


Skin microvascular reactivity correlates to clinical microangiopathy in type 1 diabetes: A pilot study

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Abstract

Aim: The aim of this study was to investigate the correlation between skin microvascular reactivity and clinical microangiopathy in patients with type 1 diabetes.

Methods: We included 61 patients with type 1 diabetes, that is, 31 patients with and 30 without clinical microangiopathy, and 31 healthy controls. A microangiopathy scoring system was introduced for comparison of data between patients with microangiopathy. Responses to iontophoresis of acetylcholine and sodium nitroprusside were assessed by laser Doppler imaging.

Results: Patients with microangiopathy had reduced acetylcholine- and sodium nitroprusside-mediated flux in forearm skin microcirculation compared to healthy controls ($p=0.03$ and $p<0.001$, respectively, repeated measures analysis of variance), whereas no significant differences were found between patients without microangiopathy and controls. Skin reactivity was reduced in patients with microangiopathy compared to patients without microangiopathy: 1.43 ± 0.38 versus 1.59 ± 0.39 arbitrary units for acetylcholine-mediated peak flux and 1.44 ± 0.46 versus 1.74 ± 0.34 arbitrary units for sodium nitroprusside-mediated peak flux ($p<0.05$ for both). A tendency of gradual decrease in acetylcholine and sodium nitroprusside responses was found in patients with increasing microangiopathy scores.

Conclusion: We conclude that skin microvascular reactivity is associated with clinical microangiopathy in patients with type 1 diabetes. Impaired skin microvascular function in type 1 diabetes seems to be multifactorial and involves both endothelial-dependent and endothelial-independent pathways. We introduce a novel microangiopathy score that could easily be used in a clinical setting for comparison of patients with various degrees of microangiopathy.

Keywords

Type 1 diabetes, clinical microangiopathy, skin microvascular function, iontophoresis

Introduction

Microvascular complications are common in patients with type 1 diabetes, and may develop as early as within 10 years of disease onset. Diabetic microangiopathy affecting the retina, kidneys and nerves causes gradual loss of organ function, and is one of the leading causes of blindness, end-stage kidney failure, peripheral neuropathy and autonomic dysfunction worldwide.^{1,2}

The dynamics of microvascular dysfunction can be studied in vivo by means of skin microvascular reactivity.^{3,4} Skin microcirculation is easy to investigate and provoke by non-invasive methods. In patients with type 1 diabetes, disturbances in skin microvascular function have been shown compared to healthy controls.^{5–8} However, only a few studies have investigated possible associations between skin microvascular reactivity and clinical microangiopathy in type 1 diabetes, and the results in those studies have been contradictory.^{9,10}

The primary aim of this cross-sectional study was to compare skin microvascular function between patients with type 1 diabetes with and without diabetic microangiopathy. Our hypothesis was that responses in skin microvascular reactivity are reduced in patients with microvascular complications. As a pilot study, we have

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Table 1. Microangiopathy score.

	Degree of clinical microangiopathy	Points
Retinopathy	No signs of retinopathy	0
	Background retinopathy	1
	Non-proliferative moderate retinopathy	2
	Proliferative retinopathy	3
Nephropathy	No proteinuria	0
	Microalbuminuria	1
	Macroalbuminuria	2
	Chronic kidney disease ^a	3
Neuropathy	No signs of neuropathy	0
	Reduced sense of vibration	1
	Reduced superficial sensation	1
	Autonomic dysfunction	1

^aChronic kidney disease stage 3B or higher, defined as estimated glomerular filtration rate (eGFR) < 45 mL/min/1.73 m².

also introduced a novel microangiopathy scoring system in which each patient is provided a total microangiopathy score based on clinical degree of retinopathy, nephropathy and neuropathy.

Research design and methods

A cross-sectional study was conducted including patients with type 1 diabetes with or without clinical microangiopathy and healthy controls.

Subjects

The study included three groups as follows: group 1 – 30 patients with at least 30 years of type 1 diabetes duration and no clinical signs of diabetic microangiopathy except for background retinopathy (which is an early and reversible stage of retinopathy); group 2 – 31 patients with type 1 diabetes and diabetic microangiopathy; and group 3 – 31 healthy controls. All patients were recruited from the adult outpatient clinic at the Department of Endocrinology and Diabetology at Danderyd University Hospital, Stockholm, Sweden.

Patients and controls arrived at the laboratory between 8 and 9 a.m. after a 10-h fast and had been instructed to avoid smoking, use of snuff and exercise in the morning. Investigation of skin microvascular reactivity was performed at a room temperature of 22°C–24°C.

Microangiopathy score

Patients with microangiopathy were given a microangiopathy score between 0 and 9 based on their degree of clinical microangiopathy in clinical examinations and information obtained from medical records (see Table 1). Severity of retinopathy and nephropathy was graded between 0 and 3 points, respectively, while the neuropathy points were added together to a total of 0–3 points.

The sum of all points generated the patient's microangiopathy score.

Retinopathy was categorized into four stages based on funduscopic findings, according to the International Clinical Disease Severity Scale for Diabetes Retinopathy:¹¹ no retinopathy (0 points); mild non-proliferative (background) retinopathy (1 point) defined by a few microaneurysms and/or dot bleedings; moderate to severe non-proliferative retinopathy (2 points) including findings of soft and hard exudates, venous beading and intraretinal microvascular abnormalities; and proliferative retinopathy (3 points) defined by neovascularization, haemorrhages and retinal detachment.

Nephropathy was categorized into four stages, based on medical records: no proteinuria (0 points); microalbuminuria (1 point) defined as urinary albumin–creatinine ratio of 3.4–33.9 mg/mmol in at least two morning samples or albuminuria of 30–300 mg in a 24-h urine collection; macroalbuminuria (2 points) defined as urinary albumin–creatinine ratio >34 g/mmol in at least two morning samples or albuminuria >300 mg in a 24-h urine collection; and kidney failure (3 points) defined as at least moderate chronic kidney disease stage 3B with reduced estimated glomerular filtration rate (eGFR) < 45 mL/min/1.73 m².

Neuropathy scoring was based on medical records and signs of distal sensorimotor neuropathy in the lower extremities according to the American Diabetes Association guidelines 2005.¹² A 128-Hz tuning fork was used to examine vibration perception at the dorsum of the interphalangeal joint of hallux. Patients with reduced (compared to vibration tuning fork against the dorsal wrist) or absent sensation of the vibrating tuning fork placed at the interphalangeal joint of the hallux were given 1 point. The superficial sensation of the plantar surface of the hallux and centrally at the heel was tested with a 5.07 Semmes–Weinstein monofilament. Patients with reduced or absent sensation of the Semmes–Weinstein monofilament in three trials at each location were given 1 point. Patients with known autonomic neuropathy including gastroparesis and/or erectile dysfunction were given 1 point. The neuropathy scores were added together to a total of 0–3 points.

Blood sampling and clinical data

After at least 20 min of rest in the supine position, brachial blood pressure was measured as a mean of three readings by an oscillometric device (OMRON 705IT, OMRON Healthcare, Kyoto, Japan). Venous blood samples were then taken with no or minimal stasis. Prevalence of albuminuria on day of investigation was assessed with urinary dipstick tests (Clinitek®, Bayer HealthCare LLC, Elkhart, IN, USA).

Iontophoresis

Skin microcirculation was investigated through iontophoresis, which is a non-invasive method for drug application

across the skin using a small electric current.¹³ Acetylcholine (ACh, Sigma-Aldrich AB, Stockholm, Sweden) and sodium nitroprusside (SNP, Hospira, Inc., Lake Forest, IL, USA), diluted in deionized water, were used to investigate endothelium-dependent and endothelium-independent microvascular reactivity, respectively. Electrode chambers (LI611 Drug Delivery Electrode Imaging; Perimed, Järfälla, Sweden) were attached to the volar side of the left forearm, avoiding hair, broken skin and visible veins, and filled with a small volume of either ACh (2%) or SNP (2%). A battery-powered iontophoresis controller (Perilont 382b; Perimed) provided a direct current of 0.1 mA for 60 s for drug iontophoresis. ACh was delivered using an anodal charge and SNP with a cathodal charge. Laser Doppler imaging (PeriScan PIM II; Perimed) was used to measure the skin microvascular flux, which is expressed in arbitrary units (AU). Each image has a duration of 36 s and consists of approximately 150 measuring points. Microvascular flux was recorded continuously up to 10 and 14 min after iontophoresis of ACh and SNP, respectively. At our laboratory, mean coefficient of variation (CV) for peak microvascular flux after iontophoresis of ACh and SNP was 11% and 20%, respectively, assessed in seven healthy individuals on 3 separate days.

Nailfold capillaroscopy

Videophotometric capillaroscopy of nailfold capillaries of the big toe was used to assess capillary blood flow at rest and following arterial occlusion. Capillaries with good optical signals, that is, with visible red blood cell movements and plasma gaps, were chosen for examination. Subjects with poorly visualized capillaries, too thick capillaries or with very high capillary blood flow in which red blood cell movement cannot be calculated were excluded from the analyses. Capillary blood cell velocity (CBV) was registered continuously at rest, during and after 1-min arterial occlusion by inflating a miniature cuff at the proximal phalanx of the digit to a cuff pressure of 200 mmHg. The CBV was determined by a computerized, videophotometric, cross-correlation technique (CapiFlow AB[®], Stockholm, Sweden) generating following variables: CBV rest value (mm/s); CBV peak (mm/s) after 1-min arterial occlusion; CBV ttp, time to peak (s); and CBV prh, post-occlusive reactive hyperaemia calculated as percentage increase of CBV from rest to peak flow. The coefficients of variation for repeated measurements of peak CBV and time to peak CBV at our lab are 13% and 11%, respectively.¹⁴

Laser Doppler fluxmetry

Laser Doppler fluxmetry (LDF, Periflux, Perimed[®]) was used to measure total skin microcirculation in the distal phalange of the big toe. The laser Doppler probe was placed within the skin area immediately adjacent to the microscopic field where video capillaroscopy was performed, and skin flux is expressed in AU. Subjects with too thick or

damaged skin in which LDF signals could not be obtained were excluded from the analyses. The following variables are calculated: LDF rest value; LDF peak flow following 1-min arterial occlusion at the proximal toe phalange; LDF ttp, time to peak (s); and LDF prh, post-occlusive reactive hyperaemia calculated as percentage increase of LDF from rest to peak flow.

Skin temperature

Skin temperature of the investigated toe nailfold was continuously recorded with an electronic thermistor (Exacon, Copenhagen, Denmark).

Biochemical analyses

HbA_{1c} levels were assessed by high-performance liquid chromatography (Variant II; Bio-Rad Laboratories, Hercules, CA, USA) and expressed in % according to the National Glycohemoglobin Standardization Program (NGSP) and in mmol/mol according to the International Federation of Clinical Chemistry (IFCC) standardization. eGFR was determined according to the revised Lund-Malmö equation.¹⁵

Statistics

Sample size calculations based on ACh-mediated peak microvascular flux showed that 27 patients were required in each group in order to detect a 25% difference between patients with and without microangiopathy, given a power of 80% and a significance level of 0.05 in a two-sided *t*-test. We therefore included at least 30 subjects in each group. Our results are presented as mean values \pm standard deviation (SD) or 95% confidence intervals (CIs) for normally distributed data, and median values with lower–upper quartiles for skewed data or number of patients. One-way or repeated measures analyses of variance (ANOVA) with contrasts were used to compare data between groups for data with normal distribution. Mann–Whitney was used to compare skewed data between groups. Simple regression analyses were used to test correlation between variables.

Ethical consideration

The protocol of this trial was approved by the local Ethics Committee in Stockholm. Written informed consent was obtained from all the patients.

Results

The characteristics of patients and controls are summarized in Table 2. The three groups were comparable regarding age and sex. Patients without clinical microangiopathy had lower body mass index (BMI), lower systolic blood

Table 2. Subject characteristics.

	Patients without microangiopathy (n=30)	Patients with microangiopathy (n=31)	Healthy controls (n=31)	p (patients vs controls)	p (between patient groups)
Age (years)	54 ± 10	56 ± 14	56 ± 13	0.72	0.55
Women, n (%)	18 (60)	16 (52)	16 (52)	0.83	0.61
BMI (kg/m ²)	23.4 ± 4.2	25.9 ± 3.8	24.6 ± 3.0	0.95	0.01
Tobacco user, ^a n (%)	4 (13)	9 (29)	2 (6)	0.08	0.21
Systolic BP (mmHg)	129 ± 13	144 ± 22	125 ± 16	0.004	0.001
Diastolic BP (mmHg)	71 ± 8	73 ± 12	73 ± 9	0.53	0.41
Toe–arm pressure ratio	0.92 (0.88–1.09)	0.88 (0.81–1.00)	0.95 (0.88–1.02)	0.24	0.046
Diabetes duration (years)	41 ± 11	40 ± 13	–	–	0.57
HbA _{1c} (mmol/mol)	55 ± 8	66 ± 13	–	–	<0.001
HbA _{1c} (%)	7.2 ± 3.0	8.2 ± 3.3	–	–	
fp-glucose (mmol/L)	10.3 (8.5–12.9)	9.9 (6.9–13.6)	5.4 (5.0–5.8)	<0.001	0.74
Cholesterol (mmol/L)	4.5 (4.1–5.1)	4.4 (3.9–5.0)	5.0 (4.6–5.5)	<0.001	0.33
LDL (mmol/L)	2.5 (2.0–2.6)	2.4 (1.9–2.9)	3.4 (2.9–3.9)	<0.001	0.98
HDL (mmol/L)	1.7 (1.4–1.9)	1.4 (1.2–1.7)	1.3 (1.1–1.6)	0.004	0.02
Triglycerides (mmol/L)	0.5 (0.4–0.8)	0.9 (0.6–1.2)	0.9 (0.6–1.1)	0.04	<0.001
eGFR (mL/min/1.73 m ²)	78 ± 8	65 ± 20	73 ± 13	0.92	0.001
Medication, n (%)					
ACEi or ARB	8 (27)	21 (68)	–	–	0.006
Beta blockers	1 (3)	8 (26)	–	–	0.13
Calcium antagonist	1 (3)	11 (35)	–	–	0.03
Statins	8 (27)	17 (55)	–	–	0.06

BMI: body mass index; BP: blood pressure; HbA_{1c}: haemoglobin A_{1c}; fp: fasting plasma; LDL: low-density lipoprotein; HDL: high-density lipoprotein; eGFR: estimated glomerular filtration rate; ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin II receptor blocker.

Data are presented as mean values ± SD, median with interquartile ranges or in numbers (n). p is based on contrasts between patients and controls or patient groups in one-way ANOVA.

^aCurrent smoker or snuff user.

pressure and better long-term glycaemic control (HbA_{1c}) compared to patients with microangiopathy, while diabetes duration and fasting plasma glucose levels were similar in both groups. Tobacco use (smoking or use of snuff) tended to be more common among patients than among controls ($p=0.08$). Most patients (80% of patients without microangiopathy and 68% of patients with microangiopathy) were treated with intermittent insulin doses, that is, long-lasting insulin once or twice per day and short-lasting insulin with meals, while the rest had continuous subcutaneous insulin infusion and bolus doses with meals. Total insulin doses per day did not differ between the two patient groups ($p=0.83$, data not shown). Lipid levels were lower among patients than in controls, probably due to the use of statin treatment. Patients without microangiopathy had higher high-density lipoprotein (HDL) levels and lower triglyceride levels compared to patients with microangiopathy. All subjects included in the study were Caucasians.

Forearm ACh and SNP iontophoresis

Differences in ACh-induced (endothelial-dependent) microvascular flux over time between the three groups are shown in Figure 1(a). Compared to healthy controls,

ACh-mediated flux was not significantly different in patients without microangiopathy ($p=0.30$, repeated measures ANOVA), while patients with microangiopathy had reduced flux values ($p=0.03$). No significant differences were found between the two patient groups with and without microangiopathy ($p=0.23$). The patients were divided into five different groups based on their microangiopathy score. Comparison of microvascular flux between patients with different microangiopathy scores showed a non-significant tendency of gradual decrease in ACh-mediated responses with increased microangiopathy scores (Figure 1(b), repeated measures ANOVA).

Responses to SNP-induced (endothelial-independent) microvascular flux over time in the three groups are shown in Figure 1(c). Compared to healthy controls, no differences in SNP-mediated microvascular flux were found in patients without microangiopathy ($p=0.40$, repeated measures ANOVA), while SNP-mediated flux was significantly reduced in patients with microangiopathy ($p<0.001$). Patients with microangiopathy also had significantly lower responses to SNP iontophoresis compared to patients without microvascular complications ($p=0.01$, repeated measures ANOVA). Comparison of SNP-induced responses between patients with different microangiopathy scores showed gradually decreased

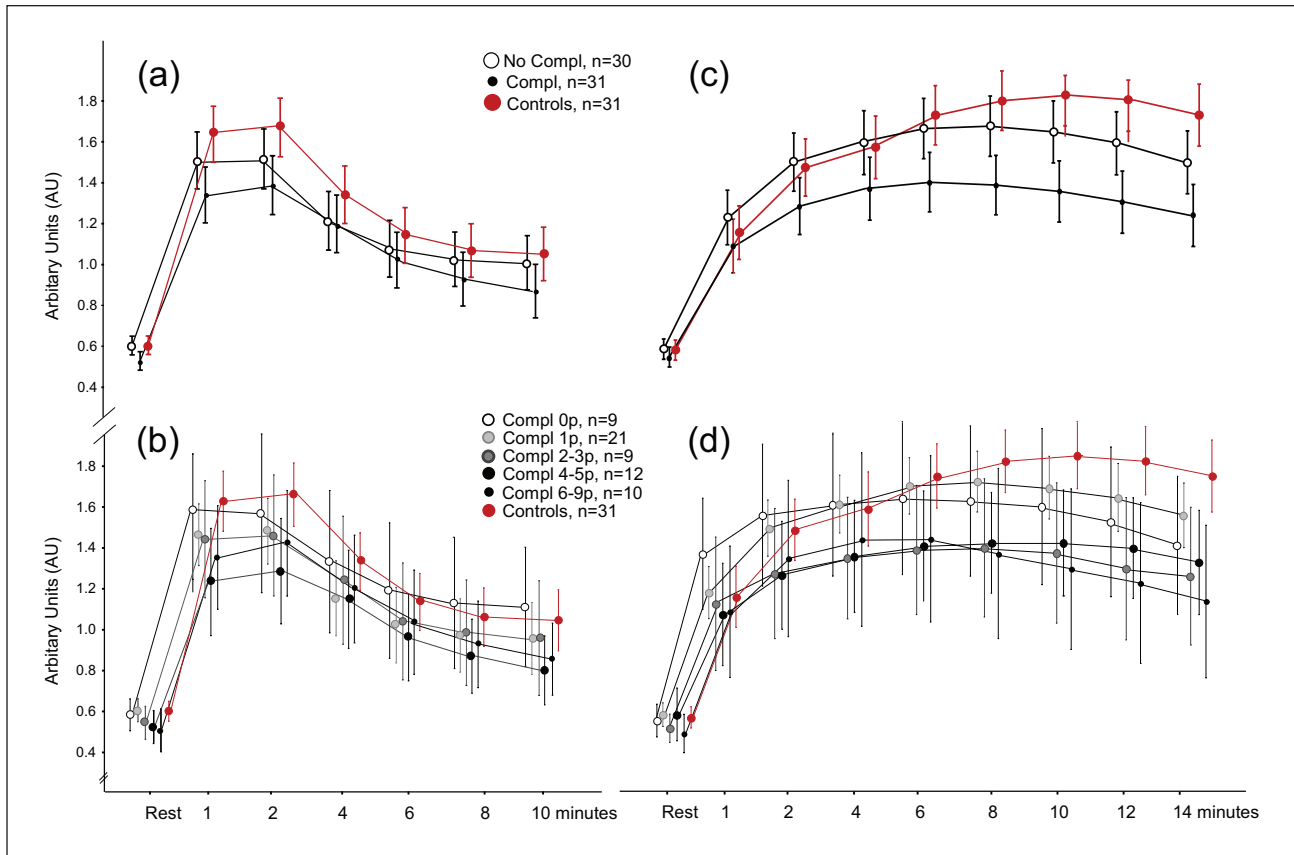


Figure 1. Increases in skin microvascular flux in response to acetylcholine and sodium nitroprusside iontophoresis in patients with type 1 diabetes and healthy controls.

Data show mean values and 95% CI of skin perfusion in arbitrary units (AU) before and after iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP). Figure 1(a) and (c) shows results of patients with no microvascular complications (white circles), patients with microvascular complications (black circles) and healthy controls (red circles). Patients with microangiopathy had, compared to controls, reduced microvascular responses to ACh ($p=0.03$, repeated measures ANOVA) and SNP ($p<0.001$). Patients with microangiopathy also had reduced SNP-mediated responses compared to patients without complications ($p=0.01$), while ACh-mediated responses were not significantly different. Subgroup analyses of patients with various microangiopathy scores (Figure 1(b) and (d)) show a gradual decrease in both ACh- and SNP-mediated responses in patients with increasing microangiopathy scores.

values with increased microangiopathy scores (Figure 1(d), $p<0.001$ repeated measures ANOVA).

ACh- and SNP-mediated peak microvascular flux levels and peak ACh/SNP index for the three groups are presented in Table 3. Peak responses to both ACh and SNP iontophoresis were reduced in patients with type 1 diabetes with or without microangiopathy compared to healthy controls. Patients with microangiopathy had reduced SNP-mediated peak flux levels compared to patients without microangiopathy ($p=0.006$), while peak ACh flux levels were not significantly different between the patient groups ($p=0.12$). ACh/SNP index values did not differ between the three groups. No significant correlations were found between ACh- or SNP-mediated peak flux and diabetes duration, HbA_{1c} levels, systolic/diastolic blood pressure, plasma lipid levels or toe/arm blood pressure index (data not shown). ACh- and SNP-mediated peak flux was weakly correlated to BMI ($r=-0.22$ for ACh and $r=-0.25$ for SNP, $p<0.05$ for both) and fasting plasma glucose levels ($r=-0.29$ for ACh and $r=-0.25$ for SNP, $p<0.05$ for both).

both). No significant differences were found between tobacco users and non-tobacco users (data not shown).

Nailfold capillaroscopy and LDF of big toe

Unfortunately, capillaroscopy and LDF were only possible in two-thirds of the patients due to thick or damaged skin, poorly visualized capillaries and poor LDF signals. Results from CBV and total microcirculatory flux assessed by LDF within the same skin area on the big toe are shown in Table 3. Compared to controls, patients with type 1 diabetes had higher CBV levels at rest ($p=0.03$), while peak CBV values were not significantly different. No significant differences were found in time to peak or post-occlusive reactive hyperaemia (prh) levels between patients and controls. Compared to patients without microangiopathy, patients with microvascular complications had lower CBV prh ($p=0.03$), while peak CBV levels were not significantly different.

Table 3. Skin microvascular responses to ACh/SNP iontophoresis and arterial occlusion in patients with type 1 diabetes and healthy controls.

	Patients without microangiopathy (n = 30)	Patients with microangiopathy (n = 31)	Healthy controls (n = 31)	p (patients vs controls)	p (between patient groups)
Forearm skin flux					
ACh peak (AU)	1.59 ± 0.39	1.43 ± 0.38	1.70 ± 0.42	0.04	0.12
SNP peak (AU)	1.74 ± 0.34	1.44 ± 0.46	1.87 ± 0.42	0.003	0.006
ACh/SNP peak	0.87 (0.77–1.01)	0.93 (0.78–1.11)	0.91 (0.82–1.06)	0.96	0.20
Big toe CBV					
CBV rest (mm/s)	0.09 (0.04–0.16) ^a	0.10 (0.06–0.23) ^b	0.06 (0.03–0.09) ^a	0.03	0.59
CBV peak (mm/s)	0.39 (0.12–0.59) ^a	0.16 (0.10–0.34) ^b	0.15 (0.09–0.29) ^a	0.14	0.20
CBV ttp (s)	16 (11–24) ^a	15 (13–22) ^b	20 (14–22) ^a	0.40	0.94
CBV prh (%)	167 (74–469) ^a	57 (16–124) ^b	181 (95–275) ^a	0.24	0.03
Big toe LDF					
LDF rest (AU)	15 (10–39) ^c	16 (8–42) ^c	9 (5–14) ^b	0.009	0.72
LDF peak (AU)	40 (29–92) ^c	71 (25–97) ^c	29 (19–56) ^b	0.03	0.49
LDF ttp (s)	8 (6–12) ^c	9 (5–11) ^c	10 (7–13) ^b	0.13	0.85
LDF prh (%)	181 (86–353) ^c	230 (115–331) ^c	304 (125–465) ^b	0.36	0.64
Big toe skin temperature (°C)	26.6 ± 2.0	27.3 ± 2.7	25.7 ± 1.9	0.03	0.44

ACh: acetylcholine; SNP: sodium nitroprusside; AU: arbitrary units; CBV: capillary blood cell velocity; ttp: time to peak; prh: post-occlusive reactive hyperaemia; LDF: laser Doppler fluxmetry.

Data are presented as mean values ± SD or medians and interquartile ranges. Changes in forearm skin perfusion following ACh/SNP iontophoresis were assessed by laser Doppler imaging. Post-reactive hyperaemia after arterial occlusion in big toe was assessed by functional capillaroscopy and laser Doppler fluxmetry. p is calculated through contrasts between groups in one-way ANOVA.

^an = 18–19.

^bn = 20–21.

^cn = 22–23.

Baseline and peak LDF levels were higher in patients than in controls, whereas no differences in LDF values were found between the two patient groups. Levels of peak LDF and peak CBV had no correlation. Skin temperature, measured at the same toe nailfold, was higher in patients with type 1 diabetes than in controls ($p=0.03$), but did not differ between the patient groups.

Discussion

This study shows that disturbances in skin microvascular function in patients with type 1 diabetes are associated with clinical microangiopathy. As responses to both ACh and SNP iontophoresis were decreased in patients with microangiopathy, it seems that both endothelial-dependent and endothelial-independent pathways are involved in the development of diabetic complications. In addition, we introduce a novel microangiopathy scoring system based on easily accessed clinical data, which enables comparison of data between patients with microvascular complications in different vascular beds. Patients with increased microangiopathy scores have a tendency of gradual decrease in ACh-mediated flux and a statistically significant gradual decrease in SNP-mediated flux. These data support the use of skin microvascular reactivity as an indicator of severity of clinical microangiopathy.

It is known that ACh iontophoresis mediates an endothelial-dependent vasodilatation, although the contribution of nitric oxide (NO), prostanoids and endothelial-derived hyperpolarizing factor in mediating this response remains unclear.⁴ SNP, on the other hand, acts directly on vascular smooth muscle cells and induces relaxation without involvement of endothelial cells.⁴ A reduction in vascular response to ACh with no concurrent reduction in SNP response is therefore indicative of endothelial dysfunction. Reduction in response to SNP can be interpreted as a structural change within the vessel wall, causing reduced vasodilator capacity or diminished NO bioavailability. High oxidative stress is one cause of reduced NO bioavailability, as reactive oxygen species and free radicals react with NO.¹⁶

In this study, we found that responses to both ACh and SNP iontophoresis were reduced in patients with type 1 diabetes compared to healthy controls, and that patients with microangiopathy had lower responses to ACh and SNP iontophoresis than patients without microangiopathy (Figure 1(a) and (c), repeated measures ANOVA). Peak ACh/SNP index did not differ between the groups (Table 3). Peak flux responses to SNP iontophoresis were significantly different between patients with and without microangiopathy, while peak ACh-mediated responses tended to be lower in patients with microangiopathy (Table 3). In

addition, comparison of data between patients with various microangiopathy scores showed a gradual decrease in ACh- and SNP-mediated responses (statistically significant only for SNP) with increasing microangiopathy scores (Figure 1(b) and (d)). Altogether, our data suggest that skin microvascular reactivity can be used as an indicator of clinical microangiopathy in patients with type 1 diabetes.

Our results also indicate that impaired skin microvascular function in type 1 diabetes is multifactorial and involves endothelial dysfunction, vessel structural changes and/or reduced NO bioavailability. Differences in flux responses to iontophoresis between patients with and without microangiopathy were statistically significant for SNP but not for ACh. This could indicate that disturbances in the endothelial-independent pathways are more important than endothelial dysfunction in development of diabetic microangiopathy. One possible confounder affecting our results between the patient groups could be the higher prevalence of hypertension and greater use of antihypertensive drugs among patients with microangiopathy. However, recent studies investigating effect of hypertension and antihypertensive drugs have failed to show that these factors affect skin microvascular reactivity.^{17,18} Of note is that neither long-term glycaemic control (HbA_{1c}) nor diabetes duration were correlated to peak ACh- or SNP-induced microvascular flux, which indicates that impaired skin microcirculation in patients with type 1 diabetes is not explained by chronic hyperglycaemia and long diabetes duration per se.

In accordance with our results, previous studies investigating skin perfusion in patients with type 1 diabetes have shown that responses to both ACh and SNP iontophoresis are reduced compared to healthy subjects.^{7-10,19,20} However, further comparison between our data and these studies is difficult to make due to use of different laser Doppler techniques for assessment of skin perfusion, examination of different skin areas (upper vs lower extremities) and differences in iontophoresis drug concentrations and administration (one vs repeated doses). Importantly, the few studies that have compared skin microvascular reactivity to diabetic microangiopathy in patients with type 1 diabetes have presented contradictory results.^{9,10}

Functional capillaroscopy of big toe nailfold in our study showed that patients with type 1 diabetes had increased CBV at rest and a tendency towards lower peak CBV levels compared to controls (Table 3). Accordingly, total skin perfusion in the same skin area measured by single-point LDF demonstrated increased basal and peak post-occlusive microcirculatory flux in patients compared to controls (Table 3). Unfortunately, we had a dropout of about one-third of the subjects due to damaged skin or thick skin with poorly visualized capillaries and undetectable LDF signals. The number of dropouts was similar in all three groups, and could result in type II error in the interpretation of these data. Nevertheless, the acquired data imply that type 1 diabetes is associated

with increased basal skin blood flow while the vasodilatory capacity is reduced. Of note is that skin temperatures in big toe of patients were higher than in controls, which also indicates that basal microvascular blood flow is increased in the patients. Indeed, it has been proposed that the early phases of diabetic microangiopathy are characterized by a paradoxical increase in microvascular flow and capillary pressure, causing basement membrane thickening and development of vascular sclerosis, limited vasodilatory reserve and reduced autoregulatory capacity.^{1,21,22}

While microangiopathy severity scores are commonly used for diabetic retinopathy and nephropathy, our study is the first to introduce a total microangiopathy scoring system on diabetic microangiopathy for different microvascular beds. First, the severity of diabetic complications in the retina, kidneys and nerve function is graded between 0 and 3 points. Then, the points are added together and a microangiopathy score is generated ranging between 0 and 9 points, in which the three vascular beds are equally weighted. Importantly, this scoring system is based on common clinical variables that are routinely measured at diabetic outpatient clinics, and therefore easy to introduce in a clinical practice. In addition, retinopathy, nephropathy and neuropathy are weighted equally since all three are common diabetic complications that cause major patient suffering and costs for the healthcare system. This study was a pilot project in which we introduce the microangiopathy score in a clinical study in small subgroups of patients with different microangiopathy scores. In the future, the scoring system needs to be modified and verified in a larger population. We argue that a validated diabetic microangiopathy score could be a useful tool for comparison of data in clinical research as well as for patient management and risk stratification in clinical practice.

This study has several strengths. The study compares results in three groups of patients with type 1 diabetes with/without microangiopathy and healthy controls. The groups are well-characterized and comparable in age and sex. While several previous studies have measured skin microvascular reactivity in patients with type 1 diabetes and healthy controls, only a few have investigated possible correlation to clinical microangiopathy. Our study is the first study that has been designed to investigate differences in ACh-mediated responses between patients with and without microangiopathy. In addition, we have compared data between patients with different microangiopathy scores, and although the groups in this subgroup analysis were small, we could show that the patients' microangiopathy scores tended to correlate with responses in skin microvascular reactivity. For the assessment of skin perfusion, we have used laser Doppler imaging, which is considered to be a more reliable and reproducible method for assessment of skin microcirculation during

iontophoresis than single-point LDF that has been used in many studies.^{23–26} We have also studied total skin perfusion and capillary blood flow following arterial occlusion. Unfortunately, CBV and LDF data could only be acquired in about two-thirds of the study population, and these data should therefore be interpreted with some caution.

Conclusion

In conclusion, our study shows that skin microvascular reactivity can be used as an indicator of clinical microangiopathy in type 1 diabetes as it is related to retinopathy, nephropathy and neuropathy. We introduce a novel microangiopathy score that could easily be used in a clinical setting for comparison of patients with various degrees of clinical microangiopathy in different organs. A diabetic microangiopathy scoring system could be a valuable tool in future research projects and clinical patient management of type 1 diabetes, but first needs to be carefully validated in larger study populations.

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