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Use of laser speckle contrast imaging to assess digital microvascular function in primary Raynaud's phenomenon and systemic sclerosis: A comparison with infrared thermography and subjective assessment using the Raynaud's condition score diary

John D. Pauling [1,2], Jacqueline A. Shipley [1], Darren J Hart [1], Anita McGrogan [2], Neil J. McHugh [1,2]

[1] Department of Rheumatology, Royal National Hospital for Rheumatic Diseases, Bath, UK

[2] Department of Pharmacy and Pharmacology, University of Bath, Bath, UK

Abstract

Objectives

Evaluate objective assessment of digital microvascular function using laser Speckle Contrast Imaging (LSCI) in a cross-sectional study of patients with primary Raynaud's phenomenon (RP) and systemic sclerosis (SSc); comparing LSCI with both infrared thermography (IRT) and subjective assessment using the Raynaud's 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 using a cold challenge on two occasions, two 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 differencesbetween primary RP and SSc, and the impact of gender.

Results

There was moderate-to-good correlation between LSCI and IRT (Spearman's 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 (ICCs 0.51-0.63) and immediately following cold challenge (ICCs 0.56-0.86) but lower during re-perfusion (ICCs 0.3-0.7). Neither subjective nor objective assessments differentiated between primary

RP and SSc. Males reported lower median daily frequency of RP attacks (0.82 vs. 1.93, *p*=0.03). Perfusion using LSCI/IRT was higher in males for the majority of assessments.

Conclusions

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 gender on subjective and objective assessment of RP warrants further evaluation.

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Name of department(s) and institution(s) to which the work should be attributed.

[1] Department of Rheumatology, Royal National Hospital for Rheumatic Diseases, Bath, UK

[2] Department of Pharmacy and Pharmacology, University of Bath, Bath, UK

Initials, surnames, appointments and highest academic degrees of all authors.

J. D. Pauling BMedSci BMBS MRCP PhD [1,2], J.A. Shipley BSc PhD [1], D. J. Hart BSc PhD [1], A McGrogan PhD [2], N.J. McHugh BMBS FRCP FRCPath MD [1,2]

[1] Department of Rheumatology, Royal National Hospital for Rheumatic Diseases, Bath, UK

[2] Department of Pharmacy and Pharmacology, University of Bath, Bath, UK

Corresponding author:

Dr J D Pauling

Royal National Hospital for Rheumatic Diseases,

Upper Borough Walls,

Bath,

BA1 1RL

John.Pauling@rnhrd.nhs.uk

Short title:

LSCI in the assessment of RP and SSc

Introduction

The episodic nature of Raynaud's phenomenon (RP) precludes objective assessment in the clinical setting leading to reliance on patient self-report. The Raynaud's Condition Score (RCS) diary collects daily information on the frequency, duration and severity of RP attacks over a 2-week period (1-5). 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'). Furthermore, the frequency, duration and severity of RP attacks might be influenced by seasonal variation and 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 SSc (6, 7). Laser speckle contrast imaging (LSCI) is an emerging non-invasive microvascular imaging modality providing real-time dynamic assessment of perfusion over large areas of tissue (8-10). Recent work has evaluated LSCI in primary RP and SSc with conflicting results (11, 12).

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 anastamoses [AVAs]) and non-glabrous regions of the digits in healthy controls (13). The specific objectives of this study were to assess the correlation between subjective (RCS diary) and objective (IRT and LSCI) assessments of digital vascular function in primary RP and SSc. We report the reproducibility of LSCI and IRT, and evaluate the impact of disease state and gender on subjective and objective assessment of RP.

Methods

Study population

Patients with SSc fulfilled either the American Rheumatology Association (ARA) 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 one week of examination and negative anti-nuclear autoantibody reactivity using immunofluoresence 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 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 two occasions, two weeks apart for microvascular imaging. At visit 1, participants were given tuition completion of a 2-week RCS diary which they returned at visit 2.

Microvascular Imaging Protocol

At each visit participants underwent a standardised cold challenge identical to previously in healthy controls (13). In brief, followed acclimatisation for 20 minutes 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 the submerged both hands (in gloves to avoid evaporative cooling) to the level of the radio-carpal joints into a water-bath cooled to 15°C (+/-0.1°C) for 60 seconds. IRT and LSCI images were taken of both hands immediately following cold challenge, and at 13-second intervals for 15 minutes.

Microvascular imaging equipment

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

Microvascular image analysis

Perfusion should be strictly defined as volume per unit area per unit time, however, as no laser instrument is capable of directly measuring blood flow, measurements derived from laser imaging tools (e.g. Doppler and flowmetry) are typically described in arbitrary flux units. In LSCI, digital imaging and processing allows real time quantification of time-varying speckle contrast with the generation of a false colour map of speckle contrast. Speckle contrast is quantified by calculating the ratio of the standard deviation to the mean of the intensities recorded for each pixel within delineated squares e.g. 5x5 or 7x7 pixels (9). The values themselves are influenced by many factors including time-constant, exposure gain, laser wavelength etc., hence the importance of observing a standardised approach to assessment.

Perfusion was calculated over three pre-defined regions of interest (ROI) as previously described (13): the dorsal aspect of right middle fingertip (ROI1), dorsal aspect of the middle phalanx of the right middle finger (ROI2), and the palmer aspect of left middle fingertip (ROI3). An example IRT and LSCI images demonstrating ROIs is available online (Figure 1). Mean perfusion (skin temperature [°C] using IRT and arbitrary flux values [fu] obtained using LSCI) at each ROI was calculated during baseline assessment (abbreviated hereafter as B), immediately following cold challenge (t0) and at 5, 10 and 15 minutes following cold challenge (t5, t10 and t15 respectively).

Statistical analysis

Data is presented as median values (and inter-quartile range [IQR]) unless otherwise stated. Correlation between continuous data was assessed using Spearman's rank correlation coefficient (r_s). Between group comparisons of unpaired data was undertaken using the Mann Whitney U test. Comparison of paired data (e.g. comparison between different ROIs for both IRT and LSCI) was undertaken using the Wilcoxon signed-rank test. Reproducibility (between assessments 1 and 2) was assessed using intra-class correlation co-efficient (ICC) (16). All data was analysed using SPSS version 18.0. A *p* value of <0.05 was considered significant.

Results

Patient demographics

Twenty five subjects (5 male) with SSc and 18 (4 male) 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 years, p=0.03), had a lower age at RP onset (20 vs. 35 years, p=0.02) and lower use of ACE inhibitors (0% vs. 28%, p=0.03) compared to SSc. There were no differences in gender, vasodilatory therapy use or smoking history between groups. The majority of SSc patients 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 ROI3 was unavailable for one subject with primary RP (shoulder discomfort prevented forearm supination). Data was unavailable for ROI1 of a subject with SSc due to previous amputation. Four (9.3%) subjects 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 ROIs at baseline and at all time-points 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 parameters of the RCS score diary and non-invasive 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 0.55-0.70 (Table 4). Reproducibility of LSCI was comparable, if not superior, to IRT. The highest ICC values were found immediately following cold challenge using LSCI at ROIs 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 minutes post cold challenge (e.g. 0.30 for ROI2 at 15 minutes and 0.34 for ROI at 10 minutes) suggesting greater variation in digital vascular responses during re-warming in comparison to 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 minutes [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.

Impact of gender on subjective and objective assessment of digital microvascular function

Males reported a lower frequency of RP attacks than females (median daily frequency 0.8 vs. 1.9, p=0.031) with trends for lower median daily duration of RP attacks (13.8 vs. 26.1 minutes, p=0.33) and RCS score (1.1 vs. 2.0, p=0.38) in males. Digital perfusion assessed using IRT and LSCI was significantly higher in males 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 ROI3 at baseline [median perfusion 758.2 vs. 491.6, p=0.065], and similar trends were observed for ROI1 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 ICCs were lower than we previously reported in healthy controls, possibly due to 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 re-perfusion (t5 and t10) than at baseline but do not feel this negates 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 (whilst 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 can not provide. We would therefore advocate retaining a provocation test (such as the cold challenge or post-occlusive reactive hyperaemia studies) in future studies of LSCI (and other laser derived techniques such as single point LDF) until such time that 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 parameters 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 impact 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 using the ROIs chosen in this 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 non-dominant ring finger) (7). Ruaro et al. used LSCI to identify lower perfusion of the volar aspect of the finger tips 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 due to 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 (ROI2) at baseline in SSc compared to 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 parameters to enhance the

capacity to differentiate between primary RP and SSc. The evaluation of re-warming 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 males, 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.

Conclusions

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 non-invasive 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.

Key messages:

- There is poor agreement between subjective and objective assessment of digital microvascular dysfunction in RP/SSc.

- LSCI is a reproducible method for the dynamic assessment of digital microvascular function in RP/SSc.

- Gender may influence outcome of subjective and objective assessment of RP.

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Conflict of interest statement

The authors declare no conflicts of interest relating to this work.

References

1.   Wigley FM, Korn JH, Csuka ME, Medsger TA, Jr., Rothfield NF, Ellman M, et al. Oral iloprost treatment in patients with Raynaud's phenomenon secondary to systemic sclerosis: a multicenter, placebo-controlled, double-blind study. Arthritis Rheum 1998;41:670-7
2.   Merkel PA, Herlyn K, Martin RW, Anderson JJ, Mayes MD, Bell P, et al. Measuring disease activity and functional status in patients with scleroderma and Raynaud's phenomenon. Arthritis Rheum 2002;46:2410-20
3.   Merkel PA, Clements PJ, Reveille JD, Suarez-Almazor ME, Valentini G, Furst DE. Current status of outcome measure development for clinical trials in systemic sclerosis. Report from OMERACT 6. J Rheumatol 2003;30:1630-47
4.   Khanna D, Lovell DJ, Giannini E, Clements PJ, Merkel PA, Seibold JR, et al. Development of a provisional core set of response measures for clinical trials of systemic sclerosis. Ann Rheum Dis 2008;67:703-9
5.   Black CM, Halkier-Sorensen L, Belch JJ, Ullman S, Madhok R, Smit AJ, et al. Oral iloprost in Raynaud's phenomenon secondary to systemic sclerosis: a multicentre, placebo-controlled, dose-comparison study. Br J Rheumatol 1998;37:952-60
6.   Pauling JD, Shipley JA, Harris ND, McHugh NJ. Use of infrared thermography as an endpoint in therapeutic trials of Raynaud's phenomenon and systemic sclerosis. Clin Exp Rheumatol 2012;30:S103-15
7.   Murray AK, Moore TL, Manning JB, Taylor C, Griffiths CE, Herrick AL. Noninvasive imaging techniques in the assessment of scleroderma spectrum disorders. Arthritis Rheum 2009;61:1103-11
8.   Briers J. Laser Doppler and time-varying speckle: a reconciliation. J Opt Soc Am A 1996;13:345-50
9.   Briers J, editor Laser speckle contrast imaging for measuring blood flow. Proceedings of the symposium on photonics technologies for 7th framework program; 2006; Wroclaw
10.   Briers JD. Laser Doppler, speckle and related techniques for blood perfusion mapping and imaging. Physiol Meas 2001;22:R35-66
11.   Ruaro B, Sulli A, Alessandri E, Pizzorni C, Ferrari G, Cutolo M. Laser speckle contrast analysis: a new method to evaluate peripheral blood perfusion in systemic sclerosis patients. Ann Rheum Dis 2014;73:1181-5
12.   Della Rossa A, Cazzato M, d'Ascanio A, Tavoni A, Bencivelli W, Pepe P, et al. Alteration of microcirculation is a hallmark of very early systemic sclerosis patients: a laser speckle contrast analysis. Clin Exp Rheumatol 2013;31:109-14

13.   Pauling JD, Shipley JA, Raper S, Watson ML, Ward SG, Harris ND, et al. Comparison of infrared thermography and laser speckle contrast imaging for the dynamic assessment of digital microvascular function. Microvasc Res 2012;83:162-7

14.   Masi. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum 1980;23:581-90

15.   LeRoy EC, Medsger TA, Jr. Criteria for the classification of early systemic sclerosis. J Rheumatol 2001;28:1573-6

16.   Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychol Bull 1979;86:420-8

17.   Clark S, Hollis S, Campbell F, Moore T, Jayson M, Herrick A. The "distal-dorsal difference" as a possible predictor of secondary Raynaud's phenomenon. J Rheumatol 1999;26:1125-8

18.   Anderson ME, Moore TL, Lunt M, Herrick AL. The 'distal-dorsal difference': a thermographic parameter by which to differentiate between primary and secondary Raynaud's phenomenon. Rheumatology 2007;46:533-8