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Dynamic contrast-enhanced ultrasound for assessment of therapy effects on skeletal muscle microcirculation in peripheral arterial disease: Pilot study

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ABSTRACT

Objective: To assess with dynamic contrast-enhanced ultrasound (CEUS) and transient arterial occlusion whether the muscular micro-perfusion in patients with peripheral arterial disease (PAD) is improved after angioplasty or surgery.

Materials and methods: This study had local institutional review board approval. Written informed consent was obtained from all 20 patients with PAD, Fontaine stage IIb (mean age, 64 years), who participated in the study. Low-MI CEUS (7 MHz; MI, 0.28) was applied to the mainly affected lower leg after start of a continuous automatic intravenous injection of 4.8 mL SonoVue®. Muscle-perfusion was monitored by CEUS before, during, and after provocation by arterial occlusion at the thigh level lasting for 60 s. CEUS examination was performed a second time within 14 days after angioplasty ($n = 15$), thrombendarterectomy ($n = 2$), angioplasty and thrombendarterectomy ($n = 1$), or bypass ($n = 2$). Clinical amelioration was re-evaluated within 6 months after the intervention using a 4-point scale.

Results: Ankle-brachial-index (ABI) increased from 0.8 ± 0.2 to 0.9 ± 0.3 after treatment ($p = 0.01$). Time to maximum CEUS signal (t_{max}) shortened from 26 ± 14 s to 14 ± 4 s ($p = 0.004$). The slope to maximum after transient occlusion (m_2) changed to steeper values ($6.4 \pm 5.8 \sim$mL/s versus $10.2 \pm 5.0 \sim$mL/s; $p = 0.04$). Shortened t_{max} predicted improvement in the patients' intermittent leg pain and therefore successful therapy outcome.

Conclusion: Dynamic CEUS with transient arterial occlusion can visualize the treatment-induced improvement of muscular micro-perfusion in patients with PAD.

1. Introduction

Peripheral arterial disease (PAD) is a result of long-lasting atherosclerosis. The chronic inflammatory disease of the vessels' walls is progressive and may result in critical limb ischaemia if not treated correctly. Diagnostic routine includes clinical examination, evaluation of ankle-brachial index (ABI) and walking distance, as well as radiological exams (e.g. magnetic resonance angiography, computed tomography angiography, conventional angiography and colour-guided duplex ultrasound). Common non-invasive imaging modalities sufficiently reveal macro-vascular changes like stenoses and occlusions [1–3]. However, radiological standard modalities cannot evaluate the micro-perfusion. With low-mechanical index contrast-enhanced ultrasound (low-MI CEUS) combined with transient arterial occlusion changes in the perfusion of skeletal muscles can be evaluated [4–7].

Therapy approaches in PAD are based either on supervised exercise and training programmes, pharmaceutical treatment (e.g. acetylsalicylic acid, phenprocoumon, clopidogrel, cilostazol) or surgical/radiological interventions (e.g. thrombendarterectomy, neo-vascular surgery, angioplasty) [2]. Obviously, it is necessary

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to establish methods that allow for monitoring of these therapy effects in the end organ itself (i.e. muscle) on a microcirculatory level. Initial studies aimed to assess therapy effects in patients with PAD using CEUS at rest evaluating the time to peak intensity after contrast injection [8]. Recently developed CEUS methods which analyse the replenishment of contrast media in the lower legs' muscles after transient arterial occlusion may decrease the error rate caused by passage of contrast media through the venous and arterial circulation [9,10]. Aim of the current study was to analyse whether the muscular micro-perfusion measured by such dynamic low-MI CEUS and provocation by transient arterial occlusion is improved after endovascular therapy using angioplasty or surgical therapy (i.e. bypass or thrombendarterectomy) in patients with PAD, and to compare these CEUS parameters assessed at early follow up with the clinical outcome.

2. Materials and methods

2.1. Study population

Informed written and oral consent was obtained from all subjects after the nature of the procedure had been fully explained to them. The study was approved by the local review board and was performed according to the declaration of Helsinki. Within this single-centre study, 20 patients with PAD, Fontaine stage IIb (mean age, 64 years; range, 53–79 years; mean weight, 81.8 kg; range, 55–108 kg) were included. Ten of the twenty patients with PAD were treated with acetylsalicylic acid only (100 mg/day); 6 patients were treated with acetylsalicylic acid and clopidogrel (Plavix®, Sanofi-aventis, Paris, France; 75 mg/day); 1 patient was treated with acetylsalicylic acid and phenprocoumon (Marcumar®, Meda, Solna, Sweden; INR 2.5–3); 1 patient was treated with phenprocoumon only (Marcumar®, Meda, Solna, Sweden; international normalized ratio (INR) 2.5–3); 1 patient was treated with clopidogrel only (Plavix®, Sanofi-aventis, Paris, France; 75 mg/day); 1 patient did not receive pro-rheological medication. None of the subjects met exclusion criteria for this study, such as any diagnosed recent acute coronary syndrome or clinically unstable ischaemic cardiac disease, severe pulmonary hypertension, uncontrolled systemic hypertension, adult respiratory distress syndrome or severe orthopaedic problems of the lower extremity or spine that would limit mobility and activity. ABI was evaluated before therapy and within 14 days after therapy.

2.2. Therapy

All PAD patients suffered from intermittent leg pain that aggravated with the walking distance. As medication and additional moderate physical exercise failed to improve the individual disorders, the included subjects were referred to and treated at a vascular and endovascular surgery centre of a university hospital. All 20 patients had at least one haemodynamic relevant stenosis (>80% diameter) of either the common/superficial femoral artery or popliteal artery. Fifteen of the twenty included PAD patients were assigned to percutaneous transluminal angioplasty of the superficial femoral artery. Two patients were assigned to thrombendarterectomy of the common femoral artery. One patient was assigned to angioplasty and thrombendarterectomy of the superficial femoral artery, and 2 patients were assigned to surgical treatment via femoropopliteal bypass (Table 1). All patients were re-evaluated within 6 months after therapy at the department of vascular surgery. This evaluation was conducted using a four-point scale: 4, disappearance of leg pain; 3, improvement of leg pain; 2, no difference before and after treatment; 1, aggravation of leg pain.

Table 1
Therapy in PAD patients.

Angioplasty of SFA	$n = 15$
Thrombendarterectomy of CFA	$n = 2$
Femoropopliteal bypass	$n = 2$
Angioplasty and thrombendarterectomy of SFA	$n = 1$

SFA: superficial femoral artery; CFA: common femoral artery; PAD: peripheral arterial disease.

2.3. Contrast-enhanced ultrasound

The ultrasound examination was carried out 1 day before and within 14 days after surgical therapy or angioplasty using a Siemens-Acuson Sequoia 512 US device with a linear transducer 15L8w (Siemens-Acuson, Mountain View, CA) and contrast pulse sequencing (CPS®) technique. The triceps surae muscle of the primarily affected lower limb was examined. The transducer was kept at the same position during the whole examination. Further details concerning the CEUS examination have been published previously [10,11].

The protocol included the following parameters: transmit frequency, 7 MHz; MI, 0.28; gain, 75 dB and minimal persistence; depth 40 mm, two foci set at 2.25 and 3.5 cm. Continuous intravenous injection of 4.8 mL suspension with microbubbles containing sulphur hexafluoride (SonoVue®, Bracco Imaging SpA, Milan, Italy) within 5 min. Length of video clips, 5 min; frame rate, 1/s. Regions of interest (ROIs) were positioned at a depth of 2 cm with a size of 1.75 cm × 5 cm. The provocation (i.e. transient arterial occlusion) was performed with a cuff connected to an automated occlusion plethysmograph Periquant® 803 (Gutmann, Eurasburg, Germany). The cuff was tied around the middle of the thigh, and was inflated 120 s after beginning of contrast media injection. The provocation lasted 60 s. The US-signal-intensity values in the ROI were calculated via Axius® software (preinstalled on the US device) and the linearized data were exported to a personal computer as Excel files. With the recorded CEUS values, a real-time measure of local blood volume in the microcirculation A(t) was possible in a.u. (arbitrary units) or [∼mL]. Signal-intensity–time curves visualized alterations of the local blood volume A [∼mL/s] due to occlusion. The areas under the signal-intensity–time curves (AUC) mirror changes of the local blood volume over time [∼mL s]. Max, i.e. the maximum CEUS signal after provocation, m_2 (slope to max), t_{max} (time difference between end of occlusion and maximum signal) and AUC_{post} (integral under the CEUS curve during the first 60 s after stop of provocation) were calculated as shown in Fig. 1. The primary hypothesis was that max, t_{max}, m_2 and AUC_{post} would differ in the

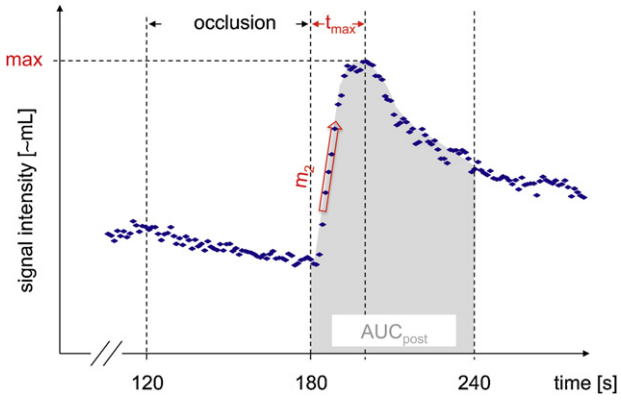

Fig. 1. Example of a signal-intensity-over-time curve. Maximum signal during reperfusion (max), time to maximum signal after occlusion (t_{max}), the maximum slope of reperfusion to max (m_2) and the area under the curve post occlusion (AUC_{post}) were extracted.