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Homocysteine Levels, Haemostatic Risk Factors and Patency Rates after Endovascular Treatment of the Above-Knee Femoro-Popliteal Artery

E. Laxdal,[1*] G. E. Eide,[2] J. Wrsching,[4] G. L. Jenssen,[4] T. Jonung,[3] G. Pedersen,[1] S. R. Amundsen,[1] E. Dregelid[1] and S. Aune[1]

[1]*Department of Vascular Surgery, Haukeland University Hospital,* [2]*Centre for Clinical Research, Haukeland University Hospital and Section for Epidemiology and Medical Statistics, Department of Public Health and Primary Care, University of Bergen,* [3]*Department of Surgical Research, University of Bergen, and* [4]*Department of Radiology, Haukeland University Hospital, Bergen, Norway*

Objectives. *To investigate the relationship between plasma homocysteine and other haemostatic variables and restenoses or reocclusions after endovascular treatment of symptomatic atherosclerosis of the above-knee femoro-popliteal artery.*
Design. *Prospective observational study.*
Setting. *University hospital.*
Patients and methods. *The study included 103 patients (116 limbs), treated with subintimal angioplasty in 58 cases (50%) and with intraluminal PTA in 58 (50%): 39 (34%) patients were treated for critical limb ischaemia. Blood samples for analyses of fasting plasma values of homocysteine, fibrinogen, D-dimer, activated protein C resistance were drawn upon admission. Median follow-up for all procedures was 11 months (range 0–42 months). Outcome events (arterial patency) were defined as ≥50% restenosis or reocclusion in the treated arterial segment. Patency rates were estimated with the product limit method and Kaplan–Meier curves. Variables found to be related significantly to patency were included in multivariate analysis performed with the Cox proportional hazard model.*
Results. *The 1-year cumulative primary patency rate for all procedures was 48%. One-year limb salvage rate in cases of critical ischaemia was 74%. Multivariate analysis demonstrated significant independent associations between patency rates and plasma D-dimer, diabetes mellitus, the nature of the lesion treated (stenosis vs. occlusion) and antithrombotic therapy with aspirin after the procedure. Plasma levels of homocysteine, fibrinogen or activated protein C resistance were not associated with patency rates. Homocysteine levels were higher in patients with critical limb ischaemia than those with intermittent claudication.*
Conclusions. *Early restenosis or reocclusion after endovascular intervention of lesions in the above-knee femoro-popliteal artery was more frequent following treatment of occlusion (versus stenosis), for patients with diabetes, patients with elevated D-dimer and those without antithrombotic therapy after the procedure. Plasma homocysteine did not appear to influence the outcome of endovascular intervention.*

Introduction

The incidence of restenosis or reocclusion after endovascular treatment of symptomatic above-knee femoro-popliteal atherosclerosis is reported to vary between 30 and 60%.[1–3] The risk factors for development of a restenosis or a reocclusion following endovascular interventions are largely unknown. The progression of atherosclerosis has been suggested as an important contributor to this problem, since the lesions display histological similarities to those of atherosclerosis.[4,5] Identification of risk factors for restenosis or reocclusion would be an aid to clinical decision making and guidance as to which pharmacological agents should be evaluated in future clinical trials for prevention of restenosis and reocclusion.

The level of plasma homocysteine has been demonstrated to be an independent risk factor for peripheral vascular and coronary artery atherosclerosis.[6] Results from studies on the relationship between elevated homocysteine levels and restenosis after coronary or peripheral vascular interventions remain controversial.[7–13]

Platelet aggregation and thrombosis, observed at the site of angioplasty in animal models, suggest a role for the coagulation and fibrinolytic systems in

*Corresponding author. E. Laxdal, Department of Vascular Surgery, Haukeland University Hospital, Bergen, Norway.

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restenosis. However, there exists no clear evidence of the benefit of antithrombotic treatment on restenoses or reocclusions after endovascular therapy on peripheral arteries.[14]

Elevated pre-treatment levels of fibrinogen have been found to be associated with restenosis after coronary angioplasty. Blood levels of D-dimer have been found to be correlated to atherosclerotic burden, but any predictive value with respect to restenosis or reocclusion has not been demonstrated.[15] Activated protein C resistance has been associated with venous thromboembolism but recently was found to be associated with atherosclerosis and restenosis following angioplasty.[16]

The aim of this study was to investigate the relationship between plasma levels of homocysteine and other haemostatic and clinical risk factors and restenosis or reocclusion after endovascular treatment of symptomatic atherosclerosis in the above-knee femoro-popliteal artery.

Patients and Methods

From October 1999 to October 2002, 186 patients (205 limbs) were subjected to endovascular treatment of symptomatic atherosclerosis of the above-knee femoro-popliteal artery in our institution. Subintimal angioplasty was performed in 88 cases and percutaneous transluminal angioplasty (PTA) in 117. Twenty-four cases (16 subintimal angioplasties and eight PTAs) were excluded because the procedure was deemed technically unsuccessful. The definition of an unsuccessful procedure was as follows: PTA: residual stenosis $\geq$50%, subintimal angioplasty: impaired flow through the reconstruction with or without residual stenosis $\geq$50% (as seen on the post-treatment angiogram). Thirteen cases were lost to follow-up, three patients who died within 30 days of the procedure and 26 who did not have the relevant blood tests performed also were excluded. Eighteen redo-procedures on cases subjected to more than one procedure of the same segment of the femoro-popliteal artery in the same extremity during the study period were excluded, i.e. the first procedure was included and subsequent procedures were excluded. Five endovascular procedures on anastomotic sites of above-knee femoro-popliteal bypasses were not included. Thus the study included the treatment of 116 limbs in 103 patients. There were 41 women and 62 men with a mean age of 74 years (range 49–94). Risk factors are listed in Table 1.

Table 1. Risk factors in 103 patients subjected to endovascular treatment of 116 above-knee femoro-popliteal stenoses or occlusions. COPD denotes obstructive pulmonary disease

Risk factor	%
Heart disease	32
Hypertension, medically treated	42
Diabetes mellitus	26
COPD	10
Stroke	16
Smoking	60
Se-creatinine $>$125 μmol/l	17

The indication for treatment was intermittent claudication in 77 cases (66%) and critical ischaemia in 39 (34%). Critical ischaemia was defined according to the second European Consensus Document on chronic critical leg ischaemia.[17] The quality of run-off was defined on basis of the number of patent crural vessels. It was not possible to retrieve information on run-off in two cases.

Occlusions longer than 4 cm were treated with subintimal technique as described by Bolia *et al.* in 58 cases (50%) and stenoses (55 cases) or occlusions shorter than 4 cm (three cases) with intraluminal percutaneous transluminal angioplasty (PTA) as described by Dotter and Judkins in 58 (50%).[18,19] The mean length of occlusions treated with subintimal angioplasty was 11.5 cm (range 3–35 cm). The mean length of lesions treated with PTA was 3.8 cm (range 0.5–20 cm).

Blood for analysis of fasting plasma homocysteine (Hcy) (normal range 0–15 μmol/l), fibrinogen (Fibr) (normal range 2–4 g/l), D-dimer (D dim) (normal range 0.0–0.5 mg/l), activated protein C resistance, with exception of patients on oral anti-coagulation (normal value $<$2) was drawn upon admission and analyzed as described earlier.[20–22] During the procedure the patient was given 5000 i.u. of heparin intravenously and if tolerated, then 160 mg aspirin daily thereafter. If aspirin was not tolerated, no other platelet-inhibiting medication was prescribed. Thus 32 of 116 cases (28%) did not take aspirin after the procedure.

Follow-up of patients treated with subintimal angioplasty was performed at 1, 3, 6, 9, 12 and 18 months, with ankle/arm pressure measurement and duplex ultrasound examination of the treated arteries. Follow-up of patients treated with intraluminal PTA was performed routinely at 1 and 12 months with ankle arm pressure measurement and clinical evaluation. However, the majority of the patients were subjected to more frequent follow up: in cases of critical ischaemia, recurrence of symptoms or because of atherosclerotic disease in the contralateral leg. A reduction in ankle-arm index of more than 10% combined with reoccurrence of symptoms was

considered to be a sign of a significant restenosis or reocclusion and the finding confirmed either by angiography or duplex ultrasonography. At the end of the study period, all patients with a patent reconstruction were invited for a further examination with ankle-pressure measurement and ultrasound assessment. If symptoms had reappeared in the period from the last follow up and ultrasonography confirmed a restenosis or reocclusion in the treated segment, an event was registered at the time of reoccurrence of symptoms. For patients declining the end of study follow up, data were censored at the previous follow-up, when the status of patency was known. Patency was defined as freedom from either reocclusion or restenosis $\geq 50\%$, both of these criteria being used as endpoints. Median follow-up time for all procedures was 11 months (range 0–42 months). Median follow-up time for event-free procedures was 18 months (range 0–39 months) and for procedures with events 4 months (range 0–42 months). All data were prospectively entered in a vascular registry run by the department and analysed with SPSS 10.0.7 for Windows. Statistical significance was accepted at $p < 0.05$. Patency rates were estimated with the product limit method and illustrated as Kaplan–Meier curves, using the log-rank test for comparison of patency rates between groups. Variables found to be significantly correlated to patency rates were subjected to multivariate analysis performed with Cox proportional hazard model. Comparison of continuous variables between two groups was done with Student's *t*-test and more than two groups was done with one way analysis of variance (ANOVA). Mann–Whitney test was applied for comparison of non-parametric data. Restenoses or reocclusion rates, limb salvage rates and calculation of haemostatic and clinical factors affecting patency rates are based on the number of treated legs. Calculations concerning homocysteine values and haemostatic values with respect to other risk factors than patency, limb salvage and amputation were based on number of patients.

Results

The technical success rate for all procedures was 88, 82% for subintimal angioplasty and 92% for PTA. Three patients (1.4%) died within 30 days of the procedure of causes unrelated to the procedures. Procedure-related complications are listed in Table 2. The 1-year cumulative primary patency rate for all procedures included in the study was 48, 65% for PTA and 31% for subintimal angioplasty. The 1-year limb salvage rate for limbs suffering critical ischaemia was 74% for all procedures, 78% for limbs treated with PTA and 63% for those treated with subintimal angioplasty. Univariate analysis demonstrated significantly inferior patency in limbs of patients with elevated plasma levels of D-dimer (>0.5 mg/l) ($p = 0.019$) (Fig. 1), diabetes mellitus ($p = 0.038$) (Fig. 2) and of occlusions as compared to stenoses ($p = 0.026$) (Fig. 3). Patency was significantly worse in limbs of patients not treated with aspirin compared to those treated with aspirin ($p = 0.043$) (Fig. 4). However, separate univariate analysis of occlusions and stenoses, respectively, showed that aspirin treatment significantly improved patency only after re-canalisation of occlusions ($p = 0.027$) and not after PTA of stenoses ($p = 0.184$). Multivariate analysis adjusting for the length of the lesions, run-off, indication, age, gender, risk factors, smoking, aspirin treatment and the nature of the lesion (occlusion or stenosis) showed independent significant association of all four parameters with patency rates (Table 3). Plasma levels of Hcy, Fibr or act prot C res were not found to be associated with patency rates.

Of 103 patients thirty (26%) had plasma Hcy values above the upper limit of the normal range (0–15 μmol/l), including 16/39 (41%) patients with critical ischaemia. The mean plasma concentrations of Hcy, Fibr and D-dim were significantly higher in patients with critical ischaemia as compared to patients with intermittent claudication ($p = 0.028$, 0.007 and 0.018, respectively). In cases of critical ischaemia resulting in amputation, the mean plasma Hcy was significantly higher than in cases of critical ischaemia resulting in limb salvage ($p = 0.016$). There was no relationship between the length or the nature of the lesions (occlusions versus stenoses) and either run-off status or the plasma variables Hcy, Fibr, D-dimer or act prot C res.

Discussion

The relationship between elevated homocysteine concentration and atherosclerosis was first observed by McCully in patients with homocysteinuria, a metabolic deficiency resulting from an autosomal recessive disorder, most commonly due to a cystathionine β synthetase deficiency.[23] Homozygotes are more severely affected than heterozygotes and may be categorised according to responsiveness to supplementation therapy with vitamins B6, B12 or folate.[24] Mild hyper-homocysteinemia can be caused by deficiency of the aforementioned vitamins, but the implications of supplement therapy are yet

Table 2. Procedure-related complications to 116 above-knee femoro-popliteal endovascular procedures in 103 patients

Procedure	*n*	Complication	*n*	Treatment	*n*
Subintimal angioplasty	58	Distal embolisation	4	Aspiration	4
		Distalisation of level	1	Bypass surgery	1
PTA	58	Distal embolisation	3	Aspiration	2
				Anticoagulation	1
		Dissection	1	None	1
		Haematoma	2	None	2
		Bleeding	1	Operation	1

unsettled.[25–28] The correlation between elevated homocysteine and coronary, cerebrovascular as well as peripheral vascular atherosclerosis has been effectively demonstrated. The role of homocysteine in vascular disease, whether as a causative factor or an indicator, has not been clarified.[7,28,29] Cell culture and animal model experiments have elucidated several potential mechanisms by which hyper-homocysteinemia may stimulate myointimal hyperplasia: inhibiting endothelial cell proliferation and stimulating vascular smooth cell growth,[30] endothelial cell damage by generation of free radicals,[31] in combination with destruction of endothelial cell protective mechanisms such as nitric oxide and glutathione.[32,33] Furthermore, homocysteine has been found to cause a decrease in compounds potentially inhibiting vascular smooth muscle proliferation,[34] and to create a procoagulant milieu through modulation of the coagulation and fibrinolytic pathway.[35–38] The nitric oxide inhibiting mechanism of homocysteine has been linked to an inhibition of endothelium-dependent vasodilatation found in vivo and in vitro studies.[39,40]

The association between restenoses or reocclusions and elevated homocysteine has not been determined, although results of several studies on cardio-vascular interventions have suggested an association. Studies of the effect of hyper-homocysteinemia on the outcome of peripheral vascular interventions have been inconclusive. Irvine and Mireskandari found a significant relationship between hyper-homocysteinemia and vein graft stenosis after infrainguinal bypass grafting whereas Beattie did not, although his study showed a significant relationship between elevated homocysteine values and myointimal hyperplasia in the vein used for grafting.[11,12,40] Results from the Dutch BOA Study, including 150 patients with occluded infrainguinal bypass grafts (synthetic as well as vein grafts) randomly matched with 220 cases of patent grafts, revealed no significant differences between the groups with respect to

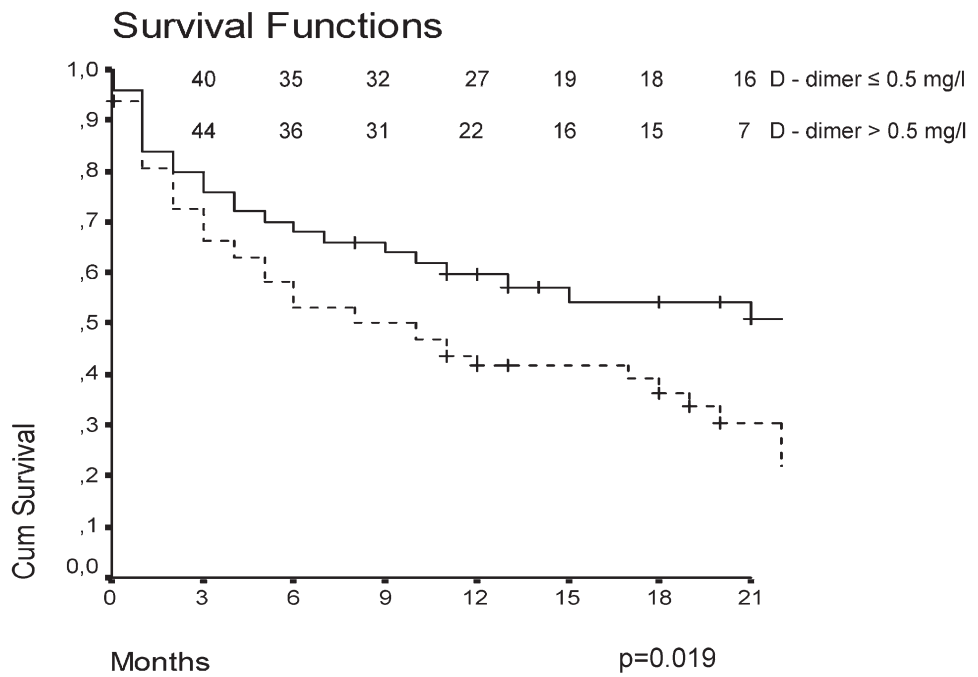

Fig. 1. Comparison of patency rates after 116 above-knee femoro-popliteal endovascular procedures in patients with D-dimer >0.5 mg/l (broken line) and patients with D-dimer ≤ 0.5 mg/l (unbroken line).

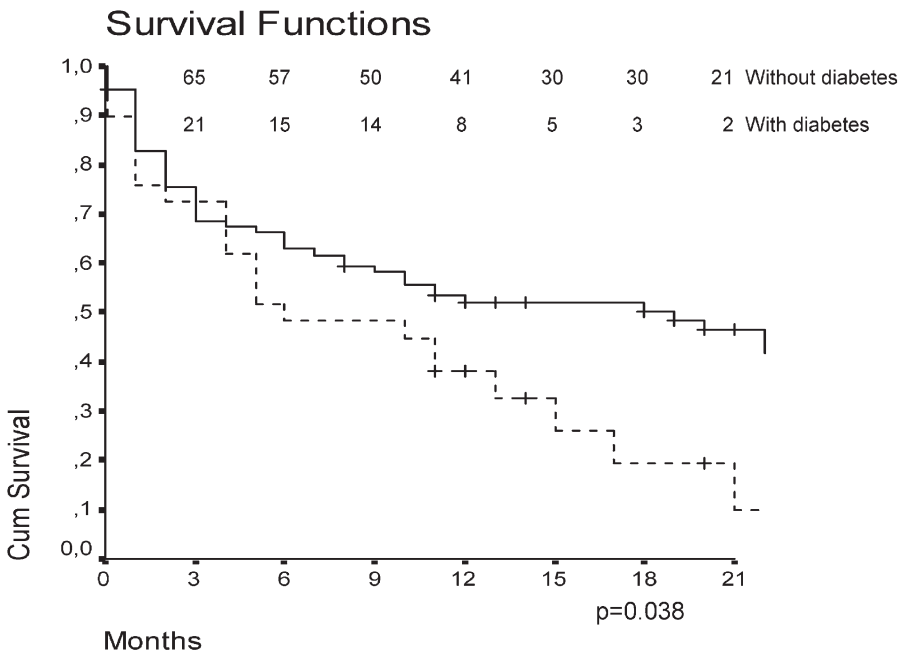

Fig. 2. Cumulative primary patency rates in patients with diabetes mellitus (broken line) compared to patency rates in non-diabetic patients (unbroken line). The numbers above the curve indicate patients at risk.

homocysteine values.[10] Results from studies of endovascular interventions are also controversial. Currie *et al.* reviewed 66 cases and found, using multiple logistic regression, that hyper-homocysteinemia was an independent risk factor for failure of all forms for peripheral vascular interventions.[13] However, Tsakiris *et al.* could not reproduce this finding in 81 cases treated with PTA.[41]

In our study, homocysteine-levels were not associated with the development restenoses or reocclusions after endovascular treatment of the above-knee femoro-popliteal artery. However, patients with

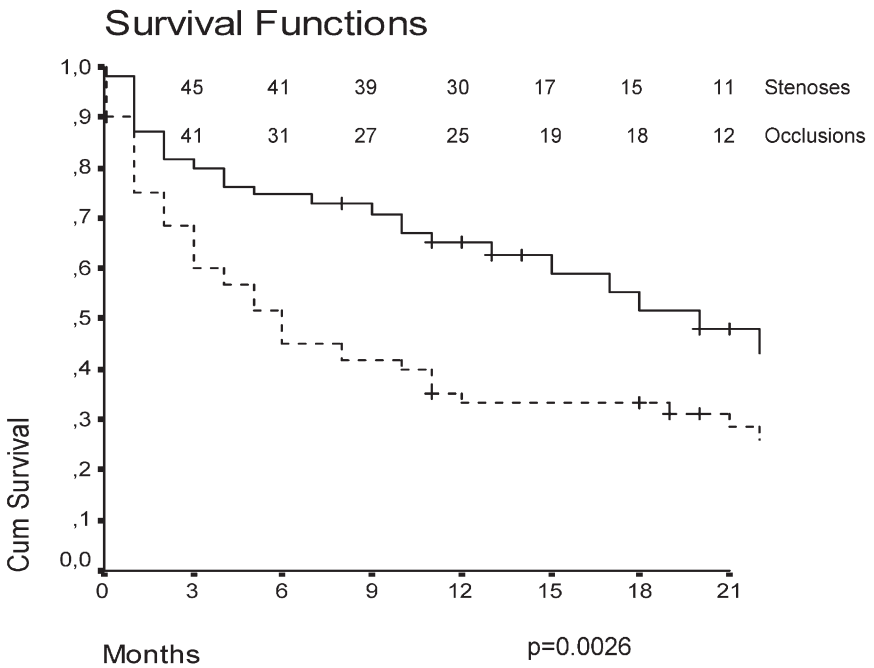

Fig. 3. Comparison of patency rates (Log rank) after endovascular treatment of occlusions (broken line) and stenoses (unbroken line). The numbers above the curve indicate patients at risk.

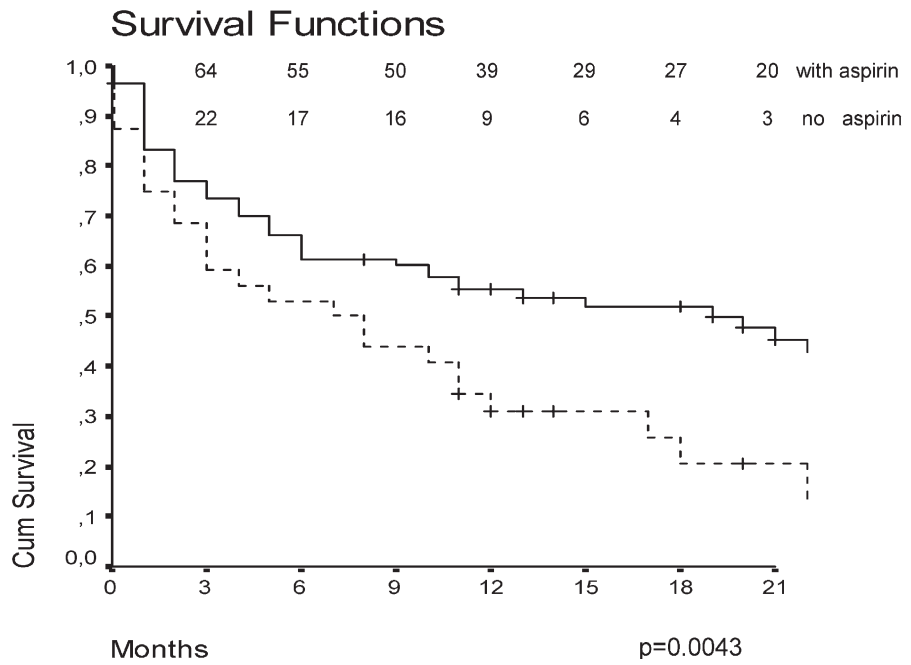

Fig. 4. Comparison of patency rates (Log rank test) in cases treated with aspirin (unbroken line) and not treated with aspirin (broken line) after 116 above-knee femoro-popliteal endovascular procedures in 103 patients. The numbers above the curve indicate patients at risk.

critical ischaemia had significantly higher values of homocysteine than patients with intermittent claudication. Furthermore, the patients with critical ischaemia resulting in amputation had even higher homocysteine values than patients with critical ischaemia whose limbs were salvaged. This indicates a possible correlation between homocysteine levels and the degree of atherosclerotic disease of the lower limb. However, there was no association between homocysteine levels and the status of run-off, quantified as number of open crural arteries as seen on the pre-treatment angiogram. Neither was there a correlation between patency rates and run-off. These observations suggest that the manifestation of vascular disease in hyper-homocysteinemia might be localised to the smaller vessels such as in collaterals, small branching arteries and/or arterioles.

Patients with diabetes had significantly lower patency rates compared to non-diabetic patients, in accordance with earlier reports.[1,42] This is in contrast to bypass surgery as a treatment of femoro-popliteal atherosclerosis, where patients with diabetes do not have lower graft patency rates.[43] Nevertheless, endovascular intervention is generally the preferred treatment for patients with diabetes, probably because of their increased co-morbidity and operative risk. Our findings emphasise the need to identify methods to prevent restenosis and reocclusion after angioplasty in the diabetic patient as well as the importance of meticulous patient follow-up, particularly after angioplasty for critical ischaemia.

The importance of anti-platelet therapy to prevent restenoses or reocclusions after PTA in peripheral arteries has not been settled.[14] We found significantly inferior patency rates in patients not receiving aspirin therapy after the procedure. When analysing subgroups, i.e. stenoses versus occlusions, the beneficial effect of aspirin was significant only for cases with occlusions. However, as this was not a randomised study of the effect of aspirin, this result cannot be regarded as conclusive.

The significant association between pre-procedural

Table 3. Multivariate analysis (Cox' proportional hazard model) of risk factors demonstrated with univariate analysis to be significantly associated with patency rates in 116 above-knee femoro-popliteal endovascular reconstructions

Variables	β	Standard error	95% CI	*p*-Value	Hazard ratio
Occlusion vs. stenosis	0.720	0.269	1.212–3.482	0.007	2.055
Diabetes mellitus	0.905	0.289	1.401–4.358	0.002	2.471
D-dimer >0.5 mg/l	0.564	0.270	1.037–2.983	0.036	1.758
No aspirin	0.597	0.258	1.095–3.011	0.021	1.816

plasma D-dimer concentration and patency following endovascular therapy of peripheral arteries has not been reported previously. The implication of our finding is, that patients who are at increased risk of restenosis or reocclusion after endovascular therapy, may be those patients with more extensive atherosclerotic disease with concomitant increase in fibrinolytic activity. This finding may not be causative, but it suggests the need to explore the possible benefits of post-procedural antithrombotic therapy in patients with altered activity of their coagulation system.

The 1-year patency rate of 48% for the whole group may appear low for a study including intraluminal PTA and subintimal angioplasty. The term patency in our study is defined as a freedom from $\geq 50\%$ stenoses as well as occlusions, in cases that were submitted to a thorough follow-up. The 1-year primary patency rate procedures performed with intraluminal PTA was 65%, comparable to a previous report.[3] The poor 1-year patency rates for cases treated with subintimal recanalisation has been demonstrated in our earlier studies.[1–3] Thus the relatively high proportion of procedures done with subintimal technique (50%) is reflected in the low overall 1-year patency rate. A probable explanation for the difference in the patency rates is the nature of the lesion treated, longer occlusions demanding subintimal angioplasty whilst stenoses or short occlusions were treated with PTA. This explanation is supported by the improved patency rates following the treatment of stenoses compared to occlusions in this study.

The aetiology of development of restenoses and reocclusions after endovascular therapy is probably multifactorial. Our study indicates that the levels of homocysteine and activated protein C do not play a major role. We conclude that the major factors affecting development of restenoses or reocclusions are related to the activity of the patient's fibrinolytic system, the presence of diabetes mellitus and antithrombotic therapy after the procedure. However, there seems to be a relationship between the degree of atherosclerotic disease of the lower limb and levels of plasma homocysteine. The need for randomised interventional studies of homocysteine lowering therapy on restenosis after endovascular treatment of peripheral arteries with is questionable. We suggest that randomised studies on the possible effect of homocysteine-lowering therapy on peripheral circulation on the progression to or of critical ischaemia would be more important. Studies of the effect of peri and post-procedural anticoagulation or antithrombotic therapy in patients with diabetes mellitus and/or elevated fibrinogen and D-dimer also could be considered.

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References

1 Laxdal E, Jenssen GL, Pedersen G, Aune S. Subintimal angioplasty as a treatment of femoro-popliteal occlusions. *Eur J Vasc Endovasc Surg* 2003; **25**(6):578–582.
2 London NJM, Srinivasan R, Sayers RD, Naylor AR, Hartshorne T, Ratliff DA, Bell PRF, Bolia A. Subintimal angioplasty of femoropopliteal artery occlusions: the long-term results. *Eur J Vasc Surg* 1994; **8**:148–155.
3 Gordon IL, Conroy RM, Arefi M, Tobis JM, Stemmer EA, Wilson SL. Three-year outcome of endovascular treatment of superficial femoral artery occlusion. *Arch Surg* 2001; **136**(2): 221–228.
4 Glacov S. Intimal hyperplasia, vascular modelling and restenosis problem. *Circulation* 1994; **89**:2888–2891.
5 Haudenschild CC. Pathobiology of restenosis after angioplasty. *Am J Med* 1993; **94**(Supp. 4A):40s–44s.
6 Clarke R, Daly L, Robinson K, Cahalane S, Fowler B *et al.* Hyperhomocysteinemia: an independent risk factor for vascular disease. *N Engl J Med* 1991; **324**:1149–1155.
7 Taylor LM, DeFrang RD, Harris EJ, Porter JM. The association of elevated plasma homocysteine with progression of symptomatic peripheral artery disease. *J Vasc Surg* 1991; **13**: 128–136.
8 Giannetti N, Herity NA, Alimolla A, Gao SZ, Schroeder JS, Yeung AC, Hunt SA, Valantine HA. Mild hyperhomocysteinemia is not associated with cardiac allograft coronary disease. *Clin Transplant* 2001; **15**:247–252.
9 Iwama Y, Mokuno H, Watnabe Y, Shimada K, Yokoi H, Daida H, Hosoda Y, Yamaguchi H. Relationship between plasma homocysteine levels and saphenous vein graft disease after coronary artery bypass grafts. *Jpn Heart J* 2001; **42**:553–562.
10 Borst GJ, Tangelder MJD, Algra A, Lawson JA, Banga JD, Eikelboom BC, van der Vliet JA, Dutch BOA Study Group. The influence of hyperhomocysteinemia on graft patency after infrainguinal bypass surgery in the Dutch BOA Study. *J Vasc Surg* 2002; **36**:336–340.
11 Beattie DK, Sian M, Greenhalg RM, Davies AH. Influence of systemic factors on pre-existing intimal hyperplasia and their effect on the outcome of infraiguinal arterial re-construction with vein. *Br J Surg* 1999; **86**:1441–1447.
12 Irvine C, Wilson YG, Currie IC, McGrath C, Scott J, Day A *et al.* Hyper-homocysteinemia is a risk factor for vein graft stenosis. *Eur J Vasc Endovasc Surg* 1996; **12**:304–309.
13 Currie IC, Wilson YG, Scott J, Day A, Stansbie D, Baird RN *et al.* Homocysteine: an independent risk factor for the failure of vascular intervention. *Br J Surg* 1996; (83):1238–1241.
14 Study group on pharmacological treatment after PTA, Platelet inhibition with ASA/dipyridamole after percutaneous angioplasty in patients with symptomatic lower limb arterial ischaemia. A prospective double-blinded trial. Study group on pharmacological treatment after PTA. *Eur J Vasc Surg* 1994; **8**: 83–88.
15 Price JF, Mamode N, Smith FB, Woodburn KR, Rumley A, Lowe GI, Fowkes FG. Haemostatic and rheological factors as

predictors of restenosis following percutaneous angioplasty. *Eur J Vasc Endovasc Surg* 1997; **14**:392–398.
16 Sampram ESK, Lindblad B, Dahlbäck B. Activated protein C resistance in peripheral vascular disease. *J Vasc Surg* 1998; **28**: 624–629.
17 Second European Consensus Document on chronic critical leg ischaemia. *Eur J Vasc Surg* 1992; **6**: 1–32.
18 Bolia A, Miles KA, Brennan J, Bell PRF. Percutaneous transluminal angioplasty of occlusions of the femoral and popliteal arteries by subintimal dissection. *Cardiovasc Intervent Radiol* 1990; **13**:357–363.
19 Dotter CT, Judkins MP. Transluminal treatment of atherosclerotic obstructions. Description of a new technique and a preliminary report of its application. *Circulation* 1964; **30**:654.
20 Donnelly JG, Pronovost C. Evaluation of the Abbott IMx fluorescence polarization immunoassay and the bio-rad enzyme immunoassay for homocysteine: comparison with high performance liquid chromatography. *Ann Clin Biochem* 2000; **37**:194–198.
21 Carter AM, Mansfield MW, Stickland MH, Grant PJ. Beta-fibrinogen gene-455 G/A polymorphism and fibrinogen levels. Risk factors for coronary artery disease in subjects with NIDDM. *Diabetes care* 1996; **19**:1265–1268.
22 Moresco RN, Vargas LC, Voegeli CF, Santos RC. D-dimer and its relationship to fibrinogen/fibrin degradation products (FDPs) in disorders associated with activation of coagulation or fibrinolytic systems. *J Clin Lab Anal* 2003; **17**:77–79.
23 McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of atherosclerosis. *Am J Pathol* 1969; **56**:111–128.
24 Fowler B, Kraus J, Packman S, Rosenberg LE. Homocysteinuria. Evidence for three distinct classes of cystathionine β synthase mutants in cultured fibroblasts. *J Clin Invest* 1978; **61**: 645–653.
25 Doshi SN, McDowell IFW, Moat SJ, Payne N, Durrant HJ, Lewis MJ, Goodfellow J. Folic acid improves endothelial function in coronary artery disease via mechanisms largely independent of homocysteine lowering. *Circulation* 2002; **105**: 22–26.
26 Scnyder G, Roffi M, Flammer Y, Pin R, Eberli FR, Meier B, Turi ZB, Hess OM. Effect of homocysteine-lowering therapy on restenosis after percutaneous coronary intervention for narrowings in small coronary arteries. *Am J Cardiol* 2003; **91**:1265–1269.
27 Schnyder G, Roffi M, Flammer Y, Pin R, Hess OM. Effect of homocysteine-lowering therapy with folic acid, vitamin B 12, and vitamin B 6 on clinical outcome after percutaneous coronary intervention: the Swiss Heart study: a randomized controlled trial. *JAMA* 2002; **288**:973–979.
28 Kumbasar SD, Dincer I, Ertas F, Gulec S, Erol C, Akyurek O, Kilickap M, Oral D, Sipahi E, Laleli Y. Hyperhomocysteinemia and restenosis. *J Cardiovasc Risk* 2001; **8**:9–13.
29 Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis. A comparison of C-reactive protein, fibrinogen, homocysteine lipoprotein (a), and standard cholesterol screening as predictors for peripheral arterial disease. *JAMA* 2001; **285**:2481–2485.
30 Tsai JC, Perella MA, Yoshizumi M *et al*. Promotion of vascular smooth cell growth by homocysteine: a link to atherosclerosis. *Proc Natl Acad Sci USA* 1994; **91**:6369–6373.
31 Hultberg B, Anderson A, Isaksson A. The cell damaging effects of low amounts of homocysteine and copper irons in hum cell line cultures are caused by oxidative stress. *Toxicology* 1997; **123**:33–40.
32 Upchurch Jr GR, Welch GN, Loscalzo J. Homocysteine, EDRF, and endothelial function. *J Nutr* 1996; **126**:1290S–1294S.
33 Loscalzo J. The oxidant stress of hyperhomocysteinemia. *J Clin Invest* 1996; **98**:5–7.
34 Vadiveloo PK, Filonzi EL, Stanton HR, Hamilton JA. G1 phase arrest of human smooth muscle cells by heparin, Il-4, and cAMP is linked to a repression of cyclin D 1 and cdk2. *Atherosclerosis* 1997; **133**:61–69.
35 Nishinaga M, Ozawa T, Shimada K. Homocysteine, a thrombogenic agent, suppresses anticoagulant heparin sulphate expression in cultured porcine aortic endothelial cells. *J Clin Invest* 1993; **133**:61–69.
36 Hajjar KA. Homocysteine-induced modulation of tissue plasminogen activator binding to its endothelial cell membrane receptor. *J Clin Invest* 1993; **91**:2873–2879.
37 Lentz SR, Sadler JE. Inhibition of thrombomodulin surface expression and protein C activation by the thrombogenic agent homocysteine. *J Clin Invest* 1991; **88**:1906–1914.
38 Lentz SR, Sadler JR. Homocysteine inhibits von Willebrand factor processing and secretion by preventing transport from the endoplasmatic reticulum. *Blood* 1993; **81**:683–689.
39 Twacol A, Omland T, Gerhard M, Wu JT, Creager MA. Hyperhomocysteinemia is associated with impaired endothelium-dependent vasodilatation in humans. *Circulation* 1997; **95**:1119–1121.
40 Mireskandari M, Schachter M, Timms IGO, Sever P, Wolfe JHN. Plasma homocysteine is an independent risk factor for vein graft stenoses. *Br J Surg* 1998; **85**:27.
41 Tsakiris DA, Tschöpl M, Jäger K, Haefeli WE, Wolf F, Marbet GA. Circulating cell adhesion molecules and endothelial markers before and after transluminal angioplasty in peripheral arterial occlusive disease. *Atherosclerosis* 1999; **142**:193–200.
42 Capek P, McLean GK, Berkowitz HD. Femoropopliteal angioplasty. Factors influencing long-term success. *Circulation* 1991; **83**(2 Suppl):I70–I80.
43 Wolfle KD, Bruijnen H, Loeprecht H, Rumenapf G, Schweiger H, Grabitz K, Sandmann W, Lauterjung L, Largiader J, Erasmi H, Kasprzak PM, Raithel D, Allenberg JR, Lauber A, Berlakovich GM, Kretschmer G, Hepp W, Becker HM, Schulz A. Graft patency and clinical outcome of femorodistal arterial reconstruction in diabetic and non-diabetic patients: results of a multicentre comparative analysis. *Eur J Vasc Endovasc Surg* 2003; **25**(3):229–234.

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