Use of Laser Speckle Contrast Imaging to Assess Digital Microvascular Function in Primary Raynaud Phenomenon and Systemic Sclerosis: A Comparison Using the Raynaud Condition Score Diary

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ABSTRACT. ***Objective.*** Evaluate objective assessment of digital microvascular function using laser speckle contrast imaging (LSCI) in a cross-sectional study of patients with primary Raynaud phenomenon (RP) and systemic sclerosis (SSc), comparing LSCI with both infrared thermography (IRT) and subjective assessment using the Raynaud Condition Score (RCS) diary.

Methods. Patients with SSc (n = 25) and primary RP (n = 18) underwent simultaneous assessment of digital perfusion using LSCI and IRT with a cold challenge on 2 occasions, 2 weeks apart. The RCS diary was completed between assessments. The relationship between objective and subjective assessments of RP was evaluated. Reproducibility of LSCI/IRT was assessed, along with differences between primary RP and SSc, and the effect of sex.

Results. There was moderate-to-good correlation between LSCI and IRT (Spearman rho 0.58–0.84, $p < 0.01$), but poor correlation between objective assessments and the RCS diary ($p > 0.05$ for all analyses). Reproducibility of IRT and LSCI was moderate at baseline (ICC 0.51–0.63) and immediately following cold challenge (ICC 0.56–0.86), but lower during reperfusion (ICC 0.3–0.7). Neither subjective nor objective assessments differentiated between primary RP and SSc. Men reported lower median daily frequency of RP attacks (0.82 vs 1.93, $p = 0.03$). Perfusion using LSCI/IRT was higher in men for the majority of assessments.

Conclusion. Objective and subjective methods provide differing information on microvascular function in RP. There is good convergent validity of LSCI with IRT and acceptable reproducibility of both modalities. Neither subjective nor objective assessments could differentiate between primary RP and SSc. Influence of sex on subjective and objective assessment of RP warrants further evaluation.
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The episodic nature of Raynaud phenomenon (RP) precludes objective assessment in the clinical setting, leading to reliance on the patient self-report. The Raynaud Condition Score (RCS) diary collects daily information on the frequency, duration, and severity of RP attacks over a 2-week period[1,2,3,4,5]. Self-report assessment of RP is subjective, influenced by health beliefs and psychological factors. The RCS necessitates prolonged assessment and can be laborious (with potential for "diary fatigue"). Further, the frequency, duration, and severity of RP attacks might be influenced by seasonal variation and the effectiveness of coping strategies adopted by patients to avoid conditions responsible for RP attacks and ameliorate attacks when they occur.

Objective methods for quantifying digital microvascular function, such as infrared thermography (IRT), overcome certain limitations of self-report and have been used to differentiate between disease states and assess therapeutic response in RP and systemic sclerosis (SSc)[6,7]. Laser speckle contrast imaging (LSCI) is an emerging noninvasive microvascular imaging modality providing real-time dynamic assessment of perfusion over large areas of tissue[8,9,10]. Several works have evaluated LSCI in primary RP and SSc with conflicting results[11,12].

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We previously reported good correlation between LSCI and IRT, excellent reproducibility of LSCI and IRT, and the potential capacity of LSCI to identify differences in perfusion between glabrous (densely populated with arteriovenous anastomoses) and non-glabrous regions of the digits in healthy controls[13]. The specific objective of our study was to assess the correlation between subjective (RCS diary) and objective (IRT and LSCI) assessments of digital vascular function in primary RP and SSc. We reported the reproducibility of LSCI and IRT, and evaluated the effect of disease state and sex on subjective and objective assessment of RP.

MATERIALS AND METHODS

Study population. Patients with SSc fulfilled either the American Rheumatology Association and/or the LeRoy and Medsger classification criteria for early SSc[14,15]. Primary RP was defined as at least 2 episodes of fingertip localized notable blue and/or sequential white/blue discoloration, in conjunction with pain upon cold exposure or emotional stress within 1 week of examination and negative antinuclear autoantibody reactivity using immunofluorescence on HEp-2 cell substrate and serum diluted to > 1:160. Exclusion criteria included pregnancy/breastfeeding, surgical sympathectomy within 12 months, or new medication for the treatment of RP within the preceding 2 months. Vasodilators were maintained at a constant dose. All participants provided informed written consent in accordance with the Declaration of Helsinki. The study was approved by the South West 3 Research Ethics Committee.

Study design. Participants attended on 2 occasions, 2 weeks apart for microvascular imaging. At visit 1, participants were taught how to complete a 2-week RCS diary, which they returned at visit 2.

Microvascular imaging protocol. At each visit, participants underwent a standardized cold challenge identical to that described in an earlier study undertaken in healthy controls[13]. In brief, following acclimatization for 20 min at 23°C (± 0.5°C), baseline imaging using IRT/LSCI was undertaken of the dorsal aspect of the right hand and volar aspect of the left hand. Participants submerged both hands (in gloves to avoid evaporative cooling) to the level of the radiocarpal joints into a water-bath cooled to 15°C (± 0.1°C) for 60 s. IRT and LSCI images were taken of both hands immediately following cold challenge, and at 13-s intervals for 15 min.

Microvascular imaging equipment. IRT images were obtained using a Thermovision camera (FLIR systems). All images were processed using the commercially available CTHERM software (Version 2.3; University of Glamorgan). The LSCI camera (Moor Instruments FLPI) was placed 30 cm (± 2 cm) from the hands at an angle of 30° (± 2.5°), and image analysis was undertaken using the moorFLPI Imager software (version 2.0; Moor Instruments). The time constant and exposure time were set at 1.0 s and 8.3 ms, respectively.

Microvascular image analysis. Perfusion should be strictly defined as volume per unit area per unit time; however, because no laser instrument was capable of directly measuring blood flow, measurements derived from laser imaging tools (e.g., Doppler and flowmetry) were typically described in arbitrary flux units.

In LSCI, digital imaging and processing allowed real-time quantification of time-varying speckle contrast with the generation of a false color map of speckle contrast. Speckle contrast was quantified by calculating the ratio of the SD to the mean of the intensities recorded for each pixel within delineated squares (e.g., 5 × 5 or 7 × 7 pixels)[9]. The values themselves were influenced by many factors including time-constant, exposure gain, laser wavelength, etc., hence the importance of observing a standardized approach to assessment.

Perfusion was calculated over 3 predefined regions of interest (ROI) as previously described[13]: the dorsal aspect of right middle fingertip (ROI 1), dorsal aspect of the middle phalanx of the right middle finger (ROI 2), and the palmar aspect of left middle fingertip (ROI 3). Figure 1 is an example of IRT and LSCI images demonstrating ROI. Mean perfusion [skin temperature (°C) using IRT and arbitrary flux values (fu) obtained using LSCI] at each ROI was calculated during baseline assessment, immediately following cold challenge (t0) and at 5, 10, and 15 min following cold challenge (t5, t10, and t15, respectively).

Statistical analysis. Data are presented as median values [and interquartile range (IQR)] unless otherwise stated. Correlation between continuous data was assessed using Spearman rank correlation coefficient (r_s). Between-group comparisons of unpaired data were undertaken using the Mann-Whitney U test. Comparison of paired data (e.g., comparison between different ROI for both IRT and LSCI) was undertaken using the Wilcoxon signed-rank test. Reproducibility (between assessments 1 and 2) was assessed using ICC[16]. All data were analyzed using SPSS version 18.0. A p value of < 0.05 was considered significant.

RESULTS

Patient demographics. Twenty-five subjects (5 men) with SSc and 18 (4 men) with primary RP were enrolled. Detailed demographics of the cohort are presented in Table 1. Patients with primary RP were younger (51.7 vs 59.2 yrs, $p = 0.03$), had a lower age at RP onset (20 vs 35 yrs, $p = 0.02$), and lower use of angiotensin-converting enzyme inhibitors (0% vs 28%, $p = 0.03$) compared with SSc. There were no differences in sex, vasodilatory therapy use, or smoking history between groups. The majority of patients with SSc had limited cutaneous SSc (22/25, 88%).

Missing data. Two patients (1 with primary RP and 1 with SSc) did not attend the second assessment. Data for ROI 3 were unavailable for 1 subject with primary RP (shoulder discomfort prevented forearm supination). Data were unavailable for ROI 1 for a subject with SSc because of previous amputation. Four subjects (9.3%) did not adequately complete the RCS diary (3 SSc, 1 primary RP).

Correlation between subjective (RCS diary) and objective (IRT and LSCI) assessments of digital vascular function. Descriptive data for both IRT and LSCI for each ROI at baseline and following cold challenge are presented in Table 2. Using pooled data from primary RP and SSc, there was moderate to excellent correlation between assessments of digital vascular function using IRT and LSCI at all ROI at baseline and at all timepoints following cold challenge (r_s 0.58–0.84, $p < 0.01$, Table 3A). The lowest correlations were identified immediately post-cold challenge (r_s 0.58–0.65 at t0). In contrast, no correlation existed between any of the variables of the RCS score diary and noninvasive microvascular imaging assessment using either LSCI or IRT assessment at any ROI, at baseline, and/or following cold challenge (Table 3B).

Reproducibility of LSCI and IRT. Reproducibility of both IRT and LSCI was moderate to excellent, with the majority of ICC values between 0.55 and 0.70 (Table 4). Reproducibility of LSCI was comparable, if not superior, with IRT. The highest ICC values were found immediately following cold

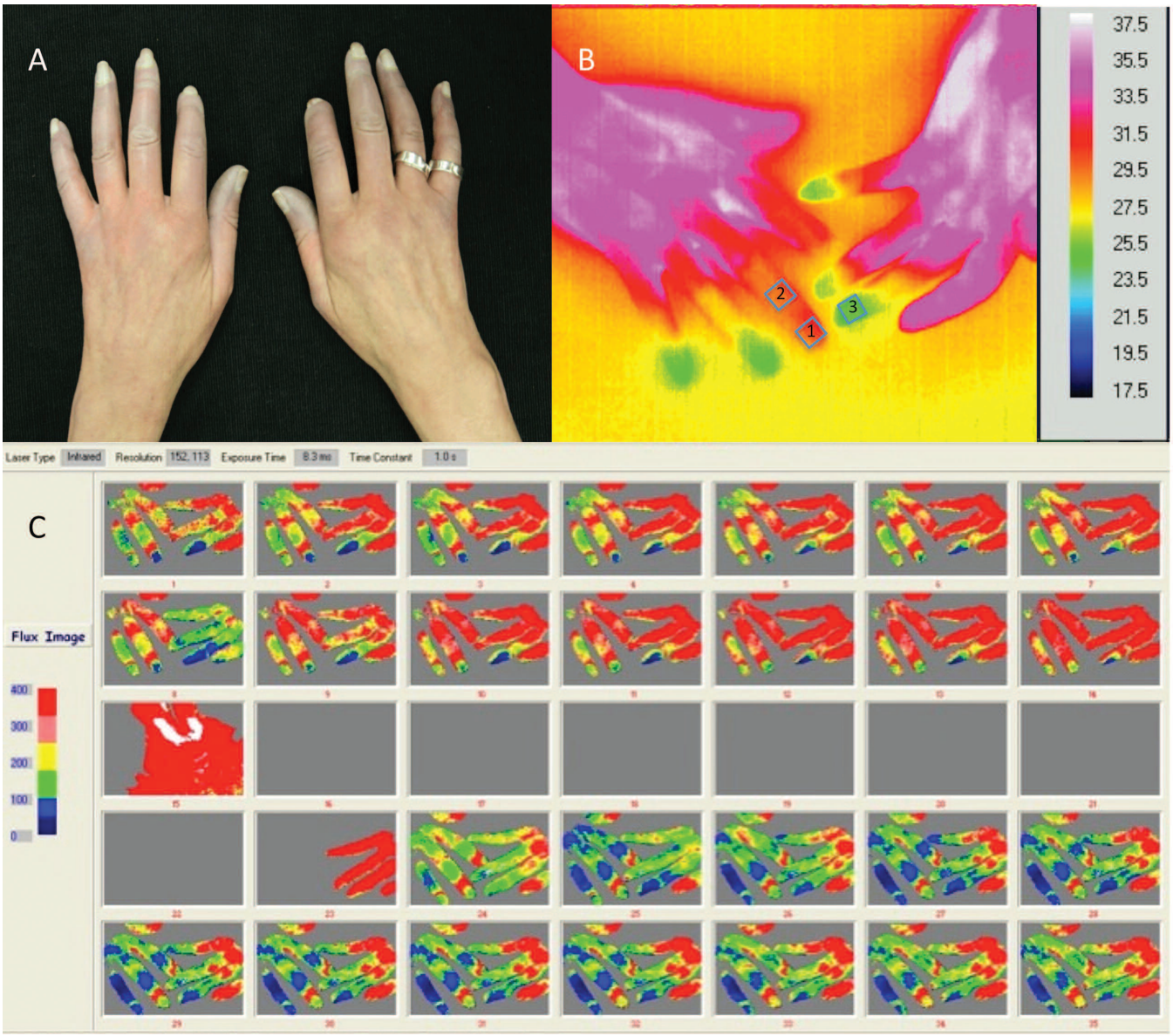

Figure 1. Objective assessment of digital vascular function in a patient with SSc complicated by recurrent digital ulceration. A. A photograph demonstrating the visual appearances of the hands on arrival for assessment with evidence of digital cyanosis and shortening of index fingers because of previous necrotic episodes. B. An IRT image of baseline assessment of digital vascular function with the 3 ROI highlighted. C. LSCI images at 13-s intervals during baseline and following cold challenge (the hands disappearing from view during cold challenge). SSc: systemic sclerosis; IRT: infrared thermography; ROI: region of interest; LSCI: laser speckle contrast imaging.

challenge using LSCI at ROI 1 and 3 (0.86 and 0.79, respectively), suggesting reproducible nadirs of digital perfusion following cold challenge (irrespective of other factors that might influence vascular function). The lowest ICC values were recorded for LSCI at 10 and 15 min post–cold challenge (e.g., 0.30 for ROI 2 at 15 min and 0.34 for ROI 1 at 10 min), suggesting greater variation in digital vascular responses during re-warming in comparison with more stable perfusion at baseline.

Differences between primary RP and SSc. There were no significant differences between SSc and primary RP for the mean daily RCS score (median 1.9 vs 2.0, p = 0.87), the mean daily duration of RP attacks (23.9 vs 22.1 min, p = 0.9), or the mean daily frequency of RP attacks (2.0 vs 1.4 attacks, p = 0.47). Similarly, neither LSCI nor IRT allowed differentiation between primary RP and SSc using the endpoints chosen for analysis.

Effect of sex on subjective and objective assessment of digital microvascular function. Men reported a lower frequency of RP attacks than women (median daily frequency 0.8 vs 1.9,

Table 1. Baseline demographics of patients. Values are median (IQR) unless otherwise specified.

Characteristics	Primary RP, n = 18	SSc, n = 25	p
Age, yrs	51.7 (19.3)	59.2 (19.8)	**0.03**
Age at RP onset, yrs	20 (24)	35 (19)	**0.02**
Age at diagnosis, yrs	43 (6)	45 (19)	0.61
Male, n (%)	4 (22.2)	5 (20)	1.00
Female, n (%)	14 (77.8)	20 (80)	1.00
Vasodilatory therapy			
Any, n (%)	5 (27.8)	12 (48)	0.22
Calcium antagonist	3 (16.7)	6 (24)	0.71
ACEi	0 (0)	7 (28)	**0.03**
SSRI	2 (11.1)	1 (4)	0.56
ERA	0 (0)	2 (8)	0.50
Smoking history, n (%)			
Never	10 (55.6)	13 (52)	1.00
Ex	7 (38.9)	9 (36)	1.00
Current	1 (5.5)	3 (12)	0.63
Season enrolled, n (%)			
Autumn/winter	10 (55.6)	13 (52)	1.00
Spring/summer	8 (44.4)	12 (48)	1.00

Significant data are in bold face. IQR: interquartile range; RP: Raynaud phenomenon; SSc: systemic sclerosis; ACEi: angiotensin-converting enzyme inhibitor; SSRI: selective serotonin reuptake inhibitors; ERA: enthesitis-related arthritis.

Table 2. IRT and LSCI data for patients with SSc and primary RP at assessment 1. All values expressed as median (IQR).

Variables	ROI	B	t0	t5	t10	t15
IRT, °C						
SSc						
	1	29.8 (5.5)*	21.5 (2.6)†	23.7 (7.1)*	25.4 (9.2)*	27 (9.4)*
	2	30.8 (7.3)	21.4 (2.9)	24.4 (7.9)	25 (9.7)	26.7 (11.1)
	3	28.8 (6.4)	20.6 (3.0)*	22.4 (6.2)*	24 (8.8)*	25.9 (9.4)
Primary RP	1	31.4 (8.0)†	21.1 (3.2)†	25.1 (9.5) *†	31.4 (11)	32.4 (10.8)
	2	30.8 (6.6)	21.5 (3.5)	25.2 (7.2)	30.4 (10.5)	31.7 (10.6)
	3	29.5 (6.7)	20.7 (3.1)*	23.5 (9.6)	30.5 (10.5)	32 (10)
LSCI, fu						
SSc	1	364 (299)†	188 (177)*	205 (282)*	243 (257)†	298 (288)†
	2	235 (306)**	79 (84)	100 (269)	132 (391)	128 (364)
	3	632 (757)*	213 (424)*	320 (642)*	422 (955)*	479 (770)*
Primary RP	1	372 (378)*	179 (128)*	274 (346)*	452 (430)*	488 (446)*
	2	124 (93)	81 (61)	81 (81)	104 (161)	120 (128)
	3	536 (483)*	202 (135)*	261 (577)*	484 (604)*	487 (672)*

* $p < 0.05$ vs ROI 2. † $p < 0.05$ vs ROI 3. ** $p = 0.007$ vs primary RP. IRT: infrared thermography; LSCI: laser speckle contrast imaging; SSc: systemic sclerosis; RP: Raynaud phenomenon; IQR: interquartile range; ROI: region of interest; B: baseline assessment at 23°C; t0: assessments immediately following cold challenge; t5: perfusion 5 min post cold challenge; t10: perfusion 10 min post cold challenge; t15: perfusion 15 min post cold challenge; fu: flux unit values.

p = 0.031) with trends for lower median daily duration of RP attacks (13.8 vs 26.1 min, p = 0.33) and RCS score (1.1 vs 2.0, p = 0.38) in men. Digital perfusion assessed using IRT and LSCI was significantly higher in men for the majority of assessments at each ROI, at both baseline and during re-warming (data not reported). While failing to achieve statistical significance, strong trends were typically present [e.g., at ROI 3 at baseline (median perfusion 758.2 vs 491.6, p = 0.065), and similar trends were observed for ROI 1 at t5, t10, and t15 (p values between 0.07 and 0.13)].

DISCUSSION

To our knowledge, this is the first study to simultaneously evaluate subjective (RCS diary) and objective assessment (LSCI and IRT) of digital vascular function in primary RP and SSc. There was close agreement between IRT and LSCI in the dynamic assessment of digital vascular function in RP and SSc, but poor correlation between these techniques and the RCS diary. We have demonstrated moderate reproducibility of both LSCI and IRT over 2 weeks, although ICC were lower than we previously reported in healthy controls,

Table 3. Correlation between objective and subjective assessment of RP at baseline and following cold challenge for both primary RP and SSc. Values are r_s.

	B	t0	t5	t10	t15
Correlation between IRT and LSCI. $p < 0.01$ for all comparisons.					
ROI 1	0.73	0.62	0.81	0.84	0.75
ROI 2	0.70	0.58	0.84	0.83	0.81
ROI 3	0.81	0.65	0.82	0.82	0.83
Correlation between the RCS diary and LSCI assessment at ROI. 1 $p > 0.05$ for all comparisons.					
Duration of RP attacks	0.05	–0.20	0.01	0.08	0.07
Frequency of RP attacks	0.02	–0.19	–0.08	0.02	0.02
RCS	–0.02	–0.01	0.01	0.00	0.00

RP: Raynaud phenomenon; SSc: systemic sclerosis; IRT: infrared thermography; LSCI: laser speckle contrast imaging; ROI: region of interest; B: baseline assessment at 23°C; t0: assessments immediately following cold challenge; t5: perfusion 5 min post cold challenge; t10: perfusion 10 min post cold challenge; t15: perfusion 15 min post cold challenge; RCS: Raynaud Condition Score.

Table 4. Reproducibility of IRT and LSCI for each ROI at baseline and following cold challenge for both primary RP and SSc. Values are ICC (95% CI). N = 41, unless stated otherwise.

ROI	Camera	B	t0	t5	t10	t15
1	IRT*	0.51 (0.24–0.71)	0.61 (0.36–0.78)	0.52 (0.25–0.72)	0.5 (0.23–0.7)	0.54 (0.28–0.73)
	LSCI	0.62 (0.39–0.78)	0.86 (0.75–0.92)	0.52 (0.25–0.71)	0.34 (0.04–0.58)	0.47 (0.19–0.68)
2	IRT*	0.51 (0.24–0.71)	0.56 (0.3–0.75)	0.54 (0.28–0.73)	0.54 (0.28–0.73)	0.55 (0.28–0.73)
	LSCI	0.63 (0.4–0.78)	0.59 (0.33–0.76)	0.65 (0.43–0.79)	0.54 (0.28–0.73)	0.30 (0–0.55)
3	IRT**	0.59 (0.33–0.76)	0.6 (0.35–0.77)	0.55 (0.28–0.73)	0.63 (0.4–0.79)	0.70 (0.5–0.83)
	LSCI*	0.55 (0.29–0.73)	0.79 (0.63–0.89)	0.5 (0.22–0.7)	0.42 (0.13–0.65)	0.55 (0.29–0.73)

* n = 40. ** n = 39. IRT: infrared thermography; LSCI: laser speckle contrast imaging; ROI: region of interest; RP: Raynaud phenomenon; SSc: systemic sclerosis; B: baseline assessment at 23°C; t0: assessments immediately following cold challenge; t5: perfusion 5 min post cold challenge; t10: perfusion 10 min post cold challenge; t15: perfusion 15 min post cold challenge.

possibly because of a shorter interval between assessments (median 8 days, IQR 7–15) in the previous work. Reproducibility of LSCI assessment in SSc has been evaluated in 2 previous studies[7,11]. Murray, *et al* reported poor reproducibility of LSCI (ICC 0.15), although repeatability was only assessed in a relatively small number of subjects (n = 5)[7]. In contrast, Ruaro, *et al* demonstrated excellent reproducibility following a second LSCI assessment within an hour of the initial assessment with an ICC of 0.95[11]. We noted that reproducibility of LSCI was lower during reperfusion (t5 and t10) than at baseline, but this does not negate the value of the cold challenge. Additional work to refine LSCI endpoints is required and might improve reproducibility before and after cold challenge. The cold challenge (while imperfect in its attempts to recreate the conditions responsible for RP attacks *in vivo*) does provide information on dynamic microvascular function that baseline assessment alone cannot provide. We would, therefore, advocate retaining a provocation test (such as the cold challenge or postocclusive reactive hyperemia studies) in future studies of LSCI (and other laser-derived techniques such as single point laser Doppler flowmetry) until studies have rendered their contribution redundant. At this stage, LSCI is primarily a research tool and is not yet warranted for routine use in clinical practice. Further validation studies are required to establish how best LSCI assessment might be applied in the assessment of RP.

The RCS diary variables lacked the capacity to differentiate between primary RP and SSc, despite a strongly held conviction that the degree of vascular dysfunction in SSc is significantly greater than in primary RP. The effect of habituation and psychosocial factors on self-report assessment of RP, particularly in the context of SSc, may account for this finding. We were also unable to differentiate between primary RP and SSc using either LSCI or IRT with the ROI chosen in our study. Murray, *et al* identified significant differences between primary RP and SSc using IRT (mean temperature across dorsal aspect of all 8 fingers), but not LSCI (perfusion at nailfold of nondominant ring finger)[7]. Ruaro, *et al* used LSCI to identify lower perfusion of the volar aspect of the fingertips in SSc compared with healthy controls[11]. In contrast, Della Rossa, *et al* reported higher basal perfusion

of the dorsal digits in SSc compared with healthy controls and primary RP, possibly because of a large number of patients with SSc receiving vasodilatory therapy (which included a regime of monthly IV iloprost)[12]. A more pronounced microvascular response to cold exposure and delayed recovery following cold challenge was demonstrated in patients with SSc compared with primary RP and healthy controls[12]. We identified higher perfusion in more proximal regions of the dorsal digits (ROI 2) at baseline in SSc compared with primary RP. This finding may account for the high dorsal digital perfusion identified in the study by Della Rossa, *et al*[12]. Future studies should attempt to refine LSCI protocols and variables to enhance the capacity to differentiate between primary RP and SSc. The evaluation of rewarming curve characteristics, longitudinal flux gradients, or the use of composite scores derived from the simultaneous assessment of multiple digits may achieve this goal, as has been successfully applied using IRT[17,18]. We did not undertake nailfold capillaroscopic (NC) studies as part of this work, and additional work exploring the relationship between NC abnormalities in SSc and digital vascular perfusion assessed using IRT and LSCI is needed.

The lower burden of RP symptoms in men, accompanied by higher digital perfusion on assessment using IRT and LSCI, was of interest, and additional work to explore the clinical significance of this apparent trend is warranted should these findings be replicated in larger studies powered to detect such associations.

Improved objective methods for assessing peripheral microvascular function in RP will aid early disease classification and might overcome limitations of subjective assessment of RP. LSCI is a novel, safe, and reproducible noninvasive technique for assessing digital microvascular dysfunction in RP/SSc. Further work is needed to establish how best to apply methods, such as LSCI, both in clinical practice and as an outcome measure in interventional trials.

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