Eur J Vasc Endovasc Surg **10**, 272–278 (1995)

Fibrinogen and Markers of Fibrinolysis and Endothelial Damage Following Resolution of Critical Limb Ischaemia*

K. R. Woodburn, A. Rumley, G. D. O. Lowe and J. G. Pollock

Vascular Surgery Unit and University Department of Medicine, Royal Infirmary, Glasgow, Scotland, U.K.

Objectives: *To assess the effects of resolution of critical limb ischaemia on the elevated plasma fibrinogen, cross-linked fibrin degradation products (FDP), and von Willebrand factor antigen (vWF) levels, reported in peripheral arterial occlusive disease.*
Design: *A prospective study of patients undergoing surgery for chronic critical limb ischaemia.*
Setting: *Two vascular surgery units providing tertiary referral services for the West of Scotland.*
Materials: *Venous blood samples were assayed for plasma fibrinogen, FDP D-dimer, and vWF levels, prior to surgery, together with fibrinolytic and rheological parameters, in 82 patients. Sampling was repeated 4 months after resolution of critical limb ischaemia.*
Outcome measures: *Levels of these parameters following successful resolution of critical limb ischaemia were compared with pre-operative levels, and with an age-matched random population sample.*
Main results: *Plasma fibrinogen and vWF levels were significantly lower (both* $p < 0.005$*, Wilcoxon matched pairs) following successful resolution of critical limb ischaemia in the 56 patients available for review, although levels remained higher than in population controls* ($p < 0.01$*, Mann-Whitney U-test). FDP levels were unchanged following surgery, remaining higher than in age-matched population controls* ($p < 0.01$*).*
Conclusions: *Resolution of critical limb ischaemia fails to reduce plasma fibrinogen, fibrin turnover, and vWF levels to those seen in population controls. This implies that increased fibrinogen and fibrin turnover in peripheral arterial disease is not solely a consequence of tissue ischaemia, while the persisting prothrombotic state following resolution of critical limb ischaemia has potentially important implications for graft and patient survival.*

Key Words: Fibrinogen; Endothelial damage; Critical limb ischaemia.

Introduction

A number of studies have indicated that blood rheology and plasma levels of fibrinogen and markers of fibrin turnover (cross-linked fibrin degradation products, FDP), together with the endothelial product von Willebrand Factor antigen (vWF), are abnormal in patients with symptomatic and asymptomatic peripheral arterial disease,[1–6] while there is evidence that the elevation in levels of these potential thrombotic mediators is related to the severity of arterial disease.[5,7,8] There is also evidence that elevated plasma fibrinogen and vWF levels are associated with, and predictive of, graft occlusion.[9–12] Alterations in potential thrombotic mediators may contribute to progression of atherosclerosis and arterial occlusion by a variety of means,[13–17] although if they merely reflect biochemical derangement secondary to tissue ischaemia, then resolution of critical limb ischaemia should reverse these potentially harmful alterations in blood rheology and levels of thrombotic mediators.

In order to investigate this possibility, patients with critical limb ischaemia[18] have been studied to determine the effects of revascularisation or amputation surgery on the abnormal blood rheology observed in patients with peripheral arterial occlusive disease, and to determine whether or not levels of plasma fibrinogen, FDP, vWF, factor VII, plasminogen activator inhibitor type-1 (PAI), and tissue plasminogen activator (tPA), are returned to normal population levels following surgical resolution of critical limb ischaemia.

*Presented at the 8th annual meeting of the European Society for Vascular Surgery, Berlin, Germany (September 1994).

Please address all correspondence to: Mr K. R. Woodburn, Senior Registrar, Surgical Unit, Eastern General Hospital, Seafield Road, Edinburgh, Scotland, U.K.

1078–5884/95/070272 + 07 $12·00/0

Materials and Methods

Eighty-two in-patients with a diagnosis of critical limb ischaemia[18] were enrolled in the study, and venous blood was sampled with minimal venous stasis prior to surgical intervention. Nine millilitres of blood from each sample were added to a plastic tube containing 1ml of trisodium citrate (0.109 M) and subsequently handled, stored, and assayed as previously described[6] for plasma fibrinogen (Clauss assay), cross-linked FDP (D-dimer antigen, ELISA, Agen Ltd., Parsippany, New Jersey, U.S.A.), vWF (ELISA, Dako, High Wycombe, U.K.), Plasminogen Activator Inhibitor (chromogenic activity assay, KabiVitrum Ltd. Uxbridge, Middx., U.K.), and tissue Plasminogen Activator antigen (ELISA, Biopool TintElize, Biopool AB, Umeä, Sweden). Rheological variables were measured as previously described[5] in blood anticoagulated with dipotassium edetate (1.5 mg/ml). The coefficients of variation for all of the above assays are below 10% in our laboratory. Serum albumin levels were determined on an Olympus AU 5200 analyser, using reagents supplied by Olympus and Boehringer Mannheim (U.K. Ltd.). White cell count and haemoglobin were determined in a Coulter S counter, and red cell aggregation by a photometric assay (Myrenne GmbH, Roetgen, Germany). Plasma carboxyhaemoglobin levels were measured on dipotassium EDTA anticoagulated blood on a Co-Oximeter 282 (Instrumentation Laboratory Ltd., Warrington, Cheshire, U.K.), within 6 h of sampling, non-smokers being confirmed by a plasma carboxyhaemoglobin of less than 2.0%.[19]

Patients were invited to attend for review 16 weeks after surgical treatment, and resolution of critical limb ischaemia was confirmed by relief of rest pain, resolution of infection and tissue healing, together with an increase in the ankle systolic pressure to above 50 mmHg. In patients who had undergone amputation, stump healing and relief of rest pain were taken as confirmatory of resolution of critical ischaemia. Venous blood samples were then obtained and processed in the same manner as preoperative samples.

Control samples were obtained from 80 subjects aged between 65 and 75 years of age (mean age 69 years), who were assessed in local population studies (the first, second, and third WHO MONICA surveys). These studies were carried out in random samples of the population of North Glasgow, who, in this age range, would all be expected to have some atherosclerotic arterial disease. Sample handling and storage were as previously described, and all assays were performed in the same laboratory, by the same methods, as samples from the patient population.

All statistical analyses were performed on microcomputer using the CSS: Statistica package (Statsoft, Tulsa, U.S.A.). Pre and postoperative results were compared using non-parametric Wilcoxon matched pairs testing, with pairwise deletion where data values were missing. Results were compared with controls using the Mann-Whitney U-test.

Results

There were 82 patients enrolled in the study (51 male, 31 female), with a mean age of 70 years. Sixty one patients survived to review at 16 weeks postoperatively, giving an overall 16-week mortality rate of 26%. Five of the 61 survivors were unable to attend for review, but were confirmed to be alive by their General Practitioners. There were therefore 56 patients available for review, who made up the study population, 39 of whom had undergone a prior revascularisation procedure.

There was no evidence of recurrence of critical limb ischaemia in the study population, the majority having undergone a reconstructive procedure (Table 1). The mean age of the patients attending for review was 69 years, with a range of 50–89 years, and other patient characteristics are indicated in Table 2. Infrainguinal revascularisation was performed using

Table 1. Operations carried out in 56 patients with critical limb ischaemia

Operation	No. of patients
Femoropopliteal/femorodistal graft	45
Limb amputation	4
Axillobifemoral graft	3
Patch profundaplasty	2
Femorofemoral crossover graft	2

Table 2. Patient characteristics in 56 cases of critical limb ischaemia, excluding 21 patients dying within 4 months of surgery, and five patients lost to follow up. Cardiovascular disease refers to patients with symptomatic ischaemic heart disease or cerebrovascular disease

Characteristic	No. of cases (%)
Male	33 (59)
Female	23 (41)
Current/recent smoker	38 (68)
Prior vascular surgery	34 (61)
Cardiovascular disease	31 (55)
Infected or gangrenous limb	25 (45)
Diabetes	13 (23)
Hypertension	8 (14)

ipsilateral autogenous leg vein where available, but as a consequence of previous revascularisation procedures 29 patients required insertion of a synthetic graft (PTFE or Dacron), while a suitable vein was available in only 23 patients. There were no significant differences in smoking habits between patients in whom vein grafts were used, and those in whom synthetic grafts were used (Chi-Square test 0.77(1)).

Rheological variables

Following resolution of critical limb ischaemia relative blood viscosity fell ($p < 0.05$, Wilcoxon matched pairs), while there was a trend towards a fall in the corrected blood viscosity ($p = 0.07$), together with a significant increase in serum albumin ($p < 0.005$, Table 3). Postoperative blood rheology in patients was comparable with age-matched population controls, with the exception of the white cell count, which remained elevated following surgery ($p < 0.0005$). Exclusion of the 25 patients with preoperative limb sepsis or necrosis did not alter these observations, with the exception of the postoperative fall in red cell aggregation, which attained statistical significance in the smaller subgroup.

Table 3. Rheological variables following successful surgical treatment for critical limb ischaemia in 56 patients, compared with levels in an age-matched random population sample. Figures are median values (interquartile range)

Variable	Preoperative level	Postoperative level	Population controls
Whole blood viscosity (mPa.s)	3.21 (2.76–3.50)	2.99 (2.72–3.60)	3.24 (2.99–3.70)
Haematocrit (%)	41 (37–45)	42 (38–45)	44 (41–47)
Corrected blood viscosity (mPa.s)	3.43 (3.22–3.70)	3.27 (3.06–3.58)†	3.40 (3.12–3.60)
Plasma viscosity (mPa.s)	1.38 (1.31–1.50)	1.39 (1.33–1.48)	1.36 (1.3–1.44)§
Relative blood viscosity (mPa.s)	2.50 (2.35–2.66)	2.37 (2.13–2.51)*	2.42 (2.24–2.61)
Red cell aggregation (units)	4.1 (3.0–5.5)	3.7 (3.0-4.4)	4.4 (3.5–5.0)§
White cell count ($x10^9/l$)	9.6 (8.0–12.2)	8.6 (7.0–10.2)*	6.2 (5.1–7.9)‡
Serum albumin (g/l)	38 (32–42)	41 (37–44)*	n.a.

* $p < 0.006$.
† $p = 0.07$, all Wilcoxon matched pairs testing.
‡ $p < 0.0001$.
§ $p = 0.07$, both Mann-Whitney U-test.
n.a. = result not available.

Table 4. Changes in haemostatic variables following successful surgical treatment for critical limb ischaemia in 56 patients, compared with levels in an age-matched random population sample. Figures are median values (interquartile range)

Variable	Preoperative level	Postoperative level	Population controls
Factor VII activity (IU/dl)	106 (79–116)	113 (98–119)*	113 (93–135)
Fibrinogen (g/l)	4.7 (3.6–5.5)	3.8 (3.0–4.9)*	3.1 (2.7–3.8)†
von Willebrand factor antigen (IU/dl)	177 (144–226)	153 (108–197)*	120 (90–147)†
Tissue plasminogen activator antigen (ng/ml)	8.5 (6.5–11.8)	6.9 (5.5–9.0)*	8.4 (5.4–10.7)
Plasminogen activator inhibitor activity (% pool)	103 (77–130)	90 (69–121)	78 (60–92)†
Fibrin degradation products (ng/ml)	285 (225–510)	316 (192–620)	220 (115–310)†

* $p < 0.006$, all Wilcoxon matched pairs testing.
† $p < 0.005$.
‡ $p = 0.02$, both Mann-Whitney U-test.

Haemostatic variables

Levels of the potential thrombotic mediators plasma fibrinogen, vWF, and tPA, all fell significantly following resolution of critical limb ischaemia (Table 4). Factor VII levels (analysed after exclusion of patients on warfarin therapy) rose significantly following surgery, while there was no significant change in the levels of cross-linked FDP. Levels of most potential thrombotic mediators remained elevated in comparison with the control population.

Exclusion of patients with preoperative sepsis or tissue necrosis from the analysis did not alter these observations, with the exception of the fall in tPA levels on resolution of critical limb ischaemia, which failed to reach statistical significance after exclusion of sepsis and gangrene ($p = 0.07$). There was, however, a trend towards a fall in the postoperative levels of cross-linked FDP following insertion of a vein graft, from a median (interquartile range) of 296 ng/ml (215–575) to 219 ng/ml (141–534) ($p = 0.09$), in contrast to the trend towards a rise observed after insertion of a synthetic graft (median preoperative level 255 ng/ml (196–463), postoperative 401 ng/ml (252–724), $p = 0.09$, Fig. 1).

Discussion

Although the patients with critical limb ischaemia were not randomly selected, the bias towards infrainguinal disease is in keeping with the distribution of

occlusive arterial disease,[20,21] while most patients with critical limb ischaemia would be expected to have undergone prior revascularisation surgery. The early mortality rate of 26% is high, but there are few figures available for comparison, although the mortality rate from amputation surgery is reported to range from 8–18%[22] depending on the site of amputation and there is evidence that at least 20% of patients with critical limb ischaemia die within 1 year of diagnosis.[23] This high mortality probably reflects the high incidence of symptomatic cardiovascular disease in patients with end-stage peripheral arterial disease, and the referral pattern to vascular surgical tertiary referral centres.

The 23% incidence of diabetes in the patients studied is approximately five times the incidence of diabetes in the general population,[24] and reflects the high incidence of gangrene in these patients,[25] while the observation that over two-thirds of the patients were current or recent smokers confirms the strong association between smoking and arterial disease.[27,28]

Blood rheology

There were surprisingly few changes in rheological parameters following resolution of critical limb ischaemia, with only relative blood viscosity (blood viscosity corrected for the effects of haematocrit and plasma viscosity) showing a significant fall postoperatively. This fall in relative blood viscosity (which is a measure of the cellular contribution to whole blood viscosity) reflects an increase in red cell deformability following resolution of critical limb ischaemia, and has previously been observed after reconstructive vascular surgery.[28]

Serum albumin levels rose significantly following resolution of critical limb ischaemia, and this observation, together with the lower haematocrit levels observed in patients with critical limb ischaemia are in contrast to findings in claudicants,[3] suggesting that patients with critical limb ischaemia have a haematological picture more typical of patients with a chronic disease state.[29] This reduction in serum protein levels may result in the lowering of plasma viscosity, which has previously been shown to be elevated in patients with claudication.[3,6]

Plasma fibrinogen

The association between elevated plasma fibrinogen and atherosclerotic vascular disease has been reported by a number of authors,[1,30–32] and there is evidence that the degree of elevation is related to both the

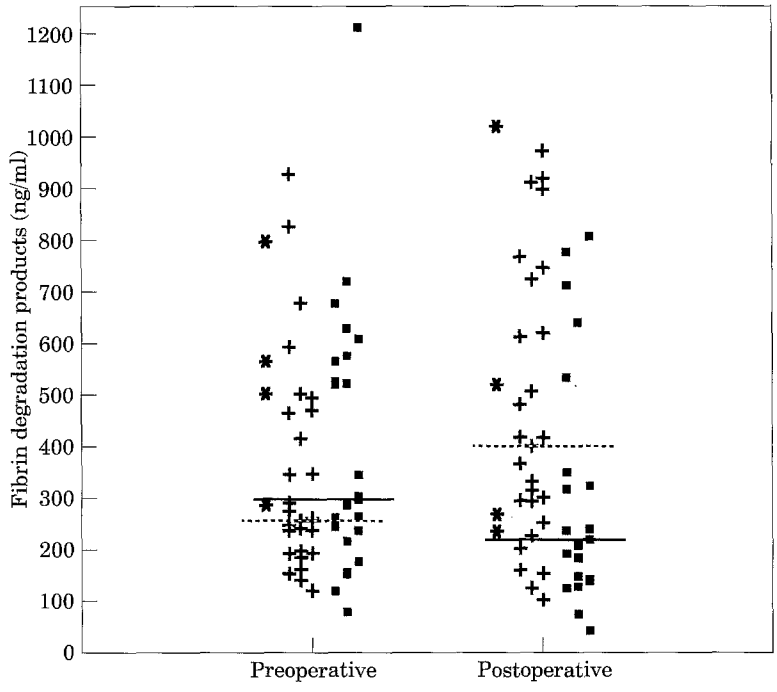

Fig. 1 Pre and postoperative FDP levels in 56 patients undergoing surgery for critical limb ischaemia, by type of operation performed. (- - -) synthetic median; (—) vein median; (*) amputation; (+) synthetic graft; (■) vein graft.

severity, and progression, of ischaemia.[8,33–35] This study suggests that relief of critical limb ischaemia reverses some of the elevation in plasma fibrinogen that is associated with advanced arterial occlusive disease, implying that biochemical changes stimulating fibrinogen synthesis occur in critically ischaemic tissues. This fibrinogen synthesis is probably hepatic in origin, in response to stimulation of hepatocytes by interleukin-6 (IL-6),[35,36] produced by activated monocytes[37] and the fall in plasma fibrinogen following revascularisation may be related to a reduction in white cell activation following resolution of critical limb ischaemia. However, correction of critical limb ischaemia fails to reduce either white cell count or fibrinogen to the levels encountered in an age-matched population, and this may contribute to the sustained elevation in plasma viscosity.

Persisting elevation in plasma fibrinogen levels may be due to a number of factors including continued smoking in the patients studied,[38] and perhaps this also explains the continued increase in white cell count. The presence of widespread arterial disease at other sites may also be associated with elevated fibrinogen levels, while there is evidence of a genetic influence on plasma fibrinogen[39–41] which may predispose certain individuals to elevated fibrinogen levels regardless of the state of their arterial circulation.

The postoperative fall in plasma fibrinogen was not solely due to resolution of limb sepsis and tissue necrosis, although preoperative fibrinogen levels were lower in patients where these features were absent, confirming that the presence of sepsis and tissue necrosis results in elevated plasma fibrinogen.[41] This fall in plasma fibrinogen following resolution of critical limb ischaemia has a number of potential clinical benefits: elevated plasma fibrinogen is known to be an independent risk factor for stroke and myocardial infarction,[32] as well as cardiovascular mortality,[30] and also to be associated with arterial graft occlusion.[11] The reduction of plasma fibrinogen levels associated with correction of critical limb ischaemia may reduce the risk of these events occurring post-operatively.

Fibrin degradation products

Cross-linked FDP reflect an increase in fibrin turnover.[42] Recent studies have suggested that FDPs are elevated in peripheral arterial disease,[35] and that the elevation in FDPs is related to the severity of occlusive arterial disease.[7,12] Initial analysis of our results indicates that fibrin turnover is unaffected by successful resolution of critical limb ischaemia. However, graft type appears to have an effect on FDP levels following resolution of critical limb ischaemia.

The use of autologous vein to revascularise a critically ischaemic limb produces a trend towards a fall in FDPs, with postoperative levels comparable to those in controls, indicating that increased fibrin turnover is reversed when the limb is revascularised with an autogenous vein graft. This may result from a reduction in thrombus formation secondary to stasis, as a consequence of an improved arterial inflow. In contrast, the use of synthetic graft materials was associated with a rise in cross-linked FDP levels, that failed to attain statistical significance. A rise in FDP levels following insertion of a synthetic graft has previously been reported,[35] and this increase in fibrin turnover may occur in all patients where a synthetic graft is inserted. Previous studies have shown that the innermost layer coating the luminal surface of a synthetic graft consists almost entirely of fibrin,[43] and these findings suggest that this layer is continually being lysed and reformed, resulting in increased FDP levels in patients revascularised with a synthetic graft. This increase in fibrin turnover may contribute to the greater risk of occlusion in synthetic grafts.

von Willebrand factor

Between 75 and 85% of circulating vWF is derived from endothelial cells, the remaining plasma von Willebrand factor being derived from platelet activation.[44] Elevated levels of vWF are therefore indicative of endothelial damage, and have been shown to be elevated following direct arterial injury,[45] and also in the presence of peripheral arterial disease.[6,46,47] Although little is known about the regulation of vWF levels,[44] it has been reported that vWF is elevated in inflammatory conditions.[48] However the postoperative reduction in vWF levels observed in this study occurred independently of resolution of limb sepsis and tissue necrosis.

Levels of vWF remain higher than in population controls following resolution of critical limb ischaemia, and this may be due to widespread disease elsewhere in the vascular tree promoting increased plasma levels, or perhaps as a consequence of persisting platelet activation[44] and fibrin turnover.[49] However, regardless of aetiology, the persisting elevation in vWF levels may have a harmful influence on long-term graft survival, as vWF is an essential requirement for the development of occlusive arterial thrombi,[50] and elevated levels appear to promote increased

thrombogenesis.[16] There is also preliminary evidence linking increased vWF levels to a poor outcome following infrainguinal revascularisation surgery.[12]

PAI, tPA, and factor VII

Resolution of critical limb ischaemia leads to a fall in tPA and PAI levels, but only the fall in tPA is observed after exclusion of cases with preoperative sepsis and necrosis. This suggests that conversion of plasminogen to plasmin could be reduced following resolution of critical limb ischaemia, although further studies are required.

Factor VII levels show an inverse relationship with fibrinogen levels,[51] and a rise in Factor VII levels would be expected to accompany any fall in fibrinogen after resolution of critical limb ischaemia. It appears that surgical resolution of critical limb ischaemia results in the return of Factor VII levels to normal population levels, presumably as result of resolution of inflammatory changes associated with critical limb ischaemia,[52] that reduce Factor VII levels.

Summary

This study confirms that increased fibrinogen, fibrin turnover, and vWF levels are associated with critical limb ischaemia. These alterations may promote a thrombogenic environment that contributes to an increased risk of cerebrovascular, cardiovascular, and peripheral vascular occlusive events in patients with critical limb ischaemia. Many of the changes observed in this study indicate that critical limb ischaemia results in biochemical alterations that are typical of chronic inflammatory conditions. Although these alterations are partly reversible, there appear to be persisting elevations in plasma fibrinogen, FDP, and vWF levels following revascularisation that may adversely affect longer-term graft and patient survival, while the trend towards increasing fibrin turnover following insertion of a synthetic graft requires further study.

Acknowledgements

The authors would like to thank the consultant surgeons in the Unit for Peripheral Vascular Surgery, Royal Infirmary, Glasgow, and the Level 5 Surgical Unit, Gartnavel General Hospital, Glasgow, for permission to study patients in their care. We thank the British Heart Foundation, and Chief Scientists Office, Scottish Home and Health Department, for financial support.

References

1 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* 1973; **4**: 576–581.
2 Reid HL, Dormandy JA, Barnes AJ, Lock PJ, Dormandy TL. Impaired red cell deformability in peripheral vascular disease. *Lancet* 1976; **i**: 666–668.
3 Lowe GDO, Saniabadi A, Turner A, Leiberman DP, Pollock JG, Drury JKD. Studies on haematocrit in peripheral arterial disease. *Klin Wochenschr* 1986; **64**: 969–974.
4 Nash GB, Thomas PRS, Dormandy JA. Abnormal flow properties of white blood cells in patients with severe ischaemia of the leg. *Br Med J* 1988; **296**: 1699–1701.
5 Lowe GDO, Fowkes FGR, Dawes J, Donnan PT, Lennie SE, Housley E. Blood viscosity, fibrinogen and activation of coagulation and leucocytes in peripheral arterial disease and the normal population in the Edinburgh Artery Study. *Circulation* 1993; **87**: 1915–1920.
6 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**: 155–162.
7 Lassila R, Peltonen S, Lepantalo M, Saarinen O, Kauhanen P, Manninen V. The severity of atherosclerosis is associated with fibrinogen and degradation of cross-linked fibrin. *Arterioscler Thromb* 1932; **13**: 1738–1742.
8 Woodburn KR, Rumley A, Love J, Lowe GDO, Pollock JG. Fibrinogen, fibrin turnover, and the extent of peripheral arterial occlusive disease. *Int Angiol* 1994; **13**(suppl. 1): 95.
9 Hamer JD, Ashton F, Meynell MJ. Factors influencing prognosis in the surgery of peripheral arterial disease: platelet adhesiveness, plasma fibrinogen, and fibrinolysis. *Br J Surg* 1973; **60**: 386–389.
10 Harris PL, Harvey DR, Bliss BP. The importance of plasma lipid, glucose, insulin and fibrinogen in femoropopliteal surgery. *Br J Surg* 1978; **65**: 197–200.
11 Wiseman S, Kenchington G, Dain R *et al.* Influence of smoking and plasma factors on patency of femoropopliteal vein grafts. *Br Med J* 1989; **299**: 643–646.
12 Woodburn KR, Rumley A, Lowe GDO, Pollock JG. Thrombotic mediators and patient selection in infrainguinal revascularisation surgery. *Int Angiol* 1994; **13**(suppl.1): 51.
13 Smith EB, Staples EM. Haemostatic factors in human aortic intima. *Lancet* 1991; **ii**: 1171–1174.
14 Smith EB, Keen A, Grant A, Stirk C. Fate of fibrinogen in human arterial intima. *Arteriosclerosis* 1990; **10**: 263–275.
15 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**: 109–121.
16 Zwaginga JJ, Ijsseldijk MJW, Beeser-Visser N, de Groot PG, Vos J, Sixma JJ. High von Willebrand Factor concentration compensates a relative adhesion defect in uraemic blood. *Blood* 1990; **75**: 1498–1508.
17 Badimon L, Fuster V. von Willebrand Factor and atherosclerotic cardiovascular disease. In: Francis RB ed. *Atherosclerotic cardiovascular disease, haemostasis, and endothelial function*. New York: Marcel Dekker Inc., 1992: 53–85.
18 European Working Group on Critical Limb Ischaemia. European consensus document on critical limb ischaemia. In: Dormandy J, Stock G, eds. *Critical leg ischaemia*. Berlin: Springer-Verlag 1990.

19 Vesey CJ, Saloojee Y, Cole PV, Russell MAH. Blood carboxyhaemoglobin, plasma thiocyanate, and cigarette consumption: implications for epidemiological studies in smokers. *Br Med J* 1982; **284**: 1516–1518.
20 Bloor K. Natural history of arteriosclerosis of the lower extremities. *Ann R Coll Surg Eng* 1961; **28**: 36–52.
21 Hertzer NR. The natural history of peripheral vascular disease: Implications for its management. *Circulation* 1991; **83**(suppl. 1): I12–I19.
22 Harris P, Moody P. Amputations. In: Dormandy JA, Stock G, eds *Critical leg ischaemia: its pathophysiology and management*. Berlin: Springer-Verlag, 1990: 87–98.
23 Norgren L. Definition incidence and epidemiology. In: Dormandy JA, Stock G, eds. *Critical limb ischaemia: its pathophysiology and management*. Berlin: Springer-Verlag, 1990: 7–16.
24 Krone W, Müller-Wieland D. Special problems of the diabetic. In: Dormandy JA, Stock G, eds. *Critical leg ischaemia: its pathophysiology and management*. Berlin: Springer-Verlag, 1990: 145–161.
25 Gutman M, Kaplan O, Skornick Y, Klausner J, Lelcuk S, Rozin RR. Gangrene of the lower limbs in diabetic patients: a malignant complication. *Am J Surg* 1987; **154**: 305–308.
26 Hughson WG, Mann JI, Garrod A. Intermittent claudication: Prevalence and risk factors. *Br Med J* 1978; **i**: 1379–1381.
27 Fowkes FGR. Aetiology of peripheral atherosclerosis. *Br Med J* 1989; **298**: 405–406.
28 Irwin ST, Rocks MJ, McGuigan JA, Patterson CC, Morris TCM, O'Reilly MJG. Effect of reconstructive vascular surgery on red cell deformability — preliminary results. *J Clin Pathol* 1983; **36**: 1136–1139.
29 Reizenstein P. The haematological stress syndrome. *Br J Haematol* 1979; **43**: 329–334.
30 Meade TW, Mellows S, Brozovic M *et al.* Haemostatic function and ischaemic heart disease: Principle results of the Northwich Park heart study. *Lancet* 1986; **ii**: 533–537.
31 Kannel WB, Wolf PA, Castelli WP, D'Agostino RB. Fibrinogen and risk of cardiovascular disease. *JAMA* 1987; **258**: 1183–1186.
32 Ernst E. Fibrinogen: An independent risk factor for cardiovascular disease. *Br Med J* 1991; **303**: 596–597.
33 Dormandy JA, Hoare E, Khaitab AH, Arrowsmith DE, Dormandy TL. Prognostic significance of rheological and biochemical findings in patients with intermittent claudication. *Br Med J* 1973; **4**: 581–583.
34 Lowe GDO, Wood DA, Douglas JT *et al.* Relationships of Plasma Viscosity, coagulation and fibrinolysis to coronary risk factors and angina. *Thromb Haemost* 1991; **65**: 339–343.
35 Reid DB. The clinical role of fibrinogen and fibrin in peripheral arterial disease. *MD Thesis: University of Glasgow* 1991.
36 Cook NS, Ubben D. Fibrinogen as a major risk factor in cardiovascular disease. *T.I.P.S.* 1990; **11**: 444–451.
37 Cruickshank AM, Fraser WD, Burns HJG, Van Damme J, Shenkin A. Response of serum interleukin-6 in patients undergoing elective surgery of varying severity. *Clin Sci* 1990; **79**: 161–165.
38 Meade TW, Chakrabarti R, Haines AP, North WRS, Stirling Y. Characteristics reflecting fibrinolytic activity and plasma fibrinogen concentrations. *Br Med J* 1979; **i**: 153–156.
39 Hamsten A, Iselius L, de Faire U, Blombäck M. Genetic and cultural inheritance of plasma fibrinogen concentration. *Lancet* 1987; **iv**: 988–990.
40 Fowkes FGR, Conner JM, Smith FB, Wood J, Donnan PT, Lowe GDO. Fibrinogen genotype and risk of peripheral atherosclerosis. *Lancet* 1992; **339**: 693–696.
41 Meade TW. Fibrinogen and other clotting factors in cardiovascular disease. In: Francis RB, ed. *Atherosclerotic cardiovascular disease, haemostasis, and endothelial function*. New York: Marcel Dekker Inc., 1992: 1–34.
42 Whittaker AN, Masci P, Rowbotham B *et al.* Applications of plasma assays of cross-linked fibrin degradation products (XL — FDP's) in the diagnosis of thromboembolic disease. In: Lowe GDO, Douglas JT, Forbes CD, Henschen A, eds. *Fibrinogen 2: Biochemistry, physiology, and clinical relevance*. Oxford: Excerpta Medica, 1987: 205–208.
43 DeBakey ME, Jordan GL, Abbott JP, Halpert B, O'Neal RM. The fate of dacron vascular grafts. *Arch Surg* 1964; **89**: 757–782.
44 Ingerslev J. von Willebrand Factor, factor VIII and the factor VIII/von Willebrand factor complex. *Dan Med Bull* 1990; **5**: 385–406.
45 Woodburn KR, Lowe GDO, Pollock JG, Rumley A, Reid AW. Percutaneous angioplasty, endothelial markers, and fibrin turnover. *J Endovasc Surg* 1994; **1**: 53–60.
46 Christe M, Delley A, Marbet GA, Biland L, Duckert F. Fibrinogen, factor VIII related antigen, Antithrombin III and A-2 Antiplasmin in peripheral arterial disease. *Thromb Haemost* 1984; **52**: 240–242.
47 Blann AD, McCollum CN. Von Willebrand Factor in atherosclerotic vascular disease. *Br J Haematol* 1992; **80**: (suppl.1): 11.
48 Blann AD, Hopkins J, Winkles J, Wainwright AC. Plasma and serum von Willebrand factor antigen concentrations in connective tissue disorders. *Ann Clin Biochem* 1992; **29**: 67–71.
49 Ribes JA, Francis CW, Wagner DD. Fibrin induces release of von Willebrand Factor from endothelial cells. *J Clin Invest* 1987; **79**: 117–123.
50 Brinkhous KM, Reddick RL, Read MS, Nichols TC, Bellinger DA, Griggs TR. Von Willebrand Factor and animal models: Contributions to gene therapy, thrombotic thrombocytopenic purpura, and coronary artery thrombosis. *Mayo Clin Proc* 1991; **66**: 733–742.
51 Meade TW. Fibrinogen. In: Fowkes FGR, ed. *Epidemiology of peripheral vascular disease*. London: Springer-Verlag, 1991: 263–270.
52 Lowe GDO. Pathophysiology of critical leg ischaemia. In: Dormandy JA, Stock G, eds. *Critical Leg Ischaemia: its Pathophysiology and Management*. Berlin: Springer-Verlag, 1990: 17–40.

Accepted 28 December 1994