Digital ulcers in systemic sclerosis: investigating the outcome measures of treatment efficacy, pathophysiology, and the development of local treatments

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Biology, Medicine and Health

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School of Biological Sciences
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<th>Description</th>
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<tbody>
<tr>
<td>ABPI</td>
<td>Ankle brachial pressure index</td>
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<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
</tr>
<tr>
<td>ACR</td>
<td>American College of Rheumatology</td>
</tr>
<tr>
<td>ANA</td>
<td>Antinuclear antibody</td>
</tr>
<tr>
<td>AVA</td>
<td>Arteriovenous anastomoses</td>
</tr>
<tr>
<td>CSURI</td>
<td>Capillaroscopic skin ulcer risk index</td>
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<tr>
<td>CTD</td>
<td>Connective tissue disease</td>
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<tr>
<td>DcSSc</td>
<td>Diffuse cutaneous systemic sclerosis</td>
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<tr>
<td>DU</td>
<td>Digital ulcer</td>
</tr>
<tr>
<td>DUO</td>
<td>Digital ulcer outcomes</td>
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<tr>
<td>ERA</td>
<td>Endothelial receptor antagonist</td>
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<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
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<tr>
<td>ET-1</td>
<td>Endothelin-1</td>
</tr>
<tr>
<td>EULAR</td>
<td>European League Against Rheumatism</td>
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<tr>
<td>EUSTAR</td>
<td>European Scleroderma Trials and Research</td>
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<tr>
<td>GAVE</td>
<td>Gastric antral vascular ectasia</td>
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<tr>
<td>GTN</td>
<td>Glyceryl trinitrate</td>
</tr>
<tr>
<td>HFUS</td>
<td>High-frequency ultrasound</td>
</tr>
<tr>
<td>LeSSc</td>
<td>Limited cutaneous systemic sclerosis</td>
</tr>
<tr>
<td>LDI</td>
<td>Laser Doppler imaging</td>
</tr>
<tr>
<td>LED</td>
<td>Light emitting diode</td>
</tr>
<tr>
<td>MCTD</td>
<td>Mixed connective tissue disease</td>
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<tr>
<td>NAC</td>
<td>N-acetylcysteine</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>PAH</td>
<td>Pulmonary arterial hypertension</td>
</tr>
<tr>
<td>PDE5</td>
<td>Phosphodiesterase type-5</td>
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<tr>
<td>PRP</td>
<td>Primary Raynaud’s phenomenon</td>
</tr>
<tr>
<td>RP</td>
<td>Raynaud’s phenomenon</td>
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<tr>
<td>RHC</td>
<td>Right heart catheterisation</td>
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<tr>
<td>SLE</td>
<td>Systemic lupus erythematosus</td>
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<tr>
<td>SRC</td>
<td>Scleroderma renal crisis</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>SRP</td>
<td>Secondary Raynaud’s phenomenon</td>
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<td>SSc</td>
<td>Systemic sclerosis</td>
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<tr>
<td>TGF-β</td>
<td>Transforming growth factor-beta</td>
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<tr>
<td>UKSSG</td>
<td>UK Scleroderma Study Group</td>
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<tr>
<td>VEDOSS</td>
<td>Very early diagnosis of systemic sclerosis</td>
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<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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Abstract

The University of Manchester

Michael Hughes

PhD (Medicine)

Digital ulcers in systemic sclerosis: investigating the outcome measures of treatment efficacy, pathophysiology, and the development of local treatments

August 2016

Introduction: Digital ulcers (DUs) are responsible for much of the pain and disability associated with systemic sclerosis (SSc), and are a biomarker of internal organ involvement and poor prognosis. DUs are often used as the primary end-point in SSc clinical trials, although the reliability of rheumatologists in grading DUs is poor to moderate at best. Fingertip DUs are believed to be ischaemic in aetiology, whereas, extensor DUs are thought to occur due to mechanical factors and recurrent microtrauma. Treatments for DUs are often poorly tolerated due to systemic vasodilation. The overarching aim was to investigate the definition and objective measurement of SSc-related DUs, their pathophysiology, and a new light treatment.

Method: Five studies were undertaken. (1) A web-based study in which photographs of digital lesions were graded, all either with or without clinical context. (2) A pilot study to assess the feasibility and tolerability of high-frequency ultrasound (HFUS) imaging to measure DUs. (3) A retrospective study examining whether thermographic abnormalities are associated with DUs. (4) A double-blind, randomised, crossover, controlled study of glyceryl trinitrate (GTN) to explore the pathophysiology of DUs in SSc. (5). A feasibility study of a novel light (red, infrared and blue) device to treat SSc-related DUs.

Results: (1) 51 rheumatologists graded ≥4500 images. The clinical context (without vs with, weighted kappa statistic) did not significantly improve the intra- (0.32,0.36) or inter-rater (0.64,0.71) reliability. (2) HFUS was performed on 15 DUs and was well tolerated and feasible in the majority, DU measurement was possible in most (n=13) DUs, the mean DU depth and width were 0.99mm and 5.74mm, respectively. (3) Patients (n=138) with abnormal (compared to normal) thermography were more likely (adjusted odds ratio = 2.84) to develop future DUs, including multiple episodes. (4) 16 DUs were studied; the microvessels of the DU centre were responsive to GTN, with an increase in perfusion, with a similar effect in both fingertip and extensor DUs. There was less of a clear signal in the DU periphery. (5) Light treatment was safe, feasible and well tolerated (46 light treatments administered in 8 patients, one studied on three separate occasions). There was a significant improvement (change in visual analogue score per visit) in DUs as assessed by both patient (-7.1, P=<0.001) and clinician opinion (-5.2, P=<0.001). DU perfusion (measured by LDI) significantly increased post-treatment.

Conclusion: The reliability of DU grading did not improve with clinical context. HFUS was feasible and well tolerated, and measurement was possible in most DUs. Our data suggests that many DUs might have an ischaemic drive, including extensor DUs. A novel light treatment was safe, feasible and well tolerated, with a tentative suggestion of treatment efficacy.
Declaration

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Rationale for submission of the PhD thesis in the alternative format

The rationale for submitting this thesis in the alternative ‘journal’ style is that the five included studies fit well with this type of format. The key themes in the research presented in this thesis relate to the measurement of DUs (to facilitate future clinical trials) and to develop new treatments, which are locally acting and free of side effects, based upon a better understanding of the pathophysiology of DUs in SSc.

To date, two of these studies have been published in peer-reviewed journals, one has been submitted and is under peer-review, and the other two studies are formatted with a view to submission in the near future. In addition, I have published a number of review articles on digital vascular disease in SSc. Where these articles are used within the introduction section, I have indicated this and provided the relevant reference. My supervisory team were all in agreement about the suitability of the presented work to be submitted in the alternative format. I have received the required prospective approval from the University of Manchester (Faculty of Medical and Human Sciences) to submit the thesis in the alternative format.

The structure of the thesis is as follows. The introduction has been written in the same style as a traditional thesis with a corresponding reference list towards the end of the thesis. Then follow five experimental chapters, which are self-contained, with a separate reference list at the end of each one (some, if not all, of the references may be duplicated in the final reference list). The two chapters, which have been published, and the chapter which is under peer-review, are presented in the individual journal style/formatting, whereas, the other chapters are presented in a style suitable for submission to peer-reviewed journals. Finally, the thesis concludes with a focussed discussion and concluding remarks to pull together the overarching themes, with a strong focus on the future research that is indicated on DUs in SSc.
Summary of my contribution to the research presented in the PhD thesis

I was involved in all aspects of the research as described below.

- Development of the research questions and design of all the studies.
- Successful funding application to complete all the studies.
- Establishing collaborations to conduct the studies, including (but not limited to) Medical Photography and Medical Physics at Salford Royal Hospital.
- Completing all the required ethical and regulatory approvals (Appendix 9.1).
- Patient recruitment/coordination of all the studies including the associated administrative work.
- Data acquisition in all the studies:
  - Study 1: Collection of the patient clinical, including DU, information, involvement in the design of the website (constructed by Mr Andrew Tracey and colleagues, The University of Manchester), and inviting expert rheumatologists to participate in the study.
  - Study 2: Collection of patient information, performing high-frequency ultrasound (HFUS) on all patients, and through consensus agreement (with Andrea Murray and Graham Dinsdale) making measurements on the HFUS images.
  - Study 3: Data acquisition through blinded review of the case notes.
  - Study 4: Prescription completion for pharmacy to dispense the randomised study drug and performing laser Doppler imaging (LDI).
  - Study 5: Collection of patient clinical, including DU related information, performing LDI before and after light treatment, application and monitoring of the light-based treatment.
- Statistical analysis in all the studies:
  - Study 1: I performed the analysis under the supervision of Professor Christopher Roberts.
  - Studies 2, 4 and 5: I performed the analyses under the supervision of Mr Jack Wilkinson.
  - Study 3: I performed the initial analysis and descriptive statistics. However, Mr Jack Wilkinson performed the final analysis included in this thesis.
- Interpretation of the results in all the studies.
- Presentation of the findings at local, national and international meetings.
- Writing of all the manuscripts submitted for publication in peer-reviewed journals.
Acknowledgments

First and foremost, I want to thank Professor Ariane Herrick for her guidance and encouragement during the PhD, and further inspiring my passion to pursue a career as a Clinical Academic Rheumatologist. I also am deeply grateful to my other two co-supervisors, Dr Andrea Murray and Professor Chris Roberts, for their support.

I was very lucky indeed to work (and constantly learn) as part of the multidisciplinary scleroderma research team during my PhD, and these studies would not of been completed without such a great team effort. I thank Mrs Tonia Moore and Mrs Joanne Manning for their help with the studies, including performing some of the laser Doppler imaging. I also want to thank Dr Graham Dinsdale for being a great source of knowledge on a wide range of topics, and Mr Jack Wilkinson for his tutoring and support on the statistical analyses. I also acknowledge Professor William Dixon for his wise counsel as my Advisor.

I am very grateful to have received the funding to complete my clinical research fellowship leading to submission of this PhD, initially from the Manchester NIHR Biomedical Research Unit, and from Arthritis Research UK. In particular, I would like to acknowledge the input from Medical Physics at Salford Royal NHS Foundation Trust in the design of the light device. I learnt a great deal from our many discussions about the device and the conduct of medical device studies. I am overwhelming indebted to the many patients who have kindly participated in the studies during my PhD. Without their input I would not have any findings to present in this thesis.

I would like to thank my family for their support in my medical career to date. I thank my fiancéé’s family for welcoming me in to their home. Finally, I would like to thank Rachael, my fiancée, for her endless support and kind words through my PhD, and who is now well versed in digital ulcers and systemic sclerosis!
The author

I passionately aspire to pursue a career as a Clinical Academic Rheumatologist.

I graduated from St Bartholomew’s and the Royal London Medical School (MBBS, Bachelor of Medicine and Bachelor of Surgery) in 2007, after intercalating to graduate BSc (First Class Honours) in Human Biosciences in 2006. I gained the MRCP (Membership of the Royal Colleges of Physicians of the United Kingdom) in 2009. I developed an interest in academic rheumatology at medical school, and during my Foundation and Core Medical Training in the North and South London rotations, respectively.

I was appointed an NIHR Academic Clinical Fellow in the North West deanery in 2011 and completed an MSc (with Merit) in Clinical Rheumatology. During this time I developed a keen interest in systemic sclerosis and in particular, digital vascular disease.

In 2013 I was appointed to a NIHR Clinical Training Fellowship (NIHR Manchester Musculoskeletal Biomedical Research Unit) and subsequently an Arthritis Research UK Clinical Research Fellowship. This PhD thesis is the result of both these fellowships.

To pursue my passion as a Clinical Academic Rheumatologist, I intend to apply for an NIHR Academic Clinical Lecturer position at the University of Manchester towards the end of 2016.
Publications arising from the work included in this thesis*


*Includes publications related to SSc and digital vascular disease, including RP, arising during my clinical research fellowship.


Book chapters


Conference contributions arising from this thesis*


*Includes contributions related to SSc and digital vascular disease, including RP, arising during my clinical research fellowship.


Prizes awarded for the work undertaken in this thesis

January 2016 1st place (oral presentation). PhD Showcase. The University of Manchester. Lighting the way for digital ulcers in systemic sclerosis: the development of local treatments, pathophysiology and outcome measures of efficacy.

1. Introduction

1.1. Background to SSc

Systemic sclerosis (SSc) is a complex autoimmune connective tissue disease (CTD), characterised by microvascular abnormalities, immune system activation and fibrosis of the skin and other internal organs (1–3). In recent years there have been great advances in the understanding of the aetiopathogenesis of SSc, which has translated into the availability of a number of effective treatments for many of the organ-based complications. Despite this progress, SSc is associated with significant morbidity and mortality. Positively, there is an increasing international interest in SSc, which is fostering the development of high-quality basic and clinical collaborative research.

1.1.1. Epidemiology

The worldwide prevalence and incidence of SSc has varied significantly in the reported literature (4–6). In a systematic literature review, which included 32 articles (published between 1969 and 2006), the prevalence of SSc ranged between 7 to 489 per million and the incidence of the disease was 0.6 to 122 per million/year (4). There is also a marked reported geographical variation in the epidemiology of SSc (4). For example, the prevalence of SSc in the United States of America is 276 per million (in 1990) (7) compared to 88 per million (in 2000) in the United Kingdom (8). Akin to other rheumatic diseases, SSc is more common in females than males (ratio 3:1 to 8:1) (9,10), with a peak age of onset between 20 and 50 years (7,10). Later onset SSc is associated with a higher frequency of internal organ complications and mortality (10). Several studies have reported that males have a more severe disease course than females (11,12). Race appears to have an important impact on SSc, with black patients compared to their Caucasian counterparts often presenting both earlier and with diffuse cutaneous SSc (dcSSc) (7,13), which has a worse prognosis than limited cutaneous SSc (lcSSc).
1.1.2. The pathogenesis of SSc

Although a full description of the pathogenesis of SSc (Figure 1.1) is beyond the remit of this thesis, a focused overview helps the reader to understand the mechanisms for many of the organ-based complications of the disease and the rationale for their treatment.

Figure 1.1. Overview of the pathogenesis of SSc. In genetically susceptible individuals, environmental factors trigger the onset of SSc. There is a complex interplay of vascular, immune and fibrotic abnormalities in the pathogenesis of SSc. It is likely that epigenetic factors play a key role in determining the onset of disease, linking genetic and environmental factors. Figure taken from Katsumoto (2).
Genetic

A number of genetic risk factors for SSc have been described, although the absolute contribution to the development of the disease is considered to be minimal. A family history of SSc is associated with a high risk of the disease, although the majority of patients with SSc will lack a family history. In a study which included three SSc cohorts in the United States of America, although family members were more likely to develop SSc than in the general population, the risk was comparatively low (1.6% vs 0.026%, respectively) (14). Furthermore, in a study which investigated the genetics of SSc in monogenetic and dizygotic twins, no significant difference in the concordance of the disease was observed (4.2% vs 5.6%, respectively) (15). In recent years using advanced genetic technologies (i.e genome wide association and immunoChIP [chromatin immunoprecipitation]) studies, a number of genetic variants (single nucleotide polymorphisms) have been described in SSc (3). These variants have been linked to both the susceptibility of the disease and specific autoantibody and disease profiles (3). Of mechanistic interest, many of these variants have a functional role in immune regulation (3), including within the major histocompatibility complex (which has a major role in antigen presentation) (16).

Epigenetics

Given the relatively small contribution of genetic risk to the development of SSc, attention has turned to the emerging field of epigenetics. Epigenetics relates to the changes in gene expression which are not directly caused by changes in genetic (deoxyribose nucleic acid [DNA]) sequence (17,18). The mechanisms involved in epigenetics include (but are not limited to) changes in ‘DNA methylation, histone code modifications and changes in microRNA expression levels in SSc cells’ (18). A number of factors (e.g. diet and hypoxia) have been reported to be associated with the development of epigenetic modifications (17,18). In particular, oxidative stress (which has been implicated in the pathogenesis of SSc) appears to be a key inducer of epigenetic changes (17,18). Various epigenetic alterations have been described in SSc including in key pro-fibrotic pathways (e.g. Transforming growth factor-beta [TGF-β]), with a number of microRNAs differentially expressed (e.g. increased miR-21) in
patients with SSc (17,18). Epigenetics likely plays an important role linking genetic susceptibility and environmental factors in the complex pathogenesis of SSc (18).

**Environmental factors**

A number of environmental factors have been implicated in the pathogenesis of SSc including (but not limited to) silica dust, vinyl chloride and organic solvents (2). However, no definitive causative relationship between environmental factors and SSc has been established (2,3). A number of viruses (e.g. Epstein-Barr and cytomegalovirus) have also been implicated in the pathogenesis of SSc, although no direct causal relationship has been identified to date (2,3). Of mechanistic interest to SSc, the eosinophilia-myalgia syndrome and nephrogenic systemic fibrosis (both systemic fibrosing disorders and ‘scleroderma mimics’) are triggered through ‘environmental’ agents; namely, adulterated rape seed oil and gandolinium contrast in renal dialysis patients, respectively (2).

**Vascular**

The key importance of vascular disease is easy for both the patient and clinician to appreciate, involving digital vascular disease (Raynaud’s phenomenon [RP] and digital ulcers [DUs]), pulmonary arterial hypertension (PAH) and the scleroderma renal crisis (SRC). A number of vascular mechanisms have been implicated in the pathogenesis of SSc (Figure 1.2), and vascular injury and dysfunction is believed to be central (and perhaps the initiating event) (1–3).
Furthermore, skin biopsies from patients with early SSc have revealed evidence of endothelial cell death (2,20). Endothelial dysfunction likely plays a key role (21). In addition, autoantibodies directed toward endothelial cells have also been described in SSc (22,23). Increased expression of endothelial cell adhesion molecules (and increased vascular permeability) results in the local influx of inflammatory cells (2,3), which are associated with the production of potentially tissue damaging, pro-fibrotic cytokines. As vascular damage accumulates there is a loss of vessels with structural changes to the wall (in particular intimal and smooth muscle hypertrophy), which reduces the size of the lumen, resulting in local hypoxia and increased oxidative stress, both of which are pro-fibrotic triggers (1–3). Platelet abnormalities (e.g. activation) have also been described (20). In SSc, there is a defective vascular repair response despite loss of blood vessels and elevated pro-angiogenic factors (e.g. vascular endothelial growth factor) (2,20). Both elevated and reduced endothelial progenitor cells (involved in vascular repair) have been reported in SSc (24–26). Nailfold...
capillaroscopy (described later) allows the progressive vascular alterations (number and shape of vessels) to be examined in vivo.

**Fibrosis**

Tissue fibrosis (scarring) is a hallmark of SSc and follows the initial oedematous phase of the disease. Normal tissue is replaced with a fibrous matrix consisting of collagen, elastin, glycosaminoglycan and fibronectin (3). The key effector cell in fibrosis is the myofibroblast (3). TGF-β is considered to be the master cytokine in the development of tissue fibrosis (3). Other molecular triggers which have been implicated in the mechanism of fibrosis in SSc (in alphabetical order) include angiotensin-1, endothelin-1 (ET-1), interleukins 6 and 13 and platelet derived growth factor (2,3). Progressive collagen deposition results in increasing tissue stiffness and mechanical stress directly impacts on fibroblast function (3). The WNT pro-β-catenin intracellular signalling pathway is likely to play a key role in tissue fibrosis in SSc (including through TGF-β independent mechanisms) (3). Fibrillin-1 has also been highly implicated in the pathogenesis of SSc. The stiff skin syndrome (a form of congenital scleroderma), the high incidence of SSc in the Choctaw native Indian population, and the tight skin mouse model of SSc, have all been attributed to abnormalities in fibrillin-1 (27–29).

**Immune**

Activation of the immune (both innate and adaptive) system is evident throughout the course of the disease in SSc. In early SSc, skin biopsies show a rich, perivascular inflammatory infiltrate of a number of cells including B and T lymphocytes, monocytes and mast cells (1–3). In addition, both circulating immune cells and tissues show a prominent interferon type 1 signal, denoting immune activation (3,30). A range of cytokines and chemokines have been implicated in the pathogenesis of SSc, in particular interleukin-6 (3,31). In addition, the presence of characteristic circulating SSc-associated autoantibodies (discussed later) in the majority of patients with SSc highlights the activation of the humoral (antibody-mediated) immune system. It is increasingly recognised that in a subset of patients with SSc and anti-RNA polymerase III antibody, the disease is a paraneoplastic phenomenon to an underlying cancer (32,33).
1.2. The diagnosis and classification of SSc

1.2.1. The diagnosis of SSc

The diagnosis of SSc is made mainly through a combination of clinical features, with a key role for targeted investigations; in particular, nailfold capillaroscopy and SSc-associated autoantibodies. Classification criteria for SSc have been jointly developed by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) (Table 1.1) (34,35). These criteria are a useful tool for clinicians when assessing patients with possible SSc, although it must be highlighted that these are not intended as diagnostic criteria. These criteria have higher sensitivity and specificity (0.91 and 0.92, respectively) than the previous 1980 ACR preliminary classification criteria for SSc (0.75 and 0.72, respectively) (34,35). Of note, however, is a recent study from Norway which included patients with both possible SSc (n = 425) and mixed connective tissue disease (MCTD) (n = 178) (a SSc-spectrum disorder, discussed later), although almost all (96%) of patients with SSc met the ACR/EULAR criteria, around 10% of MCTD patients also fulfilled the criteria (36). With an increasing international interest in the earlier diagnosis of SSc, and in part to facilitate studies of early therapeutic intervention, criteria for both the early (37) and very early diagnosis (38) of SSc have been proposed. Using the Very Early Diagnosis of SSc (VEDOSS) criteria, SSc should be suspected in the presence of one of three ‘red flags’: RP, puffy fingers or the presence of antinuclear antibodies (ANA), with the diagnosis being made in the presence of either abnormal nailfold capillaroscopy and/or SSc-associated autoantibodies (38).
<table>
<thead>
<tr>
<th>Item</th>
<th>Sub-item(s)</th>
<th>Weight/score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin thickening of the fingers of both hands extending proximal to</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>the metacarpophalangeal joints (sufficient criterion)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin thickening of the fingers (only count the higher score)</td>
<td>Puffy fingers</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Sclerodactyly of the fingers (distal to the metacarpophalangeal joints but proximal to the proximal interphalangeal joints)</td>
<td>4</td>
</tr>
<tr>
<td>Fingertip lesions (only count the higher score)</td>
<td>Digital tip ulcers</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Fingertip pitting scars</td>
<td>3</td>
</tr>
<tr>
<td>Telangiectasia</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Abnormal nailfold capillaries</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>PAH and/or interstitial lung disease</td>
<td>Pulmonary arterial hypertension</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Interstitial lung disease</td>
<td></td>
</tr>
<tr>
<td>RP</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>SSc related autoantibodies (maximum score is 3)</td>
<td>Anticentromere</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Anti-topoisomerase I</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-RNA polymerase III</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.1. The ACR/EULAR classification criteria for SSc. Patients with a score of $\geq 9$ are classified as SSc. In addition, and as stated by the authors (34,35): ‘These criteria are applicable to any patient considered for inclusion in an SSc study. The criteria are not applicable to patients with skin thickening sparing the fingers or to patients who have a scleroderma-like disorder that better explains their manifestations (e.g. nephrogenic sclerosing fibrosis, generalized morphoea, eosinophilic fasciitis, scleredema diabeticorum, scleromyxedema, erythromyalgia, porphyria, lichen sclerosis, graft-versus-host disease, diabetic cheiroarthropathy)’. PAH: Pulmonary arterial hypertension. RP: Raynaud’s phenomenon. SSc: Systemic sclerosis.
1.2.2. The classification of SSc

A spectrum of SSc disorders is recognised from RP (with abnormal nailfold capillaroscopy and/or SSc-associated autoantibodies) to localised scleroderma (e.g. morphea) and other autoimmune CTDs (e.g. MCTD). Two subsets of the disease (lcSSc and dcSSc) are recognised (Table 1.2), the distinction between the two is currently based largely upon the extent of the skin sclerosis. Around 20% of patients have overlap SSc with evidence of a second defined CTD (e.g systemic lupus erythematosus [SLE] or myositis) (39,40). Rarely, SSc may occur in the notable absence of skin sclerosis (SSc sine SSc) (41,42), and this clinically resembles, in terms of organ-based complications and prognosis, lcSSc (42).

<table>
<thead>
<tr>
<th>LcSSc</th>
<th>DcSSc</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP for many years before the onset of the skin change</td>
<td>Abrupt onset of RP within a year (before or after) of the skin change</td>
</tr>
<tr>
<td>Skin involvement limited to the hands, forearms, feet, distal lower limbs (to the level of the knees) and face</td>
<td>As per limited disease, but with also truncal and proximal (acral) skin involvement</td>
</tr>
<tr>
<td>Late onset of PAH, trigeminal neuralgia and telangiectases</td>
<td>Early onset of pulmonary fibrosis, renal failure, myocardial infarction and diffuse gastrointestinal disease</td>
</tr>
</tbody>
</table>

Table 1.2. The subtypes of SSc (43). PAH: Pulmonary arterial hypertension. RP: Raynaud’s phenomenon.

As previously described, almost all patients with SSc have the presence of an SSc-associated (anticentromere, anti-topoisomerase I or anti-RNA III polymerase) antibody. These antibodies are diagnostically useful as reflected through their inclusion in both the ACR/EULAR SSc classification and VEDOSS criteria. In addition, the SSc-associated autoantibodies can help in determining the subset of SSc (i.e. anticientromere is associated IcSSc and anti-topoisomerase I and anti-RNA polymerase III with dcSSc). Also, the SSc-associated autoantibodies are highly useful biomarkers in clinical practice of disease course, including the development of organ-based complications.
For example, anticentromere and anti-RNA polymerase III autoantibodies are associated with the development of PAH and the SRC, respectively.

1.3. The clinical course of SSc and approach to treatment

SSc denotes a highly heterogeneous, autoimmune CTD with additional involvement of the other internal organs: clinical course can be highly variable. Figure 1.3 summarises an overview of the contemporary management of SSc. Patients with SSc should be managed as part of a dedicated multidisciplinary team including colleagues from (but not limited to) specialist nursing, physiotherapy, occupational therapy and podiatry. Table 1.3 summarises some of the key organ-based complications (with examples of drug treatments). Key targeted drug therapies include phosphodiesterase type-5 (PDE5) inhibitors and endothelin-receptor antagonists (ERAs) for PAH and digital vascular disease (RP and DUs), and angiotensin receptor enzyme (ACE) inhibitors for SRC. Patients with early dcSSc need to be identified as a priority, with a view to the introduction of immunosuppressive therapy which may positively modify both the subsequent skin and internal organ involvement (44). In general, the currently used drug therapies in SSc have either a vascular or immunosuppressive mechanism of action. However, of relevance to the key role of tissue fibrosis in the pathogenesis of SSc, two drug treatments (pirfenidone and nintedanib) have been recently licensed for the treatment of idiopathic interstitial lung disease. Research into anti-fibrotic agents in SSc is ongoing, including clinical trials of novel therapies. The subset of patients with early dcSSc who are believed to be at the highest risk of disease progression (including death) should be considered for autologous stem cell transplant (45,46). However, due to concerns about the potential serious toxicity (including death) associated with the technique, this needs to be considered by the clinician on an individual case basis (44).

Whereas previously the leading course of death from SSc was the SRC this has now been superseded by lung involvement (PAH and interstitial lung disease) (47). Life expectancy is reduced in patients with SSc by between 16 and 34 years (approximately), compared to age and sex-matched healthy controls (48).
Figure 1.3. Overview of the management of patients with SSc. GI: Gastrointestinal (GI). LcSSc: Limited cutaneous systemic sclerosis. DcSSc: Diffuse cutaneous systemic sclerosis. Figure taken from the British Society for Rheumatology and the British Health Professionals in Rheumatology guideline for the treatment of SSc (44)
<table>
<thead>
<tr>
<th>Organ system</th>
<th>Clinical manifestation</th>
<th>Drug treatment (where appropriate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin and musculoskeletal</td>
<td>Scleroderma (including early dcSSc)</td>
<td>Immunosuppressive therapy (e.g. cyclophosphamide, methotrexate, mycophenolate mofetil)</td>
</tr>
<tr>
<td></td>
<td>Inflammatory arthritis</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Inflammatory cardiac disease (e.g. myocarditis)</td>
<td>Immunosuppressive therapy (e.g. steroid and/or cyclophosphamide)</td>
</tr>
<tr>
<td></td>
<td>Heart failure</td>
<td>Cardiovascular therapies traditionally used in heart failure (e.g. ACE inhibitors and diuretics)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Oesophageal reflux disease</td>
<td>Proton pump inhibitors</td>
</tr>
<tr>
<td>Peripheral vascular</td>
<td>RP and DUs</td>
<td>Vascular therapies (e.g. angiotensin II receptor blockers, calcium channel blockers, ERAs, prostacyclin analogues and PDE5 inhibitors)</td>
</tr>
<tr>
<td>Respiratory</td>
<td>Interstitial lung disease</td>
<td>Immunosuppressive therapy (e.g. cyclophosphamide, methotrexate, mycophenolate mofetil)</td>
</tr>
<tr>
<td></td>
<td>PAH</td>
<td>Vasoactive therapies (e.g. ERAs, prostacyclin analogues and PDE5 inhibitors, soluble guanylate cyclase agonists)</td>
</tr>
<tr>
<td>Renal</td>
<td>SRC</td>
<td>ACE inhibitors</td>
</tr>
</tbody>
</table>

1.4. Vascular disease in SSc

As previously described, vascular disease is strongly implicated in the pathogenesis of SSc, and is responsible for many of the early and later complications of the disease. The key vascular organ-based complications in SSc will now be described. DUs, which are the subject of the experimental component of this thesis, shall be discussed afterward.

1.4.1. Digital vascular disease in SSc

A spectrum of digital vascular disease, i.e. RP, DUs and critical digital ischaemia, is almost universally observed in patients with SSc. Both microvascular and macrovascular disease have been implicated in the development of digital vascular disease in SSc. As previously described, the progressive microvascular structural disease in SSc (which can be observed by capillaroscopy) results in impaired digital perfusion. In addition, abnormalities in the larger blood vessels, in particular the digital and ulnar arteries (49–54), have been reported in patients with SSc. Although an increased risk of cardiovascular disease has been reported in the literature (55,56), this remains a controversial issue.

Raynaud’s phenomenon

This section has been adapted from Hughes and Herrick (57)

RP (Figure 1.4) refers to the episodic colour change typically of the extremities (hands and feet) in response to cold exposure (including even a slight drop in ambient environmental temperature) and/or emotional stressors. Other vascular beds, which are commonly involved in attacks of RP, include the nose, lips and ears. The skin progresses through a stereotypical series of colour changes (physiological mechanisms in parentheses) of white (ischaemia), blue (cyanosis) and red (hyperaemia), the last of which may be associated with significant pain.
Figure 1.4. Raynaud’s phenomenon. Photographs of an attack of Raynaud’s taken by a patient with SSc using a smartphone camera. A: There is whiteness (pallor of all four fingertips). B: Normal colour has been restored to the fingers. The time between the two photographs was approximately two and a half minutes. Figure taken from Hughes and Herrick (57).

The majority of patients with RP will have primary (idiopathic) RP (PRP), which is ‘benign’ (i.e. does not result in irreversible ischaemic tissue damage). However, the clinician must be aware of the wide range of causes of secondary RP (SRP) as presented in Table 1.4, including autoimmune CTDs, and in particular SSc. Key investigations in the assessment of patients presenting with RP are nailfold capillaroscopy and testing for autoantibodies (in particular, those associated with SSc). Clinicians should be aware of the criteria for PRP proposed by LeRoy and Medsger (58), when assessing patients with RP as presented below.

- Episodic attacks of acral pallor or cyanosis with strong and symmetric pulses, in the absence of digital pitting, ulceration or gangrene.
- Normal erythrocyte sedimentation rate (ESR).
- Negative ANA titre (titre < 1:100).
- Normal nailfold capillaries.
<table>
<thead>
<tr>
<th>Primary (idiopathic) RP</th>
<th>Secondary RP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vascular (usually proximal large vessel disease, often unilateral symptoms)</strong></td>
<td>Compressive (e.g. cervical rib)</td>
</tr>
<tr>
<td></td>
<td>Obstructive: non-inflammatory (i.e. atherosclerosis)</td>
</tr>
<tr>
<td></td>
<td>Inflammatory vascular disease (e.g. thromboangiitis obliterans [Buerger’s disease])</td>
</tr>
<tr>
<td><strong>Hand–arm–vibration syndrome (vibration white finger)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Autoimmune conditions</strong></td>
<td>SSc</td>
</tr>
<tr>
<td></td>
<td>SLE</td>
</tr>
<tr>
<td></td>
<td>Sjogren’s syndrome</td>
</tr>
<tr>
<td></td>
<td>MCTD/overlap syndromes</td>
</tr>
<tr>
<td></td>
<td>Undifferentiated connective tissue disease</td>
</tr>
<tr>
<td></td>
<td>Idiopathic inflammatory myopathies</td>
</tr>
<tr>
<td><strong>Drug-related</strong></td>
<td>Amphetamines</td>
</tr>
<tr>
<td></td>
<td>Beta-blockers</td>
</tr>
<tr>
<td></td>
<td>Bleomycin</td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
</tr>
<tr>
<td></td>
<td>Clonidine</td>
</tr>
<tr>
<td></td>
<td>Cyclosporine</td>
</tr>
<tr>
<td></td>
<td>Interferon a and b</td>
</tr>
<tr>
<td></td>
<td>Methysergide</td>
</tr>
<tr>
<td></td>
<td>Polyvinyl chloride</td>
</tr>
<tr>
<td><strong>Conditions associated with increased plasma viscosity and reduced digital perfusion</strong></td>
<td>Cryoglobulinaemia</td>
</tr>
<tr>
<td></td>
<td>Cryofibrinogenaemia</td>
</tr>
<tr>
<td></td>
<td>Paraproteinaemia</td>
</tr>
<tr>
<td></td>
<td>Malignancy (including as a paraneoplastic phenomenon)</td>
</tr>
<tr>
<td><strong>Other causes and associations</strong></td>
<td>Carpal tunnel syndrome</td>
</tr>
<tr>
<td></td>
<td>Frostbite</td>
</tr>
<tr>
<td></td>
<td>Hypothyroidism</td>
</tr>
</tbody>
</table>

Table 1.4. The differential diagnosis of Raynaud’s phenomenon. Table from Hughes and Herrick (57).

Almost all patients with SSc have RP, which is often the presenting feature of the disease. In an analysis of patients from the European Scleroderma Trials and Research (EUSTAR) group which included 7655 patients with SSc, over 95% of patients had a history of RP, with no difference observed between patients with lcSSc and dcSSc disease subtypes (96.6% and 96.1%, respectively) (59). Patients
with lcSSc often present with a long history (often decades) of RP, whereas, in patients with dcSSc, the onset of RP typically occurs within a year (either before or after) of the onset of skin sclerosis (Table 1.2) (43). The importance of RP in patients with SSc is reflected by its inclusion in the ACR/EULAR classification criteria for SSc (presence of RP scoring three points out of the 9 required for the classification of SSc) (34,35). As previously described, RP is one of only three key features or ‘red flags’ which should alert the clinician to suspect the diagnosis of VEDOSS (38). In an analysis of 469 patients with RP enrolled in the VEDOSS cohort (60), those patients who were ANA positive (compared to ANA negative) were more likely to have a SSc pattern on capillaroscopy (53.6% versus 13.4%, respectively). Additionally, vascular complications (telangiectasias, current DUs and pitting scars) were more common in ANA positive RP patients. In patients with SSc, RP is often very severe, and can progress to digital ulceration and sometimes to gangrene.

RP has a marked impact on an individual's quality of life, irrespective of aetiology. Patients with SSc rated RP the second most severe feature of the disease (fatigue being first) in terms of frequency and moderate to severe impact on daily activities (61). Furthermore, in an online survey which included responses from 443 individuals with self-reported RP, most (78%) had made at least one life change due to RP, with more in subjects with SRP compared to PRP (87% vs 71%) (62). Similarly, current quality of life with RP was impaired in both PRP and SRP (6.5 and 5.2 out of a possible 10, respectively) (62).

The UK Scleroderma Study Group (UKSSG) best practice treatment algorithm on the management of RP in SSc is presented in Figure 1.5. Patient education is absolutely essential in the management of RP. Patients should be advised to keep warm and to avoid cold ambient environments. Smoking cessation should be strongly encouraged as this promotes vasoconstriction. Drug treatment is often indicated where conservative measures are insufficient to control symptoms, particularly in patients with SRP. In general, drug treatments rely upon systemic vasodilation, with the aim to try and increase perfusion to the extremities. However, as a result, these drug therapies are often poorly tolerated. Drug therapies are started
at the lowest dose and gradually increased if not effective and/or associated with significant side effects resulting in dose reduction or drug discontinuation. First line drug therapy for RP (including in SSc) is often with calcium channel blockers, although with changing health care economics, it is likely that in the future there will be earlier use of PDE5 inhibitors.
Figure 1.5. The UKSSG best practice recommendations on the management of Raynaud’s phenomenon. ACE: angiotensin converting enzyme. ARB: Angiotensin receptor blocker. CCB: calcium channel blocker. IV: intravenous. PDE5: Phosphodiesterase type-5. SSRI: Selective serotonin receptor inhibitor. Figure taken from Hughes et al (63).
Critical digital ischaemia

This section has been adapted from Hughes and Herrick (57)

Critical digital ischaemia (Figure 1.6) is a true medical emergency. Critical digital ischaemia (along with DUs) represents the most severe end of the spectrum of digital vascular disease in patients with SSc. Patient education on this matter is crucial and all patients with RP should be counselled to seek urgent medical attention if any of the digits becomes permanently discoloured. In a large retrospective study (1168 patients with SSc, 18 months follow-up) from the Royal Free Hospital, London, 19 (1.6%) and 16 (1.4%) patients developed critical digital ischaemia or gangrene, respectively, which resulted in loss of the digit (either by autoamputation or surgical amputation) in 11 (0.9%) of patients in the cohort (64).

Figure 1.6. Critical digital ischaemia in a patient with SSc. Left: Critical digital ischaemia of the left middle, ring and little fingers. Top right: Histology specimen of the amputated specimen showing intimal sclerosis with complete occlusion of the digital artery. Bottom right: Resultant mummification of the middle finger due to critical digital ischaemia. Figure taken Sharp et al (65).
Both anti-centromere and anti-beta-2-glycoprotein antibodies have been reported to be associated with the development of critical digital ischaemia in patients with SSc (66–68). Smoking is a risk factor for more severe digital vascular disease in SSc (69). Patients with SSc may be at an increased risk of macrovascular disease (described later); therefore, the peripheral pulses must always be examined in patients presenting with ischaemic digits. If there is ongoing concern, vascular imaging (with Doppler arterial ultrasound in the first instance) should be performed early in patients with critical digital ischaemia to exclude significant large vessel disease, as this is potentially amenable to revascularisation procedures. In patients with autoimmune CTDs, the clinician must remain vigilant and consider the range of other causes of critical digital ischaemia (Table 1.5).

<table>
<thead>
<tr>
<th>SSc related</th>
<th>Non-SSc related</th>
</tr>
</thead>
<tbody>
<tr>
<td>• SSc non-inflammatory angiopathy (affecting the microvessels and digital arteries)</td>
<td>• Proximal large vessel disease</td>
</tr>
<tr>
<td>• Vasculitis (e.g. overlap syndromes with rheumatoid arthritis and SLE)</td>
<td>• Smoking</td>
</tr>
<tr>
<td>• Cryoglobulinaemia</td>
<td>• Thrombophilia: Congenital and acquired (e.g. antiphospholipid syndrome)</td>
</tr>
<tr>
<td></td>
<td>• Embolic disease: thrombotic and septic emboli (rare)</td>
</tr>
<tr>
<td></td>
<td>• Paraproteinaemia</td>
</tr>
<tr>
<td></td>
<td>• Paraneoplastic phenomenon</td>
</tr>
</tbody>
</table>

Table 1.5. The causes of critical digital ischaemia in patients with SSc. Table from Sharp et al (65). SLE: Systemic lupus erythematosus. SSc: Systemic sclerosis.

The UKSSG best practice treatment algorithm on the management of critical digital ischaemia in SSc is presented in Figure 1.7. The evidence base for guiding the management of critical digital ischaemia in SSc is lacking at present. As a generalisation, the management of patients with SSc presenting with critical digital ischaemia is similar to those patients without an underlying rheumatological
condition. Patients should be admitted on an emergency basis and their analgesic regimen reviewed and optimised early during the course of their admission, often requiring opioid-based analgesia. Critically ischaemic digits are often infected, and clinicians should have a low threshold to prescribe appropriate (often intravenous) antibiotic therapy, as per local microbiological prescribing practice. Many clinicians also consider antiplatelet therapy and anticoagulation in patients with critical digital ischaemia; however, there is no evidence at present to support either of these interventions. Patients are often prescribed intravenous prostanoid therapy (to promote dilatation of the digital vessels), in an attempt to save the digit. In patients with an associated vasculitic process, steroid and/or immunosuppressant therapy should be considered.

The critically ischaemic digit often will spontaneously autoamputate (commonly with an improvement in pain); however, surgical debridement and/or amputation of necrotic tissue may be required, including for cosmesis. Botulinum toxin injection and digital sympathectomy may be considered in patients with critical digital ischaemia, although the evidence to support these interventions is limited at present, and high-quality research is required in this area.
Management of Critical Digital Ischaemia

1. Establish diagnosis and identify any treatable contributory cause

2. Treat any contributory cause
   - Large (proximal) vessel disease
   - Vasculitis
   - Coagulopathy
   - Thromboembolism
   - Smoking

3. Admit for IV prostanoid and analgesia
   + Antiplatelet therapy

4. Effective

5. Consider statin
   + Antibiotic if any possibility of infection

6. Ineffective

7. Optimise oral vasodilator therapy (consider PDE5 inhibitor)

8. Consider digital sympathectomy
   + Surgical debridement if necrotic tissue

9. Short term anticoagulation
   ±
1.4.2. Macrovascular disease

An increased prevalence of proximal (large) vessel disease has been reported in patients with SSc, including the digital artery (49–52), superficial palmar arch (50) and with selective involvement of the ulnar artery (50,53,70,71), the last of which is associated with DUs (described later). Macrovascular involvement has been studied in SSc using a number of techniques including (but not limited to) invasive (catheter) and non-invasive magnetic resonance angiography, and Doppler ultrasound to measure the ankle brachial pressure index (ABPI) (a marker of peripheral vascular disease). In SSc, diffuse involvement of both the arterial and venous systems has been described (50–52), and with similar reported involvement in the lower (compared to the upper) limbs (50). In particular, worsening ABPI has been associated with anti-centromere antibody positivity (72–74). Increasing disease duration (52,74) and age (74) have also been associated with increased ABPI in SSc, whereas there is conflicting evidence for smoking and the limited cutaneous subtype (72–74). Lower limb amputation secondary to large vessel disease has been reported in patients with SSc (75,76).

1.4.3. Other organ-based vascular complications

Pulmonary arterial hypertension

PAH is a serious, life-threatening complication, which occurs in approximately 8% to 13% of patients (as diagnosed on right heart catheterisation [RHC]) (77–79). PAH is defined (on RHC) as a mean pulmonary arterial pressure of ≥ 25 mmHg with a pulmonary wedge pressure of ≤ 15 mmHg (80). Risk factors for the development of PAH have been reported to include lcSSc, longer disease duration and RP, the presence of anti-centromere autoantibodies and a higher burden of cutaneous telangiectases (3). Patients are often asymptomatic in the early stages of PAH, which highlights the importance of screening with a view to therapeutic intervention. As the pressure insidiously elevates, the patient develops a number of clinical features including progressive dyspnoea and symptoms of right heart failure (cor pulmonale). A number of clinical risk calculators have been proposed to predict patients’ risk of the development of PAH (81–83). Meune et al (81) propose a simple risk calculator
based upon clinical parameters including pulmonary function testing, with referral for RHC if over a defined threshold. The Australian Scleroderma Interest Group have developed a tool using both pulmonary function parameters and N-terminal pro b-type natriuretic peptide (NTproBNP) with referral for RHC if either of these are considered abnormal (of note, transthoracic echocardiogram is only recommended if clinically indicated) (82). The DETECT algorithm (83) is a two-step process in which clinical risk factors for PAH are first identified (pulmonary function abnormalities, anti-centromere autoantibody positivity, serum urate, right axis deviation and NTproBNP). In patients at risk of PAH, transthoracic echocardiogram is subsequently performed, with onward referral for RHC if particular abnormalities suggestive of PAH are found (83). PAH has a dramatic impact on patient survival and is a leading cause of death in SSc (47,84), with a mortality of 50% within 3 years of diagnosis (85). There are now a number of effective treatments (Table 1.3) available for PAH including (but not limited to) ERAs, PDE5 inhibitors and soluble guanylate cyclase inhibitors.

**Scleroderma renal crisis**

SRC is another potentially life-threatening vascular organ-based complication in SSc, affecting 5% to 10% of patients (86). SRC presents with acute kidney injury and features of a systemic hypertensive emergency including pulmonary oedema, encephalopathy, retinopathy and microangiopathic haemolysis (86). Risk factors for the development of SRC include early disease duration (≤4 years), dcSSc subtype, rapidly progressing skin sclerosis, steroid therapy (≥15 mg/day), the presence of anti-RNA polymerase III autoantibodies, new cardiac involvement (e.g. pericardial effusion or congestive heart failure) and new anaemia (86–88). Historically, the mortality associated with SRC was high although, over the course of the past few decades, this has significantly improved, largely due to treatment with ACE inhibitor therapy. In the landmark study by Steen et al (89) which included 108 patients with SSc and SRC, between 1972 and 1987, treatment with (compared to without) ACE inhibitors was associated with a significant increase in one-year survival (76% vs 15%, respectively). Patient education is absolutely essential and those at highest risk should be under regular clinical review, with at least once weekly blood pressure measurement (44). Prompt recognition of the development of SRC with the early
introduction of ACE inhibitor therapy is important to maximise the chance of achieving the best outcome (44). Other anti-hypertensive agents may be required if there is difficulty in achieving control of systemic hypertension (86). Renal failure often (but not invariably) requires the introduction of renal replacement therapy (peritoneal dialysis or haemodialysis); although renal recovery may be seen up to several years after the onset of the SRC (88). In a study from the Royal Free cohort in London, which included 110 patients with SRC (between 1990 and 2005), dialysis was not required in 36% and was only required temporarily in 23% of patients (88). However, almost half of patients (41%) required dialysis permanently as a result of the SRC. Upregulation of the ET-1 axis has been described in SRC and could represent a future target for therapeutic intervention (90).

**Cardiovascular disease**

Akin to many other rheumatic diseases (e.g. rheumatoid arthritis and SLE) an increased risk of cardiovascular disease has been described in patients with SSc. In particular, an increased risk of myocardial infarction and stroke has been reported in patients with SSc (55,56). In a study which included 865 patients with SSc, the hazard ratios (compared to age, sex and time-matched controls) for myocardial infarction and stroke were 1.8 and 2.61, respectively (56). In addition, among the 858 patients with SSc included in the analysis, the hazard ratio for the development of peripheral vascular disease in SSc was 4.35 (56). Furthermore, in a systematic review, which included 14 studies, patients with SSc compared to healthy controls had both higher carotid intima-media thickness (mean difference 0.11 mm) and lower flow-mediated dilation (-3.1%) (55). Although in comparison, intima-media thickness, a marker of ‘early’ atherosclerosis (and other measures of endothelial activation) have also been reported to be normal in patients with SSc (91). As summarised by Ngian et al (92), there is conflicting evidence as to whether patients with SSc have increased arterial stiffness (another marker of an increased risk of cardiovascular disease). With similarities to other autoimmune rheumatic conditions, it has been suggested that increased atherosclerosis in SSc may be independent of traditional cardiovascular risk factors (e.g. hypertension and hypercholesterolemia) (91). It remains unclear whether cardiovascular disease occurs due to either
accelerated atherosclerosis or true macrovascular involvement in SSc (92–94), and future high-quality research is required on this subject, as this has important clinical implications.

**Gastric antral vascular ectasia**

Gastric antral vascular ectasia (GAVE) is probably unrecognised by clinicians, with a reported prevalence in patients with SSc between 1% and 22.3% (95,96). GAVE is often called ‘watermelon stomach’ due to its characteristic appearance on endoscopy, consisting of dilated blood vessels and parallel rugal folds arising from the gastric antrum and converging in the antrum (Figure 1.8A) (97,98). In general, the limited literature has suggested an association between GAVE and anti-RNA polymerase antibody positivity (95,99,100), although one study did not find such an association (96). In an analysis from the EUSTAR database, which included 49 patients with SSc and GAVE and 93 matched controls with SSc, multivariate analysis revealed that GAVE was associated (odds ratio) with reduced diffusion capacity (12.8) and the presence of anti-RNA polymerase III antibodies (4.6) (100). Furthermore, GAVE in this patient population was a major clinical problem with the majority of patients (82%) having an associated anaemia requiring blood transfusion, and almost half (45%) requiring therapeutic endoscopic procedures (100). In addition, although survival was similar between patient groups, SRC was overrepresented in patients with (vs without) GAVE (12% vs 2%, respectively) (100). Early dcSSc with rapidly progressive disease has also been reported as a risk factor for GAVE (95,99). Management consists of blood transfusion/s if anaemia is severe and/or symptomatic, and endoscopic intervention (e.g. argon coagulation laser therapy) (97,98). Clinicians must maintain a high index of suspicion to diagnose GAVE early to improve patient outcomes as this optimises the chance of endoscopic procedures being successful (97,98). Of mechanistic interest to the pathophysiology of GAVE in SSc (which is poorly understood and in general considered ‘vascular’ in aetiology), complete and sustained resolution in GAVE was reported from the use of immunosuppressive therapy (cyclophosphamide and methylprednisolone) in a patient with SSc and interstitial lung disease (101).
**Telangiectases**

Cutaneous telangiectases (Figure 1.8B) are visible (with the naked eye) superficial vascular lesions, which are obliterated with pressure and refill slowly upon release (102). Telangiectases are formed from the permanent dilatation of postcapillary venules, mainly of the subpapillary plexus (103). Telangiectases are common in patients with SSc and typically occur on the fingers and face. Although the pathophysiology of telangiectases is not completely understood, a vascular drive is strongly suspected. In hereditary haemorrhagic telangiectasia (an inherited condition with similar cutaneous lesions to SSc), elevated proangiogenic factors have been reported (e.g. vascular endothelial growth factor [VEGF]) (104,105). An association between the presence of telangiectases and microvascular disease as assessed by nailfold capillaroscopy in SSc has been reported (102,106); however, one study did not find such an association (107). In a recent cross-sectional study by Hurabielle et al (102), which included 87 patients with SSc (75 with telangiectases), both the number and size of telangiectases were associated with the severity of nailfold capillaroscopy. In addition, telangiectases tended to be associated with more severe vascular complications (e.g. DU s) (102). Telangiectases have a marked impact on patients' quality of life and are one of the most visual and distressing aspects of the disease. Ennis and colleagues (108) reported that patients with (compared to without) telangiectases had significantly higher levels of body image dissatisfaction. Further qualitative analysis identified four themes in relation to patients’ attitudes: ‘changes in behaviour as a result of telangiectases, public and private self-image, negative emotional impact of telangiectases and appreciation of life’ (108). The clinical importance of telangiectases in SSc is also reflected through the inclusion of the manifestation in the ACR/EULAR 2013 SSc classification criteria (34,35) and the DETECT algorithm for PAH (83).
Figure 1.8. Gastric antral vascular ectasia and telangiectases. A: Gastric antral vascular ectasia as seen on endoscopy. Image taken from (109). B: Facial telangiectases, in particular, over the nose. Image taken from Hughes and Herrick (110).
1.5. Digital ulcers in SSc

This section has been adapted from Hughes and Herrick (19)

DU (Figure 1.9) are common in patients with SSc and responsible for much of the pain and morbidity associated with the disease. It is increasingly recognised that DUs are also a biomarker of internal organ involvement and mortality in patients with SSc, including in early disease. However, there are now a number of effective drug therapies available to both prevent and treat DUs in SSc.

Figure 1.9. Digital ulcers. The spectrum of DU disease in patients with SSc. Fingertip (A), extensor (B), overlying subcutaneous calcinosis as seen on a plain radiograph (C and D, respectively), at the lateral aspect (E) and the nailbed of the fingers (F). Figure taken from Hughes and Herrick (19).

1.5.1. Pathophysiology

As previously described, vascular disease plays a central role in the pathogenesis of SSc including DUs and many of the organ based complications (3,111), and in the digits involves both the microvessels and digital arteries (49). Fingertip DUs are
believed to be ischaemic, whereas those over the extensor aspect of the hands (in particular over the small joints) are commonly believed to be mechanical, due to recurrent microtrauma and increased skin tension (Figure 1.9) (112). Dysfunctional arteriovenous anastomoses (AVAs) may be implicated in the pathogenesis of DUs (113). As previously described, an increased prevalence of macrovascular disease proximal to the digital artery has been reported in SSc (72), in particular affecting the ulnar artery and is associated with an increased risk of DUs (50,71). Kato et al (114) reported in a small study of 10 patients that a reduced ankle brachial index (reflecting macrovascular disease) is associated with DUs of the feet and with (lower) skin perfusion pressure. In a recent study, which included 63 consecutive patients, the presence of DUs was associated with medium and small vessel involvement (as assessed by augmentation index of the reflected wave by radial applanation tonometry), but not large vessel disease (as assessed by aortic pulse wave velocity, a measure of large vessel injury/aortic stiffness) (115). DUs may also develop in relation to subcutaneous calcinosis, and are not uncommonly associated with a marked local inflammatory response and the discharge of calcinotic material (1.9).

1.5.2. Epidemiology

DUs are common in SSc, around half of patients reporting a history of DUs (112,116–118) and approximately 10% have a current DU (118,119). DUs may often occur early in the course of the disease, within the first year after the first non-RP symptoms (112). Patients with a shorter duration between their first and second DUs (in particular if the second is within two years) have a higher (yearly) DU burden (112). In a single centre longitudinal retrospective study including 103 patients with SSc, 43% and 73% of patients developed their first DU within one and 5 years, respectively, of their first non-RP symptoms (112). In a prospective longitudinal analysis including 695 patients from the EUSTAR database, 70% of patients reported a history of DU after 10 years follow-up (120). Data from the Digital Ulcer Outcomes (DUO) registry (a European, prospective, multicentre, observational, registry of SSc patients with DU disease, irrespective of treatment) demonstrated that patients with anti-Scl-70 antibody developed DUs around 5 years earlier than those who were anti-centromere positive (121).
1.5.3. Clinical burden

Between one and two thirds of patients with SSc develop recurrent DUs (112,116,122). In a recent study from the DUO registry, which included 1459 patients with SSc, four categories of DU occurrence were identified: ‘no DU’ (33.2%), ‘episodic’ (only 1 follow-up visit with either ≥ 1 DU or new DU) (9.4%), ‘recurrent’ (≥ 2 follow-up visits with DU and/or new DU, and ≥ 1 visit with no DU or new DU) (46.2%) and ‘chronic (≥ 1 DU and/or new DU at every visit) (11.2%)’ (123). Patients often develop multiple DUs per episode (112,122), with involvement of both hands and multiple fingers (112,124). DUs are often slow to heal, especially if there is underlying calcinosis, and can be associated with underlying bone infection (which might be detected early by MR imaging) (125). In a prospective study including 100 patients with SSc and 1614 digital lesions, average time to DU healing was 76.2 days, whereas in calcinosis-related DUs this was 93.6 days (126). Other factors reported to delay DU healing include: infection, the presence of perilesional oedema, wet or dry necrosis, eschar, bone and tendon exposure, and gangrene (126).

DUs often occur on the thumb, index and middle fingers, although any finger can be involved (112,124). Amanzi et al (126) reported fingertip DUs were more common than extensor DUs (52.5% vs 30%); whereas, in a prospective study over 12 months, both types had an equal prevalence of 6% (and were equally disabling) (119). DUs less commonly occur on the lateral edges, nail base and palmar aspect of the digits (Figure 1.9) (126).

DUs are often exquisitely painful (119,124) and associated with high levels of hand and global disability (119,127,128). Those patients with DUs require greater support in the activities of daily living than those without DUs (127,129). In a study including 2327 patients from the DUO registry, increasing number (0, 1-2 and ≥ 3) of DUs at enrolment, was associated with greater work impairment during the preceding month (28%, 42% and 48%, respectively), inability to perform activities of daily living (35%, 54% and 63%, respectively) and hours of paid help (mean number of hours 17.0, 35.9 and 63.7 respectively) (129).
DU are often infected (121), most commonly by *Staphlococcus aureus* (130). In addition, Giuggioli et al found that around 25% of DU were infected with intestinal organisms (in particular, *Escherichia coli* and *Enterococcus faecalis*) highlighting the need for patient education to ensure meticulous wound care (130). Thirty-two per cent of patients enrolled in the DUO registry reported previous antibiotic therapy for soft tissue infection complicating DU (121). In a retrospective study, which included 248 patients with SSc, 19 of 45 (42%) infected DU were associated with osteomyelitis, as defined by clinical and plain radiographic features (131). In the previously described recent study from the DUO registry, those patients with a ‘chronic’ pattern to DU occurrence had a higher incidence (compared to the other ‘categories’) of the DU complications: gangrene (22.0%), amputation (15.9%), infection (61.6%), hospitalisation (52.4%) and requirement for pain medication (77.4%) (123). Furthermore, independent factors associated (hazard ratio) with DU complications included gastrointestinal manifestations (3.73) and previous soft tissue infection (5.86) (123).

### 1.5.4. Digital ulcers as a biomarker of disease severity

It is increasingly recognised that DU are associated with a severe disease course (including internal organ complications), including in early disease. In a multivariate analysis of 3196 patients from the EUSTAR database, history of DU was predictive of death (odds ratio 1.53) (132). DU are often present in patients fulfilling the VEDOSS criteria (60), and are associated with gastrointestinal (oesophageal) involvement (133).

### 1.5.5. Clinical associates of digital ulcers

The clinical and serological/vascular associates of DU development in patients with SSc are summarised in Tables 1.6 and 1.7, respectively. Many of these have been proposed as biomarkers of DU and deserve future prospective studies to validate their predictive ability.
<table>
<thead>
<tr>
<th>Increased risk</th>
<th>Disease related</th>
<th>Internal organ involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>History of DUs (134,135)</td>
<td>Lung disease: including interstitial lung disease (117,118,137,139)</td>
</tr>
<tr>
<td></td>
<td>Higher skin score (112,117,118,136,137)</td>
<td>Gastrointestinal (including) oesophageal disease (136,137,140)</td>
</tr>
<tr>
<td></td>
<td>Younger age at SSc onset (112,117,118,138)</td>
<td>Cardiac disease (140)</td>
</tr>
<tr>
<td></td>
<td>Longer RP (136) and disease duration (117,118)</td>
<td>Musculoskeletal involvement (including joint contractures) (137,138)</td>
</tr>
<tr>
<td></td>
<td>Elevated ESR (136)</td>
<td>Cutaneous peripheral vascular manifestations: RP and telangiectases (139)</td>
</tr>
<tr>
<td></td>
<td>Elevated HAQ score (118)</td>
<td>Calciosins and acro-osteolysis (139)</td>
</tr>
<tr>
<td></td>
<td>Not receiving or delay to vasodilator therapy (112,138)</td>
<td></td>
</tr>
</tbody>
</table>

| Autoantibodies | Anti-Scl-70 (topoisomerase-1) (116–118,120,136,137,140) | Smoking: for (138) and against (118,137)                                               |
|                | Anti-centromere (116)             | PAH: conflicting, for (136) and against (118,139) an association                       |
|                | Anti-fibrillarin (in African Americans) (141) | Gender: Conflicting, no difference between sexes (118) and positive associations described: male (117,136,143), female (116,140) |
|                | Anti-endothelial cell (142)       |                                                                                     |

<table>
<thead>
<tr>
<th>Conflicting evidence (for and against)</th>
<th>No association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scleroderma renal crisis (118,139)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.6. The clinical associates of digital ulcers in patients with SSc. ESR: Erythrocyte sedimentation rate. HAQ: Health assessment questionnaire. PAH: Pulmonary arterial hypertension. RP: Raynaud’s phenomenon. SSc: Systemic sclerosis. Table taken from Hughes and Herrick (19).
| Serological (e.g. vascular markers and immunological) | Asymmetric dimethylarginine increased (144)  
Angiopoitin-2 increased and Angiopoetin-like protein 3 increased (145,146)  
Clusterin increased (reduced frequency of DUs) (147)  
Soluble endoglin increased (144,148)  
Endothelial progenitor cells decreased (134)  
ET-1 increased (144) and autoantibodies toward ETA receptor (149)  
Galectin-1 increased (reduced frequency of DUs) (150)  
Interferon type 1 response gene expression increased (151)  
Mean platelet volume increased (152)  
Pentraxin-3 increased (153)  
PIGF increased (134)  
Platelet-activating factor acetylhydrolase increased (reduced frequency of DUs) (154)  
VEGF increased (144)  
sCD163/sTWEAK ratio increased (reduced frequency of DUs) (155)  
sCD40L increased (156)  
TIMP-1 rs4898 polymorphism (reduced frequency of DUs) (157) |
|---|---|
| Vascular (physiological assessment and imaging) | Nailfold capillaroscopy (135,158–162)  
Renal artery stiffness increased (163)  
Local (digital) thermal hyperemia peak: plateau ratio ≥1 (as assessed by Laser Doppler flowmetry) (reduced frequency of DUs) (164) |

Capillaroscopy to predict the risk of future digital ulcers

Nailfold capillary abnormalities have been reported to predict future DUs (135,158–161). The application of capillaroscopy by rheumatologists is likely to expand because of increasing interest in the technique following the inclusion of capillaroscopic abnormalities in the ACR/EULAR SSc classification criteria (34,35).

In a study which included 66 patients with SSc, worsening capillaroscopy pattern (as defined by Cutolo (165)) was associated with significantly increased risk of severe peripheral vascular disease, including DUs, at 18 to 24 months (adjusted odds ratios for severe peripheral vascular disease compared to normal capillaroscopy: early = 2.52, active = 6.37 and late = 16.07) (161). The capillaroscopic skin ulcer risk index (CSURI) has been proposed as a quantitative tool to predict SSc-related DUs and is calculated using the total number of capillaries in the distal row, maximum loop diameter and number of megacapillaries (158). In a multi-center validation study, the CSURI at three months had a specificity of 81.4% and a sensitivity of 93.0% to predict DUs (160). Smith et al (159) proposed a simple system based upon the mean score of capillary loss over 8 fingers, with specificity and sensitivity each around 70% of predicting present/future digital trophic lesions (including DUs). Manfredi et al (135) proposed a predictive risk chart including DU appearance and male gender, DU history, altered CSURI and ESR to stratify DU risk. The ‘CAPS’ (videoCAPillaroscopy) study, which included 623 patients from 59 centers (14 countries), reported that abnormal capillaroscopy, as well as number of DUs and the presence of critical digital ischaemia at baseline, were the strongest predictive factors of future DUs (166).
1.5.6. The definition and measurement of digital ulcers

The reliability of rheumatologists grading DUs is poor to moderate at best (167,168), which is of concern given that DUs are often a primary end point in SSc-related clinical trials, and are now included in the ACR/EULAR classification criteria for SSc (34,35). In a web-based study in which rheumatologists with an interest in SSc graded images of SSc digital lesions, intra-rater reliability was high (mean weighted kappa value of 0.81), whereas, inter-rater reliability was much poorer (0.46) (167).

The varying definitions of SSc-related DUs that have been used in several recent multi-centre, placebo-controlled trials are provided in Table 1.8. These have often related to a loss of epithelium and with a discernible depth (169–173). Most recent trials have only included DUs considered ischaemic (distal to the proximal interphalangeal joint and on the volar aspect of the fingers) (169,171–173). Previous studies have usually required the presence of at least one ‘ischaemic’ DU for patients to be included into therapeutic trials (171–173).

DU surface measurements have been reported as an outcome measure (169) and assessment of hand function is an indirect measure of DU status (174). A North American working group reported moderate reliability of DU surface area measurement using digital callipers (intra- and inter-rater intraclass correlation coefficient of 0.57 and 0.48, respectively) (168).

Hachulla et al (112) in their prospective study on the natural history of DUs proposed three distinct types of DUs as presented below:

1. DUs occurring at bony prominences, usually at metacarpophalangeal or interphalangeal joints of the fingers, promoted by microtraumatic events and by traction exerted on the sclerodermatous skin when the fingers are flexed.

2. DUs occurring at the level of subcutaneous calcifications where mechanical and inflammatory phenomena are also involved.
3. Ischaemic DU that occur most frequently on distal areas of fingers, involving pulp or sometimes lateral edges.

Laser speckle contrast analysis has been reported to enable monitoring of DU status in patients with SSc by measurement of lesion size and perfusion (175). DU definition and measurement therefore both deserve further research with a view to development of guidelines and/or recommendations.
<table>
<thead>
<tr>
<th>Trial</th>
<th>Primary outcome measure/s</th>
<th>DU definition</th>
<th>Type of DU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bosentan (RAPIDS-2) (171)</td>
<td>1. Number of new DUs. 2. Time to healing of cardinal DU.</td>
<td>‘Active’ DU = Painful area, ≥ 2 mm in diameter with visible depth and loss of dermis, amendable to healing and in a location judged compatible with a vascular aetiology (onset between 1 week and 3 months prior to randomisation). Healing was defined as complete epithelialisation, regardless of residual pain.</td>
<td>Volar surface of the digit distal to the proximal interphalangeal digital crease).</td>
</tr>
<tr>
<td>Quinapril (170)</td>
<td>Rate of occurrence of new ischaemic DUs on the hands.</td>
<td>‘Active’ DU = No epithelial surface, except for those due directly to trauma, which heal normally.</td>
<td>Any lesion on the finger.</td>
</tr>
<tr>
<td>Sildenafil (SEDUCE) (172)</td>
<td>Time to healing for each DU.</td>
<td>‘Active’ DU = Break in the skin with a loss of epithelialisation on the distal finger surface of ischaemic origin according to the physician.</td>
<td>Distal to the proximal interphalangeal joint.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In the case of an underlying scab, the presence of a DU was considered based on the clinical judgement of the physician.</td>
<td>Not located over subcutaneous calcifications or over extensor surfaces of joints.</td>
</tr>
<tr>
<td>Macitentan (DUAL 1&amp;2) (173)</td>
<td>Number of new DUs.</td>
<td>‘Active’ DU = A finger lesion with visually discernible depth and a loss of continuity of epithelial coverage associated with pain not attributable to other aetiologies.</td>
<td>At or distal to the proximal interphalangeal joint.</td>
</tr>
</tbody>
</table>

Table 1.8. Digital ulcer definitions used in recent multi-centre, placebo-controlled trials.
1.5.7. Management of digital ulcers

1.5.7.1. General approach
Patients with SSc and DU disease should be managed by a dedicated multi-disciplinary team. Patient education is crucial, and patients developing DUs should be encouraged to seek medical advice early. DUs are often infected and there should be a low threshold to prescribe appropriate antibiotic therapy. DUs can be exceptionally painful and patients should be prescribed sufficient (often opioid based) analgesia. The UKSSG best practice recommendations on the management of DUs are a useful reference tool for clinicians (Figure 1.10).
Figure 1.10. The UKSSG Best Practice Recommendations on the management of DUs in patients with SSc. ERA: Endothelial receptor antagonist. IV: intravenous. PDE5: Phosphodiesterase type-5. Figure taken from Hughes et al (63).
1.5.7.2. Non-pharmacological interventions

Patients should avoid trauma to the digits (in particular to the extensor aspects in patients with flexion contractures) to prevent DUs. Patients should be counselled as to the importance of smoking cessation. In a study including 101 patients with SSc who responded to a questionnaire, current smokers were significantly more likely than never smokers to require debridement and admission for intravenous prostanoid therapy for digital vascular disease (odds ratios 4.5 and 3.8, respectively) (69). Any contributory cause (e.g. large vessel disease) should be identified early and treated (where appropriate) (Figure 1.10). Meticulous attention should be directed toward wound care including keeping the DU clean and using a suitable dressing: if the DU is dry then attempt to wet (alginites and antimicrobials e.g. Suprasorb and Aquacel Ag, respectively) and vice versa for wet DUs (hydrogel and hydrocolloids, e.g. Intrasite gel and Duoderm, respectively) (63). Other non-pharmacological therapies that have been reported to be associated with DU healing in patients with SSc include hyperbaric oxygen (176), negative pressure and acoustic therapies (177,178), and intermittent compression (179). The authors of a meta-analysis on non-pharmacological therapies for SSc-related DUs highlighted the limited evidence base (at present) to support these interventions, and the need for further high-quality research in this area (180).

1.5.7.3. Pharmacological interventions

The development of (digital) vascular disease in SSc is believed to be multi-factorial (Figure 1.2), and many of these factors are fortunately amenable to pharmacological intervention.

Vasoactive therapies

Vasoactive therapies are central to the pharmacological treatment of DUs. Hachulla et al (112) reported that vasodilator therapy significantly delayed the development of DUs (HR=0.17, 95% CI 0.09 to 0.32). In the United Kingdom, the National Health Service England has recently approved sildenafil (now off patent) for patients with
refractory or severe DU disease, followed by add-on intravenous prostanoid, and then if these are not efficacious, bosentan (an ERA) (181).

Calcium-channel blockers
Calcium channel blockers have been little studied in DU healing/prevention, although many clinicians prescribe calcium channel blockers in this context (often for concomitant severe RP). In a randomised, double-blind study comparing oral nifedipine (30 mg daily for four weeks and then 60 mg daily for 12 weeks) and intravenous iloprost for RP (both with concomitant placebo infusions or capsules, respectively), the mean number of DUs was reduced from 4.3 to 1.4 after 16 weeks of treatment with nifedipine (182).

ACE inhibitors
Although there is a strong therapeutic rationale for the role of ACE inhibition in DU disease (and RP), including as vascular remodelling agents (as used in patients with ischaemic heart disease), there is no current evidence base to support this intervention (183). In a multi-centre, double-blind, randomised clinical trial including 210 patients with lcSSc or autoimmune RP (with an SSc-specific autoantibody), two to three years’ treatment with quinapril was not associated with a significant reduction in the number (quinapril-placebo) of new DUs (-0.08, 95% CI 0.23, 0.06) (170).

Prostanoids
Prostanoids are potent vasodilators, and also inhibit platelet aggregation and vascular smooth muscle cell proliferation (184,185). Prostanoids can be administered by various routes: oral, intravenous and subcutaneous, with side effects common to all including systemic hypotension, dizziness, flushing, gastrointestinal disturbance, jaw pain, and myalgia.
Intravenous prostanoid therapy has been reported to significantly improve DU healing and reduce the number of new DUs. In two multi-centre, double-blind, randomised trials, intravenous prostanoid therapy (0.5 to 2.0 ng/kg/minute over 6 hours, on 5 consecutive days) was associated with significantly greater DU healing than placebo (186,187). The second of these studies included 126 patients who completed the course of infusions: after three weeks’ treatment 14.6% more patients who received iloprost than placebo had ≥ 50% healed DUs (187). Side effects were very common, with 92% of patients receiving iloprost experiencing one or more prostanoid-related side effects, although 57% of patients receiving placebo also reported side effects (187).

In a multi-centre, double-blind, randomised trial of continuous intravenous epoprostenol for pulmonary hypertension in SSc-spectrum disorders, patients who received epoprostenol were noted to develop 50% fewer new DUs than those receiving placebo (188).

A single-centre, open label trial suggested that treatment with subcutaneous treprostinil was associated with a reduction in new DUs (189). However, in a subsequent multi-centre, double-blind, randomised trial in 148 patients, treprostinil, compared to placebo, was not associated with a significant reduction in the number of DUs (1.37 vs 1.51) at 20 weeks (although treprostinil conferred benefit in some of the secondary endpoints) (190). Furthermore, in a subsequent multicenter, retrospective study, which included 51 patients, after discontinuation of treprostinil there was a significant increase in DU (number) burden, both at 3-6 months (2.1) and >6-12 months (1.45) (191).

In a meta-analysis of therapies for DU healing and prevention, oral prostanoids (iloprost, beraprost, cisaprost and treprostinil) were not associated with a reduction in new DUs compared to placebo (192).
Endothelin receptor-1 antagonists

ET-1 is not only a potent vasoconstrictor, but also has a marked proliferative effect on smooth muscle cells and fibroblasts, acting via two receptors: ETA and ETB (193). In general ETA and ETB are both found on smooth muscle cells and promote vasoconstriction and hyperplasia; whereas, ETB is also found on endothelial cells and promotes vasodilation (193,194).

Bosentan is a dual ET-1 ERA and is licensed in Europe for the treatment of PAH and the prevention of recurrent DUs. Two large multi-centre, double-blind, randomised, controlled trials have demonstrated that treatment with bosentan significantly reduced the number of new DUs in patients with SSc (169,171). In the ‘RAPIDS-2’ study, which included 188 patients with SSc (from 41 centres), 24 weeks’ treatment with bosentan (62.5 mg twice daily for four weeks and 125mg twice daily thereafter) was associated with a 30% reduction in the number of new DUs (171). The treatment effect was greatest with a higher DU burden at baseline, and there was no impact on the healing of existing DUs (171). Bosentan requires regular blood monitoring (haematology and liver function tests), as treatment can be associated with abnormalities in these domains.

Macitentan is a dual ERA licensed for the treatment of PAH. In two multi-centre, double-blind, randomised, controlled trials, over 16 weeks of treatment, the number of new DUs (3 mg macitentan vs 10 mg macitentan vs placebo) did not significantly differ in either ‘DUAL-1’ (0.94 vs 1.08 vs 0.85) or ‘DUAL-2’ (1.44 vs 1.46 vs 1.21) (173).

Ambrisentan (licensed for the treatment of PAH) is a selective ETA ERA, and in two small studies has been associated with a reduction in new DUs (195,196). The larger of the two was a single centre, open-label study in which 20 patients with SSc received ambrisentan for 24 weeks (196). The mean number of DUs (per patient) significantly decreased (mean number at baseline 3.1 vs 1.3 at 24 weeks), and
complete healing of all baseline DUs was observed in 14 (out of 16) of patients who completed 24 weeks of treatment.

Phosphodiesterase type-5 inhibitors

PDE5 inhibitors inhibit the degradation and therefore increase the bioavailability of cyclic GMP, with subsequent vasodilation (197). In a meta-analysis of DU therapies which included 31 randomised controlled trials (1989 patients), PDE5 inhibitors (based upon three included RCTs with a total of 85 patients) were associated (relative risk) with DU healing (3.28) and improvement (4.29), although the authors comment that the studies were underpowered to individually detect a significant benefit (192). In a recent multi-centre, double blind, randomised, controlled trial (the ‘SEDUCE’ study), which included 84 patients, treatment with sildenafil over 12 weeks was associated with a significant reduction compared to placebo in the number of new DUs (0.86 vs 1.51). However, the time to DU healing (the primary end point of the study) was not reduced, which the authors attribute to the unexpectedly high rate of DU healing in the placebo group (172).

Combination therapy

There are no randomised controlled trials addressing vasoactive therapies in combination for DUs. Two case reports have suggested that the combination of PDE5 inhibitors and ERA therapies, either both in low dose, or initial PDE5 inhibitor with subsequent ERA, is efficacious in refractory DU disease (198,199).

Other drug therapies that have been explored

Statins

Statins (3-hydroxy-3-methyl-glutarylecoenzyme A reductase inhibitors) are believed to have a wide range of positive effects on the cardiovascular system, independent of a cholesterol lowering effect, including (but not limited to) improvement in endothelial function and increased nitric oxide (NO) bioavailability, and with
modulation of the fibrotic and immune responses (200–202). In a randomised trial, which included 84 patients with SSc, four months’ treatment with atorvastatin (40mg daily) compared to placebo was associated with a reduction in the number of new DUs (1.6 vs 2.5) during the treatment period (203). However, further research is required before statin therapy can be widely recommended.

Anticoagulant and anti-platelet agents

In a prospective parallel group, randomised study, 24 weeks’ open-label treatment with subcutaneous low molecular heparin was not associated with a significant difference in new DUs (204). Although many clinicians prescribe anti-platelet therapy for DUs (and severe RP) there is a lack of good evidence base to support this intervention. The fact that many patients with SSc have gastrointestinal involvement with a propensity for bleeding (e.g. GAVE) should be taken into account when assessing the risk:benefit ratio for any individual patient. In a randomised, double-blind, placebo-controlled study in which patients (n=28) received dual anti-platelet therapy (aspirin and dipyridamole), no difference in new DUs was observed with over one to two years’ of treatment (205). In a recent study which included 13 patients with SSc, compared to before treatment for 14 days with clopidogrel (an anti-platelet agent), there was a significant reduction in platelet activation (as assessed by aggregometry) (206). In addition, although there was no change in serotonin (activated platelets release serotonin which can lead to fibroblast activation), and there was a significant increase in soluble vascular cell adhesion molecule 1 (a marker of endothelial dysfunction) (206). Furthermore, three patients developed new DUs during the study, which led to the early termination of the study (206). Again, further research is required to inform treatment decisions, although studies of antiplatelet agents will be logistically difficult to mount.

Antioxidants

A reduction in the number of DUs with open-label treatment with intravenous N-acetylcysteine (NAC) has been reported (207,208). The larger study (208) was a prospective, open label, observational study, which included 50 patients with SSc.
Treatment with NAC (median duration of three years) was associated with a significant reduction in the number of DUs from 4.5 pre-treatment to 0.81 on NAC. As previously described, oxidative stress has been implicated in the pathogenesis of SSc and therefore antioxidant therapy warrants further study.

1.5.7.4. Surgical strategies

General approach

Surgical intervention is required only in a minority of patients with DUs, usually in those who have failed medical management. Practice varies internationally. In a survey of 334 SSc-interested rheumatologists (with 137 responders to this question), 85% of North Americans compared to 37% of Europeans reported that they never or rarely debrided DUs (168). The main aim of debridement is to relieve pain by removing necrotic tissue and/or pus. Despite therapeutic intervention, amputation of the affected digit may be necessary (112,121). Denton and colleagues have previously reported (from the DUO registry) that a significant number of patients reported a history of previous debridement (9.5%) or of gangrene (22.6%) complicating DU disease (121), in keeping with the results of the recent publication from the same registry as previously described (123). Indications for surgical intervention include failure of DU healing, severe pain, osteomyelitis and underlying calcinosis. MR imaging is a useful tool for the early detection of osteomyelitis (125). Perioperative pain management during debridement often requires opioid-based therapy (209).

Sympathectomy and botulinum toxin injection

There is increasing experience worldwide with these techniques for the treatment of DUs, although the evidence base in SSc-related digital vasculopathy is relatively poor (mainly retrospective case series) (210,211). Thoracic sympathectomy is no longer recommended for RP or DU disease for reasons including the high rate of adverse effects. Peripheral (digital) sympathectomy has been reported to prevent and heal DUs in patients with SSc (212–214). In a retrospective analysis of 26 patients with DUs, improvement in pain was reported in 92.3% post-sympathectomy, with
DU healing in the majority, and with only two patients later requiring surgical intervention (at 6 months and 4.5 years) (214). Digital sympathectomy is a highly specialised procedure available only in certain centres.

Botulinum toxin has also been reported to be associated with the healing and prevention of DUs in SSc (215–217). In a prospective study of botulinum toxin, in five patients with refractory DU disease, all DUs had healed by 12 weeks, and one within two weeks (215). The results of controlled trials of botulinum toxin therapy are awaited.

1.5.7.5. Other therapies

Local therapies

There is a strong therapeutic rationale to develop locally-acting therapies that are well tolerated (from a lack of significant systemic vasodilation), reducing the need for hospitalisation.

In one open label study, 27 patients were randomised to receive standard wound care +/- topical vitamin E gel. Vitamin E gel was associated with faster DU healing (13.2 weeks treated vs 20.9 weeks untreated), faster resolution of pain and fewer medications, including reduced cost of therapies (218). Ozgocmen et al reported that topical lignocaine was effective in controlling pain from severe DU disease in a patient with SSc, and thereby allowing the application of topical antibiotic therapy (219). Digital iontophoresis (using an electrical current to drive drug delivery through the skin) of treprostinil was reported to be a safe and effective method to increase skin blood flow in healthy controls and patients with SSc (220) and may in the future offer a novel approach to therapy. Waon therapy (essentially a high temperature sauna) over 11 weeks was effective in treating a refractory toe DU (221). Inoue et al reported the use of oral psoralen and ultraviolet A therapy in a patient with severe DU disease (222). Treatment three times daily over four weeks was well tolerated, associated with an improvement in the DU, and with loosening of
collagen bundles on skin biopsy. In a phase two pilot study which included 9 patients with SSc and DUs, extracorporeal shock wave therapy was administered once per week for 9 weeks (223). At the end of treatment (week 9), there was a marked reduction in the number of DUs compared to baseline (5.4 vs 1.1, respectively) (223). Interestingly, of the 18 large DUs (defined as >5 mm) found in 7 patients, 10 completely healed and the remainder significantly reduced in size (10.9 mm vs 2.5 mm, respectively) (223). In a recent study using an animal model of SSc (University of California, Davis – 206 chickens), treatment with a VEGF<sub>121</sub>-fibrin variant was associated with clinically meaningful improvement in ischaemic skin lesions and promotion of neoangiogenesis (with notably structurally normal capillaries) (224). In summary, current experience of local therapies relates mainly to anecdotal reports and small series, but the recent increasing interest in local therapies is encouraging.

Autologous fat grafting and stem cell transplant
Two small studies have reported an improvement in DUs with fat grafting (225,226). In a small case series of 9 patients (with 15 DUs), autologous fat grafting (to promote tissue repair) was performed between 2 and 8 months post DU development (225). Grafting was associated with complete healing of 10 DUs and ≥ 50% improvement in 2 DUs within 8 to 12 weeks, although of note, patients also received intravenous iloprost (225). In a single-centre, open label, pilot study, of 40 patients (11 with SSc and ischaemic DUs), bone marrow mononuclear cells were administered into their ischaemic limbs (227). Transplantation was well tolerated with good improvement in pain VAS; although at two years’ follow-up recurrence of ischaemic disease was not uncommon (two patients) and one patient had required digital amputation.

Immunosuppression
Khor et al (228) describe a case report in which treatment with rituximab (for severe internal organ disease) was associated with completing healing of refractory DUs. In a small case series of 3 patients, 6 months’ treatment with tocilizumab was associated with clearance of all DUs in the two patients with active DUs (3 and 8 DUs at baseline) (229). Of 10 patients with SSc who received treatment with
cyclosporin A, one patient was noted to have complete healing of their DUs (230). There is insufficient evidence at present to recommend immunosuppressive therapy for DU, and infected DUs are a relative contra-indication.

1.6. Structure and measurement of the digital vascular system

1.6.1. Macrovascular anatomy of the hand
Figure 1.11 depicts the arterial supply to the digits. The hand has two major arterial supplies: the radial and ulnar arteries, which are both formed from the division of the brachiocephalic artery at the level of the antecubital fossa at the elbow (231). At the level of the wrist, the radial artery then progresses distally along the (radial) aspect of the hand, turning medially after passing through the anatomical snuffbox to form the deep palmar arch (231). The ulnar artery descends (from the level of the wrist) distally along the (ulnar) aspect of the hand to form the superficial palmar arch (232). The three common digital arteries arise from the superficial digital arch, each then branches into two proper digital arteries at the web spaces to supply the fingers (one on either side) (231). The arterial supply to the dorsal aspect of the finger is via the proper digital artery, whereas, the palmar aspect is either from the dorsal metacarpal artery (at the level of the metacarpophalangeal joint) or via branches of the proper digital artery (distal to the proximal interphalangeal joint) (231). At the nailbed there are both proximal and distal arches, which are formed through the anastomoses of the branches arising from the digital arteries (231).

The venous system mirrors the arterial system of the hand (termed in Latin ‘vena comitans’ meaning ‘accompanying vein’). The dorsal digital veins on either side of the finger terminate at the level of the metacarpophalangeal joint to form three metacarpophalangeal veins (231). These form the dorsal venous network (arch), which extends proximally toward the elbow as the (corresponding artery in parentheses) cephalic (radial) and basilic (ulnar) veins (231).
Figure 1.11. The arterial supply to the digits of the hands originating from the level of the wrist. Figure taken from (233).

1.6.2. **Structure and function of the microcirculation**

The microcirculation (Figure 1.12) consists of arterioles (smaller branches of arteries), capillaries and venules (smaller branches of veins). Control of vascular tone is highly complex. Nervous system control of the vasculature consists of both sympathetic (constriction) and parasympathetic (dilatation) input, acting upon vascular smooth muscle cells. The endothelium and locally produced factors (in particular, NO) also have a key role in the control of vascular tone (234,235). In addition, a number of circulating factors including (but not limited to) hormones (e.g. adrenaline and noradrenaline), vasopressin (antidiuretic hormone) and products of the renin-angiotensin system have marked effects on vascular physiology (the mechanisms of which are beyond the scope of this thesis) (234).
The primary role of the microcirculation is to effectively distribute oxygen and nutrient rich blood to cells, with the simultaneous removal of waste products from cellular respiration (234). These processes occur at the level of the capillaries, which are the smallest form of blood vessel, consisting of only endothelial cells (albeit with surrounding pericytes) to facilitate their role (234). The microcirculation also plays a key role in thermoregulation to maintain body haemostasis. The skin contains AVAs, which connect arterioles and venules, thereby bypassing the skin’s nutritional capillaries (237,238). In cold ambient environments, the AVAs are closed to preserve heat, and vice versa in prevailing warmer conditions (237,238). Cold exposure induces sympathetic nervous system activity (237,238), in particular, the smooth muscle cell alpha 2c adrenoceptor, which appears to have a key role in cold-induced vasoconstriction (237,238).

1.6.3. Methods to assess the microcirculation

There are a number of methods available to assess both the structure and function of the microcirculation in vivo. This is of great interest considering the central role of vascular disease in the aetiopathogenesis of SSc and many organ-based complications, including DUs, as previously described.
**Assessment of the structure of the microvascular system**

This thesis will focus on capillaroscopy, in particular, because this is highly relevant to DU disease in SSc. As previously described, capillaroscopic abnormalities have been reported to be a strong predictor of DU disease. In addition, through its inclusion in the ACR/EULAR SSc classification criteria (34,35), there is increasing international interest and access to capillaroscopy.

Nailfold Capillaroscopy

*This section has been adapted from Hughes and Herrick (57)*

Capillaroscopy is non-invasive imaging technique, which allows the microcirculation (capillaries) to be visualised in *situ*. The capillaries at the nailfold run in parallel (rather than perpendicular) to the skin, which allows them to be visualised along their long axes. Nailfold capillaroscopy can be performed using a number of different techniques. The seminal work by Mariq and LeRoy used widefield microscopy (magnification in the order of 12-14x) (239,240). The development and increasing availability of high magnification videocapillaroscopy (magnification in the order of 200-600x) (Figure 1.13) is one of the factors, which have led to increased interest in capillaroscopy in recent years (241).

For those clinicians without access to standard wide-field microscopy or to videocapillaroscopy, the nailfold capillaries may be visualised using a dermatoscope (magnification in the order of 10x) (242,243) or with an ophthalmoscope (244). The disadvantages of the ophthalmoscope include the narrower field of view and inability to record an image for future comparison. Our recent study (243) suggested that dermoscopy compared favourably to videocapillaroscopy (to detect abnormal nailfold capillaries). However, videocapillaroscopy images were more likely to be classifiable (and were graded more severely) than dermoscopy images. It is likely that in the future, the ‘USB’ (Universal Serial Bus) microscope, a low-cost digital microscope (magnification in the order of 10x) which connects to any computer without requiring specialist software, will also be used.
LeRoy and Maricq (239) described the ‘scleroderma pattern’, which includes the enlargement and loss of capillaries with haemorrhages. Cutolo et al (241) subsequently described a method of qualitatively scoring the ‘scleroderma-pattern’ (‘early’, ‘active’ and ‘late’). The early pattern is especially relevant to the early diagnosis of a SSc-spectrum disorder in the patient presenting with RP, and is characterised by the presence of capillary enlargement including a small number of giant capillaries and with microhaemorrhages, without obvious capillary loss.

Nailfold capillary abnormalities have been reported to be predictive of DUs (as previously described) and are associated with other internal organ involvement in patients with SSc (e.g. pulmonary hypertension) (245,246). Although nailfold capillary abnormalities have been described in CTDs other than SSc, the changes tend to be non-specific, other than in inflammatory muscle disease (in particular, dermatomyositis) in which ‘scleroderma-pattern’ abnormalities are well described (247,248).

Normal nailfold capillaries (Figure 1.13A) are reassuring in the patient with RP. Conversely dilated loops, areas of avascularity, distortion of the normal capillary architecture, and multiple haemorrhages are all pointers to an underlying SSc-spectrum disorder (Figure 1.13B) [10].
Figure 1.13. Nailfold capillaroscopy. A: Normal capillaroscopy. The capillaries are regular (‘hairpin’ like) in appearance and this is reassuring in patients with RP. B: Abnormal nailfold capillaroscopy in a patient with SSc. Several capillaries are enlarged, with areas of avascularity. Figure taken from (57).

**Nailfold capillaroscopy as an outcome measure in clinical trials**

Objective outcome measures are needed in SSc digital vascular disease clinical trials. In RP clinical trials, at present, assessment of efficacy is based upon patient self-report and is therefore subject to the episodic nature of the condition, and reliant upon the successful completion of patient diaries. Several authors have reported that nailfold capillaroscopy might be a biomarker of treatment response, including in patients who have received iloprost and bosentan (249–251). Measurement of red cell velocity (at the level of the nailfold) (252) is an exciting (but highly complex) potential outcome measure in clinical trials, and deserves further research.
Peri-ulcer capillaroscopy

We have previously described in a small pilot study that the capillaries (using high magnification videocapillaroscopy) surrounding both fingertip and extensor DUs (Figure 1.14) reveal similar capillary abnormalities, suggesting that microangiopathy contributes to both (253). This may represent the underlying microvascular disease in SSc, wound healing (either normal or aberrant in SSc), or a combination of both, and future research is warranted to further investigate these findings.

Figure 1.14. Digital ulcer capillaroscopy. Capillaroscopy of peri-lesional skin surrounding a (extensor) DU in a patient with SSc. The images demonstrate the heterogeneity of capillary morphology surrounding DUs with normal (left-hand images) and enlarged (bottom right) capillaries, and with neoangiogenesis (top right). Figure taken from (253).
Assessment of microvascular function

There are a number of methods to assess microvascular function; however, this thesis will only cover laser-based techniques and thermography, firstly, because there is increasing interest in these techniques, and secondly, these are directly related to a number of the studies included in this thesis. Other functional vascular techniques, which have been investigated, include (but are not limited to) plethysmography and finger systolic pressure (254).

Laser-based techniques

A number of different laser-based techniques have been investigated in RP and SSc. All of these rely upon detection of the ‘Doppler shift’; whereby, laser light is Doppler-shifted through interaction with moving red blood cells in situ (252). The change in wavelength induced in retro-reflected/scattered light is related to the speed of the red blood cells (252).

This thesis will focus on laser Doppler imaging (LDI) as this technique is used in two of the studies described (Chapters 5 and 6). Other laser-based techniques which have been investigated include laser Doppler flowmetry (single point measurement) and laser speckle contrast imaging (measuring perfusion over an area, akin to LDI although without point-by-point scanning). Laser speckle contrast imaging is based upon the scattering of coherent light (i.e. the laser) to form a ‘speckle pattern’ (described below) (252).

The three laser-based techniques exploit particular features of laser light, which is different to other light sources (such as that from daylight or a normal light bulb), to enable blood flow to be measured, as described below.

1. Narrow bandwidth: Lasers usually emit only a small range of wavelengths/colours.
2. Spatial coherence: Lasers produce a tightly concentrated beam, which can be focussed on a very small area.
3. Temporal coherence: The output ‘waves’ are synchronised with each other due to the way the light is produced and amplified within the laser device.

For laser Doppler flowmetry and LDI points one and two are most important. A tight focal spot means that only a small area is being investigated at a time. When the light interacts with a moving object (e.g. a red blood cell), it is Doppler-shifted (i.e. the wavelength/colour is slightly altered) when scattering back towards the detector. With a narrow bandwidth (point one above), any different wavelengths detected must be due to movement in the object under test. The wavelength change is proportional to the speed of the object along the axis of the laser beam.

For laser speckle contrast imaging, it is point three that is the most important. The laser beam is expanded (so thereby counteracting point two). When coherent light interacts with a perfect mirror (i.e. a perfectly flat relative surface) it reflects back and maintains coherence. However, in the ‘real world’, where structures are never truly flat, the distance travelled by some of the light is either relatively reduced or increased. Since these two wavelengths were temporally coherent they are reflected at different points in their cycle and are therefore no longer ‘in step’ with each other. On a macroscopic scale, this results in the ‘speckle’ pattern (bright and dark spots due to constructive and destructive interference, respectively). This speckle pattern is static providing that the laser output is stable, and that there is no relative motion between the laser and the object under test. The movement of blood cells below the surface influences the speckle pattern since the light penetrates into the skin, and therefore the speckle pattern changes over time with a frequency indicative of the blood cell velocity beneath the skin.

**Laser Doppler imaging**

LDI (Figure 1.15A) has been used in research on both the pathophysiology of RP and SSc and clinical studies of treatment response. LDI measures blood flow over an area building a perfusion map (1.15B), which helps to deal with the problem of point-to-point variability in laser Doppler flowmetry (254). LDI has been used to
explore endothelial-dependent and independent vasodilation in patients with RP and SSc (255,256). Using LDI, the abnormal microvascular response (including in response to cold stimulation) in patients with SSc has been reported to be localised to the digits only (257) and to only include the dorsum of the hand in the presence of advanced microangiopathy (258).

LDI has been used as an outcome measure in a number of therapeutic trials. In a double-blind, randomised, placebo-controlled crossover trial, ORM-12741 (an alpha-2c adrenoceptor antagonist) was found not to expedite recovery from a cold challenge in the fingers (259). Although an initial clinical trial of MQX-503 (a novel preparation of glyceryl trinitrate [GTN]) showed an improvement in Raynaud’s condition score, which collects information on the frequency, severity and duration of RP attacks, averaged over one to two weeks (260,261). However, there was no difference observed in the frequency or severity of RP attacks (262). However, in a subsequent multi-centre, double-blind, randomised, placebo-controlled, laboratory-based study, MQX-503 was associated with a significant increase in skin blood flow compared to placebo after cold challenge testing (both the time to achieve and the proportion of patients achieving baseline blood flow) (263). Cutolo et al (264) reported an increase in finger perfusion (as assessed by LDI) following treatment with iloprost. Taken together, these LDI-based studies demonstrate the potential role for objective vascular outcome measures in early clinical trials in SSc (and RP), including the identification of those drug therapies which are likely to be efficacious, and which should be taken forward into larger, later stage clinical trials.
Figure 1.15. Laser Doppler imaging. A. Photograph depicting the LDI equipment at Salford Royal Hospital. The hand is placed underneath the laser housing to perform the imaging. B: Imaging of the hand in a control subject. The perfusion (‘flux’) map is presented in arbitrary units, graphically red being the highest and blue the lowest perfusion. Figure taken from Dinsdale and Herrick (254).

Thermography

Thermography (Figure 1.16) utilises an infrared camera to measure skin surface temperature, and therefore is an indirect measure of skin blood flow (assessing both small and large blood vessels) (254). Thermographic abnormalities have been reported to enable the distinction between patients with PRP and SRP (e.g. SSc-related). As a generalisation, patients with RP often have cooler fingertips (compared to the dorsum of the hand) than healthy controls (Figure 1.16). Measurement of the rewarming response of the digits in response to cold exposure (‘a cold challenge’) is useful in distinguishing between healthy controls, PRP and SRP (Figure 1.16). In addition, further assessment using a warming challenge (20 minutes at 30°C) can help to distinguish further between PRP and SRP (Figure 1.16). The ‘distal-dorsal’ (>1°C) difference (fingertips cooler than the dorsum of the hand) has been proposed as a thermographic marker to distinguish between PRP and SRP (e.g SSc-related RP) (265,266). However, because thermographic equipment is fairly expensive and a temperature-controlled laboratory is required to perform a cold challenge, its clinical use at present, is limited to specialist centres. In addition, Pauling et al (267),
questioned the added benefit from cold challenging testing, reporting that baseline thermographic abnormalities were discriminatory between PRP and SRP, and this was lost after cold challenge (likely due to the marked variation in response between the digits). Furthermore, in a meta-analysis, which included 32 studies (and 654 patients with RP), no single thermographic parameter emerged as the definitive outcome measure which should be used in future therapeutic trials (268), and this needs to be investigated in future research. In addition, van der Weijden et al (269), report their clinical experience of using thermography to identify infection (of the toe) on a background of peripheral ischaemia of the feet.
Figure 1.16. Thermography. Thermographic imaging of the hands during dynamic temperature challenge. Left column: thermal images at 23°C, middle column thermal images at 30°C, right column: rewarming curves after cold challenge. At 23°C the fingertips are cooler in patients with both PRP and SRP (B and C) unlike in healthy controls in whom the fingertips are warm (A). At 30°C, unlike in PRP (D), there are persistent temperature gradients (fingers cooler than the dorsum of the hand) in SRP (E). Rewarming curves demonstrate prompt rewarming in a healthy control subject (top), complete but delayed rewarming in a patient with PRP (middle) and no rewarming in a patient with SSc (bottom). Figure taken from Hughes and Herrick (57).

Combining structural and functional vascular imaging

Several authors have reported that structural abnormalities (as assessed by capillaroscopy) are associated with functional microvascular disease (i.e. lower perfusion) in SSc (264,270–272). Future research is warranted to ascertain the added benefit in combing both structural and functional vascular assessment, including as an outcome measure in SSc (and RP) clinical trials.
1.7. Light-based treatment for skin ulcers

1.7.1. Introduction to light-based treatment

Light-based treatment has been investigated for a diverse range of medical applications including (but not limited to) dermatological disease, regional musculoskeletal conditions and chronic pain syndromes, and more serious life-threatening diseases (including cardiovascular disease and traumatic brain injury) (273,274). This thesis shall focus on the literature pertaining to light treatment for skin ulcers, as this is relevant to our novel light-based treatment device for the treatment of DUs in patients with SSc (Chapter 6). In general, light treatment can either be administered alone, relying on endogenous mechanisms involved in ‘biostimulation’ (described later in this chapter), or in combination with a photosensitising agent (i.e. photodynamic therapy). Photodynamic therapy is used clinically in patients with severe psoriasis, in which psoralen (a photosensitising agent) is administered orally with subsequent irradiation of the skin with ultraviolet A light, to promote clearance of psoriatic skin disease.

In this thesis I shall refer to ‘light-based therapies’; however, it is important to highlight that the nomenclature used (often interchangeably) in the literature is broad and includes (and is not limited to) ‘low-intensity laser therapy’, ‘low-level laser therapy’, ‘low-level light therapy’, ‘photobiomodulation’, ‘photobiostimulation’ and ‘phototherapy’. Light-based therapy can be defined as the use of light (often in the red and near infrared spectrum) to alter biological activity (e.g. gene transcription and protein synthesis), independent of a direct thermal effect (274).

As a basic principle, a LASER (Light Amplification by Stimulated Emission of Radiation) emits a focussed non-divergent beam of light; whereas, an LED (Light Emitting Diode) emits (without additional optics) light in all directions. Both laser and LED-based light therapies have been explored in the treatment of cutaneous ulcers. The possible differences in the therapeutic application between laser and LED light-based therapies for cutaneous ulcers will be further discussed when considering
the ‘dose’ delivered by light-based treatment. It remains controversial whether the two different light sources differ in their potential clinical benefit in wound healing (273). As a practical consideration in the design of light-based therapies, modern LEDs are often considerably less expensive than their laser counterparts, and are also widely available in a wide range of wavelengths.

1.7.2. The electromagnetic spectrum

It is useful to review the arrangement of the electromagnetic spectrum of radiation to orientate the reader to the nomenclature regarding the wavelengths of LEDs, which were chosen for the custom-built light-based treatment device to treat DUs in patients with SSc (Chapter six). The electromagnetic spectrum (by decreasing order of wavelength) is comprised of radio waves, microwaves, terahertz radiation, infrared radiation, visible light, UV radiation, X-rays and gamma rays (Figure 1.17). Previous light-based treatment studies have utilised (wavelengths defined in parentheses) both red (620-750 nm) and infrared radiation (700 nm to 1 mm). In addition, although blue light (450-495 nm) has been traditionally studied for its antibacterial properties, there is increasing evidence for an additional wound healing effect (275–277).

Figure 1.17. The electromagnetic spectrum. Figure taken from (278).
1.7.3. How to record the ‘dose’ of light-based treatment

Before discussing how to report the ‘dose’ delivered by light-based therapy, it is first important to define both ‘energy’ and ‘energy density’ as these are two of the most commonly reported dose parameters in previous light treatment studies.

\[ \text{Energy (J) = Power (W) x Time (s)} \]

\[ \text{Energy density (irradiance/fluence) (J/cm}^2\text{) = Energy (J)/ Area (cm}^2\text{)} \]

As a generalisation, lasers are able to produce higher power than LEDs, and can deliver higher energy over a shorter period of time. In addition, because laser light is more focussed (than LEDs) the total energy delivered per area is higher; however, practically speaking lasers are in general relatively limited to the treatment of smaller areas due to limits in beam size.

At present there is no agreed consensus on how to record the delivered ‘dose’ of light-treatment (including when reporting the results of treatment trials). The three most commonly reported dose parameters are energy, energy density and time (279). Jenkins and Carroll (279) proposed recommendations (Table 1.9) on how to design and report the dose (treatment) and beam (irradiation) parameters in clinical and laboratory studies. They conclude that the 8 key beam parameters which must be reported are the wavelength, power, irradiation time, beam area at the skin or culture surface, pulse parameters, anatomical location, number of treatments and the interval between treatments. In addition, the authors suggest that the further addition of other dose parameters (coherence, application technique, beam profile and spectral width) would be of additional benefit when reporting the results of light-based research.
Table 1.9. Proposed criteria for how to report the ‘dose’ of light used in both basic and clinical research (279).

<table>
<thead>
<tr>
<th>Treatment parameters</th>
<th>Irradiation parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beam spot size at target (cm²)</td>
<td>Centre wavelength (nm)</td>
</tr>
<tr>
<td>Irradiance at target (mW/cm²)</td>
<td>Spectral bandwidth (nm)</td>
</tr>
<tr>
<td>Exposure duration (sec)</td>
<td>Operating mode (e.g. continuous mode)</td>
</tr>
<tr>
<td>Radiant exposure (J cm²)</td>
<td>Frequency (Hz)</td>
</tr>
<tr>
<td>Radiant energy (J)</td>
<td>Pulse on duration (sec)</td>
</tr>
<tr>
<td>Numbers of points irradiated</td>
<td>Pulse off duration (sec) or duty cycle (%)</td>
</tr>
<tr>
<td>Area irradiated (cm²)</td>
<td>Energy per pulse (J)</td>
</tr>
<tr>
<td>Application technique (e.g. skin contact)</td>
<td>Peak radiant power (mW)</td>
</tr>
<tr>
<td>Number and frequency of treatment sessions</td>
<td>Average radiant power (mW)</td>
</tr>
<tr>
<td>Total radiant energy (J)</td>
<td>Polarization (yes, no or linear)</td>
</tr>
<tr>
<td></td>
<td>Aperture diameter (cm)</td>
</tr>
<tr>
<td></td>
<td>Irradiance at aperture (mW/cm²)</td>
</tr>
<tr>
<td></td>
<td>Beam divergence (rad or deg)</td>
</tr>
<tr>
<td></td>
<td>Beam Shape</td>
</tr>
<tr>
<td></td>
<td>Beam profile</td>
</tr>
</tbody>
</table>
1.7.4. Biostimulation

The mechanism underpinning light-based therapies still remain(s) incompletely understood. ‘Biostimulation’ (or ‘photobiomodulation’) is often used in the literature as an ‘umbrella term’ to describe a range of photochemical changes at the molecular, cellular and tissue level from treatment with red and near infrared light (273,274).

An overview of the key processes implicated in the mechanism of biostimulation from light-based treatment is depicted in Figure 1.18. The key biological process in biostimulation is believed to be the absorption of red and near infrared light by intracellular molecules (chromophores or photoacceptors) within the respiratory chain of mitochondria, in particular, cytochrome c (273,274,280). NO is considered to be a key mediator of biostimulation. NO inhibits the activity of cytochrome c and light-based therapy is believed to induce photodissociation of this inhibitory molecule. Therefore the result is stimulation in mitochondrial respiratory activity, including the generation of adenosine triphosphate (ATP) (273,274). Separate increased NO generation (through stimulation of NO synthase) by light treatment has also been described (281). The generation of reactive oxygen species has been implicated in the mechanism of light-based therapy (273,274). Due to a change in the redox (reduction vs oxidation) state of the cell, there is subsequent activation of various intracellular signalling pathways (273,274,280,282). This in turn activates downstream transcription factors, resulting in the expression of genes (and resultant protein synthesis) involved in a diverse range of both cellular (proliferation and survival) and tissue (repair and regeneration) processes, all of which are likely to be beneficial to wound healing (273,274).

Akin to normal wound healing, the fibroblast is likely to play a central role in biostimulation from light-based treatment. Light-based therapy in vitro improves wound healing (including stimulation of fibroblast function) and increases angiogenesis, with a reduction in the local inflammatory response (283–290). Other cell types that have been implicated in the mechanism of light-based therapy include (but are not limited to) mast cells, macrophages, keratinocytes, lymphocytes and endothelial cells (273,274).
1.7.5. Biphasic dose response from light-based treatment

Of great interest, a biphasic response (i.e. both stimulatory and inhibitory effects depending upon dose) has been reported with light-based treatment in vitro (291). This effect is often referred to as the ‘Arndt-Schulz law’, which refers to the work by the two authors examining the initial stimulatory (low dose) and subsequent inhibitory (high dose effect) of various poisons on yeast (273). Possible explanations for the inhibitory effect of light treatment at higher doses include (but are not limited to) excess reactive oxygen species and NO, both of which are potentially beneficial in low concentrations (291). A graphical representation of the biphasic dose response in light-treatment is depicted in Figure 1.19. Therefore, in light-based treatment there

Figure 1.18. Biostimulation. The key mechanisms implicated in biostimulation from red and near infrared light-based treatment. ATP: Adenosine triphosphate. Cyt c oxidase: Cytochrome oxidase C. NO: Nitric oxide. ROS: Reactive oxygen species. Figure taken from (274).
is likely an optimal ‘dose’ (a composite of the irradiance and duration of treatment), in which the maximal stimulatory wound healing is achieved.

Figure 1.19. Biphasic dose response to light. Graphical representation of the biphasic dose response to light-therapy. Initially there is a stimulatory effect; however, as the dose of light treatment increases this becomes inhibitory in nature. Figure taken from (273).

1.7.6. Other mechanisms of wound healing from light-based treatment
With regard to the design of our light-based treatment device (Chapter six) it is important to highlight other possible mechanisms (in addition to ‘biostimulation’), which also could benefit DU healing in patients with SSc. There is some emerging evidence that blue light may have a role in wound healing. In a skin flap model, blue light increases tissue perfusion by NO release (275) and improves wound healing in a wound excision model (276,277). In addition, a thermal effect (i.e. an increase in tissue temperature) is likely to occur from both infrared light and by using LEDs, both of which could potentially benefit wound healing.
1.7.7. The antibacterial properties of light

The antibacterial effect of ultraviolet light treatment is well recognised (292). However, longer-term exposure to ultraviolet light is potentially associated with serious detrimental side effects including a propensity toward skin ageing and malignancy through the accumulation of DNA damage, which limits its therapeutic application.

More recent work has demonstrated that blue light also possesses an antibacterial effect (293–297). In the study by Maclean et al (297), inactivation of *Staphylococcus aureus* from exposure to blue light occurred between 400 nm and 420 nm, with maximal inactivation occurring at 405 nm. Subsequently, Guffey and Wilborn (294) confirmed the bactericidal effect of 405 nm, and in addition, 470 nm blue light. However, of note, the bactericidal efficacy for 470 nm was only confirmed at higher doses for *Staphylococcus aureus*. It should be highlighted that these wavelengths of blue light are located in fairly close proximity to the ultraviolet region (200 nm to 400 nm), which as previously described is potentially hazardous with long-term exposure. Of note, unlike ultraviolet light, which often requires the presence of an exogenous photosensitising agent, blue light inactivates bacteria through the stimulation of endogenous photosensitising agents (i.e. porphyrins) and the production of highly dangerous reactive oxygen species (293–297).

1.7.8. Light-based treatment studies

The major previous treatment studies pertaining to light-based therapy for diabetic, venous and pressure skin ulcers are presented in Table 1.10. Almost all of these studies have adopted either a randomised, single- or double-blind, controlled approach, often with a placebo light ‘treatment’. It is important to highlight that many of these studies have often included patients with chronic skin ulcers, which were refractory to standard medical management. Light treatment in previous studies has either investigated combined red and infrared or broadband light therapy, including both LED and laser-based systems (Table 1.10). Treatment duration has
ranged between one and three months (Table 1.10). The primary outcome measure of light treatment efficacy in previous studies has been a measure of ulcer healing: either the number of ulcers completely healed, or a measure of ulcer surface area compared to baseline (Table 1.10). Methods to assess ulcer status have included physical measurement of the maximum ulcer length and width (298), marking of ulcer shape (using planimetry) (299) and independent clinician assessment (including of photographs) (300–302). Several studies have used digital camera systems with software analysis of (including of digitized) images (301,303–307). Other reported outcome measures of efficacy have included (but are not limited to) mean ulcer granulation (306) and patient visual analogue score (301). Specific ulcer healing scales have also been utilised (Pressure Sore Status Tool and the Pressure Ulcer Scale For Healing) (308,309). In one study, ulcer area was assessed using an infrared thermal camera (310).

Whether light-based therapy is or is not an effective treatment for skin ulcers remains a controversial issue. Overall, the majority of previous treatment studies have suggested a positive treatment effect with light-therapy (298–300,302–306,309,310). However, it is difficult to directly compare between the studies due to differences in design (e.g. treatment strategies and outcome measures) to summarise the overall efficacy of light treatment. In general, the majority of studies have suggested that light treatment was associated with an additional 50% in improvement in ulcer status compared to the comparator group (298–300,303,306,309,310). A number of studies have reported up to 90% improvement in ulcer status (complete healing or area) compared to baseline (298,300,303,306). Of interest, in the study by Taradaj et al (310), which included four patient groups who received treatment with one specific wavelength (940 nm, 808 nm or 658 nm) or placebo, only 618 nm light treatment was found to have a significant benefit compared to placebo. In addition, two studies have reported no overall difference in ulcer healing with light treatment (307,308).

Two recent systematic reviews have addressed the efficacy of light-treatment for diabetic and pressure ulcers (311,312). The former, which included 131 participants
from four randomised controlled trials, concluded a beneficial effect (Oxford Centre for Evidence-based Medicine, grade of recommendation B) with light treatment for diabetic ulcers (312). However, in the second systematic review, from the Cochrane group, the authors concluded that they were ‘very uncertain as to the effects of phototherapy in treating pressure ulcers’ (311). Both the systematic reviews expressed clear concerns about the design of the included studies, including (but not limited to) the small sample sizes, method of randomisation and blinding of the assessors, all of which need to be addressed in the design of future clinical trials (311,312).

There are several issues of importance to the design of future clinical trials of light-based treatment. In previous light studies, ulcers have demonstrated an overall tendency to improve irrespective of treatment. For example, in the study by Minatel et al (306), at 90 days, although the majority (>90%) of diabetic ulcers had completely healed in the light treatment group, almost half (43%) had also healed in the placebo group. Therefore, future studies must be appropriately designed and powered (with size and cost implications) to demonstrate a treatment effect with light therapy. The improvement seen in the comparator group may represent the natural history of ulcers to heal in the absence of complicating factors (e.g. infection), but also highlights the benefit of high quality wound care (irrespective of ulcer aetiology). Several authors have reported that over 50% of the total improvement from light treatment was observed during the first month of treatment (298,306). However, in the study by Lagan et al (301), although no significant difference between light treatment and placebo therapy was seen during the first month of treatment, at 4 and 8 weeks post treatment, a separation (treatment better than placebo) in ulcer healing emerged. Of interest (but not likely to be directly relevant considering the size of DUs in patients with SSc), in the study by Caetano et al (305), all the ‘small’ (<5cm²) venous ulcers had fully healed within 60 days, irrespective of treatment. Reassuringly, from the previous studies, light-based treatment was well tolerated and no significant safety signals were reported.
Table 1.10. Previous light-based clinical trials. The major previous treatment studies permitting to light-based therapy for diabetic, venous and pressure skin ulcers. Note, that additional information regarding some of the studies is provided in the figure legend. LED: Light emitting diode.

<table>
<thead>
<tr>
<th>Study</th>
<th>Ulcer type/s</th>
<th>Study design</th>
<th>Light treatment ‘dose’</th>
<th>Number of ulcers/participants</th>
<th>Treatment frequency and duration</th>
<th>Summary of main efficacy findings</th>
</tr>
</thead>
</table>
| Minatel et al 2009 (306) | Diabetic | Randomised, double-blind, placebo-controlled | **Treatment group**<sup>A</sup> Standard wound care + LED red (660 nm) and infrared (890 nm) light: 3 J/cm²  
**Placebo group**<sup>A</sup> Standard wound care + only LED 660 nm light (3/4 LEDs disconnected): <1.0 J/cm² | **Treatment group**  
13 ulcers  
7 patients  

**Placebo group**  
10 ulcers  
7 patients | Once weekly for 9 weeks | **Ulcer healing (as assessed digital photography) compared to baseline**  
Treatment vs placebo (mean)  
Day 30 = 66.9% vs -12.3%  
Day 60 = 82.7% vs 22.9%  
Day 90 = 90.8% vs 43.3 | **Ulcer granulation compared to baseline**  
Treatment vs placebo (mean)  
Day 30 = -2.3% vs 58.6%  
Day 60 = 10.8% vs 79.2%  
Day 90 = 30.8% vs 87.0% |
<table>
<thead>
<tr>
<th>Study</th>
<th>Ulcer type/s</th>
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<th>Summary of main efficacy findings</th>
</tr>
</thead>
</table>
| Kaviani et al 2011 (302) | Diabetic | Randomised, double-blind, placebo-controlled | **Treatment group** Laser 685 nm: 10 J/cm²  
**Placebo group** ‘Sham’ light therapy | Treatment group⁸ 13 patients  
Placebo group⁸ 10 patients | Six times per week (for at least two successive weeks) and then every other day until ulcer healing | Complete ulcer healing (as assessed by digital photography (week 20)) Treatment vs placebo (number) = 8 vs 3 (P = 0.47)  
Mean ulcer time to ulcer healing Treatment vs placebo = 11 weeks vs 14 weeks |
| Kajagar et al 2012 (299) | Diabetic | Randomised, controlled | **Treatment group** Standard wound care + Light (LED) treatment (commercially available, Thor International Ltd). Wavelength not clear in the manuscript. Variable ‘dose’ of 2-4 J/cm² (depending on the size of the ulcer)  
**Control group** Standard wound care | Treatment group 34 patients  
Control group 34 patients | Daily treatment for 15 days | Mean reduction in ulcer area Treatment vs control, 1043.20±266.62 mm² vs 322.44 ± 85.84 mm² (P = <0.010) |
<table>
<thead>
<tr>
<th>Study</th>
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<th>Summary of main efficacy findings</th>
</tr>
</thead>
</table>
| Landau et al 2011 (298) | Diabetic & venous | Randomised, double-blind, controlled | **Treatment group**<sup>C</sup>  
Broadband light: 400-800 nm, 180mW/cm<sup>2</sup>  
**Placebo group**  
‘Non-therapeutic’ light irradiation (10 mW/cm<sup>2</sup>) | **Treatment group**<sup>C</sup>  
19 ulcers  
10 patients (majority diabetic, 2 venous)  
**Placebo group**  
6 ulcers (diabetic) /6 patients | Twice daily  
Followed up to 12 weeks | Complete ulcer healing  
Treatment vs placebo = 9/10 patients vs 2/6 patients  
Reduction in ulcer size  
Treatment vs placebo = 89% vs 54% |
| Gupta et al 1998 (300) | Venous | Placebo-controlled, double-blind | **Treatment group**  
Two separate probes (monochromatic light source) were used:  
Red (660 nm: 4 J/cm<sup>2</sup>) & infrared (880 nm: 4 J/cm<sup>2</sup>)  
**Placebo group**  
‘Light of the same colour given using the same delivery system’ (300) | 12 ulcers<sup>D</sup>  
9 patient<sup>D</sup> | Twice daily  
10 weeks | Percentage of initial ulcer area remaining  
Treatment vs placebo = 24.4% vs 84.7% (P = 0.0008)  
Decrease in ulcer area (compared to baseline)  
Treatment vs placebo = 190 mm<sup>2</sup> vs 4.7 mm<sup>2</sup> (P = 0.0002) |
<table>
<thead>
<tr>
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<th>Light treatment ‘dose’</th>
<th>Number of ulcers/participants</th>
<th>Treatment frequency and duration</th>
<th>Summary of main efficacy findings</th>
</tr>
</thead>
</table>
| Lagan et al 2002 (301) | Venous       | Randomised, double-blind, placebo-controlled | **Treatment group** Standard wound care + Light (LED) device: 660–950 nm: 12 J/cm² | 16 ulcers 15 patients (treatment = 8 and placebo = 7) | Treatment once weekly for four weeks. Follow up at weeks 4 and 8 | Ulcer healing (as assessed by digital photography)  
Treatment vs placebo  
End week 4: No difference between groups (values not given) (P = 0.14)  
Week 8: 54.9% vs 7.0%  
Week 12: 61.3% vs + 11.6% |
| Medenica & Lens 2003 (304) | Venous       | Prospective, case-series            | Polarised, non-coherent light: 480-3400 nm: 19.2 J/cm² | 25 patients                  | Treatment once daily over four weeks | Number of ulcers  
Start vs end of the study = 73 vs 51 (P = <0.01)  
Mean ulcer healing (as assessed by digital photography) 3.53 cm²/week |
<table>
<thead>
<tr>
<th>Study</th>
<th>Ulcer type/s</th>
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<th>Summary of main efficacy findings</th>
</tr>
</thead>
</table>
| Caetano et al 2009 (305) | Venous       | Randomised, double-blind, placebo-controlled | Group 1: Standard wound care + placebo light (LED): <0.3 J/cm<sup>2</sup>  
Group 2: Standard wound care + light (LED) treatment: 3 J/cm<sup>2</sup>  
Group 3: Standard wound care only | 32 ulcers 20 patients | Treatment twice weekly and followed up until ulcer healing | Mean ulcer healing rate  
Day 30 vs 60 vs 90  
Group 1: 20% vs 30% vs 40%  
Group 2: 30% vs 40% vs 50%  
Group 3: -10% vs 10% vs 40% |
<table>
<thead>
<tr>
<th>Study</th>
<th>Ulcer type/s</th>
<th>Study design</th>
<th>Light treatment ‘dose’</th>
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<th>Treatment frequency and duration</th>
<th>Summary of main efficacy findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leclère et al 2010 (307)</td>
<td>Venous</td>
<td>Randomised, Comparative</td>
<td>Treatment group&lt;sup&gt;E&lt;/sup&gt; Laser 980 nm: 90 J/cm&lt;sup&gt;2&lt;/sup&gt; Control group&lt;sup&gt;E&lt;/sup&gt; Standard wound care. No placebo light</td>
<td>Treatment group 18 patients Control group 16 patients</td>
<td>Treatment once weekly for 9 weeks</td>
<td>Number of patients with complete ulcer healing during treatment Treatment vs control group = 3 (16.7%) vs 4 (25%) Ulcer healing compared to baseline at the end of study (as assessed digital photography) Treatment vs control group = 74.2% vs 94.3% (P = 0.6) Mean ulcer VAS between each treatment Treatment vs control group = 2.7 vs 3.8 (P = &lt;0.86) Mean ulcer VAS during each treatment Treatment vs control group = 1.8 vs 3.8 (P = &lt;0.67)</td>
</tr>
<tr>
<td>Study</td>
<td>Ulcer type/s</td>
<td>Study design</td>
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</tbody>
</table>
| Schubert et al 2001 (303)     | Pressure     | Randomised, controlled              | **Treatment group** Standard wound care + monochromatic (LED) therapy: Red (637 nm) and infrared (956 nm) | 74 patients (n = 37 in both groups) \( ^{F} \) | Frequency (n) per week: Week 1 = 5, Week 2 = 4, Week 2 = 3, Week 4 and thereafter = 1 Followed up to 10 weeks | **Ulcer area** Reduced to 10% (of baseline) within 5 weeks of treatment and 9 weeks in the control group  
*Healing rate* Treatment group had a 49% higher ulcer healing rate than the control group |
| Taly et al 2004 (308)         | Pressure     | Randomised, double-blind, placebo-controlled | **Treatment group** Variable light (LED and laser) wavelengths: between 660 and 950 nm: 4.5 J/cm\(^2\) | **Treatment group \(^{G}\)** 35 ulcers | 14 light treatments were given, 1 every alternate day, 3 times a week (until ulcer healing or had received 14 treatments) | **Ulcer healing** Treatment vs control (number) = 18 vs 14 (P = 0.802)  
*Time to ulcer healing* Treatment vs control = 2.45 weeks vs 1.78 (P = 0.330)  
**Placebo group \(^{G}\)** 29 ulcers |
<table>
<thead>
<tr>
<th>Study</th>
<th>Ulcer type/s</th>
<th>Study design</th>
<th>Light treatment ‘dose’</th>
<th>Number of ulcers/participants</th>
<th>Treatment frequency and duration</th>
<th>Summary of main efficacy findings</th>
</tr>
</thead>
</table>
| Durović et al 2008     | Pressure     | Randomised, single-blind, placebo-controlled | Treatment group
Broadband light: 400-2000 nm, 40 mW/cm²/2.4 J/cm²
Placebo group
Standard wound care | Treatment group
20 ulcers                  | Light treatment was administered once daily, 5 times per week for 1 month | Ulcer surface area between groups at the end of treatment
Treatment vs placebo = 10.8 m² vs 23.0 m² (P = 0.0005)

Pressure Ulcer Scale for Healing (PUSH)
Treatment vs placebo = 7.4 vs 11.9 (P = 0.0003) |
<table>
<thead>
<tr>
<th>Study</th>
<th>Ulcer type/s</th>
<th>Study design</th>
<th>Light treatment ‘dose’</th>
<th>Number of ulcers/participants</th>
<th>Treatment frequency and duration</th>
<th>Summary of main efficacy findings</th>
</tr>
</thead>
</table>
| Taradaj et al 2013 (310) | Pressure | Randomised, single-blind, placebo-controlled | **Group 1:** 940 nm  
**Group 2:** 808 nm  
**Group 3:** 658 nm  
Groups 1-3: 4 J/cm²  
**Group 4:** Placebo light treatment | Patients (number) included in the final analysis  
Group 1: n = 18  
Group 2: n = 18  
Group 3: n = 17  
Group 4: n = 18 | Light treatment was administered once daily, 5 times per week for 1 month | **Healed ulcers (as assessed by a thermal infrared camera) at one and three months**  
Group 1: 11.1% and 16.7%, respectively  
Group 2: 11.1% and 16.7%, respectively  
Group 3: 47.1% and 58.8%, respectively  
Group 4: 11.1% and 16.7%, respectively  
**Decrease in ulcer area after one month (of treatment)**  
Group 1: 31.2%  
Group 2: 29.9%  
Group 3: 71.1%  
Group 4: 28.3% |
**Comments**

A. Thirty-six LEDs in total (4 X 660 nm and 32 X 880 nm). For the placebo treatment all the 880 nm LEDs were disabled and only one central 660 nm was utilised.

B. Likely LED-based. Five patients could not complete the study follow-up visits. Two patients from the placebo group underwent amputation due to gangrene. One patient in the light treatment group died due to myocardial infarction.

C. Twenty patients recruited into the study: 3 patients (2 placebo: 1 treatment) excluded due to poor compliance using the device at home and 1 patient excluded due to renal disease.

D. One patient excluded in the final analysis because they elected to discontinue the study due to a perceived lack of efficacy (after breaking the randomisation the patient had been in the placebo arm).

E. Ulcer debridement at start of the study in both patient groups. Ulcer surface temperature during light treatment was ‘controlled’ at 45-50°C using a thermal infrared camera.

F. Light treatment not completed for 10 ulcers (treatment and control group: 8 & 2 respectively) in 5 patients (2 deaths, one patient discontinued the study and two ulcers became infected).

G. Before each light treatment, the ulcer was ‘splashed’ with oxygen therapy. Two patients withdrawn on medical reasons and two patients died in the treatment and control groups, respectively.

H. Light treatment was turned off in patient group 4 (placebo). Only the applicator (non-coherent red light) was turned on. One death and one patient discontinued (due to an unrelated medical issue).
1.8. Aim, hypotheses and objectives

Against the background presented, the overarching aim, hypotheses and objectives of the thesis are as follows.

Aim

The overarching aim is to investigate the definition and objective measurement of SSc-related DUs, their pathophysiology, and a new approach to local (light-based) treatment. Specifically, the following hypotheses will be tested:

Hypotheses

*Overarching hypothesis*

That improving the measurement and understanding of the pathogenesis of DUs in SSc will facilitate the development of targeted, locally acting therapies for DU disease.

*Individual hypotheses*

1. That the addition of ‘real-world’ clinical information will improve the reliability of the grading of DUs by rheumatologists.
2. That high-frequency ultrasound (HFUS) can be used safely to objectively measure DU status in patients with SSc.
3. That functional microvascular disease is associated with the development of DUs in patients with SSc.
4. That DUs in patients with SSc are ischaemic in aetiology, including those located on the extensor aspect of the hand, and this is potentially reversible through NO donation through topical application of GTN compared to placebo.
5. That light-based treatment is a safe and effective treatment for SSc-related DUs.
Objectives

My specific objectives were to investigate, in patients with SSc:

1. The inter- and intra-rater reliability of DU assessment, with and without accompanying contextual clinical information, as assessed by rheumatologists using a web-based interface.
2. The feasibility and tolerability of HFUS to measure DUs, and secondly, to tentatively assess criterion and construct validity.
3. Whether functional microvascular disease as assessed by thermography is associated with the development of DUs?
4. To assess the responsiveness of the microvessels in the DU centre and periphery to GTN compared to placebo as assessed by LDI, and whether this differs between fingertip and extensor DUs.
5. The safety, feasibility and tolerability of light-based treatment for SSc-related DUs, and, tentatively, treatment efficacy.
2. Does the clinical context improve the reliability of rheumatologists grading digital ulcers in systemic sclerosis?

2.1. Abstract

Objectives: Digital ulcers (DUs) are often a primary end point in systemic sclerosis (SSc) clinical trials, although the reliability of rheumatologists grading DUs is poor to moderate at best. DU assessment in recent trials has been based upon visual inspection alone, which potentially misses ‘real-world’ clinical contextual information. Our aim was to investigate whether this clinical information improves the reliability of rheumatologists grading DUs. A secondary aim was to assess agreement between patients and rheumatologists.

Method: Eighty images of a range of digital lesions were collected from patients with SSc with the clinical context: pain (severity and temporal relationship), lesion duration and discharge (patient reported and clinician observed). Raters received all images either with or without the clinical context, and graded these images (using a custom-built interface) on an ordinal scale of severity: ‘no ulcer’, ‘inactive ulcer’ or ‘active ulcer’. Patients also graded their lesion/s on the same scale.

Results: Fifty-one rheumatologists from 15 countries completed the study (26 without and 25 with context): 4590 (including 510 repeated) image gradings were obtained. Context did not significantly increase (without and with context) either intra- (0.64,0.71) or inter-rater (0.32,0.36) reliability. Pain (VAS and temporal relationship) and discharge (patient reported and clinician observed) were associated with increased lesion severity, and duration with reduced severity. Agreement between individual patients and rheumatologists was poor without and with context (0.19,0.28).

Conclusion: The overall intra and inter-rater reliability of DU grading did not significantly improve with the clinical context. Agreement between patients and rheumatologists was poor.
2.2. Introduction

Digital ulcers (DUs) are common in patients with systemic sclerosis (SSc) and are responsible for much of the pain and disability associated with the disease. Half of patients with SSc may report a history of DUs (often early in the course of the disease) [1]. These DUs are associated with high levels of hand (and global) disability, impacting negatively on the activities of daily living (including occupation) and health related quality of life [2]. Patients with DUs have a worse clinical outcome than those without [3], including an association with internal organ involvement in very early SSc [4]. Despite effective drug therapies (e.g. endothelial receptor antagonists and phosphodiesterase type-5 inhibitors) [5-7], patients may still develop new DUs on treatment.

DUs are also important for two other reasons: firstly, they are often a primary outcome measure in SSc-related clinical trials and secondly, ‘fingertip lesions’ (including DUs) are now included in the 2013 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria for SSc [8-9]. Of concern, the reliability of rheumatologists with an interest in SSc (i.e. those who are likely to assess patients with SSc in clinical trials) grading DUs has been reported to be poor to moderate at best [10-11]. The assessment of DUs, including in several recent multi-centre, placebo-controlled trials, has generally been based upon visual inspection of the lesion alone [5-7,12]. A potentially key issue is that this misses important clinical contextual information (e.g. whether the lesion has recently developed associated pain, and whether there has been discharge), which many clinicians use in their routine practice when assessing DUs. If the inclusion of the clinical contextual information were to improve the reliability of DU grading, then its incorporation should be strongly considered in the design of future SSc-related clinical trials.

Against this background, our primary aim was to investigate the intra- and inter-rater reliability of the grading of photographs of digital lesions (chosen to include mainly
lesions likely to be classed as DUs) by rheumatologists with an interest in SSc, both without and with the accompanying contextual clinical information. A secondary aim was to examine the agreement between individual patient and rheumatologist opinion.

2.3. Patients and methods

Study design and participants

Eighty clinical images of a range of digital lesions (location of lesion [number]: ‘fingertip’ = 34, ‘extensor’ = 32 and ‘other’ = 14) were prospectively collected from 36 patients with SSc-spectrum disorders (the majority of whom had SSc); either at their routine clinic attendance or during an episode of hospitalisation. A trained medical photographer took all the images, placing a small graded scale (length of one centimetre) in close proximity to the digital lesion to give raters an indication of the size of the lesion. For each digital lesion the clinical contextual information was collected: the pain associated with the lesion on a visual analogue score (100 being most severe) and the temporal relationship (whether the pain was less, the same or worse than a week previously), the duration of the lesion (patient reported) and the presence of discharge (both patient reported and clinician observed). Patient and digital lesion characteristics are presented in Supplementary material. The study was approved by the National Research Ethics Committee East of England – Hatfield and all patients provided signed informed consent.

To facilitate the study, a custom built, secure, web-based interface was constructed to display and to record the grading of the images. Individuals (raters) with an interest in SSc (i.e. representative of those clinicians who would be scoring DUs in clinical trials) were invited through SSc-based organizations (the UK Scleroderma Study Group and the Scleroderma Clinical Trials Consortium) to participate in the study. Raters were randomised to receive all the images either without or with the clinical contextual information. Each rater graded 90 images: 80 unique images (in a random order) and then 10 repeated images (from the first 50 images), to allow an assessment of intra-rater reliability. Raters graded the images on a 3-point ordinal scale of
severity: either ‘no ulcer’ (0) or ‘ulcer’, and if ‘ulcer’ then either ‘inactive’ (1) or ‘active’ (2). No exemplar images or definitions (of the grading system) were provided at any point during the study. Raters were not able to return to previously graded images once they had selected their chosen answer for an individual image.

**Patient grading of digital lesions**

At the same time as when the clinical photograph was obtained, patients were also asked to grade their own digital lesion/s on the same ordinal scale of severity as was subsequently used by rheumatologists to grade the photographic images.

**Statistical analysis**

Reliability of categorical scales can be assessed using kappa coefficients that measure the measure of agreement between raters. The categories of the rating scale being used were ordered in terms of severity ‘no ulcer’, inactive ulcer’, ‘active ulcer’. For ordered scales a weighted kappa coefficient can be used, which is also an intra-class correlation coefficient. Overall intra-rater reliability was assessed using a weighted kappa coefficient with quadratic weights. Similar to our previous study [10], the reliability of ratings between pairs of categories was assessed using the interclass kappa coefficients [13]. We assessed the reliability by combining adjacent categories on the ordinal scale: ‘no ulcer vs. ‘ulcer (‘active’ and ‘inactive’ combined), and for ‘no ulcer’ and ‘inactive ulcer’ vs. ‘active ulcer’ [10]. Although somewhat arbitrary it has been suggested that kappa statistic can be interpreted as less than by chance alone (<0.0), poor (0.01-0.20), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80), almost perfect (0.81-0.99) and perfect (1.0) agreement between raters [14], so that a change in the kappa statistic of +/- 0.1 could therefore be considered a meaningful change in reliability. Confidence intervals for the kappa coefficient (means) were generated by the non-parametric bootstrap method, with random resampling (n = 1000) by rater. The association between the clinical context components was investigated using ordinal logistic regression, and agreement between the individual patient and rheumatologists was explored using a weighted
kappa coefficient with quadratic weights. All statistical analyses on the data were performed using STATA version 13.

2.4. Results

Fifty-one clinicians (raters) completed the study: 26 without and 25 with the clinical context. The raters came from 15 countries, the majority from the US [33%), UK [22%, Canada [10%] or Italy [8%]. A total of 4590 (4080 unique and 510 repeated) image gradings were obtained. The mean score (SD) both without and with the clinical context for the first (unique) images was 1.08 (0.85) and 1.01 (0.87) (P=0.32), and for the second (repeat) image was 1.13 (0.82) and 1.05 (0.90) (P=0.28), respectively. Figure 2.1 depicts a range of digital lesions and the results of the grading by raters, with and without the clinical context.

Intra-rater reliability

Intra-rater reliability is summarised in Table 2.1. The overall intra-rater reliability was high with no significant difference observed between those graders without and with the clinical context. The overall weighted kappa coefficient was 0.64 (95% CI 0.53 to 0.75) and 0.71 (95% CI 0.64 to 0.78) for without and with context respectively. The analyses comparing pairs and when combining adjacent categories is presented in Table 2.1.

Inter-rater reliability

Inter-rater reliability is summarised in Table 2.1. The overall inter-rater agreement as measured by a weighted kappa coefficient was poor with no major difference without (κ = 0.32, 95% CI 0.25 to 0.39) or with (κ = 0.36, 95% CI 0.28 to 0.44) the clinical context. The analyses comparing pairs and when combining adjacent categories is presented in Table 2.1.
Impact of the clinical contextual information

Table 2.2 presents the results of the ordinal logistic regression (combined/pooled odds ratios) between the individual clinical context components and the overall grading of the digital lesions by raters (including those who did and did not receive the clinical context).

Subgroup analysis of digital lesion anatomical location

The clinical context was associated with an increase in the intra-rater reliability (without and with) for ‘fingertip’ ($\kappa = 0.58, 0.73$) and for ‘other’ lesion ($\kappa = 0.67, 0.75$) but not of ‘extensor’ lesions ($\kappa = 0.68, 0.66$). There was however no notable change in inter-rater reliability for ‘fingertip’ ($\kappa = 0.37, 0.42$), ‘extensor’ ($\kappa = 0.26, 0.30$) or ‘other’ ($\kappa = 0.31, 0.39$) digital lesions with the addition of the clinical context.

Agreement between the individual patients and rheumatologists

Individual patients and rheumatologists infrequently graded the digital lesions as the same category (on an ordinal scale of severity), without or with the clinical context (42% vs 48%, respectively). There was evidence of a marked disparity between patients and rheumatologists with little (clinically relevant) improvement with the addition of the clinical context (weighted kappa value of 0.19 vs 0.28), which may reflect the uncertainty of the raters, rather than differences of opinion.

2.5. Discussion

The key finding of our study is that adding clinical context did not significantly change the overall intra- or inter-rater reliability of the grading of digital lesions on an ordinal scale of severity by rheumatologists with an interest in SSc. As might be expected and in keeping with our previous study [10], intra-rater reliability was significantly higher (both without and with context 0.64, 0.71) than inter-rater
reliability (0.32,0.36). The poor inter-rater reliability is of concern because this suggests that individuals with an interest in SSc are likely to disagree, even on a three-point ordinal scale of severity.

Several patterns emerged from the grading of the images as depicted in Figure 2.1. Firstly, there were a number of images that the raters were in complete (or almost complete) agreement about the assigned category, particularly at the ‘extremes’ of the scale (‘no ulcer’ or ‘active ulcer’), independent of the clinical context. Secondly, there were a second group of images in which raters were divided in opinion between the ends of the scale (i.e. ‘no ulcer’ and ‘active ulcer’), with no significant improvement in agreement with the context (exemplified by Figure 2.1C and D). Thirdly, there were images (exemplified by Figure 2E and F) where the overall lesion grading was shifted (either lower or higher) with the context.

Intra-rater reliability was much greater with the addition of clinical context for ‘no ulcers’ vs. ‘inactive ulcers’ (0.36 vs 0.67) and ‘no ulcers vs. ‘inactive ulcers’/‘active ulcers’ (0.71 vs 0.82), which suggests that some clinicians might use the clinical context in particular to distinguish ‘non-ulcer’ lesions from ‘ulcers’. This effect is lost when assessing inter-rater reliability due to the significant disagreement between raters, including a negative kappa statistic for the inter-rater reliability of ‘no ulcers’ vs. ‘inactive ulcers’ (which suggests that agreement was less than chance irrespective of context). For ‘inactive ulcers’ vs. ‘active ulcers’ there was a decrease (0.53 vs 0.41) in intra-rater reliability with the addition of context, which suggests that the clinical context might also introduce confusion between adjacent (similar) categories.

As might be expected, pain (VAS and temporal relationship) and discharge (patient reported and clinician observed) were associated with increased severity, and lesion duration with reduced severity. Rater scoring was associated with clinical context (including in those graders who did not receive context), which in part suggests that the clinical context is either visible or associated with other visible features. It is likely that DU assessment is complex, and rheumatologists obtain a wealth of clinical cues
from visual assessment of the lesion, that these are inter-related, and that the clinical context may help to inform their classification.

There was a significant increase in intra-rater reliability for ‘fingertip’ lesions with the clinical context (0.58 vs, 0.73). Only those DUs, which occur at the fingertips, are believed to be ischaemic [15]. Several recent studies have only included DUs that occur distal to the proximal interphalangeal joints (including those at the fingertip) [5-7]; therefore, raters (including through participation in SSc clinical trials) might be more familiar in assessing ‘fingertip’ lesions.

Agreement between the individual patients and rheumatologists was poor, with only a small increase between those rheumatologists who did not (0.19) or did receive (0.28) the clinical context. This is important for two reasons; firstly, does the patient DU construct potentially hold components that might improve clinician DU assessment, and secondly, implications for clinical practice (e.g. does the patient seek medical attention appropriately for the development of DUs and/or treatment escalation)?

A North West American working group recently developed by consensus a classification of DUs in SSc [11]. Although there are important differences between the two studies, including the inclusion of ‘healed’ and ‘indeterminate’ DUs in the study by Baron et al, the reliability of DU assessment in ‘live’ patients was not significantly higher compared with our results.

Our study has limitations. This was a web-based study; therefore, although this approach allowed large numbers of raters to participate, it could be argued that this misses ‘real-world’ clinical cues used by clinicians when assessing DUs in routine practice. We used a simple, pragmatic scoring system which did not include either ‘healed’ or ‘indeterminate’ categories which have been included in recent multi-centre studies [5-7], although the definition ‘inactive’ might be interpreted as ‘healed’ (or healing). Similarly to our previous study, inter-rater reliability for ‘no ulcer’ vs. ‘inactive ulcer’ produced a negative kappa statistic, which suggests that the inclusion of a ‘healed’ or ‘inactive’ ulcer category is unlikely to be helpful in clinical trials with
multiple raters. We also did not provide the raters with definitions of the categories or exemplar images, as we did not intend to test the reliability of a particular grading system. In addition, there are other clinical context factors, which we did not include which should be further explored (e.g. history of DUs and trauma). It could be argued that the high level of intra-rater reliability is in part due to recall by the grader; however, each participant viewed a large number of unique (80) images and the repeats were taken from the first 50, thereby reducing the likelihood of significant recall.

In conclusion, the addition of clinical context was not associated with a significant increase in either the overall intra- or inter-rater reliability of DU grading by rheumatologists. Agreement between the individual patients and rheumatologists was also poor. Further work to facilitate future SSc trials is needed including (but not limited to) the development of DU grading systems (potentially encouraging clinicians to use clinical contextual information in particular to help distinguish ‘no ulcer’ from DU), exploring the patient DU construct, and the investigation of (objective) measurement techniques.

Acknowledgements
Fredrick M Wigley, Frank A Wollheim. We acknowledge the contribution made by Mr Phil Steer in the construction of the web-based interface. We would also like to thank Mr Steve Cottrell and colleagues from the Medical Illustration Department, Salford Royal NHS Foundation Trust, for collecting the images.
2.6. Significance and innovations

• The overall intra- and inter-rater reliability of digital ulcer grading did not significantly improve with added clinical context.

• There was a trend that some clinicians may use the clinical context to help classify lesions as ‘no ulcer’.

• Pain (VAS and temporal relationship) and discharge (patient reported and clinician observed) were associated with increased lesion severity, and lesion duration with reduced severity.

• Future research is warranted to improve the reliability of rheumatologists grading digital ulcers as an end point in SSc-related clinical trials.
2.7. References


<table>
<thead>
<tr>
<th></th>
<th>INTRA-RATER RELIABILITY (95% CI)</th>
<th>INTER-RATER RELIABILITY (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without context</td>
<td>With context</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td>0.64 (0.53,0.75)</td>
<td>0.71 (0.64,0.78)</td>
</tr>
<tr>
<td><strong>Pairwise</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ulcers vs. inactive ulcers</td>
<td>0.36 (0.08,0.60)</td>
<td>0.67 (0.50,0.84)</td>
</tr>
<tr>
<td>No ulcers vs. active ulcers</td>
<td>0.95 (0.90,1.0)</td>
<td>0.90 (0.84,0.95)</td>
</tr>
<tr>
<td>Inactive vs. active ulcers</td>
<td>0.53 (0.38,0.67)</td>
<td>0.41 (0.22,0.60)</td>
</tr>
<tr>
<td><strong>Dichotomised</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No ulcers vs. inactive/active ulcers</td>
<td>0.71 (0.60,0.81)</td>
<td>0.82 (0.75,0.89)</td>
</tr>
<tr>
<td>No ulcers/inactive ulcers vs. active ulcers</td>
<td>0.74 (0.66,0.82)</td>
<td>0.72 (0.65,0.79)</td>
</tr>
</tbody>
</table>

Table 2.1. The intra- and inter-rater reliability of the grading of digital lesions, without and with the clinical context.
<table>
<thead>
<tr>
<th>Univariate</th>
<th>Without context</th>
<th>With context</th>
<th>Interaction</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain VAS</td>
<td>1.05 (1.03, 1.07)</td>
<td>1.23 (1.18, 1.28)</td>
<td>1.17 (1.12, 1.22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pain temporal</td>
<td>1.02 (0.97, 1.06)</td>
<td>1.53 (1.41, 1.67)</td>
<td>1.51 (1.37, 1.66)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of the lesion</td>
<td>0.82 (0.79, 0.86)</td>
<td>0.76 (0.71, 0.80)</td>
<td>0.92 (0.86, 0.98)</td>
<td>0.015</td>
</tr>
<tr>
<td>Discharge: Patient reported</td>
<td>5.37 (4.02, 7.17)</td>
<td>8.64 (6.02, 12.38)</td>
<td>1.61 (1.02, 2.55)</td>
<td>0.042</td>
</tr>
<tr>
<td>Discharge: Clinician reported</td>
<td>3.00 (2.13, 4.24)</td>
<td>4.21 (2.86, 6.20)</td>
<td>1.40 (0.84, 2.35)</td>
<td>0.200</td>
</tr>
</tbody>
</table>

Table 2.2. Ordinal logistic regression between the clinical context and the image grading. Combined (pooled) odds ratios are presented with 95% confidence intervals. Pain VAS given for a 10% increase on a 0-100 scale.
A

Pain VAS = 13/100
Pain temporal = Same
Lesion duration = 4 weeks
Patient discharge = Yes
Clinician discharge = Yes

B

Pain VAS = 16/100
Pain temporal = Same
Lesion duration = >12 months
Patient discharge = No
Clinician discharge = No

C

Pain VAS = 90/100
Pain temporal = Worse
Lesion duration = 3 weeks
Patient discharge = No
Clinician discharge = No

D

Pain VAS = 100/100
Pain temporal = Same
Lesion duration = 5 weeks
Patient discharge = Yes
Clinician discharge = Yes

E

Pain VAS = 0/100
Pain temporal = N/A
Lesion duration = >12 months
Patient discharge = No
Clinician discharge = No

F

Pain VAS = 55/100
Pain temporal = Less
Lesion duration = 3 months
Patient discharge = Yes
Clinician discharge = No
Figure 2.1. Grading of the images of digital lesions by rheumatologists. Left: Images of the digital lesions, centre: clinical contextual information and right: the results of the grading (blue and red bars without and with clinical context, respectively). Images with: high agreement irrespective of context (A&B), low agreement (with little improvement with context) (C&D) and substantial change in grading with the context (E&F).
2.8. Supplementary material

<table>
<thead>
<tr>
<th>Patient characteristics (n = 36)</th>
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</tr>
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<tbody>
<tr>
<td>Sex: female (number, %)</td>
<td>28 (80%)</td>
</tr>
<tr>
<td>Age in years, mean (SD)</td>
<td>56.4 (14.1)</td>
</tr>
<tr>
<td>Disease subtype (limited)</td>
<td>19 (54%)</td>
</tr>
<tr>
<td>RP duration in years, mean (SD) *</td>
<td>18.2 (14.6)</td>
</tr>
<tr>
<td>Disease duration in years, mean (SD) *</td>
<td>12.4 (10.8)</td>
</tr>
<tr>
<td>History of intravenous vasodilator therapy, number (%)</td>
<td>18 (51%)</td>
</tr>
<tr>
<td>History of debridement, number (%)</td>
<td>14 (40%)</td>
</tr>
<tr>
<td>History of amputation, number (%)</td>
<td>4 (11%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Digital lesion characteristics (n = 80)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain VAS (x/100), mean (SD)</td>
<td>36.3 (31.4)</td>
</tr>
<tr>
<td>Pain temporal (number)</td>
<td></td>
</tr>
<tr>
<td>Not applicable</td>
<td>20</td>
</tr>
<tr>
<td>Less</td>
<td>15</td>
</tr>
<tr>
<td>Same</td>
<td>32</td>
</tr>
<tr>
<td>Worse</td>
<td>13</td>
</tr>
<tr>
<td>Duration of the lesion (number)</td>
<td></td>
</tr>
<tr>
<td>&lt;1 month</td>
<td>12</td>
</tr>
<tr>
<td>1-3 months</td>
<td>21</td>
</tr>
<tr>
<td>3-6 months</td>
<td>7</td>
</tr>
<tr>
<td>6-9 months</td>
<td>6</td>
</tr>
<tr>
<td>9-12 months</td>
<td>3</td>
</tr>
<tr>
<td>&gt;12 months</td>
<td>31</td>
</tr>
<tr>
<td>Discharge: Patient reported, ‘yes’ * (number)</td>
<td>10</td>
</tr>
<tr>
<td>Discharge: Clinician observed, ‘yes’ * (number)</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2.3. Patient and digital lesion characteristics. *Five lesions had patient reported and clinician observed pus.
3. A pilot study using high-frequency ultrasound to measure digital ulcers - a possible outcome measure in systemic sclerosis clinical trials?

3.1. Abstract

Objective: Digital ulcers (DUs) are often the primary end point in systemic sclerosis (SSc) clinical trials, although the reliability of rheumatologists grading DUs is poor to moderate. High-frequency ultrasound (HFUS) has been used to reliably measure skin thickness in SSc. Our primary aim was to assess the feasibility and tolerability of HFUS to measure DUs in patients with SSc. A secondary aim was to tentatively assess criterion and construct validity.

Methods: HFUS was performed on 15 DUs from 11 patients with SSc-spectrum disorders (mainly SSc). Patient opinion on the feasibility and tolerability (including pain VAS) of HFUS was collected. By consensus agreement, where ‘classifiable’, DU width and depth were measured. DU HFUS measurements were correlated with those from clinical photographs (criterion validity) and DU VAS as assessed by patients and two clinicians (construct validity).

Results: HFUS was considered ‘feasible’ in almost all patients (n=10), with low reported associated pain (median [IQR] 0 [0-35]). DU width and depth were measured in the majority (n=13) of DUs. The mean DU width and depth were 5.74mm and 0.99mm, respectively. There was moderate correlation between DU HFUS and photograph surface measurements. DU HFUS measurements were moderately correlated with independent DU severity grading of DU (clinical photographs), with little correlation with patient or HFUS operator DU severity.

Conclusion: DU HFUS was feasible and well tolerated, and DU width and depth was measurable in the majority of DUs. Future research is warranted to develop DU HFUS as an objective outcome measure in SSc-clinical trials.
3.2. Introduction

Digital ulcers (DUs) are often the primary end point in systemic sclerosis (SSc)-related clinical trials (especially those of digital vasculopathy). However, the reliability of SSc-interested rheumatologists (and therefore those most likely to participate in SSc trials) in grading DUs is poor to moderate at best [1-3]. In previous multi-centre, randomised, controlled trials, clinician assessment of DUs has been based upon visual inspection alone [4-6]. Key challenges in the assessment of DUs by clinicians (with the human eye) are that lesions are often very small compared to other (e.g. diabetic and pressure) ulcers, and that there may often be a crust-like cover of necrotic tissue and exudate (‘scab’); therefore, assessment of DU ‘width’ and ‘depth’ could be significantly under or over-estimated. In addition, because progression of infected DUs to involve subcutaneous tissue can lead to osteomyelitis (with possible digital loss), DU ‘depth’ is also an important clinical question.

DUs commonly occur on the fingertips (most likely due to ischaemia) and over the extensor aspects of the hands, in particular over the small joints (most likely due to recurrent microtrauma and increased skin tension) [7]. DUs may also occur in relation to underlying subcutaneous calcinosis [7]. Despite a number of effective treatments to prevent and heal DUs [5,6], in some patients recurrent DUs remain a major source of morbidity [8]. It is also increasingly recognised that DUs are a biomarker of a poor prognosis, being associated with development of internal organ complications and mortality (including in patients with the Very Early Diagnosis of SSc) [9,10]. Therefore a method of accurately assessing DUs is badly needed, to facilitate future clinical research.

High-frequency ultrasound (HFUS) operates at a higher frequency (typically 10-90 MHz) than diagnostic medical ultrasound, and so has higher image resolution, but at the cost of reduced tissue penetration. HFUS in healthy controls using skin punch biopsies as experimental models of ‘wounds’ has been reported to measure successfully temporal changes in the dimension of the wound base [11]. In patients
with SSc, HFUS has been used to measure skin thickness (currently as a research tool only) with reported high intra- and inter-observer reliability [12,13]. HFUS is also able to successfully delineate different phases of the disease process (e.g. dermal oedema vs atrophy) [12], and with a discriminatory ability to detect change over time (e.g. correlating with changes in the skin score) [12]. HFUS is an imaging technique that is being increasingly used in the assessment of a wide range of clinical conditions including cutaneous ulceration, in particular, the investigation of the pathophysiological mechanisms driving pressure ulcer development [15].

Against this background, the primary aim of our study was to examine the feasibility and tolerability of HFUS to measure DUs in patients with SSc. A secondary aim of our study was to tentatively assess possible markers of criterion and construct validity.

### 3.3. Methods and materials

**Study design and participants**

Eleven patients with systemic sclerosis-spectrum disorders (Table 3.1) and 15 DUs (4 fingertip, 7 extensor, 2 nailbed, 1 lateral and 1 palmar aspect) were recruited in the study (exemplars provided in Figure 3.1). One DU occurred in relation to underlying calcinosis (Figure 3.1) and four patients had two DUs imaged at the single study visit. Clinical and demographic information was collected for each patient, including a history of severe digital vascular disease (defined as either previous DUs and/or intravenous vasodilator therapy). For each DU (Figure 3.2), HFUS imaging (Episcan I-200, frequency 35 MHz) was performed (using sterile ultrasound gel) along the visually apparent ‘long’ (the longest surface dimension) and ‘short’ axes (defined as being 90° perpendicular to the ‘long’ axis) (as represented in Figure 3.1D). This was then repeated immediately by the same operator, and then by a second operator. The depth and lateral resolution of HFUS is approximately 40µm and 15µm, respectively (which is appropriate considering the typical size of DUs in SSc). The National
Research Ethics Service: Committee North West-Preston approved the study, and signed patient consent was obtained.

Patient opinion on the feasibility (‘not feasible’, ‘indifferent’, ‘very’ or ‘completely feasible’) and amount of time to complete the HFUS (‘too little time’, ‘just the right amount of time’, ‘too long’) were collected. Patient reported pain on a visual analogue scale (0-100, where 100 was the most severe pain imaginable by the patient) associated with the HFUS procedure was documented.

A clinical photograph of the DU was obtained by a trained medical photographer, including a small graded scale (length of one centimetre) in close proximity to the DU to give the independent assessor an indication of the size of the lesion, and to allow correlation between the acquired DU HFUS and actual physical surface measurements. Individual patients, the (first) HFUS operator, and (later) an independent assessor graded the ‘severity’ of the DU on a VAS scale between 0-100 (where 0 was ‘not severe’ and 100 ‘most severe’).

**DU HFUS and clinical photograph measurements**

Firstly, three raters (MH, GD and AM) decided by consensus agreement whether the HFUS image was ‘classifiable’ (i.e. that a measurement of width and/or depth could be made). The raters then, together, again by consensus, identified the edges of the DU at the surface of the skin by identifying changes in the surface layers or an appreciable change in surface profile (Figure 3.1). Two lines were then drawn perpendicular to the surface of the skin at each edge (width), and a third was drawn perpendicular to the surface of the skin at the deepest level of skin structure disruption (depth) (Figure 3.1).

DU photograph surface measurement of ‘width’ (the mean of two orthogonal ‘widths’, each performed three times) in which an ellipse was fitted around the ‘long’ and ‘short’ axes of the lesion, were obtained using a custom built programme written in MATLAB.
Statistical analysis

Descriptive statistics on the data were analysed using STATA version 13 (StataCorp, College Station, TX, USA). To assess criterion and construct validity all the DU HFUS images were used to measure DU width and depth. Criterion validity was assessed by performing the correlation coefficient between the DU HFUS mean width (of the ‘long’ and ‘short’ axes) and the clinical photograph surface width (as previously defined). Construct validity was assessed by performing the correlation coefficient between the DU HFUS mean width and depth with the patient, HFUS operator and independent assessor DU VAS severity scores (note, the sample size was too small to justify estimation of confidence intervals). A correlation coefficient of around 0.5 (or higher) could be interpreted as indicative of a moderate positive relationship between two variables.

3.4. Results

Patient demographics

The characteristics of the 11 patients included in the study are described in Table 3.1. The majority of patients (n = 9) had a history of severe digital vascular disease (i.e. previous DUs and/or intravenous vasodilator therapy), and were receiving treatment (n = 9) for digital vasculopathy, with over half (n = 5) receiving advanced vasoactive therapies (Table 3.1).

Assessment of feasibility and tolerability

HFUS was considered by the majority (n = 10) of patients to be feasible (either ‘completely’ or ‘very feasible’), with only one patient reporting it to be ‘not feasible’. Most patients (n = 7) considered the HFUS examination to take ‘just the right amount of time’, with three patients reporting it took either ‘too little’ (n = 3) or ‘too long (n = 1), respectively. The patient-reported pain associated with HFUS was very low with a median VAS value (IQR) of 0 (0-35).
**DU HFUS Measurements**

In all patients the HFUS examination was completed and an image acquired of both axes. At least one measurement of both DU width and depth could be made in nearly all DUs (n = 13). Both the ‘unclassifiable’ DUs were located on the extensor aspect of the hands. The mean (SD) DU width was 5.74 (2.16) mm and depth was 0.99 (0.45) mm.

**Assessment of criterion and construct validity**

Criterion validity. Correlation between HFUS DU and clinical photograph width was moderate ($r=0.55$). The mean (SD) DU clinical photograph width was 2.46 (1.09) mm.

Construct validity. HFUS DU measurements (width and depth) were moderately correlated with independent assessor of a clinical photograph DU severity ($r =0.49$ and 0.74), with little evidence of correlation with patient ($r =0.08$ and 0.23) or HFUS operator ($r =0.25$ and 0.21) DU severity.

3.5. **Discussion**

The key findings of our study are that DU HFUS was considered feasible and was well tolerated in most patients, and that the majority of DUs had at least one HFUS image that was rated ‘classifiable’, allowing the measurement of both DU width and depth. We were able to successfully measure a range of DU types, including those that occurred at the fingertip and extensor aspects, and ulceration in relation to subcutaneous calcinosis.

The majority of patients included in our study had a history of severe digital vascular disease (previous DUs and/or intravenous vasodilator therapy) and were receiving
drug treatment for digital vasculopathy, with five patients prescribed advanced vasoactive therapies. Therefore in terms of generalisability, our patients are likely to be ‘typical’ of those participating in clinical trials of digital vasculopathy.

We attempted to examine DU HFUS criterion and construct validity, based upon pragmatic measures of possible ‘DU severity’. Our data suggests that DU HFUS and clinical photographs (surface measurements and independent subjective assessment) both correspond to ‘real’ aspects of the DU. Whereas, patient and operator DU VAS are both potentially influenced by unavoidable knowledge of other factors (e.g. reported pain and the duration of the lesion), to which the independent assessor (of a clinical photograph) would be blinded. From our measured values, DU HFUS appears to systematically overestimate ulcer width (compared to the clinical photograph measurements). It should be noted that surface measurements do not necessarily reflect the underlying structure of these complex three-dimensional lesions (highlighting a need to develop a multimodal approach to assess DUs).

Our study has a number of limitations, mostly related to the fact that this was a small pilot study, primarily addressing the feasibility and tolerability of HFUS to measure DUs in SSc. The two ‘unclassifiable’ DUs were located on the extensor aspect of the hands, which are technically challenging as they are subject to the curvature of the underlying joint (which may impede successful image acquisition). Future studies should include optimisation of DU image acquisition, possibly utilising higher frequency (with increased depth resolution) HFUS systems. As this was a pilot study of measuring DUs in SSc with HFUS, three raters decided by consensus agreement whether the image was first, ‘classifiable’ and secondly, where measurements of DU width and depth should be made. In future studies, structured protocols of how to perform DU measurements should be devised. It should also be noted that our choice of ‘measurement axes’ (i.e. long and short) was solely based upon visual inspection of the DU, and should not be interpreted as being the absolute longest and shortest dimensions (i.e. possibly subject to measurement error, although the difference is likely to be very small). We chose this method as a pragmatic approach to HFUS of these small lesions, as this could potentially be useable in clinical trials. In addition,
lesions often have superficial crust and exudate, which could be interpreted as the surface of the DU on HFUS (resulting in the under- or overestimation of DU measurements). Although we did initially intend to assess intra- and inter-rater reliability (the rationale for the second HFUS operator), there were too few pairs of images to perform any statistically meaningful analysis (therefore the data is not presented here).

In conclusion, DU HFUS was feasible and tolerated in most patients, and we were able to measure both width and depth in most DUs. Because the reliability of rheumatologists grading DUs is only poor to moderate, there is a need for objective measures of treatment efficacy in SSc-related clinical trials. Future research is warranted and should include studies with larger numbers of DUs (including the development of protocols to guide performing measurements), to optimise the HFUS examination (in particular, to improve image acquisition of extensor DUs), and prospective studies to examine the sensitivity to detect DU progression.
3.6. References


Table 3.1. Patient characteristics of the 11 patients (with 15 DUs) included in the study. CCB: Calcium channel blocker; dcSSc: Diffuse cutaenous systemic sclerosis; ERA: Endothelial receptor antagonist; lcSSc: limited cutaenous systemic sclerosis. PDE5: Phosphodiesterase type-5 inhibitor. *One patient imaged in the study had a systemic sclerosis-spectrum disorder with clinical features including Raynaud’s phenomenon, abnormal nailfold capillaries and gastrointestinal involvement.
Figure 3.1. Clinical photographs of DUs. Clinical photographs (with the DU indicated by a red arrow) are presented for fingertip (A), extensor (B) and ulceration in relation to subcutaneous calcinosis DUs (C). D: Photograph depicting the surface orientation of the ‘long’ (blue) and ‘short’ (green) DU HFUS axes. Note: The corresponding DU HFUS images of the fingertip and extensor DUs are presented in Figure 3.2.
Figure 3.2. DU HFUS. HFUS images of the ‘long’ (left column) and ‘short’ (right column) for fingertip (A & B) and extensor (C & D) DUs. The coloured bars on the HFUS images indicate the measured DU width (red) and depth (yellow). The scale bar on the HFUS images (white, bottom left) represents 1mm in each direction.
4. Thermographic abnormalities are associated with future digital ulcers and death in patients with systemic sclerosis

4.1. Abstract

**Objective:** Capillaroscopic abnormalities are predictive of future DUs. Our aim was to investigate whether functional digital vascular disease (thermographically-assessed), is also associated with future DUs.

**Methods:** A retrospective case note review of patients with SSc undergoing thermography and who were followed for up approximately three years.

**Results:** 138 patients (equal mixture of normal/abnormal thermography) were included. Patients with abnormal thermography were more likely to develop DUs (clinician observed and/or patient reported); (odds ratio [OR 2.84], P=0.021), including multiple episodes, and more likely to die (OR 5.42, P=0.050).

**Conclusion:** Abnormal thermography is associated with DUs and disease severity in patients with SSc.
4.2. Introduction

Digital ulcers (DUs) are a serious manifestation of the underlying vascular disease in patients with systemic sclerosis (SSc) [1]. Around half of patients with SSc report previous DUs, often occurring early in the course of the disease [2,3]. DUs can be exquisitely painful and impact negatively on hand and global function (including occupation) [4].

Thermographic abnormalities have been reported to enable the distinction between patients with primary (idiopathic) and secondary (e.g. SSc-related) Raynaud’s phenomenon (RP) [5]. Examples of normal and abnormal thermograms are depicted in Figure 4.1. Several authors have proposed that capillaroscopic change (assessing microvascular structure) is predictive of future DUs [6-10]. Against this background, our aim was to investigate whether functional digital vascular disease (as assessed by thermography) is also associated with future DUs.

4.3. Method

Data collection

This was a retrospective study based upon the case note review of all patients with SSc who underwent thermography at a tertiary referral centre for SSc (with a particular interest in digital vascular disease) over a five-year period between 1st January 2005 and 31st December 2009 (by an observer unaware of the thermography findings). Patients were categorised into limited or diffuse cutaneous SSc (lcSSc and dcSSc) on the basis of the extent of skin involvement [11]. The study had NHS Research and Development (R&D) approval from Salford Royal NHS Foundation Trust. Written patient consent was not necessary as we only used previously collected, non-identifiable information obtained during routine clinical practice, within the confines of the direct health care team. The study was sponsored by The University of Manchester.
Thermography

At Salford Royal NHS Foundation Trust, thermography is part of the routine assessment at a patient’s first visit when a diagnosis of SSc is either made or suspected. Patients are requested not to smoke nor to consume any caffeine-containing beverage for at least four hours before attendance. After a period of initial acclimatization (at 23°C for 20 minutes) in a climate-controlled room, thermographic images (Agema 570 elite, FLIR Systems Limited) are taken of the dorsal aspect of both hands. The hands are then immersed (wearing latex gloves) into water (15°C) for one minute. Immediately after this (and with removal of the gloves), thermographic data of the dorsal aspect of the hands are obtained. If a temperature difference (of >1°C) exists between one (or more) of the fingertips and dorsum of the hand, then the room temperature is elevated to 30°C for a further 20 minutes, with repeat imaging at the end of this period. Abnormal thermography was defined as a temperature gradient between one or more of the fingertips and the dorsum of the hand of >1°C (fingers cooler than dorsum) at 30°C [5]. Baseline patient demographics and disease characteristics were documented.

Digital ulcer documentation

DU episodes and indicators of severity were documented for up to three years post thermography. DU ‘episodes’ were either recorded as clinician observed and/or patient reported (the latter included DUs which were present only in the interim between interaction with health care professionals – to have included only ‘clinician observed’ ulcers would have underestimated ulcer frequency by omitting these DUs). Each DU ‘episode’ (including the occurrence of multiple DUs simultaneously) was considered temporally distinct from the next DU ‘episode’ (i.e. clinician observed or patient reported healing between episodes). Indicators of DU severity: intravenous prostanoid therapy (given for DU disease only), surgical debridement and digital amputation, were also documented. Loss to follow up and death were also recorded.
Statistical analysis

Logistic regression was performed with ulcer status (any ulceration on any finger in the follow up period) as a binary outcome variable and this was performed with a) any ulceration (clinician-observed and/or patient-reported) and b) only clinician-observed ulceration as the outcome variable. Adjustment was made for prognostic variables: smoking status, disease subtype, autoantibody status (anti-centromere and anti-Scl-70), age, and disease duration (as defined as time from the first non-RP manifestation). Number of episodes of ulceration was included as the outcome in a Poisson regression model, which adjusted for differences in follow-up time. Due to the small number of DU complications multivariable analysis was not possible. We present frequency data for these outcomes (including clinician observed and/or patient reported DU). All statistical analyses on the data were performed using STATA version 13.

4.4. Results

Patient characteristics

138 patients: 69 patients with normal and 69 with abnormal thermography (those with abnormal thermography were randomly selected from 90 patients during the time period using a computer-generated list) were included in the analysis. Patients’ baseline demographics and clinical characteristics are summarised in Table 4.1. All the included patients in the study had RP. Follow-up time in the normal and abnormal thermography groups were similar (Mean [SD] =2.6 [0.9] and 2.8 [0.6] years, respectively) (Table 4.1). DU severity outcome was not available for one patient who developed DU in the normal thermography group.

Occurrence of digital ulceration and number of episodes

Patients with abnormal compared to normal thermography were more likely to develop future DUs (Table 4.1). For clinician observed and/or patient reported the adjusted odds ratio (OR) was 2.84 (95% CI 1.17 to 6.86, P=0.021), whereas, for
clinician observed alone the OR was 1.79 (95% CI 0.70 to 4.55, P=0.224). After adjusting for other variables and follow-up time using Poisson regression, the DU rate ratio (95% CI, abnormal vs normal thermography) was 2.85 (1.61 to 5.04) (P=<0.001), denoting an estimated increase in the rate of observed or reported DU episodes of around three times for patients with abnormal thermography.

**Digital ulcer severity**

Of those patients developing DUs, patients with abnormal (compared to normal) thermography had a relatively higher frequency of surgical debridement, with similar intravenous prostanoid use, and with only one case of digital amputation in the normal thermography group (Table 4.1).

**Loss to follow up and death**

Patients with abnormal (compared to normal thermography) were substantially more likely to die (OR 5.42, 95% CI 1.00 to 29.40, P=0.050) (Table 4.1). Patients with abnormal thermography were also more likely to be lost to follow up (death or loss to follow up (although this did not reach statistical significance): OR 2.32, 95% CI 0.84 to 6.40, P=0.104) (Table 4.1).

**4.5. Discussion**

Patients with abnormal (compared to normal) thermography were significantly more likely to develop future DUs, including multiple episodes, post thermography. In addition, there is a suggestion that DU severity was greater in the abnormal thermography group, with a greater frequency of surgical debridements performed (although this should be interpreted with caution because there were only a small number of patients who required surgical intervention). Patients with abnormal thermography were more likely to be receiving treatment for RP at time of thermography, which might suggest that they have more severe digital vascular
disease, although again, this has to be interpreted in the context of a retrospective study.

Several authors have proposed different predictors of DU, including capillaroscopic abnormalities, in patients with SSc [6-10]. Recently Smith et al described a scoring system to predict DU within 6 months using three variables (number of capillaries in the middle finger of the dominant hand, DU count and the presence or absence of critical digital ischaemia) with positive and negative predictive values of 54.9% and 83.8% respectively) [9]. Future research is warranted to investigate the added prognostic benefit in predicting future DU from the addition of thermographic abnormalities. Relevant to this is that Blaise et al [12] recently suggested that in patients with SSc digital thermal hyperaemia pattern (another index of vascular function) is predictive of DU.

An unexpected important finding of the study was that patients with abnormal thermography were significantly more likely to die within the follow-up period of up to three years. It was not the intention of the study to investigate the cause of death post thermography and therefore this data was not available. It is increasingly recognised that DU are a biomarker of disease severity including of internal organ involvement in patients with SSc, including in early disease [13,14]. In addition, there have been recent reports that patients with RP (compared to without) have higher mortality [15,16], particularly (at least in female patients) if the nailfold capillaries are abnormal [16]. Thermographic abnormalities should be further explored as these may represent a novel, non-invasive biomarker of disease severity, allowing the identification of patients with a particularly poor prognosis. We used the definition of abnormal thermography (‘the dorsal distal difference’) as proposed by Clark et al [17]; however, other thermographic perimeters have been proposed in the assessment of RP and these should also be explored in future research.

Our study has a number of limitations, most of which are due to its retrospective nature. To reduce any possible bias, the person collecting the data from review of
patients’ medical records, was unaware of the result of the thermography. Because there may be differences between what rheumatologists and patients define as a DU, we minimised the impact of this by collecting both clinician observed and patient reported DU. If abnormal thermography is associated with more severe disease then there is the possibility of a detection bias (i.e patients with more severe disease are more likely to interact with healthcare professionals, increasing the likelihood of DU detection). In relation to patient reported ulceration, patients with more severe disease may be more likely to report complications, resulting in recall bias. These factors may cause the relationship between abnormal thermography and ulceration to be overstated. Prospective studies should include a more robust scheme of DU measurement. Both loss to follow up and death were greater in the abnormal thermography group; therefore this may have resulted in the relative underestimation of DU in the abnormal thermography group. Future research is warranted to investigate the added benefit of thermography in addition to clinical features of severe digital vascular disease (e.g history of DUs), including in those patients with early disease who may already have thermographic abnormalities, but who due to their short disease duration are less likely to have developed ischaemic complications. In addition, it is worth noting in regards to the excess deaths observed (and therefore the possible underestimating of DU detection) that the proportion of patients with dcSSc (which is often associated with a more severe disease course and worse prognosis than lcSSc) was similar between the two groups.

In conclusion, patients with abnormal thermography were significantly more likely to develop future DUs (including multiple episodes) and abnormal thermography was associated with more severe digital vascular disease. In addition, patients with abnormal thermography were more likely to die. Future research including prospective studies is warranted to investigate thermography, a non-invasive imaging technique, as a prognostic marker of future DUs and as a biomarker of digital vascular disease activity and/or severity in patients with SSc.
4.6. References


Table 4.1. Baseline patient demographics, DU occurrence and severity, and death or loss to follow up post thermography. ACA: Anti-centromere antibody, CCB: Calcium channel blocker, TG: thermography, RP: Raynaud’s phenomenon. *Frequency of DU severity outcomes given per number of any (clinician observed and/or patient reported) DUs per normal (n = 10) and abnormal (n = 22) thermography groups. Note although there were 11 DUs in the normal thermography group, markers of severity were unknown in one patient; therefore, frequencies are given for 10 DUs.
Figure 4.1. Thermography. ‘Normal’ (A & B) and ‘abnormal’ (C & D) thermograms. For a thermogram to be defined as ‘abnormal’, at 30°C there is a persistent temperature (>1°C) gradient (fingertip cooler than dorsum of the hand) (D) along at least one of the fingers.
5. Reduced perfusion in systemic sclerosis-related digital ulcers (both fingertip and extensor) can be increased by topical application of glycercyl trinitrate.

5.1. Abstract

**Introduction:** In patients with systemic sclerosis (SSc) fingertip digital ulcers (DUs) are believed to be ischaemic, and extensor surface DUs a result of mechanical factors/microtrauma. Our aim was to assess blood flow response to topical glyceryl trinitrate (GTN) compared to placebo in SSc DUs, looking for differences in pathophysiology between fingertip and extensor lesions.

**Methods:** This was a double-blind, randomised, crossover, placebo-controlled study. Sixteen (6 fingertip, 10 extensor) DUs were each studied twice (one day apart): once with GTN and once with placebo ointment. Perfusion at the DU centre (‘DUCore’) and periphery (‘DUPeriphery’), as measured by laser Doppler imaging was performed before and immediately after ointment application, then every 10 minutes, up to 90 minutes post-application. We calculated the area under the response curve (AUC) and the ratio of peak perfusion to baseline, before comparing these between GTN and placebo.

**Results:** Perfusion was lower in the DUCore compared to the DUPeriphery (ratio of 0.52). The microvessels of the DUCore were responsive to GTN, with an increase in perfusion, with a similar effect in both fingertip and extensor DUs. There was less of a clear signal in the DUPeriphery. The AUC and peak/baseline perfusion difference in means (ratio, 95% confidence interval) between GTN and placebo at the DUCore was 1.2 (1.0-1.5) and 1.2 (1.0-1.5), and the DUPeriphery was 1.1 (0.7-1.6) and 1.0 (0.8-1.2).

**Conclusion:** DUs were responsive to topical GTN, with a particular increase in perfusion to the ischaemic DU centre. If fingertip and extensor DUs share a (potentially reversible) ischaemic aetiology, this has important treatment implications.
5.2. Introduction

Digital ulcers (DUs) are a major course of pain and disability in patients with systemic sclerosis (SSc) (1,2), and are a biomarker of disease progression, including death (3). However, relatively little is known about SSc-related DU pathophysiology. It is currently believed that fingertip DUs are ischaemia-driven, while those which occur over the extensor surfaces are related to mechanical abnormalities and microtrauma (4). Previous studies using laser-based techniques are supportive of an ischaemic component to DUs, with reduced perfusion to the DU compared to the periphery (5), including in extensor surface DUs (6). In addition, the tissue adjacent to DUs has been reported to be relatively hyperaemic compared to normal skin (6).

Whether DUs are ischaemic is a key clinical question because drug therapies, in general, rely upon vasodilation, to increase perfusion to the DU. A number of recent randomised controlled trials have excluded extensor DUs (7–9), presumably on the basis that if these ulcers are not ischaemic, then they are unlikely to benefit from vasoactive therapies.

Supplementation of the nitric oxide (NO) pathway is an important therapeutic strategy in the management of digital vascular disease in SSc (e.g. with phosphodiesterase type 5 inhibitors for Raynaud’s phenomenon [RP]). In addition, we have previously reported that topical glyceryl trinitrate (GTN) (a NO donor) increases blood flow in intact SSc skin (9) as measured by laser Doppler imaging (LDI).

Against this background, our aim was to assess the responsiveness of the microvessels in the centre (‘DUCore’) and adjacent tissue (‘DUPeriphery’) of SSc-related DUs to topical NO donation from GTN, compared to placebo, and whether differences between fingertip and extensor DUs exist, to better inform our understanding of the pathogenesis (with therapeutic implications) of DUs in SSc. Our rationale was that if DU blood flow increased with GTN, then this would imply that the microvessels
within DUs were capable of endothelial-independent vasodilation and hypothesise that topical NO donation might be an effective therapy.

5.3. Methods

Patients

Patients’ demographic and clinical characteristics are provided in Table 5.1. Sixteen patients with SSc, with 6 fingertip and 10 extensor ulcers were studied. The study was approved by the National Research Ethics Committee – Preston and all patients provided signed informed consent.

Study protocol

Patients were randomised (with a balanced allocation within DU subgroup - fingertip versus extensor) to receive either GTN or placebo ointment on day one, and the alternative the following day. Sealed envelopes contained the patients’ allocation schedule, allowing the medication to be dispensed. Patients, and the two operators, one of whom applied the study medication and the second of whom performed the LDI (and later extracted LDI data), were all blinded to the randomisation. This approach with two operators minimised any bias from any potential information gained from applying the ointment (e.g. patient opinion, including any side effects). One patient received intravenous iloprost on day one after the first study visit; however, the second visit was over 12 hours after completion of the infusion.

Patients were advised to refrain from caffeine containing beverages and smoking for a period of at least four hours before each study visit. No changes were made to patients’ existing vasodilatory therapy. After a 20 minute period of acclimatisation at 23°C, baseline LDI of the DU was performed, using a modified MoorLDI-vr (Moor Instruments, Axminster, United Kingdom) LDI (red, 633 nm). Immediately after the initial image (or ‘flux map’) was acquired, either 200mg GTN (2% Percutol ®) or
placebo ointment (of similar appearance and consistency to GTN preparation) was applied to the DU, using a sterile applicator, and with a circular motion, for one minute. Any visible excess ointment was promptly removed using gauze. LDI was performed immediately (time 0) after application of the ointment, and then every 10 minutes, up to 90 minutes, at each study visit. Imaging was terminated if the patient indicated a desire to stop. Patients’ were asked to report any side effects experienced during the study visits.

**Image analysis**

Perfusion data was extracted from the captured images. Using the LDI grey-scale image of the DU (to avoid bias from seeing the perfusion image), regions of interest (Figure 5.1) of the same size were created to extract perfusion data from the DUCore and the DUPeriphery for each treatment visit. We chose to examine the DUPeriphery because of the relative hyperaemia (as previously described), which could be important in DU healing.

**Statistical analysis**

Summary measures of each patient’s response to GTN and placebo: area under the curve (AUC) and ratio of peak perfusion compared to baseline (9). We calculated the difference in means and a 95% confidence interval (95% CI) for each of these summary measures, accounting for the correlation between paired measurements. For both measures we calculated the difference in means and 95% CI on a logarithmic scale due to distributional skewness, before back-transforming to the original scale. This yielded values representing the ratio of GTN response compared to placebo. All statistical analyses were performed using STATA version 13.
5.4. Results

Fourteen (6 fingertip and 8 extensor) DUs were included in the final analysis. LDI perfusion data could not be extracted for two extensor DUs, or from the GTN day for a third extensor DU, because the DU could not be confidently identified on the grey-scale images. An example of the LDI perfusion data (flux maps) for an extensor DU is provided in Figure 5.2. In most (n=8) DUs all the planned LDI measurements were completed on both days. In three patients a single LDI measurement (i.e. at one time point only e.g. 60 minutes) was unavailable for analysis on either one or both of the days. Three patients had incomplete LDI data (i.e. fewer measurements than intended in the study design) and/or LDI was performed at different (but known and recorded) time points; however, these data were still included in the final analysis. The reasons for these deviations in study protocol were either due to technical issues (e.g. LDI equipment failure) and/or patient preference.

Two patients reported local side effects (e.g. pain and dysesthesia) after application of GTN ointment: these were mild, and did not require any action. One patient experienced marked local and systemic vasodilatory side effects (including a sensation of light headedness), with an objective (by LDI) increase in perfusion of the other digits, although this did not necessitate discontinuation of the study.

DU response to GTN and placebo

At baseline, all 14 DUs had reduced perfusion in the core relative to the periphery, with a mean (standard deviation) ratio of DUcore/DUperiphery = 0.52 (0.27), including both fingertip (0.44 [0.29]) and extensor (0.59 [0.26]) DUs. Table 5.2 presents the summary measures broken down by DU type and treatment. Box plots are provided to describe the AUC (Figure 5.3) and peak compared to baseline (Figure 5.4) response of DUs.
The AUC (difference in means [ratio], 95% confidence interval) between GTN and placebo at the DUCore was 1.2 (1.0 to 1.5), whereas at the DUPeriphery it was 1.1 (0.7 to 1.6). Similarly, the peak compared to baseline perfusion (difference in means [ratio], 95% confidence interval) for the DUCore was 1.2 (1.0 to 1.5) and the DUPeriphery was 1.0 (0.8 to 1.2). For the DUCore there was evidence that both fingertip and extensor DUs showed an increased response to GTN compared to placebo, whereas this was less clear at the DUPeriphery (Figures 5.3 and 5.4).

5.6. Discussion

The key finding of this study is that we have demonstrated the responsiveness of the DU microvessels to GTN compared to placebo, particularly at the ischaemic centre of the DU, and that this response occurs in both fingertip and extensor DUs. In addition, all the analysed DUs had relatively low perfusion at their centres when compared to the periphery, further confirming that DUs are likely to be ‘ischaemic’ in aetiology. These findings have important clinical implications, namely: if extensor (like fingertip) DUs have a potentially reversible ischaemic aetiology, they could potentially benefit from treatment with vasoactive therapies (including local NO donors) to increase blood flow and likely improve DU healing. This could warrant the inclusion of extensor DUs into future clinical trials.

Our data further supports that the tissue adjacent to the DU is relatively hyperaemic, the pathophysiology and significance of which remains unclear. Although there was some evidence that GTN increased the perfusion of adjacent tissue, this was less marked than at the DU itself, with a greater heterogeneity in response, including between fingertip and extensor DUs. If the hyperaemia observed adjacent to DUs is involved in healing, then augmentation of this mechanism (e.g. neoangiogenesis with increased perfusion) could be an additional therapeutic target, although this may be subject to a ‘ceiling’ effect (described later).
There was heterogeneity in the response by DUs (as evidenced by relatively large standard deviations relative to the mean), which could suggest that a spectrum of relative DU ischaemia might exist. In SSc, although impaired endothelial-dependent vasodilation has been fairly consistently reported, some studies have found that endothelial-independent vasodilation is also compromised (11,12). Therefore, some DUs might be unable to respond to NO supplementation, from failure of endothelial-independent vasodilation, which has important therapeutic implications.

Topical GTN has previously been investigated as a ‘local’ treatment for RP (including SSc-related RP) and is currently being revisited (13,14). In a small crossover study of topical GTN for secondary RP (mainly SSc), Franks (15) observed there was a marked improvement in DUs with GTN, but not with placebo. In our study we did not expect to observe any potential ‘therapeutic’ (i.e. healing) effect with a single dose of GTN. However, the increased DU perfusion from GTN is encouraging, and suggests that supplementation of the NO pathway could be a promising target to be re-explored in future research. However, of note, GTN is not uncommonly associated with systemic effects, as observed in one patient in this study, and the current preparations of topical GTN are fairly unpleasant (e.g. greasy) for patients to apply, both of which limit the current potential of GTN as a ‘local’ treatment for DUs.

Our study has a number of important considerations. Although this was a small study, it benefited from a robust experimental design (double-blind, randomised, crossover, placebo-controlled), with objective physiological measurements. One patient received intravenous prostanoid during the study; however the second study visit was distant enough from discontinuation, so that any haemodynamic consequences were unlikely to be significant. We used a single dose of 2% GTN to study DU pathophysiology, in our previous study (10); a similar dose of GTN increased perfusion in intact SSc skin. However, in future studies, different dose/s of GTN could be considered, as well as other experimental models of DU disease. In addition, it will be important to examine the degree of within patient variation to GTN as our data suggests that DU response is heterogeneous, and to determine whether a ‘ceiling’ effect exists, in both the DU centre and periphery, where the microvessels display no additional response to GTN.
In conclusion, the microvessels of both fingertip and extensor DUs were responsive to GTN compared to placebo, particularly in the relatively ischaemic DU centre. Further research is warranted to better explore our findings, understanding the ischaemic drive to the formation of all DUs, including extensor aspect DUs, and whether this is potentially reversible (including through topical NO donation), which has important treatment implications, including on the design of future clinical trials.

**Acknowledgement**

We would like to thank the Clinical Trials Pharmacy at Salford Royal NHS Foundation Trust for their participation in the study.
5.6. References


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<td><strong>Sex (female, n)</strong></td>
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<tr>
<td><strong>Age (mean, SD) (years)</strong></td>
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<td><strong>Disease duration, from first non-RP clinical manifestation (years)</strong></td>
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<td><strong>History of severe digital vascular disease (n)</strong></td>
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<tr>
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<tr>
<td>SRC</td>
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<td><strong>Vascular active therapies (n)</strong></td>
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<tr>
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</tr>
<tr>
<td>ERA</td>
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Table 5.1. Demographic and clinical characteristics of the 16 patients. CCB: Calcium channel blocker; ERA: Endothelial receptor antagonist; ILD: Interstitial lung disease; PDE5: Phosphodiesterase type-5; PH: Pulmonary hypertension; PAH: Pulmonary arterial hypertension. SRC: Scleroderma renal crisis. *Disease subtype as defined by LeRoy et al (16).
<table>
<thead>
<tr>
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<th>Fingertip digital ulcers</th>
<th>Extensor digital ulcers</th>
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<td>PBO</td>
<td>COMPARISON</td>
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<td><strong>AUC</strong> (arbitrary perfusion units)</td>
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<td>24200.4 (18383.1)</td>
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<td>2.2 (1.5)</td>
<td>1.0 (0.8 to 1.2)</td>
</tr>
</tbody>
</table>

Table 5.2. Summary statistics for the DUCore and DUPeriphery. The area under curve (AUC) and ratio of peak perfusion compared to baseline are presented for all, fingertip and extensor DUs. Comparisons are the ratio of means (95% CIs) for the AUCs and peak perfusion compared to baseline. Ratios with values greater than 1 indicate an increased response from glyceryl trinitrate (GTN) compared to placebo (PBO).
Figure 5.1. LDI regions of interest. Illustration of how the regions of interest (ROI) were extracted to measure DU perfusion. Left and middle: Identical grey scale images of a fingertip DU, the middle image illustrate the ROI of the DUCore and DUPeriphery. Right: The corresponding LDI perfusion (flux map) image of the DU. Blue indicates low perfusion, whereas, red is relatively higher perfusion. The perfusion to the DUCore is lower (i.e. ischaemic) compared to the DUPeriphery.
Figure 5.2. DU LDI. Example of LDI perfusion data for an extensor DU with placebo (top row) and glyceryl trinitrate (GTN) (bottom row). Grey scale images of the DU on the respective days are provided on the left hand section of the rows. LDI perfusion data is presented prior (-1 minute), immediately after (0 minute) application of the study ointment, and then every 10 minutes for 30 minutes. GTN compared to placebo was associated with a marked increase in perfusion to the DUCore and DUPeriphery.
Figure 5.3. Area under the curve: GTN vs placebo. Box plots are provided for the area under the curve (AUC) (log transformed) for the DUCore (top row) and DUPeriphery (bottom row). The responses of all (A&D), fingertip (B&E) and extensor (C&F) to GTN (blue) and placebo (pink) are presented. Median and interquartile ranges are displayed (boxes) together with the range (whiskers).
Figure 5.4. Peak to baseline perfusion: GTN vs placebo. Box plots are provided for the peak/ baseline perfusion (log transformed) for the DUCore (top row) and DUPeriphery (bottom row). The responses of all (A&D), fingertip (B&E) and extensor (C&F) DUs to GTN (blue) and placebo (pink) are presented. Median and interquartile ranges are displayed (boxes) together with the range (whiskers).
6. A Feasibility study of a novel light treatment for digital ulcers in systemic sclerosis

6.1. Abstract

**Background:** Locally acting, well-tolerated treatments for digital ulcers (DUs) in patients with systemic sclerosis (SSc) are needed. Our primary aim was to investigate the safety, feasibility and tolerability of a novel light treatment. A secondary aim was to tentatively assess treatment efficacy.

**Method:** A custom built device was constructed consisting of red, infrared and blue light-emitting diodes. Treatment was administered twice weekly for three weeks, with follow-up at weeks 4 and 8. Any safety concerns were documented at each visit. Patient opinion on the time to deliver ('too little time', 'just the right amount of time', 'too long'), feasibility ('not feasible', 'indifferent', 'feasible') and pain (0-100, 100 most severe) with light treatment was collected. Patient and clinician DU visual analogue score (VAS) (0-100, 100 most severe) were documented. An independent assessor graded the change in DU appearance from photographs (-2 [much worse] to +2 [much better]). Laser Doppler (perfusion) imaging was performed immediately before and after treatment (DU+/-contralateral site).

**Results:** 8 patients with 14 DUs were recruited. 46 light treatments were administered, with no safety concerns. All patients considered light treatment was 'feasible' and 'took just the right amount of time', with a low associated mean (SD) of 1.6 (5.2). Patient and clinician VAS (mean change, at each visit compared to the last) improved during the study (-7.1 and -5.2, respectively, both P=<0.001). Independent assessment of (mean) change in DU appearance per visit was 0.14 (P=0.01). DU Perfusion significantly increased post treatment.

**Conclusion:** Light treatment for DUs is safe, feasible and well tolerated. There was an early tentative suggestion of treatment efficacy.

**ClinicalTrials.gov Identifier:** NCT02472743
6.2. Introduction

Digital ulcers (DUs) are common in patients with systemic sclerosis (SSc) and are responsible for much of the pain and disability associated with the disease (1–6). Around half of patients with SSc report a history of DUs, often occurring early in the course of the disease (2,6–9). DUs, in particular those located on the fingertip, are believed to be ischaemic in aetiology (6,7,10,11). Patients with SSc also commonly have marked finger contractures, which may predispose to recurrent trauma, and can make wound care challenging for patients. Despite there being treatments available to prevent DUs (e.g. endothelin receptor antagonists and phosphodiesterase type-5 inhibitors) (12,13), recurrent ulceration remains a major source of morbidity in some patients with SSc. In addition, DUs are often superficially infected, in particular, with Staphylococcus aureus (14), and can progress to deeper bony progression (i.e. osteomyelitis) (13). Unfortunately, despite targeted intervention, in some patients, digital amputation may be necessary for refractory DUs (15).

Current drug therapies (e.g. intravenous prostanoids) (16,17) used to treat existing DUs in patients with SSc, tend to rely upon systemic vasodilation (with the aim to increase perfusion to the DU). These treatments are often poorly tolerated by patients, leading to dose reduction and/or discontinuation. Therefore there is a strong therapeutic rationale to develop ‘locally’ acting treatments for DUs. Such treatments would likely be well tolerated by patients (i.e. no systemic vasodilatation) and could avoid the need for hospitalization to administer intravenous prostanoid therapy.

Light-based therapy (often referred to as ‘low-level laser therapy’) has been reported in a number of studies to be a safe and effective treatment for refractory skin ulcers (diabetic, pressure and venous) (18–27). Light treatment within the red and near-infrared spectrum is believed to stimulate a number of cellular processes (e.g. gene transcription and protein expression) beneficial to wound healing (28,29). Blue light increases perfusion through stimulation of local nitric oxide release with relaxation of vascular smooth muscle, and increases wound healing in a skin excision model.
(30,31). In addition, it is well recognised that blue light has an anti-bacterial effect, including against *Staphylococcus aureus* (32).

Against this background, the primary aim of the study was to assess the safety, feasibility and tolerability of a novel light treatment, combining red, infrared and blue light, for DUs in patients with SSc. Our secondary aim was to tentatively assess whether light therapy might have a beneficial effect on DU healing: firstly, by patient and clinician opinion, and secondly, by measuring perfusion as assessed by laser Doppler imaging (LDI).

### 6.3. Patients and Methods

Patients > 18 years of age with SSc-spectrum disorders (mainly SSc) were recruited into the study. Patients with serious infection of the DU (e.g. osteomyelitis requiring antibiotic therapy) were excluded. The study was approved by NRES Committee North West - Liverpool Central, and all patients gave written consent. Treatment study visits (including LDI) were conducted in a temperature-controlled (at 23°C) laboratory.

**Visit protocol**

Patients with DUs were prospectively recruited at Salford Royal NHS Foundation Trust (a tertiary referral centre for SSc) either at their routine clinic attendance or during an episode of hospitalisation. We included DUs located at both the fingertip and extensor aspect of the hands, as well as other sites on the hands. Two DUs on the same (treated) hand were included if they were both illuminated easily within the light treatment area. Patients attended a total of 8 study visits over two months. Light treatment was administered twice weekly for three weeks (i.e. the first 6 study visits) with follow-up visits at week 4 (visit 7) and week 8 (visit 8). Patients were asked to abstain from caffeine-containing drinks and from smoking (as these both cause vasoconstriction) for at least 4 hours prior to attending each study visit. Sterile gauze
and/or water could be used (based upon the clinical judgement of the operator), using gloves, to clean the surface of the DU of any debris, which could potentially interfere with the light treatment. Patient baseline demographics and characteristics were collected. At each study visit, any changes to drug treatment were documented, as well as DU visual analogue score (VAS) (patient and clinician opinion) as described below. At each treatment study visit LDI was performed to include the site of the DU and at a contralateral site, immediately before and after the light-treatment was administered. At each study visit, a clinical photograph of the DU was taken, which was graded by an independent assessor at a later date (described later). Our light treatment protocol is described immediately below.

**Light-based treatment**

A custom-built light treatment device (Figure 6.1) was designed and constructed in house at Salford Royal NHS Foundation Trust. The relative high power device (5W optical output) consists of 32 light emitting diodes (LEDs): 10 infrared (850nm), 11 red (660nm) and 11 blue (405nm). The target ‘dose’ for the light treatment was 10 J/cm². The configuration of the device ensured that the LED outputs ‘smeared’ together sufficiently and that any ‘hotspots’/’darkspots’ essentially disappeared. Before each treatment there was an initial period of calibration with an in built power meter and software, to ensure that the administered dose was +/- 10% of the intended dose. The operator had full command of the light device through a control interface (built specifically for this study) on an attached computer. The light treatment area was appropriately 15cm², which meant that more than one DU could be treated simultaneously per hand, and allowed some freedom of movement for the patient for comfort. The other hand was covered throughout the duration of light treatment (the rationale for which is described later). During development of the light device, our preliminary data (and therefore not shown in the manuscript) indicated that the skin (as measured by an attached thermocouple) increased in temperature by 1.5°C to 2.0°C within one to two minutes of commencing light treatment. This stabilised (i.e. remained elevated compared to baseline) after approximately 5 minutes, and persisted up to 10 minutes when the measurements were stopped. This effect was
observed with all the wavelengths, not just infrared, which could cause a direct thermal effect resulting in increased perfusion.

The light treatment took approximately 15 minutes in total to administer. Both the patient and operator wore appropriate safety goggles at all times whilst the light-based device was in operation, because blue light is potentially damaging after prolonged exposure to the retina. There was no contact between the DUs and the LEDs (Figure 6.1). Before and after each patient contact the light device treatment area was cleaned with an alcohol based wipe.

**Outcome measures**

**Assessment of safety, feasibility and tolerability**

At each treatment study visit any safety issues were documented (e.g. new concerns about the appearance, including new infection, of the DU). Patient opinion on the time taken to administer (‘too little time’, ‘just the right amount of time’, ‘too long’) and overall feasibility (‘not feasible’, ‘indifferent’, ‘feasible’) of light treatment was collected. Patient reported pain on a VAS scale (0-100, where 100 was the most severe pain imaginable by the patient) directly associated with the light treatment was recorded, to assess tolerability.

**Patient and clinician DU assessment**

At each study visit (before the light treatment was administered), patient and clinician global assessments of the DU were (independently) performed on a VAS (0-100, 100 being most severe). In addition, a clinical photograph of the DU was obtained by a trained medical photographer, including a small graded scale (length of one centimetre) in close proximity to the DU; to give an indication of the size of the DU. Patients and clinicians with ≥ 1 DU were asked to rate each DU separately. For each DU an independent assessor (AH) compared the baseline photograph with the subsequent study visit photographs. The assessor was only aware of the time
point of the baseline photograph, and all the others were provided in a random order. Only the photographs of the second treatment visit per week (i.e. visits 2, 4 and 6) and both follow up visits (7 and 8) were compared to baseline. The perceived change in DU appearance was graded on a Likert scale ranging from -2 (much worse) to +2 (much better).

**LDI (visits 1-6)**

LDI measures blood flow, which allows a perfusion map (in arbitrary units) to be produced. LDI was performed immediately before and after the light treatment was administered at the site of the DU(s) and also at a contralateral site. We used a MoorLDI-vr (Moor Instruments, Axminster, United Kingdom) laser Doppler imager (red 633 nm). Perfusion measurements from the LDI images (Figure 6.2) were assessed using a standard region of interest (ROI) for the ‘DU core’ (centre of the DU), ‘DU periphery’, ‘DU distant’ sites (on the same finger at a fixed distance relative to the DU core) and ‘DU contralateral’ (to the DU core) position. The rationale of imaging these sites was to demonstrate an objective local increase in DU perfusion with light treatment effect, without a significant distant effect, including on the same (treated) finger.

**Statistical analysis**

Descriptive statistics are provided for the safety, feasibility and tolerability data. Individual patients (n = 8, one studied on three occasions) and DUs (n = 14) were considered as unique entities in the analyses. A linear mixed effects model was used to assess change in DU status for both patient and clinician opinion (including the independent assessment of photographs) and LDI. This approach accounts for the correlation of repeated measurements on a single individual and gives the rate of change across the study period (that is, from one visit to the next). For LDI, we added a pre/post treatment indicator variable, to assess the improvement immediately following treatment. LDI data were log-transformed for the analysis. A P-value of < 0.05 was considered as statistically significant. All analyses on the data were performed using STATA version 13 (StataCorp, College Station, TX, USA).
6.4. Results

Patients

Eight patients (7 female: 1 male) were recruited into the study, one of whom was studied on three occasions (re-entered the study twice with new DUs). A 10 ‘series’ of treatments was undertaken. A total of 14 DUs were treated. Two DUs were treated at the same time in four patients (with 2 unique patients providing four DUs) to be studied. The majority of patients (n = 7) had SSc: four with limited and three with diffuse cutaneous disease (33). One patient had a SSc-spectrum disorder, the clinical features in whom included abnormal nailfold capillaroscopy and Raynaud’s phenomenon, with significant gastrointestinal dysmotility. The mean (SD) age of the patients was 48.5 (15.2) years. Participant progression throughout the study is presented in Figure 6.3. Two patients withdrew due to reasons unrelated to the study, and one patient was withdrawn due to clinical concern (about the severity and aetiology) regarding the DU. There were two occasions on which the light treatment could not be administered as intended due to technical failure of the device, and this was early in the course of the study: patient 1 visit 4 and patient 2: visit 3, the latter of which was no longer studied after this visit. We treated similar numbers of fingertip (n = 4), extensor (n = 5) and lateral aspect of the finger (n = 5) DUs. The baseline demographics and clinical characteristics of the patients included in the study are summarised in Table 6.1. Two patients received antibiotic treatment during the course of the treatment phase of the study, and one patient commenced antibiotic therapy during the period of follow up. In addition, one patient commenced intravenous iloprost before the third light treatment for DU disease, and went on to receive all the intended 6 light treatments as scheduled. Patients were recruited between 20th January 2015 and 15th January 2016, and the last study visit was 15th March 2016.
Outcome measures

Assessment of safety, feasibility and tolerability

A total of 46 light treatments were successfully administered and no safety concerns were encountered during the course of the study. All the participants considered that the light treatment (n = 45, data not available for one study visit) took ‘just the right amount of time’ and was ‘feasible’. Patient reported pain associated with light treatment (n = 45 sessions, data not available for one study visit) the mean (standard deviation) was low at 1.6 (5.2), with the majority of sessions (n = 40) being considered completely painless, and for the remainder of treatments was 14.8 (7.7).

Patient and clinician DU assessment

Figure 6.4 depicts both the patient and clinician DU VAS over the course of the study for each of the 14 ulcers. DU patient and clinician VAS both improved throughout the course of the study compared to baseline. There was a trend of improvement over the study period; patient DU VAS improved by -7.1 (95% CI -8.6 to -5.7) units at each visit compared to the last (P = <0.001) and for clinicians by -5.2 (95% CI -6.5 to -3.8) (P = <0.001). Compared to baseline, the mean reduction (SD) in DU VAS at the end of the 6 light treatments (week 3, visit 6) for patients was 58.5% (29.3%) and for clinicians was 57.7% (26.0%). At the final study visit (week 8, visit 8) the reduction in DU VAS compared to baseline for patients was 82.8% (37.6%) and for clinicians was 74.9% (57.4%). The mean (SD) perceived change in DU appearance as assessed by photographs improved by 0.14 (95% CI 0.0 to 0.3) (P = 0.01) units at each visit compared to baseline. An example of serial DU clinical photographs and LDI images is presented in Figure 6.5.

LDI

For the analysis of LDI measurements, 37 (pre-treatment) and 32 (post-treatment) LDI measurements were available for both the DU Core and DU Periphery sites, 32 (pre-treatment) and 25 (post-treatment) for the DU site Distant, and 19 (pre-treatment) and 17 (post-treatment) for the DU Contralateral site. There was a
significant increase in the relative mean perfusion after (compared to before) the light treatment was administered at the DU core site; of 0.32 (95% CI 0.13 to 0.52) (P = 0.0013) and also the DU periphery of 0.15 (95% CI 0.0 to 0.30) (P = 0.04). However, perfusion to other sites overall did not significantly change. There was a mean relative change at the DU distant site of -0.06 (95% CI -0.28 to 0.16) (P = 0.57) and the DU contralateral site of -0.05 (-0.26 to 0.15) (P = 0.61).

6.5. Discussion

The key findings of our study are that our novel light-based treatment is a safe, feasible and well-tolerated treatment for DUs. We successfully administered the light treatment to DUs located on a variety of locations on the hands, including in patients with marked finger contractures. To our knowledge, this is the first study to examine light-based treatment for DUs in patients with SSc.

Although it was not the primary intention of our study to examine treatment efficacy, there was an early suggestion that light-based treatment may have a beneficial effect on DU healing, although we accept that this was a small study without a control group. Throughout the study there was a significant improvement in DU status, including the independent grading of photographs by a blinded assessor. Of interest, over half the improvement in DUs from baseline was observed during the light-treatment period (i.e. within three weeks), both as assessed by patient and clinician opinion (58.5% and 57.7%, respectively). In addition, as a possible ‘local’ therapy to increase perfusion to ‘ischaemic’ DUs to improve healing, our light treatment was associated with a significant increase in DU perfusion, in particular to the centre, but not at a similarly treated site on the same finger, or at a contralateral (untreated) site to the DU.

Previous studies of light-based treatment have investigated red and infrared (either broadband or combined) light, and have usually utilised either LED-based or low-
level laser systems. It remains controversial whether there is any difference in possible efficacy between these two different light sources. We chose to develop a LED-based system for several reasons including (but not limited to) that LEDs are readily available in a range of wavelengths, relatively inexpensive compared to lasers, and future treatment devices (which potentially could be used by patients at home) would be safer, smaller, more portable and cheaper using LEDs. The wavelengths we chose of red and infrared light are comparable to those used in previous light-based treatment studies, and the blue light utilised has well described anti-bacterial action against *Staphylococcus aureus*. Of interest, Inoue et al (34) reported the successful use of oral psoralen and ultraviolet A therapy (PUVA) in a patient with diffuse cutaneous SSC and progressive DU disease. PUVA therapy was administered three times weekly for a month (cumulative dose 23 J/cm²) and was associated with improvement in DUs, as well as in skin sclerosis and revascularization, both as assessed by skin biopsies.

All the patients included in our study had had previous DUs, and the majority had previously received intravenous prostanoid therapy for DU disease. In addition, most were receiving vasoactive therapy for digital vascular disease, with several patients prescribed ‘advanced’ therapies (phosphodiesterase inhibitors or endothelin receptor antagonists). Therefore, our patients are likely to be representative of those patients with SSC and the greatest clinical need for the development of light-based treatment for DU disease.

There are a number of aspects to highlight about our study. This was a feasibility study primarily addressing the safety, feasibility, and tolerability of light-based treatment for DUs in patients with SSC. The wavelengths and treatment frequency of light treatment are comparable to previous studies; however, in future research, these may be reviewed and potentially optimised. This was an open study, and although there was a possible suggestion of treatment efficacy, this needs to be confirmed through the scientific rigor of a double-blind, randomised, placebo-controlled trial. We adopted a pragmatic approach to the study and allowed one patient to re-enter the study twice with new DU disease. One patient commenced iloprost during the
study due to evolving medical concerns about the severity of the DU, but given this was a primarily a feasibility study, she continued in this. In addition, we used a simple grading system for firstly, patients and clinicians to grade DUs, and secondly, an independent (blinded) assessor to grade photographs of DUs. In future studies, other outcome measures of efficacy may be utilised (e.g. an independent clinician physically assessing the DU).

In conclusion, our novel light-based treatment combining red, infrared and blue light, which has the advantage of both improving blood flow and, potentially, being antibacterial (32,35) was safe, feasible and well tolerated by patients. In addition, there was an early suggestion of treatment efficacy, with a significant improvement in DUs during the course of the study. The local increase in DU perfusion with light treatment occurred in the absence of a systemic effect, suggesting that this approach to therapy will obviate the systemic vasodilation inherent in most current treatment approaches to SSc-related DU. Future research is warranted to develop light-based treatment as a locally acting therapy for DUs in patients with SSc.

Acknowledgement

We would like to thank Mr Steve Cottrell and colleagues from the Medical Illustration Department, Salford Royal NHS Foundation Trust, for collecting the images.
6.6. References


Maclean M, McKenzie K, Anderson JG, Gettinby G, MacGregor SJ. 405 nm
Figure 6.1. The light-based treatment device. Left: Side profile of the light device. Right: The light device in operation treating an extensor aspect DU in a patient with SSc. The distance from the panel of LEDs to the treatment area is approximately 15 cm.
Figure 6.2. DU LDI. A and B: Grey scales images of a lateral aspect DU, with (B) depicting the ‘DU core’ and ‘DU periphery’ regions of interest. A and B are the same image, but B is annotated. The corresponding LDI perfusion map is presented underneath for both before (C) and after (D) the light treatment was administered. Blue represents low perfusion, whereas, red is relatively higher perfusion. There is an increase in the ‘DU Core’ and ‘DU Periphery’ perfusion post light treatment.
<table>
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</table>

Figure 6.3. Patient progression throughout the study. The number of administered (and scheduled) light treatments is indicated for each study visit.
Figure 6.4. Patient and clinician DU opinion. For individual DUs both the patient and clinician visual analogue score (VAS) are presented according to study visit. Black arrow: The patient reported trauma to the DU (number one) before the study visit. Red arrows: Details about antibiotic therapy. Antibiotic therapy was prescribed prior to entry into the study in one patient (DU 6), and was started after light therapy was completed, during follow up, in two patients (DUs 5 and 8).
Figure 6.5. DU clinical photographs and LDI. Photographs are presented for a patient with SSc with a lateral aspect DU. Throughout the course of the study there was an improvement in both patient and clinician DU opinion (Figure 6.4, DU 14) and independent clinician opinion (data not shown). A clinical photograph could not be performed at week 7 (visit 7) due to operational issues. Selected corresponding LDI images are presented in the bottom row. Over the duration of the study, there is a relative increase in perfusion to the ‘DU Core’, with the tissue perfusion becoming more homogenous.
7. Final discussion and conclusions

The findings of the 5 studies have been discussed in their respective chapters, including in the context of previous research, and the limitations of the experimental work. There now follows a discussion of the broader themes of the research, including the strengths and weaknesses, with a strong focus on the future research that is warranted on DU disease in SSc.

7.1. The measurement of digital ulcers in clinical trials

Study one confirmed that the reliability of rheumatologists with an interest in SSc, and therefore most likely to participate in clinical trials, in grading DUs is poor to moderate at best. This is a major concern in the design of future SSc clinical trials. We hypothesised that the clinical context (which clinicians use in daily practice) might increase the reliability of DU grading by rheumatologists. Although there was no significant increase in either the intra- or inter-rater reliability with clinical context, there was a suggestion that some rheumatologists might use clinical information to help classify that lesions are not a DU. In future clinical trials, it could be argued that at least some rheumatologists (raters) might find the incorporation of the clinical context beneficial. Another key issue operationally in future clinical trial design is that (ideally) the same clinician should perform repeat DU assessments, because of the high level of intra-rater reliability.

Patient reported outcome measures are being increasingly used in rheumatology research and DU specific measures could be developed for use in future clinical trials. However, in our study, agreement between patients and rheumatologists was poor to moderate, with only a small increase with the clinical context. This does not necessarily mean that either patients or rheumatologists are ‘wrong’. The current DU gold standard is clinician opinion, which we know varies significantly. Indeed, there
may well be key components of the DU construct to be learned from the patient perspective. Furthermore, it could be argued that the patient DU perspective (e.g. pain and disability) is ultimately the more important outcome measure. A number of recent randomised controlled trials have used varying definitions of DUs. In our reliability study we chose not to provide either written definitions or exemplar images of DUs, but to assess clinician opinion based upon experience, including participation in SSc-related clinical trials.

Through the analysis of study one, and the confirmed concerns about the reliability of DU grading, towards the end of 2015 a DU consensus meeting was convened at the University of Manchester, with members of the UKSSG, and colleagues from dermatology, orthopaedic hand surgery and specialist nursing, as well as patient representatives. As a result a number of proposed DU definitions (Appendix 9.2) have been developed. The plan is to rerun the reliability study (in the first instance on a UK level only) towards the end of 2016. A key theme that emerged from the meeting was that there is likely to be a difference in the concept, and therefore different tools are likely needed, in preventive studies compared to studies relating to treatment (i.e. healing) DUs. That is, when considering a simple ordinal scale of digital lesion severity as an outcome measure in clinical trials, the analyses of treatment efficacy would differ between preventative (i.e. ‘no ulcer’ vs ‘inactive ulcer’/‘active ulcer’) and treatment studies (i.e. ‘active ulcer’ vs ‘inactive ulcer’/‘no ulcer’).

In parallel, there is an international effort to develop new DU definitions, and it is the intention that the UK-based DU definitions, as discussed above, will be complementary, and inform this larger on-going effort.

In addition to the DU reliability study, we have run a related study (lead by Mr Vincent Simpson, who was a final year medical student at the time) investigating the measurement of DU surface area by free hand or from fitting an ellipse to the shape of the digital lesion (abstract provided in Appendix 9.3). This study used the clinical
photographs from the DU reliability study and also serial photographs from the light treatment study. The agreement of both techniques (free hand vs. photography, and ellipse vs. photography) compared to the surface measurements obtained from the clinical photographs was overall very high, although the ellipse method tended to overestimate the size compared to the photograph. A key issue, however, is that before the lesion can be measured, it must first be deemed to be a DU. Furthermore, in the HFUS study, we highlighted the fact that DUs are likely complex geometric structures, therefore, surface measurements are not necessarily indicative of the deeper aspects within the DU.

An alternative or additional method to assess DUs in clinical trials might involve the use of clinical photographs, which could be graded by a remote panel of independent experts. Although some might argue that clinical photographs are not a substitute for physically examining the DU, in the study by Baron et al (168), the reliability of SSc experts physically examining DUs was not significantly better than in the DU reliability study, which was based on clinical photographs.

The second study examined HFUS as a possible outcome measure in SSc clinical trials. HFUS was well tolerated and we were able to measure DU depth and width in the majority of patients, with an early tentative suggestion of moderate criterion and construct validity. The average depth and width of DUs was around 1mm and 6mm, respectively, which illustrates why DUs are so challenging to assess with the human eye. In addition, DUs are often covered with a crust-like cover of necrotic tissue and exudate, which means that the depth (including the base) of the DU often cannot be examined. This could potentially be addressed through imaging techniques such as HFUS. To further improve imaging tissue resolution, in future research higher frequency HFUS systems should be considered. In addition, other imaging techniques might be explored including (but not limited to) three dimensional digital photography and confocal microscopy.
In summary, there is a clear need to develop better methods to measure DUs in SSc clinical trials, and it is hoped that these two studies will both inform future DU definition development, and suggest a possible imaging technique to objectively measure DUs.

7.2. The pathophysiology of digital ulcers in SSc

Considering the significant impact that DUs have on patients’ quality of life and function, and an emerging role as biomarker of poor prognosis, relatively little is known about the pathophysiology of DU disease in SSc. It is currently believed that DUs which occur on the fingertips are due to ischaemia; whereas, those which occur over the extensor aspect of the hands are mechanical, including due to recurrent microtrauma. Furthermore, even less is known about DUs, which occur at different sites of the hands (e.g. at the nailbed and lateral aspect of the finger), or in relation to underlying subcutaneous calcinosis.

A number of vascular associates (Table 1.7) have been associated with DUs in SSc. In particular, nailfold capillaroscopy has been reported to be highly predictive of future DUs. Whereas, capillaroscopy assesses microvascular structure, thermography measures functional vascular disease. Chapter four was a retrospective case note review study of patients with SSc and normal and abnormal thermography in relation to incident DUs. As might be suspected, those patients with abnormal thermography were more likely to develop DUs (including multiple episodes), and there was a suggestion that DU severity was greater, further supporting that vascular disease drives development of DUs. As discussed in the conclusion to that paper, the study had a number of limitations (mainly in relation to its retrospective design) and future prospective research is warranted to explore these findings further. Future studies should also investigate the additional predictive benefit from combining capillaroscopic and thermographic abnormalities. Furthermore, an unexpected finding from the study was that patients with abnormal (compared to normal) thermography were significantly more likely to die during the period of follow-up.
over three years. Future research is also indicated to investigate this observation further, in particular, through a prospective, observational study.

Study four (Chapter five) was a double-blind, randomised, crossover, placebo-controlled trial of GTN to explore the pathophysiology of DUs in SSc. We used GTN, which has a well-known mechanism of action, to study blood flow response in and around both fingertip and extensor DUs. In keeping with previous studies (175,313), perfusion at the DU centre was reduced (i.e. relatively ischaemic) compared to the periphery, including extensor DUs. The microvessels at the DU centre responded (with an increase in perfusion) to GTN compared with placebo, including both fingertip and extensor DUs. Although there was some evidence that GTN increased the perfusion of adjacent tissue, this was less marked than at the DU centre itself, with a greater heterogeneity in response, including between fingertip and extensor DUs. Our data suggests that (at least some) DUs could have a potentially reversible ischaemic aetiology, and therefore these might benefit from treatment with vasoactive therapies (including local NO donation) to increase blood flow, which could improve DU healing. Overall, there was heterogeneity in the response by DUs, which could suggest that, a spectrum of (relative) DU ischaemia exists. Indeed, an improvement in the relative ischaemia to the DU centre has been reported with healing (175,313). Although we did not collect data on DU duration, the majority of DUs were recruited ‘acutely’ either during the course of hospital admission or in the outpatient clinic, therefore, most of the DUs studied were likely to be of recent onset. It is possible that the reason why perfusion in some DUs responded less well than in others was that in patients with advanced disease (with irreversible vascular damage); there may be a failure of endothelial-independent dilation, despite exogenous supplementation of the NO pathway.

Overall, the two studies (Chapters four and five) taken together further add to the growing literature, which suggests that DUs in SSc are ischaemic in aetiology, and further question if extensor (compared to fingertip) DUs also share a significant ischaemic drive. It may well be that despite extensor DUs being primarily ‘mechanical’ in aetiology; there is a significant ischaemic component, which
impedes healing. Future research is required to further understand the pathogenesis of DUs in SSc, including DUs, which occur at different sites of the hands (e.g. the lateral aspect and the base of the nail), as this has important clinical implications, including both the treatment of DUs, and the design of future clinical trials.

7.3. The development of locally acting treatments for digital ulcers in SSc

There is a clear unmet clinical need to develop locally acting treatments for SSc-related DUs, free of systemic side effects. Furthermore, patients could likely administer such treatments at home, potentially avoiding the costs and inconvenience associated with hospitalisation. Light-based treatment has previously been reported to be a safe and effective treatment for a wide range of skin ulcers in a number of previous randomised controlled trials. However, at present, the evidence for local treatments for DUs in SSc is limited to case reports and small series. Topically applied therapies are one such possible approach.

At Salford Royal over the past few years a novel light based device to treat DUs in patients with SSc has been designed and constructed. This is the first light treatment device (within the reported literature) to combine three specific wavelengths (others have combined either red and infrared, or were broadband). These wavelengths and the ‘dose’ of light were chosen based on the design of previous light treatment devices and the biological rationale. Previous dosing regimens have varied significantly (e.g. the frequency of light treatments and number/duration of the study). Pragmatically (including cost implications), patients were studied for 8 weeks (three weeks treatment), as this was a likely timeframe in which the study’s primary objectives would be answered, namely the safety, feasibility and tolerability of light-based treatment for DUs in SSc.
Again, it was challenging to recruit patients into the study (described later in the sections on the strengths and weaknesses). Nonetheless, the study was completed and the device was found to be safe, effective and feasible. In addition, there was a tentative signal of treatment efficacy with a localised increase in DU perfusion, as assessed by LDI. This adds to the proof of principle of the device.

It is hoped that development will continue on the light-treatment device, as this is not only a promising local treatment for SSc-related DUs, but also has potential for far-reaching benefit to other more common ulcer types. Future research is needed to further understand the mechanism/s of action of the device, although, this is likely to be multifactorial in origin. Dose-ranging studies and ultimately, a randomised, placebo-controlled trial are required to determine treatment efficacy. Key to the development of all future, possible locally-acting therapies will be patient involvement, including identifying the needs and barriers to development of such treatments.

7.4. Strengths and weaknesses of the studies

The strengths and weaknesses of the individual studies have been discussed in the relevant chapters. In the remainder of this thesis, the main issues in relation to the individual studies, and the key overarching themes, with a view to future research will be discussed.

In relation to the reliability study, it could be argued that the high-level of intra-rater reliability was due to recall bias by the grader. However, this was unlikely, as the graders received a large number (80) of unique images, and then 10 repeated images (from the first 50) at the end of the study, which reduced the risk of graders remembering their previously assigned category. Whether clinical photographs are comparable to the physical inspection of DUs shall be described later when considering issues, which are relevant across different studies. Although we used a
range of clinical context features (duration, pain and discharge), it could also be argued that other types of clinical context are also important in the ‘real-life’ grading of DUs by clinicians (e.g. a history of DUs).

Study two (three) was a small pilot study primarily addressing the tolerability and feasibility of HFUS to measure DUs. An important consideration is that the DU HFUS images were graded (and ‘measured’) by the consensus opinion of three individuals. In future, larger studies investigating DU HFUS, scoring systems need to be devised, with assessment, not only of image acquisition, but also image mark-up and measurement, both of which are important in medical imaging. Although we initially intended to assess intra- and inter-rater reliability (results provided in Appendix 9.4), the number of ‘paired’ images were small, and this should be examined further in future research, with larger numbers of DUs.

The main limitations relating to the study exploring whether thermographic abnormalities are associated with DUs have been discussed in Chapter four, many of which relate to its retrospective nature. It is important to highlight that only DUs documented in the case notes were recorded, either clinician observed and/or reported by the patient. A strength of the study is that all the patients were under close clinical review at Salford Royal Hospital, where it is part of routine clinical practice to enquire, and to examine for the presence of DUs. We also collected patient-reported DUs to include those lesions, which occurred between interactions with health care professionals, in particular, those patients with less severe disease, who might be reviewed less often than patients with more severe disease.

The ‘GTN’ study (Chapter five) has a number of points to consider as discussed in the conclusion to that chapter. Logistically it was challenging to recruit patients into the study, because we treated patients according to current best practice (e.g. with intravenous prostanoids for severe DU disease), which if commenced in close (e.g. within several hours) proximity to the study visit, would have precluded inclusion. Although GTN has recently been revisited as a treatment for RP, it is unlikely that in
its current formulations this would be a candidate as a locally-acting treatment for DUs. We studied a single fixed dose of GTN based upon the known pharmacology of the medication; however, in future studies, a range of doses should be considered in the experimental design. In addition, there may be a ‘ceiling’ effect by which GTN is unable to increase blood flow further. However, of potential caution, several patients developed local side effects, and one patient developed systemic vasodilation, all of which further suggest that the dose used is very important, to allow local but not systemic vasodilation.

The final light-based treatment study (Chapter six) was a small feasibility study primarily examining the safety, feasibility and tolerability of the light treatment for DUs in SSc. It is important to highlight that this was not a pilot study in which we would be testing the protocol intended for a randomised controlled trial with a smaller number of patients. As discussed in the final section of this discussion, patient recruitment into this study was difficult, and one patient was studied on three occasions. However with our data, we were able to answer the primary aim of our study (i.e. the safety, feasibility and tolerability of light-based treatment for DUs). Patients’ clinical care was not changed due to participation in the study in any way, for example, we allowed patients to continue their usual medication, including advanced vasoactive therapies (e.g. intravenous prostanoid). In an early phase study, primarily addressing the safety of the device, it would be unethical to change patients’ treatment in order to study an experimental device. A strength of the study was that by using LDI as an objective (secondary) outcome measure, we were able to demonstrate a local, with no systemic, increase in perfusion, which adds to the proof of principle of the device.
Important issues across the studies

The definition of digital ulcers

A key theme in this thesis has been the definition of DUs as an outcome measure in clinical trials. The current DU ‘gold standard’ is clinician opinion; the reliability of which is poor to moderate at best, therefore this is a potential issue in all the experimental work presented in this thesis. Throughout the studies we adopted a pragmatic approach when recruiting patients with DU disease to participate in the studies. We did not use a strict definition of DUs in our protocol for any of the studies. Again this was a pragmatic approach as all the previous studies have used slightly varying definitions of DUs, and in particular, in study one; we chose to examine the reliability of expert rheumatologists (and not the performance of a specific set of DU definitions). Most of the patients who participated in our studies had a history of severe DU disease (e.g. recurrent DUs, including the need for surgery), many of whom were receiving advanced vasoactive therapies, specifically for digital vascular disease. Therefore, it is likely that the patients, who were recruited into the studies, were typical of those who would be included in DU clinical trials.

Clinical photographs of digital ulcers

This is an important issue as three of the studies (Chapters two, three and six) included the use of clinical photographs as an outcome measure. In particular, clinical photographs were central to facilitating the DU reliability study (Chapter two) and the serial photographs in the light-based treatment study (Chapter six).

Specific protocols for the acquisition of the clinical photographs were developed, initially, for the reliability study (Appendix 9.5), with no significant change for either the HFUS or light-based treatment studies. A small graded ruler was placed in close proximity to the digital lesion to give the viewer an indication of the size. It could be argued that clinical photographs are inferior to clinical inspection of the
DU, and that clinicians obtain more information from physical examination of the lesion. However, as previously described in this chapter, the reliability of expert rheumatologists grading DUs in person was not reported to be significantly higher than in our study.

Patient participation

The patient cohort at Salford Royal consists of over 350 patients with SSc under regular clinical review. From previous studies, it has been reported that around 10% of patients are likely to have a DU at any particular time. However, recruitment to clinical research is unpredictable, in particular, into those studies that require more than one, and in particular multiple, study visits. This was a particular concern with the ‘GTN’ and light-based treatment studies. Pragmatically, to facilitate the studies we allowed patients the opportunity to participate in more than one of the one DU studies included in this thesis. Indeed, many patients kindly participated in more than one of the studies. In the light-based treatment study, it would have been preferable to recruit completely unique patients. However, this was a difficult study to recruit into, in particular, due to the heavy commitment required by patients (i.e. 8 study visits over 8 weeks, with each visit lasting around one hour), which no doubt affected patient recruitment and retention.

7.5. Conclusion

This thesis highlights the challenges of the measurement of DUs as an outcome measure in clinical trials, and the need to develop locally acting therapies, free of systemic side effects, driven by an improved understanding of the pathophysiology of DU disease in SSc. It is hoped that the work presented in this thesis is a useful contribution to the understanding of DUs in SSc, and that future research will build upon this, so that future DU clinical trials are conducted in a more effective manner, and that well tolerated, effective medications can be developed to treat DUs.
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9. Appendices

9.1. List of the regulatory approvals for the individual studies

<table>
<thead>
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<th>Study name</th>
<th>Salford Royal R&amp;D ID</th>
<th>REC reference number</th>
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<td>Digital ulceration as an outcome measure: does contextual information improve reliability between rheumatologists?</td>
<td>2013/127DERM</td>
<td>13/EE/0255</td>
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<td>Imaging digital ulcer status in patients with systemic sclerosis: High frequency ultrasound and peri-ulcer capillaroscopy</td>
<td>2014/130DERM</td>
<td>14/NW/1125</td>
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<td>Does abnormal thermography predict future digital ulceration in patients with SSc?</td>
<td>2015/042DERM</td>
<td>Not applicable</td>
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<td>A mechanistic study of topical glyceryl trinitrate to understand the pathophysiology of digital ulcers in systemic sclerosis</td>
<td>2013/154DERM</td>
<td>13/NW/0684</td>
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<td>Light-based therapy as a novel treatment for digital ulcers in patients with systemic sclerosis</td>
<td>2014/196DERM</td>
<td>14/NW/1400</td>
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</table>
9.2. Proposed digital ulcer definitions

**Digital ulcer:** A lesion (on the finger on or distal to the MCPJ) with loss of surface epithelisation and a visually discernible depth. The ulcer bed is often wet in appearance with surface slough.

The peri-lesional skin surrounding digital ulcers is not uncommonly erythematous and/or macerated (including in the absence of superadded infection). Patients often report pain (which may be severe) associated with digital ulcers. Digital ulcers often have an overlying scab (eschar) and if there is a high index of suspicion of an underlying digital ulcer, then the lesion should be classified as such. Common sites for digital ulcers include the fingertips and over the extensor (dorsal) aspects of the hands, and in relation to subcutaneous calcinosis. Less often digital ulcers may occur at other sites on the hands (e.g. over the lateral aspects of the digits and at the base of the nail).

**Healed ulcer:** A lesion with complete surface epithelisation (otherwise the lesion would be classified as a ‘digital ulcer’).

**No ulcer:** Any lesion which does not fulfil the definitions of either a ‘digital ulcer’ or ‘healed ulcer’ including (but not limited) to: digital pitting scars, hyperkeratosis, and fissures.
9.3. Quantifying digital ulcers in SSc: Repeatability and reproducibility of software-based surface area measurement

Simpson V, Hughes M, Herrick AL, Dinsdale G.

**Background:** Digital ischaemic lesions, in particular digital ulcers (DUs) are common in systemic sclerosis (SSc). DUs mark a significant point in a patient’s life, as they can impede mobility/dexterity, are extremely painful, and are associated with internal organ involvement and poor prognosis. Effective management of DUs requires reliable monitoring in order to tailor care. Currently there are no standardised, repeatable methods in use for assessing quantifiable digital lesion characteristics. Furthermore, SSc-related clinical trials often use DUs as an end point, but rely on subjective, qualitative measures, such as clinician opinion. We propose a method of analysing digital photographs of digital ischaemic lesions using custom made software which allows image feature marking and tracking. This can provide non-invasive, reliable and repeatable measurements of DU characteristics (in this case ulcer area as estimated by (1) freehand drawing and (2) ellipse-fitting), which could potentially be used as a surrogate for DU status (e.g. progression or healing). The aim of this study was to compare the reliability and reproducibility of the two methods of DU area estimation.

**Method:** Software written in MATLAB allowed 107 images of digital ischaemic lesions mainly DUs collected from patients with SSc (as part of two ongoing studies) to be marked-up. Free hand and ellipse area measurements were taken for comparison after image calibration using 1cm reference scales, allowing measurements in millimetres rather than pixels. Three assessors measured the digital lesions (first assessor five times, second and third assessor once). Intraclass correlation coefficient (ICC) was used to test the intra- and inter-rater reliability of measurements and a Bland Altman Plot to test agreement of ellipse vs free hand measurements.
**Results:** There was excellent reliability in both the intra-rater and inter-rater ICC for both ellipse and free hand area measurements as shown in the table below. Bland Altman plot showed good agreement between ellipse and free hand measurements, although ellipse measurements tended to be larger (mean difference: $+2.2\text{mm}^2$ (+12%)). Mean ulcer areas (ellipse and free hand) were 20.7 mm$^2$ and 18.5 mm$^2$ respectively.

**Conclusion:** The software-based method described is both a repeatable and reproducible measurement tool for digital lesions. This may ultimately lead to a more objective and reproducible measure of DU healing, rather than the subjective categorisation currently used. Future work should focus on using a similar software-based approach on images captured by patients themselves (e.g. using smart-phone cameras).

<table>
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<td></td>
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<tr>
<td><strong>Inter-rater</strong></td>
<td>0.771</td>
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9.4. Digital ulcer high-frequency ultrasound reliability data

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<th>Intra-rater (95% CI)</th>
<th>Inter-rater (95% CI)</th>
</tr>
</thead>
<tbody>
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<td>Long depth</td>
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<td>0.22 (-0.24, 0.83)</td>
</tr>
<tr>
<td>Long width</td>
<td>0.65 (-0.04, 0.93)</td>
<td>0.26 (-1.08, 0.90)</td>
</tr>
<tr>
<td>Short depth</td>
<td>0.81 (0.36, 0.96)</td>
<td>0.50 (-0.34, 0.88)</td>
</tr>
<tr>
<td>Short width</td>
<td>0.62 (-0.03, 0.91)</td>
<td>0.53 (0.27, 0.89)</td>
</tr>
</tbody>
</table>

**Description**

The intra-rater and inter-rater reliability for the DU HFUS study presented in Chapter three. Data are presented as the intraclass coefficient (mean, 95% confidence interval). Although the intra-rater reliability in general appears to be high, the confidence intervals are very wide, with a true uncertainty about the true estimate. This is likely largely based upon the small number of ‘paired’ images that were used to calculate the intraclass coefficient for both the intra-rater reliability (long and short axes = 7 and 8 observations, respectively) and the inter-rater reliability (long axes = 5 and 8 observations, respectively). Again, although overall the ‘short axis’ had higher intra- and inter-rater reliability, this is more likely to be due to the small sample size, as opposed to the ‘short’ axis being intrinsically more reliable than the ‘long’ axis.
9.5. Protocol for digital ulcer clinical photographs

Photography

Patients will be asked to attend the Clinical Photography department at Salford Royal Hospital. A clinical photography form will be completed including an anatomical description of the digital lesion/s to be photographed.

Photography equipment

- Bowens studio flash units (reflectors at photographers discretion)
  - 2 subject lights providing key and fill light
  - 2 rear lights providing backdrop illumination.
- D300s / D700 camera
- 105mm Micro lens
- Disposable 1cm adhesive scale

The photographer will use his or her discretion to best record the ulcer in relation to lighting and angle of view for the close

- The whole of the finger will be photographed for orientation with generalized lighting against a pale studio wall background. Scale will vary dependent on the size of finger.
- Close-up view will always be to a set magnification of 1:3 on a D300 (1:2 on D700)
- The 1cm scale placed near the edge of the field of view but in the same plane of focus
Image processing

Images from various patients will be viewed together so it is important to for all photographers to process the images identically.

- White Balance
  - Photograph grey reference card immediately prior to taking the clinical photograph

- Croping
  - Crop in camera. Retain original camera image proportions of 2:3

- Output
  - Jpeg copy for study
    - Image size: 20x30 cm / 72 dpi
  - PACS version for patient record