Dysfunctional arteriovenous anastomoses in the hands of systemic sclerosis patients with digital ulcers

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ABSTRACT

Objective. *Previous studies indicate that the arteriovenous anastomoses (AVAs) and the arterioles with the nutritive flow are involved in the pathophysiologic process disturbing hand blood flow in systemic sclerosis (SSc). However, impact of different part of the microvascular system involved in digital ulcers (DU) is not well known. Here, we aimed to assess the vasomotor activity of the AVAs in the hands of patients with and without DU in SSc.*

Methods. *Simultaneous recordings were made of laser Doppler flux in the finger pulp and thenar eminence, together with ipsilateral radial artery blood velocity and mean arterial blood pressure (MAP) in 22 non-smoking SSc patients and 13 aged-matched healthy controls.*

Results. *AVA responses in the finger pulp to spontaneous vasoconstrictor nerve impulses were abolished in 64% of the SSc patients. Correlation and cross-spectra analysis showed positive correlation between blood flow changes and MAP changes, indicating a passive vascular bed in the SSc finger pulp with blood flow variations depending on short-term variability in MAP. Dysfunctional AVAs were identified in all the patients with a history of DU (n=8), while none of the patients with normal AVA function had episodes of DU (n=8) (p= 0.017).*

Conclusion. *We found that in SSc patients with DU there is a dysfunction of the AVAs of the finger pulp. This proof-of-concept study supports the notion that AVA dysfunction may play a critical role in SSc-related DU. AVA dysfunction may be a part of autonomic dysfunction in SSc.*

Introduction

Systemic sclerosis (SSc) is a multisystem autoimmune disease characterised by progressive fibrosis and vascular dysfunction. Raynaud's phenomenon (RP), with episodic vasospasms elicited by cold or stress, is the most common symptom of vascular dysfunction in SSc. RP occurs with a frequency of approximately 90–95% (1), and it may contribute to development of ischemic digital ulcers (DU) (2, 3). DUs are frequent in (SSc) (4) and causes pain, loss of hand function (5), secondary infections and may lead to auto-amputation of the distal phalanges (6, 7). SSc is associated with increased mortality; most related to heart, kidney and lung failure (7, 8). Any correlation between disturbed hand blood flow and internal organ failure in SSc is still not clear.

There is evidence that both the AVAs (9-11) and the arterioles with the nutritive flow are involved in the pathophysiologic process disturbing hand blood flow in SSc. However, impact of different part of the microvascular system involved on DU is not well known. The magnitude of hand blood flow depends continually on body temperature and is controlled by the arteriovenous anastomoses (AVAs). The AVAs link arterioles to the venules, bypassing the nutritive flow of the capillaries. The AVAs are large thick-walled shunt vessels lacking internal elastic laminae located in acral skin at the same depth as the eccrine sweat glands (12, 13). In the hands, the high density of the AVAs in finger pulp and nail fold decreases proximally to the thenar eminence with a factor of 0.5 whereas the capillaries increases with a factor of 3 (14). The vasoconstrictions of the AVAs are caused by bursts of efferent sympathetic impulses which are synchronous in all skin areas (14, 15). In a thermoneutral situation, the AVAs constrict 2–3 times per minute, causing large, rapid synchronous blood velocity fluctuations in the afferent arteries (16, 17). In warm or cold conditions, the AVAs

are mainly open or closed respectively. A such tight autonomic control of each vasoconstriction episode is not present for arterioles and the nutritive flow.

In the hand of SSc patients, fibrosis of the skin is caused by enhanced fibroblast activity giving rise to increased deposits of dermal collagen, which are more severe distally than proximally (18). Nailfold capillaroscopy shows structural damage with disorganisation and loss of capillaries (19, 20) which demonstrate the involvement of the nutritive flow (1). Previous laser Doppler studies further demonstrated signs of vascular structural changes of the arterioles with lower blood flow in patients with SSc than in healthy subjects (9, 20-22). A defective function of AVAs is suggested, since fingertip blood flow in patients with SSc reacted on local cooling in the same way as skin without AVAs (9) and with a loss of the synchronous blood flow pattern (11, 23, 24).

In the present study the function of AVAs located in finger pulp is compared to the function of AVAs located in normal, more proximal skin. AVA function is investigated in patients with and without history of DU and is compared to healthy controls. AVA dysfunction was defined as loss of synchronous vasomotor activity and loss of negative correlation to blood pressure variations.

Materials and methods

Subjects

Twenty-two patients were recruited from the Norwegian systemic connective tissue and vasculitis registry (NOSVAR) at the Department of Rheumatology, Oslo University Hospital. Patients were excluded if they smoked, had calcinosis of the finger pulp, diabetes or prior sympathectomy of the upper limb. They were also excluded if they had received intravenous prostanoides, bosentan or other endothelin receptor antagonists or phosphodiesterase inhibitors during the preceding three months. Thirteen healthy, non-smoking sex- and age-matched controls were recruited from the hospital staff. The subjects were asked not to drink coffee or tea on the experimental day, or to exercize or eat for at least two hours before the experiment. Informed consent was obtained from all subjects and the study protocol was approved by the regional ethics committee.

Experimental setting

The experiments were carried out in a climatic chamber. The subjects were dressed in T-shirts and trousers and rested in a supine position on a bench. The chamber temperature was adjusted to obtain large fluctuations in arterial blood velocity, indicating that the subjects were in their thermoneutral zone (room temperature 26–27ºC). The subjects were acclimatised for 30 minutes before the following simultaneous continuous measurements were recorded for 15 minutes: blood velocity from the right radial artery, laser Doppler flux from the right third finger pulp, or index finger if the third finger had DU, laser Doppler flux from the right thenar eminence, mean arterial pressure (MAP) from the left fourth finger, and tympanic temperature. All measurements were performed by the same investigator (TKB).

Blood velocity was measured in the right radial artery using a pulsed Doppler ultrasound system (SD-100, Vingmed Sound, Horten, Norway) with an operating frequency of 10 MHz. The transducer had a fixed angle of 45º between the ultrasound beam and the underlying skin surface and were fastened with adhesive tape some 3 cm proximal to the wrist. Laser Doppler probes (MBF3D; Moor Instruments, Devon, UK) were fastened with tape to the right third finger pulp (or the index finger if the third finger had an ulcer) and to the thenar eminence. The noise-limiting filter on the instrument was set at its highest level (21 kHz), and the emitted wave length was 820 nm. The flux output signal was filtered at time constant 0.1s. Instantaneous arterial blood pressure was obtained from the left fourth finger (Ohmeda 2300 Finapres, Madison, WI, The Netherlands). All signals were sent to the computer for beat-by-beat averaging gated by ECG R waves. The tympanic temperature was recorded using an ear probe (Exacon model 8940, Scientific Instruments Aps, Denmark).

Nailfold videocapillaroscopy (NVC) was performed using a videomicroscope (VideoCap 3.0 DS Medica, Italy). The NVC pattern was defined as published previously (25):

1. Early NVC pattern: few enlarged/giant capillaries, few capillary haemorrhages, relatively well-preserved capillary distribution, and no evident loss of capillaries.
2. Active NVC pattern: frequent giant capillaries, frequent capillary haemorrhages, moderate loss of capillaries, mild disorganisation of the capillary architecture, absent or mildly ramified capillaries, and presence of oedema.
3. Late NVC pattern: irregular enlargement of the capillaries, few or no giant capillaries and haemorrhages, severe loss of capillaries with extensive avascular areas, disorganisation of the normal capillary array, and presence of bushy capillaries.

Data analyses and statistics

The correlation and cross-spectra analyses were made using 7.5 min recordings. Correlation analyses were performed between blood velocity fluctuations in the radial artery and laser Doppler flux in the finger pulp, and between blood velocity fluctuations in the radial artery and laser Doppler flux in the thenar eminence. Correlation between the blood velocity values in the radial artery and MAP and between laser Doppler flux in the finger pulp and MAP was also studied.

Power density spectra were calculated for each of the variables in the separate time intervals to obtain variability ~0.05 Hz (0.03–0.07 Hz; corresponds to fluctuations with periodicity between 14.2 and 33.3 s) covering the 2–3 AVA vasomotion episodes per min (15). Cross-spectral analysis provided the phase angles and coherence between the two signals approximately at 0.05 Hz.

Since phase angles are on a closed curve, circular statistics were applied when estimating mean direction and variance. Averaged phase angles were computed by weighting the phase angles with their squared coherence, and standard deviations for the phase angles were calculated according to cir-

cular variance (26). We considered two variables to be in phase if the phase angle between them was less than 45°, and to be in inverse phase if the phase angle was more than 135°.
The Wilcoxon signed rank sum test against a two-sided alternative was used to test for differences between situations, with significance level p=0.05. The Wilcoxon median and upper and lower limits of the 95% confidence interval are reported, which corresponds to the non-parametric one-sample test (27).

Results

Demographic and clinical variables

The non-smoking study cohort included 19 females and three males (Caucasians), who fulfilled the ACR classification criteria for SSc (ACR classification, 1980) and/or the Medsger & LeRoy criteria (28). Clinical features are summarised in Table I. Of the patients, 13 were being treated with nifedipin; none of the patients had received other vasodilatative medication for the three months prior to the examination. All subjects had symptoms of RP, and eight subjects were identified with previous DU.

Table I. Demographics, disease subgroups and clinical features of the study cohort.

	Study cohort (n=22)
Demographics	
Female (%)	19 (86)
Male (%)	3 (14)
Mean age (yrs) at examination (SD)	59 (10.0)
Mean disease duration (yrs)** (SD)	9.7 (7.5)
Disease subgroup	
Limited cutaneous (%)	19 (86)
Diffuse cutaneous (%)	3 (14)
Clinical features	
RP (%)	22 (100)
Digital ulcers (%)	8 (36)
Pulmonary hypertension (%)	2 (9)
Lung fibrosis (%)	8 (36)
ACA (%)	14 (64)
ATA (%)	1 (5)

ACA: anti-centromer antibody; ATA: anti-topoisomerase antibody; RP: Raynaud's phenomenon. *Defined by the first non-Raynaud symptom. **Defined as the period between first non-Raynaud symptom and examination. n: number. yrs: years. SD: standard deviation.

Blood flow measurements in healthy subjects

Spontaneous synchronous fluctuations were evident in the finger pulp flux, the thenar eminence flux and the radial artery blood velocity in the healthy subjects. These synchronous fluctuations, invariably seen in skin areas containing AVAs, are presented in Figure 1A-C. These panels show 80 s sections from a 15 min recording from one healthy subject. The high vasomotor activity, with 4 vasoconstrictions per minute (open arrows), indicates that the subject is in the thermoneutral zone. The high correlation between blood velocity fluctuations in the radial artery and laser Doppler flux in the finger pulp (median r=0.88, 95% CI: 0.80, 0.92), and between blood velocity fluctuations in the radial artery and laser Doppler flux in the thenar eminence (median r= 0.92, 95% CI: 0.89, 0.94) were not significant different (p=0.13). The real-time beat-by-beat MAP recording from the same subject (Fig. 1D) indicates the familiar slight negative correlation between blood flow fluctuations and MAP variability (finger pulp flux – MAP: median r=-0.18, 95% CI -0.33, -0.06) (15).

Blood flow measurements in SSc patients

The recordings from SSc patients showed similar healthy AVA function in the finger pulp and thenar eminence in 8 of the 22 subjects. However, in the remaining 14 patients, dysfunctional AVA vasomotion was seen in the finger pulp, while AVA vasomotion in the thenar eminence was still almost normal. Figure 1E-H shows this pattern in one SSc patient with a history of DU. The 80 s sections are from a 15 min recording of finger pulp flux (E), thenar eminence flux (F), radial artery blood velocity (G) and MAP (H). The trace from the thenar eminence (F) shows three spontaneous vasoconstriction episodes (closed arrows) that are closely correlated to blood velocity fluctuations in the radial artery (G) (correlation coefficient in this subject is r=0.77, and for the total patient group (n=22), median r=0.77 95% CI 0.71, 0.83). Since simultaneous synchronous vasoconstriction episodes are observed in the thenar eminence and the radial artery, we would expect the same pattern from the finger pulp. As clearly seen from Figure 1E, the AVAs in the finger pulp do not respond normally to the vasoconstrictor impulses. Instead, simultaneous vasodilatory responses (closed arrows) are observed in the finger pulp, positively correlated to MAP (H) (r=0.68) and negatively correlated to radial artery blood velocity (G) (r=-0.58). The correlation analysis based on all 22 subjects showed a slightly positive correlation between finger pulp flux and radial artery blood velocity (median r=0.13, 95% CI -0.13, 0.41) and a positive correlation between finger pulp flux and MAP (median r=0.37, 95% CI 0.25, 0.51).
In the patients, the correlation between finger pulp flux and radial artery velocity fluctuations was statistically significantly lower than the correlation between thenar eminence flux and radial artery velocity fluctuations (Wilcoxon signed test p<0.001).
There was no statistically significant difference between the tympanic temperatures of patients and controls or between the room temperatures necessary to keep the subjects in the two groups in their thermoneutral zone.

Cross-spectral analysis

The cross-spectral analysis presented in Figure 2 illustrates the timing of the oscillations in the hand at ~0.05Hz. In the healthy subjects (upper panel), the changes in finger pulp flux coincided with changes in thenar eminence flux and blood velocity in the radial artery (1° and 4°, 95% CI -12°, 7°), while changes in MAP were out of phase with these vasoconstriction episodes (-104° deg, 95% CI -139°, -85°). In the SSc patients (lower panel), the changes in blood velocity in the radial artery and the thenar eminence flux coincided similarly to the healthy subjects, (13°, 95% CI 8°, 15°), but here the changes in MAP were coincident with changes in finger pulp flux (-8°, 95% CI -23°, 23°).

AVA dysfunction and digital ulcer

The findings from both the correlation analysis and the cross-spectral analysis show that the finger pulp has become

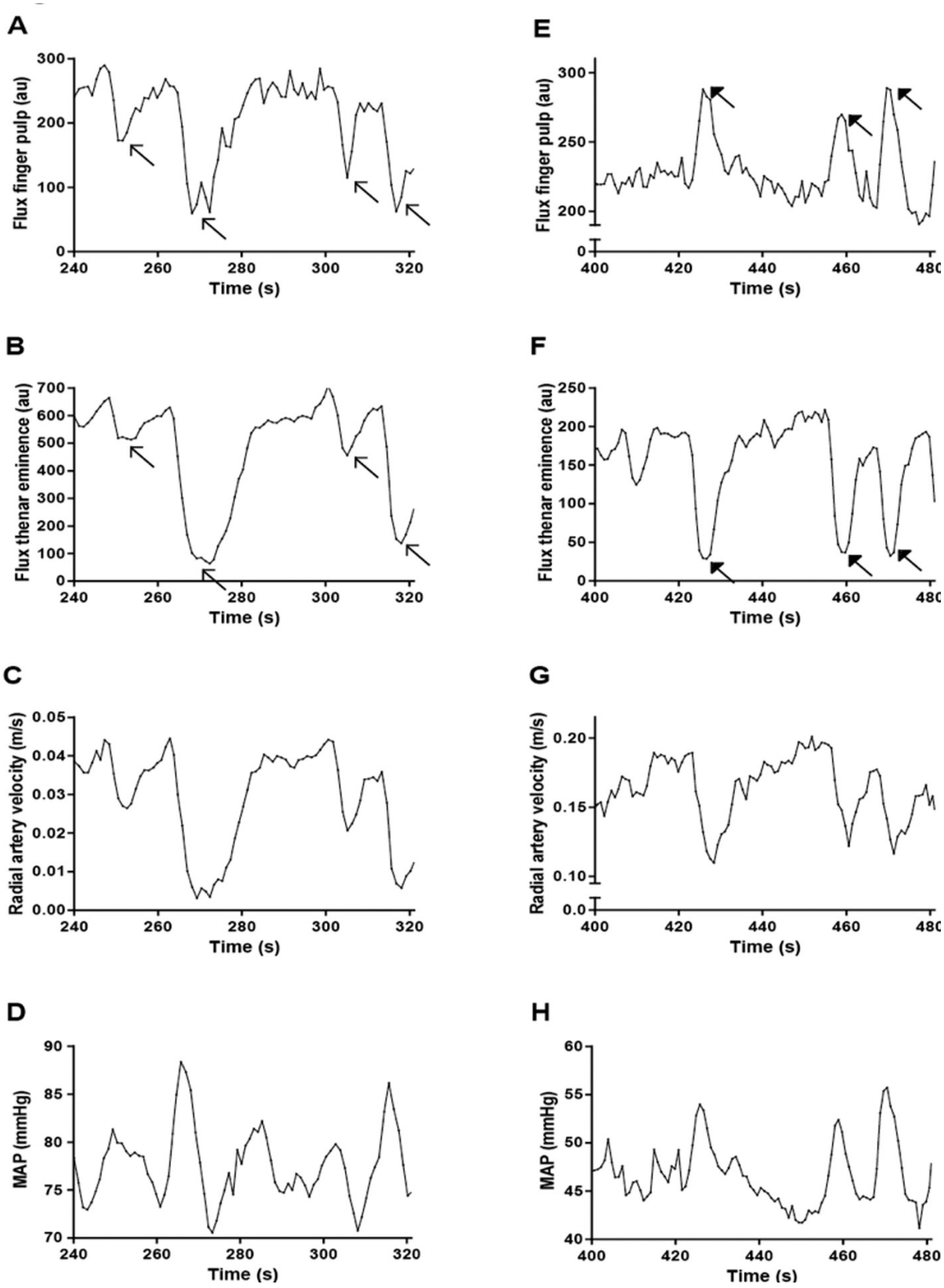

Fig. 1. Simultaneous beat-by-beat averaged finger pulp flux, thenar eminence flux, radial artery blood velocity and MAP from one healthy subject (**A-D**) and one SSc patient (**E-H**). Open arrows indicate synchronous vasoconstrictions of the AVAs in the finger pulp and in thenar eminence in a healthy subject (**A, B**). Closed arrows (**E, F**) indicate AVA dysfunction in a SSc patient with a negative correlation between finger pulp flux and thenar eminence flux (**E, F**) and a positive correlation between finger pulp flux and MAP (**E, H**). Graphs show, from top to bottom, 80 s sections of laser Doppler flux in the finger pulp (**A, E**) and thenar eminence (**B, F**), blood velocity in the radial artery (measured by ultrasound Doppler) (**C, G**) and mean arterial pressure (**D, H**).
LDF: laser Doppler flux, MAP: mean arterial pressure, au: arbitrary unit, AVA: arteriovenous anastomosis, MAP: mean arterial pressure.

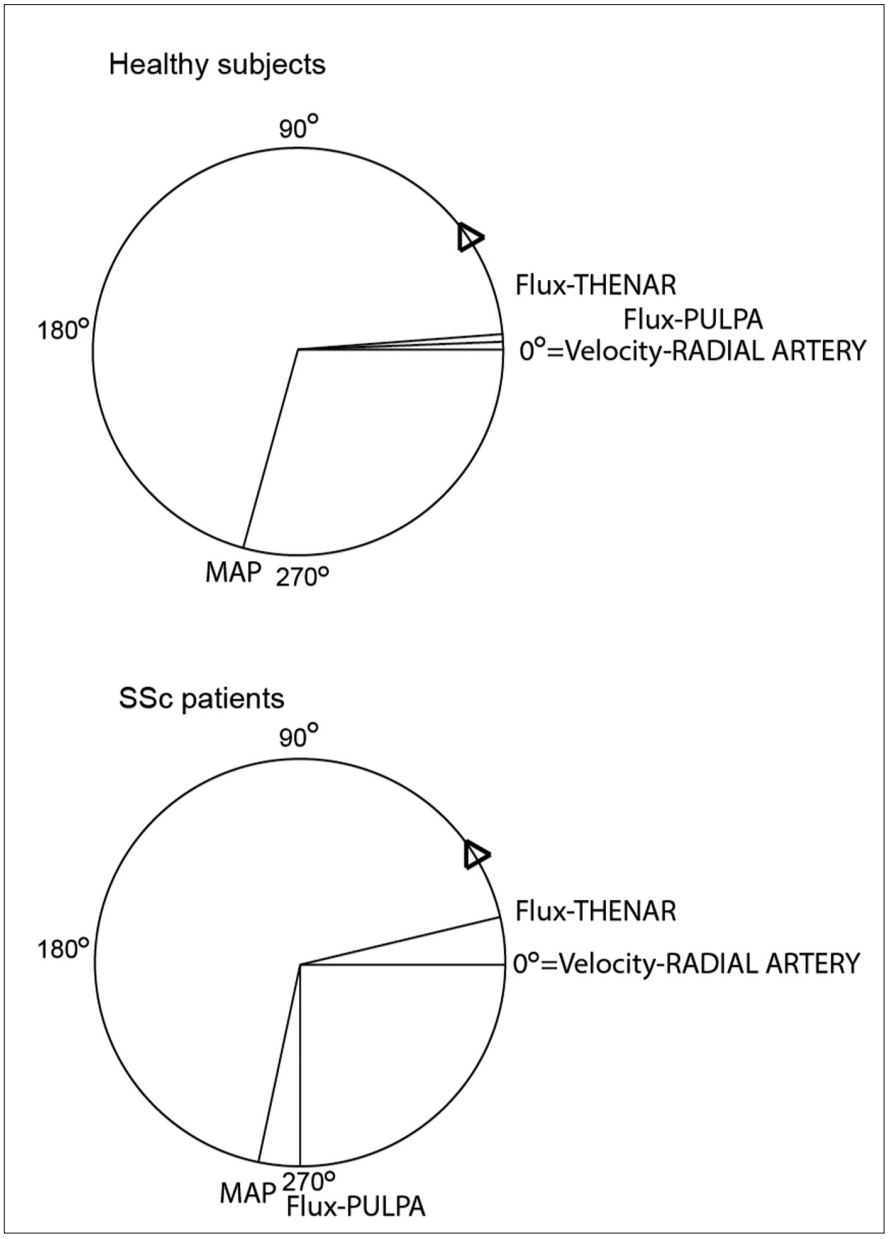

Fig. 2. Phase relationship between corresponding finger pulp flux, thenar eminence flux, radial artery blood velocity and MAP.
In healthy subjects (top figure) finger pulp flux changes coincide with flux changes in thenar eminence and in radial artery blood velocity, while MAP changes are out of phase. In SSc patients (bottom figure) thenar eminence flux changes coincide with radial artery blood velocity changes, while finger pulp flux changes coincide with MAP changes. The arrows: read the circle counterclockwise. Flux-THENAR: laser Doppler flux thenar eminence. Flux-PULPA: laser Doppler flux finger pulp. MAP: mean arterial pressure. Velocity-RADIAL ARTERY: ultrasound Doppler radial artery.

a passive vascular bed in SSc patients, and that blood flow variations in the finger pulp are dependent on blood pressure changes. Dysfunctional AVAs were seen in all the eight patients with a history of DU. Furthermore, none of the patients with normal AVA function had a previous history of DU (n=8) (Fisher's exact test, p=0.017). The study thus shows a statistically significant relationship between a history of DU and AVA dysfunction. In the patients with DU, the median correlation coefficient was negative between finger pulp flux and radial artery blood velocity, r=-0.34 (95% CI -0.62, 0.16) and positive between finger pulp flux and MAP, r=0.37 (95% CI: 0.11, 0.62).

There was no relationship between NVC pattern and AVA dysfunction: early pattern (n=3): 67% AVA dysfunction, active pattern (n=5): 40% AVA dysfunction, late pattern (n=14): 71% AVA dysfunction. There was no relationship between presence of AVA dysfunction and treatment with Nifedipine (Fisher's exact test, p>0.5).

Discussion

We investigated the AVA function in SSc patients. The novel finding in this study is the transformation of the AVAs of the finger pulp from a vascular bed under strong central nervous control in healthy subjects into a passive vascular bed in SSc patients with a history of DU. This was observed as the loss of response to vasoconstrictor nerve impulses, so that blood flow changes reflect MAP changes, in 64% of the SSc patients studied. In contrast, AVAs in the thenar eminence were found to function almost normally. This discrepancy was not demonstrated in the healthy controls. However, the correlation between thenar eminence flux and radial artery velocity fluctuations was statistically significantly lower in the patient group than in the control group, suggesting that there was also some disturbance of AVA functioning also in the skin of the more proximal part of the hand in the SSc patients.

Blood flow in hands of healthy subjects shows large, rapid, rhythmic fluctuations that are closely correlated and are caused by synchronous opening and closing of the AVAs (17, 29, 30). Rosato *et al*. (23) recorded blood flow in all five fingers of one hand in patients with SSc and found that finger blood flow was not correlated in 67% of the subjects. This is in accordance with the findings in the present study and strongly supports that dysfunctional AVAs are present in the hands of SSc patients.

However, the results in the present study further show that blood flow changes in these patients are correlated to short-term variability in MAP. We show that short-term variability in MAP is significantly negatively correlated with velocity fluctuations in the radial artery and finger pulp flux in healthy humans (15). In contrast, the flux variations in the finger pulp of the SSc subjects were positively correlated

with MAP fluctuations, indicating the presence of a passive vascular bed. We believe that the vasoconstrictor ability of AVAs in the skin of all parts of the palm may be disturbed, more severely in the distal parts of the hand, where the density of AVAs is higher. This is in agreement with the clinical finding that fibrosis is more severe in the distal part of the hand in SSc patients (31).

In our study population, 36% of the patients had DU, consistent with findings in the literature (1, 7, 32, 33). AVA dysfunction was observed in all these patients. In contrast, no patients with normal AVA function had DU or a history of DU. Six patients showed dysfunctional AVAs without any history of DU. In patients with dysfunctional AVAs, a steal phenomenon may occur whereby blood is shunted through the paralysed AVAs and bypasses the nutritional capillaries without delivering oxygen to the surrounding tissue. This gives rise to hypoxia and the development of hypoxic DU. However, the background for DU is multifactorial (2, 7). Reduced function of the resistance vessels in the phalanges of the hands may also have impact on hand edema in SSc. We hypothesise that AVA dysfunction is one of several mechanisms involved in the development of DU in SSc patients.

AVA dysfunction was found in patients with all three types of capillaroscopic pattern in the present study. This is in accordance with the results of Rosato *et al*. (23), who found dyshomogenous digital blood flow patterns in subjects with early, active and late NVC patterns. Reduced baseline flow and reduced vasodilatatory capacity in the hands of SSc patients (21, 22, 34) are probably explained by structural abnormalities of the vessels.

The pathological processes in SSc, involving activated immunological mediators, activated fibroblasts and activated endothelial cells, result in various types of tissue destruction such as fibrosis, vasculopathy and autonomic dysfunction. There are differences in anatomy and nervous control between different types of vascular beds, so that the pathological processes may be completely different in the deeper AVAs and in the ordinary arterioles and superficial capillaries. AVA dysfunction may be caused by autonomic dysfunction, which is common in SSc (33) and includes abnormal oesophageal motility (33), gastrointestinal dysfunction, arrhythmia and reduced heart rate variability (35). Destruction of the sympathetic nerves to the AVAs would be compatible with the finding that in SSc patients, the AVAs in the finger pulp are transformed into a passive vascular bed without spontaneous vasomotor activity. A higher frequency of other dysautonomic phenomena such as arrhythmia and prolonged QTc interval are reported in the SSc patients with DU than those without DU (32).

Since the nutritional needs of the skin are very low, skin perfusion depends mainly on the heat balance of the body (36). In order to achieve a thermoneutral situation, a room temperature of 24-29°C is necessary (14, 37). However, previous studies were often conducted in room temperatures below 24ºC (20, 34, 38). Our experience is that subjects are in their lower part of thermoneutral zone at these temperatures, and that finger blood velocity is low and non-fluctuating. In studies of the vasomotor activity of AVAs, it is therefore important to monitor room temperature carefully to ensure that the subjects are in their thermoneutral zone.

In conclusion, we found that in SSc patients with DU there is a dysfunction of the AVAs of the finger pulp. The AVAs of the finger pulp are transformed from a vascular bed under strong central nervous control in healthy subjects into a passive vascular bed in SSc patients. AVA dysfunction may be part of the autonomic dysfunction associated with SSc. Impaired finger blood flow in SSc patients may thus be part of autonomic dysfunction.

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