Introduction

The incidence of early (<12 months) restenoses after endarterectomy of the internal carotid artery after primary closure has been reported to vary between 0 and 21%.1–3 Patch closure of the arteriotomy has been found to reduce the tendency to restenoses.2 Although operative technique may be a cause of restenosis after carotid endarterectomy, it has been suggested that progression of atherosclerosis may also be a contributor to this problem.3 The level of plasma homocysteine has been demonstrated to be an independent risk factor for peripheral vascular as well as coronary atherosclerosis.5,6 A study of adults asymptomatic of atherosclerosis revealed a significant relationship between intimal-media carotid artery wall thickening and homocysteine levels above 10.5 μmol/L.7 Another study failed to demonstrate a significant relationship between elevated homocysteine levels and the progression of known carotid artery atherosclerosis.8 Results of studies on the relationship between elevated plasma homocysteine levels and tendency to restenoses after cardiovascular or peripheral vascular interventions are controversial.9–16 The aim of this study is to investigate the relationship between early restenoses after carotid endarterectomy and levels of plasma homocysteine as well as haemostatic and clinical risk factors possibly affecting patency rates.

Patients and Methods

From October 1999 to October 2002, 102 patients were subjected to 112 carotid endarterectomies at our institution because of internal carotid stenoses. Two
patients suffered perioperative stroke and two patients died within 30 days of surgery, leaving a combined stroke-mortality rate of 3.6%.

Operations were excluded from the study if patch closure of the arteriotomy was applied (two operations, both on male patients), a residual stenosis was identified at the first post-operative duplex ultrasound (one operation), study blood tests not performed (five operations), the patient either died or was lost to follow up within 9 months of the operation (eight operations). Thus the study included 86 patients subjected to 96 internal carotid endarterectomies.

Preoperative assessment of extra cranial arteries was done with triplex ultrasound supplemented with a two plane digital subtraction angiography or MRI for evaluation of the degree of stenosis, the extent of atherosclerotic disease of the precerebral circulation as well as the anatomy of the intracranial circulation. Fasting values of 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 (act prot C res, with exception of patients on oral anticoagulation) (normal value <2) were analysed upon admission the day before the surgery.

There were 27 women and 59 men, aged 40–88 years (mean 69 years). Risk factors, as listed in Table 1, were defined as follows: hypertension when medically treated, diabetes mellitus types I and II, renal impairment when values of serum-creatinine, exceeded 125 μmol/l and chronic obstructive pulmonary disease (COPD) when receiving continuously or periodically medication for that condition. Smokers were classified as such when still smoking or having smoked within the last 5 years. The indications for operation are listed in Table 2. All but one case had COPD, chronic obstructive pulmonary disease.

Asymptomatic >70% 10 10
Amaurosis fugax 19 20
TIA 34 35
Stroke 33 35

All data were censored at the date of last follow-up. The presence of a restenosis ≥50% was considered an event. Calculations of factors with respect to restenosis rates were done on basis of treated carotid arteries.

All data were prospectively registered in a vascular registry run by the department and analysed with SPSS 10.0.7 for Windows. Freedom from restenosis rates were estimated and illustrated with the Kaplan–Meier method, using the log-rank test for comparison of groups. The influence of plasma homocysteine levels on time to endpoint occurrence (restenosis) was evaluated from the standpoint of the high versus low levels in a stepwise manner by division into groups of 1/3. The cut-off point of the lower 1/3 of the homocysteine level in the study population was found to be 10 μmol/l. A ROC-curve analysis of homocysteine values versus restenosis deemed a homocysteine value of 10.1 μmol/l as the point with the highest additive value of sensitivity and specificity ($p = 0.044$). Multivariate analysis adjusting for age, gender and risk factors was performed with Cox proportional hazards 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). $p$-Values below 0.05 were accepted as significant.

**Results**

Eleven of 96 cases (11%) progressed to restenosis but none to reocclusion. So far none of the restenoses have

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**Table 1. Risk factors in 86 patients subjected to 96 carotid endarterectomies**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Number of patients (%)</th>
</tr>
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<tbody>
<tr>
<td>Ischaemic heart disease</td>
<td>41/47</td>
</tr>
<tr>
<td>Hypertension</td>
<td>39/45</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>9/10</td>
</tr>
<tr>
<td>COPD</td>
<td>9/10</td>
</tr>
<tr>
<td>Smoker</td>
<td>47/49</td>
</tr>
<tr>
<td>Hc-creatinine &gt;125 μmol/l</td>
<td>14/16</td>
</tr>
</tbody>
</table>

COPD, chronic obstructive pulmonary disease.
been symptomatic, demanding reoperation or reintervention. The median time from operation to diagnosis of restenosis was 8 months (range 5–17 months). The patients who suffered restenosis were younger (mean 64 years) than the patients who did not (mean 70 years), but this difference was not statistically significant.

Comparison of cases with and without restenosis (log-rank test) with respect to risk factors, plasma parameters, symptoms and degree of affection of the precerebral circulation (graded as 1, 2, 3 or 4 arteries affected by significant stenoses or occlusions as seen on the preoperative angiogram) revealed that the rates of restenosis in cases with Hcy ≥ 10 µmol/l were significantly higher than in patients with Hcy > 10 µmol/l (p = 0.0076) (Fig. 1). Restenosis rates in patients free from clinically known ischaemic heart disease (IHD) were significantly higher compared to patients with IHD (p = 0.0059) (Fig. 2). Multivariate analysis adjusting for age, gender and risk factors with exception of smoking revealed independent significant higher restenosis rates in patients with Hcy ≤ 10 µmol/l compared to patients with Hcy > 10 µmol/l (p = 0.046). The relationship between freedom from IHD and restenosis rates was not found to be significant on multivariate analysis (p = 0.051). Plasma values of Fibr, D-dim or presence of act prot C res was not significantly associated with restenosis rates. Neither were any of the other risk factors listed in Table 1.

Mean values of Hcy, Fibr, D-dim and activated protein C resistance were not significantly associated with symptoms (classified into three categories as asymptomatic, stroke, tia or amaurosis fugax). Comparison of mean values of Hcy, Fibr and D-dim with respect to affection of the precerebral circulation (classified as 1, 2, 3 or 4 precerebral arteries affected either by significant stenoses or occlusion(s) as seen on the pre-operative angiogram) revealed no differences of statistical significance between the groups.

**Discussion**

The relationship between elevated homocysteine concentrations and atherosclerosis were first observed by McCully in patients with homocysteinuria, a metabolic deficiency resulting from an autosomal recessive error, most commonly due to a cystathionine β synthetase deficiency.21 Homozygotes are more severely affected than heterozygotes and may be categorized according to responsiveness to supplementation therapy with vitamins B6, B12 or folate.22 Mild hyperhomocysteinemia can be caused by deficiency of the aforementioned vitamins, but the implications of supplement therapy are yet unsettled.23–30 The relationship between elevated homocysteine and cardiovascular, cerebrovascular as well as peripheral vascular atherosclerosis has been demonstrated. But the role of homocysteine in vascular disease, as a causative factor or an indicator, has not been clarified.31–39 Cell culture and animal model experiments have elucidated several potential mechanisms by which hyperhomocysteinemia may stimulate myointimal hyperplasia: inhibition of endothelial cell proliferation and stimulation of vascular smooth cell growth,40,41 endothelial cell damage by generation of free radicals42 combined with destruction of endothelial cell protective mechanisms such as nitric oxide and glutathione.43,44 Furthermore, homocysteine has been found to cause a decrease in compounds potentially inhibiting vascular smooth muscle proliferation,45 and to create a procoagulant milieu through modulation of the coagulation and fibrinolytic pathway.46–49 The nitride oxide depressing mechanism of homocysteine has been linked to an inhibition of endothelium-dependent vasodilatation found in vivo and in vitro studies.50,51

The causal relationship between restenosis and elevated homocysteine has not been determined. Only two studies of five on the effect of hyperhomocysteinemia on the results of peripheral vascular interventions have demonstrated a significant relationship between failure and levels of homocysteine.13–16,52

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Fig. 1. Comparison of cumulated rates of freedom from restenosis (log-rank) of 96 carotid endarterectomies in patients with plasma homocysteine values ≤ 10 µmol/l and >10 µmol/l. The numbers below the curve indicate patients at risk.
Hyperhomocysteinemia is found to increase intimal hyperplasia in rat carotid endarterectomy model, suppressible by folate supplementation. In a group of 287 pairs of adults asymptomatic of atherosclerosis, Malinow et al. found that subjects with increased intimal-medial thickness of the carotid wall had significantly higher plasma homocysteine levels than controls. Selhub et al. found in a cross-sectional study of 1041 subjects an odds-ratio for carotid stenosis of 2.0 for subjects with the highest plasma homocysteine concentrations as compared with those with the lowest concentrations. In the ‘Homocysteine and Progression of Atherosclerosis Study’, Taylor et al. followed 351 subjects with known atherosclerotic disease in a mean period of 37 months. Deaths from any cause as well as from cardiovascular disease were significantly more likely in subjects with the highest 33% of plasma homocysteine levels compared to those with the lowest 33%. The subjects with the highest levels of homocysteine were significantly more likely to have non-fatal clinical progression of coronary heart disease. However, there was no progression of cerebro-vascular disease related to homocysteine levels as assessed by increasing carotid stenosis. Until now, there exist no publications on the relationship between homocysteine and restenosis or reocclusions after carotid endarterectomy in humans.

In our study, hyperhomocysteinemias did not increase the risk of restenosis after carotid endarterectomy. On the contrary, we found a significant and independent association between values in the lower two-thirds of the normal range (the lowest 1/3 of the range of homocysteine in the study population) and the tendency to restenosis. Freedom from clinically known ischaemic heart disease was also a significant, but dependent, predictor of restenosis. Homocysteine levels could neither be related to symptoms nor the degree of atherosclerosis in the precerebral circulation.

Our findings suggest that the process of restenosis after carotid endarterectomy possibly differs from the process of atherosclerosis related to or induced by hyperhomocysteinemia in some respect. If so, it may be that the process of restenosis in endarterectomized arteries is basically different from the one seen after endovascular interventions: In the endarterectomized artery that of aggravated trauma response or inflammatory reaction to a sectional removal of intima and exposure of the media to circulating blood. In arteries traumatized by balloon angioplasty, that of intimal damage resulting creation of clots adhering to the traumatized site, triggering or aggravating an ongoing process of atherosclerosis. The biological properties of the human carotid endothelium can perhaps provide an explanation for the result. It is known that the extrinsic properties of endothelial cells can vary depending on their location. An example is the heterogeneous distribution of von Willebrand factor and von Willebrand factor propeptide in the porcine vascular tree. One can hypothesize that the biological effect of homocysteine on endothelial cells may depend on their localization.

Our results state rather the opposite of the expected, why the results must be interpreted with appropriate caution. The material is small with a relatively short follow-up time. However, it seems that the majority of the restenosis develop within the first two years of the operation. Studies on the biological properties of human endothelial cells from different types of vasculature and different locations, with respect to homocysteine metabolism, different types of arterial trauma and their effect, are warranted.

Acknowledgements

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