Plasminogen Activator Inhibitor-1 Levels and Activity Decrease After Intervention in Patients with Critical Limb Ischaemia

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WHAT THIS PAPER ADDS

We hypothesised that patients with critical and acute limb ischaemia would have increased levels of plasminogen activator inhibitor-1 (PAI-1), leading to a prothrombotic state. The investigation showed a great individual variability in PAI-1 levels, with a large proportion of the patients having increased levels prior to treatment. An effective treatment of the ischaemic state normalised those levels, and it seemed that open surgery was more effective than endovascular treatment in this respect. Further studies on the prothrombotic state during and after treatment may result in better adjuvant treatment.

Objective/background: Patients with peripheral arterial occlusive disease (PAOD), in particular critical limb ischaemia (CLI), carry a high risk of thrombotic events. We hypothesised that patients undergoing conservative, endovascular, or open surgical treatment for CLI have increased levels of plasminogen activator inhibitor-1 (PAI-1), leading to a prothrombotic state. The objective was to determine levels of PAI-1 in patients with acute or chronic PAOD/CLI.

Methods: Thirty-two patients with a median age of 74 (49–90) years were included. Three underwent thrombolysis for acute limb-threatening ischaemia. Twenty-six patients with chronic ischaemia received endovascular (n = 20) or open (n = 6) surgical treatment. Three were treated conservatively. Biomarkers and ankle brachial index (ABI) were measured before and up to 1 month after intervention. Patency was studied with repeated duplex ultrasound.

Results: Ankle pressure and ABI improved after intervention (p < .001). C-reactive protein (CRP) increased from a median of 7.90 mg/L at baseline to 31.5 on day 1 (p < .001), 28.0 on day 6 (p < .001), and returned to baseline levels on day 30. PAI-1 antigen and activity decreased from day 6 and onwards post-intervention compared with baseline (p < .05). A great individual variability in PAI-1 antigen and activity was observed. Although most actively treated patients had normal PAI-1 activity, 11/29 (38%) were above that level of normality at baseline, 10/24 (42%) on day 1, 3/23 (13%) on day 6, and 5/27 (19%) on day 30 after intervention.

Conclusion: Endovascular and open surgical treatment resulted in improved ankle pressure and ABI. The intervention was followed by a transient increase in CRP and a sustained reduction in PAI-1 levels and activity.

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INTRODUCTION

Patients with peripheral arterial occlusive disease (PAOD) and, in particular, those with the most severe form of disease, critical limb ischaemia (CLI), carry a high risk of thrombotic events. The prothrombotic state results in an increased risk of general cardiovascular events and local arterial thrombosis after intervention. Antiplatelet therapy with 75–325 mg acetylsalicylic acid (ASA)/day reduced the number of vascular events by 25% and the number of reocclusions after coronary artery bypass graft (CABG), percutaneous transluminal coronary angioplasty, percutaneous transluminal angioplasty (PTA), or femoro-popliteal bypass by 40%. The Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) study showed a further risk reduction by using clopidogrel instead of ASA. Although the relative risk reduction was similar in different subgroups, the absolute risk reduction was greater among patients with multiple risk factors, which is typical
for PAOD patients. The importance of coagulation was shown in the Dutch Bypass Oral Anticoagulants or Aspirin study, which randomised 2,621 patients after femoropopliteo-distal bypass to receive ASA or anticoagulation. Patients with anticoagulation had better patency when a vein graft was used, but ASA was more effective with a prosthetic graft. That postoperative antiplatelet therapy is better with a prosthetic graft was recently verified in the Clopidogrel and Acetylsalicylic Acid in bypass Surgery for Peripheral Arterial Disease trial. In other therapeutic situations, such as after endovascular treatment or venous bypass, coagulation and fibrinolysis can be equally important targets for secondary prophylaxis.

Critical for fibrinolysis is the activation of plasminogen to plasin, the active enzyme responsible for the degradation of fibrin. Fibrin provides the basic framework for the formation of a blood clot. The role of plasminogen activator inhibitor-1 (PAI-1) is to stabilise a formed clot by inhibiting the plasminogen activator (t-PA). In a diseased state this activity can become prothrombotic.

Recent data from patients with thrombosis, such as those with acute myocardial infarction, as well as patients with risk factors for thrombosis, such as obesity, show elevated PAI-1 levels. Progression of PAI-1 levels over time, as well as high baseline levels, is also associated with incident type 2 diabetes—possibly the most important risk factor for PAOD/CLI. A direct pathogenetic connection between these elevated PAI-1 levels and the risk of thrombosis has, however, not yet been established in humans. Theoretically, inhibition of PAI-1 could be beneficial for a patient at elevated risk of thrombosis/vascular occlusion. Additionally, animal experiments with models of thrombosis have shown that thrombus formation can be prevented by inhibiting PAI-1 with antibodies or with PAI-1 antagonists.

The primary aim of this study was to characterise the absolute levels and changes in circulating concentration of PAI-1 and PAI-1 activity among patients with acute or chronic PAOD/CLI before and after conservative, endovascular, or open surgical treatment. As there were no previous data on this group of patients no power calculation was possible and the study was hypothesis-generating. A secondary aim was to study the logistics of including patients with CLI into clinical trials on PAOD.

**PATIENTS AND METHODS**

**Study design**

This was a prospective study in patients with limb-threatening lower limb ischaemia referred to the Department of Vascular Surgery in Uppsala for possible revascularisation. The study protocol was approved by the Regional Ethics Committee of the Uppsala/Orebro region. All patients gave oral and written informed consent. The study design is described in Fig. 1.

**Inclusion/exclusion criteria**

The aim was to include all eligible patients with limb-threatening acute or chronic lower limb ischaemia, who had infrainguinal disease. Predefined exclusion criteria were age below 18 years; need for immediate amputation; intolerance to blood sampling owing to anaemia (haemoglobin < 100 g/L) or lack of peripheral veins suitable for blood-sampling; previous intervention for lower limb ischaemia within the last 3 months; mental condition not allowing informed consent; or practical problems preventing follow-up and/or blood-sampling.

Enrolled patients in whom blood samples could not be obtained at baseline or after intervention were excluded from analysis.

**Subgroups based on planned interventions**

Based on the planned interventions, four predetermined subgroups were defined (Table 1). Although open surgical embolectomy, thrombectomy, and bypass surgery are still performed, the standard contemporary treatment for acute lower limb ischaemia is catheter-guided thrombolysis, followed by subsequent PTA and/or stenting. This subgroup of patients has a high risk of cardiovascular thrombotic events, and was thought to be of particular interest to study.

Chronic CLI was defined as Fontaine’s stages III–IV or Rutherford’s categories 4–6. Rest pain (Rutherford 4) had a minimum duration of 4 weeks and required morphine-like analgesia. In patients with chronic CLI, where revascularisation was not possible, the intervention used was pain relief and, if necessary, amputation at a later date. The

<table>
<thead>
<tr>
<th>Pre-intervention assessments:</th>
<th>Types of intervention:</th>
<th>Post intervention assessments:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline blood sampling</td>
<td>Conservative</td>
<td>Blood sampling biomarkers</td>
</tr>
<tr>
<td>Patient status and demographics</td>
<td>Endovascular</td>
<td>Routine labs (day 6 and day 30)</td>
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<tr>
<td>Vital signs and ABI</td>
<td>Open surgery</td>
<td>Vital signs and ABI (day 6 and day 30)</td>
</tr>
<tr>
<td>Vessel status</td>
<td></td>
<td>Clinical vascular events and vessel status (day 6 and day 30)</td>
</tr>
</tbody>
</table>

Figure 1. Study design: clinical assessments and blood sampling. Note. ABI = ankle brachial index.
natural history of untreated CLI is difficult to assess in a contemporary context as in most centres approximately 90% undergo active revascularisation. Based on historical data it is estimated that 1 year after diagnosis 25% of patients have died, 30% are amputated, and 45% are still alive with two intact limbs.12,13

Most patients in the current study underwent revascularization. Endovascular treatment (PTA/stent/subintimal angioplasty, or a combination of these modalities) was the first-choice treatment. Open surgical treatment was performed when endovascular treatment was difficult, or even impossible, and if the patient was considered to tolerate an open procedure. Open procedures included femoropopliteal or femoro-crural bypass (with either autologous vein or expanded polytetrafluoroethylene graft), sometimes combined with trombendarterectomy in the common femoral artery, or PTA to improve inflow or outflow of the bypass.

Six patients in the endovascular group and two in the group treated conservatively had an additional endovascular intervention during the 6-month study period, but not within 1 month of recruitment. Thus, in total, 40 interventions were carried out in 32 patients, but laboratory samples specific for this investigation were only taken in conjunction with the first intervention.

Biomarker assessments

Blood was collected before and after intervention, and stored frozen (−70 °C) until analysis. All blood samples were taken between 8.00 a.m. and 10.00 a.m., to avoid confounding due to the circadian variation in PAI-1 levels. Standard clinical blood chemistry was measured at the Clinical Chemistry Laboratory at Uppsala University Hospital, Uppsala, Sweden. PAI-1 antigen and activity, D-dimer, and C-reactive protein (CRP) in plasma were all determined at the Clinical Chemistry Laboratory at Sahlgrenska University Hospital, Gothenburg, Sweden, using standard methods.

For this investigation the definition of the upper limit of normality for PAI-1 activity is crucial, and was measured with a Chromolize immunoassay test (Trinity Biotech, Bray, Ireland). The Clinical Chemistry Laboratory at Sahlgrenska University Hospital uses an upper limit of 21 IU/mL, based on approximately 40 healthy individuals, whose blood samples were obtained in 1996–97. In a recent publication, 1,016 patients aged 70 years were investigated in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study,13 in which obesity and metabolic syndrome were associated with elevated PAI-1 levels. In the PIVUS study, 319 patients were identified to have a normal body mass index (BMI) (<25), and no signs of metabolic syndrome. This subgroup had a mean level of PAI-1 activity of 5.1 IU/mL (SD: 6.6), measured using the same laboratory method. Based on this larger experience of verified healthy individuals in a similar age group from the same geographic area, we decided to define the upper limit of normality as 5.1 + 2 × 6.6 = 17.7 IU/mL, approximated to 18 IU/mL.

Another important factor for the interpretation of the results is the coefficient of variation (CV) of the assays used. For the a Chromolize immunoassay test used to calculate PAI-1 activity the CV in the interval 2–36 IU/mL was 3.7% at the 2 IU/mL level, 2.7% at the 22 IU/mL level, and 2.6% at the 36 IU/mL level. The TriniLize PAI-1 antigen immunoassay (Trinity Biotech, Jamestown, NY, USA) has a CV of 1.9% at 20 ng/mL and 2.9% at 40 ng/mL.

The blood sampling was scheduled for a total of 8–9 occasions (Fig. 1). Blood sampling on days 2 and 3 was only performed if the patient was still in hospital, which was not always the case after conservative or endovascular treatment.

Clinical assessments and duplex ultrasound

Vessel status assessment was performed pre-intervention, at 1 week and 1 month post-intervention based on clinical examination, ankle and brachial blood pressure measurements, and duplex ultrasound. Calculation of ankle brachial index (ABI) was performed. Toe-pressure measurements were not performed. Assessments at 3 and 6 months were made via telephone calls and review of the patients’ medical records (Fig. 1).

Study patient recruitment

Seventy-four patients were screened for participation with an aim to include 40 and to be able to evaluate at least 32. Thirty-five consecutive patients were found eligible and were enrolled in the study between September 2009 and April 2010 (Fig. 2). Uppsala is a regional tertiary care centre, serving a sparsely populated region. Some patients could not be included owing to transport problems. Dementia and other mental conditions precluding informed consent were other common exclusion criteria.

Table 1. Predetermined subgroups in the 32 patients studied.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombolysis for acute lower limb ischaemia</td>
<td>3</td>
<td>8.8</td>
</tr>
<tr>
<td>Pain relief/amputation for CLI</td>
<td>3</td>
<td>8.8</td>
</tr>
<tr>
<td>Endovascular treatment for CLI</td>
<td>20</td>
<td>64.7</td>
</tr>
<tr>
<td>Open surgical treatment for CLI</td>
<td>6</td>
<td>17.6</td>
</tr>
</tbody>
</table>

Note. CLI = chronic critical limb ischaemia.
Eight screened patients had prior interventions for CLI within 3 months, and were not recruited as the prior intervention could have affected coagulation/fibrinolysis. Four patients were not included because of pre-intervention haemoglobin <100 g/L, and one patient lacked veins suitable for blood-sampling. Four patients could, from a medical point of view, have been recruited, but emergency situations resulted in failure of pre-intervention blood sampling.

Two enrolled patients, both in the endovascular group, were excluded because of problems with blood-sampling. In the first patient, who was enrolled in an emergency situation, the baseline blood sample was lost. The other patient, who was on renal replacement therapy, lacked suitable veins so that post-intervention blood sampling had to be discontinued from an ethical stand-point. One patient withdrew consent to participate in the study.

Statistical methods
The study sample size was not based on any formal sample size calculation owing to the explorative character of the study. The longitudinal changes in the primary variables, PAI-1 antigen and PAI-1 activity, were explored graphically and by descriptive statistics. The degree of intra-individual change between baseline (pre-intervention) and post-intervention levels of biomarkers at different time points were assessed by p-values of statistical tests. In the group of all patients and those with invasive intervention the logarithms of the biomarkers were approximately normal, and a paired t-test was used. For the groups and variables where the normality was questioned, for example in the small open surgery group, Wilcoxon signed-rank test was used for statistical comparisons. No correction for multiple comparisons was performed.

RESULTS
Study population and baseline characteristics
Thirty-two patients were included who were conservatively (n = 3), endovascularly (n = 23), or surgically treated (n = 6). Baseline characteristics are summarised in Table 2. Median age was 74 years (range 49—90 years), the endovascular group was older (median 77 years) than the other groups (median 70—71 years). The patients with chronic CLI were rather equally distributed between the Rutherford 4, 5, and 6 categories. The high mean ankle pressure of 81 mmHg is explained by a large number of patients with incompressible arteries due to diabetes. In six patients the ankle pressure was zero.

Eight patients (25%) were active and 16 (50%) were former smokers. BMI was >25 in 20 patients (59%), and >30 in seven (21%). Eighteen had diabetes (56%) treated with insulin, often in combination with oral medication.

Twenty patients (59%) had a medical history of cardiac disease, 14 (41%) had coronary artery disease, and 6 (18%) had atrial fibrillation or valvular disease. Three had undergone CABG; two had percutaneous coronary intervention. Mean creatinine level was 136 μmol/L; three were on renal replacement therapy. Previous vascular surgical history included 11 endovascular and six open procedures for lower limb ischaemia, two major amputations, two operations for abdominal aortic aneurysms, and one carotid artery endarterectomy. At entry, 25 patients (74%) were treated with low dose ASA, three with dinkumarol (8.8%), and 10 were on low-molecular weight heparin (29%).

Surgical outcome and major complications
The median ankle pressure increased from 75 mmHg (range 0—250 mmHg) at baseline to 140 mmHg (range 60—220 mmHg, p < .01 compared with baseline) 6 days after
intervention and 138 mmHg (range 0–250 mmHg, \( p < .001 \) compared with baseline) 30 days after intervention among the 23 patients who underwent endovascular treatment. The median ABI increased from 0.46 (range 0–1.57) at baseline to 1.02 (range 0.38–1.69, \( p < .01 \) compared with baseline) 6 days after endovascular intervention and 1.00 (range 0–1.83, \( p < .01 \) compared with baseline) 30 days after endovascular intervention (Fig. 3).

One patient suffered a myocardial infarction, another a stroke. There was no 30-day mortality, but during the 6-month follow-up one patient died from combined renal and heart failure. There was no major amputation, but two toe amputations. One patient was re-operated on for a postoperative bleeding.

**Biomarkers**

The three patients who were treated conservatively had similar levels of the targeted biomarkers at baseline as the 29 who underwent active therapy, and their biomarkers were virtually unchanged during the 30-day follow-up period. They are not further included in the analysis, which is focused on the two main intervention groups: endovascular treatment (\( n = 23 \)) and open surgical treatment (\( n = 6 \)).

The median (range) high sensitive CRP measured in serum in the 23 patients who underwent endovascular treatment increased from 5.80 mg/L (0.68–85.0 mg/L) at baseline, to 25.0 mg/L (1.10–110 mg/L, \( p < .001 \)) the first day after intervention and 24.0 mg/L (3.30–130 mg/L, \( p < .01 \)) 6 days after intervention. One month post-endovascular intervention the CRP values had returned to baseline levels 10.0 mg/L (1.20–94.0 mg/L), \( p = .746 \) (Fig. 4).

Neither PAI-1 antigen nor PAI-1 activity increased post-intervention compared with baseline at the measured time points at a group level, as shown in Table 3. In contrast, from day 6 onwards there was a significant reduction in PAI-1 levels and activity (Figs. 5 and 6).

The variability of PAI-1 antigen and PAI-1 activity are described at the main assessment time points, in the two treatment groups, as well as for all treated patients, as boxplots in Figs. 5 and 6. The upper limit of normality for PAI-1 activity is given as a reference line in Fig. 6.

Although most patients were within the limit of normality for PAI-1 activity, it can be noted that among the actively treated patients, 11/29 (38%) were above that limit at baseline, 10/24 (42%) on day 1, 3/23 (13%) on day 6, and 5/27 (19%) 30 days after intervention.

**Figure 3.** Ankle-brachial index (ABI) at baseline and 6 and 30 days after intervention or surgery. (A) Patients who had endovascular treatment (including thrombolysis); (B) patients who had open surgery; (C) all patients. Significance (\( p \)-values) indicates change from baseline (paired \( t \)-test for A and C; signed-rank test for B). The boxes represent median and interquartile range (IQR). The notches extend to \( \pm 1.58 \text{IQR/sqrt}(n) \). Outliers are excluded from the figure statistics.
In this exploratory study on patients with CLI, the longitudinal changes in the primary variables, PAI-1 antigen and PAI-1 activity, did not show the expected increase post-intervention at a group level. There was, however, a significant reduction in PAI-1 levels and activity compared with pre-treatment levels, indicating that the ischaemic state was an important factor explaining high levels prior to therapy. This is in line with a previous investigation that reported an up-regulation of PAI-I by hypoxia.14

Furthermore, great variability in the PAI-1 levels was observed, and a substantial fraction of the patients had levels of PAI-1 activity above the defined threshold value. That was an expected finding, given the fact that conditions associated with increased levels of PAI-1,8 such as overweight/obesity and diabetes/metabolic syndrome, are common in this group of patients.15

If individual patients with elevated levels of PAI-1 are associated with an increased risk of thrombotic complications, and if therapeutic measures aimed at lowering PAI-1 levels may affect outcome in this group of patients, remains to be shown. The study was not designed to determine whether elevated levels of PAI-1 activity were associated with an increased risk of adverse events.

The pro-thrombotic state of patients who have undergone revascularisation for CLI is multifactorial. All of the following factors are consequences of ischaemia/reperfusion, as well as surgical trauma: immobilisation, inflammation, activation of thrombocytes, elevated levels of fibrinogen, and the studied inhibition of fibrinolysis. It is interesting to note (in Figs. 4 and 6) that the response to open surgery seems to be somewhat different to that of endovascular intervention. The early inflammatory response was greater after open surgery, but after 30 days all the patients operated on had normalised CRP, whereas a large proportion of the endovascularly-treated patients still had elevated levels 30 days post-intervention (Fig. 4). The effect on PAI-1 activity, however, did not vary depending on the type of treatment (Fig. 6). One possibility is that open surgery results in a more effective reversal of the ischaemic state. The size of the current study, however, limited the possibility of performing subgroup analyses.

One limitation is that, for ethical reasons, we had to limit the blood sampling. We aimed to study the entire post-operative period (30 days). As a consequence, the possibility of a short-term elevation of PAI-1 occurring during the first few hours after surgery or intervention cannot be excluded. This is a period, however, when thromboprophylaxis is maintained with multiple drugs. The patients were
on ASA or anticoagulation preoperatively, and they received heparin during surgery/intervention and low molecular weight heparin during the postoperative period as prophylaxis for venous thrombo-embolism. Thus, an early peak in PAI-1 is unlikely to constitute a clinical problem, and this was the rationale for focusing the blood sampling on the later postoperative period. All the drugs affecting thrombosis and haemostasis are potential confounders, but it was

Table 3. Differences in biomarker levels between baseline and post-intervention.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Day</th>
<th>Median (range)</th>
<th>Patients (n)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer (ELISA) (mg/L)</td>
<td>Baseline</td>
<td>1.00 (0.33−7.50)</td>
<td>29</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Change day 1-0</td>
<td>0.15 (−4.80 to 1.94)</td>
<td>24</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td>Change day 6-0</td>
<td>0.60 (−5.40 to 2.20)</td>
<td>23</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Change day 14-0</td>
<td>0.74 (−4.80 to 4.10)</td>
<td>20</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Change day 21-0</td>
<td>0.46 (−5.50 to 2.60)</td>
<td>22</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Change day 30-0</td>
<td>0.16 (−5.60 to 2.41)</td>
<td>27</td>
<td>0.017</td>
</tr>
<tr>
<td>P-PAI-1-activity (kIE/L)</td>
<td>Baseline</td>
<td>14.0 (0.60−69.00)</td>
<td>29</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Change day 1-0</td>
<td>−0.35 (−31.00 to 122.00)</td>
<td>24</td>
<td>0.791</td>
</tr>
<tr>
<td></td>
<td>Change day 6-0</td>
<td>−0.05 (−58.00 to 12.60)</td>
<td>23</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Change day 14-0</td>
<td>−3.6 (−55.00 to 29.00)</td>
<td>20</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>Change day 21-0</td>
<td>−4.7 (−49.00 to 46.00)</td>
<td>22</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>Change day 30-0</td>
<td>−8.0 (−36.00 to 43.00)</td>
<td>27</td>
<td>0.000</td>
</tr>
<tr>
<td>P-PAI-1-antigen (μg/L)</td>
<td>Baseline</td>
<td>19.53 (7.40−53.80)</td>
<td>29</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Change day 1-0</td>
<td>2.00 (−17.00 to 84.80)</td>
<td>24</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>Change day 6-0</td>
<td>−60 (−36.00 to 9.80)</td>
<td>23</td>
<td>0.102</td>
</tr>
<tr>
<td></td>
<td>Change day 14-0</td>
<td>−2.8 (−22.00 to 19.80)</td>
<td>20</td>
<td>0.026</td>
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<tr>
<td></td>
<td>Change day 21-0</td>
<td>−6.2 (−32.00 to 30.40)</td>
<td>22</td>
<td>0.071</td>
</tr>
<tr>
<td></td>
<td>Change day 30-0</td>
<td>−4.9 (−32.00 to 21.70)</td>
<td>27</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Note. ELISA = enzyme-linked immunosorbent assay; PAI-1 = plasminogen activator inhibitor-1.

a Only 29 patients who underwent surgery/endovascular intervention are included in this analysis.
b p refers to comparisons between baseline and the day indicated.

Figure 5. Plasminogen activator inhibitor-1 (PAI-1) antigen at baseline, and 1, 6, 14, 21, and 30 days after intervention or surgery. (A) Patients who had endovascular treatment (including thrombolysis); (B) patients who had open surgery; (C) all patients. Significance (p-values) indicates change from baseline (paired t-test for A and C; signed-rank test for B). The boxes represent the median and interquartile range (IQR). The notches extend to ±1.58 IQR/√n. Outliers are excluded from the figure statistics, but not from the significance testing.
not possible to perform a multivariate analysis with so few patients. However, the patients were treated in a very similar way, as this was a single-centre study in an academic unit with strict management guidelines. Furthermore, most comparisons were made in a matched design, on the same patients before and after treatment, thus minimising the risk of confounding.

Patients with CLI are often elderly with multiple comorbidities, including mental disorders. The CAPRIE study showed that patients with PAOD had more generalised atherosclerosis than other subgroups of patients with cardiovascular disease, and this finding was verified in many other investigations. Designers of clinical trials often prefer to study claudicants instead of patients with CLI, with the rationale that this group is easier to recruit owing to the higher prevalence and more stable nature of their disease, and the fact that they are younger. A secondary aim of this investigation was to study the logistics of including patients with CLI. Approximately half of the patients screened were recruited, resulting in 32 patients being studied over 6 months at this hospital, which has a primary catchment area of 320,000 people. Some of the exclusion criteria, such as prior intervention or anaemia, may not apply to other trials. This information may be of interest to researchers planning trials on patients with PAOD. The inclusion of patients with CLI often results in more cardiovascular events and thus could reduce the sample size, depending on the studied outcome measures and the mechanism of action of the investigational drug.

In summary, patients treated with open or endovascular surgery for acute or chronic CLI demonstrated an inflammatory response, but no signs of generally inhibited thrombolysis defined as an increased activity of PAI-1 during the postoperative period. In contrast to the hypothesis that the intervention would increase PAI-1 levels the study showed a sustained reduction after revascularisation. A fairly large proportion of the patients did, however, have increased PAI-1 activity, both at baseline and throughout the postoperative period. The clinical significance of this latter finding remains uncertain. Further studies are needed to understand the thrombogenic environment in patients with PAOD.

CONFLICT OF INTEREST

B. Carlsson, M. Lepkowska Eriksson, and D. Bock are employees of AstraZeneca, and A. Bylock was an employee of AstraZeneca at the time the study was conducted.

FUNDING

This study was funded by AstraZeneca.
REFERENCES


