Pilot study assessing pathophysiology and healing of digital ulcers in patients with systemic sclerosis using laser Doppler imaging and thermography

A.K. Murray[1,2], T.L. Moore[1], E. Wragg[3], H. Ennis[1], A. Vail[4], G. Dinsdale[1], L. Muir[5], C.E.M. Griffiths[6], A.L. Herrick[1,7]

[1]Institute of Inflammation and Repair, The University of Manchester, Manchester Academic Health Science Centre, Salford Royal NHS Foundation Trust, Salford, UK; [2]Photon Science Institute, University of Manchester, UK; [3]Rheumatology Directorate, Salford Royal NHS Foundation Trust, Salford, UK; [4]School of Community Based Medicine, The University of Manchester, Manchester Academic Health Science Centre, Salford Royal NHS Foundation Trust, Salford, UK; [5]Department of Hand Surgery, Salford Royal NHS Foundation Trust, Salford, UK; [6]Dermatology Centre, The University of Manchester, Manchester Academic Health Science Centre, Salford Royal NHS Foundation Trust, Salford, UK; [7]NIHR Manchester Musculoskeletal Biomedical Research Unit, Central Manchester NHS Foundation Trust, Manchester Academic Health Science Centre, UK.

Andrea K. Murray, PhD
Tonia L. Moore, BSc
Elizabeth Wragg, RGN
Holly Ennis, MSc
Andy Vail, BSc
Graham Dinsdale, PhD
Lindsay Muir, FRCS
Christopher E.M. Griffiths, FRCP MD
Ariane L. Herrick, FRCP MD

Please address correspondence to:
Andrea Murray, PhD,
Institute of Inflammation and Repair,
The University of Manchester, Manchester Academic Health Science Centre,
Salford Royal NHS Foundation Trust,
M6 8HD Salford, UK.
E-mail: andrea.murray@manchester.ac.uk

Received on March 4, 2016; accepted in revised form on July 4, 2016.

Clin Exp Rheumatol 2016; 34 (Suppl. 100): S100-S105.

Key words: systemic sclerosis, digital ulcer, laser Doppler imaging, thermography, healing

Funding: see page S-104.
Competing interests: none declared.

ABSTRACT

Objective. *Systemic sclerosis (SSc)-related digital ulcers (DU) cause significant pain and disability and are often a primary endpoint in clinical trials. However, their pathophysiology has been little studied. The objectives of this prospective study were to determine whether laser Doppler imaging (LDI) and thermography can identify ischaemic components in both fingertip and extensor surface DU and assess ulcer healing.*

Methods. *Patients prospectively reported new DU over a year. Patients' DU underwent imaging until the ulcer had healed. Ischaemia was defined as lower blood flow or skin temperature (and inflammation as higher) within the ulcer, compared to a non-affected site.*

Results. *53 ulcers (19 fingertip, 18 extensor, 16 'other' sites) in 17 patients were imaged (53 with LDI, 52 with thermography). For LDI data 32 (60%) ulcers were ischaemic; median perfusion ulcer/unaffected area; 0.79 (range 0.11-2.9). For thermography data 35 (66%) were ischaemic; 0.98 (0.89 to 1.1). Inflammation in the surrounding area was identified for all ulcers by LDI but not thermography. In the 36 ulcers with repeat imaging, LDI showed trends (with healing) towards increased ulcer perfusion (p=0.23) and decreased hyperaemia in adjacent areas (p=0.59). Skin temperature at the ulcer site showed no significant change (p=0.13) but adjacent area showed decreased temperature (p=0.04 signifying decreased blood flow).*

Conclusion. *LDI and thermography are sufficiently sensitive to measure ischaemia in both fingertip and extensor ulcers. LDI was better suited to monitoring change in perfusion with healing (due to higher imaging resolution, or vascular changes occurring in more superficial skin layers).*

Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterised by fibrosis and microvascular abnormality. Digital ulcers are one of its most common, painful and debilitating manifestations, and are reported to occur in between 8% and 50% of patients (1-4). Discrepancies in reported frequency may be due, in part, to the difficulty in defining digital ulcers (5, 6).

SSc-related digital ulcers often occur early in the disease process; in one study 73% occurred within 5 years of the first non-Raynaud's phenomenon feature (7). Digital ulcers may occur distally at the fingertips, where they are commonly believed to be due to ischaemia, or over the extensor aspects of the metacarpophalangeal and interphalangeal joints where they are commonly believed to be 'traumatic' (due to repetitive low grade trauma). They are associated with early age of SSc onset, increased Rodnan skin score, longer disease duration (1, 2, 7) and the presence of anti-topoisomerase (anti-Scl-70) and anticentromere (ACA) antibodies (1, 2, 8, 9). Patients who experience digital ulcers have higher global scores of disability, higher scores of hand disability, and increased anxiety than those without; they also experience more limitation in daily activities and require more domestic help (10-12).

Relatively little is known about the pathophysiology of SSc-related digital ulcers and of the factors influencing healing time, despite their associated pain and effect on quality of life. Non-invasive imaging techniques such as laser Doppler imaging (LDI) and thermography are possible objective meth-

ods to study pathophysiology (in terms of perfusion) and to monitor digital ulcer progression (and healing). The aim of this prospective study was to increase understanding of the pathophysiology of digital ulcers, and to assess the sensitivity of LDI and thermography in monitoring change in DU perfusion over time, in a cohort of patients with SSc attending Salford Royal NHS Foundation Trust, a specialist referral centre for patients with SSc.

Specific objectives of the present study were: 1) Testing the hypothesis that all SSc-related digital ulcers (*i.e.* extensor as well as fingertip) have ischaemic components; 2) Determining whether LDI and thermography are sensitive methods for measuring change in perfusion (directly and indirectly respectively) as ulcers heal.

Patients

Patients attending for annual review over a 12-month period at a specialist SSc clinic were invited to participate in the study.

Methods

Patients were examined initially at the annual review visit, which included an assessment for digital ulcers. If one or more ulcers were present at the baseline visit, patients attended for regular visits (1 to 4 weekly, depending on patient preference and clinical need) and underwent imaging and treatment as described below. These patients were a subgroup of the cohort of patients included in a previously reported prevalence study (4). Following recruitment, patients with or without ulcers were asked to contact the department if any new ulcers developed within the following 12 months. If at any time more than one ulcer was present, each ulcer was assessed and included in the study. At the final (12 month) visit the ulcer had healed, was so near to healing that no further visit was necessary or lasted longer than the 12 month study period. Those patients whose ulcers lasted longer than the 12-month study period continued to undergo treatment but no further study data was obtained; their data was taken and included up to 12 months. All patients gave written consent; the study was approved by the North West Greater Manchester National Research Ethics Service Committee.

Skin perfusion imaging and temperature measurement of ulcers (baseline and follow-up visits)

All imaging was performed in a low-lit temperature controlled room at 23°C. Patients were acclimatised for 20 minutes before imaging. All patients were asked to refrain from smoking and caffeine for 4 hours prior to the study as these can affect perfusion. At each visit LDI (MoorLDI-vr, 633 nm, Moor Instruments, Axminster, UK) and thermography (Agema Thermavision 570; FLIR, Kent, UK) were used to record the perfusion and surface temperature (an indirect measure of perfusion). A single image was taken with each technique to include the ulcer site, an adjacent area of skin and an area of skin a small distance away from the ulcer (<2 cm) (representing perfusion at non ulcerated skin, Fig. 1). LDI images were taken at a distance of 45 cm. Thermal images were taken at a distance of 30 cm. No dressing or creams were present/had been applied at the site of imaging on the day of the study. Adhesive arrows were used to highlight the position of the ulcer in the images. Photographs of the ulcers were taken by Salford Royal Hospital Clinical Photography as a record of status and location.

Specialist wound care

All patients reporting active digital ulcers attended a specialist nurse-led clinic where digital ulcers were treated according to best clinical practice by a specialist wound care or Raynaud's and Scleroderma specialist nurse. The main elements of management were irrigation and application of hydrocolloid dressing (Duoderm extra thin). Dressings were changed, when necessary between visits, by the patient after 2-3 days. If the skin surrounding the ulcer appeared to be macerated at the visit then a barrier cream was applied prior to the dressing. If, using the hydrocolloid dressing, the wound became too wet then this was substituted by a dry dressing.

Data analysis

LDI data (analysed in MoorLDI v5.0D) was measured as a ratio of perfusion at the ulcer site/normal (U_L) and adjacent/normal (A_L). The site of the ulcer was identified by the location of the arrow, and the border of the ulcer was visible in the accompanying greyscale DC images (equivalent to low resolution photographs of the area imaged). The same areas in thermal images (analysed in Agema Research v. 2.1) were measured in °C giving U_T and A_T. When U or A <1 (*i.e.* blood flow or skin temperature were lower than unaffected skin) then this was taken to represent ischaemia and when U or A>1 hyperaemia. The same sized region of interest was taken for all LDI and thermography images. Comparing blood flow and skin temperature at the site and adjacent to the ulcer to that of an area of normal skin allows each ulcer to have its own control and minimises the effect of differences in blood flow and skin temperature across differing anatomical areas. For multiple visits the first and last visit data were taken for analysis.

Statistical analysis

Analysis was carried out in STATA v. 10 using standard tests: Pearson chi-squared to compare proportions; *t*-test for continuous data; and log-rank test to compare durations. Ratios (U and A described above) were log-transformed prior to analysis. Significance values should be interpreted as indicative only, given the low number of patients; multiple ulcers for a participant were analysed assuming independence.

Results

During the 12-month recruitment period 17 patients attended with 61 ulcers (14 (82%) female, median age 61 (range 41 to 88) years, 7 (41%) limited cutaneous SSc (lcSSc) subtype (13), Raynaud's duration 16 (1 to 44) years, disease duration 13 (1 to 27) years, 3 (18%) smokers, 5 (29%) ACA positive, 3 (18%) anti-Scl70 positive).

The number of ulcers per patient were: 8 patients with one ulcer, one with two ulcers, three with three ulcers, two with five ulcers, one with 8 ulcers, one with 11 ulcers and one with 13 ulcers. Four pa-

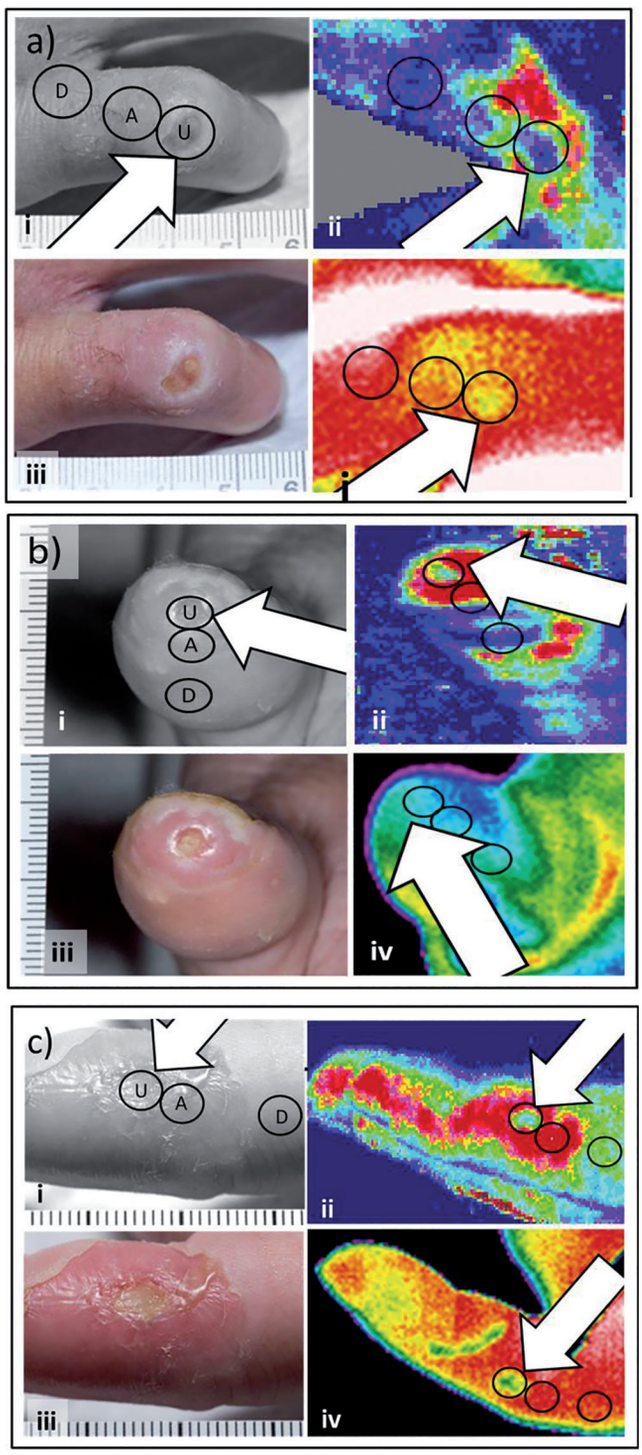

Fig. 1. Examples of (at first visit) a) extensor, b) digital tip and c) 'other' digital site ulcers, i) Black and white photographic example with regions of interest marked (U=ulcer, A=adjacent, D=distant [unaffected]), ii) LDI (blue and green represent lower blood flow than red), iii) colour photograph and iv) thermographic (yellow and green represent lower blood flow than red).

tients attended for one visit, five for two, two for three, one for 7, one for 9, one for 10, one for 13, one for 14 and one for 15 visits. Eight ulcers were not imaged due to technical reasons or patient time constraints; 53 ulcers were imaged.

Of these 53 imaged ulcers (53 with LDI, 52 with thermography), nineteen ulcers occurred at the digital tip, 18 on the extensor surface and 16 at 'other' sites (*i.e.* not at the tip or extensor surface, *e.g.* side of finger or below nailbed). A composite of photographs, LDI and thermographic examples of the ulcers at different sites are shown in Figure 1.

Objective 1:
Ischaemia in digital ulcers

LDI measurements

Data were taken for 53 ulcers. Median (range) LDI measurements for all 53 ulcers, as ratios of perfusion (initially in arbitrary perfusion units), U_L and A_L, at baseline were 0.79 (0.11 to 2.9) and 3.22 (1.2 to 24) respectively. Thirty two (60%) of all 53 ulcers were ischaemic (13/19 (68%) digital tip, 7/18 (39%) extensor and 12/16 (75%) other). All adjacent areas were hyperaemic. Data are shown, grouped for location, in Figure 2a.

Thermography

Data were available for 52 ulcers. Median (range) thermography measurements, for all 52 ulcers as ratios of skin temperatures, U_T and A_T, at baseline were 0.98 (0.89 to 1.1) and 1.0 (0.93 to 1.1) respectively. Thirty five (66%) of the 53 ulcers were ischaemic (15/18 (83%) digital tip, 10/18 (56%) extensor and 10/16 (63%) other). In contrast to the LDI data some of the adjacent areas were also found to be ischaemic (13/18 (72%) digital tip, 7/18 (39%) extensor and 9/16 (56%) other). Data are shown, grouped for location, in Figure 2b.

Objective 2:
Longitudinal change during healing as assessed by LDI and thermography

Thirty six of the 52 ulcers were imaged at more than one visit (from 11 patients; 13 digital tip, 10 extensor and 13 other), giving separate initial and final visits (median (range) duration between first and final visits of 62 (12 to 336) days). Longitudinal examples of photographs, LDI and thermographic images of ulcers are shown in Figure 3.

LDI measurements

Median (range) perfusion of the ulcer area (U_L) rose from 0.68 (0.11 to 2.9) to 0.78 (0.11 to 4.9) indicating a trend towards increased blood flow at the ulcer site with healing (although this was not significant, p=0.23). The blood flow in the adjacent areas (A_L) decreased from 3.2 (1.2 to 21) to 2.6 (1.3 to 20) indicating a reduction in hyperaemia (again with no statistical significance, p=0.59). Data are shown, at baseline and final visit, in Figure 2c.

Thermography

Median (range) skin temperature in

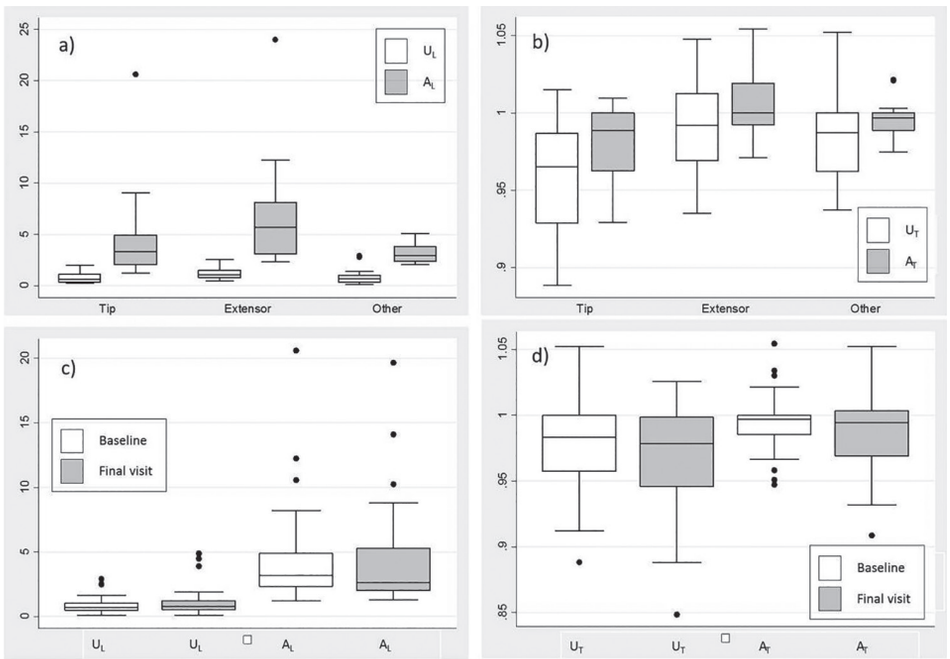

Fig. 2. Baseline and longitudinal data. Baseline imaging data shown for location; a) LDI perfusion data (ulcer site/normal (UL, white boxes) and adjacent/normal (AL, grey boxes)), outliers due to areas of high perfusion in adjacent areas, b) thermography skin temperature data (ulcer site/normal (UT, white boxes) and adjacent/normal (AT, grey boxes)). Longitudinal data with ulcer healing (baseline white boxes, final visit grey boxes); c) LDI perfusion data (ulcer site/normal (UL) and adjacent/normal (AL)), d) thermography skin temperature data (ulcer site/normal (UT) and adjacent/normal (AT)). Central line represents the median, box limits the interquartile range and whisker limits the range (excluding outliers). If outliers are present these are marked separately with 'o'.

the area of the ulcers (U_T) was 0.98 (0.89 to 1.1) at the initial and 0.98 (0.85 to 1.0) at the final visit; no significant change between baseline and final visits (p=0.13). The adjacent area skin temperature (A_T) decreased from initial (1.0 (0.95 to 1.1)) to final visit (0.99 (0.91 to 1.1)) indicating significant (p=0.04) reduction in blood flow adjacent to the ulcer. Data are shown, at baseline and final visit, in Figure 2d.

Discussion

Our main finding, demonstrated by non-invasive imaging techniques, is that both extensor and digital-tip ulcers can be ischaemic, confirming our hypothesis (Objective 1), albeit that a smaller proportion of extensor than digital-tip ulcers are ischaemic. LDI has shown that ischaemia occurs in digital tip, extensor and 'other' (digital site) ulcers; 60% of ulcers were found to be ischaemic (*i.e.*, as discussed above that blood flow is lower at the ulcer than unaffected, non-ulcerated skin). Areas adjacent to all ulcer sites studied here were hyperaemic, likely indicative of inflammation in the surrounding tissue.

Thermography and LDI results were consistent; most ulcers were ischaemic. However, the degree of ischaemia at ulcer sites, and of hyperaemia at adjacent sites, was less with thermography than with LDI. The differences between LDI and thermography data are most likely due to differences in the skin depths that the techniques image. Firstly, LDI utilises a low powered laser scanned over the skin to measure relative blood flow in the upper layers of the skin, which are more likely to reflect differences/be sensitive to change in ulcerated skin. Thermography measures skin temperature as an indirect measure of blood flow; the infrared signal collected from the skin surface by the thermal camera is potentially caused by residual inflammation deeper within the tissue, further increasing the signal. The ischaemic signal from a superficial ulcer may therefore be overwhelmed by heat radiating from deeper tissue. Secondly, whereas ulcers are clearly visualised on LDI images, they are not on thermography images due to the lower resolution of the thermal camera at the focal length-restricted imaging distance. Boundaries of smaller ulcers are not demarcated and cannot be resolved with the thermal camera.

Our second objective was to determine whether LDI and thermography were sufficiently sensitive to detect changes in perfusion and temperature, respectively, as ulcers heal. LDI data indicated overall blood flow increased at the ulcer site and decreased in the adjacent area with healing, suggesting that LDI is sensitive to change. Thermography detected no change in ulcers with healing but did show a decrease in hyperaemia of the surrounding tissue. These findings suggest that LDI is more sensitive than thermography in detecting changes in blood flow within ulcers over time. This is again most likely due to the higher imaging resolution of LDI that the changes occur in the upper layers of the skin, more exclusively imaged by LDI. This study suggests that LDI may be the better technique for future studies.

A previous study has documented short term follow-up of digital ulcer healing with speckle imaging (a variation on LDI which images the superficial capillaries in the skin). Our study lends further support to the observation by Ruaro *et al*. (14) of lower blood flow at the ulcer site and increased blood flow upon healing. In addition, our study also used thermography to measure ischaemia and inflammation and followed ulcers through to healing.

There are two other previous reports of the use of LDI in monitoring ulcer/ischaemia response to treatment. The first was a case report, describing the response of a patient with ischaemic necrosis (in contrast to ulceration), to adventitiectomy (15), showing the utility of LDI in monitoring changes in blood flow. The second monitored blood flow

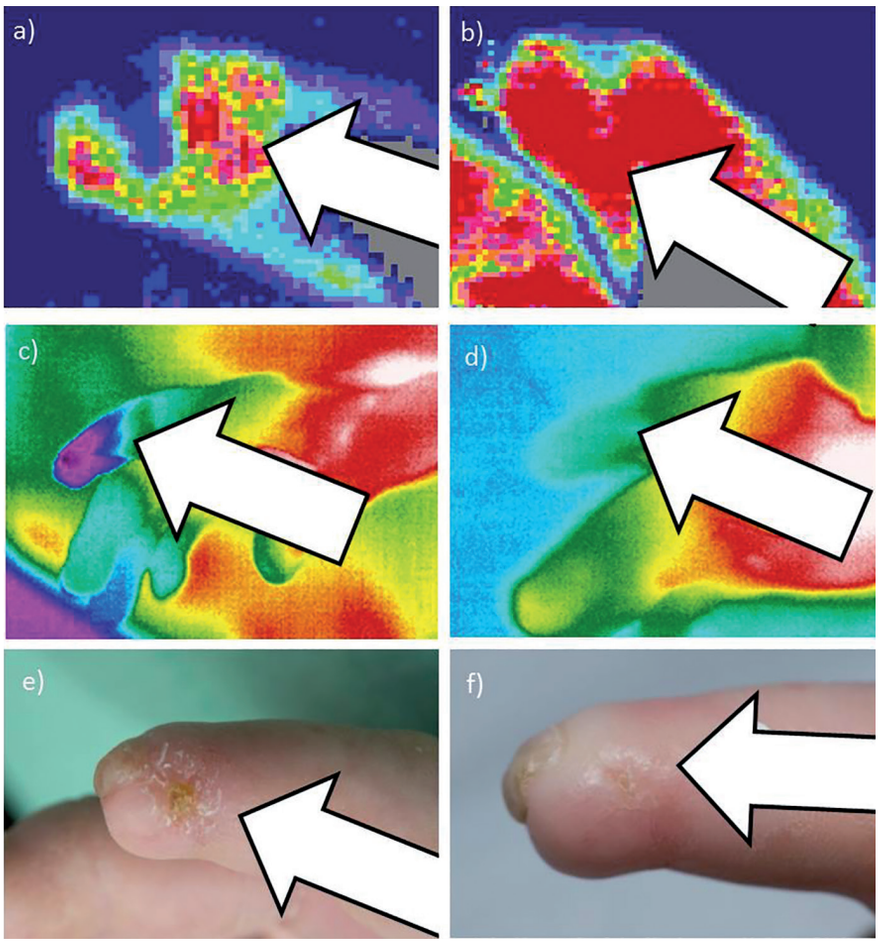

Fig. 3. Longitudinal example of an ulcer undergoing healing (left at baseline and right after 3 months). a) Baseline LDI flux image showing perfusion (blue represents relatively low blood flow and red high, arrow to mark the area of interest); b) LDI flux at 3 months (on same scale); c) thermography image showing skin temperature at baseline, blue is low and red/white relatively high temperature; d) thermography image at 3 months (same scale); e) photograph of ulcer at the first visit; f) photograph of ulcer at 3 months.

response to bosentan over 12 weeks (16); however, ulcer sites themselves were not studied, only hand blood flow. Our study shows that LDI and thermography have the capacity to identify the region of ulceration and surrounding inflammation and track these with time. In conclusion, our key findings are that extensor as well as digital-tip and 'other' ulcers can be ischaemic, albeit less frequently, and that LDI and thermography can be used to monitor this ischaemia, which reduces with ulcer healing. Therefore LDI and thermography show potential as objective measures of ulcer healing (*i.e* as outcome measures). Although, at present the detectable effect size is unknown, data shown here demonstrates that there is variation of the images captured with ulcer improvement. These findings, for LDI in particular, may have significant future impact on clinical trials of digital ulceration. This is particularly relevant with the current interest in developing new therapies for SSc-related digital ulcers. Future research applications could include combining these techniques with modalities that image skin structure such as optical coherence tomography or high frequency ultrasound in order to monitor ulcer size and depth during healing and to examine relationships with changes in blood flow and skin temperature.

Key messages

- Extensor as well as digital-tip and 'other' ulcers can be ischaemic
- LDI and thermography can be used to monitor this ischaemia, which reduces with ulcer healing.
- LDI, in particular, may have significant future impact as a clinical trial outcome measure.

Acknowledgement

We are grateful to Adrienne Taylor, tissue viability nurse, for her help in this study. We thank the Scleroderma and Raynaud's UK for funding this study. The authors would like to acknowledge the assistance given by IT Services and the use of the Computational Shared Facility at The University of Manchester.

Funding

This study was funded by the Scleroderma and Raynaud's UK. This report includes independent research supported by (or funded by) the National Institute for Health Research Biomedical Research Unit Funding Scheme. The views expressed in this publication are those of the authors and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health. A.K. Murray is funded by Arthritis Research UK.

References

1. KHIMDAS S, HARDING S, BONNER A, ZUMMER B, BARON M, POPE J: Canadian Scleroderma Research Group Registry. Associations with digital ulcers in a large cohort of systemic sclerosis: results from the Canadian Scleroderma Research Group Registry. *Arthritis Care Res* 2011; 63: 142-9.
2. TIEV KP, DIOT E, CLERSON P *et al*.: Clinical features of scleroderma patients with or without prior or current ischemic digital ulcers: post-hoc analysis of a nationwide multicenter cohort (ItinerAIR-Sclerodermis). *J Rheumatol* 2009; 36: 1470-6.
3. FERRI C, VALENTINI G, COZZI F *et al*.: Systemic sclerosis: demographic, clinical and serologic features and survival in 1,012 Italian patients. *Rheumatology* 2002; 81: 139-53.
4. ENNIS H, VAIL A, WRAGG E *et al*.: A prospective study of systemic sclerosis-related digital ulcers: prevalence, location, and functional impact. *Scand J Rheumatol* 2013; 42: 483-6.
5. HERRICK AL, ROBERTS C, TRACEY T *et al*.: Lack of agreement between rheumatologists in defining digital ulceration in systemic sclerosis. *Arthritis Rheum* 2009; 60: 878-82.
6. BARON M, CHUNG L, GYGER G *et al*.: Consensus opinion of a North American Working Group regarding the classification of digital ulcers in systemic sclerosis. *Clin Rheumatol* 2014; 33: 207-14.
7. HACHULLA E, CLERSON P, LAUNAY *et al*.: Natural history of ischemic digital ulcers in systemic sclerosis: single-center retrospective longitudinal study. *J Rheumatol* 2007; 34: 2423-30.

8. GUILLEVIN L, HUNSCHE E, DENTON CP *et al*.: Functional impairment of systemic scleroderma patients with digital ulcerations: results from the DUO Registry. *Clin Exp Rheumatol* 2013; 31: 71-80.
9. DENTON CP, KRIEG T, GUILLEVIN L *et al*.: DUO Registry investigators. Demographic, clinical and antibody characteristics of patients with digital ulcers in systemic sclerosis: data from the DUO Registry. *Ann Rheum Dis* 2012; 71: 718-21.
10. BÉREZNÉ A, SEROR R, MORELL-DUBOIS S *et al*.: Impact of systemic sclerosis on occupational and professional activity with attention to patients with digital ulcers. *Arthritis Care Res* 2011; 63: 277-85.
11. MOUTHON L, MESTRE-STANILAS C, BEREZNE A *et al*.: Impact of digital ulcers on disability and health-related quality of life in SSc. *Ann Rheum Dis* 2010; 69: 214-17.
12. BRAND M, HOLLAENDER R, ROSENBERG D *et al*.: An observational cohort study of patients with newly diagnosed digital ulcer disease secondary to systemic sclerosis registered in the EUSTAR database. *Clin Exp Rheumatol* 2015; 33: S47-54.
13. LEROY EC, BLACK C, FLEISCHMAJER R *et al*.: Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988; 15: 202-5.
14. RUARO B, SULLI A, SMITH V, PAOLINO S, PIZZORNI C, CUTOLO M: Short-term follow-up of digital ulcers by laser speckle contrast analysis in systemic sclerosis patients. *Microvasc Res* 2015; 101: 82-5.
15. ROSATO E, ROUMPEDAKI E, PISARRI S, SALSANO F: Digital ischemic necrosis in a patient with systemic sclerosis: the role of laser Doppler perfusion imaging. *Vasa* 2009; 38: 390-3.
16. ROSATO E, MOLINARO I, BORGHESE F, ROSSI C, PISARRI S, SALSANO F: Bosentan improves skin perfusion of hands in patients with systemic sclerosis with pulmonary arterial hypertension. *J Rheumatol* 2010; 37: 2531-9.