Acta Derm Venereol 2016; 96: 641–644

INVESTIGATIVE REPORT

Non-invasive Imaging of Localised Scleroderma for Assessment of Skin Blood Flow and Structure

Andrea K. MURRAY[1,2], Tonia L. MOORE[1], Joanne B. MANNING[3], Graham DINSDALE[1,2], Jack WILKINSON[4], Monica BHUSHAN[5], Christopher E. M. GRIFFITHS[5] and Ariane HERRICK[1]

[1]Centre for Musculoskeletal Research, Institute of Inflammation and Repair and [4]Centre for Biostatistics, Institute of Population Health, The University of Manchester, Manchester Academic Health Science Centre, Salford Royal NHS Foundation Trust, Salford, [2]Photon Science Institute, The University of Manchester, Manchester, [3]Rheumatology Directorate, Salford Royal NHS Foundation Trust, Salford, and [5]Dermatology Centre, Salford Royal NHS Foundation Trust, The University of Manchester, Manchester Academic Health Science Centre, Manchester, UK

Extensive morphoea causes major morbidity, disability and disfigurement; pathophysiology is poorly understood. The aim of this study was to investigate, with non-invasive imaging, the relationship between localised abnormalities of skin structure and perfusion, which characterise morphoea. Thirty-two patients with morphoea underwent imaging at affected and unaffected sites. Skin thickness was imaged with optical coherence tomography (OCT) and high-frequency ultrasound (HFUS). Perfusion was imaged with dual-wavelength laser Doppler imaging (LDI) and thermography. Epidermal thickness showed a small increase from affected to unaffected site (OCT, active and inactive plaques [$p=0.005$ and $p=0.004$], HFUS active plaques only [$p=0.03$]). Deeper perfusion was higher within affected than unaffected sites (LDI $p<0.001$, thermography $p<0.0001$, active and inactive plaques). Epidermal thickness was inversely related to superficial (but not deeper) perfusion. This novel study of OCT, HFUS, LDI and thermography confirms loss of epidermal thickness and increased deeper perfusion in morphoea plaques. *Key words: morphoea; scleroderma; imaging; perfusion; skin thickness.*

Accepted Dec 16, 2015; Epub ahead of print Dec 22, 2015

Acta Derm Venereol 2016; 96: 641–644.

Andrea Murray, Centre for Musculoskeletal Research, Institute of Inflammation and Repair, University of Manchester, MAHSC, Salford Royal NHS Foundation Trust, Salford M6 8HD, United Kingdom. E-mail: andrea.murray@manchester.ac.uk

Morphoea (localised scleroderma) refers to thickening and tightening of the skin and underlying tissues (1, 2). Active lesions show increased angiogenesis resulting in erythema and increased blood flow as compared to surrounding skin (2). Inactive plaques tend to show lower blood flow. The relationship between both the fibrotic and vascular components of the skin in active and dormant morphoea needs to be elucidated. In some patients, morphoea can be extensive and a cause of major morbidity, disability and disfigurement. There is a need to increase our understanding of the pathophysiology of morphoea in order to identify key targets for therapeutic intervention, because current treatments are far from ideal. The aim of this study was to investigate the relationship between increased blood flow and the localised abnormalities of skin structure which characterise morphoea.

PATIENTS AND METHODS

Patients with a confirmed diagnosis of plaque morphoea were recruited into the study. One plaque was measured for each patient; if more than one plaque was present the newest and most active plaque was selected. The duration, location and status (active/dormant as assessed by the patient) of the imaged plaques were noted by the observers taking measurements (TM, GD). All patients gave written consent. The study was approved by National Research Ethics Service North West Committee.

All measurements were made in a low−lit, temperature controlled laboratory (23ºC). All participants were asked to refrain from caffeine and nicotine for 4 h before the study and were acclimatised for 20 min before imaging.

Techniques for assessment of skin structure

Skin structure was measured using two imaging techniques; optical coherence tomography (OCT) and high frequency ultrasound (HFUS). OCT (Fig. 1a) is a relatively new light-based imaging technique analogous to ultrasound (3). A Thorlabs OCM 1300 SS (Thorlabs Inc. NJ USA, 1300 nm), which images into the skin up to approximately 2 mm at a resolution of approximately 7 microns was used to take B scans of skin (epidermis only).

HFUS (Fig. 1b) is an established technique for measurement of skin thickness in patients with SSc (4). The EPISCAN–I-200 system (Longport Inc., PA, USA; 35 MHz) used has a resolution of 40 µm. HFUS penetrates more deeply than OCT (up to approximately 3 cm) allowing measurements of both epidermis and dermis to be taken. OCT and HFUS are therefore complementary techniques: OCT has the advantage of very high resolution but low penetration, whereas HFUS has higher penetration but lower resolution.

Techniques for assessment of skin blood flow

Blood flow was assessed directly by laser Doppler imaging (LDI) (Fig. 1c) and indirectly by skin temperature (thermography; Fig. 1d). The dual wavelength LDI used is a modified MoorLDI (Moor Instruments, Axminster, UK; as described previously [5]) and uses low power scanning lasers (red wavelength laser, 633 nm and green wavelength laser, 532 nm) to image two layers of superficial skin blood flow (red laser; deeper, larger vessels and green laser; superficial, smaller vessels).

 doi: 10.2340/00015555-2328
 ISSN 0001-5555

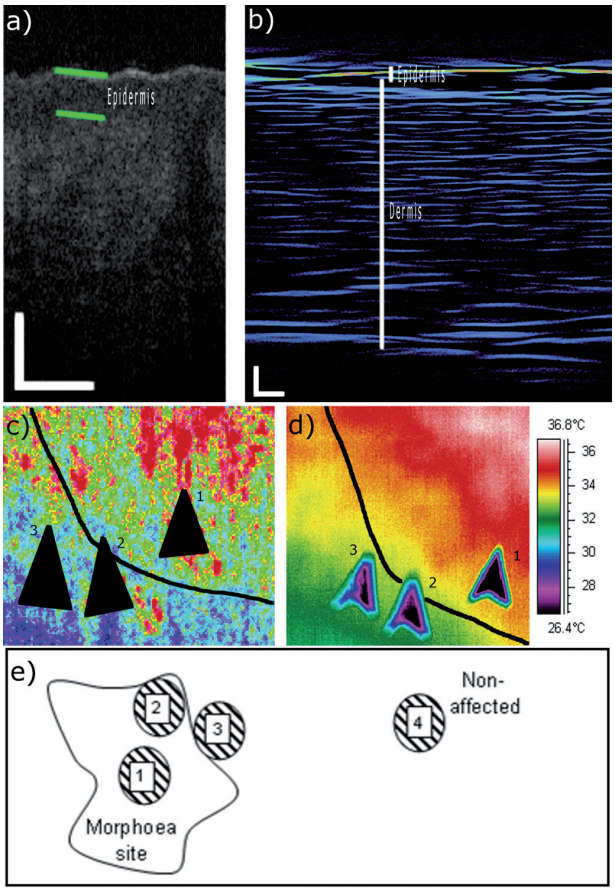

Fig. 1. (a) Optical coherence tomography image of skin in the centre of the plaque, epidermis boundaries marked with green lines. White horizontal and vertical scale bars represent approximately 250 µm. (b) High frequency ultrasound image of skin in the centre of the plaque, scale bars are approximately 250 µm. (c) Red laser Doppler imaging (LDI) image of blood flow (deeper vessels) within and around the plaque. Red represents increased blood flow and blue relatively lower blood flow (arbitrary perfusion units). The boundary of the plaque is shown with the thin black line (plaque is on the right hand side of the line). Arrows mark the location of measurements taken in the 1) centre, 2) inside and 3) outside edges of the plaque (d) Thermal image of the morphoea site. Temperature scale shown on the right hand side. Boundary of the plaque shown by the thin black line (Plaque lies on the right of the line), arrows mark the areas of measurements. 1) is centre of the plaque, 2) inside edge and 3) is outside edge. (e) Measurement areas (and representative regions of interest for the LDI and thermography measurements), hatched areas represent centre of morphoea 1), edge of morphoea, 2) area adjacent to morphoea, 3) and area distant (10 cm) from morphoea, 4).

Thermography is the process of imaging infra-red radiation (heat) emitted by the body. Skin temperature has been shown to be representative of a combination of both superficial skin and deeper muscular blood flow within tissue (6). Images were taken with an Agema Thermavision 570 (FLIR, Kent, UK).

Areas of interest

These were defined as shown in Fig. 1c–e at the centre of the plaque, on the inside and outside edges of the plaque and at a site away from the plaque (10 cm).

Image analysis

For OCT and HFUS, 3 measurements were taken across the image to provide a mean thickness (µm). OCT images were analysed using a bespoke Matlab (MATLAB version R2012a, Mathworks Inc., MA, USA) interface and analysis routine which allowed the layers of the skin to be manually identified and marked up. In both OCT and HFUS the skin's surface appears as a hyper-reflective (bright) layer; the dermal–epidermal junction (DEJ) as hypo-reflective (dark band of pixels) and then the dermis below as a second hyper-reflective layer. For OCT epidermal thickness was recorded (number of pixels) as shown in Fig. 1a (including the dark band of the DEJ) and as described elsewhere in the literature (7–10). Pixel-distance calibration was determined from a sample of known size and the same optical density as skin. Epidermal and dermal thickness measurements were extracted for HFUS using EPISCAN Ultrasound scanner software version 4.0.0.030 (Longport Inc., Silchester, UK). For HFUS epidermis was measured as shown in Fig. 1b as previously described (including the DEJ) (4). In HFUS images the whole of the dermis can be observed. The lower boundary of the dermis is marked by a dark, hypo-reflective layer. The dermis was measured as the total depth of the second hyper-reflective layer (as shown in Fig. 1b). For LDI mean blood flow (arbitrary perfusion units, moorLDLS Research, version 5.0D (Moor Instruments) was taken at each site using a standardised area of interest for each subject (as shown in Fig. 1c [*arrows*] and 1e). For thermography mean skin temperature (ºC) over a standardised area was extracted on Agema Research, version 2.1 (FLIR, Sweden) as shown in Fig. 1d [*arrows*] and 1e. Thus for each plaque one measurement at each site was generated for each imaging technique.

Statistical analysis

Linear mixed effects modelling was used to investigate the difference in blood flow and skin thickness across the sites, as well as the relationship between blood flow and skin thickness. A patient-specific random term was used to account for the multiple observations on each person. Interaction terms were used to investigate whether the relationship between skin thickness and blood flow varied across the sites. A 5% significance level was used throughout. Analysis was conducted in Stata version 12 (11).

RESULTS

Patients

Thirty two patients took part in the study (5 [16%] male; median age 39.5; interquartile range 28.4–61.4 years; duration and location of the plaques are shown in Table I. Of the 32 plaques 15 (47%) were classed

Table I. *Duration of active (*n = *15) and inactive (*n = *17) morphoea plaques (frequencies and percentages given) and anatomical location of morphoea in the 32 patients studied*

	n (%)	Inactive:active, *n*
Duration of plaque		
<5 years	13 (41)	6:7
5–10 years	8 (25)	3:5
10–15 years	7 (22)	5:2
>15 years	4 (13)	3:1
Location		
Head (en coup de sabre) or face	3 (9)	
Chest	1 (3)	
Abdomen	8 (25)	
Posterior trunk	8 (25)	
Upper limb	6 (19)	
Lower limb	6 (19)	

as active. Three patients had systemic sclerosis in addition to morphoea.

Skin thickness

Epidermal thickness showed a small but statistically significant increase moving from the centre of the plaque to the uninvolved site when measured by OCT (for both active and inactive plaques [$p=0.005$ and $p=0.004$ respectively]) and HFUS (for active but not for inactive plaques [$p=0.03$ and $p=0.11$, respectively]). Dermal thickness as measured by HFUS showed no significant differences between areas of interest (Fig. 2a–c).

Blood flow

Deeper blood flow increased moving from the uninvolved site to the centre of the plaque for measurements taken with the red LDI ($p<0.001$ for both active and inactive plaques) and thermography ($p<0.0001$ for both active and inactive plaques) but not superficial blood flow as measured by green LDI ($p=0.63$ and $p=0.95$ for active and inactive plaques respectively) (Fig. 2d, e).

Relationships between skin thickness and blood flow

No associations were observed between HFUS dermal measurements and red LDI and thermal imaging (representing deeper blood flow, $p=0.76$ and $p=0.72$, respectively), and this lack of association was consistent across the sites. Looking for associations between OCT and HFUS epidermal measurements and green LDI (representing superficial blood flow), perfusion measured with green LDI decreased as epidermal thickness increased when using HFUS measurements ($p=0.04$) but not when using OCT measurements ($p=0.29$). Again, these relationships did not vary across the sites.

DISCUSSION

The aim of this study was to learn more about the relationship between fibrosis and vascularity in morphoea plaques. Increased perfusion in morphoea plaques may be due to: Changes in the vasculature within the plaque; changes in the cutaneous layers above or surrounding those vessels (decreased thickness may make blood flow easier to image as vessels are closer to the skin's surface or changes to skin may affect the skin's optical properties, making it less opaque to light); or a combination of both. Our finding of increased perfusion in morphoea plaques is consistent with our previous work and that of others (12–17). Increased blood flow was more marked in larger (deeper) rather than in smaller (superficial) vessels, suggesting that vascular changes due to morphoea preferentially affect

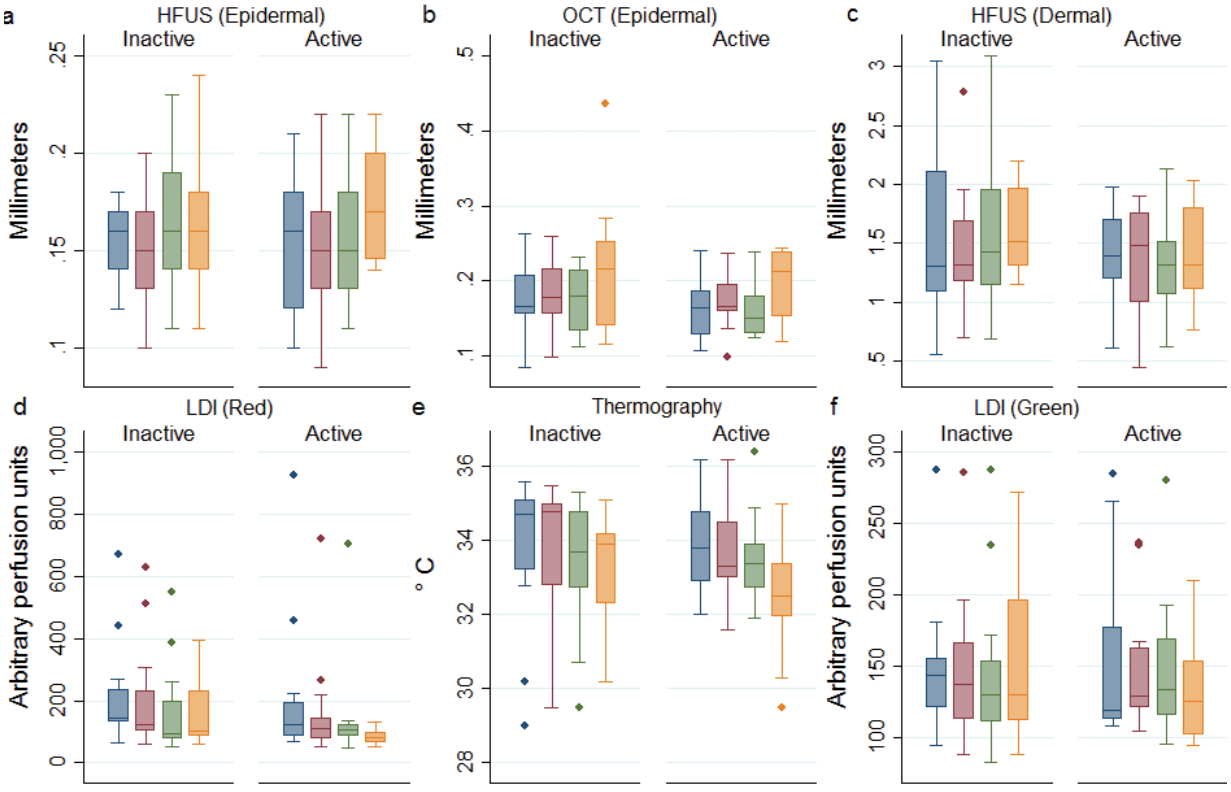

Fig. 2. Box plots of each site (split into inactive and active plaques) for a) LDI (red, deeper blood flow [arbitrary perfusion units]; b) thermography (skin temperature [ºC]); c) LDI (green, superficial blood flow); d) HFUS (epidermis [mm]); e) OCT (epidermis [mm]); f) HFUS (dermis [mm]). Box edges represent interquartile range, central line is median, whiskers are 95% limits and dots represent outliers. Sites are centre (blue), inside edge (red), adjacent outside (green) and distant (amber).

larger vessels, rather than capillaries. It also suggests that there are changes to these vessels, rather than this being due solely to their proximity to the skin's surface. That increased perfusion was found in both active and inactive plaques suggests that microvascular changes may be permanent and therefore may be structural rather than functional.

Epidermal thickness was reduced at the centre of the plaque as compared to the uninvolved skin as measured by OCT (both active and inactive plaques) and HFUS (active plaques only). This suggests that OCT may be more sensitive due to increased resolution. No dermal thickness differences were observed between affected and unaffected sites, possibly implying that surface layers are more involved. That epidermis was thinner in the centre of the plaque adds further weight to the suggestion that the observed increase in deeper blood flow/skin temperature may be multifactorial, and at least in part, due to the increased blood flow/heat conduction from deep tissue through epidermis which has atrophied.

Several studies have been carried out to image morphoea, however they have measured either skin thickness or perfusion (12–18). The novelty of this study lies in the investigation of morphoea plaques with a combination of techniques to examine the inter-relationships between dermal and epidermal thickness and blood flow.

The limitations of this study were the small patient numbers and that our study was cross-sectional and could not therefore address causation. Taking data bilaterally, where, for example, a limb was involved may provide extra data to assess differences from baseline in future studies. OCT techniques have now improved, allowing 'angiography' of cutaneous vessels to be performed and this would be of interest in order to assess whether increased blood flow is due to functional or structural changes in vessels. Now that these imaging techniques have demonstrated a relationship between blood flow and skin thickness what are now required are larger, longitudinal studies to clarify the relationship. These need to include patients with very early plaques (all our patients had well-established disease) to examine whether abnormalities of blood flow occur early, and might therefore contribute to pathogenesis.

ACKNOWLEDGEMENTS

We are grateful to Dr. Hazel Bell, Consultant Dermatologist and Dawn Marsh, Research Nurse of The Royal Liverpool and Broadgreen University Hospitals NHS Trust, and to Dr. Marina E. Anderson, Senior Lecturer and Honorary Consultant in Rheumatology, University of Liverpool and Paula Pyrkotsch, Vascular Nurse Technician, Aintree University Hospital NHS Foundation Trust for the facilitation of their patients into the study. We are grateful to Longport Inc. for the loan of the ultrasound equipment and their advice.

Funding: This work was supported by the Scleroderma Society.

The authors declare no conflicts of interest.

REFERENCES

1. Laxer RM, Zulian F. Localized scleroderma. Curr Opin Rheumatol 2006; 18: 606–613.
2. Chung L, Lin J, Furst DE, Fiorentino D. Systemic and localized scleroderma. Clin Dermatol 2006; 24: 374–392.
3. Fercher AF, Drexler W, Hitzenberger CK, Lasser L. Optical coherence tomography – principles and applications. Rep Prog Phys 2003; 66: 239–303.
4. Moore TL, Lunt M, McManus B, Anderson ME, Herrick AL. Seventeen-point dermal ultrasound scoring system-a reliable measure of skin thickness in patients with systemic sclerosis. Rheumatol 2003; 42: 1559–1563.
5. Murray AK, Gorodkin R, Moore TL, Gush RJ, Herrick AL, King TA. Comparison of red and green laser Doppler imaging in healthy controls. Lasers Surg Med 2004; 35: 191–200.
6. Clark S, Dunn G, Moore TL, Jayson MIV, King TA, Herrick AL. Comparison of thermography and laser Doppler imaging in the assessment of Raynaud's phenomenon. Microvasc Res 2003; 66: 73–76.
7. Gambichler T, Pljakic A, Schmitz L. Recent advances in clinical application of optical coherence tomography of human skin. Clin Cosmet Investig Dermatol 2015; 8: 345–354.
8. Gambichler T, Matip R, Moussa G, Altmeyer P, Hoffmann K. In vivo data of epidermal thickness evaluated by optical coherence tomography: effects of age, gender, skin type, and anatomic site. J Dermatol Sci 2006; 44: 145–152.
9. Gambichler T, Moussa G, Sand M, Sand D, Altmeyer P, Hoffmann K. Applications of optical coherence tomography in dermatology. J Dermatol Sci 2005; 40: 85–94.
10. Gambichler T, Valavanis K, Plura I, Georgas D, Kampilafkos P, Stücker M. In vivo determination of epidermal thickness using high-definition optical coherence tomography. Br J Dermatol 2014; 170: 737–739.
11. StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP.
12. Moore TL, Vij S, Murray AK, Bhushan M, Griffiths CE, Herrick AL. Pilot study of dual-wavelength (532 and 633 nm) laser Doppler imaging and infrared thermography of morphoea. Br J Dermatol 2009; 160: 864–867.
13. Li SC, Liebling MS. The use of Doppler ultrasound to evaluate lesions of localized scleroderma. Curr Rheumatol Rep 2009; 11: 205–211.
14. Wortsman X, Wortsman J, Sazunic I, Carreño L. Activity assessment in morphea using color Doppler ultrasound. J Am Acad Dermatol 2011; 65: 942–948.
15. Abignano G, Aydin SZ, Castillo-Gallego C, Liakouli V, Woods D, Meekings A, et al. Virtual skin biopsy by optical coherence tomography: the first quantitative imaging biomarker for scleroderma. Ann Rheum Dis 2013; 72: 1845–1851.
16. Shaw LJ, Shipley J, Newell EL, Harris N, Clinch JG, Lovell CR. Scanning laser Doppler imaging may predict disease progression of localized scleroderma in children and young adults.Br J Dermatol 2013; 169: 152–155.
17. Birdi N, Shore A, Rush P, Laxer RM, Silverman ED, Krafchik B. Childhood linear scleroderma: a possible role of thermography for evaluation. J Rheumatol 1992; 19: 968–973.
18. Martini G, Murray KJ, Howell KJ, Harper J, Atherton D, Woo P et al. Juvenile-onset localized scleroderma activity detection by infrared thermography. Rheumatol 2002; 41: 1178–1182.