Non-invasive digital thermal monitoring and flow-mediated dilation in systemic sclerosis

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ABSTRACT

Objective. *The fingers, toes, and tips of the nose and ears have specialised structural and functional features for thermoregulation, and are the most common areas of Raynaud's phenomenon in systemic sclerosis. Digital thermal monitoring (DTM) of vascular reactivity assesses Doppler ultrasound hyperemic, low frequency, blood velocity of radial artery and fingertip vascular function. Flow mediated dilation (FMD) is an indirect measure of endothelial function, perfusion, and vasodilator ability. In this study, we investigated the cross-sectional correlation of FMD and DTM variables to inform an optimised non-invasive study of SSc endothelial function. A student's T-test was used to compare means of DTM across binary variables.*

Methods. *Consented SSc registry patients were included in this analysis. The subjects were prepared for FMD and DTM per standardised guidelines. The SSc clinical features were recorded. Spearman's Rank Correlation was used to assess the strength of a relationship FMD and DTM variables.*

Results. *Thirty-four SSc subjects had FMD and DTM performed on the same day. Relative (0.42, p=<0.02), absolute FMD (0.41, p<0.02), and shear rate (0.32, p<0.07) were weakly, but significantly correlated with the DTM. Reactive hyperaemia (-0.44, p=0.000) was weakly inversely, but significantly related with DTM. Baseline diameter and flow were not significantly related to the DTM.*

Conclusion. *This non-invasive study of SSc endothelial function suggests that macrocirculation (including relative and absolute FMD, shear rate, and peak hyperaemia) and microcirculatory thermoregulation (characterised by DTM) are significantly correlated, thus warrants further prospective study.*

Introduction

Systemic sclerosis (SSc, scleroderma) is an autoimmune connective tissue disease characterised by progressive vasculopathy and fibrosis. The first symptom of systemic sclerosis in most cases is Raynaud's phenomenon (RP), which is a bi- or tri-phasic colour change of the skin in response to cold temperature or stress that is associated with pain and/or tingling (1). The arterial inflow of specific skin areas such as fingers, toes, and tips of the nose and ears are different from other skin areas in that they have specialised structural and functional features for thermoregulation. As such, a critical aspect of management for RP in SSc patients, in addition to the prescription of vasodilators, is educating patients to stay warm (2). In SSc-RP, this cold or stress induced sympathetic vasoconstriction is further amplified throughout the vascular network, including upstream arteries, which undergo vasospasm, arteriovenous anastomoses, and arterioles providing nutritional support to the skin (3). The presence of endothelial dysfunction results in reduced activity of vasodilators, nitric oxide, and prostacyclin, and increased thrombotic and inflammatory activity, which disrupts nutritional capillary blood flow. The maintenance of nutritional capillary blood flow is normally provided by the conduction of vasodilatation to upstream vessels that results from flow-mediated activation of the endothelium (3).

Peripheral arterial tonometry (PAT) technique has been introduced as a method for the measurement of vascular function in SSc, and identified that earliest endothelial changes occur in smaller arterioles and microvascular beds, but not in medium or macrovascular beds (4). The PAT technique compares pulse amplitude at the fingertips

before and after a 5 - min arm - cuff - induced reactive hyperaemia. However, the PAT probe includes a fingertip cuff that obstructs microvasculature at the point of measurement; therefore may not be able to accurately evaluate microvascular reactivity at the fingertip. Digital thermal monitoring (DTM) of vascular reactivity assesses Doppler ultrasound hyperemic, low frequency, blood velocity of radial artery and a fingertip vascular function without fingertip occlusion (5). Similar to the PAT technique, DTM is performed during an arm-cuff occlusion-induced reactive hyperaemia and both tests have modestly correlated with brachial artery reactivity (6). PAT and DTM are automated, portable, easy to perform and less user-dependent than other methodologies for bedside vascular function measurement (6). The advantage of the DTM method is that it measures both cutaneous microvascular and vascular reactivity that result in increased blood flow to the fingers because of reactive hyperaemia. While repeatability of the DTM is excellent (5), there is a limited literature on the clinical use of the DTM technique and it has not been previously reported in SSc.

Flow mediated dilation (FMD) is an indirect measure of endothelial function (brachial artery flow mediated dilation), perfusion (resting forearm blood flow), and vasodilator ability (reactive hyperaemia) and has been demonstrated as a potential early clinical marker of digital ulcer risk. Our group has previously described the role of serial FMD testing with duplex ultrasound to assess in vascular dysfunction in SSc (7, 8). We reported that FMD could be used to assess the therapeutic effect of vasoactive medications and, through the serial quantification of shear stress, possibly predict patients at risk for digital ulceration (8, 9). The use of FMD has been demonstrated to be a reproducible and operator-dependent test for non-invasive measurement of vascular function (5). In this study, we investigated the correlation of FMD and DTM variables in a cross-sectional method to inform an optimised non-invasive study of SSc endothelial function.

Methods

Subjects

Inclusion criteria was a diagnosis of SSc by 2013 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria (10) who were enrolled at the time of their routine care visit to the SSc Clinic at The University of Utah and Salt Lake Veterans Affair Medical Center, USA. The only exclusion criterion was the presence of an active digital ulcer. All participants were enrolled consecutively over a 7.5-month period and had FMD and DTM performed on the same day. The institutional review board of the University of Utah and Salt Lake City VAMC, which serves as the ethics committee, approved all procedures. Written informed consent was obtained prior to participation after an explanation of the nature, benefits, and risks of the study.

Subject characteristics

Clinical features of patients with SSc were recorded for SSc (Table I) and included RP duration, modified Rodnan skin score (mRSS), history of SSc-related vascular complications including pulmonary arterial hypertension, scleroderma renal crisis, and/or DU, antinuclear antibody, SSc-specific antibody status, tobacco use, and cardiovascular-acting medications.

Flow-mediated dilation

Preparation for endothelium-dependent dilation was assessed non-invasively by FMD in accordance of guidelines (11) and previously described by our group (12). In our SSc clinic this procedure is performed by a trained vascular technician after the care visit, thus was a minimum of 30 minutes after the DTM measurement (13). The FMD procedure involves inflating a cuff on the upper forearm to a supra-systolic external pressure for several minutes and measuring change in diameter and blood flow in the brachial artery proximal to the occlusion following rapid deflation of the cuff. The ischaemia-evoked dilation of resistance vessels distal to the occlusion produces a marked temporary increase in blood flow (reactive hyperaemia) in the proximal conduit arteries that can be quantified and, in turn, causes dilation (FMD) of those proximal conduit arteries. Thus, FMD not only assesses the ability of peripheral conduit arteries to dilate in response to the physiological stimulus of increases in intravascular shear (*e.g.* endothelial derived vasodilation), but also the vasodilator ability of the peripheral resistance arteries to a brief bout of ischaemia.

Specifically, a blood pressure cuff was placed on the right arm, distal to the ultrasound Doppler probe on the brachial artery. Simultaneous measurements of brachial artery vessel diameter and blood velocity were performed using a linear array transducer operating in duplex mode, with imaging frequency of 14 MHz and Doppler frequency of 5 MHz (Logic 7, GE Medical Systems, Milwaukee, WI, USA). All measurements were obtained with the probe properly positioned to maintain an insonation angle of ≤60°. The sample volume was maximised according to vessel size based on real-time ultrasound visualisation. The brachial artery was insonated approximately midway between the antecubital and axillary regions, and measurements of diameter and blood velocity were acquired continuously at rest and for 2 minutes after cuff deflation. End-diastolic, ECG R-wave-gated images via video output from the Logic 7 for off-line analysis of brachial artery vasodilation using automated edge-detection software (Medical Imaging Application, Coralville, IA, USA) were collects. FMD was quantified as the maximal change in brachial artery diameter after cuff release, and is described as a percent and absolute increase in diameter from rest. Shear rate was calculated according to the equation: shear rate (s^{-1}) = blood velocity · 8/vessel diameter. Cumulative shear rate (area under the curve, AUC) at the time of peak brachial artery vasodilation was determined using the trapezoidal rule, as described previously (14). To normalise vasodilation to its vasodilators stimulus (shear rate), FMD (percentage) was divided by post-cuff release shear rate AUC until peak dilation occurred and multiplied by 1000 (15).

Table I. Demographics.

Age (years)	58 ± 12
Sex (female)	32 (94%)
Duration of SSc (first non-Raynaud symptom)	10.8 ± 8.0
Limited cutaneous SSc	22 (65%)
Modified Rodman skin score	11 ± 9
White	30 (88%)
History of scleroderma renal crisis	4 (12%)
History of pulmonary artery hypertension	2 (6%)
History of digital tip ulcer	13 (38%)
Smoking	
Never	20 (59%)
In the past	8 (24%)
Current	4 (12%)
Unknown	2 (6%)
Antinuclear antibody positive	34 (100%)
RNA polymerase III antibody positive	9 (26%)
Topoisomerase antibody positive	7 (21%)
Centromere antibody positive	18 (53%
Vasodilator therapy	
• Calcium channel blocker	32 (94%)
• Angiotensin receptor blocker	1 (89%)
• Ace inhibitor	4 (12%)
• Phosphodiesterase Inhibitor	3 (9%)
• Endothelin receptor antagonist	2 (6%)
• Prostacyclin analogue	0

Digital thermal monitoring

All DTM measurements were performed in the morning SSc clinic in a quiet room at a controlled ambient temperature prior to clinic visit. This procedure begins with an automated blood pressure measurement, followed by cuff occlusion of the arm. During the cuff occlusion the fingertip temperature in the hand falls because of the absence of warm circulating blood. Once the cuff is released, blood flow rushes into the forearm and hand, causing a temperature rebound in the fingertip, which is directly proportional to the vascular reactivity. The captured temperature signals reflect blood flow changes in both skin and subcutaneous tissues and is not affected by finger motion. A single measure is reported by this automated system. This outcome, vascular reactivity index, is reported herein.

Specifically, DTM of both hands was obtained during 5 min stabilisation, 5 min cuff inflation to 50 mmHg greater than systolic blood pressure, and 5 min deflation using an automated, operator-independent protocol (VENDYS, Endothelix Inc., Houston, TX, USA). Thermal changes during a 5 min arm-cuff induced reactive hyperaemia test were monitored continuously in the fingertip of both the occluded and non-occluded arms using VENDYS software. The device contains of a computer based thermometry system (0.006°C thermal resolution) with two fingertip RTD (Resistance Temperature Detector) fast response probes designed to minimise the skin-probe contact area and fingertip pressure, attached to the pulp of the index finger on both hands. The system includes a common automated sphygmomanometer cuff, cuff-inflation pump, and release valve to permit non-invasive measurement of arterial pressure and the control of occlusive hyperaemia. Dual channel temperature data were simultaneously recorded at a 1 Hz sampling rate. Upon occlusion, as blood flow ceases, the finger skin temperature decays exponentially from its starting temperature toward the lower temperature of the adjacent ambient air. Upon release of occlusion, the original blood flow returns to the finger plus the additional volume of blood corresponding to the measure of reactivity. Temperature rebound is defined as temperature prior to cuff inflation subtracted from temperature maximum after cuff relief. Temperature rebound area under the curve is provided as a single value of digital thermal measurement, which is the vascular reactivity index (VRI) (16).

Statistics

This a cross-sectional analysis of patients seen on a single day at the SSc Clinic that had both FMD and DTM performed September 2018 through April 2019. Statistics were performed using Stata, v. 15.1. The DTM was left skewed and one outlier was omitted from analysis as the baseline value required active hand warming in order to activate the system. Therefore, the Spearman's Rank Correlation was used to assess the strength of a relationship FMD and DTM variables (34 observations). Significance was set at $p<0.02$. The difference in vascular reactivity index between those with and without a history of SSc, related complications, medication use and smoking status was examined using a student's t-test. Means and standard deviation along with *p*-values are shown.

Results

Sociodemographic

The clinical features of the 34 SSc patients that had FMD and DTM performed on the same day as their clinical care visit are described in Table I. Our population was mostly middle-aged, mean age 58 years. All but two of the participants were women. The average disease duration was nearly 11 years (10.8±8). About two-thirds had limited cutaneous SSc (n=22), the remainder had diffuse cutaneous SSc (n=12). The average modified Rodnan skin score (mRSS) was 11±9. The majority were white (n=30). A history of scleroderma renal crisis (SRC) and pulmonary artery hypertension (PAH) were rare (n=4 and n=2, respectively). Approximately a third (n=13) had a history of digital tip ulcer, but none were active during testing per exclusion criteria. Two-thirds were never smokers and 4 were current smokers. All were ANA positive, and 24 were scleroderma-specific antibody positive. All SSc patients were all on vasodilators at the time of testing; with a calcium channel blocker for the indication of Raynaud's phenomenon the most commonly prescribed medication.

Relationship of vascular reactive index with demographic and clinical variables

Although vascular reactivity index (VRI) decreased with age, higher

mRSS, and duration of SSc, the associations were not statistically significant (p>0.08) (Table II). Differences in VRI across binary clinical characteristic variables are also shown in Table II, and none were clinically significant. We examined whether vascular reactivity index was different among those with and without a history of SSc related complications, smoking status (never versus past or current), use of calcium channel blockers and gastrointestinal medication use. There were no significant differences between VRI in those with or without a history of smoking. Likewise, there were no differences those with SSc related complications (SRC, PAH, or interstitial lung disease) and those without. All patients were on vasodilator therapy, but there was no difference in VRI between those who were taking a calcium channel blocker, and those who were taking another type of vasodilator.

Relationship of flow mediated dilation measures with vascular reactivity index

Of all FMD parameters obtained, only baseline diameter and flow were not significantly correlated with VRI (Table III). Relative (0.42, p=<0.02), absolute FMD (0.41, p<0.02), baseline flow (0.37, p<0.04) and shear rate (0.32, p=<0.07) were weakly, but significantly correlated with the DTM (Table III). Reactive hyperaemia (-0.44, p=0.009) was weakly inversely, but significantly related with the VRI. There were no complications observed related to the testing.

Table II. Vascular reactivity index with clinical and demographic characteristics.

Variable	Correlation Coefficient or Mean ± SD	p-value
Age*	Spearman's rho = -.32	p=0.08
Modified Rodnan Skin score*	Spearman's rho =-0.06	p=0.7
Duration of SSc (first non-Raynaud symptom)*	Spearman's rho =-0.25	p=0.15
History of scleroderma renal crisis**		
Yes	0.58 ± 0.46	
No	0.88 ± 0.54	p=0.31
Current scleroderma renal crisis**		
Yes	0.59 ± 0.46	
No	0.90 ± 0.56	p=0.31
History of pulmonary artery hypertension**		
Yes	0.75 ± 0.48	
No	0.85 ± 0.54	p=0.80
Current pulmonary artery hypertension**		
Yes	0.70 ± 0.46	
No	0.95 ± 0.56	p=0.34
History of digital tip ulcer**		p=0.41
Yes	0.74 ± 0.58	
No	0.91 ± 0.50,	
Current ILD**		p=0.96
Yes	0.86 ± 0.62	
No	0.85 ± 0.43,	
Smoking**		p=0.59
Never	0.78 ± 0.56	
In the past or current	0.88 ± 0.52	
Vasodilator therapy**		p=0.52
Calcium Channel Blockers	0.73 ± 0.48	
Other	0.86 ± 0.56	
Gastrointestinal medications**	0.74 ± 0.44	
	0.89 ± 0.61	p=0.42

*Spearman's Rho; ** mean ± standard deviation.

Table III. Spearman correlation of flow mediated dilation measures with digital thermal measurement of vascular reactivity index.

Flow Mediated Dilation Variable	Vascular reactivity index	p-value
Baseline Flow*	0.37	<0.04
Baseline diameter	0.13	0.27
Normalised relative FMD	0.42	<0.02
Normalised absolute FMD	0.41	<0.02
Peak hyperaemia	-0.44	<0.009
Shear rate	0.32	<0.07

*n=33 one missing value.

Discussion

The most common manifestation of vasculopathy in SSc is RP. Thus, understanding this aspect of disease is imperative. Cold temperature clearly induces RP, which in its most severe form can result in DU. As such, understanding how to quantify vasculopathy related to the response to temperature by DTM could be an important research tool. In this study, we present promising data on the correlation of the vascular reactivity index generated by automated DTM to our established, previously reported vasculopathy clinical research tool, FMD.

The significance of this project is that personalised endothelium-guided therapy for SSc patients mandates an understanding of vascular dysfunction. Both FMD and DTM vascular investigations use ischaemia induced hyperaemia to assess vasodilation. The ischaemia-evoked dilation of resistance vessels distal to the occlusion produces a marked temporary increase in blood flow (reactive hyperaemia) in the proximal conduit arteries that can be quantified, and that subsequently causes dilation (FMD) of those proximal conduit arteries. While FMD assess vessel size and flow, DTM specifically evaluates the temperature of the skin (cutaneous microcirculation) in response to an ischaemia-evoked response. Since DTM is fully automated, it is important to

assess if DTM and FMD correlate, as it would be more feasible to use DTM, which reports a single value of VRI, for serial bedside testing in a multi-centre study.

In this present study, FMD reactive hyperaemia was inversely associated with DTM as would be expected. This investigation supports our previous work in the utility of FMD to assess SSc vasculopathy (8), however this report we identify that baseline artery diameter and blood flow do not significantly correlate to microcirculatory cutaneous change. This data further supports the concept that the skin areas with specialised structural and functional features for thermoregulation warrant further characterisation in SSc. Our results suggests the FMD and DTM are complimentary in studying vasculopathy.

The goal of DU management is to improve perfusion to the affected digit to facilitate healing. The significance of our study is that the data presented herein suggests vasodilators that target vascular smooth muscle may not influence cutaneous blood flow. As such, DU treatment and prevention may require both vasodilators as well as therapies that can influence cutaneous perfusion. This is an important observation since clinical algorithms for RP and DU management in SSc do not specifically address this aspect of care (2, 17). DTM and FMD for functional vasculopathy assessment may be particularly valuable in very early systemic sclerosis (VEDOSS) population where quantification of functional vasculopathy prior to DU development is imperative (18).

Our study is not without limitations. It is a single centre, cross-sectional study of a limited number of patients. The pretest preparation was the main limitation for recruitment. We did not test patients with active digital ulcers due to the concern of inducing two bouts of ischaemia in these patients during the testing. As such, differences in vascular measures between those with and without an active DU cannot be made. Our non-significant reported relationships may be related to small sample size. Additionally, chronological use of FMD and DTM, and correlation to vascular outcomes is an important future study for understanding the predictive value of these tests. Nonetheless, our reported findings are important for further clarifying the challenge of defining SSc vasculopathy, and the value of a multi-system approach.

Vascular reactivity is a vital component of vascular function that enables the circulatory system to respond to physiologic and pharmacologic stimuli that require adjustments of blood flow and alterations of vessel tone and diameter. Thus, defining vasoconstrictive and vasodilative responses at both the macrovascular and microvascular cutaneous levels is important in SSc. The optimised digital ulcer prevention and treatment strategy may require both a focus on macrocirculation (including understanding the shear rate capture by FMD) and microcirculatory thermoregulation (characterised by DTM). However, their concurrent use as investigative tools of vasculopathy is limited by subject preparation and testing time, thus may be best achieved in the research rather than clinical setting.

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