating the effect of possible fibrinogen lowering on vascular outcome in patients with myocardial infarction and claudication. If this does occur, it would confirm the hypothesis that fibrinogen is causally linked to the development of atherosclerotic disease (at least following initial clinical diagnosis) and would indicate the importance of lowering fibrinogen in clinical practice.

However, further analysis of data derived from prospective studies is required to confirm the role of fibrinogen reduction in prevention of atherothrombotic disease.

6.5.2 Measurement of fibrin D-dimer in clinical practice

Although there is increasing evidence that fibrin D-dimer has prognostic significance in the incidence of atherothrombotic events, further large-scale prospective studies are necessary before it can be confirmed as a clinical marker of intravascular thrombi formation. This is partly because raised fibrin D-dimer levels may also be indicative of extravascular fibrin turnover, such as occurs in a variety of disorders including infection or injury, renal and liver dysfunction and cardiac failure. Thus, the specificity of fibrin D-dimer in prediction of vascular disorders is uncertain.

Currently, fibrin D-dimer assays are commercially available only for the diagnosis of disseminated intravascular coagulation and venous thromboembolism in clinical practice. However, there are reservations about the usefulness of these tests. For example, a normal level of fibrin D-dimer is considered indicative that a venous thromboembolism has not occurred, but the test has a low positive predictive value if levels are raised. Elevated fibrin D-dimer has also been reported in conditions associated with thromboembolic risk, such as chronic atrial fibrillation and ventricular aneurysms and this factor may be of future use in assessing the response to anti-coagulant treatment (Lip and Lowe 1995).

In most recent epidemiological studies of arterial risk, the enzyme linked immunosorbent assay (ELISA) has been almost universally adopted for estimation of fibrin D-dimer.

However, the antibodies used in commercial kits tend to vary and reference ranges differ between individual manufacturers. Interpretation of the results regarding D-dimer from different surveys is therefore difficult at present, and it is also unlikely that an international standard will be developed in the near future to facilitate comparison of fibrin D-dimer levels.

Furthermore, in comparison to fibrinogen, few studies have examined the influence of environmental factors in determining levels of fibrin D-dimer in the general population. Knowledge of these correlates would be of value in determining the role of fibrin D-dimer as an independent marker of vascular risk, which has generally been inconsistent epidemiologically.

The studies reported in this thesis indicate that both plasma fibrinogen and fibrin D-dimer (and also other haemostatic markers) are related to the presence and progression of peripheral atherosclerotic disease. These associations indicate a possible role in causation, but this has not been confirmed in randomised controlled trials as yet. Furthermore, the measurement of these factors is not sufficiently developed, and the associations with disease not sufficiently precise, that their measurement can be widely adopted in clinical practice in the near future.

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

In this brief chapter, the principal conclusions from each study and the recommendations for further research are listed.

7.1 Sites of Atheroma Study: Conclusions

1. Subjects referred for angiography were categorised by site (aorto-iliac, femoro-popliteal or dual-site) and severity of peripheral atherosclerosis using the Bollinger scoring system. This demonstrated the diffuse distribution of disease in symptomatic peripheral arterial disease, although many patients could be categorised as having disease predominantly in the aorto-iliac or femoro-popliteal segments.

2. Levels of the three main cardiovascular risk factors (blood pressure, serum cholesterol and cigarette smoking) varied in patients depending on the main site of peripheral atherosclerosis. The differences were not statistically significant, but this may have been due to the small numbers of subjects in each site category. It is therefore conceivable that single risk factors or interactions between the risk factors may influence the initiation or progression of peripheral atherosclerosis in different leg arteries.

3. There were no significant differences in the mean levels of plasma fibrinogen and

fibrin D-dimer, or other haemostatic factors between patients with disease affecting different sites (femoro-popliteal, aorto-iliac or dual-site). However, levels of the factors (with the exception of fibrin D-dimer) tended to be higher in those with more diffuse disease. This suggests that coagulation and fibrinolytic activity in symptomatic peripheral arterial disease may be associated with differing patterns of disease within the lower limb arteries.

4. Plasma fibrinogen (measured by nephelometry), fibrin D-dimer and von Willebrand Factor were more strongly correlated with the severity of disease (additive score) in the femoro-popliteal arteries than in the aorto-iliac arteries, after adjusting for age and sex. The implication of these results is that a greater degree of endothelial dysfunction, fibrin formation and breakdown may occur in relation to disease in the femoro-popliteal arteries.

5. After inclusion of all haemostatic factors and time of venepuncture into a multiple regression model, independent relationships between nephelometric fibrinogen, fibrin D-dimer and disease severity was found only in the femoro-popliteal arteries. In contrast, the association between von Willebrand Factor and severity of disease became non-significant. This suggests that greater coagulation and fibrin turnover may contribute to the extent of atherosclerotic disease within these arteries. Alternatively, these findings may reflect an inflammatory response to the presence of atheroma.

6. Further adjustment for life-time smoking consumption, or for current smoking had little effect on the association between nephelometric fibrinogen and disease. By

comparison, life-time smoking reduced the relationship between fibrin D-dimer to non-significance. This may indicate a stronger influence of chronic smoking on increased fibrin turnover than fibrinogen in symptomatic peripheral arterial disease.

7.2 Prognostic Study of Intermittent Claudication: Conclusions

1. A high incidence of both fatal and non-fatal vascular events occurred during the six year follow-up period. This is consistent with the majority of studies of hospital patients with intermittent claudication and may be partly due to co-existing disease in the coronary and cerebral arteries. The all cause mortality rate was significantly higher in males than in females.

2. The high mortality rate associated with progression to severe chronic leg ischaemia was confirmed. Myocardial infarction and stroke accounted for most deaths and only a minority of patients died from causes directly related to peripheral atherosclerosis.

3. Correlations were found between all the haemostatic factors and increasing severity of disease (ABPI) at baseline. The strongest associations occurred with the factors associated with fibrin breakdown (fibrin D-dimer and tissue plasminogen activator). This implies that increasing severity of lower limb atherosclerosis is associated with deposition of fibrin and increased fibrinolytic activity.

4. Baseline median levels of plasma fibrinogen, fibrin D-dimer and von Willebrand Factor were significantly higher in subjects who subsequently died from ischaemic

heart disease compared to those who had no vascular events during follow-up. This suggests that increased coagulation and fibrinolytic activity may have contributed to thrombosis or progression of atherosclerosis in the coronary arteries.

5. In relation to the development of stroke, only tissue plasminogen activator antigen levels showed a significant elevation at baseline. These findings suggest that abnormal fibrinolytic activity may identify those at risk of cerebrovascular events in claudicants.

6. In multivariate analysis adjusting for age and sex, smoking, blood glucose, systolic blood pressure and baseline evidence of ischaemic heart disease, all the relationships between haemostatic factors and vascular events became weaker and were no longer statistically significant. These results indicate that the associations between the haemostatic factors and adverse outcome in symptomatic peripheral arterial disease are partly due to inter-relationships with confounding risk factors. However, haemostatic variables may still be mechanisms through which such risk factors may promote events.

7. There were no significant differences in baseline levels of any of the haemostatic factors between subjects who had deterioration of limb ischaemia and who underwent vascular investigations, in comparison to the group who experienced no deterioration of limb ischaemia. Only von Willebrand Factor levels were significantly raised in patients who developed severe chronic leg ischaemia (rest pain, ulceration and gangrene). This may indicate a higher degree of endothelial disturbance in these patients.

8. In multivariate analyses adjusting for life-time smoking, the relationships between fibrinogen, fibrin D-dimer, other haemostatic factors and the progression of peripheral arterial disease remained similar indicating that any slight effect that these factors might have had in the progression of disease was mostly independent of cigarette smoking.

7.3 Recommendations

1. The Bollinger scoring system was used to quantify the site and severity of peripheral atherosclerosis in this study, but the method has limitations. Research is required in the use of more advanced techniques such as computer-generated densitometry to improve measurement precision and reduce variability in the interpretation of angiographic images.

2. In view of the inconclusive relationship found between cardiovascular risk factors and also haemostatic factors and site of peripheral arterial disease, further research is required in larger studies to delineate the importance and independent effect of these factors on the site of atherosclerosis. Epidemiological surveys using newer non-invasive imaging techniques are also required to confirm whether relationships between risk factors and site of peripheral arterial disease occur in the general population.

3. The reasons why cigarette smoking appears to be more strongly associated with aorto-iliac disease in women than in men are unknown. Further work is needed to examine

the inter-relationships between smoking, anatomical differences and haemodynamic effects in separate samples of men and women in relation to peripheral arterial disease.

4. There is also evidence that certain haemostatic factors, such as fibrinogen may be relatively more important in determining the degree of risk of peripheral arterial disease in men than in women. Sex differences in susceptibility to haemostatic factors for the development of peripheral arterial disease should be investigated further.

5. Further studies are required to determine whether the relationships between fibrinogen, fibrin D-dimer or other haemostatic factors and the development and progression of atherothrombotic disease are likely to be causal. Randomised controlled trials (e.g. the LEADER Study) are currently in progress to test this hypothesis by assessing the effects of fibrinogen lowering agents on the prognosis and incidence of cardiovascular events in claudicants and those with established ischaemic heart disease. These will provide direct evidence on whether thrombotic risk can be modified in individuals at high risk.

6. Given that routine measurement of fibrinogen and fibrin D-dimer is not feasible at present, attention in clinical practice should be given to the use of modifying cardiovascular risk factors associated with peripheral arterial disease, such as smoking, lipids and blood pressure on the incidence of vascular events in claudicants, and the use of simple intervention agents, such as aspirin.

7. The results from the Prognostic Study suggest that fibrinogen and fibrin D-dimer may

be relatively weak prognostic factors in the development of ischaemic heart disease and stroke events in claudicants. The value of other markers in risk prediction should therefore be investigated. In particular, the relationship between inflammatory markers, such as C-reactive protein, serum amyloid A and cytokines, haemostatic function and clinical outcome in claudicants requires clarification.

8. Impaired fibrinolytic activity may enable identification of those claudicants who are more likely to suffer a stroke. However, it is not clear whether t-PA or PAI activity is the more important fibrinolytic marker in determining thrombotic risk. Therefore, the relationship between these two factors in terms of fibrinolytic potential should be studied. The extent to which plasma levels of t-PA and PAI are genetically and environmentally determined in claudicants should also be examined.

9. Elevated levels of the haemostatic factors, fibrinogen and von Willebrand Factor were significantly associated with deterioration of peripheral arterial disease in the Prognostic Study. Research is required to evaluate the effects of these factors in predicting other outcomes of treatment for symptomatic peripheral arterial disease, such as graft occlusion and angioplasty.

10. Given the developments in genetic techniques and analysis in recent years, research is required into the investigation of the particular genotypes influencing the formation and metabolism of haemostatic factors which have been shown in these and other studies to be related to the development and progression of atherothrombotic disease.

REFERENCES

Allan PLP. Duplex ultrasound. In: Fowkes FGR, ed. Epidemiology of peripheral arterial disease. London: Springer-Verlag, 1991: 41-54.

Allen DR, Browse NL, Rutt DL et al. The effect of cigarette smoke, nicotine and carbon monoxide on the permeability of the arterial wall. J Vasc Surg 1988; 7: 139-52.

Al-Zahrani H, Lowe GDO, Douglas JT et al. Increased fibrin turnover in peripheral arterial disease: comparison with a population study. Clin Haemorrheol 1992; 12: 867-72.

Antiplatelet Trialists' Collaboration. Collaborative overview of randomised trials of antiplatelet therapy. Br Med J 1994; 308: 81-106.

Aoki S, Harpel PC. Inhibitors of the fibrinolytic enzyme system. Semin Thromb Haemost 1984; 10: 24-41.

Aston NO, Lea Thomas M, Burnand KG. The distribution of atherosclerosis in the lower limbs. Eur J Vasc Surg 1992; 6: 73-7.

Astrup T. The biological significance of fibrinolysis. Lancet 1956; ii: 565-8.

Badimon JJ, Fuster V, Chesebro JH, Badimon L. Coronary atherosclerosis : a multifactorial disease. Circulation 1993; 87 (Suppl II): II-3-II-16.

Bainton D, Sweetnam P, Baker I, Elwood P. Peripheral vascular disease: consequences for survival and association with risk factors in the Speedwell prospective heart disease study. Br Heart J 1994; 72: 128-132.

Baker IA, Eastham R, Elwood PC et al. Haemostatic factors associated with ischaemic heart disease in men aged 45-64 years. Br Heart J 1982; 47: 490-4.

Balleisen L, Bailey J, Epping PH, Schulte H, van de Loo J. Epidemiological study on factor VII, factor VIII and fibrinogen in an industrial population. I. Baseline data on the relation to age, gender, body-weight, smoking, alcohol, pill-using and, menopause. Thromb Haemost 1985; 54: 475-9.

Banerjee AK, Pearson J, Gilliland EL et al. A six year prospective study of fibrinogen and other risk factors associated with mortality in stable claudicants. Thromb Haemost 1992; 68: 261-3.

Barker DJP, Meade TW, Fall CHD et al. Relation of fetal and infant growth to plasma fibrinogen and factor VII concentrations in adult life. Br Med J 1992; 304: 148-52.

Bashir SA, Duffy SW, Qizilbash N. Repeat measurements of case-control data: corrections for measurement error in a study of ischaemic stroke and haemostatic factors. Int J Epidemiol 1997; 26: 64-70.

Baumgartner HR. The role of blood flow in platelet adhesion, fibrin deposition and formation of mural thrombi. Microvasc Res 1973; 5: 167-79.

Belli AM, Cumberland DC, Know AM et al. The complication rate of percutaneous peripheral balloon angioplasty. Clin Rad 1990; 41: 380-3.

Benderly M, Graff E, Reicher-Reiss H et al. Fibrinogen is a predictor of mortality in coronary heart disease patients. Arterioscler Thromb Vasc Biol 1996; 16: 351-6.

Berg K, Kierulf P. DNA polymorphisms at fibrinogen loci and plasma fibrinogen concentration. Clin Genet 1989; 36: 226-35.

Bergqvist D, Karacagil S. Femoral artery disease. Lancet 1994; 343: 773-8.

Berstein EF, Fronek A. Current status of non-invasive tests in the diagnosis of peripheral arterial disease. Surg Clin North Am 1982; 62: 475-87.

Bini A, Fenoglio JJ, Sobel J et al. Immunochemical characterization of fibrinogen, fibrin I and fibrin II in human thrombi and atherosclerotic lesions. Blood 1987; 69: 1038-45.

Bini A, Fenoglio JJ, Mesa-Tejada R, Kudryk B, Kaplan KL. Identification and distribution of fibrinogen, fibrin and fibrin(ogen) degradation products in atherosclerosis: use of monoclonal antibodies. Arteriosclerosis 1989; 9: 109-21.

Bjerregaard P, Dyerberg J. Fish oil and ischaemic heart disease in Greenland (letter). Lancet 1988; ii: 51.

Blann AD, McCollum CN. Hemostatic factors in patients with vascular disease. Atherosclerosis 1992; 93: 255-6.

Blann AD, McCollum CN. Adverse influence of cigarette smoking on the endothelium. Thromb Haemost 1993; 70: 707-11.

Blann AD, McCollum CN. von Willebrand factor, endothelial damage and atherosclerosis. Eur J Vasc Surg 1994; 8: 10-15.

Blann AD, Waite MA. von Willebrand factor and soluble E selectin in hypertension: influence of treatment and value in predicting the progression of atherosclerosis. Coronary Artery Dis 1996; 7: 143-?

Blann AD, Miller JP, McCollum CN. von Willebrand factor and soluble E-selectin in the prediction of cardiovascular disease progression in hyperlipidaemia. Atherosclerosis 1997; 132: 151-6.

Blombäck B, Carlsson K, Fatah K et al. Fibrin in human plasma: gel architectures governed by rate and nature of fibrinogen activation. Thromb Res 1994; 75: 521-38.

Bollinger A, Breddin K, Hess H et al. Semiquantitative assessment of lower limb atherosclerosis from routine angiographic images. Atherosclerosis 1981; 38: 339-46.

Bowers BL, Valentine RJ, Myers SI, Chervu A, Clagett GP. The natural history of patients with claudication with toe pressures of 40 mmHg or less. J Vasc Surg 1993; 18: 506-11.

Bradby GV, Valente AJ, Walton KW. Serum high density lipoproteins in peripheral vascular disease. Lancet 1978; ii: 1271-4.

Brezinka V, Padmos I. Coronary heart disease risk factors in women. Eur Heart J 1994; 15: 1571-84.

Bruins Slot H, Strijbosch L, Greep JM. Inter-observer variability in single plane aortography. Surgery 1981; 90: 497-503.

Brunner EJ, Davey Smith G, Marmot M et al. Childhood social circumstances and psychosocial and behavioural factors as determinants of plasma fibrinogen. Lancet 1996; 347: 1008-13.

Buchanan A. Contributions to the physiology and pathology of the animal fluids. London Medical Gazette 1836; 18: 50-4.

Cardia G, Grisorio D, Impedovo G, Lillo A, Regina G. Plasma lipids as a risk factor in peripheral vascular disease. Angiology 1990; 41: 19-22.

Christe M, Delley A, Marbet GA, Biland L, Duckert F. Fibrinogen, factor VIII related antigen, antithrombin III and α_2-antiplasmin in peripheral arterial disease. Thromb Haemost (Stuttgart) 1984; 52: 240-2.

Clause LH, Comp PC. The regulation of hemostasis: the protein C system. N Engl J Med 1986; 314: 1298-304.

Clauss A. Gerrinnungs-physioogische schnellmethode zur bestiminung des fibrinogens. Acta haematologica 1957; 17: 237-46.

Colman RW. Factor XII activation and inhibition in inflammation. Agents Actions Suppl 1993; 42: 125-43.

Collen D, Lijnen HR. Thrombolytic therapy. Ann N Y Acad Sci 1991; 614: 259-64.

Conlan MG, Folsom AR, Finch A et al. Associations of factor VIII and von Willebrand factor with age, race, sex and risk factors for atherosclerosis. The Atherosclerosis Risk In Communities (ARIC) Study. Thromb Haemost 1993; 70: 380-5.

Connaghan DG, Francis CW, Lane DA, Marder VJ. Specific identification of fibrin polymers, fibrin degradation products and crosslinked fibrin degradation products in plasma and serum with a new sensitive technique. Blood 1985; 65: 589-97.

Connelly JB, Cooper JA, Meade TW. Strenuous exercise, plasma fibrinogen and factor VII activity. Br Heart J 1992; 67: 351-4.

Cooper J, Douglas AS. Fibrinogen level as a predictor of mortality in survivors of myocardial infarction. Fibrinolysis 1991; 5: 105-8.

Cortellaro M, Boschetti C, Cofrancesco E et al. The PLAT Study: hemostatic function in relation to atherothrombotic ischemic events in vascular disease patients. Arteriosclerosis 1992; 12: 1063-70.

Cortellaro M, Boschetti C, Cofrancesco E et al. Increased fibrin turnover and high PAI-1 activity as predictors of ischemic events in atherosclerotic patients: a case control study. Arterioscler Thromb 1993; 13: 1412-7.

Cortellaro M, Cofrancesco E, Boschetti C et al. Association of increased fibrin turnover and defective fibrinolytic capacity with leg atherosclerosis. Thromb Haemost 1994; 72: 292-9.

Cossman DV, Ellison JE, Wagner WH et al. Comparison of contrast arteriography to arterial mapping with color-flow duplex imaging in the lower extremities. J Vasc Surg 1989; 10: 522-8.

Criqui MH, Fronek A, Barrett-Connor E et al. The prevalence of peripheral arterial disease in a defined population. Circulation 1985; 71: 510-15.

Criqui MH, Langer RD, Fronek A et al. Mortality over a period of 10 years in patients with peripheral arterial disease. N Engl J Med 1992; 326: 381-6.

Cronenwett JL, Warner KG, Zelenock GB et al. Intermittent claudication. Current results of nonoperative management. Arch Surg 1984; 119: 430-6.

Curran J, Hamsten A, Fatah K et al. A genetic polymorphism in the α-fibrinogen gene at amino acid 312 and its relevance to myocardial infarction. Blood Coag Fibrinol 1994; 13(Suppl 2): (abstr O-13).

Da Silva A, Widmer LK, Ziegler HW, Nissen C, Schweizer W. The Basle Longitudinal Study: report on the relation of initial glucose level to baseline ECG abnormalities, peripheral artery disease, and subsequent mortality. J Chron Dis 1979; 32: 797-803.

Davey Smith G, Shipley MJ, Rose G. Intermittent claudication, heart disease risk and mortality. Circulation 1990; 82: 1925-31.

Davies MJ, Thomas A. Thrombosis and acute coronary artery lesions in sudden cardiac ischemic death. N Engl J Med 1984; 310: 1137-40.

Davey-Smith G, Shipley MJ, Rose G. Intermittent claudication, heart disease risk factors, and mortality. The Whitehall study. Circulation 1990; 82: 1925-31.

DeBacker IG, Kornitzer M, Sobolski J, Denolin H. Intermittent claudication - epidemiology and natural history. Acta Cardiol 1979; 34: 115-24.

De Boever E, De Bacquer D, Braeckman C et al. Relation of fibrinogen to lifestyles and to cardiovascular risk factors in a working population. Int J Epidemiol 1995; 24: 915-21.

DeJana E, Languino LR, Polentarutti N et al. Interaction between fibrinogen and cultured endothelial cells. Induction of migration and specific binding. J Clin Invest 1985; 75: 11-18.

Doolittle RF. Fibrinogen and fibrin. Sci Am 1981; 245: 92-101.

Dormandy JA, Hoare E, Colley J, Arrowsmith DE, Dormandy TL. Clinical, haemodynamic, rheological and biochemical findings in 126 patients with intermittent claudication. Br Med J 1973a; iv: 576-81.

Dormandy JA, Hoare E, Khattab AH, Arrowsmith DE, Dormandy TL. Prognostic significance of rheological and biochemical findings in patients with intermittent claudication. Br Med J 1973b; v: 581-3.

Dormandy J, Mahir M, Ascady G et al. Fate of the patient with chronic leg ischaemia. J Cardiovasc Surg 1989; 30: 50-7.

Dormandy JA, Murray GD. The fate of the claudicant - a prospective study of 1969 claudicants. Eur J Vasc Surg 1991; 5: 131-3.

Duguid JB. Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. J Pathol Bacteriol 1946; 58: 207-12.

ECAT Angina Pectoris Study Group. ECAT angina pectoris study: baseline associations of haemostatic factors with extent of coronary arteriosclerosis and other coronary risk factors in 3000 patients with angina pectoris undergoing coronary angiography. Eur Heart J 1993; 14: 8-17.

Eliasson M, Asplund K, Evrin PE, Lundblad D. Relationship of cigarette smoking and snuff dipping to plasma fibrinogen, fibrinolytic variables and serum insulin. The Northern Sweden MONICA Study. Atherosclerosis 1995; 113: 41-53.

Elwood PC, Yarnell JWG, Pickering J, Fehiby AM, O'Brien JR. Exercise, fibrinogen and other risk factors for ischaemic heart disease. Br Heart J 1993; 69: 183-7.

Emeis JJ, Kooistra T. Interleukin 1 and lipopolysaccharide induce an inhibitor of tissue-type plasminogen activator in vivo and in cultured endothelial cells. J Exp Med 1986; 163: 1260-6.

Ernst E, Matrai A, Schmölzl CH, Magyarosy L. Dose-effect relationship between smoking and blood rheology. Br J Haematol 1987; 65: 485-7.

Ernst E, Matrai A, Marshall M. Blood rheology in patients with transient ischaemic attacks. Stroke 1988; 19: 634-6.

Ernst E, Resch KL. Fibrinogen as a cardiovascular risk factor: a meta-analysis and review of the literature. Ann Intern Med 1993; 118: 963-5.

Esmon CT. Molecular events that control the protein C anticoagulant pathway. Thromb Haemost 1993; 70: 29-35.

Feyerabend C, Russell MAH. Rapid gas-liquid chromatographic determination of cotinine in biological liquids. Analyst 1980; 105: 998-1001.

Folsom AR, Wu KK, Davis CE, Conlan MG, Sorlie PD, Szklo M. Population correlates of plasma fibrinogen and factor VII, putative cardiovascular risk factors. Atherosclerosis 1991; 91: 191-205.

Folsom AR, Wu K, Shahar E, Davis CE. Association of hemostatic variables with prevalent cardiovascular disease and asymptomatic carotid artery atherosclerosis. Arterioscler Thromb 1993; 13: 1829-36.

Folsom AR. Epidemiology of fibrinogen. Eur Heart J 1995; 16(Suppl A): 21-24.

Folsom AR, Wu KK, Rosamond WD, Richey Sharrett A, Chambless LE. Prospective study of hemostatic factors and incidence of coronary heart disease: the Atherosclerosis Risk In Communities (ARIC) Study. Circulation 1997; 96: 1102-8.

Fowkes FGR. Epidemiology of atherosclerotic arterial disease in the lower limbs. Eur J Vasc Surg 1988; 2: 283-91.

Fowkes FGR. Aetiology of peripheral atherosclerosis. Br Med J 1989; 298: 405-6.

Fowkes FGR, Housley E, Cawood EHH et al. Edinburgh Artery Study: Prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. Int J Epidemiol 1991; 20: 384-92.

Fowkes FGR, Housley E, Riemersma R et al. Smoking, lipids, glucose intolerance and blood pressure as risk factors for peripheral atherosclerosis compared with ischemic heart disease in the Edinburgh Artery Study. Eur J Vasc Surg 1992; 6: 31-5.

Fowkes FGR, Lowe GDO, Housley E et al. Cross-linked fibrin degradation products, progression of peripheral arterial disease, and risk of coronary artery disease. Lancet 1993; 342: 84-6.

Fowkes FGR, Lee AJ, Lowe GDO, Riemersma RA, Housley E. Inter-relationships of plasma fibrinogen, low-density lipoprotein cholesterol, cigarette smoking and the prevalence of cardiovascular disease. J Cardiovasc Risk 1996; 3: 307-12.

Francis CW, Marder VJ. A molecular model of plasmic degradation of crosslinked fibrin. Semin Thromb Haemost 1982; 8: 25-35.

Fuster V, Stein B, Ambrose JA et al. Atherosclerotic plaque rupture and thrombosis: evolving concepts. Circulation 1990; 82(Suppl II): II-47-II-59.

Gaffney PJ. D-dimer. History of the discovery, characterisation and utility of this and other fibrin fragments. Fibrinolysis 1973; 7(Suppl 2): 2-8.

Gaffney PJ, Brasher M. Subunit structure of the plasmin-induced degradation products of cross-linked fibrin. Biochim Biophys Acta 1973; 295: 308-13.

Gaffney PJ, Perry MJ. Unreliability of current serum fibrin degradation (FDP) assays. Thromb Haemost 1985; 53: 301-2.

Gaffney PJ, Wong MY. Collaborative study of a proposed international standard for plasma fibrinogen measurement. Thromb Haemost 1992; 68: 428-32.

Gailani D, Broze Jr GJ. Factor XI activation in a revised model of blood coagulation. Science 1991; 253: 909-12.

Gaines KJ, Chesney C, van der Zwaag R, Cape C. Racial differences in coagulation studies in stroke. Neurol Res 1992; 14(Suppl 2): 103-8.

Giansante C, Fiotti N, Cattin L, Da Col PD, Calabrese S. Fibrinogen, D-dimer and thrombin-antithrombin complexes in a random population sample: relationships with other cardiovascular risk factors. Thromb Haemost 1994; 71: 581-6.

Gilchrist E, Tulloch JA. Observations on the plasma fibrinogen content after myocardial infarction. Edin Med J 1952; 59: 561-7.

Gilliland EL, Llewellyn CD, Goss DE, Lewis JD. The morbidity and mortality of stable claudicants - results of five year follow-up. Presented at 2nd International Vascular Symposium, London, September 1986.

Gillum RF, Fortmann SP, Prinease RJ, Korrke TE. International diagnostic criteria for acute myocardial infarction and acute stroke. Am Heart J 1984; 108: 150-8.

Gladal K. Thromboplastin synthesis in endothelial cells. Haemostasis 1984; 14: 378-85.

Gofin R, Kark JD, Friedlander Y, Lewis BS, Witt H et al. Peripheral vascular disease in a middle-aged population sample. Ist J Med Sci 1987; 23: 157-67.

Gonzalez-Gronow M, Stack S, Pizzo sv. Plasmin binding to the plasminogen receptor enhances catalytic efficiency and activates the receptor for subsequent ligand binding. Arch Biochem Biophys 1991; 286: 625-8.

Green F, Humphries S. Control of fibrinogen levels. Baillière's Clin Haematol 1989; 2: 945-59.

Greenberg CS, Devine DV, McCrae KM. Measurement of plasma fibrin D-dimer levels with the use of a monoclonal antibody coupled to latex beads. Am J Clin Pathol 1987; 87: 94-100.

Greenhalgh RM, Lewis B, Rosengartens et al. Serum lipids and lipoproteins in peripheral arterial disease. Lancet 1971; ii: 947-50.

Gurewich V, Lipinski B, Hyde E. The effect of the fibrinogen concentration and the leucocyte count on intravascular fibrin deposition from soluble fibrin monomer complexes. Thromb Haemost 1976; 36: 605-14.

Haimovici H. Patterns of atherosclerotic lesions of the lower extremity. Arch Surg 1967; 95: 918-33.

Haines AP, Howarth D, North WRS et al. Haemostatic variables and the outcome of myocardial infarction. Thromb Haemost 1983; 50: 800-3.

Hamsten A, Blombäck M, Wiman B et al. Haemostatic function in myocardial infarction. Br Heart J 1986; 55: 58-66.

Hamsten A, Iselius L, de Faire U, Blombäck M. Genetic and cultural inheritance of plasma fibrinogen concentration. Lancet 1987; ii: 988-90.

Handa K, Kono S, Saku K et al. Plasma fibrinogen levels as an independent indicator of severity of coronary atherosclerosis. Atherosclerosis 1989; 72: 209-13.

Hart PH, Burgess DR, Vitti GF, Hamilton JA. Interleukin-4 stimulates human monocytes to produce tissue-type plasminogen activator. Blood 1989; 74: 1222-5.

Hein HO, Suadicani P, Gyntelberg F. Ischaemic heart disease incidence by social class and form of smoking: the Copenhagen Male Study - 17 years' follow-up. J Intern Med 1992; 231: 477-83.

Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. Biochem J 1990; 265: 621-36.

Heinrich J, Balleisen L, Schulte H, Assman G, van de Loo J. Fibrinogen and factor VII in the prediction of coronary risk: results from the PROCAM study in healthy men. Arterioscler Thromb 1994; 15: 54-9.

Heinrich J, Schulte H, Schönfeld R, Köhler E, Assman G. Association of variables of coagulation, fibrinolysis and acute-phase with atherosclerosis in coronary and peripheral arteries and those arteries supplying the brain. Thromb Haemost 1995; 73: 374-9.

Hennekens CH, Buring JE. Cohort studies. In: Hennekens CH, Buring JE, eds. Epidemiology in medicine. Boston/Toronto: Little, Brown and Company, 1987: 153-77.

Herman JB, Medalie JH, Goldbourt U. Differences in cardiovascular morbidity and mortality between previously known and newly-diagnosed adult diabetics. Diabetolgia 1977; 13: 229-34.

Hermans J, McDonagh J. Fibrin: structure and interactions. Semin Thromb Hemostas 1982; 8: 11-24.

Herren T, Stricker H, Haerberli A, Do D-D, Straub PW. Fibrin formation and degradation in patients with atherosclerotic disease. Circulation 1994; 90: 2679-86.

Holm B, Brosstad F, Kierulf P, Godal HC. Polymerization properties of two normally circulating fibrinogens, HMW and LMW. Evidence that the COOH-terminal end of the α-chain is of importance for fibrin polymerization. Thromb Res 1985; 39: 595-606.

Horrevoets AJ, Smilde A, de Vries C, Pannekoek H. The specific roles of finger and kringle 2 domains of tissue-type plasminogen activator during in vitro fibrinolysis. J Biol Chem 1994; 269: 12639-44.

Howard G, Sharrett AR, Heiss G et al for the ARIC Investigators. Carotid artery intima media thickness in general population as evaluated by B-mode ultrasound. Stroke 1993; 24: 1297-1304.

Hughson WG, Mann JI, Garrod A. Intermittent claudication: prevalence and risk factors. Br Med J 1978a; i: 1379-81.

Hughson WG, Mann JI, Tibbs DJ, Woods HF, Walton T. Intermittent claudication: factors determining outcome. Br Med J 1978b; i: 1377-9.

Humphries SE, Cook M, Dubowitz M, Stirling Y, Meade TW. Role of genetic variation at the fibrinogen locus in determination of plasma fibrinogen concentrations. Lancet 1987; i: 1452-5.

Humphries SE. Genetic regulation of fibrinogen. Eur Heart J 1995; 16(Suppl A): 16-20.

Imparato AM, Kim GE, Davidson T et al. Intermittent claudication: its natural course. Surgery 1975; 78: 795-9.

Ishida T, Tanaka K. Effects of fibrin and fibrinogen-degradation products on the growth of rabbit aortic smooth cells in culture. Atherosclerosis 1982; 44: 161-74.

Iso H, Folsom AR, Sato S et al. Plasma fibrinogen and its correlates in Japanese and US population samples. Arterioscler Thromb 1993; 13: 783-90.

Jacobsen UK, Dige-Pedersen H, Gyntelberg F, Svendsen UG. 'Risk factors' and manifestations of arteriosclerosis in patients with intermittent claudication compared to normal persons. Dan Med Bull 1984; 31: 145-8.

Jansson JH, Nilsson TK, Johnson O. von Willebrand factor in plasma: a novel risk factor for recurrent myocardial infarction and death. Br Heart J 1991; 66: 351-5.

Jansson JH, Olofsson BO, Nilsson TK. Predictive value of tissue plasminogen activator mass concentration on long-term mortality in patients with coronary heart disease. Circulation 1993; 88: 2030-4.

Jarrett RJ. Diabetes mellitus. In: Fowkes FGR, ed. Epidemiology of peripheral arterial disease. London: Springer-Verlag, 1991: 187-93.

Jelnes R, Gaardsting O, Hougaard Jensen K et al. Fate in intermittent claudication: outcome and risk factors. Br Med J 1986; 293: 1137-40.

Jern C, Wadenvik H, Marr H, Hallgren J, Jern S. Haematological changes during acute mental stress. Br J Haematol 1989; 71: 156-63.

Jonason T, Bergstrom R. Cessation of smoking in patients with intermittent claudication: effects on the risk of peripheral vascular complications, myocardial infarction and mortality. Acta Med Scand 1987; 221: 253-60.

Juergens JL, Barker WW, Hines EA. Arteriosclerosis obliterans: a review of 520 cases with special reference to pathogenic and prognostic factors. Circulation 1960; 21: 188-95.

Juhan-Vague I, Alessi M-C. Plasminogen activator inhibitor 1 and atherothrombosis. Thromb Haemost 1993; 70: 138-43.

Kallero KS. Mortality and morbidity in patients with intermittent claudication as defined by venous occlusion plethysmography: a ten-year follow-up study. J Chronic Dis 1981; 34: 455-62.

Kannel WB, Wolf PA, Verter J, McNamara PA. Epidemiologic assessment of the role of blood pressure in stroke: the Framingham Study. JAMA 1970; 214: 301-10.

Kannel WB, Shurtleff D. The Framingham Study - cigarettes and the development of intermittent claudication. Geriatrics 1973; 28: 61-8.

Kannel WB, McGhee DL. Update on some epidemiological features of intermittent claudication: the Framingham Study. J Am Geriatr Soc 1985; 33: 13-18.

Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease: the Framingham Study. JAMA 1987; 258: 1183-6.

Kannel WB, D'Agostino RB, Belanger AJ. Update on fibrinogen as a cardiovascular risk factor. Ann Epidemiol 1992; 2: 457-66.

Kant JA, Fornace AJ Jr, Saxe D et al. Evolution and organisation of the fibrinogen locus on chromosome 4: gene duplication accompanied by transposition and inversion. Proc Natl Acad Sci 1985; 82: 2344-8.

Kohler TR, Nance DR, Cramer NM, Vandenburghe N, Strandess DE. Duplex scanning for aorto-iliac and femoro-popliteal disease: A prospective study. Circulation 1987; 76: 1074-80.

Kozol RA, Bredenberg CE, Fey JD et al. Dependent rubor as a predictor of limb risk in patients with claudication. Arch Surg 1984; 119: 932-5.

Krishnaswamy S, Church WR, Nesheim ME, Mann KG. Activation of human prothrombinase. Influence of factor Va on the reaction mechanism. J Biol Chem 1987; 262(7): 3291-9.

Laing S, Greenhalgh RM. The detection and progression of asymptomatic peripheral arterial disease. Br J Surg 1983; 70: 628-30.

Lassila R, Peltonen S, Lepäntolo M et al. Severity of peripheral atherosclerosis is associated with fibrinogen and degradation of cross-linked fibrin. Arterioscler Thromb 1993; 13: 1738-42.

Law MR, Wald NJ, Wu T, Hackshaw A, Bailey A. Systematic underestimation of association between serum cholesterol concentration and ischemic heart disease in observational studies: data from the BUPA Study. Br Med J 1994; 308: 363-6.

Lawson JH, Butenas S, Ribarik N, Mann KG. Complex-dependent inhibition of factor-VIIa by antithrombin III and heparin. J Biol Chem 1993; 268: 767-70.

Lawton G. Cigarette consumption and atherosclerosis. Their relationship in the aorta and iliac and femoral arteries. Br J Surg 1973; 60: 873-6.

Lea Thomas M, Andress MR. Value of oblique projections in translumbar aortography. Am J Roentgenol 1972; 116: 187-93.

Lee AJ, Smith WCS, Lowe GDO, Tunstall-Pedoe H. Plasma fibrinogen and coronary risk factors: the Scottish Heart Health Study. J Clin Epidemiol 1990; 43: 913-9.

Lee AJ, Lowe GDO, Smith WCS, Tunstall-Pedoe H. Plasma fibrinogen in women: relationships with oral contraception, the menopause and hormone replacement therapy. Br J Haematol 1993; 83: 616-21.

Lee AJ, Fowkes FGR, Lowe GDO, Rumley A. Fibrin D-dimer, haemostatic factors and peripheral arterial disease. Thromb Haemost 1995; 74: 828-32.

Lee AJ, Fowkes FGR, Rattray A, Rumley A, Lowe GDO. Haemostatic factors and rheological factors in intermittent claudication: the influence of smoking and extent of disease. Br J Haematol 1996; 92: 226-30.

Leng GC, Fowkes FGR. Lipids: Epidemiology. In: Fowkes FGR, ed. Epidemiology of peripheral arterial disease. London: Springer-Verlag, 1991: 165-79.

Leng GC, Fowkes FGR. The Edinburgh Claudication questionnaire: an improved version of the WHO/Rose questionnaire for use in epidemiological surveys. J Clin Epidemiol 1992; 45: 1101-9.

Leng GC, Fowkes FGR, Donnan Pt, Housley E. Reactive hyperaemia test in a random sample of the general population. J Vasc Surg 1993; 17: 479-86.

Leng GC, Fowkes FGR. The epidemiology of peripheral arterial disease. Vasc Rev Med 1993; 4: 5-18.

Leng GC, Lee AJ, Fowkes FGR et al. Incidence, natural history and cardiovascular events in symptomatic and asymptomatic peripheral arterial disease in the general population. Int J Epidemiol 1996; 25: 1172-81.

Leschke M, Motz W, Strauer BE. Hämorheologisch-therapeutische anwendungsmöglich keiten bei der koronaren herzkrankung. Wien Med Wochenschr 1986; Spel No 136: 17-24.

Levenson JA, Simon AC, Safar ME, Fiessinger JN, Housset EM. Systolic hypertension in arteriosclerosis obliterans of the lower limbs. Clin Exp Hypertens 1982; 4: 1059-72.

Lijnen HR, van Hoef B, Collen D. On the molecular interactions between fibrin, tissue-type plasminogen activator and plasminogen. Thromb Res Suppl 1990; 10: 45-54.

Lindgren AL, Lindoff C, Norrving B, Astedt B, Johansson BB. Tissue plasminogen activator and plasminogen activator inhibitor-1 in stroke patients. Stroke 1996; 27: 1066-71.

Lip GYH, Beevers DG. Abnormalities of rheology and coagulation in hypertension. J Hum Hypertens 1994; 8: 693-702.

Lip GYH, Lowe GDO. Fibrin D-dimer: a useful clinical marker of thrombogenesis?. Clin Sci 1995; 89: 205-14.

Lipinska I, Lipinska B, Gurewich V. Lipoproteins, fibrinolytic activity and fibrinogen in patients with occlusive vascular disease and in healthy subjects with a family history of heart attacks. Artery 1979; 6: 254-64.

Liu CY, Nossel HL, Kaplan KL. The binding of thrombin to fibrin. J Biol Chem 1979; 254: 10421-5.

Loscalzo J. The relationship between atherosclerosis and thrombosis. Circulation 1992; 86(Suppl III): III-95-III-99.

Lowe GDO, Drummond MM, Lorimer AR et al. Relationship between extent of coronary artery disease and blood viscosity. Br Med J 1980; i: 672-4.

Lowe GDO. Blood rheology in arterial disease. Clin Sci 1986; 71: 137-46.

Lowe GDO, Fowkes FGR, Dawes J et al. Blood viscosity, fibrinogen, and activation of coagulation and leukocytes in peripheral arterial disease and the normal population in the Edinburgh Artery Study. Circulation 1993; 87: 1915-20.

Lowe GDO, Rumley A, Yarnell JWG, Sweetnam PM, Thomas HF. Fibrin D-dimer, von Willebrand Factor and tissue plasminogen activator antigens are predictors of major ischaemic heart disease: the Caerphilly Study. Blood Coag Fibrinol 1995; 6: 156-7.

Lowe GDO, Yarnell JWG, Sweetnam PM et al. Fibrin D-dimer, tissue plasminogen activator, and the risk of major ischaemic heart disease in the Caerphilly Study. Thromb Haemost 1998; 79: 129-33.

Mann KG, Jenny RJ, Krishnaswany S. Cofactor proteins in the assembly and expression of blood clotting enzyme complexes. Annu Rev Biochem 1988; 57: 915-56.

Mann KG, Nesheim ME, Church W et al. Surface-dependent reactions of the vitamin K-dependent enzyme complexes. Blood 1990; 76: 1-16.

Mannucci PM. Recent progress in the pathophysiology of fibrinogen. Eur Heart J 1995; 16(Suppl A): 25-30.

Margaglione M, Di Minno G, Grandone E et al. Abnormally high circulation levels of tissue plasminogen activator and plasminogen activator inhibitor-1 in patients with a history of ischemic stroke. Arterioscler Thromb 1994; 14: 1741-5.

Marguerie GA, Plow E. The fibrinogen dependent pathway of platelet aggregation. Ann N Y Acad Sci 1983; 408: 556-66.

Mari D, Coppola R, Bottasso B, Mannucci PM. High levels of fibrinogen, factor VII and factor VIII in healthy centurians. Blood Coag Fibrinol 1994; 5(Suppl 2): (Abstr P-65).

Marckmann P, Jespersen J, Leth T, Sandström B. Effect of fish oil diet versus meat diet on blood lipids, coagulation and fibrinolysis in healthy young men. J Intern Med 1991; 229: 317-23.

Margaglione M, Di Minno G, Grandone E et al. Abnormally high circulation levels of tissue plasminogen activator and plasminogen activator inhibitor-1 in patients with a history of ischemic stroke. Arterioscler Thromb 1994; 14: 1741-5.

Markowe HLJ, Marmot MG, Shipley MJ et al. Fibrinogen: a possible link between social class and coronary heart disease. Br Med J 1985; 291: 1312-4.

Marmot MG, Adelstein AM, Robinson N, Rose GA. Changing social-class distribution of heart disease. Br Med J 1978; 2: 1109-12.

Mavor GE. The pattern of occlusion in atheroma of the lower limb arteries. The correlation of clinical and arteriographic findings. Br J Surg 1956; 43: 1352-64.

McDonald EJ, Malone JM, Eisenberg RL, Mani RL. Arteriographic evaluation of the femoral bifurcation: value of the ipsilateral anterior oblique projection. Am J Roentgenol 1976; 127: 955-6.

Meade TW, Chakrabarti R, Haines AP, North WRS, Stirling Y. Characteristics affecting fibrinolytic activity and plasma fibrinogen concentrations. Br Med J 1979; 1: 153-6.

Meade TW. Epidemiology of atheroma and thrombosis. In: Bloom AL, Thomas DP, eds. Haemostasis and thrombosis. London: Churchill Livingston, 1981.

Meade TW, Haines AP, Imeson JD, Stirling Y, Thompson SG. Menopausal status and haemostatic variables. Lancet 1983; i: 22-4.

Meade TW, Vickers MV, Thompson SG et al. Epidemiological characteristics of platelet aggregability. Br Med J 1985; 290: 428-32.

Meade TW, Mellows S, Brozovic M et al. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. Lancet 1986; ii: 533-7.

Meade TW, Imeson J, Stirling Y. Effects of changes in smoking and other characteristics on clotting factors and the risk of ischaemic heart disease. Lancet 1987; ii: 986-8.

Meade TW, Cooper JA, Stirling Y et al. Factor VIII, ABO blood group and the incidence of ischaemic heart disease. Br J Haematol 1994; 88: 601-4.

Meilahn EN, Kuller LH, Mathews KA, Kiss JE. Hemostatic factors according to menopausal status and use of hormone replacement therapy. Ann Epidemiology 1992; 2: 445-55.

Meilahn EN, Becker RC, Corrao JM. Primary prevention of coronary heart disease in women. Cardiology 1995; 86: 286-98.

Meilahn EN, Cauley JA, Tracy RP et al. Associations of sex hormones and adiposity with plasma levels of fibrinogen and PAI-1 in post-menopausal women. Am J Epidemiol 1996; 143: 159-66.

Miller GJ, Martin JC, Webster J et al. Association between dietary fat intake and plasma factor VII coagulant activity - a predictor of cardiovascular activity. Atherosclerosis 1986; 60: 269-77.

Miller TQ. High-risk studies are influenced by indirect range restriction. J Behav Med 1994; 17: 567-88.

Mitchell JRA, Schwartz CJ. Arterial disease. Oxford: Blackwell Scientific Publications, 1965.

Møller L, Kristensen TS. Plasma fibrinogen and ischemic heart disease risk factors. Arterioscler Thromb 1991; 11: 344-50.

Morris JN, Clayton DG, Everitt MG, Semmence AM, Burgess EH. Exercise in leisure time: coronary attack and death rates. Br Heart J 1990; 63: 3234.

Mosesson MW. Fibrinogen heterogeneity. Ann N Y Acad Sci 1983; 408: 97-113.

Mowbray PI, Lee AJ, Fowkes FGR, Allan PL. Cardiovascular risk factors for early carotid atherosclerosis in the general population: the Edinburgh Artery Study. J Cardiovasc Risk 1997; 4: 357-62.

Murabito JM, D'Agostino RB, Silbershatz H, Wilson PWF. Intermittent claudication: A risk profile from the Framingham Heart Study. Circulation 1997; 96: 44-9.

Naito M, Funaki C, Hayashi T et al. Substrate-bound fibrinogen, fibrin and other cell attachment-promoting proteins in a scaffold for cultured vascular smooth muscle cells. Atherosclerosis 1992; 96: 227-34.

Naschitz JE, Ambrosio DA, Chang JB. Intermittent claudication: predictors and outcome. Angiology 1988; 39: 16-22.

Nerem RM, Harrison DG, Taylor WR, Alexander RW. Hemodynamics and vascular endothelial biology. J Cardiovasc Pharmacol 1993; 21(Suppl 1): S6-S10.

Nicoloso G, Hauert J, Kruithof EKO, van Melle G, Bachmann F. Fibrinolysis in normal subjects: comparison between plasminogen activator inhibitor and other components of the fibrinolytic system. Thromb Haemost 1988; 59: 299-303.

Nilsson TK. Analysis of factors affecting tissue plasminogen activator activity and antigen concentrations before and after venous occlusion in 123 subjects. Clin Chem Enzymol Commun 1989; 1: 335-41.

Ögren M, Hedblad B, Janzon L. Biased risk factor assessment in prospective studies of peripheral arterial disease due to changes in exposure and selective mortality of high-risk individuals. J Cardiovasc Risk 1996; 3: 523-8.

Otter M, Barrett B, Bergshoeff MM, Rijken DC. Binding of tissue-type plasminogen activator by the mannose receptor. J Biol Chem 1991; 266: 13931-5.

Panchenko E, Dobrovolsky A, Davletov K et al. D-dimer and fibrinolysis in patients with various degrees of atherosclerosis. Eur Heart J 1995; 16: 38-42.

Pilger E, Pristautz H, Pfeiffer KH, Kostner GM. Retrospective evaluation of risk factors for peripheral atherosclerosis by stepwise discriminant analysis. Arteriosclerosis 1988; 3: 57-63.

Powell JT, Edwards RJ, Worrell PC et al. Risk factors associated with the development of peripheral arterial disease in smokers; a case-control study. Atherosclerosis 1997; 129: 41-8.

Price JF, Namode N, Smith FB et al. Haemostatic and rheological factors as predictors of restenosis following percutaneous transluminal angioplasty. Eur J Vasc Endovasc Surg 1997; 14: 392-8.

Price JF, Fowkes FGR. Risk factors and the sex differential in coronary artery disease. Epidemiology 1997; 8: 584-91.

Qizilbash N, Jones L, Warlow C, Mann J. Fibrinogen and lipid concentrations as risk factors for transient ischaemic attacks and minor ischaemic strokes. Br Med J 1991; 303: 605-9.

Rabbani LE, Loscalzo J. Recent observations on the role of hemostatic determinants in the development of the atherothrombotic plaque. Atherosclerosis 1994; 105: 1-7.

Radjput-Williams J, Knott TJ, Wallis SC et al. Variation of apolipoprotein-B gene is associated with obesity, high blood cholesterol levels, and increased risk of coronary heart disease. Lancet 1988; ii: 1442-6.

Radomski MW, Palmer RMJ, Moncada S. Endogenous nitric acid inhibits human platelet adhesion to vascular endothelium. Lancet 1988; ii: 1057-8.

Resch KL, Ernst E, Matrai A, Paulsen IIF. Fibrinogen and viscosity as risk factors for subsequent cardiovascular events in stroke survivors. Ann Int Med 1992; 117: 371-5.

Reunanen A, Takkunen H, Aromaa A. Prevalence of intermittent claudication and its effect on mortality. Acta Med Scand 1982; 211: 249-56.

Ridker PM, Vaughan DE, Stampfer MJ, Manson JE, Hennekens CH. Endogenous tissue-type plasminogen activator and risk of myocardial infarction. Lancet 1993; 341: 1165-8.

Ridker PM, Hennekens CH, Cerskus A, Stampfer MJ. Plasma concentration of cross-linked fibrin degradation product (D-dimer) and the risk of future myocardial infarction among apparently healthy men. Circulation 1994a; 90: 2236-40.

Ridker PM, Hennekens CH, Stampfer MJ, Manson JE, Vaughan DE. Prospective study of endogenous tissue plasminogen activator and risk of stroke. Lancet 1994b; 343: 940-3.

Rose GA. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bull WHO 1962; 27: 645-58.

Rosengren A, Wilhelmsen L, Welin L et al. Social influences and cardiovascular risk factors as determinants of plasma fibrinogen concentration in a general population sample of middle-aged men. Br Med J 1990; 300: 634-8.

Rosenson RS, Tangney CC, Hafner JM. Intraindividual variability of fibrinogen levels and cardiovascular risk profile. Arterioscler Thromb 1994; 14: 1928-32.

Ross R. The pathogenesis of atherosclerosis. A perspective for the 1990s. Nature 1993; 362: 801-9.

Ruckley CV. Symptomatic and asymptomatic disease. In: Fowkes FGR, ed. Epidemiology of peripheral arterial disease. London: Springer-Verlag, 1991: 97-108.

Rühling K, Zabel-Langhennig R, Till U, Thielmann K. Enhanced net transfer of HDL cholesteryl esters to Apo B containing lipoproteins in patients with peripheral vascular disease. Clin Chim Acta 1989; 184: 289-96.

Sackett DL, Epid MS, Gibson RW, Bross IDJ, Pickren JW. Relation between aortic atherosclerosis and the use of cigarettes and alcohol. An autopsy study. N Engl J Med 1968; 279: 1413-20.

Salomaa V, Stinson V, Kark JD et al. Association of fibrinolytic parameters with early atherosclerosis: The ARIC Study. Circulation 1995; 91: 284-90.

Schmitz-Huber U, Thompson SG, Balleisen L et al. Lack of association between haemostatic variables and the presence or the extent of coronary atherosclerosis. Br Heart J 1988; 59: 287-91.

Schroll M, Munck O. Estimation of peripheral arteriosclerotic disease by ankle blood pressure measurements in a population study of 60 year-old men and women. J Chron Dis 1981; 34: 261-9.

Schroll M. Blood pressure as a cardiovascular risk factor in a 10-year prospective study of men and women born in 1914 and examined in 1964 and 1974 in Glostrup. Dan Med Bull 1982; 28: 154-64.

Schurtleff D. An epidemiological investigation of cardiovascular disease. In: Kannel WB, Tavia Gordon T, eds. The Framingham Study. DHEW Publication, 1983.

Seiffert D, Wagner NN, Loskutoff DJ. Serum-derived vitronectin influences the pericellular distribution of type 1 plasminogen activator inhibitor. J Cell Biol 1990; 111: 1283-91.

Siitonen O, Uusitupa M, Pyörälä K, Voutilainen E, Lansimies E. Peripheral arterial disease and its relationship to cardiovascular risk factors and coronary heart disease in newly diagnosed non-insulin-dependent diabetics. Acta Med Scand 1986; 220: 205-12.

Sixma JJ. Role of platelets, plasma proteins and the vessel wall in haemostasis. Thromb Haemost 1987; 2: 283-302.

Smith EB. Fibrinogen and atherosclerosis. Wien Klin Wochenschr 1993; 105: 417-24.

Smith EB. Fibrin deposition and fibrin degradation products in atherosclerotic plaques. Thromb Res 1994; 75: 329-35.

Smith EB, Thompson WD. Fibrin as a factor in atherogenesis. Thromb Res 1994; 73: 1-19.

Smith FB, Lowe GDO, Fowkes FGR et al. Smoking, haemostatic factors and lipid peroxides in a population case control study of peripheral arterial disease. Atherosclerosis 1993; 102: 155-62.

Smith FB, Lee AJ, Rumley A, Fowkes FGR, Lowe GDO. Tissue-plasminogen activator, plasminogen activator inhibitor and risk of peripheral arterial disease. Atherosclerosis 1995; 115: 35-43.

Smith FB, Lee AJ, Fowkes FGR et al. Haemostatic factors as predictors of ischaemic heart disease and stroke in the Edinburgh Artery Study. Arterioscler Thromb Vasc Biol 1997; 17: 3321-5.

Smith I, Franks PJ, Greenhalgh RM, Poulter NR, Powell JT. The influence of smoking cessation and hypertriglyceridaemia on the progression of peripheral arterial disease and the onset of critical ischaemia. Eur J Vasc Endovasc Surg 1996; 11: 402-8.

Sprengers ED, Kluft C. Plasminogen activator inhibitors. Blood 1987; 69: 381-7.

Stern DM, Brett J, Harris K, Nawroth PP. Participation of endothelial cells in the protein C and S anticoagulant pathway: synthesis and release of protein S. J Cell Biol 1986; 102: 1971-78.

Stone MC, Thorp JM. Plasma fibrinogen - a major coronary risk factor. J R Coll Gen Pract 1985; 35: 565-9.

Stormer B, Horsch R, Kleinschmidt F et al. Blood viscosity in patients with peripheral arterial disease in the areas of low shear rates. J Cardiovasc Surg 1974; 15: 577-84.

Strandness DE, Stahler C. Arteriosclerosis obliterans, manner and rate of progression. JAMA 1966; 196: 1-4.

Strong JP, Richards ML. Cigarette smoking and atherosclerosis in autopsied men. Atherosclerosis 1976; 23: 451-76.

Stuart J, George AJ, Davies AJ, Auckland A, Hurlow RA. Haematological stress syndrome in atherosclerosis. J Clin Pathol 1981; 34: 464-7.

Suenson E, Lützen O, Thorsen S. Initial plasmin-degradation of fibrin as the basis of a positive feed-back mechanism in fibrinolysis. Eur J Biochem 1984; 140: 513-22.

Sweetnam PM, Thomas HF, Yarnell JWG et al. Fibrinogen, viscosity and the 10-year incidence of ischaemic heart disease. The Caerphilly and Speedwell Studies. Eur Heart J 1996; 17: 1814-20.

Sweetnam PM, Yarnell JWG, Lowe GDO et al. The relative power of heat-precipitation nephelometric and clottable (Clauss) fibrinogen in the prediction of ischaemic heart disease: the Caerphilly and Speedwell Studies. Br J Haematol 1998; 100: 582-8.

Swick N. Darstellung der niere und harnwege in röntgenbild durch intravenvöse kontraststoffes des urosclectans. Klin Wochenschr 1929; 8: 2087-2011.

Takano K, Yamaguchi T, Uchida K. Markers of a hypercoagulable state following acute ischemic stroke. Stroke 1992; 23: 194-8.

Thiele BL, Strandness DE. Accuracy of angiographic quantification of peripheral atherosclerosis. Prog Cardiovasc Dis 1983; 26: 223-35.

Thompson SG, Kienast J, Pyke SDM, Haverkate F, van de Loo JCW. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. N Engl J Med 1995; 332: 635-41.

Thompson WD, Smith EB. Atherosclerosis and the coagulation system. J Pathol 1989; 159: 97-106.

Töpfer-Peterson E, Lottspeich F, Henschen A. Carbohydrate linkage site in the β-chain of human fibrin. Hoppe-Seyler's Z Physiol Chemie 1972; 357: 1509-13.

Van der Bom JG, de Knijff P, Haverkate F et al. Tissue plasminogen activator and risk of myocardial infarction. The Rotterdam Study. Circulation 1997; 95: 2623-7.

Van Hinsbergh VWM, Sprengers ED, Kooistra T. Effect of thrombin on the production of plasminogen activators and PA inhibitor type-1 by human foreskin microvascular endothelial cells. Thromb Haemost 1987; 57: 148-53.

Van Pelt-Verkuil E, van de Mee P, Emeis JJ. Defibrinogenation by Arvin reduces air-drying-induced arteriosclerosis in rat carotid artery. Thromb Haemost 1989; 61: 246-9.

Varizi ND, Smith DH, Winer RL et al. Coagulation and inhibitory and fibrinolytic proteins in essential hypertension. J Am Soc Nephrol 1993; 4: 222-8.

Vasse M, Soria J, Mirshahi SS, Caen J, Vannier JP, Soria C. Positive and negative regulation of fibrinogen biosynthesis by cytokines. Blood Coag Fibrinol 1994; 5(Suppl 2): (Abstr O-7).

Veldman FJ, Vorster HH, Jerling J, ventner CS. Effects of soluble dietary fibre on fibrin clot structure. Blood Coag Fibrinol 1994; 5(Suppl 2): (Abstr P-10).

Violi F, Criqui M, Longoni A, Castiglioni C, the A.D.E.P. Group. Relation between risk factors and cardiovascular complications in patients with peripheral arterial disease. Atherosclerosis 1996; 120: 25-35.

Vogelberg KH, Berchtold P, Berger H et al. Primary hyperlipoproteinaemias as risk factors in peripheral arterial disease documented by arteriography. Atherosclerosis 1975; 22: 271-85.

Vogt MT, Wolfson SK, Kuller LH. Segmental arterial disease in the lower extremities: correlates of disease and relationships to mortality. J Clin Epidemiol 1993; 46: 1267-76.

von Rokitansky K. Abnormal conditions of the arteries. In: A manual of pathological anatomy. London: Sydenham Society, 1852: Vol IV, part II, Chapter 3.

Walden R, Adar R, Rubenstein ZJ, Bass A. Distribution and symmetry of atherosclerotic lesions of the lower extremities: an arteriographic study of 200 limbs. Cardiovasc Interv Radiol 1985; 8: 180-2.

Warlow CP. Cerebrovascular disease. In: Weatherall DJ, Ledingham JGG, Warrell DA, eds. Oxford Medical Publications: 1987, 21.155-21.170.

Watanabe K, Yoshitomi F, Tanaka K. Fibrinogen degradation products influence PGI2 synthesis by cultured porcine aortic endothelial and smooth muscle cells. Atherosclerosis 1984; 51: 151-61.

Weiss NS. Cigarette smoking and arteriosclerosis obliterans: an epidemiological approach. Am J Epidemiol 1972; 95: 17-25.

Widmer LK, Biland L, Da Silva A. Risk profile and occlusive peripheral artery disease (OPAD). In: Proceedings of 13th International Congress of Angiology. Athens, 9-14 June. 1985.

Widmer LK, Da Silva A. Historical perspectives and the Basle Study. In: Fowkes FGR, ed. Epidemiology of peripheral arterial disease. London: Springer-Verlag, 1991: 69-83.

Wilhelmsen L, Svärdsudd K, Korsan-Bengtsen K et al. Fibrinogen as a risk factor for stroke and myocardial infarction. N Engl J Med 1984; 311: 501-5.

Wiman B, Collen D. On the mechanism of the reaction between human antiplasmin and plasmin. Eur J Biochem 1978; 84: 573-8.

Wiseman SA, Powell JT, Barber N, Humphries SE, Greenhalgh RM. Influence of apolipoproteins on the anatomical distribution of arterial disease. Atherosclerosis 1991; 89: 231-7.

Wojta J, Gallicchio M, Zoeller H et al. Thrombin stimulates expression of tissue type plasminogen activator and plasminogen activator inhibitor type 1 in cultured human vascular smooth muscle cells. Thromb Haemost 1993; 70: 469-74.

Wolfe JHN. Defining the outcome of critical ischaemia. A one year prospective study. Br J Surg 1986; 73: 321.

Woodburn KR, Lowe GDO, Rumley A, Love J, Pollock JG. Relation of haemostatic, fibrinolytic and rheological variables to the angiographic extent of peripheral arterial occlusive disease. Int Angiol 1995; 14: 346-52.

Woodburn KR, Rumley A, Lowe GDO et al. Clinical, biochemical and rheologic factors affecting the outcome of infra-inguinal bypass grafting. J Vasc Surg 1996; 24: 639-46.

Woodward M, Tunstall-Pedoe H. An iterative approach for identifying smoking deceivers with application to the Scottish Heart Health Study. Prev Med 1992; 21: 88-97.

Woodward M, Lowe GDO, Rumley A, Tunstall-Pedoe H. Fibrinogen as a risk factor for coronary heart disease and mortality in middle-aged men and women: The Scottish Heart Health Study. Eur Heart J 1998; 19: 55-62.

Wosornu D, Allardyce W, Ballantyne D, Tansey P. Influence of power and aerobic exercise training on haemostatic factors after coronary artery surgery. Br Heart J 1992; 68: 181-6.

Yarnell JWG, Fehily AM, Milbank J et al. Determinants of plasma lipoproteins and coagulation factors in men from Caerphilly, South Wales. J Epidemiol Community Health 1983; 37: 137-40.

Yu S, Sher B, Kudryk B, Redman CM. Intracellular assembly of human fibrinogen. J Biol Chem 1983; 258: 13407-10.

APPENDIX 1

Hospital Record No ..... Subject No .....

SITES OF ATHEROMA STUDY

CONSENT FORM

Purpose of the Study

The purpose of this study is to obtain further information as to why narrowing of the arteries tends to occur in certain areas of the leg, with a view to improving prevention and treatment of the disease.

Research Examination

A blood sample will be taken and your blood pressure will be measured on both arms and ankles after five minutes rest. You will then be asked some questions about your health and smoking habits.

Consent Agreement

I understand the purpose of this research which has been fully explained to me by a member of the research team. The study has been given ethical approval by a Medical Ethics Sub-Committee of the Lothian Health Board.

I give my consent to the research team carrying out a medical examination on me as described, although I can withdraw from the examination at any point if I so wish.

NAME .....
(Capitals)

ADDRESS .....

.....

.....

SIGNATURE .....

SIGNATURE OF RESEARCH TEAM MEMBER .....

SITES OF ATHEROMA STUDY

VENEPUNCTURE RECORDING FORM

RECORDER FS: 1 OTHER: 2

SUBJECT NAME ..... DATE .....

TIME OF VENEPUNCTURE .....

VENEPUNCTURE

	Yes	No
Has patient had jaundice in last 12 months?	☐	☐
	Yes	No
Has patient ever had hepatitis B/serum jaundice?	☐	☐

Was venepuncture normal?	1
Was venepuncture difficult/slow?	2
Was venepuncture not possible?	3

COMMENTS

APPENDIX III

Record No ..... Study No .....

SITES OF ATHEROMA STUDY

BLOOD PRESSURE RECORDING FORM

Subject Name ..... Date .....

Systolic Blood Pressure mmHg

	R. Brachial	L. Brachial
OBSERVED	☐☐☐	☐☐☐
RANDOM ZERO	☐☐☐	☐☐☐
ADJUSTED	☐☐☐	☐☐☐

Diastolic Blood Pressure mmHg

	R. Brachial	L. Brachial
OBSERVED	☐☐☐	☐☐☐
RANDOM ZERO	☐☐☐	☐☐☐
ADJUSTED	☐☐☐	☐☐☐

Ankle Systolic Blood Pressure mmHg

	Right	Left
OBSERVED	☐☐☐	☐☐☐
RANDOM ZERO	☐☐☐	☐☐☐
ADJUSTED	☐☐☐	☐☐☐
ABPI	☐☐☐	☐☐☐

COMMENTS

SITES OF ATHEROMA STUDY

QUESTIONNAIRE

THE INFORMATION GATHERED IN THIS QUESTIONNAIRE IS STRICTLY CONFIDENTIAL AND WILL ONLY BE USED BY THE RESEARCH TEAM IN THIS STUDY. THE RESULTS WILL APPEAR ONLY AS GENERAL STATISTICS WITH NO MEANS OF INDIVIDUAL IDENTIFICATION.

PLEASE COMPLETE THE FOLLOWING IN BLOCK CAPITALS:

TITLE MR/MRS/MISS .....

SURNAME .....

FORENAMES .....

DATE OF BIRTH .....

DATE .....

IF YOU NEED ANY HELP COMPLETING THIS QUESTIONNAIRE THE RESEARCH TEAM WILL BE PLEASED TO ASSIST.

THANK YOU VERY MUCH FOR THIS INFORMATION. WE ARE GRATEFUL FOR YOUR CO-OPERATION IN THIS STUDY.

I MEDICAL HISTORY

WE SHOULD LIKE TO ASK YOU SOME QUESTIONS ABOUT ILLNESSES YOU MAY HAVE HAD IN THE PAST OR YOU HAVE AT PRESENT. HAVE YOU EVER BEEN TOLD BY YOUR DOCTOR THAT YOU HAVE OR HAVE HAD ANY OF THESE ILLNESSES? PLEASE TICK ONE BOX.

		Yes	No	Unsure
i)	ANGINA	☐	☐	☐
ii)	MYOCARDIAL INFARCTION/CORONARY THROMBOSIS/HEART ATTACK	☐	☐	☐
iii)	HIGH BLOOD PRESSURE	☐	☐	☐
iv)	STROKE	☐	☐	☐
v)	DIABETES	☐	☐	☐
vi)	BLOOD CLOT IN LEG OR LUNG	☐	☐	☐
vii)	OTHER SERIOUS ILLNESSES	.....		
		.....		

II MEDICATIONS

ARE YOU AT PRESENT RECEIVING ANY REGULAR MEDICATIONS FROM YOUR DOCTOR? PLEASE ANSWER YES OR NO AS APPROPRIATE TO ALL THE FOLLOWING QUESTIONS. PLEASE GIVE NAMES OR DRUGS IF POSSIBLE.

		Yes	No
i)	DRUGS TO IMPROVE CIRCULATION	☐	☐
Name	.....		
ii)	DRUGS TO LOWER BLOOD PRESSURE	☐	☐
Name	.....		
iii)	DIURETICS/WATER TABLETS	☐	☐
Name	.....		
iv)	INSULIN INJECTIONS	☐	☐
Name	.....		
v)	DIABETIC TABLETS	☐	☐
Name	.....		
vi)	ASPIRIN TABLETS	☐	☐

	Yes	No
HAVE YOU TAKEN ANY ASPIRIN TABLETS IN THE LAST 10 DAYS?	☐	☐

IF YES, HOW MANY? .....

	Yes	No
DO YOU TAKE ANY OTHER MEDICINES?	☐	☐

WHAT ARE THEY? PLEASE GIVE NAMES IF POSSIBLE

....

....

III OTHER MEMBERS OF YOUR FAMILY

HAVE ANY MEMBERS OF YOUR FAMILY BEEN DIAGNOSED AS HAVING ANY OF THESE ILLNESSES?

		Father	Mother	Brother or Sister	Son or Daughter
i)	HEART ATTACK	☐	☐	☐	☐
ii)	ANGINA	☐	☐	☐	☐
iii)	HARDENING OF THE ARTERIES IN THE LEG/ CLAUDICATION	☐	☐	☐	☐

	Brothers	Sisters
DO YOU HAVE ANY BROTHERS AND SISTERS?	☐	☐

	Sons	Daughters
DO YOU HAVE ANY CHILDREN?	☐	☐

IV SMOKING

	Yes	No
DO YOU SMOKE AT PRESENT?	☐	☐

IF NO, PROCEED TO QUESTION 5

1. WHAT DO YOU USUALLY SMOKE NOW?

	Yes	No
CIGARETTES	☐	☐
PIPE	☐	☐
CIGARS	☐	☐

2. HOW MANY DO YOU USUALLY SMOKE NOW?

CIGARETTES/ROLLED TOBACCO PER DAY	.....	cigarettes/oz
PIPE TOBACCO PER WEEK	.....	oz
CIGARS PER WEEK	.....	cigars

3. HOW MANY YEARS DURING YOUR LIFE HAVE YOU SMOKED CIGARETTES? ..... years

4. HOW MANY CIGARETTES HAVE YOU SMOKED ON AVERAGE PER DAY DURING THE PERIOD YOU HAVE SMOKED? ..... cigarettes

PROCEED TO SECTION V OVER PAGE

	Yes	No
5. IF YOU DO NOT SMOKE NOW, HAVE YOU EVER SMOKED REGULARLY?	☐	☐

IF NO, PROCEED TO SECTION V OVER PAGE

6. WHAT DID YOU USUALLY SMOKE?

	Yes	No
CIGARETTES	☐	☐
PIPE	☐	☐
CIGARS	☐	☐

7. HOW MUCH DID YOU SMOKE ON AVERAGE WHILE YOU WERE A SMOKER?

CIGARETTES/ROLLED TOBACCO PER DAY	.....	cigarettes/oz
PIPE TOBACCO PER WEEK	.....	oz
CIGARS PER WEEK	.....	cigars

8. FOR HOW MANY YEARS DID YOU SMOKE CIGARETTES? ..... years

9. IF YOU SMOKED CIGARETTES, HOW LONG IS IT SINCE YOU FINALLY GAVE UP? ..... years ..... months

V LEG PAIN/CLAUDICATION

		Yes	No	I Am Unable To Walk
1.	DO YOU GET A PAIN OR DISCOMFORT IN YOUR LEG(S) WHEN YOU WALK?	☐	☐	☐

IF YOU ANSWERED "YES" TO QUESTION 1, PLEASE ANSWER THE FOLLOWING QUESTIONS. OTHERWISE PROCEED TO QUESTION 8 OVER THE PAGE.

		Yes	No
2.	DOES THIS PAIN EVER BEGIN WHEN YOU ARE STANDING STILL OR SITTING?	☐	☐
3.	DO YOU GET IT IF YOU WALK UPHILL OR HURRY?	☐	☐
4.	DO YOU GET IT WHEN YOU WALK AT AN ORDINARY PACE ON THE LEVEL?	☐	☐

5. WHAT HAPPENS TO IT IF YOU STAND STILL?

USUALLY CONTINUES MORE THAN 10 MINUTES ☐

USUALLY DISAPPEARS IN 10 MINUTES OR LESS ☐

6. WHERE DO YOU GET THIS PAIN OR DISCOMFORT?

MARK THE PLACES(S) WITH AN "X" ON THE DIAGRAM BELOW

7. HOW LONG DID YOU EXPERIENCE THIS PAIN BEFORE ATTENDING THE HOSPITAL CLINIC?

UNDER 6 MONTHS ☐ ☐

MORE THAN 6 MONTHS ..... years ..... months

	Yes	No
8. HAVE YOU EVER HAD SURGERY ON THE ARTERIES OF YOUR LEGS IN THE PAST?	☐	☐

IF YES, WAS THE SURGERY

	Yes	No
TO REMOVE ANY PART OF YOUR LEG OR FOOT	☐	☐
A PREVIOUS ARTERIOGRAM?	☐	☐
ANGIOPLASTY (BALLOON DILATATION)?	☐	☐
RECONSTRUCTIVE SURGERY? (WHERE PIECES OF THE VEINS IN YOUR LEGS ARE REMOVED TO REPLACE DISEASED OR BLOCKED ARTERIES ELSEWHERE IN YOUR BODY)	☐	☐

WHEN DID THIS SURGERY TAKE PLACE? ☐☐☐☐☐☐

Blood Processing Procedure

Size of Tube	Anti-coagulant/ blood	Factor	Invert/ roller	Centrifuge	Aliquot size	Micro-tube colour	Ice	Storage	Delivery
10 ml plastic	1 ml tri-sodium citrate trasylol 9 ml blood	a) Fibrinogen b) von Willebrand Factor c) Fibrin D-dimer	Invert 5/6x No	2800 rpm 10 mins 4°C	5x0.5 ml plasma	Red	Dry	-40°C Freezer	Monthly to Glasgow Red Star
5 ml plastic	0.5 ml tri-sodium citrate 4.5 ml blood	Plasminogen activator inhibitor-Type 1	Invert Yes	2800 rpm 10 mins 4°C	2x0.5 ml plasma	Green	Dry	-40°C Freezer	Monthly to Glasgow Red Star
10 ml glass	10 ml blood	a) Total cholesterol b) HDL-cholesterol c) Serum thiocyanate	No No No	Stand for 1 hour	3 ml serum	Clear	Wet	-40°C Freezer	Monthly to Glasgow Red Star
		d) Serum cotinine	No		2x0.5 ml serum	Blue	Wet	-40°C Freezer	Monthly to Dundee Red Star

Patient :

DOB/XR No :

Date of Angio :

ABDOMINAL AORTIC SEGMENT

Occlusive Pattern

Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%	Additive Score : Location :
13	4	2	1	Single
	5	3	2	Multiple ≤ H
15	6	4	3	Multiple > H

: Vectorial Score

RIGHT LEG

COMMON ILIAC SEGMENT

Occlusive Pattern

Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%	Additive Score : Location :
13	4	2	1	Single
	5	3	2	Multiple ≤ H
15	6	4	3	Multiple > H

: Vectorial Score

LEFT LEG

COMMON ILIAC SEGMENT

Occlusive Pattern

Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%	Additive Score : Location :
13	4	2	1	Single
	5	3	2	Multiple ≤ H
15	6	4	3	Multiple > H

: Vectorial Score

EXTERNAL ILIAC SEGMENT

Occlusive Pattern

Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%	Additive Score : Location :
13	4	2	1	Single
	5	3	2	Multiple ≤ H
15	6	4	3	Multiple > H

: Vectorial Score

EXTERNAL ILIAC SEGMENT

Occlusive Pattern

Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%	Additive Score : Location :
13	4	2	1	Single
	5	3	2	Multiple ≤ H
15	6	4	3	Multiple > H

: Vectorial Score

RIGHT LEG

INTERNAL ILIAC SEGMENT

Occlusive Pattern

Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%	Additive Score : Location :
13	4	2	1	Single
	5	3	2	Multiple ≤ H
15	6	4	3	Multiple > H

: Vectorial Score

LEFT LEG

INTERNAL ILIAC SEGMENT

Occlusive Pattern

Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%	Additive Score : Location :
13	4	2	1	Single
	5	3	2	Multiple ≤ H
15	6	4	3	Multiple > H

Vectorial Score

PROFUNDA FEMORIS SEGMENT

Occlusive Pattern

Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%	Additive Score : Location :
13	4	2	1	Single
	5	3	2	Multiple ≤ H
15	6	4	3	Multiple > H

: Vectorial Score

PROFUNDA FEMORIS SEGMENT

Occlusive Pattern

Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%	Additive Score : Location
13	4	2	1	Single
	5	3	2	Multiple ≤ H
15	6	4	3	Multiple > H

: Vectorial Score

SUPERFICIAL FEMORAL SEGMENT

Occlusive Pattern

Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%	Additive Score : Location :
13	4	2	1	Single
	5	3	2	Multiple ≤ H
15	6	4	3	Multiple > H

: Vectorial Score

SUPERFICIAL FEMORAL SEGMENT

Occlusive Pattern

Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%	Additive Score : Location :
13	4	2	1	Single
	5	3	2	Multiple ≤ H
15	6	4	3	Multiple > H

: Vectorial Score

RIGHT LEG

POPLITEAL SEGMENT

Occlusive Pattern

Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%	Additive Score : Location :
13	4	2	1	Single
	5	3	2	Multiple ≤ H
15	6	4	3	Multiple > H

: Vectorial Score

LEFT LEG

POPLITEAL SEGMENT

Occlusive Pattern

Occlusion	Stenosis >50%	Stenosis ≤ 50%	Plaques ≤ 25%	Additive Score : Location :
13	4	2	1	Single
	5	3	2	Multiple ≤ H
15	6	4	3	Multiple > H

: Vectorial Score

PERIPHERAL VASCULAR CLINIC
Royal Infirmary of Edinburgh
Lauriston Place
EDINBURGH EH3 9YW

Date

Dear

I am writing to you as a patient who has been seen recently at the Peripheral Vascular Clinic in the Royal Infirmary to invite you to have a further examination. This will be part of a Medical Research Council study which will be vitally important in helping us to understand artery disease and to improve treatment. The information will also prove helpful in your case.

I should like you to attend the Peripheral Vascular Clinic for an examination to be carried out by a member of our specially trained research team. Your blood pressure will be measured on both arms and on both ankles. Also small samples of urine and blood will be collected. You will then be given a short questionnaire on your health, after which tea or coffee will be served.

Your appointment is as follows:

Date	Day	Time	Place
			Peripheral Vascular Clinic Royal Infirmary of Edinburgh

If you have any queries please telephone Mrs Anna Rattray at: 031-667 1011, Ext. 2489, 9am-2pm daily Monday-Friday.

We very much look forward to seeing you at the clinic, at which time we shall make every effort to make you feel welcome and answer any queries.

With best wishes.

Yours sincerely,

Dr E Housley
Consultant Physician

Please tear off and return in pre-paid envelope Rec. No.....

Name ..... Tel No. .....

Address ..... Hospital No. .....

.....

DELETE AS APPROPRIATE*:

* Appointment is suitable
or
* Appointment is not suitable, I will telephone Mrs Rattray 031-667 1011 ext. 2589.
or
* Appointment is not suitable, please send me another appointment

PROGNOSTIC STUDY OF INTERMITTENT CLAUDICATION

Q U E S T I O N N A I R E

THE INFORMATION GATHERED IN THIS QUESTIONNAIRE IS STRICTLY CONFIDENTIAL
The Questionnaire contains information which will only be used by the research team in this study. The results obtained will appear only as general statistics with no means of individual identification possible

Please complete the following in block capitals:

TITLE: Mr/Mrs/Miss .....

SURNAME: .....

MAIDEN/PREVIOUS NAMES: .....

FORENAMES: .....

DATE: .....

N.H.S. NO. (if known): .....

G.P. & ADDRESS .....

PLEASE COMPLETE THE REMAINDER OF THE QUESTIONNAIRE WITH THE HELP OF THE RESEARCH TEAM

THANK YOU VERY MUCH FOR THIS INFORMATION. WE ARE GRATEFUL FOR YOUR CO-OPERATION IN THE STUDY.

Subject No. .....

PERSONAL HISTORY

1. (a) Please tick one box

	Male	Female
	☐	☐

(b) Marital Status

Single	☐
Married	☐
Divorced	☐
Other	☐

2. Enter your date of birth

Day	Month	Year
☐☐	☐☐	☐☐

MEDICATIONS

3. Are you at present receiving any regular medications from your doctor. Please answer Yes or No, as appropriate, to all the following questions

	Yes	No
Drugs to improve circulation	☐	☐
Drugs to lower blood pressure	☐	☐
Diuretics/water tablets	☐	☐
Insulin injections	☐	☐
Diabetic tablets	☐	☐
Other tablets - please give names of medicines if possible	☐	☐

....

....

....

MEDICAL HISTORY

4. The questions below concern illnesses you may have had in the past or illnesses you have at present. Please answer Yes or No, as appropriate, to all of the following questions.

Have you ever been told by your doctor that you have had or have any of the conditions listed below?

	Yes	No
i. Angina	☐	☐
ii. Myocardial infarction/coronary thrombosis/heart attack	☐	☐
iii. High blood pressure	☐	☐
iv. Stroke	☐	☐
v. Diabetes	☐	☐
vi. Deep Venous thrombosis or pulmonary embolism	☐	☐

LEG PAIN

		Yes	No
5.	i. Do you get a pain in either leg on walking?	☐	☐
	ii. Do you get this pain in your right calf?	☐	☐
	iii. Do you get this pain in your left calf?	☐	☐
	iv. Does this pain ever begin when you are standing still or sitting?	☐	☐
	v. Do you get it when you walk uphill or hurry?	☐	☐
	vi. Do you get it when you walk at an ordinary pace on the level?	☐	☐
	vii. Does the pain ever disappear while you are still walking?	☐	☐

viii. What do you do if you get it when you are walking?

Stop	1	☐
Slow down	2	☐
Continue at same pace	3	☐

ix. What happens to it if you stand still?

Usually continues for more than 10 minutes	1	☐
Usually disappears in 10 minutes or less	2	☐

x. How long did you experience this pain before attending the Peripheral Vascular Clinic?

Under 6 months	1	☐
6 months or over and under 1 year	2	☐
1 year or over and under 18 months	3	☐
18 months or longer	4	☐

	Yes	No
6. Have you ever had surgery on the arteries of your legs, surgery to remove any part of the leg or foot or angioplasty (Balloon dilatation) to your leg?	☐	☐

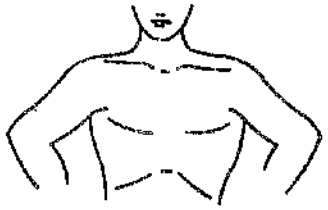
FOR OFFICE USE ONLY GRADE

CHEST PAIN

		Yes	No
7. i.	Do you ever get pain or discomfort in your chest? IF NO, PROCEED TO QUESTION 8	☐	☐
ii.	Do you get this pain or discomfort when you walk uphill or hurry? IF NO, PROCEED TO QUESTION 8(vii)	☐	☐
iii.	Do you get it when you walk at an ordinary pace on the level?	☐	☐
iv.	When you get any pain or discomfort in your chest what do you do?		
	Stop	☐	
	Slow down	☐	
	Continue at the same pace	☐	
v.	Does it go away when you stand still or sit down?	☐	☐
vi.	How soon?		
	10 minutes or less	☐	
	more than 10 minutes	☐	

vii. Where do you get this pain or discomfort? Mark the place(s) with 'X' on the diagram.

RIGHT



LEFT

		Yes	No
8. i.	Have you ever had a severe pain across the front of your chest lasting for half an hour or more?	☐	☐

ii. What was the cause? .....

FOR OFFICE USE ONLY A: GRADE MI:

SMOKING

9. Smoking has been linked with many health problems. It is important that you answer the following section as accurately as possible. Please tick appropriate boxes

	Yes	No
Do you smoke at present? IF NO, PROCEED TO QUESTION 11	☐	☐

10. i. What do you usually smoke now?

cigarettes	☐
pipe	☐
cigars	☐

ii. How many do you usually smoke now?

cigarettes/rolled tobbaco per day	..... cigarettes/oz
pipe tobacco per week	..... oz
cigars - per week	..... cigars

iii. For how many years during your life have you smoked cigarettes? ..... years

iv. How many cigarettes have you smoked on average per day during the period you have smoked? ..... cigarettes

PROCEED TO QUESTION 13

	Yes	No
11. If you do NOT smoke now, have you ever smoked regularly? IF NO, PROCEED TO QUESTION 13	☐	☐

12. i. What did you usually smoke?

cigarettes	☐
pipe	☐
cigars	☐

ii. How much did you smoke on average while you were a smoker?

cigarettes/rolled tobbaco per day	..... cigarettes/oz
oz. pipe tobacco per week	..... oz.
cigars per week	..... cigars

iii. For how many years did you smoke cigarettes? ..... years

iv. If you smoked cigarettes, how long is it since you finally gave up? ..... years ..... months

	Yes	No
13. Is any other member of your household a smoker?	☐	☐

FOR OFFICE USE ONLY A: GRADE MI:

APPENDIX IX

Dear Dr

RE:
DOB:

Your patient has been followed up for the last six years as part of a research investigation to determine whether haemorheological factors influence the prognosis of arterial disease. We would be very grateful if you could provide us with any information concerning any cardiovascular events, hospital admissions and medication that have occurred in the past six years. Please complete the enclosed form and return it to us in the envelope provided. However, if this is not convenient, a member of the research staff would be available to visit your practice to obtain this information.

If you have any queries, please telephone Ms Felicity Smith on 0131-650-3245.

Thank you very much for your help.

Yours sincerely

Dr Gillian Leng
Clinical Research Fellow

Ms Felicity Smith
Research Associate

APPENDIX X

STUDY NO.....

PROGNOSTIC STUDY OF INTERMITTENT CLAUDICATION

Patient name: .....

Address: .....

.....

Date of Birth: .....

	Yes	No
Does this patient still attend your practice?	☐	☐

Change of address (if known) .....

.....

.....

Please tick if your patient has been newly diagnosed with any of the following conditions in the last 6 years, and provide details as appropriate:

		Date	Hospital
Myocardial Infarction	☐	.....	.....
Angina Pectoris	☐	.....	.....
Stroke	☐	.....	.....
Transient Ischaemic Attack	☐	.....	.....
Critical Limb Ischaemia (please specify)	☐	.....	.....
Aortic Aneurysm	☐	.....	.....
Other Cardiovascular Event (please specify)	☐	.....	.....
Has experienced none of the above	☐		

MANY THANKS FOR YOUR HELP. PLEASE RETURN IN PRE-PAID ENVELOPE.

APPENDIX XI

Study No .....

PROGNOSTIC STUDY OF INTERMITTENT CLAUDICATION

1996 QUESTIONNAIRE

THE INFORMATION GATHERED IN THIS QUESTIONNAIRE IS STRICTLY CONFIDENTIAL. This information will only be used by the research team in this study. The results obtained will appear only as general statistics with no means of individual identification possible.

Please complete the following in block capitals:

TITLE Mr/Mrs/Miss .....

SURNAME .....

FORENAMES .....

DATE .....

GP NAME & ADDRESS .....

.....

.....

.....

THANK YOU VERY MUCH FOR THIS INFORMATION.
WE ARE GRATEFUL FOR YOUR CO-OPERATION IN THE STUDY.

A MEDICAL HISTORY

HAVE YOU HAD ANY OF THE FOLLOWING ILLNESSES OR CONDITIONS IN THE LAST 6 YEARS :

Please tick boxes as appropriate.

		Yes	No
1.	Angina	☐	☐
2.	Heart Attack (Myocardial Infarction Coronary Thrombosis)	☐	☐
3.	Stroke	☐	☐
4.	Diabetes	☐	☐
5.	Blood clot in leg veins or lungs	☐	☐
6.	Aortic aneurysm	☐	☐
7.	High blood pressure	☐	☐

If yes to any of the above conditions, please provide details of any hospital attendances that were required, either at a clinic or as an inpatient.

Condition	Hospital	Date of Attendance
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....

B MEDICATION

PLEASE LIST ANY REGULAR MEDICATIONS THAT YOU ARE TAKING AT PRESENT.

Name of Medication	Duration of Treatment	Condition Prescribed For
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....

C LEG PAIN

		Yes	No
1.	Do you still get a pain in either leg on walking?	☐	☐

IF YES, PLEASE COMPLETE THE FOLLOWING QUESTIONS, OTHERWISE PROCEED TO SECTION D OVER THE PAGE.

2.	Does the pain ever begin when you are standing still or sitting?	☐	☐
3.	Do you get this pain in your calf (or calves)?	☐	☐
4.	Do you get it when you walk uphill or hurry?	☐	☐
5.	Do you get it when you walk at an ordinary pace on the level?	☐	☐
6.	Does the pain ever disappear while you are still walking?	☐	☐

7. What do you do if you get it when you are walking?

- ☐ Stop
- ☐ Slow down
- ☐ Continue at same pace

8. What happens to it if you stand still?

- ☐ Usually continues more than 10 minutes
- ☐ Usually disappears in 10 minutes or less

9. Where do you get this pain or discomfort?
Mark the place(s) with an X on the diagrams below

D LEG PAIN TREATMENT

HAVE YOU HAD ANY OF THE FOLLOWING TREATMENTS FOR YOUR LEG PAIN IN THE LAST 6 YEARS ?

		Yes	No
1.	Angioplasty (balloon dilatation)	☐	☐
2.	Surgery, eg bypass, amputation	☐	☐
3.	Treatment for ulcer or gangrene	☐	☐
4.	Other	☐	☐

If yes to any of the above, please give details:

Treatment	Hospital	Date
.....	.....	.....
.....	.....	.....
.....	.....	.....

E CHEST PAIN

		Yes	No
1.	Do you ever get pain or discomfort in your chest?	☐	☐

IF YES, PLEASE COMPLETE THE FOLLOWING QUESTIONS, OTHERWISE PROCEED TO SECTION F.

2.	Has this pain or discomfort developed in the past 6 years?	☐	☐
3.	Do you get it when you walk uphill or hurry?	☐	☐
4.	Do you get it when you walk at an ordinary pace on the level?	☐	☐

5. When you get the pain or discomfort in your chest, what do you do?

   ☐ Stop
   ☐ Slow down
   ☐ Continue at same pace

6.	Does it go away when you stand still or sit down?	☐	☐

7. How soon does it go away?

   ☐ 10 minutes or less
   ☐ more than 10 minutes

8. Where do you get this pain or discomfort?
   Mark the place(s) with X on the diagram

9.	Have you ever had a severe pain across the front of your chest lasting for more than half an hour in the last 6 years?	☐	☐

10. What was the cause? .....

F SMOKING

1.	Do you smoke?	☐	☐

IF YES:

2. How much do you smoke on average?

cigarettes/rolled tobacco	.....	cigs/oz per day
pipe tobacco	.....	oz per week
cigars	.....	cigars per week

IF NO :

		Yes	No
3.	I have never smoked	☐	☐
4.	I used to smoke and stopped ..... months ago	☐	☐

RECORDING FORM FOR STUDY PATIENT DEATHS

A PATIENT IDENTIFICATION CARDIOVASCULAR EVENT NO _

STUDYNO _____ NAME
ADDLN1
RECNO _____ ADDLN2
ADDLN3
DOB _____
GP
DOD ___ ___ ADDLN1
ADDLN2

B INFORMATION SOURCE

DATE NOTIFIED _____
NHSCR __ 1
RELATIVE __ 2
HOSPITAL\PVC __ 3
GP __ 4
OTHER __ 5
UNKNOWN __ 9

C DEATH CERTIFICATE INFORMATION

CODES (410-414, 431-437)
_____ 1 _____ 3
_____ 2 _____ 4

PLACE HOME __ 1
HOSPITAL __ 2
OTHER __ 3
PM 1,2 OR 3 __

D PROVISIONAL DIAGNOSIS

MYOCARDIAL INFARCTION __ 1
STROKE __ 2
SUDDEN DEATH __ 3
THROMBOSIS/EMBOLISM __ 4
PVD __ 5
OTHER CV RELATED __ 6
UNKNOWN __ 9

E FINAL DIAGNOSIS OF CARDIOVASCULAR EVENT

MYOCARDIAL INFARCTION
DEFINITE __ 1
POSSIBLE __ 2
SUDDEN DEATH __ 3
STROKE - DEFINITE __ 4
POSSIBLE __ 5
THROMBOSIS/EMBOLISM __ 6
PVD RELATED __ 7
CV RELATED __ 8
NON CV RELATED __ 9
UNKNOWN __ 99

F CONFIRMATION CRITERIA

POST MORTEM __ 1
DEATH CERTIFICATE __ 2
MI CONFIRMED < 4 WEEKS __ 3
STROKE CONFIRMED < 6 WEEKS __ 4
OTHER __ 5

G CV DEATH CONFIRMATION

STUDY CRITERIA __ 1
CLINICAL IMPRESSION __ 2

H CONFIRMATORY INFORMATION FROM

DEATH CERTIFICATE __ 1
HOSPITAL PM __ 2
PROC FISCAL PM __ 3
HOSPITAL RECORDS __ 4
GP RECORDS __ 5
OTHER __ 6
UNKNOWN __ 9

I OTHER RELEVANT INFORMATION

YES __ 1
NO __ 2

APPENDIX XIII

Study No ☐☐☐☐☐

Name _____ GP Name _____

Address _____ GP Address _____

_____ _____

_____ _____

DOB ___/___/___

Cardiovascular Event Number ☐☐ Date ___/___/___

NHS Number _____

1. Information Source

Patient/Relative	☐ 1
GP	☐ 2
PVC	☐ 3
CSA	☐ 4
Other _____	☐ 5
Unknown	☐ 6

2. Provisional Diagnosis

Myocardial Infarction	☐ 1
Stroke	☐ 2
Angina	☐ 3
Transient Ischaemic Attack	☐ 4
Intermittent Claudication	☐ 5
Thrombosis/Embolism	☐ 6
Amputation	☐ 7
Other CV _____	☐ 8
Other Non CV _____	☐ 9
Unknown	☐ 10

3. Confirmation Source

Patient/Relative	☐ 1
GP Record	☐ 2
Hospital Record	☐ 3
Other _____	☐ 4
Unknown	☐ 5

4. Final Diagnosis

Myocardial Infarction Definite	☐ 1
Myocardial Infarction Possible	☐ 2
Primary Cardiac Arrest	☐ 3
Stroke Definite	☐ 4
Stroke Possible	☐ 5
Angina	☐ 6
Transient Ischaemic Attack	☐ 7
Intermittent Claudication	☐ 8
Rest Pain/Ulcer/Gangrene	☐ 9
Thrombosis/Embolism	☐ 10
Vascular Surgery (not amp)	☐ 11
Amputation	☐ 12
Other CV _____	☐ 13
Other Non CV _____	☐ 14
Unknown	☐ 15
Angioplasty	☐ 16
CABG	☐ 17

5. Confirmatory Criteria for MI

Pain	☐ 1
ECG	☐ 2
Enzymes	☐ 3
Equivocal ECG	☐ 4
Equivocal Enzymes	☐ 5

6. Confirmatory Criteria for Stroke

Clinical Criteria	☐ 1
CT Scan	☐ 2
Discharge Diagnosis	☐ 3

7. Final Diagnosis Confirmation

Study Criteria	☐ 1
Clinical Impression	☐ 2