Successful lower extremity angioplasty improves brachial artery flow-mediated dilation in patients with peripheral arterial disease

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Introduction: **Peripheral arterial disease (PAD) is associated with systemic impaired flow-mediated dilation (FMD) and increased risk for cardiovascular events. Decreased FMD may be caused by a decrease in arterial shear stress due to claudication and inflammation due to muscle ischemia and reperfusion. We assumed that endovascular revascularization of lower limb arterial obstructions ameliorates FMD and lowers inflammation through improvement of peripheral perfusion.**

Methods: **The study was a prospective, open, randomized, controlled, single-center follow-up evaluation assessing the effect of endovascular revascularization on brachial artery reactivity (FMD) measured by ultrasound, white blood cell (WBC) count, high-sensitive C-reactive protein (hs-CRP), and fibrinogen. We investigated 33 patients (23 men) with chronic and stable PAD (Rutherford 2 to 3) due to femoropopliteal obstruction. Variables were assessed at baseline and after 4 weeks in 17 patients (group A) who underwent endovascular revascularization and best medical treatment, and in 16 patients (group B) who received best medical treatment only.**

Results: **FMD did not differ between group A and B (4.96% ± 1.86% vs 4.60% ± 2.95%; $P = .87$) at baseline. It significantly improved after revascularization in group A (6.44% ± 2.88%; $P = .02$) compared with group B at 4 weeks of follow-up (4.53% ± 3.17%; $P = .92$), where it remained unchanged. The baseline ankle-brachial index (ABI) was similar for group A and B (0.63 ± 0.15 vs 0.66 ± 0.10; $P = .36$). At 4 weeks of follow-up, ABI was significantly increased in group A (1.05 ± 0.15; $P = .0004$) but remained unchanged in group B (0.62 ± 0.1). WBC counts of the two groups were comparable at baseline (group A: 7.6 ± 2.26 × 10^6/mL and group B: 7.8 ± 2.02 × 10^6/mL, $P = .81$). In group A, the leukocyte count significantly decreased after angioplasty from 7.6 ± 2.26 to 6.89 ± 1.35 × 10^6/mL ($P = .03$). For group B, WBC count did not differ significantly compared with baseline (7.76 ± 2.64 × 10^6/mL; $P = .94$). No effects were observed on hs-CRP or fibrinogen from endovascular therapy.**

Conclusion: **Endovascular revascularization with reestablishment of peripheral arterial perfusion improves FMD and reduces WBC count in patients with claudication. Revascularization may therefore have clinical implications beyond relief of symptoms, for example, reducing oxidative stress caused by repeated muscle ischemia or increased shear stress due to improved ambulatory activity. (J Vasc Surg 2008;48:1211-6.)**

Peripheral arterial obstructive disease (PAOD) is a common manifestation of atherosclerosis affecting >5% of the aged population.[1] Despite the low rate of peripheral complications and amputation, PAOD is associated with a minimal to severe impairment in functional activity and with an increased risk of future cardiovascular events.[2-5] For this reason, PAOD is considered a marker for systemic atherosclerosis. To date the most powerful prognostic indicator in PAOD patients is the ankle-brachial pressure index (ABI).[6]

Recent data suggest that increased inflammatory activity and endothelial dysfunction could be linked in PAOD patients and affect cardiovascular outcomes.[7,8] The degree of elevation of white blood cell (WBC) count within the normal range is a marker for increased risk for cardiovascular events in patients with PAOD.[9,10] This had been attributed to repeated muscle ischemia producing oxidative stress, with subsequent subclinical inflammatory activation.[7,11-13] For example, treadmill exercise in these patients is associated with a systemic inflammatory response[14,15] and acute systemic endothelial dysfunction at distant sites.[12] Thus, the ischemia–reperfusion injury associated with intermittent claudication could be among the causes for increased inflammatory activity and endothelial dysfunction in PAOD patients.[12,16,17]

Claudication also results in decreased physical activity and hence reduced shear stress.[16] Repeated shear stress has been shown to be one of the strongest stimuli for improvement of endothelial function through an increase in nitric oxide synthesis.[17,18] The two factors, postischemic muscle reperfusion and decreased shear stress, seem to be synergistic causes for deterioration of endothelial function in PAOD patients.

In recent years noninvasive ultrasound-based assessment of brachial arterial flow-mediated dilatation

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(FMD) has been used to study this specific vascular impairment (endothelial dysfunction) consisting in a decreased ability of the artery to dilated upon augmented blood flow after a transient forearm ischemia.[19] Impaired FMD is thought to be linked to nitric oxide availability in the endothelium.[20] In addition, a predictive value of FMD for cardiovascular events in patients with PAOD has been reported recently.[8,21]

The aim of this study was to prospectively assess whether the correction of intermittent leg ischemia by endovascular revascularization is associated with a reduction in inflammatory mediators and thus an improvement in endothelial function.

METHODS

Study design. The study was conducted at a tertiary referral center as a prospective, open, randomized, controlled, single-center follow-up evaluation assessing the effect of lower limb endovascular revascularization on endothelial dysfunction and inflammatory indicators (plasmatic procoagulant activity) in patients with symptomatic PAOD. Only patients with chronic and stable PAOD (Rutherford class 2 to 3) due to femoropopliteal obstruction were eligible for the study.[22] Exclusion criteria were history of lower limb or coronary revascularization, acute ischemic event within the last 3 months, chronic inflammatory disorders, moderate or severe renal insufficiency, and severe liver disease. Patients with incompressible tibial arteries and persistent claudication after angioplasty were not eligible for the study.

Randomization for angioplasty (group A) or conservative treatment (group B) was accomplished by a procedure that used a random numeric sequence. The investigators who performed FMD and laboratory analyses were blinded to patient treatment allocation. The local ethic committee approved the study, and all patients gave written informed consent.

Assessment of brachial artery FMD, WBC count, high-sensitive C-reactive protein (hs-CRP), and fibrinogen were performed at baseline and at 4 weeks after randomization in both groups. Before the investigations, patients were at rest overnight and were not allowed to consume nicotine, caffeine, or to take vasoactive drugs for at least 10 hours before the experiments and blood sampling. Patients were not exposed to any other study drug or therapeutic treatment.

Endovascular procedures. Assessment before and after the intervention included clinical examination, Doppler measurements of lower limb occlusive pressures with calculation of the ankle-brachial index (ABI), color duplex sonography, and determination of routine laboratory tests.

Endovascular treatment was performed in the routine manner. A 4F to 6F sheath that was compatible with an over the wire low-profile dilation balloon and, occasionally, an additional stenting system, which was at the discretion of the interventionalist, was introduced antegrade into the common femoral artery of the affected leg. After sheath placement and diagnostic angiography, 5000 U of unfractionated heparin was injected intra-arterially.

Postinterventional therapy lasted 4 weeks and consisted of aspirin (100 mg/d) or clopidogrel (75 mg/d), or both in case of stent insertion. To exclude any medication effects on the investigated indicators, medication remained unchanged except for clopidogrel in case of stent implantation (2 patients). This medication was initiated after baseline assessment and angioplasty with stent implantation and lasted for 28 days.

Follow-up visits were done at day 30 to 32 after the intervention. Successful angioplasty was defined by a final angiogram with residual stenosis of $<30\%$ and postinterventional ABI improvement of at least 0.1. In addition, pain free walking distance was proven the day after the procedure and at 30 to 32 days later by treadmill testing.

Assessment of FMD. Ultrasound assessment of endothelial-dependent FMD of the brachial artery was done according to recently reported guidelines.[23] The study was performed between 8 and 10 AM in a temperature-controlled room (20° to 22°C) with subjects resting in a supine position. Brachial diameter was imaged using a high-resolution (14-MHz line array) transducer ultrasound system (Siemens, Erlangen, Germany) equipped with electronic callipers, vascular software for two-dimensional imaging, color and spectral Doppler, and internal electrocardiogram.

The brachial artery was imaged at a location 2 to 5 cm above the cubital fossa. A sphygmomanometer cuff was placed on the forearm. The cuff was inflated at least 50 mm Hg above systolic pressure to occlude artery inflow for 5 minutes. All vasodilation measurements were made at the end of diastole. Off-line measurements were performed on a personal computer using the brachial reactivity analysis software (Siemens). The response of the vessel diameter to reactive hyperemia was calculated and expressed as a percentage change relative to the diameter immediately before cuff inflation. Off-line analysis was performed by one operator (A. S.) in a blinded fashion.

Assessment of ABI and treadmill testing. The ABI was calculated with the patient supine. The highest systolic pressure of the anterior or posterior tibial artery was measured in each limb and was divided by the highest brachial artery pressure. Standardized treadmill testing was performed at 3.2 km/h and 12% inclination, 1 day postprocedure and 4 days later, to assess 10 minutes of pain-free walking time in patients after angioplasty.

Biochemistry. Measurement of hs-CRP was done using an immunoturbidimetric detection method (Roche, Hitachi Modular P800, Basel, Switzerland). Fibrinogen clotting was measured according to the Clauss method on a Behring BCS coagulation analyzer using Multifibren U (Dade Behring Diagnostics, Siemens Healthcare Diagnostics, Deerfield, Ill).[24] Platelet and WBC counts were measured by an LH-750 and LH-780 System (Beckman-Coulter Inc, Fullerton, Calif) and ADVIA 120 Hematology system (Siemens Healthcare Diagnostics). Enzymatic methods were used to measure plasma total cholesterol and triglycerides (Roche). Low-density lipoprotein cholesterol was calculated using the Friedewald formula.[25]

Table I. Clinical characteristics of patients with peripheral arterial obstructive disease in group A (angioplasty) and group B (conservative treatment)

Variable	*Group A (n = 17)*	*Group B (n = 16)*	P
Age, mean (range), years	66 (47-82)	72 (53-84)	.25
Sex, No.			
Males	11	6	.12
Females	6	10	
Body mass index, kg/m^2	26.2	26.8	.69
Hypertension, No. %	12 (71)	12 (75)	.54
Diabetes mellitus, No. %	4 (24)	4 (25)	.73
Dyslipidemia, No. %	9 (56)	11 (68)	.10
Smoking, No. %			
Active	8 (47)	8 (50)	
Former	3 (18)	7 (44)	.08
Never	6 (35)	1 (6)	
Coronary artery disease, No. %	4 (23)	4 (25)	.73
Cerebrovascular disease, No. %	3 (17)	0 (0)	.10
Medication, No. %			
Aspirin/clopidogrel	16 (94)	14 (87)	.90
Oral anticoagulation	1 (6)	3 (13)	.76
Statin	7 (41)	9 (56)	.17
ACE inhibitor	7 (41)	6 (38)	.89
Calcium antagonist	2 (11)	1 (6)	.67
Angiotensin receptor blocker	3 (17)	4 (25)	.45

ACE, Angiotensin-converting enzyme.

Statistical analysis. Data are expressed as mean ± standard deviation (SD). Data were analyzed using the Mann-Whitney *U* test for intergroup comparison and the Wilcoxon signed rank test for intragroup comparison. Sample size calculation was based on data by Brendle et al,[26] who demonstrated an improvement of FMD by 61% (from 0.18 ± 0.03 to 0.29 ± 0.04 mm) after a 6-month walking exercise therapy. Assuming an effect size of 1.61 ($\alpha = 0.05$; power = 0.80, two-tailed) we calculated a sample size of 16 subjects per group. Expecting a drop-out rate of 20%, we had to evaluate 42 patients (21 per group). Data were analyzed with StatView 5.0.1 software (Adept Scientific, Acton, Mass). A value of $P < .05$ was considered to be significant.

RESULTS

Patient demographics. Nine patients were excluded from the study, four from group A because of persistent claudication after endovascular revascularization and five in group B due to cardiovascular events before the follow-up assessment. For analysis of indicators, only patients with complete baseline and follow-up assessment were included, resulting in 17 patients (group A) with successful angioplasty and subsequently pain-free walking distance during 10 minutes on the treadmill, and 16 patients in group B with best medical treatment. Clinical baseline characteristics did not differ among PAOD patients of both groups (Table I). Patients in whom endovascular therapy was not successful were not included.

Flow-mediated dilation. FMD did not differ significantly between group A (4.96% ± 1.86%) and B (4.60% ± 2.95%) at baseline ($P = .65$). After successful endovascular revascularization, FMD significantly increased in group A compared with baseline (6.44% ± 2.88%; $P = .02$; Fig 1). FMD of group B did not change during follow-up (4.53% ± 3.17%; $P = .92$) and differed from postinterventional FMD of group A, although this difference did not reach statistical significance ($P = .09$). The ABI was similar for group A and B at baseline (0.63 ± 0.15 vs 0.66 ± 0.10; $P = .36$). The ABI significantly increased in group A after revascularization (1.05 ± 0.15; $P = .0004$) but remained unchanged in group B at follow-up (0.62 ± 0.1; $P = .82$).

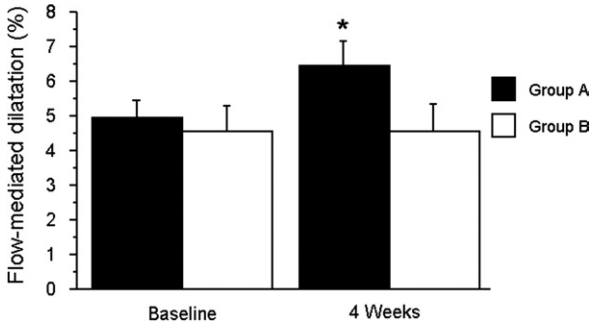

Fig 1. Endovascular revascularization in group A (*black bars*) improved flow-mediated dilation compared with baseline (*P = .02) and with group B (conservative treatment, *white bars*) at follow-up ($P = .09$). Conservative treatment did not alter flow-mediated dilation in group B ($P = .92$). *Error bars* show the standard deviation.

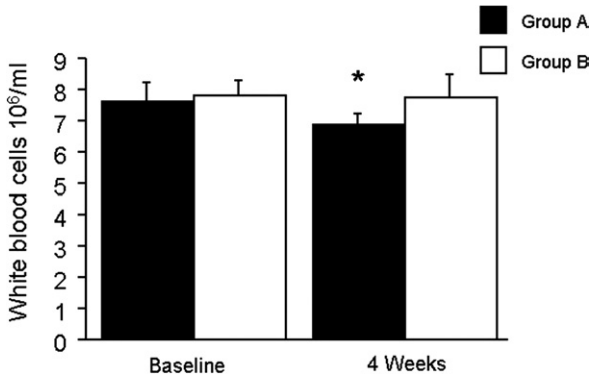

Fig 2. Effect of angioplasty on white blood cell count on group A (revascularization, *black bars*) and group B (conservative treatment, *white bars*). *Error bars* show the standard deviation. *P = .03 for group A compared with baseline.

Laboratory analysis. The WBC counts of the two groups were comparable at baseline, at 7.6 ± 2.26 × 10^6/mL for group A and 7.8 ± 2.02 × 10^6/mL for group B ($P = .81$; Fig 2). After angioplasty, a slight but significant decrease occurred in the WBC count for group A (6.89 ± 1.35 × 10^6/mL, $P = .03$). For group B, WBC count did not change significantly compared with baseline (7.76 ± 2.64 × 10^6/mL, $P = .94$). In contrast, there was no

Table II. Effect of angioplasty (group A) and conservative treatment (group B) on high-sensitive C-reactive protein and fibrinogen in patients with stable symptomatic peripheral atherosclerotic disease

Indicator	*Group A (n = 17)*	P[a]	*Group B (n = 16)*
Fibrinogen (g/L)			
Baseline	3.87 ± 0.53	.48	4.24 ± 0.69
Follow-up	3.54 ± 0.54	.52	4.0 ± 0.78
P[b]	.44		.41
Hs-CRP (mg/L)			
Baseline	2.93 ± 2.16	.46	2.67 ± 1.12
Follow-up	2.39 ± 1.46	.25	2.2 ± 1.01
P[b]	.17		.28

Hs-CRP, High-sensitive C-reactive protein.
[a]Intergroup comparison.
[b]Intragroup comparison.

relevant change of platelet count from baseline (group A, 263 ± 79 × 10^3/mL; group B, 225 ± 85 × 10^3/mL) to follow-up (group A, 260 ± 85 × 10^3/mL; group B, 212 ± 69 × 10^3/mL) or significant differences between the two groups.

Although the levels of hs-CRP and fibrinogen were slightly lower in both groups at follow-up, these differences were not significant, nor did the values differ between the groups at baseline or follow-up (Table II).

DISCUSSION

Patients with PAOD exhibit a marked deterioration of FMD and increased levels of inflammatory and oxidative stress markers, possibly due to the atherosclerotic burden, muscle ischemia and reperfusion, and decreased shear stress due to claudication.[8,13,27-29] Furthermore, higher levels of WBCs within the normal range were shown to be associated with poorer prognosis in PAOD patients.[10,30] The present study indicates that endovascular treatment of intermittent claudication ameliorates FMD and reduces WBC count.

So far, a beneficial effect has only been shown for antioxidative treatment in PAOD patients, which resulted in a transient improvement of FMD.[12,13,31] This is thought to be due to the scavenging effects of antioxidants on reactive oxygen species that result from reperfusion of ischemic muscle. Successful revascularization attenuates muscle ischemia and therefore generation of reactive oxygen species and inflammation. Inflammation and free oxygen radicals reduce nitric oxide bioavailability. The abolishment of claudication and hence generation of reactive oxygen species may partly explain our observation, because FMD has been shown to closely correlate with nitric oxide bioavailability.[20]

In addition, the limitation of physical activity by intermittent claudication is relieved through successful endovascular repair. Physical activity (ie, walking) has been shown to be the main stimulus for endothelial-dependent vasodilatation through a rise in shear stress.[32,33] An increase in endothelial nitric oxide synthesis is an important physiologic adaptation to regular exercise. Moreover, regular exercise has been shown to improve vascular function in adults independent of changes in other risk factors.[34,35] Therefore, besides being of pathophysiologic interest, our findings may also have a clinical application in that the indication for endovascular treatment in patients with intermittent claudication may be expanded beyond simple pain relief.

Epidemiologic studies have demonstrated a high prevalence of vascular events in patients with PAOD.[3,36] Several studies have clearly shown the importance of engaging in regular exercise to attenuate or reverse the disease process in patients with cardiovascular disease.[37] Continuous walking, either three times for 60 minutes or six times for 30 minutes per week, had been proposed as being sufficient for attenuation of atherosclerotic disease progression.[38] It is highly unlikely, however, that patients with PAOD and at Rutherford stages 2 or 3 are able to walk for half an hour. Studies on walking exercise in patients with PAOD demonstrate an increase of up to 400% in pain-free walking distance.[39] This increase may still be far beyond the capacity to continuously walk for half an hour to provide a sufficient stimulus for prolonged shear stress for sufficient effect on nitric oxide synthesis.

In our study, endovascular therapy resulted in an increase of walking capacity that was proven by treadmill testing 4 weeks after the procedure. Increased physical activity, although not quantified in our study, could explain the changes found already after a 4-week follow-up. It is well known that exercise training improves ambulatory function collateralization, resulting in an improved pain-free walking distance. The development of collateralization is slow, however, whereas in contrast, successful angioplasty reestablishes full walking capacity immediately. This facilitates physical activity.

Presently, it remains unclear whether the abolishment of claudication and hence reduction in oxidative stress or already increased physical activity explains a better FMD in our study population. Provided the absence of additional oxidative stress (ie, repeated muscle ischemia), it has been shown that exercise affects FMD already after a short training time of 4 weeks.[40]

Other possible factors, such as vitamin supplementation or other medications with aspirin/clopidogrel, or both, on FMD and WBC count are unlikely to have affected the present findings because patients were asked to continue their medication unchanged and to withhold any supplementation. In two patients who had stent implantation, clopidogrel medication was ceased 2 to 4 days before the follow-up assessment.

Findings of a reduced WBC count in our study may be related to the abolishment of muscle ischemia after successful revascularization. This is an important finding, because the degree of elevation of WBC count within the normal range is a marker for an increased risk of cardiovascular events.[10] In contrast, we did not find any changes in the platelet count. Similarly, hs-CRP and fibrinogen remained

unchanged in both groups, although an effect on hs-CRP due to reduction of inflammation might be expected. In accordance with our findings of unchanged hs-CRP and fibrinogen, Wahlgren et al[41] reported a transient increase in fibrinogen and hs-CRP levels within the first day after the endovascular procedure, but these markers did not differ from baseline values at the 1-month follow-up. The time period in our study may have been too short, or the study population too small, to reveal any beneficial effects of increased physical activity or decreased oxidative stress, or both, on hs-CRP and fibrinogen.

The lack of assessment of physical activity or markers of oxidative stress is a shortcoming of the present study. Future studies are needed to address these questions, both in terms of pathophysiologic understanding and therapeutic management of PAOD patients. Another limitation is the lack of structured exercise training for both groups. However, the extent of exercise training would then differ with regard to absolute walking capacity between the two groups, whereas the present scenario represents the real-world setting. Nevertheless, the possible spontaneous increase in physical activity, which is mainly attributable to relief of symptoms in the angioplasty group, demonstrates the importance of endovascular revascularization in terms of freedom from pain that might be able to counteract a sedentary lifestyle.

So far, the endovascular treatment for Rutherford stages 2 to 3 was aimed to improve patients' quality of life. If PAOD is recognized as a restriction of physical activity in a growing elderly population and endovascular or surgical revascularization can be confirmed to increase physical activity, thereby reducing cardiovascular morbidity and mortality, additional systemic benefits of revascularization in terms of public health economy may outweigh the initial costs. Future randomized studies comparing conservative vs endovascular therapy are warranted to confirm the present findings in larger patient settings incorporating cardiovascular end points.

In conclusion, endovascular revascularization for stable claudication resulting in alleviation of symptoms improves brachial artery endothelial function and decreases leukocyte count.

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AUTHOR CONTRIBUTIONS

Conception and design: MH, AS, JD
Analysis and interpretation: MH, JD, CK, ND, IB, AS
Data collection: MH, JD, AS
Writing the article: MH, JD, AS
Critical revision of the article: ND, IB, CK
Final approval of the article: MH, JD, CK, ND, IB, AS
Statistical analysis: MH, AS
Obtained funding: MH, JD, AS
Overall responsibility: MH
MH and JD contributed equally to this work

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