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Reduced intraepidermal nerve fiber density in patients with chronic ischemic pain in peripheral arterial disease

Eva Gröne [a], Nurcan Üçeyler [b], Thomas Abahji [c], Johannes Fleckenstein [a], Dominik Irnich [a], Thomas Mussack [d], Ulrich Hoffmann [c], Claudia Sommer [b], Philip M. Lang [a,*]

[a] Department of Anaesthesiology, University Hospital of Munich, Munich, Germany
[b] Department of Neurology, University Hospital of Würzburg, Würzburg, Germany
[c] Department of Angiology, University of Munich, Munich, Germany
[d] Department of Surgery, University of Munich, Munich, Germany

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ABSTRACT

Chronic ischemic pain in peripheral arterial disease (PAD) is a leading cause of pain in the lower extremities. A neuropathic component of chronic ischemic pain has been shown independent of coexisting diabetes. We aimed to identify a morphological correlate potentially associated with pain and sensory deficits in PAD. Forty patients with symptomatic PAD (Fontaine stages II-IV), 20 with intermittent claudication (CI), and 20 with critical limb ischemia (CLI) were enrolled; 12 volunteers served as healthy controls. All patients were examined using pain scales and questionnaires. All study participants underwent quantitative sensory testing (QST) at the distal calf and skin punch biopsy at the distal leg for determination of intraepidermal nerve fiber density (IENFD). Additionally, S100beta serum levels were measured as a potential marker for ischemic nerve damage. Neuropathic pain questionnaires revealed slightly higher scores and more pronounced pain-induced disability in CLI patients compared to CI patients. QST showed elevated thermal and mechanical detection pain thresholds as well as dynamic mechanical allodynia, particularly in patients with advanced disease. IENFD was reduced in PAD compared to controls ($P < 0.05$), more pronounced in the CLI subgroup (CLI: 1.3 ± 0.5 fibers/mm, CI: 2.9 ± 0.5 fibers/mm, controls: 5.3 ± 0.6 fibers/mm). In particular, increased mechanical and heat pain thresholds negatively correlated with lower IENFD. Mean S100beta levels were in the normal range but were higher in advanced disease. Patients with chronic ischemic pain had a reduced IENFD associated with impaired sensory functions. These findings support the concept of a neuropathic component in ischemic pain.

1. Introduction

Chronic ischemic pain is a leading cause for pain in the lower extremities. Initially, symptoms of peripheral arterial disease (PAD) appear with physical exercise and cease when the patient rests (intermittent claudication = CI, Fontaine stage II). With progression of the disease and insufficient circulation, pain occurs at rest (critical limb ischemia = CLI, Fontaine stage III to IV) [35]. The prevalence of critical limb ischemia is estimated to be 0.24% in women and 0.26% in men [19]. Because of an increase in vascular risk factors and advanced aging in industrialized countries, the prevalence of PAD might be even higher in the future. Although progress has been made concerning PAD diagnosis and treatment, the exact pathogenesis of ischemic pain has not been completely resolved.

Previous studies suggested a change in pain character in chronic ischemia. Patients with CI present mainly with nociceptive pain, while patients with CLI predominantly suffer from neuropathic pain, as indicated by data from validated questionnaires [40]. Furthermore, in quantitative sensory testing (QST), sensory deficits were more pronounced in CLI than in CI, indicating a PAD-associated peripheral neuropathy independent of coexisting diabetes [21]. Until now, it has not been investigated whether sensory abnormalities in PAD are associated with morphological changes such as a reduction of intraepidermal nerve fiber density (IENFD) in skin biopsies.

* Corresponding author. Address: Department of Anaesthesiology, University of Munich, Marchioninistr. 15, Munich 81377, Germany. Tel.: +49 89 7095 3429; fax: +49 89 7095 8886.
E-mail address: philip.lang@med.uni-muenchen.de (P.M. Lang).

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The aim of this study was to investigate whether painful ischemic neuropathy in PAD is associated with reduced IENFD, and if this reduction in fiber density correlates with sensory function as measured with QST. Our data substantially contribute to the understanding of the pathogenesis of peripheral ischemic pain and the development of ischemic neuropathy in PAD.

2. Methods

2.1. Study design

We performed a prospective single-center study. Forty patients with symptomatic PAD were included: 20 patients with CI (Fontaine stage II, pain with physical exercise) and 20 patients with CLI (Fontaine stage III to IV, ischemic rest pain and/or minor tissue loss due to chronic ischemia). The characteristics of the study population are given in Table 1. Patients with PAD were examined using pain questionnaires. They underwent QST at the distal calf of the affected lower extremity, approximately 10 cm above the lateral malleolus. Afterwards, a 3-mm skin punch biopsy was taken from the same location to assess the IENFD. Blood was withdrawn from the cubital vein for determination of S100beta serum levels. An age-matched control group consisting of 12 healthy subjects was tested with the same methods except for pain questionnaires.

2.2. Subjects

All patients with PAD were inpatients in the Center for Angiology and Vascular Surgery at the University Hospital of Munich (Ludwig-Maximilians-University, LMU). Diagnosis of PAD, as well as Fontaine stages, was established by complete history, ankle brachial index (ABI), and angiographic or sonographic evidence of vascular stenosis.

Inclusion criteria were confirmed diagnosis of symptomatic PAD (Fontaine II-IV) and age >40 years. Patients were not included if the medical history revealed any hints for peripheral neuropathy, chronic pain of other origin, and other diseases that could impair the sensory system, difficulties in communication and in cooperation with pain questionnaires, and QST.

Healthy controls were excluded if aged <40 years, diagnosed with or suspected to potentially have any form of PAD, diabetes mellitus, neurological disease including polyneuropathy, or any disorder such as chronic pain, cutaneous lesions in the tested area, chronic substance abuse, or intake of analgesics 24 hours prior to the investigation.

All subjects participated voluntarily and gave written informed consent. The study was performed according to the Helsinki Declaration and was approved by the local Ethics Committee.

2.3. Pain scales and questionnaires

The questionnaires consisted of different validated instruments that have been used successfully before for the survey of patients with PAD. The visual analogue scale (VAS) is a simple but effective tool assessing pain intensity at rest and during exercise. The short-form McGill Pain Questionnaire (SF-MPQ) was included to investigate different descriptions of pain [32]. It consists of 11 sensory (throbbing, shooting, stabbing, sharp, cramping, gnawing, hot-burning, aching, heavy, tender, and splitting) and 4 affective (tiring-exhausting, sickening, fearful, punishing-cruel) pain descriptors that are ranked on an intensity scale from 0 = none to 3 = severe. The sum of a rank value is represented in a sensory

Table 1
General characteristics of the subjects in the control group (CG) and those with PAD (all PAD), divided into the group with intermittent claudication (CI) and critical limb ischemia (CLI).

		All PAD	CI	CLI	CG
Number of subjects	n	40	20	20	12
Gender	Male/female	27/13	10/10	17/3	5/7
Age (year)	Mean ± SEM	69 ± 1.2	68.9 ± 1.6	69.1 ± 1.8	62.3 ± 4.6
Risk factors*					
Diabetes mellitus	Yes/no	20/20	7/13	13/7	0
Smoker/ex-smoker/nonsmoker	n	8/24/8	5/11/4	3/13/4	2/1/9
Pack-years of smokers and ex-smokers	Mean ± SEM	41.1 ± 5.1	46.3 ± 8.7	36 ± 5.1	2 ± 0.8
Arterial hypertension	Yes/no	37/3	20/0	17/3	2/10
Body mass index (kg/m^2)	Mean ± SEM	26.9 ± 0.8	27.1 ± 1.03	26.6 ± 1.1	25.8 ± 0.9
Hypercholesterolemia	Yes/no	31/9	17/3	14/6	3/9
Coronary artery disease	Yes/no	17/23	6/14	11/9	0/12
History of cardio-/cerebrovascular disease	Yes/no	12/28	4/16	8/12	0/12
Family history of vascular disease	Yes/no	15/25	8/12	7/13	7/5
Clinical examination*					
ABI	Mean ± SEM	0.69 ± 0.05	0.7 ± 0.04	0.68 ± 0.09	
Systolic ankle pressure (mm Hg)	Mean ± SEM	84.5 ± 7.8	102 ± 9.1	67.1 ± 11.7	
Sonographic evidence of PAD	Yes/no	37/3	20/0	17/3	
of which: stenosis/obstruction		20/17	15/5	5/12	
Oscillographic evidence of PAD	Yes/no	37/3	18/2	19/1	
pathologic: easy/moderate/heavy		1/17/19	1/14/3	0/3/16	
Angiographic evidence of PAD	Yes/no	33/7	14/6	19/1	
of which: DSA/CT/MRT		28/3/2	13/0/1	15/3/1	
Indication for intervention	Yes/no	38/2	18/2	20/0	
of which: PTA, stent/bypass, patch/amputation		17/17/4	17/1/0	0/16/4	
Previous interventions	Yes/no	29/11	11/9	18/2	
davon: PTA, stent/bypass, patch/amputation		15/9/5	10/1/0	5/8/5	
Duration of claudication (months)	Mean ± SEM	31.8 ± 5.9	23.1 ± 4.8	43.5 ± 11.7	
Duration of pain at rest (months)	Mean ± SEM	8.3 ± 1.9	5 ± 1	8.7 ± 2.1	
Influence of body position on pain	Yes/no	33/7	18/2	15/5	
Cold sensations	Yes/no	20/20	9/11	11/9	
Ulcerations	Yes/no	17/23	1/19	16/4	

PAD, peripheral arterial disease; ABI, ankle brachial index; DSA, digital subtraction angiography; CT, computer tomography; MRT, magnetic resonance tomography.
* Risk factors and clinical examination in the control group (CG) and the PAD group (All PAD), divided into the group with intermittent claudication (CI) and critical limb ischemia (CLI), data are number (n) per group, respectively, means ± SEM.