Intraoperative Endothelial Damage is Associated with Increased Risk of Stenoses in Infrainguinal Vein Grafts

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Objectives: Saphenous vein injury is believed to predispose to vein graft stenoses. The aims of this study were to assess the endothelial injury associated with infrainguinal vein bypass surgery by serial measurements of serum thrombomodulin concentration as well as by platelet scintigraphy, and to relate these findings with the postoperative development of stenoses.

Methods: In 35 patients undergoing vein bypass surgery serum thrombomodulin concentration was measured pre- and postoperatively. Autologous 111-indium labelled platelets were administered into the common femoral artery immediately after restoration of flow in the graft and scintigraphic images were obtained 4 and/or 24 h later.

Results: Serum thrombomodulin increased markedly from median 17 ng/ml preoperatively to 32 ng/ml 1 day after surgery (p = 0.00002). Platelet scintigraphy revealed a total of 62 focal activity accumulations, the majority being located in the anastomotic regions. Among the 30 patients with grafts remaining patent at 30 days stenoses occurred in nine (16%) of 55 regions with scintigraphic platelet accumulations as compared to only four (4%) of 94 regions without platelet accumulations (p = 0.03).

Conclusions: The very high predictive value of a negative platelet scintigraphy (96%) strongly suggests that localised perioperative endothelial injury is an important pathogenetic factor in the development of vein graft stenoses.

Key Words: Platelet scintigraphy; Thrombomodulin; Vein graft stenosis.

Introduction

Within one year after surgery approximately a quarter of infrainguinal vein bypasses develop stenoses that, uncorrected, may progress to occlusion.1-5 Vein graft stenoses are assumed to result from intraoperative damage of the saphenous vein leading to intimal hyperplasia and subsequently luminal narrowing.4-6 However, a causal relationship between such injury and stenoses has not been established. Thrombomodulin is a glycoprotein receptor expressed on the surface of endothelial cells and released into the circulation in response to injury. An increased serum level of thrombomodulin is a reliable marker of endothelial cell damage.7-11 Platelets aggregate on damaged endothelium and play a central role in vascular repair. Previously, early postoperative platelet scintigraphy has been shown to be valuable in the detection of focal endothelial injury in patients undergoing vascular surgery.12-15 In this study vein graft injury during bypass surgery was assessed by serial measurements of plasma thrombomodulin levels and by early postoperative indium-111 platelet scintigraphy. The thrombomodulin levels and the scintigraphic findings were related to the postoperative development of stenoses during follow-up.

Materials and Methods

Subjects

Forty patients scheduled for infrainguinal vein bypass surgery between January 1994 and June 1996 were included in the study. Although preoperative vein evaluation was performed by B-mode ultrasound imaging five patients were intra-operatively found to have poor quality veins. In these a polytetrafluoroethylene (PTFE) bypass was inserted and they were excluded from further analysis. The median age of the remaining 19 males and 16 females was 72 (range 44-82) years. Six patients were diabetic and seven received acetylsalicylic acid preoperatively. The
indication for surgery was intermittent claudication in six, rest pain in nine and ischaemic ulceration or gangrene in 20 patients. The study was approved by the local Research Ethics Committee and informed consent was obtained in each case.

Surgery

An in situ vein bypass was performed in 34 patients and a composite vein graft in one patient. The level of the proximal anastomosis was the femoral artery in 34 and the popliteal artery in one. The level of the distal anastomosis was the popliteal artery above the knee in one case, the popliteal artery below the knee in 15 patients, a single crural artery in 17 and a pedal artery in two patients. The surgical procedures were carried out as described by Leather et al. The proximal vein was separated from the common femoral vein and the most proximal valves were excised under direct vision. A Fogarty embolectomy catheter was introduced from the distal end and an intraluminal valve cutter was attached to it. These were drawn distally with the vein distended by a papaverine-heparin/saline solution infused at a pressure of 200–300 mmHg. After completion of the proximal anastomosis arteriovenous fistulas were identified by flowmetry and ligated. During arterial clamping intravenous heparin (50 i.u. kg\(^{-1}\) body weight) was given for thrombosis prophylaxis. From the day before surgery until mobilisation all patients received subcutaneous low molecular weight heparin (logiparin 3500 i.u. daily) and after mobilisation acetylsalicylic acid 500 mg a day.

Thrombomodulin assays

Blood samples for thrombomodulin assays were drawn preoperatively, 4 h, 1 and 2 days postoperatively. Aliquots of the sera had been stored at -20 °C until analysed. The serum thrombomodulin levels were determined by commercially available 2-site enzyme-linked immunosorbent assays (ELISA) (Diagnostica Stago, Asnières, France, distributed by Boehringer Mannheim GmbH, Mannheim, Germany) as described earlier. Briefly, according to the manufacturer’s instruction, precoated 96-well microtitre plates were incubated with diluted samples (serum-dilution: 1:5) or serial dilutions of the provided standard. After washing the ELISA-plates were incubated with the secondary HRP-conjugated monoclonal anti-thrombomodulin-antibody. Subsequently, the plates were washed again and incubated with the substrate solution. After color stabilisation the optical density was measured by an automated ELISA-plate reader with the suggested filters (Titertek Multiscan Plus MKII, ICN/Flow, Meckenheim, Germany). The concentrations of the samples were calculated in relation to the respective reference standard curve. The mean of duplicates was used for further analysis.

Platelet scintigraphy

Preoperatively a blood sample was drawn without venous stasis. The platelets were isolated and labelled with indium-111 as previously described. Following meticulous haemostasis and immediately after restoration of flow in the graft the labelled platelets were re-injected into the femoral artery just above the proximal anastomosis. Gamma camera images of the thigh and leg in the antero-posterior projection were obtained 4 h (n = 3), 24 h (n = 23) or 4 and 24 h (n = 9) after platelet injection using a GE XRT camera together with a Starcam computer. The scintigraphic studies were evaluated by two experienced examiners who were blinded with respect to clinical outcome. Focal platelet accumulations were observed in the following areas: the groin, the proximal and distal parts of the thigh, the knee, the proximal and distal parts of the leg and the foot. The number of regions assessed in each patient varied from four to six according to the level of the proximal and distal anastomoses. The length of the activity accumulations were measured and the intensity was semiquantified as moderate or intense.

Follow-up

After discharge the patients were included in a follow-up regimen consisting of ankle blood pressure measurements by Doppler technique and ultrasound duplex scanning at 6 weeks, 3, 6, 9 and 12 months. A localised increase in the peak systolic velocity exceeding 250% was taken as an indicator for a significant stenosis. Reintervention was considered in patients with stenoses associated with an interval reduction in the ankle brachial index of more than 0.15. The occurrence of bypass occlusions and of stenoses during follow-up were related to thrombomodulin levels and scintigraphic findings.
Intraoperative Injury and Vein Graft Stenosis

30
50
Time (hours) after surgery

Fig. 1. Serum thrombomodulin levels measured preoperatively, 4 h, 1 and 2 days after infrainguinal vein bypass surgery in 26 patients (median values and inter-quartile ranges. *: p<0.001 compared with preoperative values (Wilcoxon test).

Statistics

Frequencies were compared by Fisher’s exact test. Since most of the data was not normally distributed, results are mainly presented as median values with ranges, and non-parametric statistics were used for comparisons: the Mann–Whitney test for comparison of continuous variables between groups and the Wilcoxon test for comparison of continuous variables within groups.

Results

Thrombomodulin assays

For various logistical reasons blood samples for thrombomodulin assay were not obtained in six patients. In three patients a very high serum thrombomodulin level (>70 ng/ml) was observed preoperatively and these were not included in further analyses. One of the three patients had non-insulin dependent diabetes complicated by end stage renal disease. In the other two cases no cause of the elevated preoperative thrombomodulin level could be identified. Serum thrombomodulin in the remaining 26 patients increased significantly from 17 (range 10–60) ng/ml preoperatively, to 21 (range 12–59) ng/ml 4 h, p = 0.0005, 32 (range 16–59) ng/ml 1 day, p = 0.00002 and 31 (range 14–55) ng/ml 2 days, p = 0.00007 after vein bypass surgery (Fig. 1). The median increase in thrombomodulin from preoperatively to 4 h postoperatively was 3.0 (range 2–19) ng/ml and from preoperatively to day 1 15.5 (range 1–42) ng/ml. Neither the preoperative thrombomodulin levels nor the postoperative increase differed significantly between diabetics and non-diabetics.

Scintigraphic findings

A total of 62 focal platelet accumulations were identified in the groin (n = 27), proximal thigh (n = 13), distal thigh (n = 5), knee region (n = 2), proximal leg (n = 10) and distal leg (n = 5). The activity accumulations measured median 5 (range 1–25) cm in length and were graded as moderate in 46 cases and intense in 16 cases (Fig. 2).

Clinical findings

Early (<30 days) events

Five (14%) bypasses, including the composite vein graft, thrombosed within 30 days after surgery.

Events during follow-up

The thirty patients with in situ vein grafts which remained patent at 30 days were followed a median of 12 (range 1–18) months. Nine non-haemodynamically significant stenoses and three haemodynamically significant stenoses were identified in nine grafts a median of five (range 1–9) months after surgery. The stenoses were located in the groin in seven, proximal leg in one, distal leg in three and a long stenosis extended over both the proximal and the distal leg. Two stenotic grafts were revised and remained patent.
Table 1. Serum thrombomodulin levels (ng/ml) measured preoperatively, 4 h, 1 and 2 days after infrainguinal vein bypass surgery in the subgroup of 22 patients with vein grafts remaining patent during the first 30 days postoperatively. The patients are classified according to the presence or absence of bypass stenosis (median values and ranges).

<table>
<thead>
<tr>
<th></th>
<th>Absence of stenosis (n = 16)</th>
<th>Presence of stenosis (n = 6)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperatively</td>
<td>19 (10–60)</td>
<td>17 (15–41)</td>
<td>n.s.</td>
</tr>
<tr>
<td>4 h after surgery</td>
<td>22 (12–59)</td>
<td>18 (15–41)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Day 1</td>
<td>34 (16–59)</td>
<td>38 (19–52)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Day 2</td>
<td>32 (14–55)</td>
<td>36 (15–43)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

throughout the rest of follow-up. Of the seven conservatively treated grafts with stenoses one occluded 12 months after surgery.

**Correlation between events and thrombomodulin levels**

There was no difference in preoperative or postoperative serum thrombomodulin levels between patients with grafts occluding early and patients with grafts remaining patent at 30 days. Among the patients with grafts which remained patent at 30 days serum thrombomodulin levels preoperatively to 2 days postoperatively were similar in those who developed graft stenoses and those who did not develop stenoses (Table 1).

**Correlation between events and scintigraphic activity accumulations**

No difference in the number or intensity of activity accumulations between patients with grafts occluding early and patients with grafts remaining patent at 30 days was observed. Among the 30 grafts which were patent at 30 days stenoses were identified in nine (16%) of 55 regions where activity accumulations had been visualised as compared to only four (4%) of 94 regions where no activity accumulations had been observed, p = 0.03 (Tables 2 and 3). The occurrence of stenoses showed no correlation with length or intensity of the activity accumulations. The diagnostic accuracies of perioperative scintigraphy in predicting the development of postoperative graft stenoses are listed in Table 3.

**Discussion**

Graft stenoses due to intimal hyperplasia are the major cause of infrainguinal vein bypass failure. Surgical preparation of the saphenous vein for in situ vein bypass surgery causes extensive morphological and functional endothelial damage. However, the pathogenetic significance of this perioperative injury in the development of intimal hyperplasia is unknown. Only one clinical study has addressed the issue in humans. Moody et al. peroperatively recorded events assumed to lead to endothelial damage such as fistula ligation, valve disruption and clamping. It was found that these events showed no correlation with subsequent stenoses in in situ vein grafts. However, no direct assessment of the endothelial state was performed.

Thrombomodulin is the transmembranous glycoprotein receptor for thrombin mainly expressed on endothelial cells. The thrombomodulin-thrombin complex acts as an important anticoagulant resulting in accelerated activation of protein C. These functions contribute to the non-thrombogenic nature of endothelium. In-vitro studies have confirmed that soluble thrombomodulin is a reliable marker of endothelial cell damage independent of physiological activation. Thrombomodulin activity is present in veins harvested for coronary artery bypass grafting but greatly reduced in mechanically denuded vein segments and in veins exposed to arterial flow. This reduced thrombomodulin activity may result in dysfunctional endothelial anticoagulant activity and possibly contributes to graft thrombosis.

In the present study a marked increase in serum thrombomodulin was observed in the first 2 days following vein bypass surgery. The initial (within 4 h) increase is due to the direct, surgery dependent endothelial damage within the vein in addition to the general response to surgery. The further increase in thrombomodulin observed between 4 h and 1 day after surgery reflects the pathophysiological process of the removal from intraoperatively injured cells resulting in a time-dependent release of thrombomodulin. The haemodynamic changes with increased pressure and flow rate in the vein graft after bypass surgery might contribute, together with the perioperative endothelial damage, to this prolonged release of thrombomodulin. In keeping with our results plasma thrombomodulin has been found to increase after other cardiovascular procedures, including percutaneous transluminal angioplasty and open heart surgery. On day 2 the thrombomodulin values showed a tendency to decrease. Changes beyond 2 days postoperatively were not assessed in the present study but in animal experiments the endothelial thrombomodulin activity has been found to return to normal 1 week after balloon catheter denudation. The association between...
Table 2. The occurrence of graft stenoses according to focal scintigraphic platelet accumulations in 30 patients with vein grafts remaining patent at 30 days. The varying number of segments is due to the variable length of the vein grafts.

<table>
<thead>
<tr>
<th>Region</th>
<th>n</th>
<th>Platelet accumulation</th>
<th>Stenosis</th>
<th>No platelet accumulation</th>
<th>Stenosis</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groin</td>
<td>29</td>
<td>25</td>
<td>6 (24%)</td>
<td>4</td>
<td>1 (25%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Proximal thigh</td>
<td>29</td>
<td>11</td>
<td>0 (0%)</td>
<td>18</td>
<td>0 (0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Distal thigh</td>
<td>29</td>
<td>5</td>
<td>0 (0%)</td>
<td>24</td>
<td>0 (0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Knee</td>
<td>30</td>
<td>1</td>
<td>0 (0%)</td>
<td>29</td>
<td>0 (0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Proximal leg</td>
<td>17</td>
<td>10</td>
<td>0 (0%)</td>
<td>7</td>
<td>2 (29%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Distal leg</td>
<td>13</td>
<td>3</td>
<td>3 (100%)</td>
<td>10</td>
<td>1 (10%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Foot</td>
<td>2</td>
<td>0</td>
<td>0 (0%)</td>
<td>2</td>
<td>0 (0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Total</td>
<td>149</td>
<td>55</td>
<td>9 (16%)</td>
<td>94</td>
<td>4 (4%)</td>
<td>0.03</td>
</tr>
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</table>

Table 3. Occurrence of graft stenoses according to the presence or absence of focal scintigraphic platelet accumulations in 149 vein graft segments.

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>n</th>
<th>Platelet accumulation</th>
<th>No platelet accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stenosis</td>
<td>13</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>No stenosis</td>
<td>136</td>
<td>46</td>
<td>90</td>
</tr>
<tr>
<td>Total</td>
<td>149</td>
<td>55</td>
<td>94</td>
</tr>
</tbody>
</table>

Diagnostic accuracy of perioperative platelet scintigraphy in predicting postoperative development of graft stenoses.

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<tbody>
<tr>
<td>Positive predictive value</td>
<td>16%</td>
<td>(9/55)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>96%</td>
<td>(90/94)</td>
</tr>
<tr>
<td>Specificity</td>
<td>69%</td>
<td>(90/136)</td>
</tr>
<tr>
<td>Overall accuracy</td>
<td>66%</td>
<td>(99/149)</td>
</tr>
</tbody>
</table>

Platelets play a key role in the repair of vascular injuries. Immediately after an injury platelets aggregate on the luminal surface and may form thrombosis. Various factors secreted from activated platelets as well as from damaged endothelial cells stimulate the proliferation of smooth muscle cells and synthesis of extracellular matrix leading to intimal thickening. Platelet scintigraphy has proven an effective tool in vivo studies of vascular injury in humans. In the present study the labelled platelets were injected directly into the inflow artery in order to improve image quality by increasing the amount of labelled thrombocytes to the region of interest. Intravenous administration of labelled platelets might lead to their sequestration in the spleen and bone marrow. The time of scintigraphic assessment varied between 4 and 24 h postoperatively and a pilot study has demonstrated that scintigraphic findings do not change significantly during that period. Since the purpose of the scintigraphic studies was to identify the presence of localised platelet accumulations an assessment of the overall activity in the vein grafts was not performed. As previously described, we found platelet aggregations, mainly located in the regions of anastomosis, in the majority of patients undergoing vein bypass surgery. Extravascular accumulations due to intraoperative bleeding are unlikely as meticulous haemostasis was performed in all cases. Furthermore, using 111-indium labelled platelets Stratton et al. showed that non-vascular surgery does not lead to platelet accumulations. It therefore seems justified to assume that the platelet accumulations are intraluminal and represent perioperative endothelial injury.

The observed lack of association between platelet accumulations and early postoperative occlusions suggest that endothelial injury does not play a central role in these events. In patients with grafts remaining patent at 30 days the occurrence of vein graft stenoses was four times higher in regions where platelet aggregations had been identified as compared to regions without platelet aggregations. Only in four cases, out of a total of 149 regions without visible platelet binding, a stenosis complicated the following 12 months. The very high predictive value (96%) of a negative scintigraphy strongly suggests that localised perioperative endothelial injury, as demonstrated by platelet accumulation at the site of subsequent development of stenosis, is an important pathogenetic factor. The low positive predictive value shows that, in spite of endothelial injury in many patients, the bypass may remain patent without development of a clinically significant stenosis, confirming previous observations that additional factors are involved. Among these preexisting morphological changes in the saphenous vein as well as serological risk factors, including high plasma levels of fibrinogen and the presence of antibodies to...
cardiolipin, have been shown to be associated with increased risk of graft failure.

A limitation of the present study was the methods used did not allow an accurate anatomical localisation of the platelet accumulations or of the stenoses and hence a precise correlation was not possible. Furthermore, the lack of a control group of patients undergoing surgery not associated with endothelial damage makes interpretation of the thrombomodulin results difficult.

Conclusion

The intervention-dependent thrombomodulin release from endothelial cells as well as the frequent finding of focal platelet accumulations in the vein grafts confirms that infrainguinal vein bypass surgery is associated with endothelial injury. The very high predictive value of a negative scintigraphy suggests that localised peri-operative vascular injury is a prerequisite of the development of vein graft stenoses and the low predictive value of a positive scintigraphy emphasises that other factors are involved.

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