Peripheral arterial occlusive disease has been found to correlate with alterations in blood and plasma viscosity, whereas preoperative values did not affect the outcome of infrainguinal bypass grafting. After successful infrainguinal bypass grafting, Woodburn et al found that the relative blood viscosity, as a measure of erythrocyte rigidity, decreased and that the corrected blood viscosity only showed a tendency to decrease, whereas hematocrit was unaffected. In their experiment, the postoperative blood rheologic features were comparable with those of age-matched control subjects.

The aim of this study was to investigate hemorheologic variables in patients with critical limb ischemia (CLI) before and after infrainguinal reconstruction. A comparison was also made with a control group.

**Purpose:** Plasma and whole blood viscosity are elevated in patients with intermittent claudication. The objectives of this study were to investigate whether critical limb ischemia influences hemorheologic variables and whether the rheologic variables in blood from the affected limb differ from the general circulation. We also intended to study whether successful infrainguinal reconstruction improved hemorheologic variables.

**Methods:** Ten consecutive patients with critical limb ischemia (CLI) underwent arterial reconstruction, one patient with profundaplasty and nine patients with bypass procedures. Venous blood was sampled from the antecubital vein (arm) and the femoral vein (leg) of the affected limb 1 day before and 1 month after surgery. Ten control subjects (matched according to age, sex, diabetic status, and renal insufficiency) were also sampled. Whole blood viscosity, plasma viscosity, erythrocyte aggregation tendency, and erythrocyte fluidity (the latter variable describing the deformability of the erythrocytes) were measured by means of rotational viscometry. Erythrocyte volume fraction was also determined. Fibrinogen was measured in the patients with CLI.

**Results:** Erythrocyte fluidity, blood viscosity, and erythrocyte volume fraction were lower in patients with CLI than in control subjects (P < .01, P < .01, and P < .05, respectively). No major differences between cubital and femoral vein blood were seen before or after the operation in patients with CLI or in control subjects. Successful revascularization did not influence the hemorheologic variables, except for a decrease in blood viscosity in femoral vein blood (P < .05).

**Conclusion:** Hemorheologic properties were impaired in patients with CLI. Because no differences were seen between the systemic and local circulation and because no major improvement occurred 1 month after arterial reconstruction, other mechanisms besides local tissue ischemia may play a role. (J Vasc Surg 2000;31:691-5.)
group that was matched according to age, sex, diabetic status, and renal insufficiency.

MATERIAL AND METHODS

Approval for this study was obtained from the research ethics committee, Faculty of Medicine, University of Uppsala. Patients and control subjects gave their approval after receiving verbal and written information.

Patients. Ten patients (six men and four women) with a median age of 71 years (range, 52-85 years) had CLI. Except for two patients with false high pressures because of incompressible vessels, the median ankle brachial pressure index was 0.24 (range, 0-0.6). Seven patients had rest pain and nocturnal pain, and seven patients had arterial ulcers. Five patients had diabetes; three patients had a history of coronary arterial disease; two patients had sequelae after cerebrovascular lesion; three patients were receiving medication for hypertension, and one patient was dependent on hemodialysis. Six patients were smokers. Exclusion criteria were malignancies, inflammatory disease, and medication with warfarin before or after an operation. Nine infrainguinal bypass grafted (seven vein and two polytetrafluoroethylene) and one profundaplasty were performed. After the operation, all patients received 160 mg of acetyl salicylic acid daily.

Control population. Ten volunteers without clinical signs of peripheral arterial occlusive disease (six men and four women; median age, 74.5 years; range, 52-78 years) were used as control subjects for all variables. Five of these patients had diabetes, and one patient had renal insufficiency and was dependent on hemodialysis.

Blood sampling. Patients with CLI were sampled 1 day before surgery and 1 month after successful reconstruction. The control subjects were sampled once. In these two groups, blood was harvested both from the antecubital vein and the femoral vein (from the affected limb in patients with CLI) and collected in dry-heparinized vacutainer tubes (Terumo Venoject, Leuven, Belgium). A tourniquet was used during sampling from the antecubital vein because our reference values were calculated from blood harvested under similar conditions, and duplex scanning was used to guide puncture of the femoral vein.

Rheologic analyses. All rheologic analyses were performed by the same investigator (B.S.). A Couette rotational viscometer (Low Shear 30; Contraves AG, Zürich, Switzerland) with a 1 to 1 couette (bob-in-cup) system was used at 37°C. Whole blood viscosity was measured at shear rates of 100 seconds⁻¹ and 1 seconds⁻¹. Whole blood viscosity at high shear rate represented whole blood apparent viscosity (BV; reference values: median, 4.58; minimum-maximum, 3.60-5.63 MPa × second); at the low shear rate, the whole blood viscosity is mainly dependent on erythrocyte aggregation why erythrocyte aggregation tendency (AG; reference values: median, 0.98; min-max, 0.76-1.26 MPa × second) could be calculated by correction for erythrocyte volume fraction (EVF) and plasma viscosity (PV). The unit for AG is dimensionless. PV was analyzed at a shear rate of 38 seconds⁻¹ (reference values: median, 1.30; minimum-maximum, 1.16-1.49 MPa × second). The intra-assay and inter assay coefficients of variability for BV were 0.9% and 2.6% for AG were 1.4% and 7.9% and for PV were 0.7% and 3.1% respectively. Erythrocyte fluidity (Pa⁻¹ × second⁻¹) was determined with a modification of the original method with the use of only one centrifugation of whole blood instead of washing of the erythrocytes and then pipetting off the plasma anduffy coat, resuspending the erythrocytes in buffer to a standardized EVF of 55% and immediately measuring the viscosity at a shear rate of 1.0 second⁻¹. Erythrocyte fluidity was expressed as the inverted value of the erythrocyte viscosity (reference values: median, 103.3; minimum-maximum, 75.1-127.1 MPa × second), measured in pascal⁻¹ × second⁻¹. Intra-assay coefficient of variability for erythrocyte fluidity was calculated to be 4.9%.

EVF. EVF was analyzed by microhematocrit centrifugation at 11000g for 5 minutes without correction for trapped plasma.

Fibrinogen assay. Quantitative determination of fibrinogen levels in plasma was performed by the clotting method of Clauss (Fibri-Prest Automat 2; Triolab AB, Diagnostica Stago, Asnieres-sur-Seine, France). Reference interval in our laboratory was 2.0-3.6 g/ L. Coefficient of variation was 8% or less.

Statistics. Variables are presented as median and minimum-maximum, unless otherwise stated. Wilcoxon signed rank test was used for the comparison of the results before and after reconstructive surgery. Comparisons between groups were made with the Mann-Whitney U test. Results were presented as two-tailed level of probability; a P value of less than .05 was considered significant.

RESULTS

Arm versus leg. Except for lower values for whole BV because of the lower EVF and in femoral blood than in cubital blood 1 month after surgery.
(P < .05, for both), no differences were found among patients with CLI. The control subjects showed a lower EVF in femoral blood than in cubital blood (P < .05). Because there were only minimal differences in leg and arm vein blood, we preferred (to make it easier to compare patients and control subjects) to present and compare just the values of arm vein blood.

**CLI versus control subjects.** EVF was lower in CLI than in control subjects (P < .05); whole BV also was lower in patients with CLI (P < .01; Table I). PV and AG did not differ between patients with CLI and control subjects. Erythrocyte fluidity was significantly lower in patients with CLI than in control subjects (P < .01). There was no significant difference in fibrinogen concentration when seven of the patients with CLI were compared with six of the control subjects who had diabetes and renal insufficiency (5.62 g/L [3.50-7.09] vs 3.9 g/L [3.6-6.3]). Both patients with CLI and control subjects were higher than the reference interval of our laboratory (2.0-3.6 g/L).

**CLI before surgery versus CLI after surgery.** Whole BV was lower in femoral blood 1 month after surgery than before surgery (P < .05; Table II). No other differences were seen.

**DISCUSSION**

Whole BV, erythrocyte fluidity (corresponding to erythrocyte deformability), and EVF all differed significantly between patients with CLI (both before and after the operation) and control subjects, who were matched according to sex, age, diabetic status, and renal insufficiency. Because the groups consisted of only 10 patients and 10 control subjects, the statistical power in this study is low for small differences. However, there were three significant changes between patients and control subjects. These changes indicated impaired flow properties in CLI with a possible subsequent decrease in the transport of oxygen and nutritive substances to the tissues and reduced removal of metabolites from the tissues. Moreover, in a comparison between the patients with CLI and 83 healthy subjects with a median age of 38 years, EVF was lower, PV was higher, whole BV was lower, AG was higher, and erythrocyte fluidity was lower in patients with CLI (data not shown). These data support the findings that hemorheologic variables are abnormal in patients with CLI.

Only whole BV in the blood draining the ischemic and revascularized limb was significantly altered 1 month after successful revascularization, indicating no major improvement of hemorheologic variables after infrainguinal arterial reconstruction.

The use of low shear rate rotational viscometer differed from previous measurements of hemorheologic variables in peripheral arteriosclerotic occlusive disease with the use of a capillary viscometer (Coulter-Harkness) for measuring blood and PV. In a comparison of five different methods for estimating the degree of AG, viscometry together with the sedimentation test and the Paar device showed the highest range of response and sensitivity. When the capillary viscometer erythrocyte fluidity was used as a measure of erythrocyte deformability, erythrocyte deformability was calculated by a correction of the blood viscosity for the effects of hematocrit and PV. In the rotational viscometer, erythrocyte fluidity was measured in a more direct way by the inversion of erythrocyte viscosity at a standardized EVF of 55% in a buffer at a low shear rate. Viscometric methods have been mentioned by the International Committee of Standardization in Hematology as

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**Table I.** Patients with CLI versus control subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with CLI before surgery</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVF (%)</td>
<td>38.6 (29.2-50.4)</td>
<td>44.4 (39.4-49.6)*</td>
</tr>
<tr>
<td>PV (MPa × sec)</td>
<td>1.37 (1.34-1.85)</td>
<td>1.34 (1.2-1.7)†</td>
</tr>
<tr>
<td>BV (MPa × sec)</td>
<td>4.03 (3.37-5.40)</td>
<td>4.68 (4.06-5.26)‡</td>
</tr>
<tr>
<td>AG</td>
<td>1.22 (0.92-1.43)</td>
<td>1.11 (0.92-1.68)‡</td>
</tr>
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<td>1.11 (0.92-1.68)‡</td>
</tr>
</tbody>
</table>

The values are expressed as medians (minimum-maximum). *P < .05. †P = .19; not significant. ‡P < .01; the Mann-Whitney U test was used for comparison of the two independent groups. §P = .3150; not significant. ||Represents erythrocyte deformability.

**Table II.** Preoperative versus postoperative values in patients with CLI

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preoperative values</th>
<th>Postoperative values</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVF (%)</td>
<td>38.6 (29.2-50.4)</td>
<td>39.0 (33.4-49.2)*</td>
</tr>
<tr>
<td>PV (MPa × sec)</td>
<td>1.37 (1.34-1.85)</td>
<td>1.40 (1.22-1.55)*</td>
</tr>
<tr>
<td>BV (MPa × sec)</td>
<td>4.03 (3.37-5.40)</td>
<td>3.86 (3.64-4.80)*</td>
</tr>
<tr>
<td>AG</td>
<td>1.22 (0.92-1.43)</td>
<td>1.21 (0.96-1.39)*</td>
</tr>
<tr>
<td>EVF (%)</td>
<td>38.6 (29.2-50.4)</td>
<td>39.0 (33.4-49.2)*</td>
</tr>
<tr>
<td>PV (MPa × sec)</td>
<td>1.37 (1.34-1.85)</td>
<td>1.40 (1.22-1.55)*</td>
</tr>
<tr>
<td>BV (MPa × sec)</td>
<td>4.03 (3.37-5.40)</td>
<td>3.86 (3.64-4.80)*</td>
</tr>
<tr>
<td>AG</td>
<td>1.22 (0.92-1.43)</td>
<td>1.21 (0.96-1.39)*</td>
</tr>
</tbody>
</table>

The values are expressed as median (minimum-maximum). The Wilcoxon signed rank test was used for comparing repeated measurement. *P not significant. †Represents erythrocyte deformability.
one method for measuring erythrocyte fluidity.\(^9\)

In contrast to other studies\(^{10-12}\) we could not establish any significant correlation between age and hemorheologic variables when we analyzed our reference group and control group together (data not shown). Moreover, there were no differences in PV, BV, or AG between the reference group of 83 subjects with a median age of 38 years and the control group of 10 subjects with a median age of 74.5 years. These diverging results may be explained by differences in population size or different methods used; actually, such a correlation still remains to be determined.

Alterations of hemorheologic variables may depend on the existence of arteriosclerosis\(^2,4,13\) and with early lesions,\(^4\) diabetes,\(^15\) hypertension,\(^16\) and renal insufficiency. In our study, a large proportion of the patients experienced various manifestations of arteriosclerosis, diabetes mellitus, and hypertension; one patient was dependent on hemodialysis. To compensate for these factors, we used a control group that was matched for age, sex, diabetic status, and hemodialysis. However, the hemorheologic impairment caused by CLI alone may be difficult to assess. There were findings that the only significant change between preoperative and postoperative rheologic variables was a postoperative decrease in whole BV in femoral blood; the lack of difference between cubital and femoral blood may indicate that factors other than tissue ischemia caused impaired blood rheologic conditions or that the local tissue ischemia induces a generalized effect on rheologic variables.

The lack of major rheologic improvement after arterial reconstruction is supported by Woodburn et al.\(^4\) The decreased values of whole blood viscosity and EVF compared with control subjects are contradicting findings concerning claudicants\(^1\) and the early stages of atherosclerosis.\(^4\) There are also studies that indicate unaltered BV and EVF in claudicants compared with control subjects\(^2\) and decreases of BV and EVF that vary with the angiographic extent of peripheral arterial disease.\(^4\)

The erythrocyte fluidity decrease in patients with CLI persisted after revascularization, which indicated a chronic nature of erythrocyte pathologic features that may explain an increased destruction of large and stiff erythrocytes that are unable to pass through the narrow filters of the spleen with the subsequent decrease in EVF observed in this study.

With a linear increase in EVF, there is an exponential increase in whole blood viscosity\(^{18}\) that may explain the decreases in both of these variables in patients with CLI compared with control subjects.

PV has been shown to increase with the severity of arteriosclerosis\(^3\) and in claudicants.\(^1,2\) A correlation has been found between fibrinogen and PV.\(^{10}\) Our study did not show any differences in PV or fibrinogen between the groups, which supports the latter relationship. However, the small sample size makes these observations uncertain.

In conclusion, because the impaired hemorheologic variables in patients with CLI seem to be of long duration and not immediately relieved by arterial reconstruction, they may have a multifactorial cause and not be caused by tissue ischemia only. Future studies of the hemorheologic effects of drugs that improve graft patency would be of great interest.

REFERENCES


13. Franzini E, Driss F, Driss F, Daoud F, Darcet P, Chan TM.


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