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ARTICLE

Prospective study on microangiopathy in type 2 diabetic foot ulcer

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Abstract
Aims/hypothesis We investigated the significance of microangiopathy in the development of foot ulcer, which is still disputed.
Methods We assessed microangiopathy by histological analysis of the capillary ultrastructure using transmission electron microscopy and capillary density and arteriolar morphology in paraffin-embedded sections from the skin of type 2 diabetic patients: 30 neuroischaemic patients (Isc) revascularised with peripheral angioplasty and 30 neuropathic patients (Neu) with foot ulcer, compared with ten non-diabetic volunteers.
Results In the diabetic patients, capillaries in the dermal papillary layer were fewer (−22.2%, 159 ± 43 vs 205 ± 52 mm^2 in non-diabetic volunteers, $p < 0.01$). They also showed detrimental remodelling, with a 2.2-fold increase in capillary basement membrane thickness (3.44 ± 1.19 vs 1.53 ± 0.34 μm in non-diabetic volunteers, $p < 0.001$) and a 57.7% decrease in lumen area (14.6 ± 11.1 vs 34.7 ± 27.5 μm^2, $p < 0.001$). No differences were observed between the diabetic Isc or Neu patients. Isc were more prone to develop arteriolar occlusion than Neu (16.8 ± 6.9% vs 6.7 ± 3.7%, respectively, $p < 0.001$). No patient had been amputated at 30 days and healing time was significantly longer in Isc (180 ± 120 vs 64 ± 50 days in Neu, $p < 0.001$).
Conclusions/interpretation Capillary microangiopathy is present in equal measure in neuroischaemic and neuropathic diabetic foot skin. The predominance of arteriolar occlusions with neuroischaemia indicated the existence of an additional 'small vessel disease' that did not affect an effective revascularisation and did not worsen the prognosis of major amputations but slowed the healing process of the neuroischaemic foot ulcer.
Trial registration: ClinicalTrials.gov NCT02610036.

Keywords Amputation · Arteriolar occlusions · Basement membrane thickness · Capillary density · Diabetic foot · Neuroischaemic foot · Neuropathic foot · Skin biopsy · Small vessel disease · Wound healing time

Abbreviations

BPG	Bypass graft
CBMT	Capillary basement membrane thickness
CLI	Critical limb ischaemia
eGFR	Estimated GFR
H&E	Haematoxylin and eosin
Isc	Neuroischaemic (in the study)
Neu	Neuropathic (in the study)
PTA	Percutaneous transluminal angioplasty
SMA	Smooth muscle actin
$TcPO_2$	Transcutaneous oxygen tension

Introduction

The effects of diabetes mellitus on the microcirculation have long been recognised: retinopathy, nephropathy and

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neuropathy are characterised by microvascular complications affecting most type 1 diabetic patients and many type 2 patients with long duration of diabetes and poor metabolic control [1, 2]. However, the presence of microangiopathy in the development of foot ulcer remains disputed [3, 4] and very few studies exist on PubMed responding to the keywords 'microangiopathy' and 'diabetic foot' together.

Microangiopathy comprises detrimental changes in both the structure and function of the microcirculation [5]. In diabetic patients, histological abnormalities have been observed in arterioles and capillaries of the foot [6]. The main structural change is thickening of the capillary basement membrane, associated with microangiopathy when it occurs in the retina, nerve, kidney, skin, muscle and heart of diabetic patients [7, 8]. Some studies have investigated functional damage in the microcirculation of the foot skin, but histopathological features of the skin vasculature have been rarely reported [9], probably because taking a foot biopsy in patients with or without foot ulcer is an invasive method with evident negative aspects.

This study examined microangiopathy by histological analysis of the capillary ultrastructure using transmission electron microscopy and capillary density and arteriolar morphology in the skin of type 2 diabetic patients: 30 neuroischaemic patients (Isc) revascularised with angioplasty and 30 neuropathic patients (Neu) with foot ulcer, compared with ten non-diabetic volunteers.

Methods

Study design This clinical trial (ClinicalTrial.gov registration no. NCT02610036) was designed as a pilot prospective non-randomised study to verify the presence of microangiopathy by foot skin biopsy in 60 type 2 diabetic patients with foot ulcer, 30 Isc and 30 Neu patients, enrolled at the Diabetic Foot Center of Istituto di ricovero e cura a carattere scientifico (IRCCS) MultiMedica (Milan, Italy). These biopsies were compared with foot skin biopsies from ten healthy non-diabetic volunteers enrolled in the Department of Orthopedics of IRCCS MultiMedica between February 2012 and March 2015. Exclusion criteria were type 1 diabetes, diabetes duration less than 10 years, dialysis, active cancer, immunological diseases and immunosuppression/organ transplantation. The study was approved by the Ethics Committee of the IRCCS MultiMedica and adhered to the principles of the Declaration of Helsinki [10]. All the individuals agreed to participate in the study and gave informed consent.

Clinical assessment and treatment All diabetic patients referred to the Diabetic Foot Center of IRCCS MultiMedica for foot lesions were examined to check for sensorimotor neuropathy, critical limb ischaemia (CLI), infection, metabolic control and comorbidities. Sensorimotor neuropathy was detected with a vibration perception threshold >25 V using a biothesiometer (Neurothesiometer SLS, Nottingham, UK), no sensitivity in >5/9 foot points with a Semmes–Weinstein 10 g filament and no Achilles tendon reflex. Peripheral arterial occlusive disease was investigated by assessment of foot pulses, ankle arterial pressure (DIADOP 50, Mediland, Milan, Italy), transcutaneous oxygen tension ($TcPO_2$) on the dorsum of the foot (TCMTM3, Radiometer, Copenhagen, Denmark) and duplex scanning (Acuson Corporation, Mountain View, CA, USA).

According to the TransAtlantic Inter-Society Consensus II parameters [11], CLI was diagnosed if $TcPO_2$ on the dorsum of the foot was <30 mmHg and ankle pressure was <70 mmHg when assessable (not assessable if ankle pulses absent or foot arteries not compressible because of medial calcifications) [12]. All patients who met these criteria were examined by angiography and, if the vessel diameter was obstructed >50%, percutaneous transluminal angioplasty (PTA) was done in the same session as a first-choice revascularisation procedure. In patients in whom PTA was unsuccessful, a peripheral bypass graft (BPG) was considered. In these patients $TcPO_2$ was reassessed after revascularisation. Revascularisation was done approximately 1 month before first ray amputation surgical procedure.

The presence of local cellulitis, erythema, foul odour, warmth and/or purulence was used to indicate infection [13], for which intravenous broad-spectrum antibiotic therapy was immediately prescribed. At discharge, each patient was provided with a therapeutic shoe with a rigid sole and Velcro closure suitable for holding a bandaged foot (Optima Molliter, Civitanova Marche, Italy). The antibiotic therapy was adjusted as soon as the sensitivity test results were available.

For each diabetic patient, we recorded the following data: sex and age; duration of diabetes; insulin or oral therapy; history of cardiac disease, stroke and arterial hypertension; and fasting C-peptide, serum albumin, C-reactive protein and leucocyte count. Diabetic retinopathy was identified from eye examination by an ophthalmologist, diabetic nephropathy was defined as estimated GFR (eGFR) <60 ml min^{-1} 1.73 m^{-2} and serum creatinine >115 μmol/l, medial arterial calcifications by plain foot radiography, microalbuminuria between 30 and 299 mg/24 h and macroalbuminuria >300 mg/24 h.

Healthy non-diabetic volunteers were in hospital (Department of Orthopedics of IRCCS MultiMedica) for the surgical correction of hallux valgus. These volunteers underwent a complete physical examination and blood tests to verify the absence of any pathology and were also tested for the presence of foot pulses and normal Achilles tendon reflex, to exclude peripheral arterial occlusive disease and peripheral neuropathy, respectively.

Surgical procedure and specimen preparation In healthy non-diabetic volunteers, a skin biopsy approximately 2 mm thick was obtained during surgery for correction of hallux

valgus at the level of the first metatarsophalangeal joint. In diabetic patients in hospital for treatment of foot ulcer in the toe area, a 2 mm thick biopsy was taken from healthy non-ulcerated skin adjacent to the site of wound suturing at the level of the first metatarsophalangeal joint during surgical amputation at the first ray.

Samples were immediately fixed with 4% (wt/vol.) paraformaldehyde and 2% (wt/vol.) glutaraldehyde in phosphate buffer 0.12 mol/l pH 7.4 for transmission electron microscopy analysis or with 10% (vol./vol.) neutral buffered formalin for histological analysis.

Capillary ultrastructure by transmission electron microscopy Biopsies were reduced and post-fixed with 4% (wt/vol.) paraformaldehyde (PFA) and 2% (wt/vol.) glutaraldehyde in phosphate buffer 0.12 mol/l pH 7.4 overnight at 4°C, followed by incubation at room temperature for 2 h in 1% (wt/vol.) OsO_4. After dehydration in a graded series of ethanol preparations, tissue samples were cleared in propylene oxide, embedded in epoxy medium (Epoxy Embedding Medium kit; Sigma-Aldrich, St. Louis, MO, USA) and polymerised at 60°C for 72 h. From each sample, one semi-thin (1 μm) section was cut with a Leica EM UC6 ultramicrotome (Leica Microsystems, Vienna, Austria), stained with Toluidine Blue and mounted on a glass slide to identify the areas of papillary dermis containing the transverse section of capillaries. Ultra-thin (60 nm thick) sections of areas of interest were then obtained, counterstained with uranyl acetate and lead citrate, and examined with an energy filter transmission electron microscope (Libra120, Carl Zeiss, Oberkochen, Germany). Luminal, endothelial cell and basement membrane outer perimeters of cross-sectional capillaries were traced manually and assessed by iTem software (Olympus Soft Imaging Solutions, Münster, Germany) using images obtained with a yttrium aluminium garnet (YAG) scintillator slow-scan charge-coupled device (CCD) camera (Sharp Eye, TRS, Moorenweis, Germany). Basement membrane thickness and endothelial cell area were derived from the above measurements. An average of 12.3 capillaries per patient were analysed in the papillary dermis.

Capillary density Serial transverse 5 μm sections from paraffin-embedded skin biopsies were cut from the top of the skin and stained with the basic dye Toluidine Blue (0.1% (wt/vol.) in phosphate buffer 0.1 mol/l) to check when the papillary dermis was reached. At this point, the specific marker of endothelial cell lectin from *Ulex europaeus* FITC conjugate (#L9006; Sigma-Aldrich) was applied for 1 h at 37°C in an adjacent section and cross-sectional capillary profiles were counted in at least three different areas of the skin biopsy at ×400 magnification using a fluorescence microscope (Olympus IX51; Olympus, Italia). In a separate section, to express the capillary density as number of capillaries per mm^2 of papillary dermal tissue, staining with Mayer's haematoxylin for 15 min was followed by 1% (wt/vol.) eosin G for 3 min to clearly visualise and calculate the areas of single papillae containing the capillaries.

Arteriolar histology Haematoxylin and eosin (H&E) and smooth muscle actin (SMA) staining was carried out in longitudinally oriented paraffin-embedded sections to study arterioles in the sub-papillary and reticular dermis at the dermal–subcutaneous interface. Mayer's haematoxylin was applied for 15 min followed by 1% (wt/vol.) eosin G for 3 min. An average of 47 arterioles in four different sections were analysed for each patient. Arterioles were defined as occlusive when the lumen area, calculated by Cell^F software (Olympus Soft Imaging Solutions), was less than 20% of the total arteriolar area. The incidence of arteriolar occlusion was expressed as a percentage of the total number of arterioles.

SMA staining was then done to study the involvement of smooth muscle cells in the increase in tunica media thickness. After endogenous peroxidase inhibition (15 min, H_2O_2 3% (vol./vol.) solution), sections were pre-treated using heat-mediated antigen retrieval with sodium citrate buffer (0.01 mol/l, pH 6) for 10 min, incubated with a rabbit polyclonal antibody anti-human α-SMA (1:300, RB-9010 LabVision, Thermo Scientific, Loughborough, UK) for 1 h at room temperature and detected using an avidin/biotin amplification system (#PK 6100, Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA, USA) with 3,3′-diaminobenzidine (DAB) as the chromogen.

Outcome measures Diabetic patients attended visits 7, 14 and 30 days after surgery, then until healing of the surgical wound for evaluation of the healing time, or major amputation, or death. Above-the-ankle amputation was considered a major amputation and was done when gangrene extended beyond the Chopart joint. Complete wound healing was defined as complete re-epithelialisation. Vital status was recorded until the time of healing, major amputation or death.

Statistical analysis Descriptive statistics of the whole population are reported; differences between diabetic and non-diabetic, Isc and Neu patients were tested using the Student's *t* test for means and standard deviation for continuous variables, and Pearson's χ^2 test for discrete variables. The 95% level was adopted for the confidence intervals, and 5% to test the null hypothesis. All results, including linear regression and Pearson's linear correlation coefficient, were calculated with GraphPad Prism (version 4.00; GraphPad Software, La Jolla, CA, USA).

Results

Patient population Sixty type 2 diabetic adults with foot ulcer, 30 Isc and 30 Neu patients of both sexes, were enrolled

and scheduled for amputation of the first ray at the Diabetic Foot Center of IRCCS MultiMedica. Skin biopsies were obtained during the surgical procedure. For the control group, we enrolled ten healthy non-diabetic volunteers in hospital for surgical correction of hallux valgus. All were women, in agreement with the prevalence of hallux valgus in the general population. The ages of non-diabetic and diabetic participants were 64.7 ± 7.4 and 69.5 ± 11.5 years, respectively, and these were not significantly different ($p = 0.210$).

All Isc and Neu patients had biothesiometer vibration perception threshold >25 V, no sensitivity in > 5/9 foot points with Semmes–Weinstein 10 g filament and no Achilles tendon reflex. All the Neu patients had both pedal pulses palpable and no haemodynamically significant stenoses at duplex scanning. $TcPO_2$ was 48.1 ± 11.6 mmHg. The ankle pressure could not be measured in six Neu patients because of non-compressible arteries. In the other 24 Neu patients, the ankle pressure was 108.5 ± 24.6 mmHg.

All the Isc patients had at least one foot pulse absent and haemodynamically significant stenoses (>50% of vessel diameter) at duplex scanning and angiography. $TcPO_2$ before revascularisation was 17.9 ± 10.3 mmHg. This was significantly lower than in the Neu group ($p < 0.001$). Ankle pressure could not be measured in 16 Isc patients: both tibial arteries were absent in ten patients and six had non-compressible arteries. In the other 14 Isc patients, the mean ankle pressure was 77.1 ± 42.0 mmHg. This was significantly lower than in the Neu group ($p = 0.019$). All Isc patients underwent revascularisation, 27 with PTA and three with peripheral BPG. All these patients obtained at least one patent artery in the foot and a significant increase in $TcPO_2$ (38.8 ± 7.2 mmHg, $p < 0.001$). This, however, was significantly lower than the $TcPO_2$ in the Neu group ($p < 0.001$).

The main demographic and clinical characteristics of the diabetic patients (Table 1) were comparable with those of the neuroischaemic and neuropathic patients with diabetic foot ulcer previously studied [14]. The presence of macroangiopathy may explain the more frequent history of cardiac disease in Isc patients while differences in serum albumin and glycaemia at entry simply reflect variability in these two small populations of diabetic patients.

Capillary morphometry Representative images in Fig. 1 depict the ultrastructure of the capillary in the dermal papillary layer of non-diabetic (Fig. 1a) and diabetic (Fig. 1b) participants. Capillary basement membrane thickness (CBMT) in diabetic patients was 2.2-fold greater than in non-diabetic volunteers (3.44 ± 1.19 vs 1.53 ± 0.34 μm, $p < 0.001$) (Fig. 1c); there were no differences between Isc and Neu patients (3.50 ± 1.44 vs 3.39 ± 0.88 μm, $p = 0.735$) (Fig. 1d). Lumen area in the capillary was 57.7% smaller in diabetic patients (14.6 ± 11.1 vs 34.7 ± 27.5 μm^2 in non-diabetic volunteers, $p < 0.001$) but was similar in the two groups of diabetic patients (13.0 ± 12.0 μm^2 in Isc vs 16.4 ± 8.6 μm^2 in Neu, $p = 0.224$).

Table 1 Demographic and clinical characteristics of diabetic patients ($n = 60$) with neuroischaemic (Isc) or only neuropathic (Neu) foot ulcer

Variable	Isc	Neu	*p*
Age (years)	72.0 ± 10.0	66.9 ± 12.4	0.083
Women (no. [%])	4 (13.3)	7 (23.3)	0.506
Men (no. [%])	26 (86.7)	23 (76.7)	
Insulin therapy (no. [%])	17 (56.7)	22 (73.3)	0.278
Diabetes duration (years)	17.9 ± 13.2	16.1 ± 9.3	0.551
Glycaemia at entry (mmol/l)	9.1 ± 3.9	12.0 ± 4.7	0.012
Glycaemia at discharge (mmol/l)	8.4 ± 3.6	7.8 ± 1.9	0.424
HbA_{1c} (%)	8.3 ± 2.3	8.6 ± 2.4	0.134
HbA_{1c} (mmol/mol)	67 ± 23	70 ± 21	0.134
Fasting C-peptide (nmol/l)	8.2 ± 29.7	11.0 ± 45.1	0.782
Retinopathy (no. [%])	12 (40.0)	16 (53.3)	0.438
Microalbuminuria (no. [%])[a]	16 (53.3)	16 (53.3)	0.438
Macroalbuminuria (no. [%])[b]	2 (6.7)	2 (6.7)	1.000
Creatinine > 115 μmol/l (no. [%])	16 (53.3)	16 (53.3)	1.000
eGFR > 60 ml min^{-1} 1.73 m^{-2} (no. [%])	14 (46.7)	14 (46.7)	1.000
Deep infection (no. [%])	26 (86.7)	29 (96.7)	0.353
Leucocytes $>10^3$ mm^3 (no.)	9.2 ± 2.9	10.5 ± 5.0	0.234
C-reactive protein (nmol/l)[c]	705 ± 714	781 ± 810	0.097
Serum albumin (g/l)	32 ± 5	29 ± 6	0.005
Anti-hypertensive therapy (no. [%])	24 (80.0)	22 (73.3)	0.761
History of cardiac disease (no. [%])	14 (46.7)	7 (23.3)	0.032
History of stroke (no. [%])	5 (16.7)	2 (6.7)	0.424
Arterial calcifications (no. [%])	19 (63.3)	24 (80.0)	0.252
Current smokers (no.)	2	1	1.000
Ex-smokers (no.)	3	2	1.000

Data are expressed as mean ± 1 SD or %

[a] Microalbuminuria: 30–299 mg/24 h

[b] Macroalbuminuria: ≥ 300 mg/24 h

[c] Measured prior to surgical procedure after antibiotic treatment

Capillary density Capillary rarefaction, assessed by counting the green fluorescent capillary profiles stained with lectin I (Fig. 2a) in single papillae dermis areas visualised by H&E (Fig. 2b), was observed in diabetic patients (159 ± 43 vs 205 ± 52 mm^2 in non-diabetic volunteers, $p = 0.003$) (Fig. 2c). Capillary density did not differ between Isc and Neu patients (155 ± 36 vs 165 ± 47 mm^2, respectively, $p = 0.343$) (Fig. 2d).

Arteriolar occlusion The incidence of arterioles in the dermis showing lumen occlusion due to hyperplasia of smooth muscle cells in the tunica media (Fig. 3b) was significantly increased in diabetic compared with non-diabetic participants (11.7 ± 7.5% vs 0.3 ± 0.9%, respectively, $p < 0.001$) (Fig. 3c).

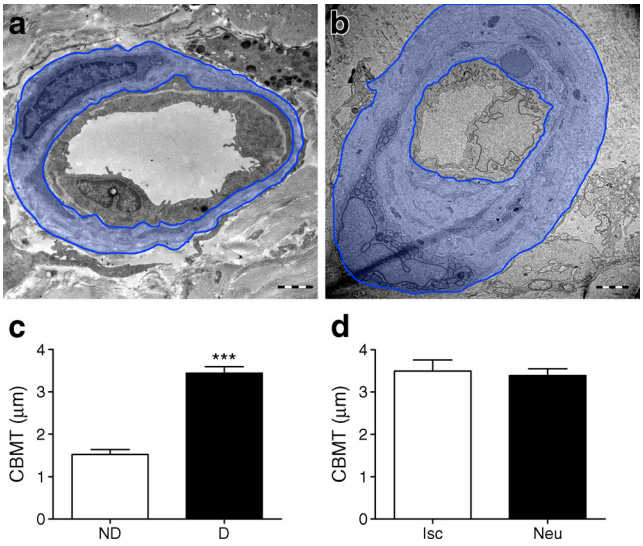

Fig. 1 Representative transmission electron microscopy images of capillary morphology in the papillary layer of samples from non-diabetic (**a**) and diabetic (**b**) participants; scale bar 2 µm. CBMT, outlined in blue, was assessed in non-diabetic and diabetic participants (**c**) and Isc and Neu patients (**d**). Data are presented as mean ± SD. $^{***}p < 0.001$. D, diabetic; ND, non-diabetic

Isc patients were more prone to develop arteriolar occlusion than Neu patients (16.8 ± 6.9% vs 6.7 ± 3.7%, respectively, $p < 0.001$) (Fig. 3d). This was significantly correlated with the reduction in transcutaneous oxygen tension at the dorsum of the foot ($r^2 = 0.286$, $p < 0.001$) (Fig. 3e).

Outcomes One Isc patient died from stroke 6 days after the first ray amputation. One Isc patient required major amputation 245 days after initial surgery because of non-treatable re-stenosis and extended wet gangrene. One Neu patient underwent major amputation because of extended infection 82 days after initial surgery. These patients were not considered in the evaluation of healing time, which was significantly longer for Isc (average 180.1 ± 119.3 days) vs Neu (64.3 ± 49.9 days), $p < 0.001$.

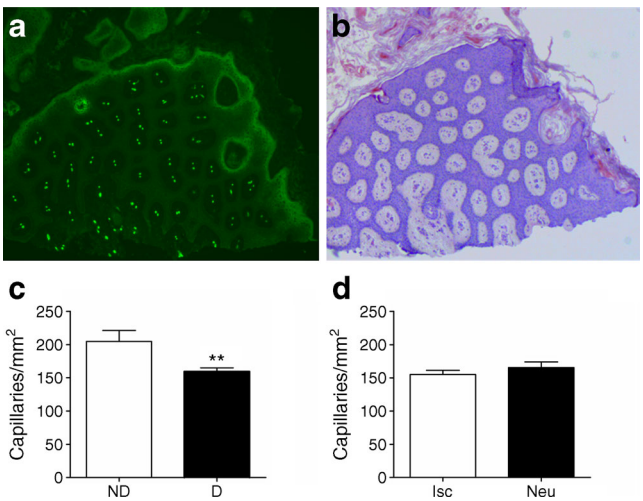

Fig. 2 Green fluorescent capillaries stained with lectin I (**a**) visualised in papillary dermis by H&E (**b**) at ×50 original magnification. Capillary density was assessed in non-diabetic and diabetic participants (**c**) and Isc and Neu patients (**d**). Data are presented as mean ± SD. $^{**}p < 0.01$. D, diabetic; ND, non-diabetic

Discussion

Our study confirms the significant increase in CBMT in the skin of aged diabetic patients with foot ulcers and long diabetes duration [7] and describes, for the first time, reduction in capillary density as an additional histopathological feature of type 2 diabetic microangiopathy. Another new finding was that microangiopathy was absolutely equal in neuroischaemic and neuropathic patients. The fact that all our ischaemic patients were also neuropathic should not be misleading and or even considered a bias as, in our experience [14] and in the literature [15–17], it is becoming increasingly clear that the population of patients with ischaemic diabetic foot corresponds exactly to these characteristics.

There are conflicting reports about skin capillary blood flow in the diabetic foot. Some studies have reported the absence of alterations in capillary blood circulation in neuropathic diabetics [18, 19], while others observed a marked reduction in capillary blood perfusion in diabetic patients with or without peripheral arterial disease [20–23]. This suggests the presence of microangiopathy of the capillaries in diabetic patients with foot ulcer but does not confer any typical characteristics to patients with neuropathy alone or with peripheral vascular disease. This indicates that it has no causal influence in foot ischaemia: $TcPO_2$ values in these two groups of patients were very different, obviously for some other reason. It follows that if one believes that microangiopathy might be an aetiopathogenic factor in the ischaemic foot, the cause of other microvascular changes still needs to be found.

In our study, we noted that arteriolar occlusions were very frequent in Isc patients, indicating the presence of occlusive microvascular disease. This idea of an arteriolar occlusive disease brings back the old question of a 'small vessel disease' and its importance in the impaired wound healing of the ischaemic foot. This was reviewed by LoGerfo and Coffman [24], who examined the differing results of Goldenberg et al [25], describing an endothelial proliferation sufficient almost to occlude the lumen of smaller vessels, and Strandness et al [26], denying the existence of arteriolar occlusive disease.

Our patients demonstrated hyperplasia of smooth muscle cells in the tunica media causing occlusive narrowing of the arteriolar lumen with no sign of alterations in the tunica intima.

Vascular occlusion due to intimal hyperplasia occurs exclusively in vessels larger than arterioles such as atherosclerotic [27] or digital arteries of diabetic patients [25], caused by the proliferation of smooth muscle cells migrating from the tunica

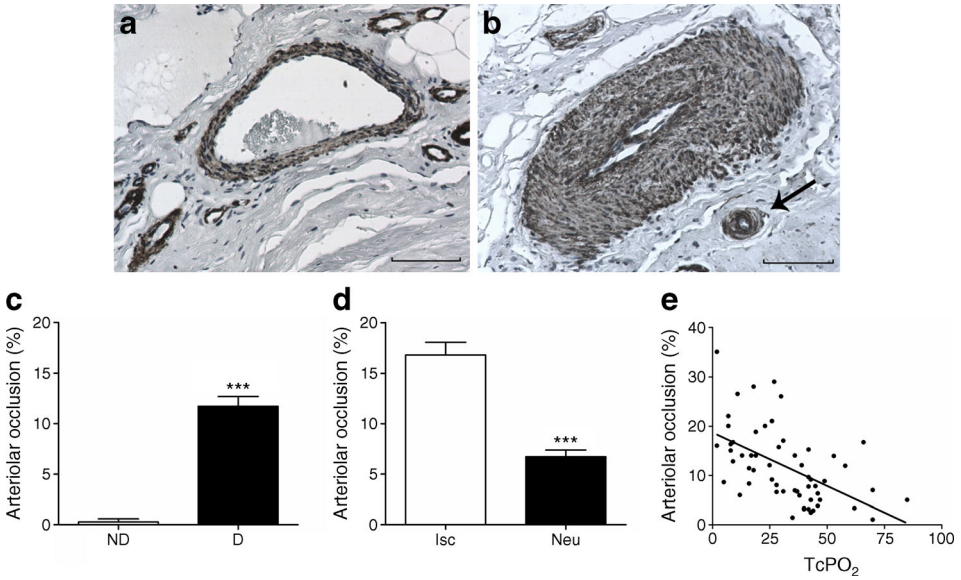

Fig. 3 An arteriole surrounded by smaller arterioles stained for SMA in reticular dermis at the dermal–subcutaneous interface of a sample from a non-diabetic volunteer (**a**). Increased tunica media thickness due to smooth muscle cell proliferation leading to lumen occlusion is observed in an arteriole and in a smaller arteriole (arrow) in the dermis of a diabetic patient (**b**); scale bar 100 μm. Arteriolar occlusion was assessed in non-diabetic and diabetic participants (**c**) and Isc and Neu patients (**d**). The correlation of arteriolar occlusion with transcutaneous oxygen tension is shown in (**e**). Data are presented as mean ± SD. $^{***}p < 0.001$. D, diabetic; ND, non-diabetic

media rather than endothelial proliferation, as initially postulated by Goldenberg et al [25].

The absence of arteriolar occlusion observed by Strandness et al [26] does not contradict our observations. Their analysis focused on arterioles in the subcutaneous and muscular tissue while we looked at smaller arterioles in the papillary and reticular dermis. These are the same arterioles in which Goldenberg et al [25] observed arteriolosclerotic lesions with duplication of fibrillar concentric laminated layers but no 'endothelial proliferation'.

The presence of an occlusive microvascular disease in diabetic patients with CLI is clear from our data. It is equally clear, however, that it is irrelevant to major amputation: at 30 days none of the patients with CLI needed major amputation, and also in the longer follow-up the percentages of participants requiring major amputation were equal. This confirms that revascularisation of at least one artery of the foot is effective in avoiding major amputation in the neuropathic patient [28]. Therefore, both Goldenberg et al [25] and LoGerfo and Coffman [24] were right: Goldenberg et al actually saw arteriolar occlusive disease and LoGerfo and Coffman showed that revascularisation was effective in preventing amputation. However, the healing time of the foot lesions differed significantly for Isc and Neu patients. Foot ulcers take a notoriously long time to heal: the International Working Group on the Diabetic Foot reports a mean healing time of 6 months for diabetic foot ulcers [29]. The foot ulcer healing time reported in many studies varies widely depending on the patients' characteristics [30, 31] and in particular were different for patients with neuropathic or ischaemic ulcers [16, 32]. This is probably because in diabetic patients revascularisation can restore normal blood flow in the large vessels, including the pedal arteries, but cannot restore normal microvascular flow [33–35]. The fact that the post-revascularisation $TcPO_2$ in the Isc group was significantly higher than at baseline but remained significantly lower than the $TcPO_2$ in the Neu group confirms this interpretation.

In conclusion, microangiopathy in patients with diabetic foot ulcer involves the capillaries and is present in equal measure in neuroischaemic and neuropathic patients. This microangiopathy is due to an increase in basement membrane thickness and a reduced number of capillaries. Arteriolar occlusions in neuropathic patients led to additional microvascular disease or so-called 'small vessel disease' that did not prevent revascularisation and did not raise the risk of major amputation, but slowed wound healing.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement All authors took part in the conception and design of the study, as well as either drafting or critically revising the manuscript. GC, MC and DM researched clinical data. SM, CB, DN and AC researched histological data. RM enrolled non-diabetic patients. ADI performed the eye examinations. FF and EF are responsible for the integrity of the work as a whole. All authors revised the article and approved the final version to be published.

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