Multifactorial Pathways Contributing to the Development and Impact of Foot Problems in Systemic Sclerosis (Scleroderma)

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Intellectual Property and Publication Statements

The candidate confirms that the work submitted is his/her own and that appropriate credit has been given where reference has been made to the work of others.

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Multifactorial Pathways Contributing to the Development and Impact of Foot Problems in Systemic Sclerosis

Abstract

Systemic sclerosis (SSc) is a heterogeneous connective tissue disease characterised by vasculopathy, immune activation and fibrosis [1-3]. The multi-system nature of the disease has a wide-ranging impact on the patient's overall health on physical, psychological and psychosocial levels [4] [5-7]. While foot problems in patients with SSc have been previously described and that their presence is associated with disability [8-10], the impact of such problems and its major contributors has yet to be determined.

The underpinning hypothesis of this thesis was that the development and impact of foot problems on the Quality of Life (QoL) of patients with SSc is multifactorial; involving a complex inter-relationship between disease, functional impairment, personal factors, environmental factors and psychosocial factors. In order to explore this hypothesis, a multiple methodological approach was employed. First, a literature review and a consultation with clinical experts was undertaken to identify the potential candidate factors that may contribute to foot problems. Second, a case-control, cross-sectional study of 121 patients with SSc and 51 healthy participants was undertaken in order to investigate the impact of factors that contribute to foot problems. Finally, the pathway by which the candidate factors that were identified as contributing to foot pathology impacted on the overall quality of life in people with SSc was explored. Using data from the same 121 patients with SSc, structural equation modelling (SEM)

was used to explore the inter-relationships between multifactorial pathways associated with foot pathology and its impact on patients with SSc.

The results from this thesis can be summarised as follows: i) patients with SSc have significant foot problems; ii) SSc has both a physical and psychological impact; iii) foot problems are affected by complex interrelations between multiple factors; and iv) foot problems are a significant contributor to the impact on the quality of life of patients with SSc.

Begonya Alcacer-Pitarch

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Abbreviations

Abbreviation	Meaning
ABPI	Ankle Brachial Pressure Index
ACE	Angiotensin-Converting Enzymes
ADLs	Activities of Daily Living
ANCA	Anti-Neutrophil Cytoplasmic Antibody
ANS	Autonomic Nervous System
CNS	Central Nervous System
CTD	Connective tissue diseases
DP	Dorsalis Pedis
DU	Digital Ulcers
ECM	Extracellular matrix
FPDQ	Foot pain and Disability Questionnaire
HADS	Hospital Anxiety and Depression Scale
HLAs	Human Leukocyte Antigens
ICC	Intraclass Correlation Coefficient
IP	Interphalangeal
kPa	KiloPascals
MFPDI.	Manchester Foot Pain and Disability Index
MHC	Major Histocompatibility Complex
MRI	Magnetic Resonance Imaging
MTP	Metatarsophalangeal
N	Newton
NFC	Nail Fold Capillaroscopy
OA	Osteoarthritis
OR	Odds Ratio
PAD	Peripheral Arterial Disease
PAH	Pulmonary Arterial Hypertension
PAOD	Peripheral Arterial Occlusive Disease
PNS	Peripheral Nervous System
PPG	Photoplethysmography
PROMs	Patient-Reported outcome measures
PVD	Peripheral Vascular Disease
QoL	Quality of Life
R&D	Research and Development
RCS	Raynaud’s condition score
RCS	Raynaud’s Condition Score
RF	Rheumatoid Factor
ROI	Regions of Interest
ROM	Range of Movement

RP	Raynaud's Phenomenon
SD	Standard Deviations
SEM	Structural Equation Model
SF-36	MOS 36-items Short-Form health survey
SHI	Scleroderma Heart Involvement
SoNS	Somatic Nervous System
SRC	Scleroderma Renal Crises
SSc	Systemic Sclerosis
SSc HAQ	Scleroderma Health Assessment Questionnaire
SSc QoL	SSc Quality of Life
TBP	Toe Brachial Pressures
TP	Tibialis Posterior
VEDOS	Very Early Diagnosis of SSc
VIF	Variance Inflation Factor
WHO	World Health Organisation

Chapter 1
Introduction

1.1 Background

Systemic sclerosis (SSc) is an autoimmune rheumatic disease characterised by three principal features: i) vasculopathy; ii) immune activation with production of autoantibodies and alteration in immune cells; and iii) fibrosis of the skin and internal organs as a result of excessive matrix deposition [2, 11-15]. The prevalence of SSc in the UK is 8.21 per 100 000 [16], with onset as early as the second decade [16-18]. The impact of the early onset of SSc is reflected in the high societal costs: in 1997 the cost associated with SSc in the United States was $1.5 billion [19] with a proportionally similar cost estimated for the UK.

Foot problems in SSc have been reported to be common and disabling [9, 20]and include: Raynaud's phenomenon, associated severe ischemia leading to apical digital ulceration, subcutaneous calcinosis, skin thickening, callus formation, foot ulcers, sensory loss, tendonitis, joint space narrowing, bone demineralization, joint subluxation, joint margin erosions and degenerative changes[9, 20, 21]. People with SSc have reported significant foot impairment and reduced foot health status [10], possibly influenced by the presence of pain, as it has been well established that pain is a predictor of physical functioning in patients with SSc [22]. The mechanisms by which foot pain is increased in people with SSc, and the factors contributing to the impact of foot problems in

this group have not been investigated systematically however. There is therefore a need to investigate and identify the factors that predict foot problems and their impact on the quality of life of patients with SSc.

1.2 Hypothesis

The research hypothesis that this programme of work explores is:
The development and impact of foot problems on the QoL of patients with SSc is multifactorial; involving a complex inter-relationship between disease, functional impairment, personal factors, environmental factors and psychosocial factors.

In order to explore this, a multiple-methodological approach was used.

1.3 Aim

The primary aim of this study was to identify the factors that contribute to the development and impact of foot problems in patients with SSc

1.4 Objectives

To achieve this aim, three objectives were identified:

i) To identify the potential candidate factors that may influence foot pathology in SSc;
ii) To investigate the candidate factors that contribute to foot pathology and explore their impact, and
iii) To explore the relationships between factors that contribute to foot problems and their impact on the quality of life of patients with SSc.

1.5 Structure of the thesis

The structure of the thesis is outlined in this section providing a summary of the content of each chapter.

Chapter two is a review of the literature. This literature review informs the hypothesis and provides background information for the thesis. The literature review covers different areas of the disease, such as disease subsets, classification of SSc, epidemiology, aetiopathogenesis, pathophysiology, clinical manifestation and associated mechanism- with a focus on foot pathology and impact of foot pathology in SSc.

Chapter three provides information about the varied methodologies employed in this thesis. This chapter explains in detail the methods underpinning each of the three phases of the study i.e. the literature review, the case-control cross-sectional study and the structural equation modelling, and describes the conceptual framework for the methods used to identify the factors contributing to the developments and impact of foot problem in SSc.

Chapter four describes the results obtained from the exploration of the factors contributing to the developments and impact of foot problem in SSc. This chapter describes in detail the results obtained from: the identification of the potential candidate factors that may influence foot pathology in SSc; the investigation of the candidate factors that contribute to foot pathology and exploration of their impact; and the exploration of the multifactorial pathways in foot pathology and its impact on quality of life.

Chapter five provides a critical appraisal of the findings from the study, the implications of these findings, and an evaluation of the limitations of the study. In addition, this chapter discusses the contribution of this work to the existing literature and the recommendations of future research based of the findings obtained from this study.

Chapter 2
Background and Review of the Literature

2.1 Background

Systemic sclerosis (SSc) is a heterogeneous connective tissue disease characterised by vasculopathy, immune activation and fibrosis [1-3]. SSc is also known as scleroderma, meaning hardening of the skin, a hallmark of the disease, [23, 24] with skin involvement seen in the vast majority of the patients [25]. Several different terms have been used to describe the disease over the years, including scleroderma, systemic scleroderma, progressive systemic sclerosis and systemic sclerosis [23].

2.2 Disease subsets

The heterogeneous nature of the disease has made the development of classification criteria and definition of the different disease subsets for SSc a challenge. This is reflected in the number of the different attempts to develop criteria over the years, as presented in Table 2.1 (and discussed in section 2.3).

Year and Author	Classification Scheme
1945 Goetz [26]	**2 subsets: acrosclerosis and diffuse:** based on skin thickening limited to extremities or includes trunk
1962 Tuffanelli and Winkelman [27]	**2 subsets: acrosclerosis:** RP, acral skin involvement**; diffuse SSc:** no RP, skin involvement beginning centrally
1964 Winterbauer [28]	**CRST syndrome:** calcinosis, RP, sclerodactyly, telangiectasia
1969 Barnett [29]	**3 subsets: limited, moderate, extensive,** based on skin involvement of the fingers only, limbs and face, and involvement of the trunk, respectively
1979 Rodnan [30]	**3 subsets: classical disease** involving skin of the trunk, face and proximal extremities, and early involvement of esophagus, intestine, heart, lung and kidney**; CREST syndrome;** and overlap syndromes including sclerodermatomyositis and **mixed connective tissue disease**
1986 Giordano [31]	**6 subsets: I: sclerodactyly only; II:** sclerodactyly and skin involvement of neck, lower eyelid, or axillae**; III:** skin involvement of hands and forearms ± legs ± face**; IV:** group III and arm and/or thigh skin involvement; **V:** group III and thorax; **VI**: group III and/or IV and/or **V** plus the abdomen**.** **3 subsets: limited:** skin involvement of fingers, face, neck, axillae**; intermediate:** skin involvement proximal to fingers**; diffuse:** truncal skin involvement
1987 Holzmann[32]	**5 subsets (Types I–IV)** based on presence/absence of RP, sclerosis, extracutaneous manifestations, ANA
1988 LeRoy [24]	**2 subsets: diffuse cutaneous SSc:** onset of RP within 1 year; truncal and acral skin involvement; tendon friction rubs; early incidence of ILD, renal failure, diffuse GI disease, myocardial involvement; absence of ACA, abnormal ND; **limited cutaneous SSc:** RP for years, skin involvement limited to hands, face, feet, forearms or absent; late incidence of PAH, trigeminal neuralgia, calcinosis, telangiectasia; high incidence of ACA, abnormal NC
1988 Masi [33]	**3 subsets: digital:** skin involvement of fingers or toes but not proximal extremity or trunk**; proximal extremity:** proximal extremities or face but not trunk**; truncal:** thorax or abdomen
2001 LeRoy and Medsger [34]	**4 subsets: limited SSc (LSSc)** consists of (1) objective RP plus any one of NC changes or SSc selective autoantibodies or (2) subjective RP plus both NC changes and SSc selective autoantibodies; **limited cutaneous SSc (lcSSc):** criteria for LSSc plus distal cutaneous changes**; diffuse cutaneous (dcSSc):** criteria for lcSSc plus proximal cutaneous changes**; diffuse fasciitis with eosinophilia:** proximal cutaneous changes without criteria for lSSc or lcSSc
2002 Scussel-Lonzetti [35]	**4 subsets: normal skin, limited:** skin involvement restricted to fingers, with RP, calcinosis**,** esophageal involvement and telangiectasia**; intermediate:** skin involvement of arms proximal to metacarpophalangeal but not trunk**; diffuse:** skin involvement of the trunk
2002 Ferri [36]	**4 subsets**: **sine scleroderma SSc**: absence of cutaneous involvement with visceral involvement, NC changes and autoantibodies; **limited cutaneous**: skin involvement of fingers with or without involvement of neck, face, and axillae; **intermediate cutaneous**: skin involvement of upper and lower limbs, neck and face without truncal involvement, **diffuse cutaneous:** distal and truncal skin involvement
2004 Maricq [37]	**6 subsets: diffuse, intermediate, digital, scleroderma sine scleroderma, undifferentiated connective tissue disease with scleroderma, CREST syndrome**

Table 2.1 Classification of systemic sclerosis subsets Modified from Johnson et al, page 1857[38] RP: Raynaud's phenomenon; NC: nailfold capillary; ILD: interstitial lung diseases; GI: gastrointestinal; ACA: anticentromere antibodies; PAH: pulmonary arterial hypertension; LSSc: limited SSc.

Improved understanding as well as recognition of the heterogeneous autoantibody subsets associated with clinical manifestations has aided the phenotyping of disease subsets and the development of more refined criteria. This has also permitted the differentiation of SSc from other conditions within the SSc spectrum [1, 38, 39].

Localized scleroderma is the most frequent form of scleroderma in childhood [40]. It is distinct from systemic sclerosis and includes areas such as plaque morphea, linear scleroderma and en coup de sabre. Varying degree of involvement and severity of skin and subcutaneous tissues occurs and may lead to significant functional and cosmetic deformity [23]. For the purpose of this literature review the focus will be on the systemic form of the disease, and the term used will be Systemic Sclerosis (SSc).

2.3 Disease Classification / diagnosis criteria

As noted previously, the heterogeneous nature of the disease has made the agreement of a disease classification difficult, and has influenced the development of the three different evolving classification/ diagnostic criteria.

The first SSc classification was published by the American Rheumatism Association (ARA) in 1980 [41], which aimed to broadly distinguish SSc from non-SSc patients. However, this classification did not adequately address disease heterogeneity [42]. Eight years later, a second classification system was proposed by LeRoy et al. [24],which took into consideration the principal distinguishing nature of the disease and introduced the two subsets of SSc: limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc). The 1988

classification was later supplemented with an additional subset of early or limited SSc which did not include the presence of cutaneous involvement; it was termed limited Systemic Sclerosis (lSSc) [43]. This subset of lSSc has not been widely accepted [42, 44]. The more widely used classification for patients who have SSc without cutaneous involvement is systemic sclerosis *sine scleroderma* (ssSSc) [45]. The addition of this subset is useful in clinical practice as some patients early in their disease may present without apparent skin involvement [38], as well as instances of established disease without the usual skin tightening.

There has however remained the need for a validated classification criteria that in particular can detect early and limited scleroderma to enable early detection of SSc; as well as to standardise the criteria for patients' inclusion into studies, enabling a more robust comparison of results across studies [1]. These needs have been fulfilled by the latest development of the 2013 American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) criteria which have demonstrated better performance over the 1980 criteria [39, 46-48], and the preliminary criteria for the very early diagnosis of SSc (VEDOS) [49] which has enabled the early detection of scleroderma.

2.4 The prevalence of systemic sclerosis

Estimates of the prevalence of SSc vary enormously from between 30 to 2,280 per 1,000,000 population [50], with higher estimates in North America and Australia when compared to Europe and Japan [51, 52]. In the northern countries of Europe (England and Iceland), the prevalence is lower, when

compared to countries in Southern Europe (France and Greece) [52, 53]. Some spatiotemporal clustering has also reported in Ontorio, Rome, and near London's airports [52, 54].

The prevalence of SSc in the UK ranges from 31 per million in the West Midlands [55] to 150 per million in South and West London [54]. There are no epidemiological data for the area of West Yorkshire, but the closest where epidemiological data have been reported from is the North East of England, with a prevalence of 88 (8.8 per 100 000) and 82 per 1,000,000 when adjusted for the entire UK [16, 56]. Of the 63.2 million people in the UK, 5,183 people potentially suffer from SSc.

Epidemiological data from different areas of the world are summarised in Table 2.2. The incidence of systemic sclerosis is lower in native Nigerians than in African Americans. Oklahoma Choctaw Indians have an incidence of 472 cases per million population, which is higher than that of the Missouri Choctaw Indians. These differences may reflect distinct environmental exposures as well as differences in genetic predisposition.

Publication (first author, journal, year)	Country/state	Case ascertainment	Inclusion criteria	Period	Incidence/ million/year	Prevalence/million
USA						
Medsger, Ann Intern Med, 1971 [37]	Minnesota	Hospital record review	Study specific	1947–68	2.7	–
				1947–52	0.6	–
				1953–68	4.5	–
Kurland, Mayo Clin Proc, 1969	Minnesota	Hospital record review	ACR	1951–67	1.2	105
Michet, Mayo Clin Proc, 1985	Minnesota	Diagnostic retrieval system	ICDA (7th)	1950–79	10	138
Medsger, J Chronic Dis, 1978	USA	Veteran Administration hospital	ACR + study specific	1963–68	2.3	19.8
Steen, Arthritis Rheum, 1997 [22]	Pennsylvania	Hospital record review	ACR + study specific	1963–82	13.9	–
				1963–72	9.6	–
				1973–82	18.7	–
Maricq, Arthritis Rheum, 1989 [9]	South Carolina	Multistage population survey	ACR + study specific	1989	–	290–1130
Laing, Arthritis Rheum, 1997 [5]	Michigan	Multiple sources (CR)	ACR + Leroy/Medsger	1985–91	14.1	–
Mayes, Arthritis Rheum, 2003 [6]	Michigan	Multiple sources (CR)	ACR and CREST	1989–91	21	276
Robinson, Cur Med Res Op, 2008 [8]	All states	2 medical/drug claims datasets	ICD diagnostic codes	2001–02	–	300
Australia						
Wigley, Soc Sci Med, 1980	Southern Half	Hospital record review	Study specific	1950–73	1.96	–
Eason, Aust NZ J Med, 1981	New Zealand	Hospital record review and specialist practices	ACR	1970–79	6.3	30
Englert, Aust NZ J Med, 1999 [11]	Sydney	Hospital record review	ACR + study specific	1974–88	12	45.2 (1975) 86.2 (1988)
Chandran, Aust NZ J Med, 1995	South Australia	Hospital record review	ACR + study specific + overlap syndrome	1987–93	–	208
Roberts-Thomson, Int Med J, 2001 [10]	South Australia	Multiples sources	ACR + study specific + overlap syndrome	1993	15.1	200
				1999	22.8	233
Roberts-Thomson, Int Med J, 2006 [23]	South Australia	Multiples sources	ACR + study specific + overlap syndrome	1993–02	20.4	232.2
Japan						
Tamaki, Arch Dermatol Res, 1991 [12]	Tokyo	Public health system	ACR	1987	7.2	38–53
Europe						
Silman, Br J Rheumatol, 1988 [13]	England (West Midlands)	Multiple sources	Study specific	1986	3.7	31
Allcock, Rheumatology, 2004	England (Newcastle)	Multiple sources	ACR + Leroy/Medsger	2000	–	88
Czirjak, Clin Exp Rheumatol, 2005	Hungary (South West)	Multistage population survey	ACR + Leroy/Medsger	2001	–	910–2370
Valter, Scand J Rhumatol, 1997	Estonia (South)	Multistage population survey	ACR + SSD	1996–97	–	350–2280
Geirsson, Ann Rheum Dis, 1994	Iceland	Multiple sources	ACR + CREST	1975–90	3.8	71
Kaipiainana, J Int Med, 1996	Finland	Multiple sources	ACR + CREST	1990	3.7	–
Le Guern, Rheumatology, 2004 [7]	France (Seine St Denis)	Multiple sources (CR)	ACR + Leroy/Medsger	2001	–	158
Alamanos, Semin Arthritis Rheum, 2005	Greece (northwest)	Multiple sources	ACR + Leroy/Medsger	1981–02	11	154
Ariaz-Nunez, Medicine, 2008 [16]	Spain (northwest)	Two-stage hospital based survey	ACR + Leroy/Medsger	1988–06	23	277

ACR: American College of Rheumatology; CR: capture–recapture method; CREST: calcinosis, Raynaud's phenomenon, oesophagus involvement, sclerodactyly, telangiectasia; ICD: International Classification of Diseases; ICDA: International Classification of Diseases, Adapted; USA: United States of America.

Table 2.2: Geographical and time variation of prevalence and incidence of systemic sclerosis. Taken from Ranque et al, Page A313[51]

As with other autoimmune disease, there is a higher female-to-male ratio in SSC, approximately 3:1 (ranging from 1 to 14:1) [50, 52, 56, 57]. In the North East of England the female to male ratio has been reported to be 3.9:1 [56].

The onset of the disease varies according to gender and ethnic background but generally reaches its peak of incidence at the fifth decade. However, SSc can occur at any age, albeit rare in children and in the very elderly [16-18, 51, 52].

2.5 Aetiopathogenesis of systemic sclerosis

The aetiopathogenesis of the disease remains unclear but epidemiological and genetic data indicate complex interactions between the host genetic background and a variable contribution by environmental factors[2, 51, 58].

2.5.1 **Scleroderma risk factors**

There are a number of risk factors for developing SSc, including genetic, ethnic, and environmental risk factors, the later including occupation, infection, and non-occupational non-infectious factors. There is however, disagreement in the literature regarding which factors are considered the strongest contributors to the development of the disease. Some authors suggest that the major risk factor is genetic [58, 59] while others indicates that environmental exposure plays a large role [51].

2.5.2 **Genetics**

A strong argument for the contribution of genetic factors in the development of SSc is made through: family studies that have shown clustering of the disease (occurring more frequently than in the general population; 1.6% compared to 0.026 % [58]); higher frequency of other autoimmune disorders as well as autoantibodies; geographical/ethnic variation and the association of certain human leukocyte antigens (HLAs) and major histocompatibility complex (MHC) alleles in the context of disease types and manifestations. In first-degree relatives, the relative risk of SSc increased by between 10 to 16-fold, (average 13), which is further increased for siblings 10 to 27-fold increased risk (average

15). However, the absolute risk for each family member remains very low, at around 1% [18, 58, 59]. This risk increases slightly for families with African American ethnicity, which has a higher percentage of familial SSc (3%) when compared with caucasian families (1.5%) [59].

In addition to sibling studies, twin studies have also been conducted and revealed that both monozygotic and dizygotic twin pairs share the same risk of developing SSc, supporting a shared environmental risk factor; although, when the positivity of antinuclear antibody (ANA) was assessed in the same twin pairs the concordance rate was 100% in monozygotic and only 64% in dizygotic twins, indicating that genetic factors may play a variable role [60].

These results suggest that genetics are involved in the development of the disease and that environmental exposure is a concomitant risk factor (Ranque and Mouthon, 2010).

While the genetic predisposition of SSc does not follow a Mendelian pattern, there is evidence supporting the assertion that genetic factors are involved in both disease susceptibility and progression [58].

A large number of genetic variants have been implicated in SSc susceptibility and pathogenesis. The genes found to be associated with the disease have a modest but reproducible effect on susceptibility through populations, and many of them map to the same biological pathway[61]. In some cases, combinations of genetic variance have been associated with an additive risk for susceptibility [61, 62].

Some of the genetic associations report single nucleotide polymorphisms (SNPs) in genes coding for:

- vasomotor regulatory factors i.e. angiotensin–converting enzyme, endothelin and nitric oxide synthase;
- B-cell markers i.e. CD19; chemokines and chemokine receptors i.e. monocyte chemotactic protein-1 (MCP-1) and CXCR2;
- cytokines i.e. interleukin-1a (IL-1a), IL-4 and tumour necrosis factor-a (TNF-a);
- growth factors and their receptors i.e bone morphogenetic protein type II receptor (BMPRII), connective tissue growth factor (CTGF) and transforming growth factor-b (TGF-b);
- an anti-oxidant i.e. glutathione S-transferase; and extracellular matrix (ECM) proteins i.e. fibronectin, fibrillin and secreted protein, acidic and rich in cysteine (SPARC) [2, 63, 64].

There are other SNPs that have been identified to be present in patients with SSc but they will not be covered in this section as it is beyond the scope of this literature review.

2.5.2.1 Ethnicity

It is not clear at the time of writing, whether the effect of ethnicity on the disease reflects a genetic or an environmental input [65]. Studies conducted in the USA observed that the prevalence of SSc was higher amongst black people than white [66, 67]: those from a black ethnic background have a worse prognosis, and are more likely to develop the disease at a younger age, have the diffuse subset, have higher incidence of inflammatory features and worse age-adjusted survival rate [66, 67].

Studies undertaken in Australia and France reported a higher prevalence of SSc among people born in continental Europe than non-European (Northern and Sub-Saharan Africans, Asians, and Caribbean) [68]. However, when compared with patients from European descent, SSc occurs more frequently in black Americans, some Asians, and some Native Americans (Choctaw Indians) [51], who were also more likely to have the diffuse subset of the disease associated with worse survival indicating worse survival [18].

2.5.2.2 Environmental risk factors

Differentiating the association between environmental exposure and SSc is challenging. To fully explain any association, the exposure to the environmental factor and the development of the disease has to occur chronologically. Most of patients with SSc have not had the exposure to the environmental factors and the majority of the exposed subjects do not develop SSc [69].

Environmental factors can be classified in three categories: occupational, infectious, and non-occupational/non-infectious [70].

i. Occupational

Several occupational factors have been identified as potential risk factors to the development of SSc.

Crystalline silica was one of the first environmental factors identified as a risk factor, when inhaled as dust. People working with rock and soil, such as mining, quarry and pottery work, have high exposure to this element and an increasing risk of contracting the disease [69-74].

Solvents are also considered a risk factor when penetrating through the skin and airways. Solvents implicated included organic solvents tetrachloroethylene, trichloroethylene, perchloroethylene, carbone tetrachloride, benzene, paint thinners/removers (e.g toluene and turpentine), nail polish and glues (e.g. acetone, methyl acetate and ethyl acetate), degreasers (e.g. hexane and petrol ether) varnish, gasoline, and aromatic hydrocarbons [75-78].

Other elements that have been reported to have an associated risk are ketones, epoxy resins, white spirit, cement, pesticides, and welding fumes [77-79].

ii. Infection

Bacterial and viral agents have been considered as a risk factor for the development of SSc, particularly helicobacteria pylori, human cytomegalovirus, herpes viruses, retroviruses, Epstein-Barr Virus, toxoplasma gondii and parvovirus B19 [80-83]. Despite the different studies undertaken there is no strong evidence to conclude that SSc has a viral or bacterial origin [69, 81].

iii. Non-occupational/ non-infectious factors

Some drugs have been identified as potential risk factors because of the scleroderma–like symptoms associated with their use [84], including oestrogen replacement therapy in post-menopausal women [76], appetite suppressants (e.g. dexamphetamine, diethylpropion, fenfluramine, fenproporex, mazindol, methaqualone, and phenmetrazine) [85], carbidopa and L-5 hydroxytryptophan (5-HTP) [86], bleomycin [87] and gadolinium [88]. The evidence supporting the role of some of these drugs in the development of SSc is weak as it comes from single case studies or studies with small numbers.

A body of evidence suggests therefore that there may be many potential environmental triggers for the development and progression of SSc, although the hosts' factors also determine the susceptibility of the host to any triggers [81].

2.6 The pathophysiology of systemic sclerosis

While much of the detail of the pathophysiology of SSc remains poorly understood, it is clear that the disease is typically characterised by three features, namely, vasculopathy, immune activation (presence of autoantibodies) and inflammation, and fibrosis. The complex interaction between these three factors leads to immunological disruption; obliterative fibrointimal proliferation of the small vessels with vasospastic episodes, resulting in ischaemia; and activation of resident connective tissue cells (e.g. fibroblasts, myofiblobasts, pericytes) with increased deposition of extracellular matrix constituents [3, 89-91]. While the exact processes associated with SSc remain to be fully elucidated, research has identified key cells, molecular processes and factors driving these pathways, resulting in changes to the vasculature, immune activation and fibrosis (Table 2.3).

Pathophysiologic Compartment	*Key Cells*	*Molecular Processes*	*Driving Molecular Factors*
Vasculature	Endothelial cells Pericytes Smooth muscle cells	• Endothelial cell injury • Endothelial cell apoptosis • Oxidative stress • Platelet activation and thrombosis • Enhanced microvascular permeability • Increased synthesis of extracellular matrix molecules • Hypertrophic and fibrotic remodelling of the blood vessels with progressive lowering of the inner vascular diameters • Defect angiogenesis and vasculogenesis	• Vasculotropic viruses • Inflammatory cytokines • Granzymes • Von-Willebrand factor • VCAM-1 • ELAM-1 • VEGF • Endothelin-1 • Reactive oxygen species
Autoimmunity and inflammation	B- and T-lymphocytes	• Local and general activation of lymphocytes • enhanced transendothelial migration of $CD4^+$ T cells • oligoclonal expansion of lymphocytes • Cytokine synthesis, release of fibrosis-promoting cytokines	• Autoantibody synthesis (endothelial cell-specific antibodies, anti-PDGF antibodies, anti-Scl70 antibodies, anti-centromere antibodies) • (Profibrotic) cytokines including IL-6, IL-4, IL-10 • sIL-2 receptors
Fibrosis	Fibroblasts Pericytes Myofibroblasts Smooth muscle cells Stem cells	• Tissue remodelling • synthesis of collagens in combination with reduced cleavage of matrix components • transdifferentiation of fibroblasts	• Growth factors (TGFβ and TGF receptors, EGF, CTGF, PDGF) • Intracellular signalling molecules (BMPs, SMADs, p38-, JNK-, MAPK, focal adhesion kinase (FAK), TGF-β activated kinase 1, lipid kinases like PI3K, AKT, calcium-dependent phosphatase calcineurin, tyrosine kinase c-ABL, early growth response 1 (EGR-1), SP3, Ets-1, FLI-1, p53, RAS • Vascular multifunctional molecules (PAI-1, ET-1) • Chemokines (MCP-1) • nuclear hormone receptors (PPARγ)

Table 2.3: Key processes of SSc pathophysiology and the respective driving cells and molecules. Adapted from Geyer et al ,Page 97 [3]

2.6.1 **Vascular dysfunction**

A hallmark of SSc is the clinical observation of Raynaud's Phenomenon (RP), which occurs in up to 95% of patients with SSc [92]. RP is an exaggerated vasoconstriction response to cold or emotions (e.g. anxiety) seen in the general population as well as in patients with SSc [93, 94]. In addition, structural changes are seen in patients with SSc, illustrated by characteristic changes in

the capillaries of the skin (with haemorrhage, megacapillaries and loss of capillary density) [95]. These features imply insufficient vasodilatory capacity and also irreparable vascular damage. These vascular changes manifest on a clinical level prior to the development of fibrotic features in the skin, gut as well as major internal organ involvement such as lung and myocardial disease. Whilst the fibrotic and cellular and humoral autoimmune processes are also central features, the exact interplay and dynamics between these processes remains challenging to define. Nevertheless, the vasculopathy is considered a key event in the early pathogenesis of SSc [96] [89][90]. Indeed, in addition to the early clinical feature of RP, much of the related morbidity and mortality in SSc is related to underlying vasculopathy.

Microvascular involvement

The vascular pathology is predominantly a microvasculopathy, characterised by fibrointimal proliferation, with initial functional vascular injury (associated with disruption of the vessel's structure and function), leading to structural changes, intimal proliferation and occlusion. This results in a decrease in blood flow and irreversible tissue damage [93, 97]. The consequences of the vasculopathy can be observed clinically in different organs, as mentioned earlier, but the most visible and earliest clinical signs are observed on the upper and lower limbs; in particular on the hands and feet with Raynaud's attacks, tissue ischaemia and ulcerations.

The cause of the initial endothelial damage and vascular injury is uncertain; several triggers have been postulated including viral infection, toxin, oxidative stress, or immune-mediated through endothelial autoantibody activity [89, 98]. The initial vascular injury is associated with a functional change with impaired

release of expected vasodilators such as nitric oxide and prostacyclins (leading to RP, reflecting an exaggerated, normal physiological response that is reversible). In contrast to healthy population however, an irreversible structural change ensues that can be largely attributed to marked release of endothelin-1 (ET-1). ET-1 levels are increased in the blood and in bronchoalveolar lavage fluids from patients with SSc [99, 100]. It is a potent vasoconstrictor that also promotes leukocyte adhesion to the endothelium as well as vascular smooth muscle cell proliferation and fibroblast activation with consequent deposition of extracellular matrix. The consequent hypertrophic and fibrotic remodelling of the blood vessels (the process summarised as a vascular fibrointimal proliferation) [101-103] leads to progressive reduction of the vessel lumen. This results in the loss of vascular elasticity and luminal space [3, 104]. This process underlies the vascular occlusion and resultant tissue hypoxia and damage observed.

The vascular damage in turns promotes expression of endothelial cell adhesion ligands. Endothelial cell damage then appears to trigger a chain of events leading to an initial innate and then adaptive response which is followed by the extravasation of inflammatory cells, initially mainly of the monocytic lineage and then a subsequently lymphocyte predominant infiltrate. Endothelial cell damage and activatation leads to the release of several regulators and mediators affecting smooth muscle cells and fibroblasts with a fibrosis of vascular and interstitial spaces [3, 104, 105]. The release of vasoactive mediators also increases microvascular permeability; these events correlating with the cutaneous oedematous phase often clinically observed in the early stage of the disease [3, 25]. The oedematous phase can be observed in both the upper and

lower limb, and the early involvement of the feet in the oedematous phase suggests the early effects of the disease on the foot pathology[9].
Furthermore, the endothelial damage impairs endothelial-dependent vasodilation which affects the balanced regulation of vessel vasoconstriction [106]; with also the underproduction of vasodilators (Nitric Oxide) [107, 108] and an increase of endothelium-derived vasoconstrictors (ET-1) [109, 110]; continuing the cycle of vascular dysfunction.

These pathological vascular processes have clear effects on the peripheries including the feet. The functional and structural changes described earlier leads to thrombotic events in the small arteries, which produce complete occlusion and can result in ischaemic insult with hypoxia, necrosis and loss of tissue [111] [3, 104]. This process presents clinically in the form of cyanotic lower limbs and painful ischemic ulcers or gangrenous lesions on the feet (most frequently seen in the toes), which in turn can lead to amputation and reduced function [9, 112, 113].
The process of fibrotic intimal hyperplasia is characteristic of the small vessel vasculopathy, and although it is not as common in larger vessels it has also been reported to occur in major vessels having severe consequences such as lower limb amputation [114].
Tissue hypoxia created by capillary and/or arteriolar occlusion or breakdown is thought to trigger physiological reactions, such as angiogenesis and vasculogenesis, which attempt to restore the homeostasis in the affected area. In SSc however, both of these processes are dysregulated leading to an inappropriate repair process in response to endothelial cell injury or insult. This

dysregulation of angiogenesis is associated with the clinical observation of reduced capillary density as a result of an inability to replace damaged vessels [3, 115, 116] [115, 117-120]. The inability to repair damaged vasculature or create new vessels has an effect on tissue viability and impairs healing, thereby increasing risk of tissue damage and chronic ulcerations for patients with SSc. The structural damage caused by the process described above has an effect on the vascular function. The vessel function is further impaired by an imbalance vasoconstriction and vasodilatation, with a preference for vasoconstriction. The vascular pathogenesis contributing to the abnormal vasoconstriction in SSc is mostly related to the Raynaud's phenomenon (RP) pathology. In patients with SSc the RP pathogenesis cannot be clearly differentiated from the structural vascular pathogenesis caused by the disease as their pathogeneses are interrelated [93, 121].

This exaggerated vasoconstriction is also affected by the neural abnormalities in the central and peripheral pathways of the autonomic nervous system. In RP the central mechanisms are thought to contribute mostly to vasospasm, but is the peripheral neural pathways which are considered more important on the effect to the vessels vasoconstriction [93]. The autonomic and sensory afferent nerves release neurotransmitters that control the vascular tone of the digits, which are in turn key factors in the mechanism of neural control and are affected in patients with RP [93, 122]. For example the calcitonin gene-related peptide (CGRP)- immunoreactive nerve fibres that supply blood vessels with vasodilators are reduced in number [123], leading to impaired vasodilation. At the same time the activation of the arteriolar smooth muscle α2-adrenoreceptors (noradrenaline receptors), which are considered very important

in the regulation of digital vascular tone, increases on exposure to cold, causing vasoconstriction [124-126].

In addition to the functional and structural vascular changes and neural abnormalities described above, there are associated intravascular factors such as defective fibrinolysis, platelet activation, increased blood viscosity, white blood cells activation, reduced red blood cells deformability and oxidant stress which will also contribute to the impairment of basal blood flow, particularly in the microcirculation, leading to tissue damage [93].

Macrovascular involvement

Whilst microvascular pathology is a central mechanism underlying SSc, tissue viability and reduced healing is also thought to be compromised by the pathological alterations in the macrovasculature [3, 127].

Although not as clear as with other autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, macrovascular involvement in SSc has been reported to be more prevalent when compared to controls [13]. Reports demonstrate increased arterial stiffness, carotid intima thickening and reduced flow-mediated dilatation, all of which are associated with increased risk of development of atherosclerosis; suggesting an excess cardiovascular risk for patients with SSc [128, 129]. The pathological alterations of the macrovasculature also lead to an increased prevalence of Peripheral Arterial Disease (PAD) in the upper and lower limb of patients with SSc [14, 15]. Macrovascular damage progresses in tandem with worsening of the microangiopathy [15] [14]. Some discrepancies exist in accounts reporting the correlation between macrovascular damage and disease duration; with both existence of correlation [14] and non-existence [15] reported.

Occlusive microvasculopathy, as described above, is considered the principal vasculopathy in SSc. However, the presence of frank vascular inflammation in the form of vasculitis, with inflammatory infiltrates damaging the vessels, has also been identified as contributor to the vasculopathy in some patients with SSc[130]. The presence of frank vasculitis in SSc is considered to be rare and when present it is a co-existent feature of the disease as opposed to being part of the central pathology [131-133](D'Angelo et al., 1969).

Different types of vasculitis affecting the various sizes of vessel have been reported to coexist with the occlusive vasculopathy [130]. The most prevalent vasculopathy present in patients with SSc is the Anti-Neutrophil Cytoplasmic Antibody (ANCA) associated vasculitis. It is considered primarily a necrotising small-vessel vasculitis, although it can also affect the medium-sized vessels [130]. The presence of vasculitis in patients with SSc has been reported to lead to severe ischaemia and peripheral neuropathy in the limbs [134] [133].

2.6.2 Immune system and Inflammation in SSc

2.6.2.1 Autoantibodies & SSc

Autoantibodies are used to aid diagnosis and to assess the disease prognosis [135]. Autoantibodies directed to nuclear autoantigens (ANA) are present in patients, with a prevalence varying from 80 to 98% [136] [89]. They are directed to various antigenic targets. Two types of ANA most frequently associated with SSc are anti-centromere (ACA) and anti-topoisomerase I antibodies (ATA). These are found in over 50% of patients with SSc [137-139]. In addition, another ANA, anti-RNA polymerase III antibody (ARA), has also been identified

in patients with SSc [135, 136, 140]. These autoantibodies have a high specificity and can be present exclusively from each other, although in some patients ACA and ATA can be present simultaneously; a small percentage (3-11%) of patients are ANA negative [135, 136, 140].

In addition to the three ANAs commonly seen in SSc, there are other autoantibodies often producing nucleolar staining by indirect immunofluorescence (IIF) on HEp-2 cells that have been identified as being involved in the disease and to have a clinical correlation [141], see Table 2.4. However, they are not SSc-specific and therefore even though they might be useful to determine disease progression and prognosis they are not specific enough to be used for diagnosis. These include antibodies to fibrillarin (AFA), PM/Scl complex (anti-PMScl), Th/To, and to the RNA polymerase (anti-RNAP) family. Other autoantibodies have been more recently reported such as anti-U1RNP, anti-Ku, and anti-Platelet-Derived Growth Factor Receptor (PDGFR) [142] [141, 143].

Autoantigen	Autoantibody frequency	Differential cutaneous involvement	Clinical correlate
Topoisomerase I	9–20%	Diffuse	More severe disease, pulmonary fibrosis, increased mortality
Centromere proteins	20–30%	Limited	Calcinosis, ischemic digital loss, pulmonary hypertension
Polymyositis/Scl	3%		Polymyositis overlap (24% have polymyositis/Scl70 autoantibodies), milder disease
Fibrillarin (U3 RNP)	7–12%	Diffuse	More common in males, increased internal organ involvement, racial variation
RNA polymerase I–III	20%	Diffuse	Renal crisis, increased mortality
B23/nucleophosmin/numatrin	11%		Pulmonary hypertension
Th/To	2–5%	Limited	Milder cutaneous involvement, increased severity of pulmonary fibrosis, increased mortality
Ku	1–14%		Polymyositis SSc overlap
Sm	Rare		SLE overlap, poor prognosis
U1-RNP	8%		Overlap and milder disease
U1-RNA	61% U1RNP positive patients		Pulmonary fibrosis in those who also have U1-RNP autoantibodies
Nucleosome	3–46%		No known associations
Histone	16–29%		Pulmonary fibrosis, cardiac and renal involvement, decreased survival
High mobility group proteins	33%		No known associations
RNA helicase protein (Gu)	7% with antinucleolar autoantibodies		No known associations
Mitochondria	8%	Limited	Primary biliary cirrhosis
Glycine/tryptophan bodies	~1%		Also occur in other SARDs, including neuropathies and Sjögren's syndrome
Proteinase 3 and myeloperoxidase	3–4%		Rare reports of SSc with vasculitis. Frequent pulmonary, GIT and renal involvement
Endothelial cell	28–71%		Digital infarcts, pulmonary hypertension
Fibroblasts	26–58%	Diffuse	Pulmonary involvement, increased mortality
Platelet-derived growth factor receptor	100%		Also found in extensive chronic graft versus host disease
Fibrillin 1	>50%	Diffuse	Ethnic variations
Matrix metalloproteinase	50%		Levels correlate with skin, blood vessel and lung fibrosis
Peroxiredoxin I	33%		Longer disease duration, pulmonary fibrosis, cardiac involvement, antitopo I
Cardiolipin	20–25%		Increased disease severity (controversial)
Tissue plasminogen activator	20%	Limited	Pulmonary hypertension
Interferon-inducible gene (IFI16)	21%	Limited	No known associations

SLE, systemic lupus erythematosus; SARD, systemic autoimmune rheumatic diseases; GIT, gastrointestinal tract.

Table 2.4: Summary of autoantibodies described in Systemic Sclerosis and their clinical correlations. Taken from Walker and Fritzler ,Page 581 [141]

Although it remains unclear whether autoantibodies have a pathogenic role or are merely epiphenomena, they can be useful in aiding diagnosis as well as risk assessment and clinical management. For example, the presence of ACA is present in approximately 70% of patients with lcSSc disease subset [144] and carries an increased risk of developing pulmonary arterial hypertension (PAH) The presence of ARA is associated with an increase of SRC and ATA antibodies and is indicative of worse prognosis (with an increased risk of developing pulmonary fibrosis and mortality [145]); both of these antibodies being present in 45% and 27%, respectively, of patients with the dcSSc disease subset [89, 146].

2.6.2.2 Activation of the immune system in SSc and evidence of early inflammation

The damage to the endothelium seen within the vascular pathway is followed by infiltration of inflammatory cells enabling the development of both innate and adaptive immune responses [147]. Histopathological evidence in early stage disease lesional skin demonstrates mononuclear cell infiltrates (mainly T cell, macrophages mast cells and occasionally B cells) before any evidence of skin fibrosis [148]. The involvement of inflammation in the pathogenesis of the disease can be observed in histological abnormalities from tissue of the different systems affected by the disease. Inflammatory infiltrates have been reported to be present in the interstitium and perivascular in muscle tissue, lower dermis and subcutis [149], subcutaneous fat [25]central and peripheral nerved lesions [150] [151], synovial membrane [152, 153] and subsynovium [154].

Evidence of T-cell activation in affected skin lesions with monoclonal and oligoclonal Tcell expansion and associated increased serum T-cell derived cytokines suggests an important role for these immune cells in the pathobiology. In addition, B-cells are also activated in the long term [142, 147, 155] leading to a chronic hyper-reactivity [156], with a hypergammaglobulinaemia and the presence of several autoantibodies in the blood. Whilst the role for SSc-specific autoantibodies is not clear, more recent studies suggest the biological activities and potential pathogenic roles of autoantibodies in patients with SSc. Antibodies specific for fibroblasts, endothelial cells, and PDGF receptors might

directly cause fibroblast or endothelial cell activation and contribute to tissue damage [3].

The inflammatory and autoimmune element of the disease therefore drives peri-vascular inflammation, release of (predominantly Th2) cytokines that promote fibrosis, and the production of stimulatory autoantibodies. Consequently, this increases fibrogenesis and contributes to progression of vasculopathy and tissue remodelling [3]. Nonetheless, it still unclear whether the involvement of the immune system in SSc is part of the disease initiation and/or disease maintenance [89].

2.6.3 **Fibrosis**

The prototypic hallmark of SSc is fibrosis, caused by excessive accumulation of collagen (produced by fibroblasts) and other connective tissue components in affected organs. It is the persistent activation of the genes encoding various collagens in SSc fibroblasts that distinguishes normal wound healing associated (controlled) repair with the uncontrolled fibrosis observed in systemic sclerosis [2].

A prolonged and exaggerated activation of fibroblasts, together with maintenance of the fibroblast-mediated effect, ultimately causes the pathophysiologic alterations, which lead to fibrosis in SSc.

Fibroblasts are recruited and activated by multiple cytokines and growth factors to generate myofibroblasts. Activation of fibroblasts and myofibroblasts leads to excessive deposition of collagen and other extracellular matrix (ECM) proteins, eventually resulting in tissue fibrosis, tissue contraction and permanent scarring [89]. Several cellular determinants of fibrosis have been identified including

pericytes, fibrocytes, other monocyte-derived fibroblast progenitors although their exact role continues to be an area of investigation. The principal molecular determinant of fibrosis however seems to be TGF-beta, secreted by monocytes/macrophages, T cells, platelets and fibroblasts. An increasing number of cytokines (IL-4/13), growth factors (CTGF, PDGF) and chemokines (MCP-1 and -3) have also been recognised in contributing to the fibrotic process [157].

The figure below describes the pathogenic features of the disease and the interrelations, which lead to the symptoms observed clinically.

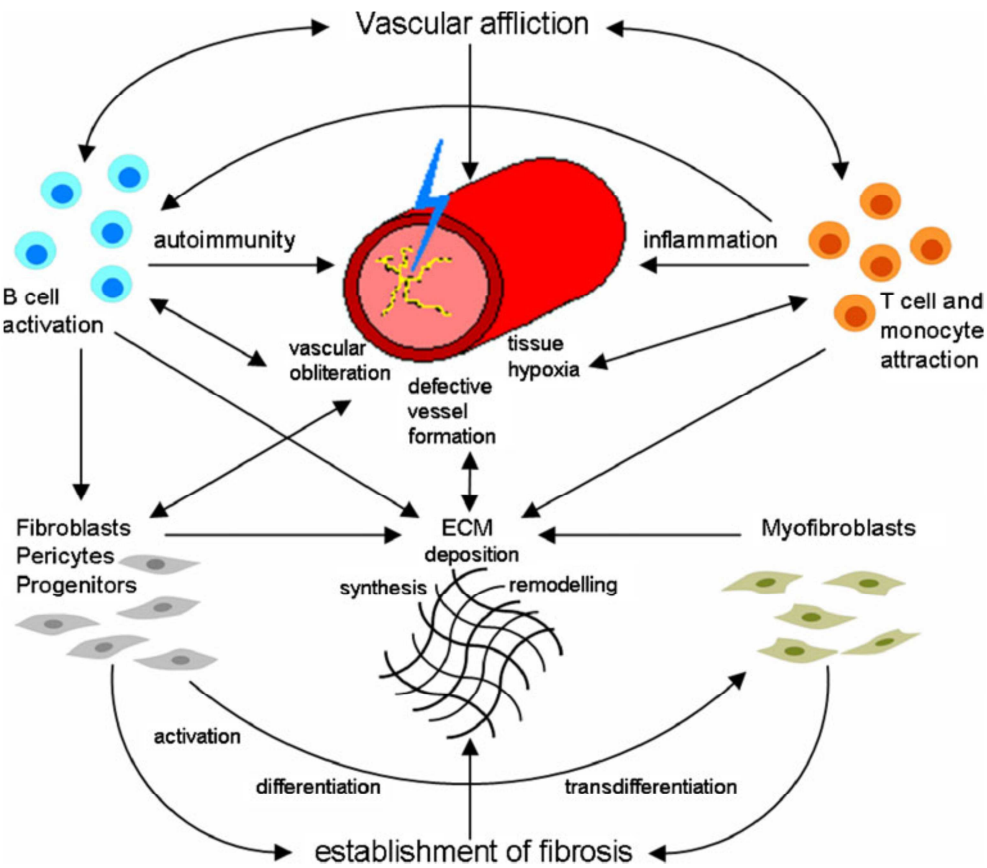

Figure 2.1 Pathogenic Features of Systemic Sclerosis. Adapted from Geyer et al, page 93 [3],

2.7 Clinical manifestations of systemic sclerosis and associated mechanisms – focus on foot pathology

SSc manifestations are multi-systemic and wide-ranging. Cardio-pulmonary, renal and gastrointestinal involvement commands attention in light of the increased morbidity and mortality associated with major internal organ involvement. These will be briefly discussed below but with subsequent emphasis on the integumentary, vascular, nervous and musculoskeletal systems as these elements of the disease have a direct and most significant effect on the foot pathologies.

2.7.1 Indirect contributors to foot pathology

2.7.1.1 Cardio-pulmonary system

Primary cardiac involvement of the heart can affect all structures of the heart. Clinically evident cardiac involvement is associated with a poor prognosis (1-6) and a large proportion of SSc-related fatalities are attributable to cardiac causes. Whilst fibrosis is a central feature of SSc, clinical and pathological evidence suggests microvascular dysfunction is a primary process and one of the earliest features of disease. Repeated ischaemia and reperfusion damage (with replacement fibrosis and contraction band necrosis) can involve the pericardium, myocardium, and cardiac conduction system [158]. The patchy fibrotic lesions in the myocardium are the hallmark of the Scleroderma Heart Involvement (SHI) [132]. As well as myocardial fibrosis, a myocarditis may also develop, may co-exit with a peripheral myositis and may lead to a secondary fibrosis following resolution of the inflammatory process. The consequence of

SHI is most frequently seen as a left ventricular diastolic dysfunction; a systolic dysfunction is not as common in SSc but may also be observed [159, 160]. The left ventricular dysfunction can be exacerbated by the presence of coronary or hypertensive heart disease and can lead to heart failure. Arrhythmias often underlie cardiac mortality. The mechanisms are multi-factorial, including direct effects of microvascular injury, the subsequent development of fibrosis as well as autonomic dysfunction. Systemic hypertension associated with Scleroderma Renal Crises (SRC) can also strain the heart while concomitant PAH can cause cor pulmonale, pulmonary overload of the right heart and failure [159, 161].

Lung involvement in SSc accounts for the major cause of mortality. The two principal pathologies are pulmonary arterial hypertension (PAH) and interstitial lung disease (ILD) or pulmonary fibrosis.

Isolated PAH is more common in patients with lcSSc, associated with the ACA. It is histologically similar to idiopathic PAH. It is characterised by pulmonary vasculopathy and possibly vasoconstriction (as a consequence of an imbalance between vasodilators and vasoconstrictors) leading to increased pulmonary vascular resistance. The vascular obstruction as detailed earlier is a result of possible vascular smooth muscle proliferation caused by remodelling of the pulmonary vessels and coagulation abnormalities with thrombosis [162].

ILD is more common in patients with dcSSc and may be complicated by the development of (secondary) pulmonary hypertension [163].

Impaired lung and heart function will have an effect on the efficacy of soft tissue oxygenation therefore contributing to the severe effect that the vasculopathy already has on soft-tissue oxygenation of the limbs.

2.7.1.2 Renal system

The scleroderma renal crises present as abrupt cardiovascular renal disease and is characterised by severe hypertension and hyperreninemia, retinopathy, microangiopathic hemolytic anemia, cardiac failure, convulsions and rapidly progressive renal insufficiency. This renal crisis with malignant hypertension and fast progressing real failure was the leading cause of death in SSc before the introduction of angiotensin-converting enzymes (ACE) [162, 164, 165].

The disease pathology affects the kidneys by creating focal lesions of intima sclerosis and hyperplasia of the interlobular arteries, and fibrinoid necrosis of the walls of afferent arterioles and glomerular loops. Originally these lesions are present in focal areas although when they become extensive the vascular damage leads to a widespread focal cortical infarction. This leads to systemic hypertension and, fatally, fibrinoid necrosis of the arterioles of the kidney and other viscera [166].

Chronic renal disease has been correlated to peripheral arterial occlusive disease (PAOD) and foot ulcers in patients with renal problems and diabetes [167, 168]. The presence of PAOD has also been reported in patients with SSc [129] however in this population the direct link between SRC, PAOD and foot ulcerations has not been reported.

Renal failure is also known to be associated with peripheral neuropathy. Literature indicates that 60% of patients with renal failure develop polyneuropathy mostly as a result of ischaemia. The polyneuropathy affects the motor, sensory, autonomic and cranial nerves; nerves which are also predominantly affected in SSc neuropathy [169-171]. In patients with renal

failure the clinical presentation is of a distal sensory loss more pronounced in the lower limb, and an electrophysiology indicating axonal loss and secondary demyelination [172]. Similar presentation has been identified in patients with SSc and polyneuropathy as they have distal sensory loss with axonal degeneration and reduction of density of the myelinated fibres [151, 173]. However, even though ischaemia is noted to be one of the possible causes for neuropathy in SSc, the pathological mechanisms for neuropathy in SSc are not fully understood and there is a lack of literature indicating that there is a relationship between renal involvement in SSc and neuropathy.

2.7.1.3 Gastrointestinal system

The gastro-intestinal complications are usually extensive involving many regions of the gut, from the oesophagus to the anus. The oesophagus has been identified as the most commonly affected, but the anorectum, small bowel, colon, and stomach can also have functional problems [174]. Gastrointestinal dysmotility appears to be as a result of smooth muscle atrophy and fibrosis.

The oesophagus is affected by oesophageal motor dysfunction and gastro-oesophageal reflux. The distal two-thirds of the oesophagus is damaged causing oesophageal dysmotility leading to reflux and nocturnal acid breakthrough. Chronic reflux can lead to oesophageal injury and intestinal metaplasia or Barrett oesophagus [175, 176], which in turn can lead to oesophageal adenocarcinoma [162].

In addition gastric, intestinal, colonic and anorectal dysmotility can cause pseudo-obstruction, bacterial overgrowth, defecatory disorders and in severe cases, malnutrition [177] [174, 176]. These gastrointestinal problems not only

carry a health risk for the patients but also have a negative impact on their quality of life [178].

The direct implication of the gastrointestinal involvement in the occurrence of DU has not been described, however together with lung fibrosis, PAH and SHI, are the organ manifestations more commonly present in patients with DU [179, 180]. It is well established that a lack of nutrients as a result of gastrointestinal dysfunction, and a lack of oxygen as a result of cardiovascular problems has an affect on the tissue viability and impaired healing [181-183].

2.7.2 **Direct contributors to foot pathology**

2.7.2.1 Integumentary system

The skin structure is multi-layered with interlayered boundaries. It is attached to underlying tissue and all the surrounding skin, and crossed by its epidermal appendages and vascular and neural tissues. The skin has different functions, providing support to internal structures, protection from mechanical trauma, acting as a barrier between the outside environment and the physiologic conditions within the body, and helping to regulate the body's internal environment with the inclusion of physiological and immunological roles [184] The layers of the skin differ slightly in structure and function depending on the location on the body, being the thinnest over the eyelids and the thickest on the plantar of the foot. Its physical properties can be affected by the amount and

duration of stress that the skin is under. In addition to these normal stresses, pathologies such as SSc will confer an additional impact [185].

In SSc, the skin is one of the organs most affected by the disease. The whole cutaneous connective tissue system is affected by the excess of fibrous collagen deposition, resulting in the accumulation of type I collagen and/ or type III collagen in the early stages of disease. The collagen-rich extracellular matrix replaces epithelial structures and specialized smooth muscle, leaving the affected tissue contracted [91].

The areas of skin affected differ between the disease subsets, with the exemption of the SSc *sine scleroderma* subset in which the skin is not affected. In the lcSSc subset, the skin involvement is limited to the face and below the elbows and knees, while in the dcSSc subset the skin involvement is acral and truncal – with involvement above elbows and knees +/- truncal area automatically categorising as dcSSc [24]. The skin of the feet can be involved in both subsets affecting 26% of the patients [9]. Histologically, skin involvement in SSc is central to the diagnosis of the disease; more recently however with a better understanding of the disease pathogenesis and clinical presentations, and in particular, to ensure early diagnosis, the involvement of the skin is no longer regarded as mandatory for the diagnosis. Defining instead subtypes that enable earlier diagnosis as well as to recognise subsets such as SSc *sine scleroderma* where many of the pathological processes are evident without skin involvement; has evolved recently [186].

The clinical features of skin involvement may vary, reflecting different phases, starting with an oedematous phase, followed by a firm induration, and fibrosis[89].

The oedematous phase is present in the early stages of the disease when the papillary and reticular dermis presents with oedema. This phase has been reported to relate with the initial onset of the disease when the endothelium is damaged initially [3] (as described in section 2.6.1).

After the oedematous phases skin is left tight and thickened as a result of the accumulation of collagen and other extracellular matrix constituents[187]. The collagen extends from the dermis to the subcutis in a compacted, homogeneous, hyalinised pattern replacing the subcutaneous fat and surrounding atrophic sebaceous glands. In addition, the lower dermis and the subcutis accumulate inflammatory infiltrates such as lymphocytes, plasma cells and macrophages [149] around the collagen bundles and blood vessels. These infiltrates also perfuse the subcutaneous fat and entrap the sebaceous glands [25]. The blood vessels in the skin are also affected by these pathological process, presenting with intimal proliferation, lumen occlusion and fibrosis[89]. With disease progression the skin lesions becomes relatively avascular and after 12 to 18 months there is usually little or no inflammation present, but the late lesions are left sclerotic. In these lesions the collagen appears closely packed and the sweat glands are atrophic or absent and the collagen may replace the fat cells in the subcutaneous tissue [25].

These different pathological elements leave the tissue fibrosed, contracted and scarred [89], features that can be observed clinically on the involved skin (Figure 2.2).

There is also evidence that the pathological changes occur in the apparently “uninvolved” skin [187]. A progressive worsening of the skin occurs within the first two to four years, after this period, the progress usually starts to plateau

and may even show a slow improvement [187], although the damaged is irreversible in the majority of the cases.

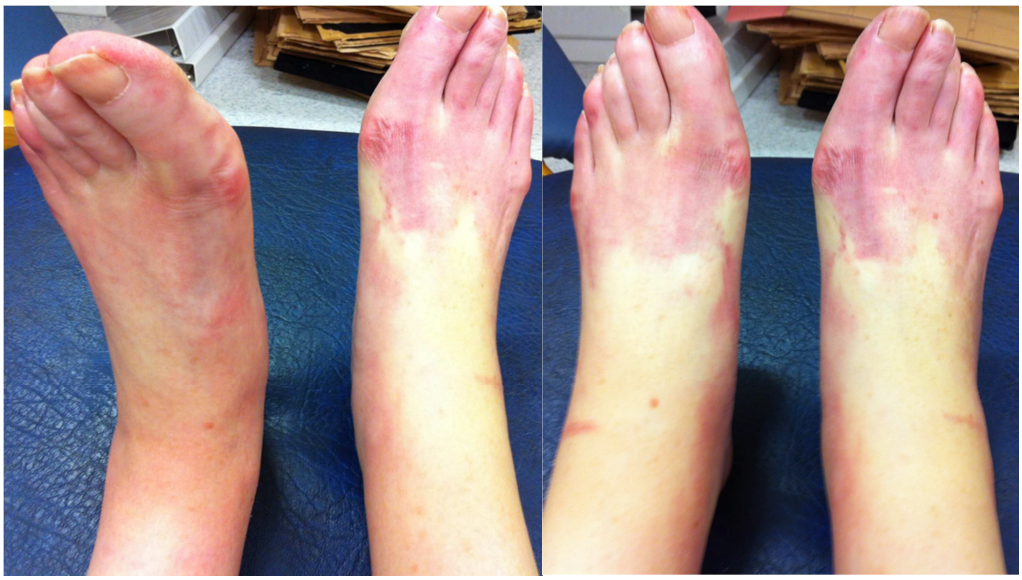

Figure 2.2 Fibrosis affecting the feet causing capillary compression during mechanical movement i.e plantarflexion

Once the pathological process is established, the integumentary system undergoes structural and biomechanical changes. The pathological process affecting the skin leads to the loss of the skin's mechanical and biological properties, which compromises the skin integrity, affecting tissue viability, healing and its ability to adapt to the pressures: for example fibrosis of the hypodermis in SSc has an impact on the mechanical properties of the skin by reducing extensibility [188]. The loss of skin extensibility together with the fibrosis of periarticular soft-tissue structures also has an effect on the joint function, which in the feet can result in impaired foot function [9, 189] [10]. The structural and biomechanical changes of the skin, together with joint pathologies can lead to development of flexor contractures. Flexor contractures leave the fingers and the toes in a fixed-flexed position which has a major impact on the patients' activities of daily living (ADLs) causing in turn a detriment to their quality of life [190]. These deformities not only impair the

movement of the affected joints, but also contribute to the risk of the development of ulcerations over the flexed joints and areas of skin tension. When ulcers develop over these regions then the ulcer aetiology becomes multifactorial. The skin is exposed to constant stress as a result of the deformities, and losses its ability to adapt to pressure due to the presence of fibrosis; this, combined with the vasculopathy causes these deformed and fibrosed areas to be at risk of ulcerations [117, 191].

Ulceration can also occur in areas without deformity. The aetiology of this type of ulceration are commonly associated with skin fibrosis, hyperkeratosis, vasculopathy and/or the presence of calcinosis [192]. Once ulcers are present they are extremely painful and have a very slow healing rate. The healing rate is negatively affected by the amount of skin pathology, and to the ulcers can become chronic, increasing the risk of amputation [192]. Lower limb amputation as a result of refractory ulcers has an incidence of 0.67% [193] in patients with SSc. Even in cases where healing is achieved, the ulcerated area experiences tissue loss and scar tissue formation. The latter together with the presence of vasculopathy, adds to the impairment of the biomechanical properties of the local skin leaving the area even more prone to re-ulceration.

Scarring of the skin not only occurs as a result of ulceration of the skin pathology, but also due to the wider pathology which causes the skin to become thicker and tighter as the disease progresses. These changes in the skin further impair the biomechanical properties affecting the musculoskeletal function of the body structures affected such as hands and feet [89, 91, 194]; skin atrophy in the feet has been reported to affect half of patients with SSc [9].

These pathological changes also affect the subcutaneous tissue causing fat-pad atrophy, and in the later stages of the disease fat cells in the subcutaneous tissue might be replaced by collagen [25, 91]. In the feet this substitution can lead to subcutaneous fat-pad atrophy on the plantar aspect of the foot, which may contribute to the increased plantar foot pressures reported in SSc [10]. High plantar pressures and prolonged duration of loading through discrete areas of the foot have been associated with pain during walking in other systemic musculoskeletal conditions such as rheumatoid arthritis [195, 196]. It is plausible therefore to hypothesise that high plantar foot pressures could subsequently cause pain and functional impairment. This hypothesis has been proved in other disease [195, 196] and in SSc this is supported by preliminary supporting evidence obtained from a pilot work undertaken while developing the program of work described in this study [10].

The impact caused by skin fibrosis is not only at a physical level but it also has a psychological impact. The skin fibrosis occurs in visible parts of the body and as the tissue becomes fibrosed, contracted and scarred [89] the patient's body appearance can change causing a psychological impact as the patient becomes concerned about their body image. This psychological impact has been described from low appearance self-esteem to depression, anxiety and social withdrawal [197] [198].

2.7.2.2 Vascular system

The vascular system experiences functional and morphological changes in the circulation as described in section 2.61.

One of the most common vascular problems present clinically in patients with SSc is Raynaud's Phenomenon (RP); between 90 to 98% of the patients with SSc suffers from this condition [199, 200]. This is a reversible, transient, vasospastic condition triggered by cold or stress. The RP can be divided into primary RP (idiopathic), or secondary RP (secondary to other disease/condition) [93]. Primary RP is associated with normal capillary architecture in contrast to secondary RP, where clear abnormalities are recognised; the most well described changes have been observed in patients with SSc. Specific SSc nail fold capillaroscopy (NFC) patterns, early, active or late vasculopathy have been reported [201-203]. The SSc NFC pattern correlates with SSc disease duration severity and is predictive of future organ damage [204, 205] [206, 207]

In SSc RP is usually the first presenting clinical feature supporting the concept that the vasculopathy is one of the earliest pathological processes. In 70% of patients with SSc the RP can be one of the first clinical symptoms observed [200], and it can manifest up to 20 years prior to the presence of other disease symptom [208].

Approximately 10% of the general population suffers from RP and never develop any disease (primary RP) [121, 199], although the risk of developing SSc increases (hazard ratio 9.34) in those patients that have RP and ACA antibodies [209].

The clinical presentation of the RP can be divided into three phases. The initial phase is the ischaemic phase, where the digits present with a pallor discolouration (white) reflecting vasospasm; this phase is followed by the deoxygenation phase, where the digits are cyanotic (blue discoloration); and in

the final phases, the reperfusion phase, the digits present rubor (red) expressing the compensatory vasodilation[93, 121, 199] (Figure 2.3).

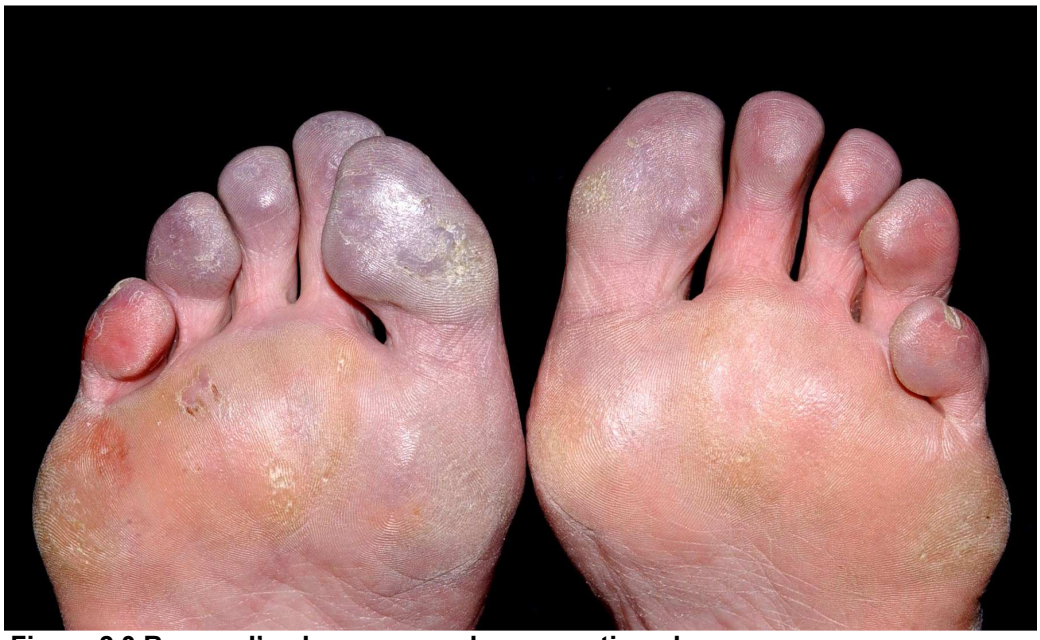

Figure 2.3 Raynaud's phenomenon deoxygenation phase.

RP is thought to also affect organs and other body parts [93, 199]. Reversible vasospasm may possibly occur in the terminal arterial supply of the gastrointestinal tract, kidneys, lungs and heart and is a precursor of the structural lesions of the microcirculation [125, 210]. Clinically RP is more visible on the external parts of the body such as the ears, tongue, nipples, nose and digits [211, 212], the latter being the most commonly and severely affected. In the feet RP has been reported to affect 43%(n=100) of the patients and necrotising RP, characterised by digital ulcers, to affect 8%.

Clinically, the severity of the SSc vasculopathy can be appreciated in fingers and toes by the presence of digital ulcers (DU) and necrosis which can lead to tissue loss, fingers and/or toes amputation and significant functional impairment [213]; heavily impacting on the patient's quality of life [214].

The proportion of patients with SSc reported as affected by DU varies depending on the study, with a reported ranging from 26.2% [180] to 50% [215]. The fingers and to a lesser extent the toes are most commonly affected by ulcerations but ulcers can occur elsewhere. Alivernini et all [180], in a study of 130 SSc participants reported that 3.8 % of the participants developed other ulcers not involving the digits. The prevalence of foot ulcers in a population of 50 patients with SSc was described to be 26% [8].

The multifactorial aetiology of ulceration reflects the complex interplay of the pathologies with significant impact on foot pathology. The structural and functional pathology affecting the microvasculature can lead to ulcers on the apices of the digits (Figure 2.4), over the extensor surfaces of the joints and on the lower leg [112, 192, 216]. Other factors contributing to the risk of developing ulceration in people with SSc include calcinosis, impaired skin biomechanics due to fibroses or hyperkeratosis [192], and neuropathy. The latter, though lacking a formal body of evidence, has been reported in a case study of neurotropic ulcerations present in a patient with peripheral sensory neuropathy and SSc [217].

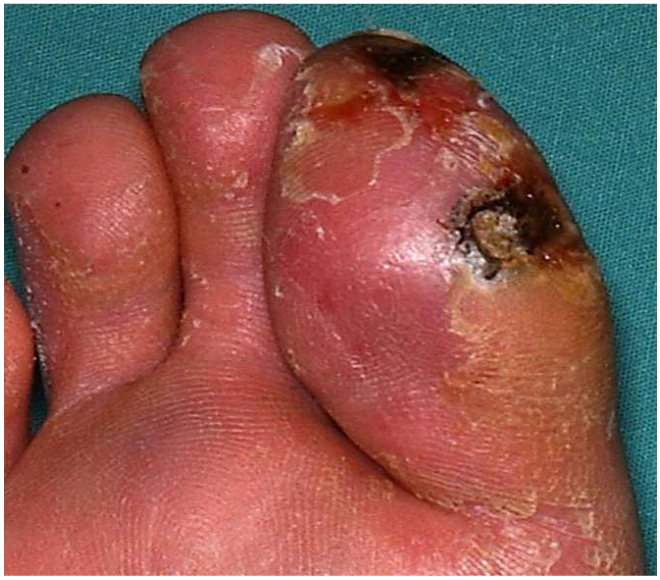

Figure 2.4 Ischaemic ulcer on the apex of the hallux.

The presence of ulcers creates a significant risk to the person with SSc- the healing process is jeopardised by the vascular and skin pathology, thus the ulcer can become chronic and/or infected. Infection further contributes to poor healing and increases the risk of deeper infection, including osteomyelitis and/or necrosis, which in severe cases can lead to septicaemia and/or ultimately amputation [112, 113, 180, 218]. Amputation can also be caused by the presence of gangrene as a result of vasculopathy[191]. Autoamputation of fingers and toes as a result of necrosis is often seen in 14-29% [219], while lower limb amputation following foot ulceration has been reported in 6% of cases in a study of 50 patients with SSc [8].

In addition, the presence of macrovascular disease has a compounding adverse effect on the blood supply to the lower limb as it leads to the presence of PAD [14, 15]. In the lower limb the PAD can be symptomatic in the form of painful intermittent claudication with a prevalence of 21% [13]. The presence of intermittent claudication has also been found to coexist with ischaemic heart disease and cerebrovascular disease with a prevalence of 15.2% and 6.5% respectively[13]. The presence of PAD can further compromising tissue viability and ulcer healing [9, 15, 113].

In contrast to the mainly neuropathic ulceration associated with conditions such as diabetes mellitus, ulcers in people with SSc are often extremely painful and can take many months to heal. The mean time to heal ranges from 25 to 281 days, depending on the type of DU, but it has been reported to be as long as 810 days in some cases[192]. The presence of pain and functional impairment

as a result of ulceration or amputation has a highly negative impact on the patient's quality of life [218, 220].

The development and the healing of the ulcers in patients with SSc can be aggravated by the presence of vasculitis with the already existent occlusive vasculopathy. Vasculitis in SSc has been reported to lead to severe ischaemia, ulcerations, necrosis and amputations; the latter more commonly seen when the vessels of the digits are involved [133].

In addition, in some cases vasculitis has also been identified as a possible factor that plays a role in the pathogenesis of the neurological lesions [221, 222]. It has been postulated that vasculitis affects the arteriae nervorum leading to necrosis of the nerve bundles. The damaged nerves present with diffuse degeneration below the lesion area [223] and depending on the nerve affected, this can cause peripheral neuropathy [151]. Clinical and pathological findings show asymmetric neuropathy or multiple mononeuropathies[151]. These neuropathic presentations are characteristic of nerve involvement in necrotizing vasculitis presentations [224].

2.7.2.3 Neurological system

In the past the involvement of the nervous system in SSc has been considered a rare event [225]. However, over the years increasing evidence of its occasional involvement in the disease has emerged. Central nervous system (CNS) involvement is rare, with the peripheral nervous system (PNS) mainly affected. The focus in this literature review will be on PNS pathology and also the psychiatric and psychological changes in SSc.

The pathophysiological mechanisms involved in the cause of neuropathy are not fully defined. However the three central components, vasculopathy, inflammation and fibrosis underlining SSc pathogenesis also appear to underlie the bases for the pathogenesis of the nerve fibre degeneration. The vasculopathic involvement comprises different pathophysiological mechanisms: mucoid degeneration of the peripheral nerves, obliterative microangiopathy and, occasionally, a co-existent vasculitis. Mucoid degeneration of the peripheral nerves starts with the pathological process in which the vessel's endothelium is injured. Injury to the endothelium of the vasa nervorum causes thickening of the basal laminal and the adventitial oedema causing microangiopathy. This microangiopathy leads to an increase in the vessel's permeability of plasma with subsequent deposition of mucoid material in the nerves causing neuropathy [173, 226, 227]. In addition, obliterative microangiopathy causes chronic lower grade ischaemia, which has been suggested to be a plausible cause of subclinical nerve dysfunction; as its presence will be expected to result in axonal loss [21]. The presence of vasculitis involving the vasa nervorum has also been identified to cause mononeuritis multiplex in patients with SSc, although vasculitis as a cause of neuropathy in SSc has been reported to be rare [131, 221].

The role of inflammation in the development of neuropathy in SSc is not clear, with conflicting reports and opinions. Some studies report that there is no inflammatory element involved in the process of neuropathy in SSc [21, 114, 173, 228] while others report evidence of inflammation involvement [151] [150, 169] . Evidence seems to indicate that the contribution of inflammation to the neuropathy might be partially through its involvement in the disease

vasculopathy, as the biopsies specimens in central and peripheral nerve lesions show presence of a chronic perivascular lymphocytic inflammation, inflammatory cells within the vessels wall and microglial activation [150] [151]. Both mononeuritis multiplex and sensorimotor neuropathy have been identified in SSc as neuropathies that might be caused by involvement of an inflammatory element. Inflammation also plays a role in trigeminal neuropathy and compression neuropathies, as both of these appear to be related to the SSc oedematous phase [150, 151]. However, the oedematous phase is not the only cause of compression neuropathies in SSc, with deposition of collagen in the carpal tunnel, calcium deposits and hypertrophied synovial sheaths and/or active arthritis have also been described as underlying contributors [21, 229]. Interestingly, in SSc compression neuropathies that present with carpal and cubital tunnel syndrome-like symptoms, it has been hypothesised that the cause could be a result of vascular or autoimmune-dependent neuropathy rather than compression as such; as imaging technology has failed to identify the diffuse or focal nerve enlargements commonly seen in carpal and cubital tunnel syndrome [229].

Finally, fibrosis affects the connective tissue that surrounds the nerve and nerve fibres, structures known as the epineurium and perineurium. Once the epineurium and perineurium are fibrosed it causes strangulation of the nerves due to incarceration in collagen, causing neuropathy [173, 226, 228].

The histopathological characteristics of damaged nerves have been evaluated in biopsy specimens of nerve lesions and of skin from the fingers, lower thigh and leg. The biopsies from the nerve lesions demonstrated a significant reduction in the density of the myelinated fibres [151, 173]; while the biopsies

from the skin showed a significant loss of myelinated and unmyelinated sensory autonomic nerve fibres (A-delta fibres and C-Fibres), associated with a reduction in the vascular bed [230]. In the latter study the authors suggested the role of ischaemia as the cause of the reduction of the number of nerve fibres, although they also suggested that immune-mediated or metabolic causes might also be relevant [230]. It should be noted that all these mechanism proposed in the development of neuropathy have been derived from case studies and/or from studies with small numbers of participants (n=2-12), and confounding factors in the development of neuropathy such as vitamin deficit, concomitant drugs and diseases [231], were not adjusted for. Therefore, further evidence supporting these proposed pathological mechanisms is needed before any firm conclusions can be drawn.

In systemic sclerosis neurological complications have been reported to occur between 1% - 40% [150].

2.7.2.3.1 Peripheral Nervous System

Within the PNS, both the autonomic nervous system (ANS) and the somatic nervous system (SoNS) are affected by the pathogenesis of the disease [150, 231, 232]. The SoNS is commonly referred to in the literature as peripheral nervous system, therefore for the purpose of this literature review and to avoid confusion, the autonomic nervous system will be referred as ANS and the somatic nervous system as peripheral nervous system (PNS).

i. Autonomic Nervous System

In SSc ANS dysfunction is characterised by a parasympathetic under-activity and sympathetic overdrive [150, 232]. The most widely reported clinical symptoms involving the ANS are increased and diminished heart rate, and blood pressure variability. The ANS dysfunction also contributes to the wide-ranging clinical sequelae including abnormal sympathetic skin responses, Raynaud's phenomenon, gastrointestinal motor dysfunction, oesophageal dysmotility, impotence, cardiac abnormalities and hypohidrosis [233] [150, 230, 234, 235].

ii. Peripheral nervous system

Peripheral nervous system abnormalities have been reported to be present in up to 50% (but only n=14 in the study) of patients with SSc[233]. However, the prevalence of PNS abnormalities varies depending on the study - 34% (n= 32) in one study [169]; and as low as 5.6% (n=125) in another [236]. In SSc, peripheral neuropathy has been documented as developing between 0-25 (mean 12.4) years from disease onset [151]. The clinical presentation of the peripheral neuropathy has been reported to be generally distal with a non-length dependent pattern to the sensory symptoms, more sensory than motor, and mostly multiple mononeuropathy or asymmetric in involvement (suggesting multifocal pathology) [151, 233]. It has also been suggested to be independent of skin fibrosis, as abnormal sensory thresholds have been observed in unaffected skin [233], and not to correlate with disease duration [171, 233]. Peripheral neuropathy has however also been reported to be present with early neurological signs of sensory-motor mononeuropathy that later extends to the

rest of the body with a typical distal polyneuropathy and a greater involvement of the lower limbs [173]. The variation in the clinical presentation of the neuropathies may be as a result of the diversity of the design and methodology of the studies describing these features.

A systematic review investigating at neurological involvement in SSc [150] reviewed 61 studies/case reports (n= 1628). The most commonly reported PNS involvement was trigeminal neuropathies (16.52 %), followed by peripheral sensorimotor polyneuropathies (14.25%) and entrapment neuropathies (9.25%). The authors also described asymptomatic peripheral neuropathy in some cases (17.14%). Subclinical peripheral neuropathy has been described in the presence of mononeuropathy and polyneuropathy with a pattern of a distal peripheral neuropathy [169-171]. The sensory deficit has been shown to be subclinical in the majority of the patients and to be worse in the feet than in the hands [237]. When sensory symptoms are present they can vary from lancinating pains, burning, allodynia, paraesthesia, pruritis, through to loss of sensation, and the symptoms have been reported to be more prominent in the extremities [233].

Amongst neuromuscular syndromes, myopathies are also frequently present, affecting 51% (n=229) of patients in one study. In studies that included electroneuromyography, myopathy was the prevailing finding (56.55%) followed by sensory abnormalities (14.83%) and motor abnormalities (13.45) [150]. The myopathies tend to affect mostly proximal muscles[150], although myopathy has also been reported in distal muscles such as the anterior tibial muscle [169]. Muscle biopsies taken from the anterior tibial muscle revealed neurogenic structural alteration such as group atrophy and fibre type grouping, leading to

hypertrophy of small and large fibre types in the anterior tibial muscle [169]. In addition electromyography revealed signs of chronic denervation in distal leg muscles, both findings indicating lower limb involvement in the myopathy. Any neurogenic and structural abnormalities in the muscle fibres can have a negative effect on the biomechanical function of the body part involved; when the muscles of the lower limb are involved it can have an impact on the patients' ambulation and consequently on their daily activities.

Peripheral neuropathy in SSc is mostly reported as affecting in cranial, truncal and upper extremity nerves. Foot involvement in the peripheral neuropathy has been reported in different studies [173, 233] but has been investigated only in one study [237]. The paucity of studies assessing peripheral neuropathic involvement of the feet may be as a result of an incomplete investigation of the patients or because as mentioned earlier, the peripheral neuropathy in the feet is mostly asymptomatic and is therefore unnoticed [21].

Schady et al [21] (n=29) reported the presence of subclinical peripheral nerve dysfunction in the feet and hands, being worse in the feet. The foot involvement was reported to be predominantly of a sensory defect, affecting more touch [171, 238] than small fibre modalities such as pain and temperature [237]. Tactile sensitivity in the feet was impaired in 50% of the patients but with mainly a subclinical presentation [237]. This study does not only highlights the elevated number of patients with SSc that have peripheral neuropathy, but more importantly it revealed the fact that the peripheral neuropathy does not necessarily present with clinical symptoms. This is potentially problematic if patients are unaware of a loss of sensitivity to harmful stimuli, which may put them at risk of injury.

2.7.2.3.2 Psychiatric syndromes and psychological symptoms

Different psychiatric syndromes and psychological symptoms have been reported in SSc. A systematic review carried out on the neurological involvement in SSc, which included 90 studies/case reports (n=6028), identified that psychiatric manifestations were present in 2712 patients. Amongst the psychiatric pathologies dysthymia, suicidal ideation, psychoticism and paranoid ideation were reported, but their presence was considered to be very rare, with the exception of the dysthymia[239]. Amongst the psychological symptoms depression and anxiety were the most predominant, with a prevalence of 30-40% for depression and 25-64% for anxiety [150]. Analysis of two SSc databases, encompassing 2780 subjects, implied a relative risk of 1.5 for both depression and anxiety [150]. The presence of these clinical features has been identified as having a negative impact on the patient's life, as impaired psychological function has been associated with reduced quality of life [240].

2.7.2.4 Musculoskeletal system

Musculoskeletal involvement in SSc is very common and some 90% of patients with SSc have musculoskeletal complaints. Complaints range from intermittent small and large joint arthralgia to chronic polyarthritis [241, 242], and muscle involvement. Joint symptoms have been reported to be present in 66% of patients with SSc [243], and muscular symptoms in half of the patients; the latter with a predominance of muscle weakness and myalgia [169].

2.7.2.4.1 Intra-articular and periarticular structural pathologies

The musculoskeletal system can be involved in the disease at an early stage. Up to 20% of patients with SSc report joint symptoms as early as one year or more prior to diagnosis and a third within the same year of diagnosis [243]. Articular manifestations in early SSc can range from minimal arthralgia to a polyarthritis of a chronic or intermittent nature. These articular manifestations may present as a stiff, painful, symmetrical arthropathy, which is clinically indistinguishable from that of Rheumatoid Arthritis (RA) [241, 243]. The arthropathy and the joint symptoms are not exclusive to early disease, they also can present during the course of the disease; in halve of the patients with SSc joint symptoms presented after diagnosis [243] [244].

The mechanisms involved in the articular changes have not been fully identified but different factors have been proposed. For example, the articular space narrowing has been reported to occur as a result of chondral atrophy secondary to chronic deformity and disuse rather than being related to joint inflammation as seen in RA [241]. Equally, there are other contradictory data supporting the role of inflammation in the SSc arthropathy. The synovial membrane has been shown to have superficial fibrin deposition, focal microvascular obliteration and chronic inflammatory cell infiltration [152, 245]. Inflammatory infiltrates have also been found in the subsynovium and perivascular tissues [154], and the amount of inflammatory infiltrates has been reported to correlate with the presence of rheumatoid factor (RF) [246]. The association of RF and arthritis in patients with SSc is not fully supported by all the studies. Schmeiser et all [247], Blocka et all [241] and La Montagna [9] found no correlation between RF

antibodies and arthritis in their studies, therefore no conclusions can be draw supporting the role of RF and arthropathy.

Clinically, joint inflammation has been reported in 91% of the patients [248]. However, this high figure from this one small study is likely to be due to selection bias, as data were obtained from patients with a current or past history of articular symptoms, and seven patients had overlap disease with RA. In a study with a larger sample size (n=7286) much lower values for the prevalence of synovitis have been reported with synovitis affecting only 1 in 8 patients with SSc [244].

An interesting finding by Auvouac et al [244] revealed the presence of synovitis is associated with increased levels of acute-phase reactants, markers of severe vascular disease and muscle weakness. The presence of raised acute-phase reactants indicates systemic inflammation and has a strong association with synovitis. This finding is supported by Schumacher et al [152] who found, in synovial biopsies of patients with SSc, the presence of inflammatory cell infiltration, associated with focal microvascular obliteration and fibrin deposition. Even though the arthropathy present in SSc has similarities with the arthropathy seen in RA on a clinical and laboratory level [249], synovial histopathological analysis has shown less proliferation of lining cells than that seen in RA arthropathy. The usual proliferation of synovial hyperplasia and pannus formation typical of RA does not occur in the SSc arthropathy. Although, in SSc this can be confounded by the presence of severe destructive joint disease [152]. Instead what can be observed in synovial lining cells is the presence of fibrin accumulation, with atrophy of the synovial cells leading to a fibrosis, similar to that present in the dermis, and obliterative microvascular angiopathy,

similar to that reported in other tissues in SSc [152, 154, 241, 250]. Such synovial joint involvement can have an effect on the patient's quality of life, as synovial fibrosis has been reported to associate with joint pain and tenderness, and/or pain during joint motion [154].

Avascular necrosis related to vasculitis and neuropathic arthropathy both also have been reported as causes for articular changes in SSc [251, 252]. These causes have been referred to as plausible mechanisms for arthropathy[241, 253], although as previously discuss vasculitis is not a common feature of SSc and there is not strong evidence in the literature supporting these theories, as these mechanisms only have been reported in case studies.

Articular and periarticular pathology in SSc affects the hands more than the feet [9, 241, 254], but the prevalence in the feet cannot be considered low. In a study of 76 patients with SSc the most common articular arthropathy in the feet was joint-space narrowing with a prevalence of 35% of the distal IP joints and 13% of the proximal IP joints, followed by subchondral sclerosis 21%, subluxation/luxation of phalanges 13%, juxtaarticular demineralisation 7%, and erosion 3% [189]. The prevalence of erosions was reported to be higher in two different studies with smaller sample size 12% (n=46, n=33) [241, 254]). Erosions were reported to affect the MTP joints and talo-navicular joints, while joint-space narrowing affected the distal and proximal IP, MTP and ankle joints [9, 254]. The prevalence of the juxtaarticular demineralisation also has been reported higher, 42%, but in this study the location in the feet was not specified [243].

The presence of erosions in the MTP joints[254] [189] could be expected to be associated with the increase of plantar foot pressures identified in patients with SSc [10], as erosions have been previously reported to be associated to abnormal plantar pressures in patients with RA [196, 255].

In 13% of the patients with SSc arthropathy is considered to be a cause of severe destruction of multiple joints of the hands and feet [256]. In the feet the arthropathy presents with a significant degenerative pattern in 18% of the patients, a fibrotic pattern in 8% and inflammatory pattern in 7%. This significantly higher prevalence of the degenerative pattern in the arthropathy of the feet might be as a result of the weight-bearing nature of the feet, which could become a risk factor for the development of arthropathy[189].
Perhaps this degenerative nature of the disease reflects the nature of other arthropathies that also have been reported in patients with SSc, such as erosive osteoarthritis (OA), which affects 18% of SSc patients and primary OA affecting 13%; both of which impair flexion of the joint affected[153, 243]. In this study the joints reported to be affected by the two types of OA were the distal interphalangeal joints (IP) [243] [153]. Erosive arthritides in the feet have been reported in between 7% [256] and 12% [254], [241]) of the patients with SSc, and are associated with the presence of arthritis in the hands.Thus patients with SSc that present clinicaly with arthitis in their hands it is likely that their feet will also be affected. Erosions associated with SSc have been described as small and discrete by Allali et al [254], and bilateraly asymmetrical and sometimes located dorsally by Bassett et al [256]. Allali et al [254] reported that the erosive changes in the joints show a radiological picture compatible with erosive OA.

Another clinical feature occurring as a result of articular and periarticular involvement in SSc is flexion contractures. Flexion contractures can have a multifactorial aetiology. As previously described skin fibrosis can contribute to the development of the flexion contractures adding to the loss of dexterity of the digits. Flexion contractures are considered one of the most predominant clinical features in SSc [256], with a prevalence as high as 90% [241] and can affect fingers, toes, wrists and less frequently shoulders, elbows, and knees [252] [241] [243] [257]. As noted previously, when data from studies with larger sample sizes were analysed, the presence of flexion contractures did not appeared to be as prevalent as other studies previously reported. Avouac et al[244] obtained data from the EUSTAR registry, involving 7286 participants, and found a prevalence of 31% for flexion contractures, which was considerably less than what was previously reported in studies with smaller sample size i.e. 90% (n=65) prevalence [241]. In Avouac et al 's study the location of the flexion contractures was not reported, thus data regarding its prevalence in the toes were missing. Only two studies have reported the prevalence of flexion contractures of the toes, showing a prevalence of 5% [189] and 15% [9]. Another study focusing on peripheral joints [241], assessed the feet but did not report data on flexion contractures because they reported found difficult to interpret these changes in the feet, possibly because of the resemblance to other foot pathologies such as claw toes and hammer toes.

Flexion contractures occur as a result of joint destruction turning into ankylosis and fibrotic changes in the skin and periarticular structures (figure 2.5); they can lead to measure functional disability [244],[189, 258]. The structural changes

caused by the flexion contractures in the feet and its impact in foot function can be observed on the impaired foot mechanics during gait.

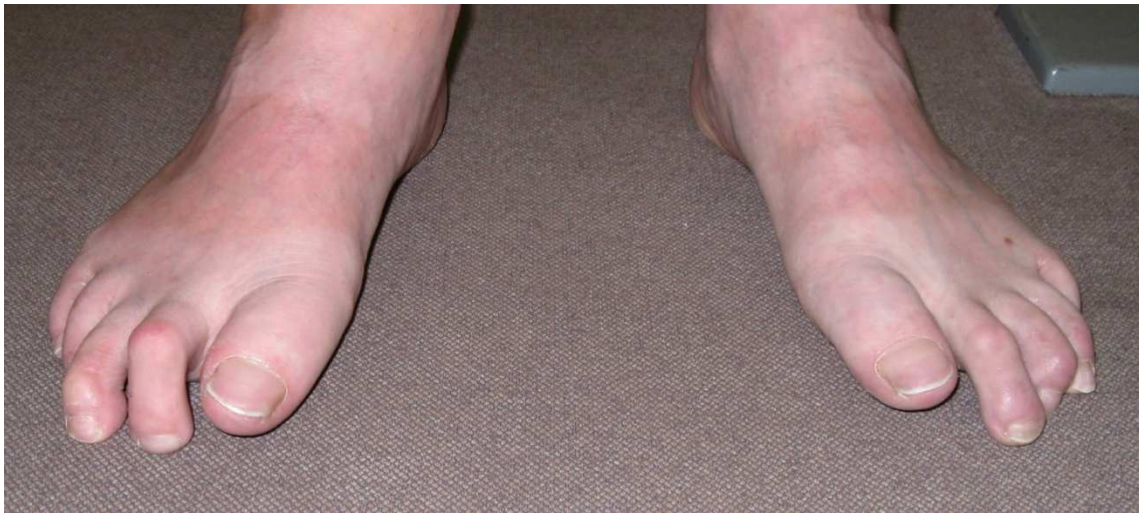

Figure 2.5 Flexion contractures of the toes and bilateral acro-osteolysis of the 1st toes.

In patients with flexion contractures the disability can be made worse by the presence of a more severe peripheral vascular and muscular disease, with elevated acute-phase reactant, and the presence of higher fibrotic propensity including pulmonary fibrosis, as all of these features are associated with flexion contractures [244] [189].

In SSc, periarticular structures in particular may be affected, causing tendon friction rubs. This feature is present in 11% of the patients with SSc and in some patients tendon friction rubs present together with flexion contractures and synovitis [244]. The presence of these three features was associated, independently of each other, with systemic inflammation and with severe vascular, muscular, renal, and interstitial lung involvement [244], indicating that those patients with musculoskeletal involvement might be a discrete subset

presenting with more aggressive disease, and consequently the disease having a greater impact on their quality of life.

The relationships between articular involvement and the different SSc disease subsets remains an area of controversy however, and some authors report that there is not a relationship with articular involvement and clinical or serological SSc subsets[9], while others report articular manifestations to be more prevalent in the dcSSc subset and to be associated with disease activity and elevated acute–phase reactants [244].

Any arthropathy/arthralgia is worsened by the involvement of the periarticular soft–tissue. Tendons are key periarticular structures affected in SSc: in flexion contractures, the tendon develops an extensive fibrosis; in tendon friction rubs, the tendon presents with fibrinous deposits on the surface of the tendon sheath; and in tenosynovitis the tendon sheath becomes inflamed [259] [9, 253]. All three clinical features have been reported to affect the feet, however the prevalence has only been reported on tendon contractures 6% and tendon friction rubs 10% [9]. In the feet the tendons affected by tendon contractures and tenosynovitis are the flexor toe tendons, while the extensor toe tendons and the anterior and posterior tibial muscle tendons have been reported to be affected by tendonitis [259] [9, 253]. Tendon friction rubs affect the anterior and posterior tibial muscle tendons, toe flexors and extensor tendons, the Achilles' tendon, and the tendons on the dorsum of the foot; from the latter the specific tendons involved were not specified [9, 248, 253]. These three clinical features contribute to the functional impairment of the joints and to the arthralgia; particularly to the latter as in the feet arthralgia is present in 36% of the patients

and is significantly more frequent than in the hands [9], possibly as a result of the weight-bearing nature of the feet, as they are exposed to greater forces and repetitive strains.

Clinically, the joints affected present with tenderness, pain on palpation and/or effusion, without synovial thickening [241]. Stiffness and arthralgia have been reported as two of the most common clinical symptom in patients with SSc; and more than two-thirds of patients have morning stiffness of 30 minutes or greater, and 32% consider their joint pain of a moderate significance, while 20% of a great significance [243, 253].

When inflammation is present the pattern of the joint inflammation is commonly symmetrical and polyarticular, with 61% of the patients presenting within these characteristics, 22% of the patients present with oligoarticular involvement and 17% with a monoarticular involvement [243]. The distribution of the clinically inflamed joints suggests a higher prevalence in the metacarpophalangeal 52%, followed by the wrist and knee 43%, distal IP 39%, proximal interphalangeal (IP) joints 34%, shoulder 30%, MTP 26%, ankle 8% and elbow joints 4%[243]. Conversely, in a study carried out by Misra et al [248], the prevalence of ankle joint inflammation was 61%, which suggests that the ankle joint may be the most affected joint by inflammation, reiterating the foot involvement in the disease and highlighting the need for exploring further foot pathologies in SSc. The presence of inflammation in the MTP and ankle joint has an impact on the joint's function and consequently on patient ambulation, who probably will adopt an antalgic gait to alleviate the pain pressures during walking [10], as also seen in disease such as RA [260].

In SSc, restricted range of movement (ROM) has been reported in several joints, 43% patients have restricted ROM of the wrist, 13% of the elbow, 26% of the shoulder, 7% of the ankle, 4% of the knee and hip [243]; the impaired ROM of the ankle, knee and hip can also have an effect in the patient's gait.

Both restricted ROM and inflammation can have a direct or indirect impact to the refined function of the joint and in some cases the impairment can cause significant disability. In patients with SSc, disuse of the joints has lead to loss of muscle-mass [257], particularly the disuse of the knees, ankle, elbows, wrists, metacarpophalangeal(MCP) and proximal IP [257] contributing further to function impairment [261]..

Joint involvement in SSc has not only been identified clinically but patients with SSc also perceive clinical symptoms, such as stiff joints together with pain and fatigue, as the symptoms most frequently associated to their disease [262]. In addition, joint involvement has been reported to contribute to the patients' physical disability.

2.7.2.4.2 Muscle pathology

Muscle pathology (a myositis or a myopathy) may occur in between 39% [257] to 96% [263] of patients. It tends to affect mostly the proximal musculature and the muscles of the forearms and hands, and presents as myopathy or, less frequently, myositis [263] [253]. However, the frequency of myopathy varies depending on its definition [253]. The appearance of muscle disease in SSc may be insidious and is frequently characterised by muscle weakness and myalgia [169].

In general the most common symptoms reported, by over 60% of the patients with muscle involvement, are fatigability and weakness [169, 257]. These two symptoms are not specific to the muscle involved however and the symptoms related to muscle are generalised muscle aching, reported in 11% of the patients, muscle tenderness in 4% of the patients and cramps in 2% [257]. Although in some cases severe muscle atrophy and weakness are present without any symptoms [257], which could be an indication of neuropathy or disuse.

The most frequent histological abnormalities reported to affect the muscles are interstitial and perivascular fibrosis affecting 25% of the cases, followed by interstitial and perivascular inflammatory infiltrates affecting 15%, and myofibril atrophy, necrosis and degeneration, all three together, affecting one fifth of all SSc patients [257]. The abnormalities that appear to be specific to muscle fibres pathology in SSc are atrophy, necrosis, and eosinophilic floccular degeneration, while the interstitial tissue shows irregularly distributed islands of peri- and epimysial fibrosis and increased interstitial fat [257] [263]. In the interstitium signs of inflammatory cellular infiltrates, such as lymphocytes and less frequently neutrophilic leucocytes have been found [257] which indicates a pathway of inflammation in SSc-related muscle disease.

In the case of active inflammation in the muscle, the inflammatory myositis is accompanied by muscle weakness, very high muscle enzyme concentrations and abnormal electromyography (EMG) results, and is characterised by its rapid progression and its disabling impact. The proportion of SSc patients affected by inflammatory myositis is low (17%) when compared to the 80% who are affected by a milder slow progressive myopathy. This slow progressive

myopathy presents with muscle weakness, interstitial fibrosis and variation in diameter of muscle fibres, and mild elevation of muscle enzymes, but does not have active inflammation, or abnormal EMG results [263].

In SSc, muscle weakness and loss of muscle mass correlates with the presence of elevated aldolase levels and creatinuria, which in turn indicate the severity of muscle cell degeneration [257]. Muscle fibre degeneration and inflammatory cellular infiltrates are present in other causes of myopathy and are not exclusive to the SSc pathology, but the presence of interstitial fibrosis in muscle fibres is found more commonly in SSc than in other causes of myopathy (Medsger et al., 1968), reflecting the nature of the pathogenesis of the disease.

Other pathological processes can contribute to the morphology alterations of the muscle fibre such as denervation or neurogenic atrophy from myopathy [169, 257]. It should be considered sometimes the cause for myopathy might not be local-driven disease pathogenesis affecting muscle tissue and that there may be secondary factors at play including disuse such as that seen as a result of arthropathy or other secondary SSc features that lead to general debilitation; such as poor nutrition (due to malabsorption), renal failure or congestive heart failure due to myocardial and pulmonary fibrosis[259] [257, 264].

The presence of co-morbidities leading to general debilitation is common in patients that have myopathy, because skeletal myopathy frequently affects patients with dcSSc, who already have organ involvement secondary to SSc. It has been shown that those patients with pulmonary fibrosis already have a higher risk of developing skeletal myopathy and that skeletal myopathy is associated with cardiac disease [265] [266]. Thus the combination of the

myopathy and organ involvement might be expected to have a greater impact on patients' function.

2.7.2.4.3 Other musculoskeletal abnormalities

In addition to the muscular, intra-articular and periarticular pathologies, other abnormalities affecting the musculoskeletal system have been identified in the feet of SSc patients. Diffuse osteopenia has been reported to affect 44% of the patients [189], valgus deformity 26% [189], calcaneal spurs 22% [189], subcutaneous calcinosis 30% [241], reabsorption of distal phalanges 12% [189], tarsal degenerative changes 4% [189],ankylosis 3% [241], pencil-in-cup deformity 1% [189] and periostitis. The latter prevalence was reported together with the hands as 12% [241].

The extent of calcinosis and reabsorption of distal phalanges in the feet has been reported to be lower in different studies, ranging from 6% to 18% [8, 9, 241, 254]. The presence of soft-tissue atrophy over the distal phalanges seems to be associated with bone reabsorption of the turfs of the distal phalanges, although the underlying bone loss is always greater than the soft-tissue change [256].

When including other areas of the body such as fingers, forearms, elbows and knees, the prevalence of soft-tissue calcifications has been reported to be present in 50% of the patients with SSc [267, 268]. Soft-tissue calcifications can be disabling for the patient, as they develop usually near bony prominences and in periarticular tissues, particularly at sites of chronic irritation or microtrauma. Occasionally, calcium gets deposited within the joint causing severe joint destruction [256]. Although sometimes the presence of soft-tissue calcification

might not necessarily be troublesome to some patients, in others it can cause local inflammation, pain and /or functional impairment, particularly when the areas affected involve periarticular areas and/or joints, as it interferes with joint range of movement and consequently daily life activities. Superficial calcinosis can ulcerate the skin and lead to chronic ulcerations and/or secondary infection [259, 269, 270] (Figure 2.6).

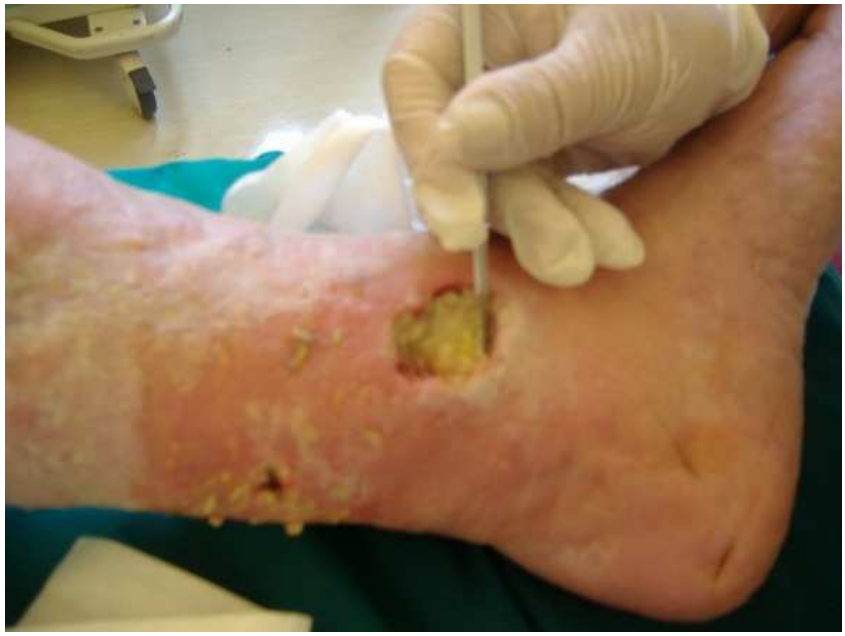

Figure 2.6 Chronic ulceration above the medial malleolus caused by calcium deposits affecting patient's walking.

The effect of functional impairment of the lower limb as a result of the musculoskeletal involvement can be observed in the patient's gait, as walking speed and stride length is reduced in patients with SSc. Both of these parameters are indicative of functional disability and patients with SSc have been found to correlate with the S-HAQ [10]. Overall the prognosis of the disease depends on the degree of visceral involvement but the musculoskeletal involvement is considered a major cause of morbidity and disability [271] and foot pathology contribute to the disability [10].

As a consequence of these interrelated pathological processes, the presence of foot impairment and reduced foot-health status has been reported in patients with SSc, with a systematic relationship between the impact of the disease and the level of foot-related impairment, measured by both patient- reported measures and objective measures of function [10, 220].

2.8 Impact of foot pathology in systemic sclerosis

In SSc the multi-system nature of the disease has wide-ranging impact on the function and structure of the systems affected, causing impairment to the patient on physical, psychological and psychosocial levels, leading to disability [4] [5-7].

However, disability does not always reflect disease severity, as clinical severity or disease factors do not have a linear relationship with the psychological impact [272-274]. This indicates that the patient's perceived impact of a disease is a complex interaction between the physical factors, psychosocial factors, the patient's personality and the patient's perception of the disease [262, 275].

This complexity is illustrated by the relationship between the factors that have been correlated with disability. Some clinical features have been reported to correlate with physical disability or with psychological disability, while others have an impact on both levels. However, these associations differ between studies [5, 22, 261, 274-276]. Physical disability has been found to correlate with patient-reported joint pain on motion, clinician–determined joint tenderness and number of tender points, joint contractures, digital ulcers, extremity ulcers

(other than digital), dyspnoea, high skin score, and gastrointestinal involvement [274] [5, 261, 276]. Some of these clinical symptoms have also been identified as being associated with impaired psychological function. For example digital ulcers have been reported to cause increased aesthetic burden [214]; lung difficulties has been associated with anxiety, and the overall disease severity of the SSc with depression[197]. Both depression and anxiety also have been associated with other physiological and psychosocial factors such as pain, fatigue, social support, emotion-focused coping helplessness, fear of disease progression, fear to negative evaluation and attractiveness [197, 277].

Even though clinical severity or disease factors do not have a linear relationship with the psychological impact [272-274], in patients with SSc functional disability has been reported to be a significant predictor of distress[278].

To capture the impact of the disease the published studies have used different participants-reported outcomes measures (PROMs) such as generic PROMs capturing physical, psychological and psychosocial impairment and disease-specific PROMs capturing elements specific to patients with that particular disease [276] [262]. However, the impact of the disease on the patients' quality of life, reflecting psychosocial impairment, has only been captured by generic PROMs as opposed to disease-specific PROMs, possibly not reflecting the true level of the psychosocial impact in this population. To capture the true psychosocial impact of the disease, a needs- base disease-specific quality of life (QoL) [279] PROM should be used. By capturing the disability at a physical, psychological and psychosocial level the overall impact of the disease is assessed at a biopsychosocial level, as recommended by the World Health Organisation (WHO) [280].

There is evidence supporting the disabling effect that different clinical features of SSc have on the patients, including the physical disability caused by the presence of foot problems [8, 10]. However, there are no published data reporting the impact of foot problems on the quality of life of patients with SSc.

2.9 Summary

SSc is a complex connective tissue disease characterised by vasculopathy, immune activation and fibrosis. The pathogenesis of the disease affects different systems such as integumentary, vascular, neurological, musculoskeletal, cardio-pulmonary, renal, and gastrointestinal systems. The multi-system nature of the disease leads to widespread secondary effects including foot problems and related disability. Foot-related disability has an impact on patients' physical and psychosocial function, affecting patient QoL. Although the empirical description of foot involvement in patients with SSc is well documented, the factors that contribute to foot pathology and its impact on the quality of life of this population has not been well reported.

Chapter 3
Methodology

3.1 Introduction

Chapter three describes the methodologies used in this thesis. First, a literature review and a consultation with experts was undertaken to identify the potential candidate factors that may contribute to foot problems (chapter 2). Then patients with SSc were compared to healthy volunteers in a case-control cross-sectional study to investigate further the candidate factors that contribute to foot problems and explore their impact on the QoL of people with SSc; the impact of foot problems was quantified via participant-reported outcome measures described in section 3.3.2.2, and the candidate factors that contribute to foot problems were identified through detailed clinical investigations described in section 3.3.2.1. Finally, structural equation modelling was used to explore the multifactorial pathways in foot pathology and its impact on quality of life (section 3.4). An outline of this study design is presented in Figure 3.1 and each phase of the study is explained in detail in this chapter.

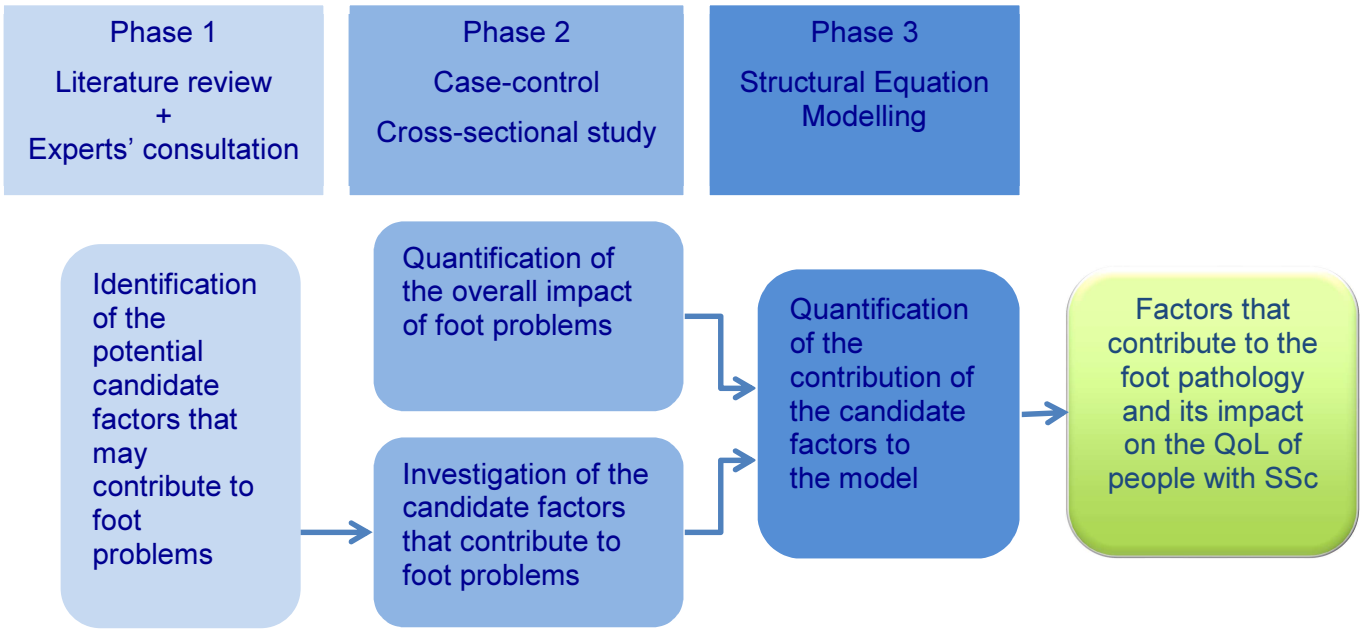

Figure 3.1: Outline of study design

Ethical approval was obtained from the Leeds (East) Research Ethics Committee (Ref **10/H1306/14)** and all the patients provided written informed consent in accordance with the Declaration of Helsinki (updated 2008).

3.2 Phase One: Identification of potential candidate factors.

The potential candidate factors that may contribute to foot problems were identified through published literature and a consultation with two clinical experts: a podiatrist and a consultant rheumatologist, both of whom, specialise in SSc. This process provided a comprehensive identification of physical and physiological potential candidate factors, the former affecting the integumentary, vascular, neurological and musculoskeletal systems. The published literature also provided the theoretical construct for the SEM. This literature was synthesised in chapter 2.

3.3 Phase Two: Investigation of the candidate factors that contribute to foot pathology and exploration of its impact

The potential factors obtained from phase one were further investigated using a case-control cross-sectional study. In this study, detailed clinical assessments and patient reported outcomes measures (PROMs) were used to explore the nature and impact of foot problems in SSc and to compare them with a group of healthy volunteers.

This case-control cross-sectional study provided data from which, after undertaking an exploratory analysis (section 4.3.2.1), the final set of candidate factors for the SEM were selected.

3.3.1 Participant recruitment

There is little consensus on the appropriate sample size for SEM. The required sample sizes reported range from 100 participants to several thousands[281], but what seems to be established is that large sample sizes are needed. SEM requires at least between 100 and 500 cases, but if the population studied is restricted in size then samples of less than 200 participants have been considered acceptable, although lower than 100 is not acceptable [282].

Given that SSc is a relatively rare condition, a planned recruitment of 150 cases was considered appropriate and feasible. After two years of active recruitment the number of patients with SSc recruited was 121, the majority of the participants that declined the invitation to take part in the study did so due to ill health. This number was considered acceptable for using SEM, as it is larger

than the minimum 100 cases. A further 51 healthy controls were also recruited for the case-control cross-sectional study yielding a total sample of 172.

In the absence of existing data on which to base the power calculation for the case-control cross-sectional study, we opted to use published guidelines for moderate effect size (Cohen's d=0.5, where "d" is the difference in means divided by the SD). Using a standard two-group comparison of means approach, this is equivalent to powering using a ratio of 1:2 for mean:SD. For example we could use values of 5 and 10 for the means in each group (difference 5), and SD=10. To achieve 80% power at the 5% level of significance, assuming a 3:1 ratio of cases to controls, this would require 42 controls and 127 cases. Note that it is immaterial which values are used for the means and SD, provided the ratio equates to d=0.5 [283].

3.3.1.1 Identification and recruitment

Recruitment of cases was undertaken at Chapel Allerton Hospital (Leeds Teaching Hospitals NHS Trust). The main health carer (consultant rheumatologist or podiatrist as appropriate) made the first approach to SSc patients attending for their routine care at the connective tissue disease clinics and/or the foot health clinic. Patients indicating a willingness in principle to participate were given a brief verbal explanation of the study, a written information sheet and the consent form. Potential participants were advised to read the information sheet and consent form and to discuss with family, friends and/or their GP as they deemed appropriate. If they decided that would like to take part in the study, they completed an expression of interest form, provided at the bottom of the patient information sheet, and returned it to the research

team in a freepost envelope. Once the team received the slip, the patient was contacted by telephone to arrange an appointment.

Recruitment of the healthy participants was undertaken through the patients with SSc, using a technique referred to as “bring a friend” where patients were asked to nominate a healthy friend/relative of the same gender and same age (+/-2years) who was willing to participate. This recruitment strategy has been described previously in the literature as an effective method of matching for socio-economic, ethnicity and other demographic factors. In order to keep a balance, age and gender was monitored but the selection was not specific.

When the SSc patients were given their information sheet and consent form they were also given a copy to help them recruit a healthy participant. The method followed subsequently was the same as described for the cases.

3.3.1.2 Inclusion/Exclusion Criteria

This case-control study targeted a broad range of people with SSc, including those with severe foot involvement as well as those with minimal or no foot involvement. As such, the inclusion criteria were broad and exclusion criteria minimal as described in Table 3.1.

Inclusion criteria for the patients with SSc	Inclusion criteria for the healthy participants
➢ Age ≥18 years old ➢ Physician diagnosis of SSc (ARA/ACR 1980 criteria) ➢ Willingness to participate in the study	➢ Age ≥18 years old ➢ Generally good health
Exclusion Criteria for both groups:	
➢ History of lower limb orthopaedic or vascular surgery in the 12 months prior to taking part in the study. ➢ Inability to understand or comply with the protocol.	

Table 3.1 Selection criteria for both groups.

As the recent ACR/EULAR classification criteria for SSc were still in development when recruitment took place, the diagnostic criteria used for patients inclusion in this study was based on a positive diagnoses of SSc as per the ARA/ACR (1980) criteria [41]or, if this criteria was not fully met, Consultant diagnosis of SSc was accepted. The criteria used for the subset classification was the lcSSc, dcSSc and SSc sine Scleroderma [24, 284] (Table 3.2), as these subsets criteria have been used for diagnostic and research proposes for the last three decades.

A summary of participant recruitment and inclusion at different phases of the study is presented in figure 3.2.

Criteria reference	Criteria for the classification of SSc		
American Rheumatology Association (ARA) preliminary Criteria for the Classification of Definite Systemic Sclerosis(1980) [41, 285]	**Major Criterion:** Proximal sclerosis	**Minor Criteria:** Sclerodactyly Digital pitting scars or loss of substance on the distal finger pad Bibasilar pulmonary fibrosis	
	To meet the criteria for the SSc diagnosis the patients has to fulfil the major criterion or any combination of 2 or more minor criterion.		
SSc Subsets according to LeRoy et al. (1988) [24]	**Limited Cutaneous SSc:** Raynaud's phenomenon for years at presentation Skin sclerosis limited to hands, feet, face, and forearms, or absent Significant late incidence of pulmonary hypertension Trigeminal neuralgia Calcinosis Telangiectasia Dilated nailfold capillary loops, usually without capillary dropouts	**Diffuse Cutaneous** SSc: Onset of Raynaud's phenomenon within 1 year of onset of skin changes Truncal and acral skin involvement Presence of tendon friction rubs Early and significant incidence of interstitial lung disease, oliguric renal failure Diffuse gastrointestinal disease Myocardial involvement Presence of anti-DNA, topoisomerase I (anti-Scl-70) antibodies Absence of anticentromere antibodies Nailfold capillary dilatation and destruction	**Overlap Syndromes** Either diffuse or limited SSc with typical features of one or more of the other connective tissue diseases
Systemic Sclerosis Sine Scleroderma [284]	**ssSSc** if the patient has all of the following features: 1)Raynaud's phenomenon or a peripheral vascular equivalent (digital pitting scars, digital-tip ulcers, digital-tip gangrene, abnormal nailfold capillaries) 2)Positive ANA 3)Any of the following: distal esophageal hypomotility, small bowel hypomotility, pulmonary interstitial fibrosis, primary pulmonary arterial hypertension (without fibrosis), cardiac involvement typical of scleroderma, or renal failure consistent with scleroderma renal crisis. 4)No other defined connective tissue or other disease as a cause of 1), 2), or 3).		

Table 3.2 Diagnostic criteria

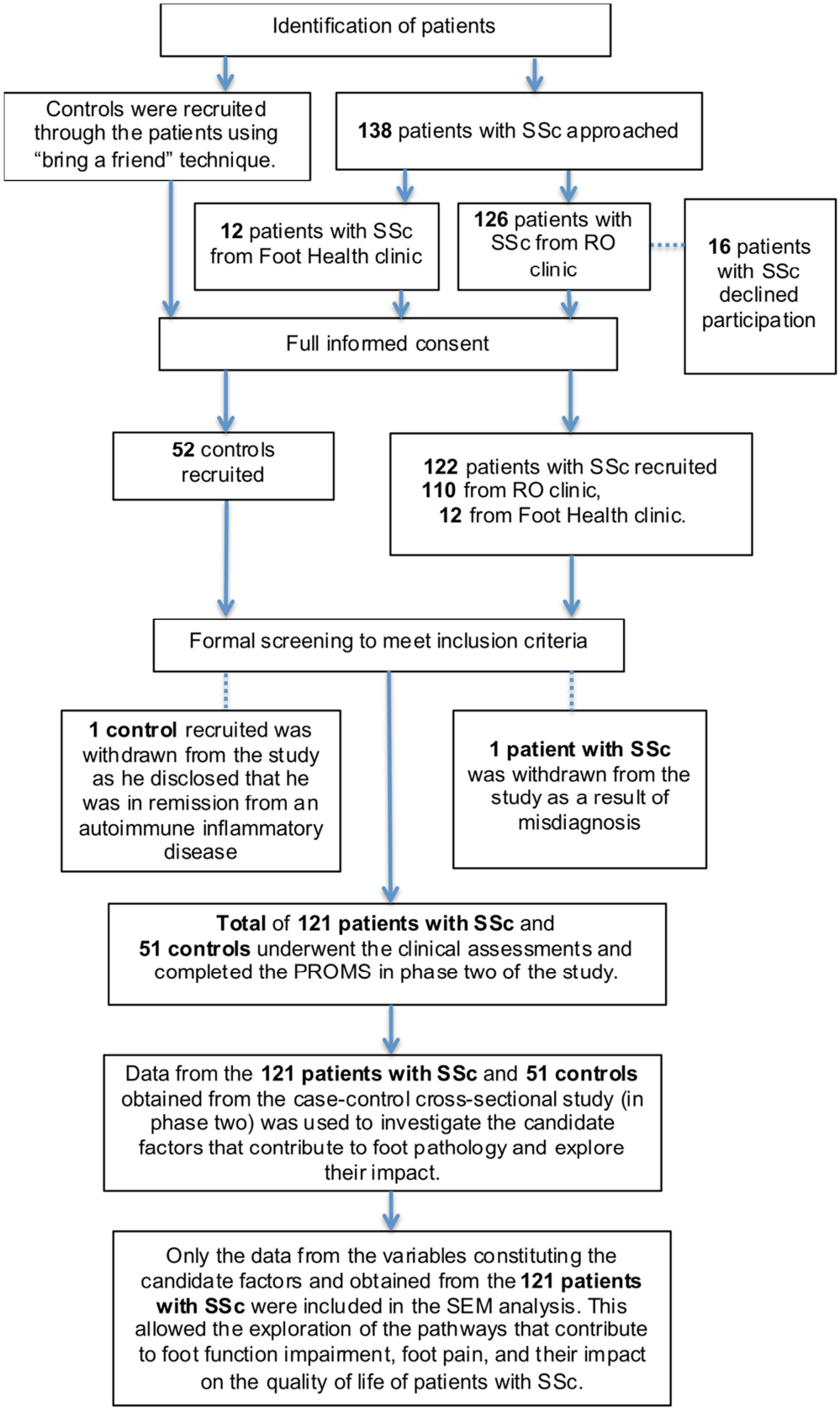

Figure 3.2: Study Flow Chart. RO= Rheumatology Outpatients

3.3.2 **Data collection**

Recruitment and data collection was undertaken over two years (February 2011- February 2013), through the four seasons so as to allow for seasonal variation, which has an effect on some of the symptoms such as RP [286] [287]. The candidate (BAP) carried out the data collection, aided by a research assistant (RAW), and all took place within one visit.

Two types of data were collected: participant-reported data and clinical data. Participant-reported data comprised of demographics, smoking history (pack years), comorbidities, medication, surgery history and standardized patient reported outcome measures (PROMs). With the exception of the PROMs, the rest of the participant reported data was obtained from all the participants including controls, and for the patient group it was cross-referenced with their medical notes. The patients also provided data regarding the presence of current or past skin ulcers and calcium deposits.

Clinical data was collected to identify the factors that contribute to foot problems. The participants undertook detailed clinical assessments of the integumentary, vascular, neurological and musculoskeletal features of the lower limb. A detailed description of the clinical assessment and the PROMs used is presented in section 3.3.2.1 and 3.3.2.2 respectively.

3.3.2.1 Detailed clinical assessments of the lower limb

The detailed clinical assessments of the lower limb assessed four different body systems: integumentary, vascular, neurological, and musculoskeletal system.

The tests used to assess these systems were the modified Rodnan Skin Score (mRSS), Ankle Brachial Pressure Index (ABPI), qualitative Doppler waveform analysis, Von Frey monofilament, vibration threshold, thermal threshold, thermal pain threshold, analysis of foot function during walking, plantar foot pressures, and ultrasound measurement of the heel and plantar 3rd MTPJ soft-tissue thickness, All these tests are summarised in Table 3.3.

System	Clinical Assessment	Assessment Overview	Target Pathology
Skin	**mRSS**	The mRSS is used to assess the extent of skin involvement in the patients with SSc by assessing skin thickness by clinical palpation [288, 289]	Skin fibrosis
Vascular	**ABPI**	Brachial pressure and ankle pressure are taken. To obtain the ankle pressure the Dorsalis Pedis (DP) and Posterior Tibial (PT) arteries' pressures are taken and the highest value amongst them is used to calculate the ABPI [290-292]	Presence and severity of peripheral arterial disease[293]
	Qualitative Doppler waveform analysis	The Doppler waveform from the DP and TP arteries is performed. The waveforms can be categorized according to appearance, which indicates the presence or absence of peripheral arterial disease [290-292] .	Peripheral arterial disease
Neurological	**Von-Frey monofilaments**	The mechanical threshold for light touch/pressure is assessed using the method of limits [294, 295]	Large fibre peripheral sensory neuropathy
	Vibrameter	The vibration detection threshold is determined by the detection of appearance of the vibration stimuli on the dorsal area of the first metatarsal [295, 296]	Large fibre peripheral sensory neuropathy
	Thermal threshold	The thermal threshold is used to detect the sensation for warmth and cold using the method of limits.[295, 297, 298])	Small fibre peripheral sensory neuropathy
	Thermal pain threshold	The thermal pain threshold is used to detect the sensation for heat-induced pain using the method of limits[295, 297, 298].	Small fibre peripheral sensory neuropathy
Musculoskeletal	**Eight camera three-dimensional, high resolution motion capture system (VICON, Oxford, UK) integrated with twin Birtek force plates for appending force data**	Joint motions and forces are feed into a multi-segment foot model (modified Oxford Foot Model [299]) defining joint motion and force interactions in detail [300] [299]	Abnormal pattern in gait and foot function
	Plantar Pressure (Novel EMED-ST)	To obtain high-resolution measures of pressure and force distributions on the plantar aspect of the foot [301].	Abnormal plantar foot pressures
	High-resolution ultrasound	To quantifies soft-tissue thickness at the plantar aspect [302]	Soft-tissue atrophy

Table 3.3 Summary of clinical assessments

3.3.2.1.1 Assessment of the integumentary system.

The modified Rodnan Skin Score

The mRSS is used to assess the extent of skin involvement in the patients with SSc. The results from this test are used to quantify the extent of skin involvement and to differentiate the SSc patients into two diagnostics groups, limited or diffuse sclerosis [43, 194]

This tool is a valid outcome measure to assess skin thickness by clinical palpation. It has been shown to have face, content, discriminant, convergent and divergent validity [288, 289, 303-306]. However, the inter-observer variability is regarded clinically to be fairly high even though it has been reported to have acceptable inter-observer and intra-observer reliability [305]. Hence, the validity of this tool has been established only when the same investigator does the assessment of the skin [289]. In this study the candidate was trained in performing mRSS and carried out the skin assessment in all the cases.

3.3.2.1.2 Assessment of the vascular system

Only the presence of macrovascular pathology was assessed as the assessment of microvascular disease proved overly challenging and ultimately not feasible to perform within the available time frame. This is further explained in the discussion.

The vascular system was assessed for Peripheral Arterial Disease (PAD). This was achieved by using the ABPI test and the qualitative Doppler waveform analysis.

The equipment used to assess the ABPI and the qualitative Doppler wave forms is a bidirectional Doppler (Smartdop 30EX, Koven Technology,Inc., St Louis, MO) with a 8 MHz probe. It is used to detect arterial blood flow in the extremities, assessing systolic pressures in the upper and lower limbs and automatically calculating the associated ABPIs.

Ankle Brachial Pressure Index

Reduction of ABPI values (<0.9mmHg) is of diagnostic value for the presence and severity of Peripheral Arterial Disease (PAD), and a good predictor of cardiovascular events [293, 307].

The validity of the ABPI in detecting stenosis of a 50% or more reduction in the diameter of the lumen has been published in the literature [308-310]. The studies done by Lijmer et al [308], Fowkes [310] and Feigelson at al[309] reported on the sensitivity and specificity of the ABPI against lower extremity angiography. The studies used an ABPI threshold of 0.91, 0.90, 0.80 (mmHg) respectively, and found that the sensitivity of the ABPI was 79%, 95%, 89% and the specificity 96%, 100%, 99% respectively. Furthermore, the ABPI has been shown to have a positive predictive value of 90%, a negative predictive value of 99%, and an accuracy of 98%[309]. This indicates that the ABPI is an accurate diagnostic measure for the presence of the PAD in the lower limb.

The use of the ABPI remains an important tool in the vascular assessment particularly those with atypical presentations [311], this being the case in this study group.

The bilateral ABPIs were acquired after the participants rested as recommended [312] on a clinical couch in a reclined position for 30 min and for 10 min in a supine position. The brachial pressure was assessed followed by the ankle pressure. To obtain the ankle pressure the Dorsalis Pedis (DP) and Posterior Tibial (PT) arterial pressures were taken. The fact of obtaining two different values from two arteries of the foot, causes some controversy on regards of which ankle pressures should be used for the calculation of the ABPIs i.e. the higher value, the lower value or the average value of the two [290-292, 313, 314].

For the purpose of this study the higher-pressure value was used. It was considered the most adequate of all three options because by using the higher value, to obtain a pathological ABPI both arteries must have significant occlusive disease. Moreover, patients with SSc can have luminal stenosis as a result of their microvascular arterial pathology [3, 218], and the higher-pressure value is more sensitive to detect luminal stenosis [290]. In addition, the American College of Cardiologists and the American Heart Association recommend using the highest value of the ankle pressure to be conservative in the diagnosis of PAD [315].

The ABPI calculation was obtained by dividing the ankle pressure by the brachial pressure. ABPI values ranging from 0.00-0.40 mmHg are considered severe PAD; from 0.41-0.90 mild to moderate PAD; 0.91-0.99 borderline (equivocal); 1.00-1.30 Normal; and >1.30 non-compressible [315].

When the ABPI values are above 1.30 or the patient has an ankle systolic blood pressure of >20 mmHg or 20% higher than the brachial systolic pressure, the patient is considered to have calcified arterial walls, which are poorly compressible [315]. If the arteries are non-compressible it can prevent accurate assessment of the ABPI [315].

Qualitative Doppler ultrasound waveform analysis

The qualitative Doppler waveform analysis provides haemodynamic information. The sensitivity and specificity of this method for detecting PAD in the lower extremities has been reported in the published literature. Walton et al [316] reported a sensitivity of 87% and a specificity of 88%; while Sensier et al [317] reported slightly higher sensitivity 95% and lower specificity 80% with an overall accuracy of 87%.The latter values for sensitivity and specificity are similar to those of Eiberg et al [318], of 98% and 81% respectively. However, the limitation of the Doppler waveform is that in the presence of stenosis of less than 50%, this modality loses sensitivity. Thus the presence of a minimal or moderate lesion as categorized by Walton et al. [316] might not be detected. This insensitivity might be as a result of the flow disturbance not being transmitted as far as the area of insonation [316].

The waveforms can be categorized by their appearance, which indicates the presence or absence of PAD. There is little consensus in how the different waveforms can be categorized however and different studies have used different systems. They do all have in common a description of the waveform in relation to the level of PAD.

The waveform categorization used in this study was one used more commonly in research and clinical settings; to categorize the waveforms as triphasic, which indicates absence of PAD; biphasic indicating presence of moderate disease; and monophasic indicating severe PAD[319, 320]. The triphasic waveform presents with a primary wave in systole (sharp systolic uprise and fall) followed by a short reverse flow, and a tertiary wave in diastole (diastolic forward flow). The spectral window through the pulse cycle is narrow in the absence of PAD; the biphasic waveform preserves the systolic upraise and fall, and the reverse flow, but the tertiary waveform in diastole is absent. In the biphasic wave, the spectral window is broadening; and in the monophasic waveform the waveform becomes damped (low amplitude and low curve upstroke and fall), the reverse flow is lost, and the spectral broadening is extensive [316-319] (Figure 3.2). In the presence of occlusion the waveform becomes extremely dampened with no reverse flow wave [316, 320].

The Doppler waveform from the DP and TP arteries were evaluated after the patient was resting for 10 min in a supine position, and three consecutive pulse waveform were acquired using the hand-held Doppler with a 8 MHz ultrasound probe (Smartdop 30EX, Koven Technology,Inc., St Louis, MO) A frequency of >5 MHz transducer (8-10 MHz transducer) is more useful to image superficial vessels such as pedal arteries [321]. Following acquisition the qualitative waveform analysis was performed by visual interpretation of continuously displayed waveforms, using the thriphasic, biphasic and monophasic description defined previously.

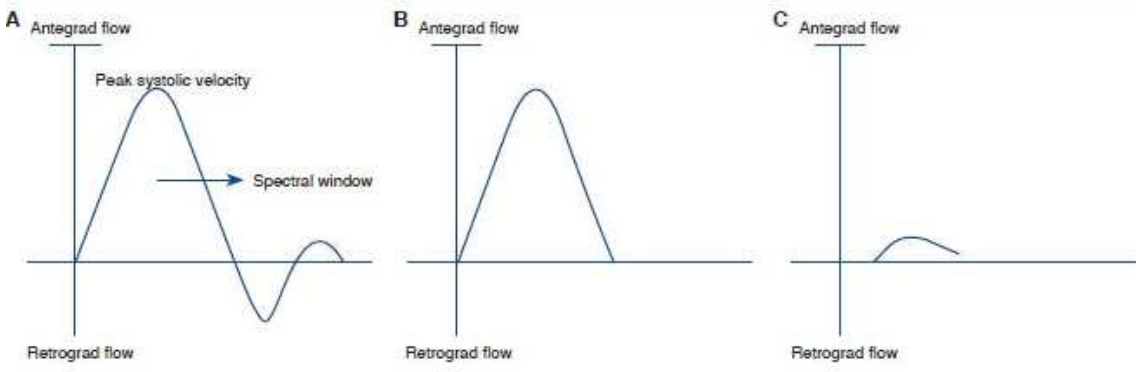

Figure 3.3 Lower limb arterial waveforms patterns: (A) thriphasic (B) biphasic (C) monophasic. Imaged modified from Verim et al, page 249 [322]

3.3.2.1.3 Assessment of the neurological system

The neurological system was assessed for the presence of peripheral sensory neuropathy. The assessments involve bilateral lower limb quantitative sensory examinations of the somatic sensory nerve fibres, the functions of which includes sensation for touch, vibration, temperature and pain. The nerve fibres tested are the large diameter myelinated (A-alpha and A-beta) which carry motor function, touch and vibration sense; and the small diameter myelinated (A-delta) and unmyelinated (C) nerve fibres, which carry temperature and pain sensation, and autonomic function [323, 324].

To assess the large diameter myelinated nerve fibres, the tests were conducted using graded **Von-Frey monofilaments** to assess touch sensation, and the **Vibrameter** to assess vibration sensation.

Von-Frey monofilaments

The mechanical threshold for light touch was determined using calibrated nylon **Von-Frey monofilaments** (SENSELab, Somedic, Sweden) according to the method of limits [294, 295]. The test was carried out on eight different foot areas: the apex of all five the toes, the dorsum of the foot, and plantar aspect of

the foot i.e heel and ball of the feet. The test was done with the participant's eyes closed. The monofilament was placed on the anatomical area that was being tested and then a force was applied till the monofilament buckled. The higher the number of the Von-Frey monofilament the greater the nominal force (grams) needed to buckle the monofilament, and the greater the pressure (g/mm^2) exerted. The environmental humidity and temperature has an effect on the structural composition of the nylon monofilament affecting the buckling force of the monofilament. The higher the humidity and temperatures, the lower the loading applied by the monofilament [325]. Nonetheless, the monofilaments recover their initial calibration when stored at the same environmental conditions[325]. The humidity and temperature were recorded before the test started to control for environmental variables, and the monofilaments were stored in the same environmental conditions to minimize the effect of these environmental factors to the monofilament performance.

The test started with number 6 Von-Frey filaments (monofilament diameter 0.18 mm, pressure exerted 3.3 g/mm^2). If the monofilament was not felt in some of the anatomical areas, the test was repeated using the monofilament in an ascending manner until the participant was able to feel the monofilament in that area. After number 6, the next Von-Frey filament grade used was number 9 (monofilament diameter 0.26 mm, pressure exerted 7.3 g/mm^2) then the numbers used were applied in ascending order by one extra grade at the time to a maximum pressure of 137.3 g/mm^2, produced by the Von-Frey filament grade number 19 (monofilament diameter 1.01mm)

The mechanical threshold for light touch using the Von-Frey monofilament has been shown to have high test-retest reliability, of 0.89, and a high inter-observer reliability 0.88 as indicated by the Pearson's product-moment correlation [297].

Vibrameter

The vibration detection threshold was assessed with a "**Vibrameter" (Type IV)** (SENSELab, Somedic, Sweden) using the method of limits. The vibration detection threshold was determined by the detection of onset of the vibration stimulus on a bony prominence [296, 326], in this study the location being the dorsal area of the first metatarsal shaft. The appearance threshold method has been shown to have less variance than that of the disappearance threshold [296].

The vibratory stimulus was delivered at a frequency of 100/120 Hz and the measurement of the stimuli ranged between 2:0 to 399.9 microns. The vibrameter delivered stimuli of increasing intensities starting from the base line value of 0μm (of displacement) at a rate of 1μm/s.

The vibration perception threshold test was used as recommended as part of the battery of the quantitative sensory testing in clinical trials [295, 296, 326, 327].

Thermal threshold and thermal pain thresholds

To quantitatively assess the small diameter myelinated (A-delta) and unmyelinated (C) nerve fibres for small fibre neuropathy the **thermal threshold** and **thermal pain thresholds** were measured objectively using the Modular Sensory Analyzer (MSA) Thermal Stimulator, (SENSELab, Somedic, Sweden). This computer-controlled device generates and documents response to highly

repeatable thermal stimuli, such as warmth, cold, and heat-induced pain. The method of limits was again used to detect the thermal threshold and thermal pain threshold. This approach is widely used in neurological research and has been shown to be a reliable method for detecting small fibre neuropathy[295, 297, 298]. The stimuli are transmitted through a thermode (25 x 50mm), which in this study was placed dorsally and plantarly over the metatarsophalangeal (MTP) joints of both feet.

The detection of the cold and warm threshold was measured, before the thermal pain threshold, by the perception threshold test [328]. This test was used with the setting “mix stimulus”, which consists of five cold stimuli followed by another five heat stimuli. The mean and standard deviation of the five different sensory thresholds was used as the final threshold value.

In the “mix stimulus”, the time intervals between the five stimuli were set randomly, time ranging between four to six seconds. The stimuli baseline temperature for both threshold tests was 32°C. The cold stimuli could decrease until reaching 10°C, and the heat stimuli could increase until reaching 50°C.. The slope i.e. the rate of change of the thermode temperature, is 1°C/s for the decrease to cold stimuli or increase of heat stimuli, and 3°C/s for returning to the baseline temperature of 32°C.

In the pain threshold test only one noxious “heat stimulus” was produced.

The thermal threshold and thermal pain threshold both have been shown to have high test-retest reliability, and high inter-observer reliability. For the cold detection threshold the correlation is 0.76 and 0.75 respectively, for the warm is 0.85 for both, and for the heat pain threshold is 0.88 and 0.87 respectively as

indicated by the Pearson's product-moment correlation; with no systematic difference between means, therefore reflecting agreement [297].

The sensitivity and specificity of the thermal threshold test and heat pain threshold are less clear [329, 330]. However, Shukla et al [298]reported a sensitivity of 72% on the thermal threshold test in detecting small fibre neuropathy, and this method has been widely used in clinical trials assessing small fibre neuropathy in different diseases, including SSc [171, 331].

3.3.2.1.4 Assessment of the musculoskeletal system

The musculoskeletal system was assessed for dynamic investigation of foot function, plantar pressures and quantification of compromise intra-articular and extra-articular structures. These features were assessed using the following tests: analysis of foot function during walking, plantar foot pressure, and ultrasound evaluation of plantar soft-tissue thickness.

Foot function during walking

To assess the dynamics of the foot function, the participants underwent a detailed analysis of foot function during normal walking. This was carried out using an eight camera, three dimensional, high resolution motion capture system (VICON, Oxford, UK) capturing at 390Hz and integrated with two Birtek force plates for appending force data sampled at 1000Hz. Joint motions and forces were fed into a multi-segment foot model (modified Oxford Foot Model [299] defining joint motion and force interactions in detail [299, 300]. Figure 3.4 shows the outputs obtained from the high-resolution motion capture system and

the Birtek force plates at three different stages during stance phase i.e heel strike, midstance, and push-off.

To define the multi-segment foot model of the foot, reflective markers were placed on the skin in specific anatomical locations, as described by Stebbins et al. in the modified Oxford Foot Model method (mOFM) [299]. The mOFM comprises a tibial segment, a hindfoot, a forefoot, and a hallux. The original OFM was developed by Carson et al [299, 300] to be used in adults. However, this multi-segment foot model was later on improved by Stebbins et al. when it was modified to be used in children, and has since been applied to foot deformities in children and adults [299]. The mOFM has been reported to be reliable during adult gait, thus making its use acceptable in assessing dynamic foot function in adults [332]. Figure 3.3 shows the placement of the reflective markers using the modified OFM method

The data capture protocol consists of one static trial and three dynamic trials. For capturing data in the static trial, the participant's foot is placed in an anatomical position defined as the Foot Posture Index (FPI) "0" [333]. For the dynamic trials, the participant walks several times along a 10m walkway until the participants assumed a natural gait, then three trials were captured and the trial deemed most representative of the participant's gait from consistency graphs was analysed.

Data was analysed using V3D software and six candidate variables were extracted: stance time, vertical ground reaction force 1 (heel-strike), vertical ground reaction force 3 (propulsion), Ankle ROM, hindfoot vs shank ROM and forefoot vs hindfoot ROM.

Figure 3.4 Participant walking through the three different stages of the stance phase of gait (i.e heel strike, midstance and push-off) during gait data capture. Reflective markers placed using the modified OFM method.

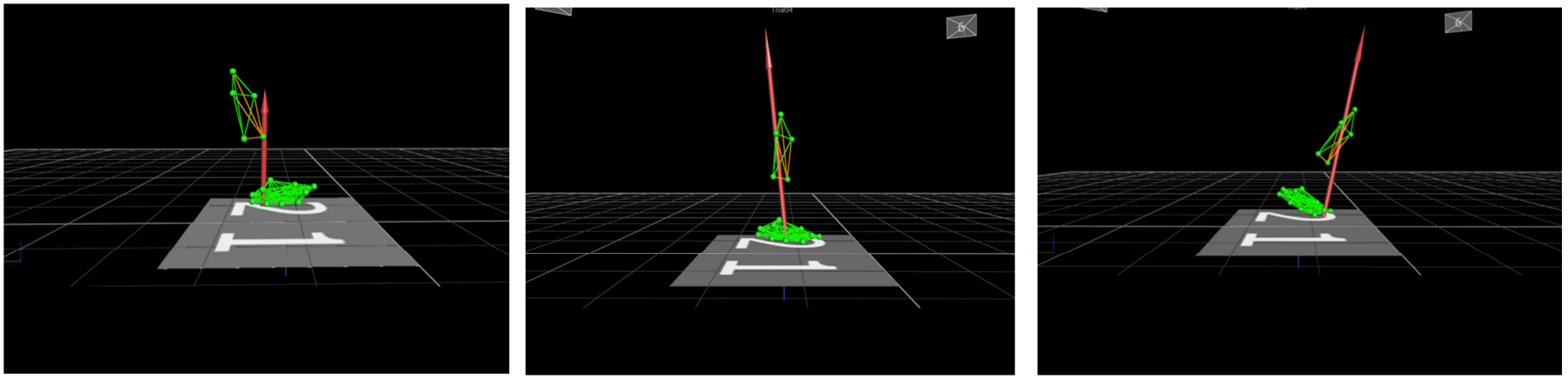

Figure 3.5 Outputs obtained from the high resolution motion capture system (VICON) and the Birtek force plates, at three different stages during stance phase; starting from left to right the photos represent heel strike, midstance and push-off. Green image represent the dynamic model created by the program from data obtained form the reflective markers and captured by the infrared cameras, the red arrow represent the direction and strength of the ground reaction force.

Plantar foot pressures

Plantar foot pressures were recorded using the Novel EMED-ST pressure platform (Novel GmbH, Munich Germany) to obtain high-resolution measures of pressure and force distributions under the foot. To obtain the plantar pressures, the patient walked barefoot over the pressure platform for six walking trials, at a self-selected normal walking speed, and three representative steps were collected from each foot and averaged and used for the analysis. A minimum of two steps gait initiation was used to ensure adequate reliability [301, 334, 335]. The use of the Novel EMED-ST pressure platform to obtain plantar pressures has been extensively reported in the literature [301, 334-337]. This plantar pressure system quantifies the plantar pressures, and discriminates between normal and abnormal loading patterns on the plantar aspect of the foot [336, 337] (Figure 3.5).

The parameters obtained from this plantar pressure system (i.e. Peak pressure, contact area, contact time, pressure–time integral, force–time integral, and instant of peak pressure) have been shown to be repeatable, thus they can be used as a part of the assessment of foot pathology [336, 337]. This method has been previously used to quantify plantar pressures in different rheumatic diseases including SSc [10, 195, 338].

The pressure parameters extracted for this study were the maximum mean pressure under the 3rd MTP joint and heel, and the mean force under the 3rd MTP joint and the heel.

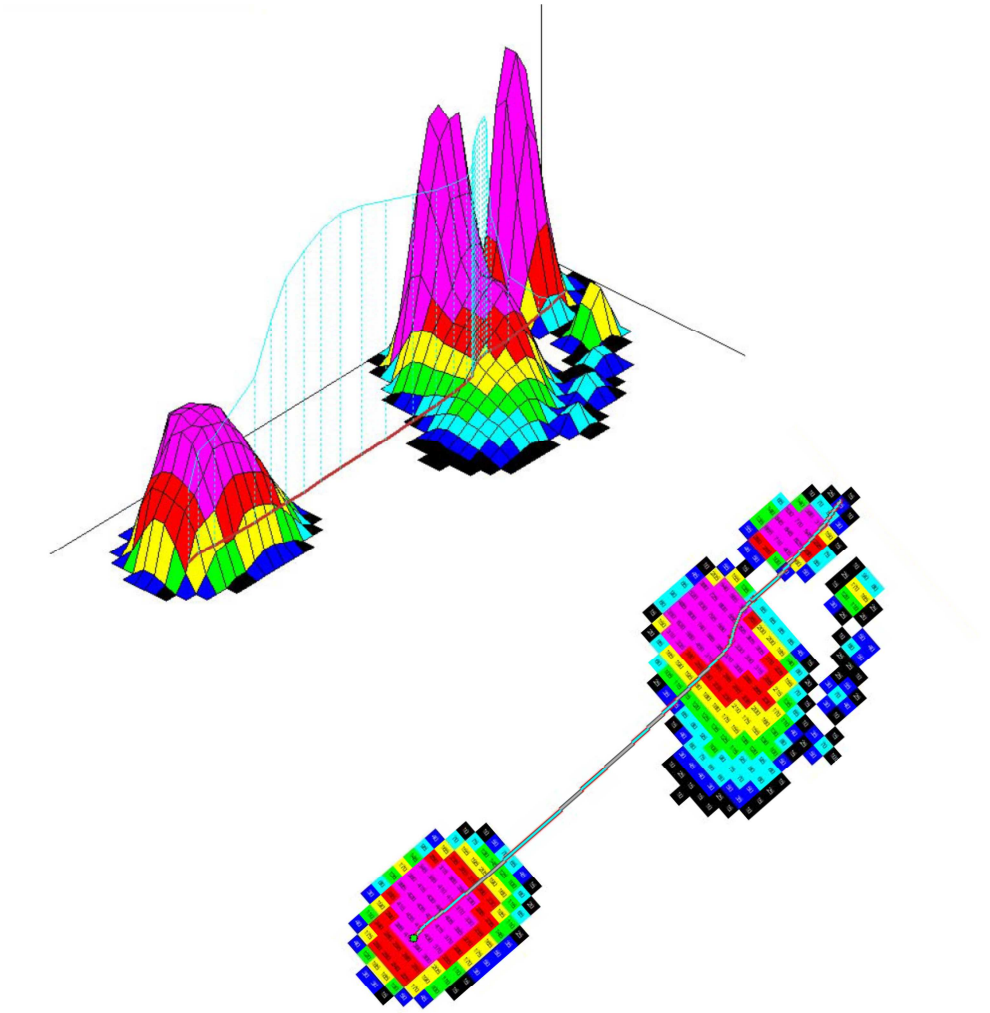

Figure 3.6 Plantar foot pressure prints. Pink areas indicate higher pressures

Ultrasound

A High-resolution diagnostic GE Voluson ultrasound machine (GE Healthcare, Chalfont, UK) with an 18 Mhz probe was used to quantify objectively, the soft tissue thickness at the plantar aspect of the MTP joints and the heel (Figures 3.6 and 3.7). Ultrasound is an established diagnostic imaging technique, widely used in patients with musculoskeletal diseases, which allows objective measurement of soft tissue dimensions at sub-millimetre resolution. Ultrasound has been reported to be a useful and objective tool for quantifying thickness of the epidermis, dermis and subcutis in patients with SSc [302, 339]. In order to

determine consistency of the US measurements, an assessment of the candidate's own intra-rater reliability was undertaken and is reported in Appendix 1.

Ultrasound, has been used previously in SSc studies to identify the musculoskeletal pathologies mentioned above i.e. thickness of the epidermis, dermis and subcutis [189, 253, 259, 340], and it plays an important role on the diagnosis of musculoskeletal pathologies [244, 341-343]

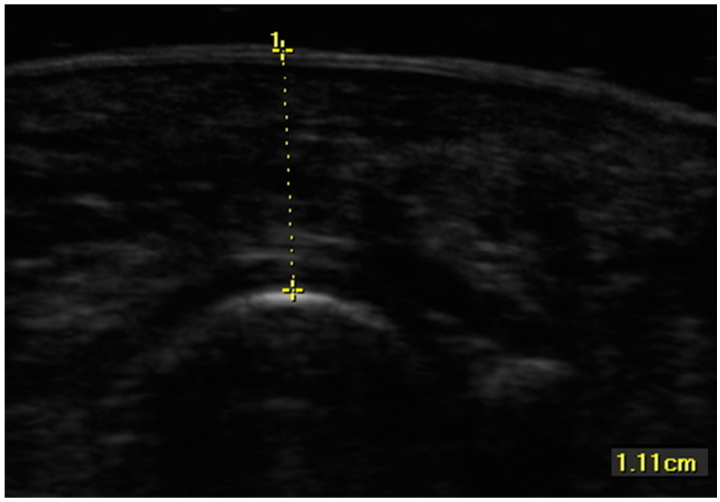

Figure 3.7 Measurement of the plantar soft-tissue thickness under the 3rd MTP joint, using ultrasound B mode image.

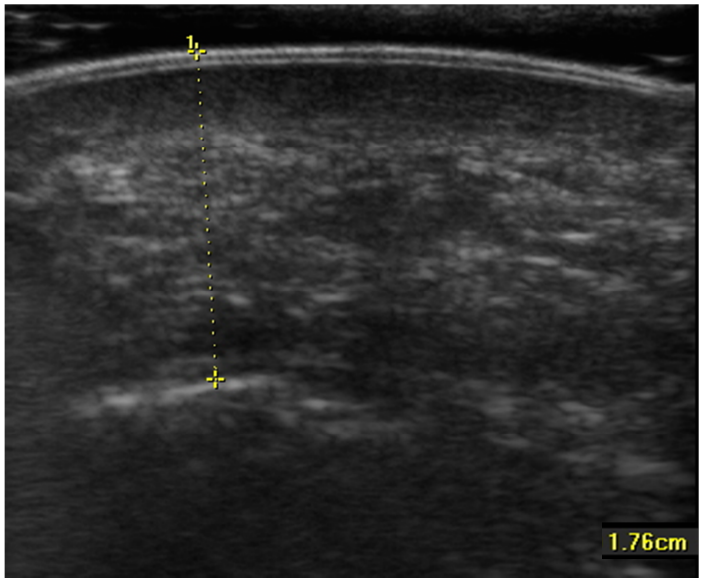

Figure 3.8 Measurement of the plantar soft-tissue thickness under the heel, using ultrasound B mode image

3.3.2.2 Exploration of the impact of SSc

To capture the impact of the disease the participants completed six PROMs exploring specific domains: the MOS 36-items Short-Form health survey (SF-36), the Hospital Anxiety and Depression Scale (HADS), the Scleroderma Health Assessment Questionnaire (SSc HAQ), the SSc Quality of Life (SSc QoL), the Raynaud's condition score (RCS), and the Manchester Foot Pain and Disability Index (MFPDI). The healthy participants completed the same questionnaires with the exception of the disease specific questionnaires i.e. SSc HAQ and SSc QoL. The six outcomes measure are summarised in Table 3.4, and described in this section.

Patient Reported Outcomes Measures	Type of outcome measure	Domains	What does it measures?
MOS SF-36 [344, 345]	Generic	1-Functional status 2-Well being 3-Overall evaluation of health	Patient's perceived general health status
Hospital Anxiety and Depression Scale [346]	Generic	1-Anxiety 2-Depresion	The symptoms and severity of anxiety and depression
SSc HAQ [347]	Disease specific	1-Function/Disability 2- Pain 3-Vascular symptoms 4-Digital ulcerations 5-Gastrointestinal symptoms 6-Lung symptoms 7-Patient's perceived overall disease severity	Disease specific health status, by assessing functional limitations general and organ-specific symptoms.
SSc QoL [279])	Disease specific	1-Disease impact on health and well-being[279]	Needs-base quality of life
RCS [348]	Symptom specific	1-Raynaud's attacks activity 2-Raynaud's attacks impact	Daily frequency and duration of the Raynaud's attacks, and the level of difficulty that the patient experience as a result of the Raynaud's attacks.
MFPDI [349]	Region specific	1-Foot & ankle pain 2-Foot & ankle disability 3-Foot & ankle appearance	Activity related foot and ankle disability and pain, and appearance.

Table 3.4 Patient-reported outcome measure

MOS SF-36

The **SF-36** was used to assess general health status in order to compare the impact of SSc with not only our healthy volunteers, but also with other conditions. This health survey is a validated generic outcome measure designed to examine a person's perceived health status, and to identify how a particular condition causes health to deviate from the "healthy standard" [344, 345]. It consists of one multi-item scale measuring eight health concepts/variables. These eight variables can be divided in three domains: i) The functional status domain which comprises physical functioning, role limitations because of physical health problems, social functioning, role limitations because of emotional problems; ii) the well-being domain containing general mental health (psychological distress and psychological well-being), bodily pain, vitality (energy/fatigue); and iii) the overall evaluation of health domain comprised by the variable general health perception. In addition to the one multi-item scale it also includes a single-item measure of the health transition or change [344, 345].

The SF-36 scales can also be grouped in two summary measures the physical component summary and the mental component summary (McHorney et al., 1993). The score obtained from the questionnaire varies from 0 worst health state measurable by the tool to 100 best health state[345, 350, 351].

The SF-36 version used in this study was version one and the software used to score the questionnaire was QualityMetric Health Outcomes(tm) Scoring Software 4.0. The licence was obtain through QualityMetric Health Outcomes

Hospital Anxiety and Depression Scale (HADS)

The HADS was used to measure domain specific health status of anxiety and depression. This questionnaire is a self-assessment scale, which measures the symptom severity and distinguish cases of anxiety and depression i.e scores ≥8 from non-cases i.e scores <8 [346, 352]. The domains of anxiety and depression symptoms are measured by 14 items, seven for each subscale measuring the two domains. Each item has four responses with scores ranging from 0 to 3. The HADS is a reliable measure of anxiety and depression and has been reported to be a valid measure of the severity of these mood disorders [346, 353]. To prevent influence from somatic disorders and from serious mental disorders, this scale excludes symptoms related to physical disorders (symptoms of somatic reference), and those that might arise from mental diseases [346]. The HADS has been used in previous studies as an outcome measure to assess anxiety and depression in patients with SSc [197, 354].

License to use the HADS was obtained from GL Assessment.

SSc HAQ

The **SSc HAQ** was used to assess disease specific health status in terms of functional ability. This tool is composed by two outcomes measure the “short” Health Assessment Questionnaire Disability Index (HAQ-DI) including the Visual Analogue Pain Scales (VAPS) [355], and five specific Scleroderma-Visual Analogue Scales (Scleroderma-VAS) [347]. The SSc-HAQ measures 14 domains: eight on function/disability measured by the HAQ-DI (dressing, arising, eating, walking, hygiene, reach, grip, and common daily activities); one on pain measured by the VAPS [356]; and five on individual scleroderma organ system symptoms which are measured by the five scleroderma-specific VAS

(vascular problems [Raynaud's Phenomenon (RP)], digital tip ulcers, Gastro Intestinal (GI) symptoms, lung symptoms (usually shortness of breath), and overall disease severity from the patients perspective) [347].

The HAQ-DI was originally developed to measure function/disability (physical disability) in people with Rheumatoid Arthritis [355, 356] but it has been shown to be a valid and reliable measure of disability in patients with SSc, as its domains pertains to activities of daily living [357-359]. The fact that the HAQ-DI [356] was not developed originally for patients with SSc creates limitations of its use, as it does not capture disabilities caused by some disease features such as muscle weakness and skin tightness [359]. Nonetheless, the five Scleroderma-VAS add incremental content validity to the HAQ-DI because it contains SSc specific domains that contribute to the conceptual framework of disability in SSc, allowing to capture disability secondary to internal organ involvement [359]. Therefore, the SSc HAQ can be used to assess general and organ–specific symptom as well as functional limitations in patients with SSc [359] [358, 360].

It needs to be noted that the SSc-HAQ is not a composite outcome measure and so the scores from the Scleroderma-VAS are reported individually, and are not added to the HAQ-DI scores (Johnson et al., 2005). On the other hand, the HAQ-DI score is a composite score falling between 0 to 3 in an ordinal scale, 0 interpreted as (no impairment in function) to 3 (maximal impairment of function). The 15cm Scleroderma-VAS are scored by measuring the centimetres from the left anchor (no pain/ no limitation) to the patient's mark. The VAS scores can be converted to equivalent scores on the no impairment to maximal impairment scale, where each centimetre is equivalent to 0.2 points converting the result

into an ordinal scale of 0 (no impairment in function) to 3 (maximal impairment of function) equivalent to the HAQ-DI (Johnson et al., 2005 ; Steen and Medsger, 1997). However, in this study the Scleroderma-VAS were converted and treated as 10cm VAS in order for a more sensitive measure to be included in the structural equation modelling.

SSc QoL

The **SSc QoL is a needs-based quality of life** outcome measure developed for patients with SSc. The needs-based model is based on the understanding that individuals are driven or motivated by their needs, and that life gains its quality from the ability and capacity of individuals to satisfy their needs [361, 362]. The needs-based definition of quality of life (QoL) is a different construct to our physical ability or health status; as QoL is a complex interaction between the ways in which people perceive their health and how it relates to other aspects of their lives that are non-medical. Therefore the construct of the SSc QoL does not depend on a medical model but reflects the issues that are important to the patient independent of the physician's understanding of quality of life [363].

The SSc QoL questionnaire is a self–assessment scale consisting of 29 items that measures the impact of SSc on health and well-being and cover four overarching themes identified by SSc patients: emotion, physical adaptation, impact on/with others and impact on self.

Each item has two responses scoring either 0 (true) or 1 (not true). The total score is obtained by adding up the scores, with a maximum score of 29; the higher the score the worse the quality of life [279]. The SSc QoL is a reliable tool which has been used previously in a randomized control trial (RCT) [364].

License to use the Scleroderma Quality of Life Scale Questionnaire© 2006 was obtained from the University of Leeds.

Raynaud's Condition Score (RCS)

The Raynaud's Condition Score (RCS) is a symptom specific outcome measure. This tool measures the daily frequency and duration of the Raynaud's attacks, and the level of difficulty that the patients experience as a result of the Raynaud's attacks. The RCS integrates the impact and activity of RP [348]. The level of difficulty is measured by a numerical scale, which scores ranges from 0 (no difficulty) – 10 (extreme difficulty). It has been shown in previous studies that the RCS is a valid and reliable outcome measure of the impact caused by the Raynaud's phenomenon attacks [289, 348].

The RCS and the RP-10cm VAS, which is incorporated into the SSc HAQ, are both validated tools to assess Raynaud's phenomenon activity in RP associated to SSc. However, the RCS has been shown to have face, content, criterion, discriminant, and construct validity, and to bare superiority over the RP-10 cm VAS [289, 348].

The use as outcome measures of the RCS, SF 36, SSc HAQ in Scleroderma and Raynaud's phenomenon clinical trials, has been recommended by the Scleroderma Clinical Trials Consortium and the Outcomes Measure in Rheumatology Clinical Trials (OMERACT) [289, 306, 348]

Manchester Foot Pain and Disability Questionnaire /index (MFPDI)

The Foot Pain and Disability Index (FPDI), more commonly referred as Manchester Foot Pain and Disability Questionnaire (MFPDQ), is used to assess the foot specific health status. The MFPDQ is a validated, multidimensional,

generic, self-administered instrument use to assess foot pain and disability. The MFPDQ measures activity related foot and ankle disability and pain, and appearance [349, 365]. There are two versions of the MFPDQ. The original version developed by Garrow et al. [349], which consists of 19 items, and a modified version which consists of 16 items [365]. For the propose of this study the original version by Garrow et al [349], the 19 item version, was used.

The total score from the questionnaire is calculated by summing the scores from each item. The minimum score obtainable is 0 and the maximum is 38, the higher the scores the grater the foot pain and disability. Each item is rated in an ordinal scale: None of the time = 0; on some days = 1; and on most/every day= 2. The participants were considered to have foot problems if they had scored at least one of the 10 MFPDI function items experienced on most /every day; as it is an indicator of disabling foot pain reflecting impaired physical function [366].

Licence to use the MFPDI was obtained from ISIS Outcomes (Oxford, Uk).

These generic and disease specific questionnaires evaluated the physical and psychological disability perceived by the patients. In this context, the term disability was used with the meaning described by the International Classification of Functioning, disability and health (ICF) in the biopsychosocial model. Disability, involves dysfunctioning at one or more of the three levels of human functioning: at the level of body or body part, the whole person, or the whole person in a social context. Therefore, disability is composed by impairments (problems in body function or structure), activity limitations (difficulties that an individual might have in executing activities) and participation

restrictions (problems an individual may experience in involvement in life situations) [280, 367].

On the next stage of phase two, after the patients completed the questionnaires, the patients underwent a series of detailed clinical assessments of the lower limbs. These assessments were used to identify the potential candidate factors that contribute to foot problems.

In summary, the data collected from the clinical assessments captured the potential factors that cause foot problems, while the data from the questionnaires captured and quantified in detail the extent of the physical, psychological and psychosocial SSc related impact. These data provided the inputs to the exploratory analysis used to identify the candidate factors for the Structural Equation Modelling (SEM) explained in phase three.

Prior to undertake the statistical analysis the candidate completed a PgCert in Health Research and a three-day course in Structural Equation Modelling with Amos.

3.3.3 **Strategy for data analysis**

Prior to the SEM testing carried out in phase three, summary statistics were used to describe the demographics and clinical features of the sample. From the clinical assessments, only data from the dominant foot was included in the statistical analysis.

Exploratory analysis and regression analysis was used to select the candidate factors for the SEM. Analysis was undertaken using SPSS (Version 20)

3.3.3.1 Comparison of SSc patients and healthy participants

The data obtained from the quantification of the impact and the clinical assessments was used to compare the patients with SSc with the controls in an exploratory analysis. The between-groups comparison of the data helped inform the identification of the candidate factors for the later modelling with those factors that showed the more significant differences or bigger effects sizes between the groups being prioritised for the SEM, along with further variables that were strongly associated with the theoretical construct of the model.

i. Summary statistics

For the case-control cross-sectional study and for the SEM data summary statistics were checked for each variable and consisted of frequency distribution, measures of central tendency of the data i.e. mean and median, and measures of dispersion of the data i.e. Standard Deviations (SD), interquartile range and ranges. Summary statistics were used together with a bootstrapping technique, which provides an estimate of the sampling distribution within our cohort. Assessment of normal distributions and outliers in the data were undertaken.

ii. Exploratory statistics

Exploratory statistics were employed to check for assumptions of normality and homogeneity of variance for each variable, and to assess which statistical tests, i.e. parametric or non-parametric, test would be more appropriate to explore the data. The assumption of normality was assessed graphically by histogram, and

box-plots and confirmed by statistical testing of significance of skewness and kurtosis. This statistical test uses the corresponding z-scores of skewness and kurtosis to compare against values that indicate whether skewness and kurtosis are not different from 0 (a normal distribution). The assumption of homogeneity of variance was assessed graphically by using a boxplot of the variable data for the two groups and statistically by using the Hartley's F_{max} known as variance ratio. The boxplot also indicated the presence of any outliers that could have influenced the results.

In order to compare the SSc patients with healthy participants, a t-test was used for interval variables that met the assumptions of normality and homogeneity of variance. For the interval variables that did not meet the assumptions, a non-parametric test for independent samples, the Mann-Whitney test, was used. The Mann-Whitney test was also used for the ordinal variables and the Pearson's Chi-Square (χ^2) test for categorical variables. For the latter test the assumptions of independence and expected frequencies ≥5 were met [368].

With the non-parametric tests the Monte Carlo (2-tailed) method was used to calculate the p-value, while the bootstrap method was used for the parametric tests.

The effect size was calculated using Cohen *d* and Pearson's correlation coefficient for normally distributed variables [368, 369]. While for the non-normally distributed variables only Pearson's correlation coefficient was used, as Pearson's correlation coefficient is the recommended test to calculate the effect size when using the non-parametric Mann-Whitney test [283, 368].

3.3.3.2 Exploratory analyses of the potential factors for the SEM

Structural Equation Modelling (SEM) is a powerful modelling approach used to explore the relationships between several independent and dependent variables. SEM is a sophisticated method for examining correlations and relationships between several dependent and independent variables. For example, correlation is used to explore the relationship between two variables, and this analysis will provide a value (r or Rho) indicating the strength of the association. The disadvantage of the correlation is that only one relationship between two variables can be evaluated at a time and the variables are each considered in isolation. Therefore, if we wanted to explore the combined impact of several independent variables (such as foot pain, foot function and depression) on a dependent variable (such as QoL) then regression analysis would be used. Regression analysis offers the ability to measure the impact of each independent variable on the dependent variables when considering all the variables together (ß weights), and it also provides an estimate of how much of the dependent variable is explained by all independent variables combined (R^2). Regression is constrained by three major issues however: the first is that only one dependent variable can be explored at a time; the second is that the direction of a relationship is treated in only one direction (such as depression impacts on QoL and not that QoL may impacts on depression); and finally, regression does not take into account error associated with the outcome measures.

SEM offers a solution to some of the issues that are inherent in multiple regression techniques. SEM is a sophisticated modelling approach, extending the power of simpler techniques such as regression to allow quantification of

complex interactions between factors. It is composed of two basic elements path analysis (i.e multiple regressions, which is concerned with the relationships between measured or observed variables of interest) and factor analysis (which considers the extent to which items or measures capture latent variables) [370].

SEM is driven by a conceptual theory about a set of variables and their relationship. This method requires a theoretical model underlined by a hypothesis that is to be tested by the model. It is very important that the model is hypothesis driven, either by evidence from the literature or by a conceptual understanding of the variables, as SEM involves an iterative analysis technique where relationships between variables can be changed in order to fit the model [371]

Conventional statistical analysis was undertaken using SPSS (Version 20) and SEM modelling using AMOS (Version 21).

3.3.3.2.1 Multiple regression analysis- Methods

The multiple regression analysis methods section is divided in two parts: data preparation for the regression model and multiple regression methods.

i. Data preparation for the regression model

To identify which factors would be included in the SEM, regression analyses were undertaken in order to understand how variables were related to foot function, foot pain and quality of life. Data were assessed for collinearity, linearity and hetroscedasticity, independent errors/residuals, outliers and missing data.

Collinearity

All variables entered into the models were assessed for multicollinearity. Three different methods were used to identify collinearity between variables: correlations coefficients, Variance Inflation Factor (VIF) and the VIF related tolerance statistic. For the correlation matrixes the following tests were used, depending on the type of variables. Spearman's correlation coefficient was used when one of the variables was continuous but non-normally distributed, and when the variables were ordinal or categorical. Point-biserial correlation was used when one of the categorical variables was a discrete dichotomous variable. Any variables that had a correlation coefficient of 0.8 or greater were considered to be collinear and were excluded from the model, as this indicates redundancy for the model. However, the correlation coefficient method misses subtle forms of collinearity therefore for the multiple regression models the VIF and the VIF related tolerance statistic, which indicates if two predictors have a strong linear relationship, were also assessed. The models were only accepted if the VIF was ≤ 10 and the tolerance statistic was > 0.20 [368].

Relationships between variables that were considered collinear were excluded, as they were redundant for the model.

Linearity and heteroscedasticity / heterogeneity of variance

The assumptions of linearity and heterogeneity of variance of the outcome variable was assessed by using a plot of standardised residuals against standardised predicted values known as *zpred* vs *zresid* plot. For the predictor variables these assumptions were assessed by partial plots, which are scatterplots of the residuals of the outcome variable and each of the predictors when both variables are regressed separately on the remaining predictors. The

partial plots were also used to identify any obvious outliers that could have influenced the predictor's regression coefficient.

Independent errors/ residuals

After the model was created the Durbin- Watson test result was checked to assess that the residuals in the model were independent, specifically whether adjacent residuals were correlated. A value of 2 was considered that the residuals were uncorrelated, greater than 2 was considered a negative correlation and lower than 2 a positive correlation.

Outliers and missing data

Data from each variable were explored graphically using a boxplot; this allowed the identification of outliers and extreme scores.

Case-wise diagnostics were also undertaken to check the residuals for evidence of bias. For each regression model, the case-wise diagnostics were examined for extreme cases and any cases that had a standardised residual less than -2 or greater than 2 were considered outliers and were investigated further. However, the model was still considered adequately accurate if 5% of the cases had a standardised residual outside ±2 or 1% had a standardised residual outside ± 2.5; although any cases with a standardised residual value above 3 were investigated further as they were caused for concern.

Any outliers were checked to see whether they were exerting undue influence over the parameters of the model. To assess the overall influence of a single outlier on the model as a whole, the Cook's distance statistic was used, and any outlier having a Cook's distance value > 1 was considered influential to the model.

To combat the effects of the outliers, and the problems with violation of the assumption of normality, a robust statistical method was used, the bootstrap. The bootstrap method overcomes these problems by estimating the properties of the sampling distribution from the sample data.

For those variables that also had problems with normality and linearity transformation of their data were also undertaken.

Missing data in the multiple regression models was addressed by excluding the cases listwise.

ii. Multiple regression models- Methods

In preparation for the SEM the multivariate relationships between each of the variables were explored using multiple regression analysis. The regression models were assessed to refine the selection of the candidate factors for the SEM.

The force entry multiple regression method was used to identify significant relationships between the predictors and the outcomes and the direction of the relationships, and the data obtained was used subsequently to inform the theoretical model for the SEM.

The force entry multiple regression method enters all the predictors into the model simultaneously. This method is appropriate when there is a lack of evidence to rank the variables in order of importance in predicting the outcome [368]. Prior to the current study there was not enough published evidence of the impact of foot problems in patients with SSc to rank the variables in order of the importance of predicting the outcome.

Sixteen different multiple regression models were constructed, four for each of the systems assessed clinically (i.e. integumentary, vascular, neurological, and musculoskeletal), and sequentially using the QoL, HAQ, MFPDI function and MFPDI pain variables as outcomes. These PROMs scores were the closest representations of relevant aspects of the conceptual variable of overall "impact", the latent variable to be assessed in the SEM. The predictor variables were comprised of the clinical assessment variables that represented each of the four different systems under investigation. From the predictor variables, those that had the greatest contribution to the regression models would be prioritized as candidate factors for the SEM.

Other variables considered potential confounders were also added into the multiple regression models so that they could be adjusted for. These variables consisted of demographic variables (age and gender), disease activity variable (disease duration and disease subset), comorbidities (number of comorbidities) and BMI.

The results obtained from the exploratory analysis, together with the regression model data were used to inform the decisions about which clinical variables would be included in the SEM.

3.4 Phase three: Multifactorial Pathways

The pathway-modelling phase was designed to explore the role of candidate factors in the multifactorial pathway of foot pathology and its impact on quality of life. This was achieved by using Structural Equation Modelling (SEM).

For this phase, only the data from the 121 patients with SSc was included, as the data from the healthy participants was collected only for use as comparator data in the case-control cross-sectional study.

3.4.1 **Overview of Structural Equation Modelling**

SEM is an analytical technique which incorporates the strength of factor analysis, path analysis and regression in a single model that can be tested statistically, permitting the evaluation of relationships amongst latent variables [281, 370]. Latent variables are variables that cannot be directly measured and SEM defines them in terms of behaviour believed to represent this variables. This model is a powerful tool to identify the direct and indirect mediating effects of multiple factors to a latent variable, such as the concept of “impact” of foot problems in patients with SSc (Figure 4.2) [372]. Furthermore, SEM permits multiple dependent variables to be explored at a time, and variables can be treated as dependent and independent variables. Thus, SEM treats the direction of the relationship between variables as potentially bidirectional. It includes reciprocal and mediating relationships and provides estimates of measurement error [370, 372, 373].

In contrast to other multivariable methods, SEM allows hypothesis testing as it offers a confirmatory rather than an exploratory approach to data analysis. Thus, data can be analysed for inferential purposes instead of descriptive purposes. The confirmatory approach applies only to pre-specified models and is lost once the initial model does not fit the data and the researcher has to modify the model. Consequently, if this occurs the subsequent SEM analysis cannot be used as evidence for causation as it has lost its strictly confirmatory

application. If the initial model requires modification then the purpose of the model becomes “model generation” with the aim of producing a model with three properties: it makes theoretical sense, it is reasonably parsimonious and its correspondence with the data is acceptably closed[282] .

The causal relationships being explored in this study was represented by a series of structural (i.e. regression) equations, and they were modelled pictorially for clearer theoretical conceptualization. The pictorial model represents the latent variables, ie those which are indirectly measured by observed variables (e.g. QoL), by an oval shape; while the directly observed variables (e.g. plantar pressures) are represented by a rectangular shape. In addition, residuals or error terms in SEM are explicitly represented in the diagram as latent variables. This is because error variance is not directly observed in the raw data and needs to be estimated using the whole model and the data [282].

Each hypothesized relationship was concurrently tested at the same time as the entire system of variables, to determine the extent to which it was consistent with the data. The goodness of fit was then used to test the acceptability of the hypothesised relationships in the model; if the goodness of fit was inadequate the model was rejected [372]. Note however, that before a model was rejected, the relationships between variables were changed to try to obtain a better model (i.e. model generation), and all the changes were made in light of evidence from the literature or using a conceptual understanding of the variables [374].

SEM was considered the best statistical multivariable model for this study as it allows the analysis of observed and latent variables, thus allowing the

evaluation of the contributory effects of different factors to the outcome variables i.e. the factors contributing to foot pathology and the factors contributing to the impact of foot pathology on the QoL of patients with SSc.

3.4.2 **Hypothesis testing**

To explore the multifactorial pathways in foot pathology and its impact on quality of life, following our initial exploration of one model (Chapter 4), three initial models were designed exploring foot function, foot pain, and the impact of foot pathology on the QoL of patients with SSc.These models were based on the International Classification of Functioning, disability and health (ICF) and supported by the literature and the results obtained from the exploratory analysis.

The hypothesis to be tested was that the impact of foot problems on the QoL of patients with SSc is multifactorial; involving a complex inter-relationship between disease, impairment, personal factors, environmental factors and psychosocial factors.

3.4.3 **Measures for the model in relation to the ICF**

Using the ICF framework as the basis for building the SE model, allowed the impact of foot problems in patients with SSc to be measured at a biopsychosocial level; as recommended by the WHO [280, 367]. The ICF framework was developed around a biopsychosocial model of disability. This model integrated the social model (where disability is a socially created problem and not an attribute of an individual) and the medical model (where disability is

a feature of the person directly caused by disease, trauma and other health conditions). The biopsychosocial model of the ICF encompasses the following components: body function and structure, activity, participation, environmental factors and personal factors (Figure 3.8).

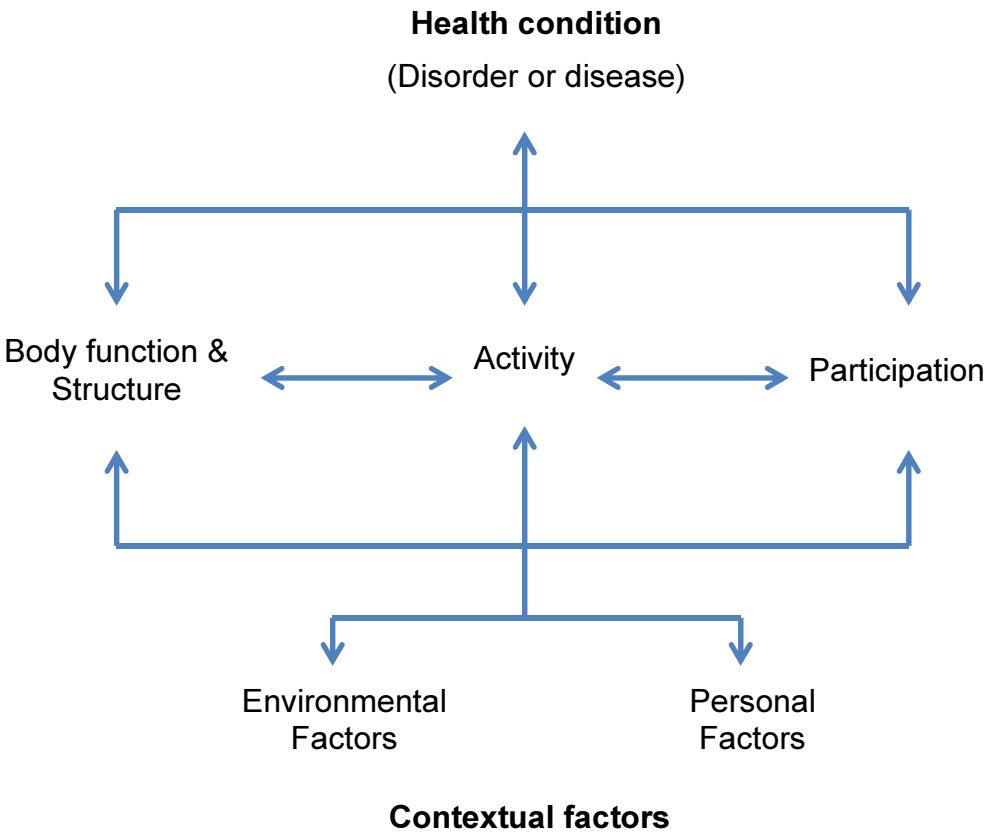

Figure 3.9 ICF framework adapted from WHO [280]

In this study the ICF frameworks was used as a structure for the identification of potential variables that contribute to foot pathology and to its impact on the QoL of patients with SSc. A summary of the constructs and outcomes measures considered in the SE models is present on Table 3.5

Construct	ICF construct	Measure
Demographics	Personal factor	Age
	Personal factor	Gender
	Personal factor	Ethnicity
Disease characteristics	Health condition & environmental factors	BMI
	Health condition	Disease duration
	Health condition	Disease subtype
	Body function and structures & health condition	mRSS
	Body function and structures & health condition	Plantar soft -tissue thickness
	Body function and structures & health condition	Vascular assessments
	Body function and structures & health condition	Neurological assessments
Disease interference characteristics	Health condition	VAS Breathing problems
	Health condition	VAS GI problems interference
	Health condition	VAS DU interference
	Health condition	VAS overall disease severity
	Health condition & environmental factors	VAS Raynaud's interference
Co-morbidities	Health condition	Number of comorbidities
Environmental factors	Environmental factors	Pack years
	Health condition & environmental factors	BMI
Pain	Body function and structures	MFPDI Pain
	Body function and structures	VAS Pain
Function	Activity	SSc HAQ
	Activity	MFPDI Function
	Body function and structures & health condition	MSK assessments
Anxiety	Body function and structures	HADS Anxiety
Depression	Body function and structures	HADS Depression
QoL	No corresponding factor, but embodies all the factors above	SSc QoL

Table 3.5 Summary of constructs and outcomes measures considered in the SE model

3.4.4 **Strategy for analysis of the data**

3.4.4.1 Data preparation for the SEM

To prepare the data for SEM modelling, the variables were explored using exploratory statistics as described in section 4.3. The variables were further explored using correlations and regression models, which together with a coherent theoretical construct of the model obtained from the literature, helped determine the candidate variables that were to be included in the original pre-specified SEM model. Statistical analysis was undertaken using SPSS (Version 20), and AMOS (Version 21).

Prior to use in the SEM, the candidate variables were tested for the required assumptions. SEM is related to multiple regression and therefore the assumptions that need to be considered are similar; the data needs to be interval and to have multivariate normality. Furthermore, there should be no missing data, extreme outliers or evidence of multicollinearity.

Assumptions and missing data

The data was explored to evaluate the validity of these assumptions as described in section 3.3.3.2.1. In addition, additional diagnostic statistics derived as part of the SEM modelling further assess the assumption of multivariate normality using Mardia's coefficient, which provides the multivariate Kurtosis values. Values between 1 and 10 indicate moderate non-normality, while values >10 indicated severe non-normality. When non-normality was

found the bootstrap statistic was used [281, 282, 372]. Missing values were estimated by AMOS using the Maximum Likelihood estimation [372].

Outliers

To detect outliers, two methods were used: a graphical and a statistical method. Data from each variable were explored graphically using a boxplot; this allowed us to identify the presence of outliers and extreme scores. While AMOS case-wise diagnostics provided the Mahalanobis distance. This statistical method identifies any cases for which the observed scores differ substantially from the centroid of scores for all cases [372].

To combat the effects of outliers and the problems with non-normality, the bootstrap method was again used.

Collinearity

Prior to entering the variables in the SEM model, all 45 potential candidate variables for the SEM were assessed for multicollinearity using correlation matrixes. For the correlation matrices the following tests were used depending on the type of variables. Spearman's correlation coefficient was used when one of the variables was continuous but non-normally distributed, and when the variables were ordinal or categorical. Point-biserial correlation was used when one of the categorical variables was a discrete dichotomous variable. Any variables that had a correlation coefficient of 0.8 or greater were considered to be collinear and one of those two variables was excluded from the model, as this indicates redundancy for the model. Relationships between variables that were considered collinear were excluded as they were redundant for the model.

3.5 Structural Equation Modelling (SEM)

After the candidate factors were selected, they were used in the different SE models developed. The data from these variables were imported to AMOS via an SPSS database, as opposed to using a matrix correlation. This data format provided more flexibility in the range of analytical tests that could be applied, such as the use of bootstrapping technique.

An initial model was applied and the SE models were assessed using a series of "fit" statistics summarised in Table 3.6 These statistics consist of Chi-Square (χ^2), Goodness of Fit Index (GFI), Comparative Fit Index (CFI) and Root Mean-Square Error of Approximation (RMSEA). The χ^2 measures the fit between the expected model and the data collected. A significant test indicates that there is a discrepancy between the expected model and the observed data, while a non-significant test indicates a good fit. The GFI measures the variance and covariance present in the data and the model being tested. Values for this test ranges from 0 to 1, any value ≥0.90 are considered an acceptable model fit and 1 a perfect fit. The CFI compares the existing model fit with an independent model /null model, which assumes that the variables in the model are uncorrelated. Results between 0.90 and 0.95 are considered a satisfactory fit and results >0.95 a good fit [371].

The RMSEA test is an absolute test, as it does not require a comparison with a null model. It is interpreted as the amount of information within the empirical covariance matrix that cannot be explained by the proposed model. A good model fit is indicated by a result of <0.05 however a result of ≤0.08 still be considered an adequate fit, and values >0.10 is an invalid model [375]. Out of

the four fit tests, the RMSEA and the CFI are considered the most robust, as they are least affected by sample size.

	Statistical test	Interpretation	Fit Test Criteria	Affected by sample size
Absolute tests	Chi-Square (χ^2)	Measures the fit between the expected model and the data collected	Non significant χ^2	Difficulty in achieving non-significance in large sample size, as small deviations are labelled as significant
	Root Mean-Square Error off Approximation (RMSEA).	Assessed the amount of information within the empirical covariance matrix that cannot be explained by the proposed model	Good fit<0.05 Adequate fit ≤0.08 Invalid model >0.10	Is one of the tests least affected by sample size It is considered a robust test
Comparative tests	Goodness of Fit Index (GFI),	Measures the variance and covariance present in the data and the model being tested	Good fit >0.95 Adequate fit ≥0.90 Invalid model <0.90	When degrees of freedom are large relative to sample size, GFI is biased downward unless the number of parameters is also very large
	Comparative Fit Index (CFI)	Compares the existing model fit with an independent model /null model, which assumes that the variables in the model are uncorrelated	Good fit >0.95 Adequate fit 0.90 - 0.95 Invalid model <0.90	Is a robust test in relatively small samples

Table 3.6 Summary table of fit statistics used in Structural Equation Model

To build the SEM model a series of iterative steps were undertaken:

1. The model was generated
2. The output notes generated by the model were assessed to check that the assumptions had been met.
3. The fit statistics were assessed to check whether the model had a good fit.
4. The diagnostic information was assessed to see whether the model could be improved:
    a. The regression weights showed whether the relationships between variables were significant. Non-significant (p=>0.05) paths were removed; commencing with those that had highest values.
    b. The modification indices suggested any modifications that would improve the model. The modifications were undertaken in a hierarchical fashion, the relationships with the highest modification indices were included first.
    c. The standardised residuals covariance matrices were checked and any value ≥ 2.50 indicated that the relationships were not well captured by the model. Thus, a new relationship between the two variables suggested was added to the model.
5. After each modification was made to the model, the model was re-evaluated by checking the fit statistics and model diagnostics.

All the modifications suggested by the model were made, applying the theoretical construct as opposed to blindly using the modifications that the

model diagnosis suggested. Thus any modification suggested that was not clinically sound was not included.

This process was repeated until the optimal model was produced.

The methods chapter has described the three methodologies used in this thesis i.e literature review, the case-control cross-sectional study and the Structural Equation Modelling. These varied methodologies allowed to achieve the three study's objectives: i) the identification of the potential candidate factor that influenced foot pathology in SSc; ii) the investigation of the candidate factors that contributed to foot pathology and exploration of their impact; ii) and the exploration of the multifactorial pathways in foot pathology and its impact on quality of life. Results from this study are described in Chapter Four.

Chapter 4
Results

4.1 Overview

This chapter presents the results of the studies that explore the multifactorial pathways contributing to the development and impact of foot problems in systemic sclerosis (SSc). The aim of this thesis was to identify the factors that contribute to the development and impact of foot problems in patients with SSc. To achieve this aim, we identified three objectives: first, to identify possible factors that may have an impact on or are associated with foot problems; second to investigate the candidate factors that contribute to foot pathology and explore their impact; and third, to explore the relationship between factors that contribute to foot problems and their impact on the quality of life of patients with SSc. The objectives were met by using clinical and statistical methods as outlined in chapter three and presented in Table 4.1

Phase	Objectives	Summary of Methodological Approach
1	To identify the potential candidate factors that may influence foot pathology in SSc	Review of literature Consultation with experts
2	To investigate the candidate factors that contribute to foot pathology and explore their impact	Cross-sectional study comparing patients with scleroderma and healthy participants
3	To explore the multifactorial pathways in foot pathology and its impact on quality of life	Structural equation modelling exploring the pathways that contribute to foot function impairment, foot pain, and their impact on the quality of life of patients with SSc

Table 4.1 Summary table of the thesis' objectives and methodological approaches

4.2 Phase One: Identification of potential candidate factors.

The potential candidate factors that may contribute to foot problems were identified through published literature and a consultation with two clinical experts: a podiatrist and a consultant rheumatologist, both of whom, specialise in SSc. Several databases, including Embase and Embase Classic (1947 to 2010), Ovid MEDLINE(R) (1946 to 2010), Allied and Complementary Medicine (1985 to 2010) Science Direct (1995 to 2010) and PubMed.gov (1920 to 2010) were searched for terms related to SSc and the foot and SSc pathology, including foot pathology, gait parameter, vasculopathy, neuropathy, musculoskeletal involvement, skin fibrosis and variations thereof. The search provided with a list of the potential candidate factors (Table 4.2), which were then reviewed through consultation with the two clinical experts. The experts were then asked to identify candidate factors that met the following criteria:

(i) Factors that were of relevance to the clinical pathology that patients with SSc commonly present with;

(ii) Factors that were considerate of the pathophysiological mechanisms that underlay the disease that potentially contributed to the development and/or impact of foot problems, and

(iii) Factors that when tested for posed no great burden or discomfort on the patient.

The consultation provided the identification of physical and physiological potential candidate factors, the former affecting the integumentary, vascular, neurological and musculoskeletal systems. A summary of the potential

candidate factors is present in Table 4.2 and a description of the test and methods used to assess these factors is present in Chapter 3

Physical, psychological and psychosocial categories	Original selection of the potential candidate factors considered prior to the experts consultation	Potential candidate factors selected in phase 1
Systems explored		
Integumentary	Skin fibrosis	Skin fibrosis
Integumentary*	Soft-tissue calcification	Number of current and past calcium deposits
Integumentary/ vascular	Ulcerations	Number of current and past ulcerations
Integumentary	Changes in skin pigmentation	*Not selected*
Integumentary	Changes in skin hydration	*Not selected*
Nails	Nail pathology	*Not selected*
Vascular	Raynaud's phenomenon	Raynaud's phenomenon
Vascular	Peripheral arterial obstructive disease	Presence and severity of peripheral arterial disease
Vascular	Microvascular disease	Blood changes in the microvascular bed of the toes
Vascular	Vascular calcification	*No selected*
Neurological	Peripheral nerve dysfunction	Small and large fibre peripheral neuropathy
Neurological	Mononeuropathy	*Not selected*
Neurological	Autonomic neuropathy	*Not selected*
MSK	Abnormal plantar foot pressures	Abnormal plantar foot pressures
MSK	Abnormal pattern in gait and foot function	Abnormal pattern in gait and foot function
MSK	Subcutaneous and fat-pad atrophy	Soft-tissue thickness
MSK	Erosions	*Not selected*
MSK	Osteopenia	*Not selected*
MSK	Osteomalacia	*Not selected*
MSK	Bone demineralisation	*Not selected*
MSK	Juxta-articular osteoporosis	*Not selected*
MSK	Calcaneal spurs	*Not selected*
MSK	Arthritis	*Not selected*
MSK	Joint space narrowing	*Not selected*
MSK	Joint cystic lesions	*Not selected*
MSK	Acro-osteolysis	*Not selected*

Physical, psychological and psychosocial categories	Original selection of the potential candidate factors considered prior to the experts consultation	Potential candidate factors selected in phase 1
MSK	Periarticular fibrosis	*Not selected*
MSK	Flexion contractures of the toes	*Not selected*
MSK	Friction rubs	*Not selected*
MSK	Tendonitis	*Not selected*
MSK	Joint subluxation	*Not selected*
MSK	Muscle weakness and atrophy	*Not selected*
PROMs		
Psychological	Symptom severity and of anxiety and depression	Symptom severity and of anxiety and depression
Physical/ Psychosocial	Health related QoL- Patient's perceived general health status	Health related QoL
Psychosocial	Needs-base QoL- Disease impact on health and well-being	Needs-base quality of life
Physical	Disease specific health status, assessing functional limitations, general and organ-specific symptoms	Disease specific health status, by assessing functional limitations general and organ-specific symptoms
Physical/ Psychosocial	Activity related foot and ankle disability and pain.	Activity related foot and ankle disability and pain.
Physical/ Psychosocial	Raynaud's attacks activity Raynaud's attacks impact	Raynaud's attacks activity Raynaud's attacks impact

Table 4.2 Summary of the potential candidate factors selected from the literature and through experts' consultation. PROMs: patient reported outcomes measures; QoL: quality of life

4.3 Phase Two: Investigation of the candidate factors that contribute to foot pathology and exploration of its impact

The results described in this section are the results obtained from the case-control cross-sectional study, where the outcomes of the detailed clinical assessments of the lower limb and the PROMs were compared between patients with SS and healthy participants. The results from this phase helped inform which of the candidate factors were selected for the structural equation modelling.

4.3.1 **Participant profile**

In order to understand the prevalence and impact of foot pathology in SSc, 174 participants were recruited from February 2011 to February 2013, from the Connective Tissue Disease Clinic at the Rheumatology Outpatients Department in Chapel Allerton Hospital, Leeds Teaching Hospitals NHS Trust. Of the 174 participants, 122 were patients with SSc and 52 were healthy volunteers. Two participants were withdrawn from the study: one SSc patient as a result of misdiagnosis and one healthy participant because shortly after recruitment he disclosed that he was in remission from an autoimmune inflammatory disease

From the remaining 172 participants, 121 were patients with SSc and 51 healthy participants. Participant details are included in Table 4.3. The majority of the participants were northern-European. Ethnic diversity was limited in the study despite recruitment being done in an area that over-represents ethnic minorities in comparison to the national average. In the patient group, there

were more female participants, which reflects the higher female to male ratio characteristic of the disease (mean ratio of 3:1 [16]). In terms of specific disease subtypes, 96 (79%) patients had lcSSc while 24 (20%) had dcSSc and only 1 (0.8%) had SSc sine scleroderma.

Characteristics		Patients with SSc n=121	Healthy participants n=51
Age (years)*		57 (12.3)	50 (14.4)
Male /Female; n		15/106	8/43
Ethnicity; n (%):			
	White British	102 (84%)	38 (74%)
	White Irish	2 (1.7%)	0
	White other	5 (4%)	3 (6%)
	Indian	6 (5%)	4 (7.8%)
	Pakistani	5 (4%)	0
	White and black Caribbean	0	1 (2%)
	Caribbean	0	1 (2%)
	African	0	2 (3.9%)
	Other ethnic group	1 (0.8%)	2 (3.9%)
BMI (Kg/m^2)*		26.29 (5.6)	25.45 (3.9)
Smoking status; n (%)			
	Current smoker	15 (12.9%)	5 (10.2%)
	Ex-smoker	41 (35%)	14 (29%)
	Never smoked	75 (65%)	35 (71%)
Pack years**		12.50 (6.8 to 23.2)	13 (4 to 35)
Number of comorbidities *		4.65 (2.4)	1.04 (1.4)
Disease duration (years)*		9.50 (7.4)	n/a
Disease subtype; n (%)			n/a
	Limited SSc	96 (79%)	n/a
	Diffuse SSc	24 (20%)	n/a
	SSc sine Scleroderma	1 (0.8%)	n/a
mRSS**		2 (0 to 5)	n/a
Number of patients with current or past history of ulcers		59 (49%)	n/a
	N^{o} of patients with current ulcers	18 (15%)	n/a
Number of patients with current or past history of calcium deposits		57 (48%)	n/a
	N^{o} of patients with current calcium deposits	39 (32%)	n/a
Number of medication		7.29 (4%)	1.27 (1.8)
Immunosuppressant: n (%)		29 (24%)	0
	Methotrexate	26 (21%)	0
	Mycophenolate Mofetil	3 (2.5%)	0
	Pulse Cyclophophamide	0	0
	Hydroxychloroquine	0	0
Steroids		26 (21%)	0
Calcium channel blockers		65 (54%)	2 (4%)
ACE inhibitors		48 (40%)	4 (8%)
Beta-Blockers		4 (3%)	4 (8%)
Bosentan		3 (2.5%)	0
Sildenafil		6 (5%)	0
Iloprost (within 3 month prior to the study)		22 (18%)	0
Other medications unclassified		5.47 (3.5)	1.02 (1.54)

Table 4.3: Demographic characteristics of the study participants. * Mean (Standard deviation); ** Median (interquartile Range); BMI: Body Mass Index; mRSS Modified Rodnan Skin Score

4.3.2 **Missing data**

Overall there was a very small amount of missing data. Out of a total of 51 variables across all participants, there were only missing data from six individual variables, of which 16 values (0.18%) were missing from 11 participants. From these 11 participants, 9 were patients with SSc from whom 14 (0.22%) values were missing.

4.3.2.1 Detailed clinical assessments of the lower limb

The results obtained from the clinical assessments were analysed using exploratory statistics to identify the clinical factors that had the potential to differentiate between patients with SSc and healthy participants. The results are grouped in sections according to the system being assessed.

4.3.2.1.1 Integumentary system

The disease specific mRSS was described using summary statistics and description of the median and the interquartile range. The skin involvement in this SSc cohort was relatively mild as the median mRSS score was 2/51. mRSS scores were not derived for the controls and so no direct comparisons are presented (Table 4.4).

Variables	Patients with SSc n=121	Healthy participants n=51	Test statistics	*P* value	Effect size
mRSS** (Score range 0-51)	2 (0 to 5)	n/a	n/a	n/a	n/a

Table 4.4: Integumentary tests statistical analysis summary.** Median (interquartile Range); mRSS Modified Rodnand Skin Score

4.3.2.1.2 Vascular system

The median ABPI values were normal for both groups. The SSc group had a median ABPI of 1.10 vs a median of 1.13 observed in the healthy participants group. The result indicated a between groups median difference in scores of 0.03. This difference was statistically non-significant but had a small effect size (r= 0.11). Analysis of the dorsalis pedis (DP) and tibialis posterior (TP) arterial waveforms showed a statistically significant difference in the proportion of participants in each group who had non-detectable/ monophasic arterial waveforms and in between group participants having biphasic/triphasic arterial waves (Table 4.5). Pearson's χ^2 was also significant indicating the presence of an association between SSc and abnormal pathological arterial waveforms. The strength of these associations was evaluated using by Cramer's statistic; which, out of a possible maximum score of 1, indicated a medium association of 0.22 (p=0.005) for DP arterial waves and 0.24 (p=0.001) for TP arterial waves. The odds of having non-detectable/monophasic DP arterial waveform was 0.32 with exposure to the disease (SSc) and 0.06 without exposure to SSc; with an Odds Ratio (OR) of 5.33. For the TP arterial waves the odds of having a non-detectable/ monophasic wave was 0.21 with exposure to the disease and 0 without exposure. Due to the odds of having a non-detectable/monophasic TP arterial wave for the healthy participants being 0 it was not possible to derive an OR for this variable. A summary of all the vascular findings is presented in Table 4.5.

All the assumptions required for the Pearson's chi-square χ^2 were met: independence was achieved by each participant only contributes to one cell of

the contingency table; the expected frequencies were ≥5 and all expected counts were >1 and no more than 20% were <5 [368].

Variables	Patients with SSc n=121	Healthy participants n=51	Test statistics	P value	Effect size
ABPI**	1.10 (1.02 to 1.18)	1.13 (1.06 to 1.20)	U = 2632	0.126	r=0.11
DP arterial waves					
No-detectable and monophasic	30(24.8%)	3(5.9%)	χ^2=8.275	0.005	CS=0.22 r=0.22
Biphasic and triphasic	91(75.2%)	48(94.1%)	χ^2=8.275	0.005	
TP arterial waves					
No present and monophasic	21 (17.4%)	0(0.0%)	χ^2=10.082	0.001	CS=0.24 r=0.24
Biphasic and triphasic	100(82.6%)	51(100%)	χ^2=10.082	0.001	

Table 4.5: Vascular tests statistical analysis summary. ** Median (interquartile Range), ABPI: ankle brachial pressure index; Effect size, r=Pearson's correlation coefficient, r= 0.10 (small effect), 0.30 (medium effect), 0.50 (large effect).CS: Cremer's statistics interpreted like person's r[368].

4.3.2.1.3 Neurological system

As room temperature and humidity influence the neurological assessments, we compared both temperature (degrees Celsius) and humidity (% relative humidity) when collecting the data for the two different groups and found that conditions were not statistically different. The mean (SD) room temperature while collecting data for the SSc patient group was 23^{o} (1.3^{0}) and relative humidity a mean (SD) 36% (9%), while when collecting data for the healthy participants the environmental temperature and humidity was a mean (SD) of 23^{o} (1.2^{o}) and 36% (9%) respectively.

All the neurological variables except pain threshold on the dorsum and plantar surfaces of the foot were non-normally distributed, therefore the Mann-Whitney

non-parametric test for independent samples and the Monte Carlo (2-tailed) method to calculate the *p*-value, was used to analyse data from these variables. The details are included in table 4.6 with median (IQR) and mean (SD) values as appropriate. When compared to healthy individuals, patients with SSc showed a reduced sensation in all tests undertaken. For the von Frey monofilament N9 test, patients felt the stimulus in a median of seven out of eight sites, while in healthy individuals the median was at all eight sites. This difference was statistically significant (p=0.001) and had a medium effect size(r =0.28). The patients with SSc also had reduced vibration perception threshold compared to healthy participants (1.7 vs 1.29; U = 2257;p= 0.005) with median difference of 0.41 microns, and a considerable reduction of heat perception threshold on the dorsum of the foot (38.46 vs 37.18; U = 2279; p= 0.006) and plantar surface of the foot (39.40 vs 38.75; U = 2432; p= 0.027) with a median difference of 1.28^{o}C and 0.65^{o}C respectively. The difference between groups for these three variables was statistically significant (p= 0.005; p= 0.006; p= 0.027 respectively) with small effect size (r =0.21; r =0.21; r =0.17 respectively). When testing for cold perception threshold on the dorsal (27.13 vs 28.35; U = 2130) and plantar surface of the foot (26.91 vs 26.63; U = 22620) a decreased perception was identified in both locations with a mean difference of 1.22^{o}C for the dorsum and a 0.28^{o}C for the plantar surface. The between-group difference in medians for the cold perception threshold on the dorsum of the foot was statistically significant (p= 0.001) and had a small to medium effect size (r =0.24), while the cold perception threshold on the plantar surface was not statistically significant (p= 0.119) with a small effect size (r =0.12).

For the normally distributed variables of pain threshold on the dorsum and plantar of the foot, the two-sample t-test with bootstrapping was used. The SSc patients showed an impaired perception of pain when compared to healthy individuals on the dorsal (45.06 vs 43.50; t =3.121) and the plantar surface (46.94 vs 46.80; t = 0.221), with a mean difference of 1.56^{o}C for the dorsum and 0.14^{o}C for the plantar surface. The mean difference between groups for the pain perception threshold on the dorsum of the foot was statistically significant (p= 0.008) and had a medium effect size (d=0.48; r =0.23), while for the plantar of the foot this difference was non-significant (p= 0.027) and had no effect size (d=0.03; r =0.01). Results described in Table 4.6.

Variables	Patients with SSc n=121	Healthy participants n=51	Test statistics	P value	Effect size
Von Frey Monofilament N9 (threshold 7.3gr of pressure/mm2, tested in 8 sites of the foot) **	7 (6 to 8)	8 (7 to 8)	*U* = 2079	0.001	r=-0.28
Vibration perception threshold (microns)**	1.7 (0.9 to 4.1)	1.29 (0.62 to 2)	*U* = 2257	0.005	r=0.21
Heat perception threshold foot dorsum (^{O}C)**	38.46 (36.6 to 41.9)	37.18 (35.4 to 39.9)	*U* = 2279	0.006	r=0.21
Heat perception threshold foot plantar (^{O}C)**	39.40 (37.3 to 43.8)	38.75 (36.9 to 40.5)	*U* = 2432	0.027	r=0.17
Cold perception threshold foot dorsum (^{O}C)**	27.13 (24.5 to 28.40)	28.35 (26.4 to 39.6)	*U* = 2130	0.001	r=0.24
Cold perception threshold foot plantar (^{O}C) **	26.91 (24.6 to 27.8)	26.63 (24.2 to 29.1)	*U* = 2620	0.119	r=0.12
Pain threshold foot dorsum (^{O}C)*	45.06 (2.7)	43.50 (2.99)	*t* = 3.121	0.008	d=0.48 r=0.23
Pain threshold foot plantar (^{O}C)*	46.94 (2.49)	46.80 (2.52)	*t* = 0.221	0.844	d=0.03 r=0.01

Table 4.6: Neurological tests statistical analysis summary. * Mean (Standard deviation); ** Median (interquartile Range); Effect size, d= Cohen's d, 0.20 (small effect), 0.50 (medium effect) and 0.80 (large effect) [376], r=Pearson's correlation coefficient, r= 0.10 (small effect), 0.30 (medium effect), 0.50 (large effect)[368].

4.3.2.1.4 Musculoskeletal system

All the variables used to assess the musculoskeletal parameters were non-normally distributed, with the exception of plantar soft-tissue thickness, and vertical ground reaction force at push-off. The variables mean force heel, hindfoot ROM with regards to shank and forefoot ROM with regards to hindfoot, became normally distributed after transformation using log10 or squared root transformation (Table 4.7). Non-normally distributed data was analysed using the Mann-Whitney test for independent samples and the Monte Carlo (2-tailed) method. Where indicated, normally distributed and transformed variables were analysed using the two-sample t-test with the bootstrap method. The details are included in table 4.7 with median (IQR) and mean (SD) values as appropriate

Tissue thickness at 3rd MTP joint and heel

When compared to healthy participants, the SSc patients had a reduced mean soft-tissues tissue thickness at the 3rd MTP joint and heel. This might indicate a structural change of all or some of the soft-tissues located in these areas and could affect the mechanical properties these soft-tissues structures. The between groups mean difference was 1mm under the 3rd MTP joint and of 0.06 mm under the heel. This mean difference was highly statistically significant (t=-1.289; p=<0.0001), but demonstrated a minimal effect size; as indicated by the low values of Cohen's d (d=0.19) and Pearson's correlation (r=0.09) respectively.

Pressure and force at the 3rd MTP joint and heel, and stance time

The maximum mean pressure for the 3rd MTP joint and for the heel variables yielded a median difference between groups of 20 kilopascals (kPa) (U=2825; p=0.374) and 10(kPa) (U=2750; p=0.265). Both differences were not however, statistically significant and effect sizes were minimal (r=0.06; r=0.08 respectively). While excessive plantar pressures can lead to tissue damage as commonly see in patients with diabetes [377], reduced plantar pressures might be an indicator of reduced foot function, impairing loading of the ROI, or increased foot pain, causing avoidance of loading of the specific painful joint/area.

The mean force on the plantar aspect of the 3rd MTP joint and heel were also reduced in patients with SSc. The mean force at the 3rd MTP joint yielded a median difference between groups of 1.6 N, (U=2418), which was a statistically significant difference (p=0.025) with a small effect size (r=0.17). The mean force at the plantar of the heel yielded a median difference between groups of 14N (t=-4.93p=0.001) and a moderate effect size (d=0.75; r=0.35.). The plantar mean forces contribute to the plantar pressures therefore as the force decreases the plantar pressures also tend to decrease. A reduction in forces might be an indicator of foot pathology, as implied and further explained in model one section 4.4.4 .

The stance time was increased in patients with SSc. The median difference between groups for the stance time was of 0.1 second (s), (U=1221, p=<0.0001) and had a medium effect size (r=0.48). The vertical ground reaction force at heel strike showed a median difference between groups of 0.07N (U=1730; p=<0.0001) and a medium size effect (r=0.35). The variable vertical

ground reaction force at push-off had a mean difference between groups of 0.09, (t=-4.30p= <0.0001), and had a size effect close to being considered large (d=0.22; r=0.33). The increase stance time and decrease ground reaction forces are indicators of reduce biomechanical function of the lower limb and/or an antalgic gait, as they imply reduce walking velocity.

Ranges of motion (ROM) at the hindfoot and forefoot

Decrease ROM of the hindfoot and forefoot was present in patients with SSc. From the three variables selected from the literature to represent ROM of the foot "segments" there were two that were statistically significant and yielded a small to medium effect size: the ankle ROM variable, which yielded a median difference between groups of 3^{o} (U=2142; p=0.003; r=0.24), and the forefoot ROM relative to the hindfoot in which there was a median difference between groups of 1^{o} (U=-2.972; p=0.003; d= 0.45; r=0.22). The third variable measuring hindfoot ROM relative to the shank demonstrated a median difference between groups of 1^{o} (U=-1.44), which was not statistically significant (p=0.166) and had a small effect size (d= 0.22; r=0.11). Joint range of motion is one of the major determinants of function. A decrease range of motion in the hindfoot or forefoot can cause foot function impairment.

It is important to clarify that for the log transformed data the median difference between groups is calculated from the raw data while the t-test and the effect size test are calculated from the transformed data. A summary of the biomechanical results is presented in Table 4.7

Variables	Patients with SSc n=121	Healthy participants n=51	Test statistics	*P* value	Effect size
Plantar soft-tissue thickness 3rd MTPJ (mm) *	1.08 (0.25)	1.17 (0.17)	*t* = -1.289	<0.0001	d=0.19 r=0.09
Soft-tissue thickness heel (mm) *	1.56 (0.28)	1.62 (0.21)	*t* = -0.884	<0.0001	d=0.13 r=0.07
Max mean pressure 3rd MTPJ (kPa) **	460 (340 to 577)	480 (370 to 605)	*U*= 2825	0.374	r=0.06
Max mean pressure heel (kPa) **	380 (312 to 480)	390 (340 to 495)	*U*=2750	0.265	r=0.08
Mean force 3rdMTPJ (%BW) **	30 (24 to 34)	31.6 (26 to 36)	*U*=2418	0.025	r=0.17
Mean force heel (%BW)**	90 (76 to 100)	104 (93 to 111)	log10 trans. *t*=-4.930	0.001	d=0.75 r=0.35
Stance time (s)**	0.7 (0.62 to 0.75)	0.6 (0.55 to 0.62)	*U*=1221	<0.0001	r=0.48
Vertical ground reaction force at heel strike (%BW) **	1.02 (1 to 1.08)	1.09 (1.05 to 1.16)	*U*= 1730	<0.0001	r=0.35
Vertical ground reaction force at push-off (%BW) *	1.02 (0.10)	1.11 (0.06)	*t*= -4.30	<0.0001	d=0.72 r=0.33
Ankle ROM (°)**	22 (20 to 25)	25 (20 to 27)	U=2142	0.003	r=0.24
Hindfoot ROM with regards to shank (°)**	9 (7 to 11)	10 (7 to 12)	SQRT trans. t= -1.446	0.166	d=0.22 r=0.11
Forefoot ROM with regards to hindfoot (°)**	13 (10 to 15.2)	14 (12 to 17)	log10 trans. t=-2.972	0.003	d=0.45 r=0.22

Table 4.7: Musculoskeletal tests statistical analysis summary * Mean (Standard deviation); ** Median (interquartile Range; ROM: range of motion; % BW: percentage body weight, s: seconds; Kpa, kilopascals Effect size, d= Cohen's d, 0.20 (small effect), 0.50 (medium effect) and 0.80 (large effect), r=Pearson's correlation coefficient, r= 0.10 (small effect), 0.30 (medium effect), 0.50 (large effect)

4.3.3 **Exploration of the impact of SSc**

The impact across a range of dimensions was captured by six PROMs as described in Chapter 3: the SF-36, the HADS, SSc HAQ, SSc QoL, RCS and the MFPDI. The RCS was removed from the analysis however, following concerns over data quality as patients struggled to answer the questions, reporting that it was difficult to remember how many Raynaud's attacks they had on the day and how long they lasted. For example one of the patients said that she felt like "the Raynaud's was constantly on".

Each of the PROMs provided numeric scores that could be used to quantify relevant dimensions of impact. As described in Chapter 3, the scores from the generic PROMs i.e. SF-36, HADS and MFPDI were compared between groups, by conducting formal statistical tests of difference. The Mann-Whitney test for independent samples and the independent t-test were used to compare healthy participants with patients with scleroderma; the p-values were calculated using the Monte Carlo (2-tailed) method or the bootstrap methods to obtain more robust results. A summary of the results is presented in Table 4.8.

4.3.3.1 Physical impact

Not surprisingly, patients with SSc reported greater physical disability than healthy participants. The SF-36 summary physical score indicated highly significant function impairment in patients with SSc when compared to healthy participants (median 30 vs 56; U=532; p=<0.0001). The variables encompassing physical disability in the SF-36, showed that physical functioning (median 29 vs 55; U=532; p=<0.0001) and role limitations because of physical

health (median 28 vs 56; *U*=825; *p*=<0.0001) was nearly double in the SSc patients compared to the healthy participants. The SSc patient group also reported significantly worse bodily pain (median 37 vs 56; *U*=784; *p*=<0.0001), vitality (median 42 vs 58; *U*=603; *p*=<0.0001) and over all worse general health (median 33 vs 55; *U*=417; *p*=<0.0001).

The physical impairment captured by the SF-36 was reflected in the functional ability that the patients reported in undertaking daily tasks. This was revealed in the results of the SSc HAQ scores (median 1.25; IQR= 0.37 to 2), which indicate that the majority of the patients had between "some difficulty" and "much difficulty" in undertaking basic daily-tasks, demonstrating a substantial negative impact on overall functional ability.

The SSc-HAQ also included several visual analogue scales which captured the interference of some disease symptoms and/or pathology in the patients' daily activities: the results varied from indicating very low interference associated with the digital ulcers VAS (median 0.1; IQR= 0 to 5) to an increase interference with GI problems, breathing problems and pain (medians ranging from 3 to 5); with the highest interference being caused by Raynaud's (medians 5; IQR= 1 to 7). While SSc HAQ scores concurred with the poor general health scores captured by the SF-36, the patients reported a relatively high overall disease severity (medians 5; IQR= 1 to 7 and 2 to 7). In addition to the general physical disability, patients with SSc reported foot related disability using the MFPDI. They reported significant problems with their foot function compared to healthy controls (11 vs 0; *U*=617; *p*=<0.0001) and much greater foot pain than the controls (5 vs 0; *U*=827; *p*=<0.0001).

4.3.3.2 Psychological and psychosocial Impact

Even though impact on the psychological well-being was not as severe as the impact on physical disability, a psychological impact was reported by patients with SSc. The SF-36 mental health summary score indicated a highly significant difference in scores between patients with SSc and healthy participants (46 vs 56; *U*=1741; *p*=<0.0001). Psychological distress was increased in patients with SSc contributing to reduced psychological well-being, and a lower score in the general mental health variable (45 vs 55; *U* = 1727; p=<0.0001). In addition, patients also reported role limitations because of emotional problems (44 vs 55; *U* = 1501; p=<0.0001).

Compared with our healthy volunteers, patients with SSc demonstrated significantly worse scores for both the anxiety (8 vs 4; *U* = 934; p=<0.0001) and depression (6 vs 1; *U* = 934; p=<0.0001) elements of the HADS. This programme of work found that levels of anxiety and depression were disturbingly prominent; with anxiety scores being twice as high and depression scores being five times higher than in healthy participants.

The reduced psychological well-being in patients with SSc seemed to contribute to an impact at the broader psychosocial level. The SF-36 scores for social functioning demonstrated that the disease interfered with social functioning (41 vs 57; *U* = 1061; p=<0.0001) and with health related QoL The impact on the patients' quality of life and psychosocial impairment was also captured by the needs-base SSc QoL, which results indicated that the patients' quality of life was affected to a considerable level, as the overall mean (15; SD= 9) score reported was higher than halve of the maximum score.

Variables	Patients with SSc n=121	Healthy participants n=51	Test statistics	P value	Effect size
SF-36 physical functioning (NBS) (Score range 0-100)	29 (19 to 44)	55 (53 to 57)	*U* = 532	<0.0001	r=0.65
SF-36 role physical (NBS) (Score range 0-100) **	28 (28 to 49)	56 (56 to 56)	*U* = 825	<0.0001	r=0.62
SF-36 bodily pain (NBS) (Score range 0-100) **	37 (29 to 46)	56 (51 to 63)	*U* = 784	<0.0001	r=0.59
SF-36 general health (NBS) (Score range 0-100) **	33 (24 to 43)	55 (53 to 60)	*U* =417	<0.0001	r=0.68
SF-36 vitality (NBS) (Score range 0-100)**	42 (32 to 51)	58 (56 to 63)	*U* = 603	<0.0001	r=0.63
SF-36 social functioning (NBS) (Score range 0-100) **	41 (30 to 52)	57 (51 to 57)	*U* = 1061	<0.0001	r=0.52
SF-36 role emotional (NBS) (Score range 0-100)**	44 (24 to 55)	55 (55 to 55)	*U* = 1501	<0.0001	r=0.44
Sf-36 mental health (NBS) (Score range 0-100) **	45 (37 to 54)	55 (50 to 59)	*U* = 1727	<0.0001	r=0.34
SF-36 summary physical (Score range 0-100) **	30 (24 to 41)	56 (54 to 59)	*U* = 429	<0.0001	r=0.68
SF-36 summary mental health (Score range 0-100)**	46 (36 to 56)	56 (53 to 58)	*U* = 1741	<0.0001	r=0.34
HADS anxiety (Score range 0-21) **	8 (4.49)	4.57 (3.59)	t = 4.832	<0.0001	d=0.74 r=0.35
HADS depression (Score range 0-21) **	6 (2 to 9)	1 (0 to 2)	*U* = 934	<0.0001	r=0.55
MFPDI function (Score range 0- 20) **	11 (4 to 16)	0 (0 to 0)	*U* = 617	<0.0001	r=0.65
MFPDI pain (Score range 0-10) **	5 (2 to 7)	0 (0 to 0)	*U* = 827	<0.0001	r=0.59
SSc HAQ (Score range 0-3) **	1.25 (0.37 to 2)	n/a	n/a	n/a	n/a
SSc QoL (Score range 0-29)*	15 (9)	n/a	n/a	n/a	n/a
VAS Pain (Score range 0-10) **	4 (2 to 7)	n/a	n/a	n/a	n/a
VAS Raynaud's interference (Score range 0-10) **	5 (1 to 7)	n/a	n/a	n/a	n/a
VAS DU interference (Score range 0-10) **	0.1 (0 to 5)	n/a	n/a	n/a	n/a
VAS GI problems (Score range 0-10) **	3 (0.1 to 6)	n/a	n/a	n/a	n/a
VAS Breathing problems (Score range 0-10) **	3 (0.3 to 7)	n/a	n/a	n/a	n/a
VAS Overall disease severity (Score range 0-10) **	5 (2 to 7)	n/a	n/a	n/a	n/a

Table 4.8: PROMs statistical analysis summary. * Mean (Standard deviation); ** Median (interquartile Range); VAS, visual analog scale; DU, digital ulcers; GI, gastrointestinal; Effect size, d= Cohen's d, 0.20(small effect), 0.50 (medium effect) and 0.80(large effect), r=Pearson's correlation coefficient, r= 0.10 (small effect), 0.30 (medium effect), 0.50 (large effect)

4.4 Phase Three: Multifactorial pathways

This section presents the results of the analyses that were undertaken to explore the pathways of foot pathology and disease influences on quality of life in patients with SSc. Using the candidate factors identified in Phase two (Section 4.3), in phase three we explored the influences of these factors on foot pathology and quality of life using models defined *a-priori,* and based on ICF framework. As described in Chapter 3, the data was explored first using exploratory analyses and the regression modelling and then tested using structural equation modelling (SEM). This study describes the relationships between quality of life and personal factors, physical and psychological impairment, factors associated with SSc and psychosocial factors using SEM, which explicitly tests the hypothesis of the thesis.

4.4.1 **Participant profile**

Data for the SEM were collected in Phase two of this programme of work and only included the 121 patients with SSc. Demographics and patient characteristics have been presented previously in Table 4.3. All data were explored and checked for distribution and presence of outliers. Data for each individual were checked and outliers were removed, as required for SEM. Of note, there was no missing data in those variables entered for the SEM so all 121 patients were included in the final analysis.

4.4.2 **Preparation of the Data**

The variables considered to be potential candidate factors after phases 1 and 2 were explored for normality through graphical and statistical methods. Graphically a histogram and box-plot was plotted for each variable, and a test of significance of skewness and kurtosis was conducted. The results from the skewness and kurtosis tests indicated that out of 44 variables which were potential candidate factors, only 8 were normally distributed: age, number of comorbidities, HADS anxiety, plantar soft-tissue thickness 3rd MTPJ, soft-tissue thickness heel, pain threshold foot dorsum, pain threshold foot plantar and vertical ground reaction force at push-off. However, all statistical tests for normality are based on null hypothesis significance testing, meaning that in large samples they can reach significance even for small and unimportant effects while in small samples they lack the power to detect important effects. For larger sample sizes (i.e. n= ≥ 30 or in some cases 100) the test for normality can be avoided by applying the central limit theorem. This theorem indicates that in some situations we can assume normality independently of the shape of the data [368]. This central limit theorem is justifiable in relation to these study data as the sample for this study was 121 patients with SSc and 50 healthy individuals.

In the multiple regression models the central limit theorem was taken into consideration in addition to the bootstrap procedure.

However, for the exploratory analysis between groups, a more conservative approach was taken when selecting the statistical tests and non-parametric tests were used to analyse the non-normal distributed variables.

Collinearity was assessed as described in section 3.4.4.1. Only three of the 44 variables that were potential candidate factors for the SEM had a Spearman's rho (r_s)value above 0.80. The collinearity existed between the variable QoL and HADS depression (r_s= 0.84) and QoL and HAQ DI (r_s= 0.81).

While values of above 0.8 can be deleted from analyses, these correlations were considered borderline as the theoretical construct is that these variables are not directly measuring the same underlying construct but because quality of life is highly affected by depression and impaired function. Consequently, HADS depression and HAQ DI were not excluded from SE model. Correlations are present on Figure 4.1.

rowtype_	varname_	Age	Gender	BMI	Diagnosis	Dise. Dura.	N. comor.	Pack years	mRSS	N. ulcers	N. calcium	Tis. thick. 3rd	Tis. thick. Heel	M.M.Press.3rd	M.M.Press. Heel	M.Forc.3rd	M.Forc.Heel	Stance Time	VGRF Heel-strike	VGRF push-off	Ankle	Hindfoot-Shank	Forefoot-Hindfoot	ABPI	DP Art.Wav	TP Art.Wav	Monofil.N9	Vibration	Heat dorsum	Heat plantar	Cold dorsum	Cold plantar	Pain dorsum	Pain plantar	Anxiety	Depression	VAS Pain	VAS Internal	VAS Breathing	VAS Raynaud's	VAS Du	VAS Dis. Sev.	HAQ-DI	SSc-QoL	MFPDI Funct.
n		121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121	121				
corr	Age	1																																											
corr	Gender	0.173	1																																										
corr	BMI	-0.062	-0.013	1																																								
corr	Diagnosis	-0.245	-0.115	-0.068	1																																								
corr	Disease duration	0.039	0.147	-0.244	-0.065	1																																						
corr	Number of comorbidities	0.213	0.144	0.104	-0.12	0.15	1																																					
corr	Pack years	0.238	-0.15	-0.103	-0.167	-0.023	0.231	1																																				
corr	mRSS	-0.12	-0.102	-0.185	0.369	0.147	-0.031	-0.121	1																																			
corr	Number of current ulcers	-0.109	0.024	-0.159	0.125	0.176	0.082	0.059	0.203	1																																		
corr	Number of current calcium deposits	0.014	0.139	-0.11	0.025	0.176	0.007	0.024	0.237	0.263	1																																	
corr	Soft-tissue thickness 3rd MTPJ plantar	-0.092	-0.239	0.217	-0.18	-0.34	-0.137	-0.021	-0.177	-0.127	-0.208	1																																
corr	Soft-tissue thickness Heel	-0.145	-0.186	0.479	-0.094	-0.306	-0.132	-0.078	-0.237	-0.342	-0.256	0.441	1																																
corr	MAX Mean Pressure 3rd MTPJ	0.016	0.026	0.178	-0.155	0.194	-0.004	-0.074	-0.09	-0.086	-0.038	-0.427	-0.034	1																															
corr	Max Mean Pressure Heel	-0.183	-0.169	-0.003	0.045	0.233	-0.189	-0.108	0.166	0.139	0.039	-0.161	-0.258	0.223	1																														
corr	Mean Force 3rd MTPJ	-0.026	-0.079	-0.192	-0.165	0.072	-0.032	0.12	-0.034	-0.044	-0.115	-0.089	-0.051	0.496	0.07	1																													
corr	Mean Force Heel	-0.207	-0.261	-0.28	-0.094	0.047	-0.313	-0.132	-0.008	0.025	-0.135	0.153	0.064	0.053	0.565	0.329	1																												
corr	Stance Time V3D	0.244	-0.02	0.269	0.034	0.01	0.278	0.283	-0.111	0.068	0.054	-0.071	-0.058	-0.056	-0.234	-0.271	-0.579	1																											
corr	Vertical Ground Reaction Force 1	-0.205	-0.105	-0.388	-0.004	-0.137	-0.303	-0.003	0.023	-0.013	-0.086	0.053	0.029	-0.076	0.133	0.234	0.516	-0.659	1																										
corr	Vertical Ground Reaction Force 3	-0.014	-0.001	-0.433	-0.093	-0.037	-0.324	-0.033	0.059	-0.089	-0.017	0.137	-0.071	-0.057	0.119	0.286	0.438	-0.577	0.611	1																									
corr	Ankle Range of Motion	-0.11	0.073	-0.048	-0.098	0.117	-0.134	-0.089	-0.088	0.018	0.044	0.219	0.126	-0.117	0.079	0.089	0.174	-0.048	0.115	0.165	1																								
corr	Hindfoot with regards to Shank ROM	-0.154	0.078	0.095	-0.228	-0.067	-0.054	0.021	-0.303	-0.18	-0.051	0.179	0.216	0.027	-0.214	0.123	-0.01	-0.129	0.025	-0.009	0.185	1																							
corr	Forefoot with regards to Hindfoot ROM	-0.072	0.205	-0.059	-0.12	0.006	-0.223	-0.07	-0.148	0.025	0.09	0.008	-0.016	0.062	0.006	0.071	0.073	-0.167	0.069	0.193	0.424	0.307	1																					
corr	Ankle brachial pressure	-0.089	-0.114	0.071	0.078	0.012	-0.066	-0.055	-0.104	-0.163	-0.045	0.064	0.051	0.014	0.157	0.119	0.086	-0.148	0.027	0.086	-0.011	-0.053	0.042	1																				
corr	DP Arterial Waves	0.067	-0.1	0.198	0.105	-0.219	-0.069	-0.008	-0.154	-0.256	-0.084	-0.035	0.102	0.147	0.086	0.05	0.047	-0.05	-0.084	-0.079	-0.018	0.144	0.059	0.168	1																			
corr	TP Arterial Waves	-0.094	-0.04	0.216	0.127	-0.103	-0.028	-0.246	-0.057	-0.116	-0.252	0.083	0.097	-0.036	0.235	0.016	0.117	-0.075	-0.05	-0.047	-0.038	-0.006	-0.029	0.367	0.293	1																		
corr	Von Frey Monofilaments N9	-0.308	0.044	-0.054	0.064	-0.093	0.064	-0.163	0.123	0.122	0.085	-0.091	-0.044	0.227	0.142	0.26	0.187	-0.329	0.118	0.13	0.049	0.076	0.107	0.147	0.116	-0.07	1																	
corr	Vibration perception threshold	0.463	-0.064	0.015	-0.163	-0.057	0.124	0.299	-0.23	-0.083	0.017	0.036	-0.085	-0.106	-0.059	-0.069	-0.133	0.25	-0.072	-0.081	-0.116	-0.046	0.011	-0.015	0.105	0.058	-0.354	1																
corr	Heat perception threshold dorsum of the foot	0.23	-0.134	0.012	-0.002	0.127	0.112	0.273	-0.116	0.078	0.054	-0.008	-0.113	-0.103	-0.041	-0.095	-0.155	0.351	-0.147	-0.177	-0.041	-0.064	-0.126	-0.028	-0.088	0.058	-0.396	0.256	1															
corr	Heat perception threshold plantar of the foot	0.05	-0.157	0.036	0.046	0.19	0.2	0.249	-0.061	0.024	-0.008	-0.05	0.007	-0.016	0.034	-0.121	-0.097	0.31	-0.048	-0.195	0.004	-0.048	-0.265	-0.052	-0.153	0.027	-0.321	0.138	0.628	1														
corr	Cold perception threshold dorsum of the foot	-0.241	0.055	0.129	0.043	-0.033	-0.066	-0.206	0.019	0.044	0.055	0.098	0.15	0.036	0.129	0.032	0.117	-0.247	0.053	0.127	0.187	0.056	0.2	0	0.132	0.041	0.251	-0.227	-0.432	-0.327	1												
corr	Cold perception threshold plantar of the foot	0.084	0.097	0.023	-0.033	-0.078	0.077	0.032	-0.004	-0.008	0.018	-0.003	-0.073	0.113	-0.078	0.121	-0.097	-0.123	-0.136	0.101	-0.009	-0.04	0.188	-0.048	0.015	-0.059	0.139	-0.058	-0.296	-0.494	0.362	1											
corr	Pain threshold dorsum of the foot	0.092	0.022	-0.05	-0.122	0.165	0.004	0.032	-0.176	-0.034	0.064	-0.041	-0.064	-0.042	-0.06	-0.037	-0.029	0.111	-0.041	-0.039	0.029	0.017	-0.031	-0.038	-0.14	-0.056	-0.259	0.188	0.447	0.38	-0.205	-0.192	1										
corr	Pain threshold plantar of the foot	-0.209	-0.114	0.198	0.057	0.032	-0.025	-0.01	-0.079	-0.032	0.031	0.218	0.159	-0.1	-0.007	-0.145	0.008	0.066	0.032	-0.108	0.206	0.055	-0.1	0.053	-0.06	0.06	-0.178	0.038	0.193	0.456	0.05	-0.297	0.343	1								
corr	HADS- Anxiety Score	0.01	0	0.199	-0.026	-0.107	0.327	0.019	-0.186	-0.086	-0.111	0.041	0.119	0.017	-0.178	-0.032	-0.204	0.227	-0.079	-0.309	-0.043	0.08	-0.04	0.129	0.071	0.107	0.043	0.094	0.098	0.161	-0.131	-0.086	0.088	0.064	1							
corr	HADS- Depression Score	0.038	-0.102	0.217	0.107	-0.048	0.418	0.2	-0.054	0.051	-0.037	0.007	-0.058	-0.101	-0.148	-0.121	-0.38	0.391	-0.274	-0.43	-0.224	-0.06	-0.175	-0.025	0.021	0.067	-0.05	0.24	0.192	0.169	-0.165	0.005	0.11	-0.002	0.683	1						
corr	VAS Pain	-0.098	0.02	0.113	0.042	0.079	0.21	0.116	0.002	0.178	0.057	0.008	-0.045	-0.103	0.008	-0.232	-0.278	0.32	-0.249	-0.355	-0.043	-0.194	-0.145	0.078	-0.102	0.003	-0.038	0.06	0.202	0.238	0.007	0.062	0.125	0.155	0.305	0.496	1					
corr	VAS Internal Problems Interference	0.045	0.023	0.171	-0.036	0.106	0.365	0.13	-0.031	0.074	0.141	-0.059	0.115	-0.112	-0.135	-0.234	-0.263	0.288	-0.237	-0.335	-0.165	-0.087	-0.153	0.032	-0.048	-0.108	-0.064	0.049	0.112	0.278	-0.065	-0.178	0.081	0.125	0.388	0.412	0.501	1				
corr	VAS Breathing Problems Interference	0.114	-0.122	0.136	0.121	0.014	0.254	0.202	0.062	0.102	-0.025	0.062	-0.053	-0.115	-0.08	-0.151	-0.259	0.374	-0.287	-0.279	-0.034	-0.124	-0.12	0.049	0.063	0.062	-0.09	0.03	0.11	0.175	-0.104	-0.074	-0.011	0.07	0.263	0.418	0.436	0.387	1			
corr	VAS Raynaud's Interference	-0.228	-0.11	-0.076	0.141	0.095	0.129	-0.014	0.124	0.217	0.119	0.03	-0.058	-0.23	-0.075	-0.198	-0.154	0.187	-0.048	-0.239	-0.031	-0.067	-0.161	-0.013	-0.199	0.022	-0.079	-0.138	0.123	0.196	-0.031	-0.099	0.199	0.232	0.384	0.381	0.475	0.433	0.382	1		
corr	VAS Du Interference	-0.121	-0.088	-0.148	0.081	0.346	-0.038	-0.005	0.303	0.402	0.299	-0.124	-0.067	-0.04	0.134	-0.072	0.071	-0.019	0.035	0.001	0.066	-0.153	-0.11	-0.021	-0.177	-0.166	0.029	-0.214	0.065	0.135	0.024	-0.104	0.119	0.142	-0.081	0.007	0.252	0.314	0.177	0.419	1	
corr	VAS Disease Severity	-0.023	-0.127	-0.007	0.013	0.028	0.274	0.127	-0.02	0.184	0.021	-0.01	-0.129	-0.14	-0.016	-0.21	-0.172	0.31	-0.17	-0.298	-0.119	-0.169	-0.185	-0.001	-0.102	0.002	-0.123	0.076	0.17	0.224	-0.128	0.03	0.101	0.114	0.374	0.544	0.713	0.489	0.613	0.612	0.271	1
corr	HAQ-DI	0.014	0.019	0.137	0.147	0.129	0.458	0.195	0.074	0.272	0.039	-0.135	-0.119	-0.107	-0.084	-0.262	-0.39	0.503	-0.355	-0.481	-0.128	-0.134	-0.187	-0.066	-0.064	0	-0.129	0.089	0.235	0.253	-0.143	-0.053	0.063	0.115	0.411	0.661	0.643	0.453	0.528	0.449	0.183	0.576	1
corr	SSc-QoL	-0.027	-0.035	0.126	0.11	-0.024	0.411	0.146	-0.107	0.164	-0.032	-0.023	-0.114	-0.153	-0.186	-0.192	-0.34	0.411	-0.257	-0.419	-0.198	-0.052	-0.163	-0.001	0.002	0.048	-0.079	0.114	0.169	0.137	-0.197	-0.02	0.099	0.009	0.669	0.843	0.591	0.509	0.51	0.511	0.102	0.664	0.813	1
corr	MFPDI Functional	0.001	0.026	0.243	0.139	0.124	0.37	0.168	-0.034	0.144	-0.01	-0.052	-0.069	-0.14	-0.114	-0.228	-0.422	0.473	-0.286	-0.502	-0.106	-0.135	-0.232	-0.024	-0.084	-0.054	-0.11	0.07	0.235	0.289	-0.123	-0.121	0.157	0.102	0.411	0.602	0.554	0.462	0.494	0.424	0.037	0.507	0.715	0.677	1
corr	MFPDI Pain	-0.097	0.099	0.162	0.073	0.105	0.206	0.05	0.018	0.234	-0.016	0.057	0.027	-0.113	-0.153	-0.092	-0.277	0.215	-0.088	-0.266	-0.064	-0.138	-0.124	0.082	-0.209	-0.173	-0.096	-0.043	0.211	0.171	-0.037	-0.103	0.082	0.023	0.288	0.403	0.503	0.389	0.306	0.359	0.173	0.382	0.521	0.499	0.708	1

Figure 4.1 Correlation matrix for the SEM candidate factors. All values are Spearman's rho. Variables with a r_s value above 0.80 are highlighted

4.4.2.1 Multiple regression analysis

Multiple regression analysis was initially used to further explore the potential suitability of the candidate factors identified in Phases 1 and 2.

Four different regression models were constructed for each of the four systems assessed clinically (i.e. integumentary, vascular, neurological, and musculoskeletal) and using PROMS as the dependent variable including the QoL, HAQ, MFPDI function and MFPDI pain. From the early iterations of these models the contribution of the clinical variables to the model was either non-existing or small, as the adjusted R^2 values for these clinical variables were within the range of 0.000 to 0.26. Of note, the adjusted R^2 value tended to increase when other the PROMs variables such as the VAS were included as independent variables. The addition of other PROMS as independent variables resulted however in models that worked statistically but were overwhelmed by the PROM-based independent variables while the clinical independent variables were having very small effect, defeating the purpose of the modelling helping the selection of the clinical variables. Ultimately it was not possible to derive robust enough multiple regression models to enable the selection of the candidate factors for the SEM. Instead the selection of the candidate factors for entry into the SEM was based on the theoretical construct and the exploratory statistics.

4.4.3 **Initial Model Analysis**

Informed by the literature review and the outcomes of the cross-sectional study in Phase 2, a structural equation model was constructed based on the theoretical construct. This mode was intended to explore the impact of the physical and psychosocial factors on quality of life. This model is presented in Figure 4.2

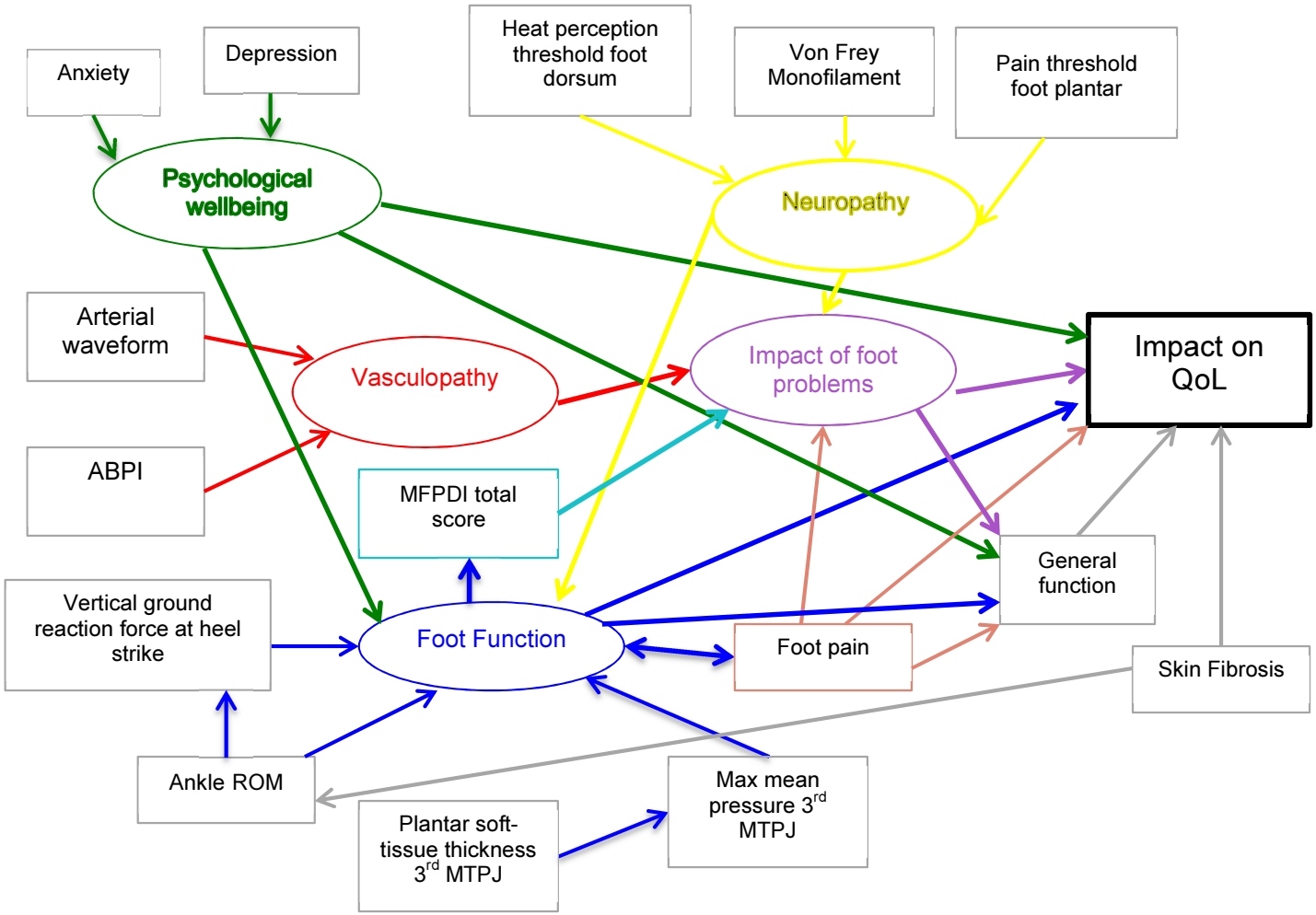

Figure 4.2 Example of an initial pre-specified theoretical model underlined by the hypothesis, that was to be tested by the SEM modelling

Using AMOS, this initial theoretical model was analysed using SEM, but even after undertaking several modifications and iterations, including modification and alterations to the latent variables the initial model could not be made to work. While this is not unusual in SEM, it highlighted that the clinical assessments included under vasculopathy, neuropathy and foot function did not map onto a shared construct, even with substantial manipulation and review of the model. From this we can summarise that our initial model, while grounded in a reasonable theoretical construct, is not supported by the data.

Of note, there are model types in SEM that do not use substantive latent variables (even though most of the SE models have error terms represented as latent variables). These type of models concern effects only among observed variables and employ a technique is known as path analysis, which is a member of the SEM family[282]. A path model is a structural model for observed variables, and a structural model represents hypothesis about effect priority i.e that X is a cause of Y [282].All the models obtained from this study data used path analysis as no substantive latent variables were obtained.

As the data did not fit the theoretical models this left only two options, either to abandon the model or modify the hypothesis on which the initial model was based. Therefore, in order to understand the pathways around foot pathology and their impact on quality of life more fully and in differing dimensions, three further models were developed: the first model was developed to capture the factors contributing to the dimension of foot pain, the second to capture the factors contributing to the dimension of foot function and the third model to capture the impact of foot problems on overall QoL in patients with SSc. As a consequence of doing this refinement and developing new models, the SEM

approached changed to model generation, as opposed to the initial model confirmation. The model generation process aims to discover a model with three properties: i) the model makes theoretical sense, ii) it is reasonably parsimonious; and iii) its correspondence with the data is acceptably closed[282]. All the modified models achieved these three properties.

The outcomes from each model are detailed in the subsequent sections. Of note, while in all three dimensions it was possible to construct working models with good fit statistics, none of the models contained latent variables.

4.4.4 Model One: exploration of multifactorial pathways that contribute towards foot function

As described above, a model was generated from the candidate variables to identify the factors that contribute to the impact of foot problems in relation to foot function. The initial model is presented in Figure 4.3. When we analysed the model, it was not supported by the data as demonstrated by a significant χ^2 statistics (χ^2=79.45, df = 51, p= 0.007) and the fit statistics, which indicated a poor model fit (RMSEA= 0.068, GFI= 0.904;CFI= 0.914). To improve the model fit the model diagnostics information was assessed. The regression weights of each variable's relationship were explored and those with a non-significant path were removed in an iterative process, starting with those paths that had the highest *p* value. After removal of the indicated path the overall model fit and the fit statistics were reviewed.

Once all the non-significant variables' relationships were removed, the modification indices were assessed for any suggested relationships between

variables that might improve the model fit. Any additional relationship that was suggested was included, starting with those relationships with the highest modification indices. During this iterative processes the standardised residuals covariance matrices were checked and any relationship that had a covariance value ≥ 2.50 was an indication that the relationship was not captured in the model, thus new a relationship was added. This iterative process was followed until the best fitting model was obtained. The iterations of the model indicating which relationships were removed or added are summarised in Table 4.9

The assumption of multivariate normality among the variables in this model was not met. The Mardia's coefficient was 57.71, while any value >10 indicates severe non-normality, therefore the use of the bootstrapping techniques was necessary to minimise bias and obtain more robust results [281, 372, 378].

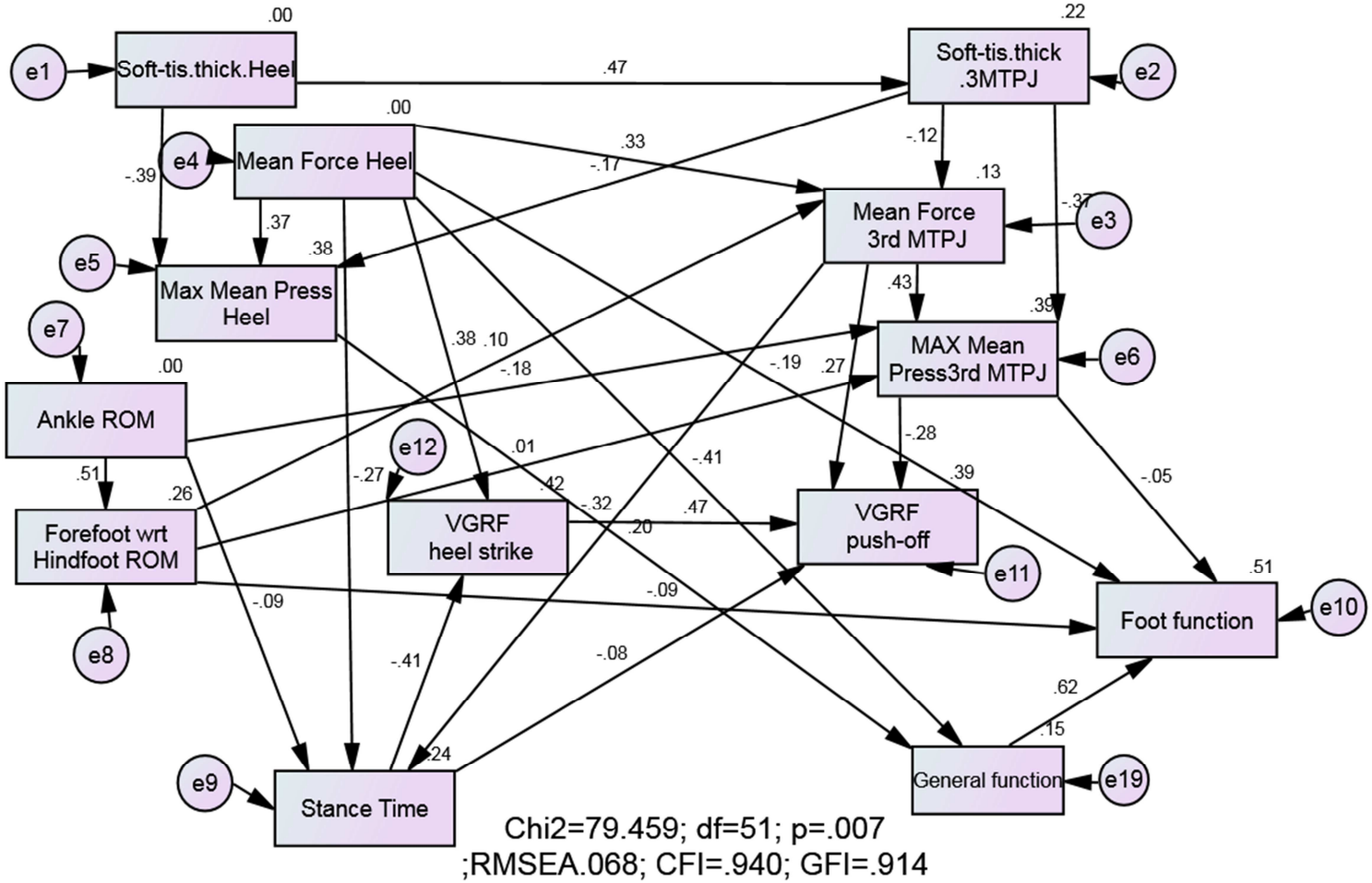

Figure 4. 3 Structural equation model testing the factors contributing to impairment of foot function in patients with SSc. The numbers at the top right corner of each variable represents the R^2 value, or percentage of variance of the variable explained by the model. The arrows represent the direction in which the relationship is and the values on top of each line are the regression weights. The larger the regression weights the better predictor the variable is of the variable that the arrow is feeding into.

Model	N	Relationships Modifications	χ^2	df	*P* Ideal: >0.05	RMSEA Ideal: <0.05	CFI Ideal: >0.95	GFI Ideal: >0.95
1	121	Initial model	79.45	51	0.007	0.068	0.940	0.914
2	121	Remove relationship 3rd MTPJ plantar pressure ➔foot function	80.17	52	0.007	0.067	0.940	0.912
3	121	Remove relationship stance time ➔VGRF push-off	80.91	53	0.008	0.066	0.941	0.910
4	121	Remove relationship forefoot ROM with regards to hindfoot ➔ 3rd MTPJ plantar pressure	80.91	54	0.010	0.064	0.943	0.911
5	121	Remove relationship forefoot ROM with regards to hindfoot ➔ foot function	82.58	55	0.009	0.65	0.941	0.909
6	121	Remove relationship ankle ROM ➔stance time	83.80	56	0.009	0.064	0.941	0.907
7	121	Remove relationship forefoot ROM with regards to hindfoot ➔ mean force 3rd MTPJ	85.08	57	0.009	0.064	0.940	0.905
8	121	Add relationship stance time ➔ general function	74.69	56	0.048	0.053	0.960	0.916
9	121	Add relationship general function ➔VGRF push-off	63.85	55	0.193	0.037	0.981	0.925
10	121	Add relationship soft-tissue thickness 3rd MTPJ ➔ankle ROM	54.21	54	0.466	0.006	1.000	0.936
11	121	Remove relationship soft-tissue thickness 3rd MTPJ ➔ mean force 3rd MTPJ	56.07	55	0.434	0.013	0.998	0.934
12	121	Remove variable forefoot ROM with regards to hindfoot	47.05	44	0.349	0.024	0.993	0.942
13	121	Add relationship VGRF push-off ➔foot function	44.23	43	0.419	0.015	0.997	0.945
14	121	Change direction relationship general function➔ stance time	47.26	43	0.303	0.029	0.990	0.941

Table 4.9 Summary table of iterations of structural equation model for foot function.

The best fitting foot function model is a complex model as it included a number of variables and parameters. Overall the model shows a good fit (χ^2=47.267, df = 43, p= 0.303, RMSEA= 0.029, GFI= 0.990, CFI= 0.941), and explains half of the variance (R^2 =0.51) that is associated with altered foot function in patients with SSc (graph presented in Figure 4.4). Eleven variables are identified in the model, two of which have a direct effect on foot pain, general function (R_{gwt}= 0.58) and ground reaction force at push-off (R_{gwt}=- 0.12), and one (mean force heel) which had both a direct (R_{gwt}= -0.16) and indirect effect.

The other eight variables had an indirect effect on foot function: soft-tissue thickness on heel and 3rd MTPJ, maximum mean pressure heel and 3rd MTPJ, mean force 3rd MTPJ, ground reaction force on heel strike and push-off, stance time and ankle ROM. As these variables had an indirect effect however, their impact on foot function was mediated through mediator variables. Thickness of the soft-tissue on the heel is associated with a direct effect on the soft-tissue thickness under the 3rd MTPJ (R_{gwt}= 0.47) and on the maximum mean heel plantar pressure (R_{gwt}= -0.39). While an increase in heel plantar pressures is associated with an increase in impairment of general function (R_{gwt}= 0.20).

Foot function was influenced by general function, as measured by the HAQ-DI. General function is also directly affected by the mean force of the heel (R_{gwt}= -0.41), thus those patients that have a decrease in the mean force going through the heel during gait are more likely to have poorer general function. An increase in the mean force going through the heel also has the effect of increasing the vertical ground reaction force going through the foot at heel strike (R_{gwt}= 0.38) and a decrease in the time spent in weight bearing (i.e. stance phase, R_{gwt}= -

0.22). In addition, a decrease in the time spent in the stance phase of gait also decreases the vertical ground reaction force passing through the foot at heel strike (R_{gwt}= -0.40). Patients with a decreased vertical ground reaction force at heel strike were more likely to have a decreased vertical ground reaction force at the push-off phase during gait (R_{gwt}= 0.44); while a decrease in the later indicates a decrease in foot function (R_{gwt}= -0.12). Conversely, a decrease in maximum mean plantar pressure under the 3rd MTPJ predicts an increase in the vertical ground reaction force at push-off (R_{gwt}= -0.26).

Those patients with an increased mean force going through the plantar aspect of the 3rd MTPJ during gait are more likely to have increased maximum plantar pressures under this area (R_{gwt}= 0.43) and an increase vertical ground reaction force at push off (R_{gwt}= 0.22). An increase in soft-tissue thickness under the 3rd MTPJ predicts a decrease in max mean plantar pressures under this region (R_{gwt}= -0.37).

Of note, a decrease in ankle ROM predicts an increased in maximum mean plantar pressures under the 3rd MTPJ is (R_{gwt}= -0.17). While a decrease in soft-tissue thickness under the 3rd MTPJ, is related to a decreasing ankle ROM (R_{gwt}= 0.28). Furthermore, a decrease in soft-tissue thickness under the 3rd MTPJ predicts an increase of maximum mean pressure under the heel during gait (R_{gwt}= -0.17), which in turn predicts a decrease in general function (R_{gwt}= 0.20). General function is one of the predictors having a direct effect on stance time (R_{gwt}= 0.23) and foot function (R_{gwt}= 0.58), so patients that reported impaired general function were more likely to have an increased stance time during gait and impaired foot function.

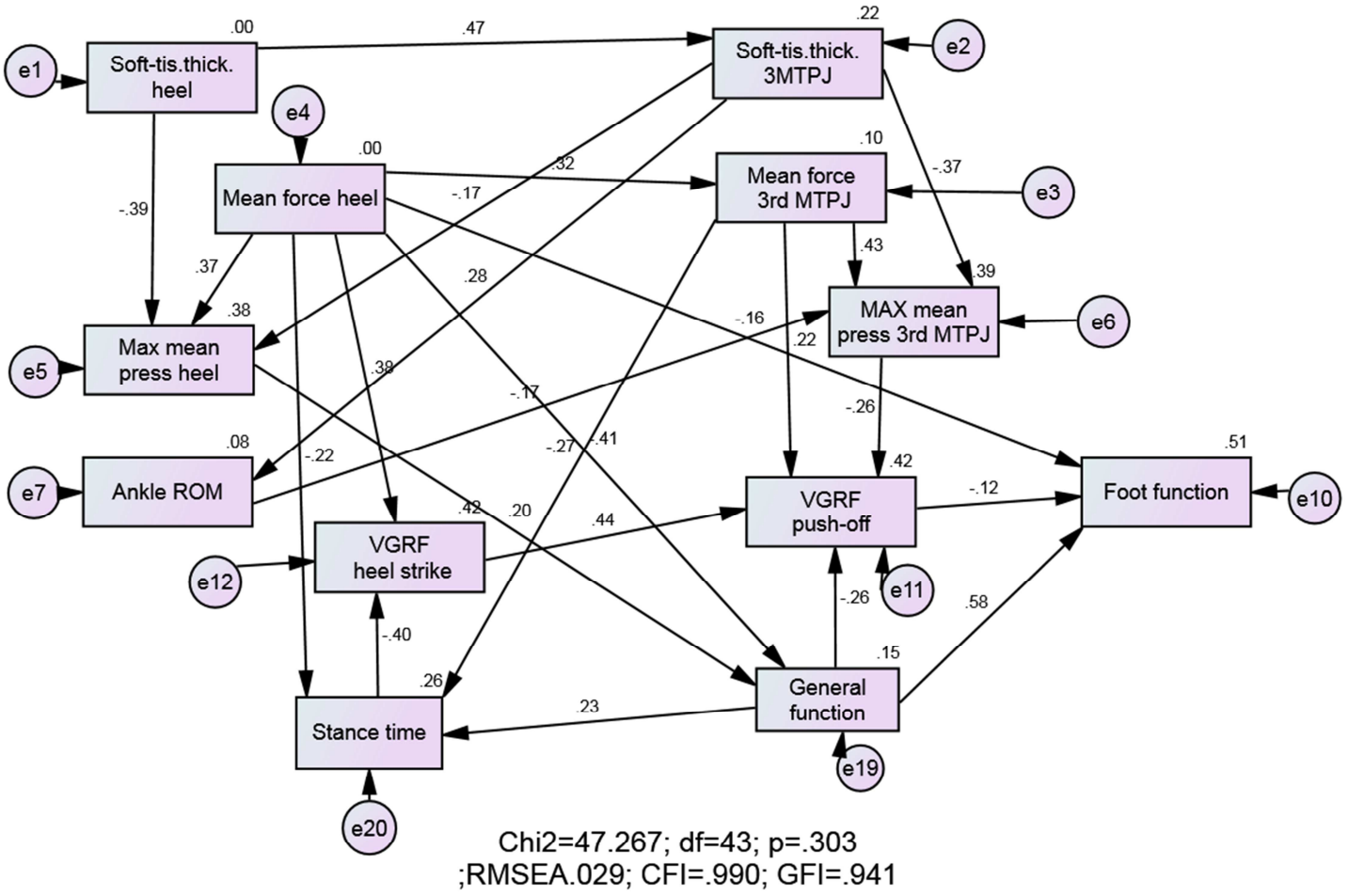

Figure 4.4 .Best fitting structural equation model for the factors contributing to impairment of foot function in patients with SSc. The numbers at the top right corner of each variable represents the R^2 value, or percentage of variance of the variable explained by the model. The arrows represent the direction in which the relationship is and the values on top of each line are the regression weights. The larger the regression weights the better predictor the variable is of the variable that the arrow is feeding into. The variables disease duration, number of comorbidities, age, gender and diagnosis subtype when entered in the model they did not contribute to the model.

In summary, when the combined direct and indirect regression weights are considered, it is clear that poor general function is the highest single predictor of poor foot function (0.62); having a direct (0.58) and indirect effect (0.04) on foot function. Patients with SSc produce lower forces during walking and spend longer time in weight bearing; both signs that are indicative of impaired foot function and/or foot pain. A summary of the direct, indirect and total standardised effects for the variables of this model is provided in Table 4.10.

Variable	Direct effect	Indirect effect	Total effect
General function	0.583	0.036	0.619
Mean force heel	-0.156	-0.238	-0.393
Max mean pressure heel	0.000	0.125	0.125
Vertical ground reaction force at push-off	-0.122	0.000	-0.122
Soft-tissue thicknes heel	0.000	-0.065	-0.065
Vertical ground reaction force at heel strike	0.000	-0.053	-0.053
Soft-tissue thicknes plantar 3rd MTPJ	0.000	-0.035	-0.035
Max mean pressure 3rd MTPJ	0.000	0.031	0.031
Stance time	0.000	0.021	0.021
Mean force 3rdMTPJ	0.000	-0.020	-0.020
Ankle ROM	0.000	-0.005	-0.005

Table 4.10 Standardised direct, indirect and total effect of the variables as they relate to foot function

4.4.5 **Model Two: exploration of multifactorial pathways that contribute to foot pain**

The previous section focused on foot function and so the dimension of foot pain was explored in a second set of models. The initial model developed to identifying the factors that contribute to the impact of foot pain is presented in Figure 4.5. Initial analysis showed a significant χ^2 statistics (χ^2=101.43, df = 24, p= 0.000) and the fit statistics indicated a poor model fit (RMSEA= 0.164, GFI= 0.808;CFI= 0.849). To improve the model fit the model diagnostics information was assessed as described for the first model. Iterations of the model indicating which relationships were removed or added are summarised in Table 4.11

The assumption of multivariate normality among the variables in this model was checked and it showed a Mardia's coefficient of 2.155 indicating a moderate multivariate non-normality. Therefore the use of the bootstrapping techniques was again necessary to maximise the robustness of results. Boot strapping also complemented the fact that the model could not be replicated with additional sample data or cross-validated with a split sample [378].

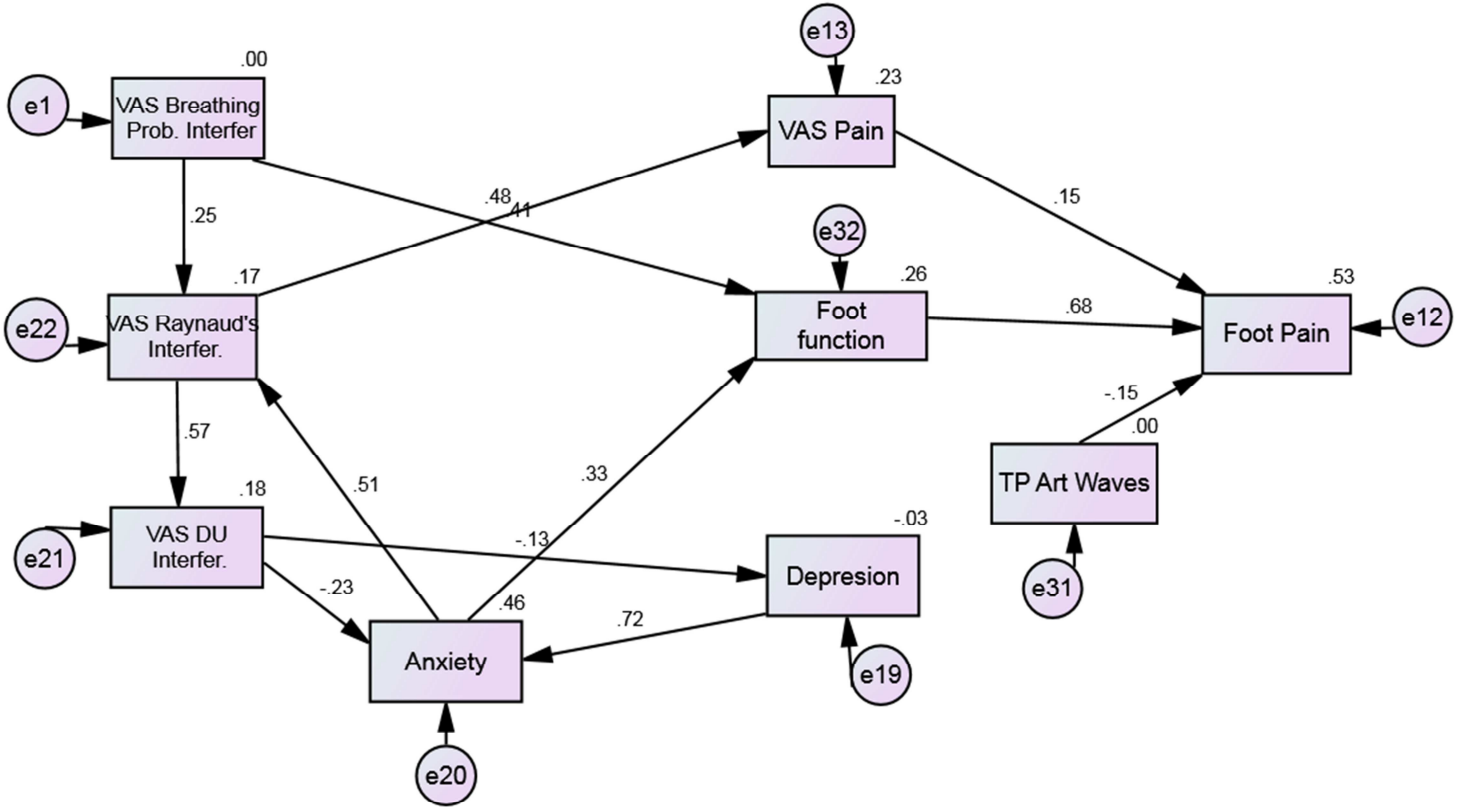

Figure 4.5 Structural equation model testing the factors contributing to foot pain in patients with SSc. The numbers at the top right corner of each variable represents the R^2 value, or percentage of variance of the variable explained by the model. The arrows represent the direction in which the relationship is and the values on top of each line are the regression weights. The larger the regression weights the better predictor the variable is of the variable that the arrow is feeding into The number of comorbidities, age, gender and diagnosis subtype when entered in the model they did not contribute to the model.

Model	N	Relationships Modifications	χ^2	df	*P* Ideal: >0.05	RMSEA Ideal: <0.05	CFI Ideal: >0.95	GFI Ideal: >0.95
1	121	Initial model	101.41	24	0.000	0.164	0.808	0.849
2	121	Add relationship general function ➔ VAS pain	191.11	32	0.000	0.204	0.696	0.776
3	121	Add relationship foot function ➔general function	113.80	31	0.000	0.149	0.842	0.857
4	121	Add relationship general function ➔depression	55.697	30	0.003	0.184	0.951	0.925
5	121	Add relationship VAS breathing problem ➔general function	41.28	29	0.065	0.059	0.977	0.941
6	121	Remove relationship VAS breathing interfere ➔foot function	69.33	30	0.000	0.105	0.925	0.906
7	121	Remove relationship anxiety ➔foot function	75.11	31	0.000	0.109	0.916	0.898
8	121	Remove relationship foot function ➔general function	124.88	32	0.000	0.156	0.823	0.864
9	121	Remove relationship foot function ➔foot pain	190.43	33	0.000	0.199	0.700	0.813
10	121	Remove variable foot function	33.08	24	0.102	0.056	0.976	0.942
11	121	Add relationship general function ➔foot pain	21.73	23	0.536	0.000	1.000	0.962
12	121	Remove VAS DU variable	11.70	17	0.847	0.000	1.000	0.976
13	121	Add relationship anxiety ➔foot pain	8.68	16	0.926	0.000	1.000	0.981

Table 4.11 Summary table of iterations of structural equation modelling for impact of problems in relation to foot pain

The best fitting model obtained for foot pain (χ^2=8.682, df = 16 p= 0.926, RMSEA= 0.000, GFI= 1.000, CFI= 0.981) demonstrated very good fit (Figure 4.6) This model explains nearly 40% of the variance (R^2 =.38) in foot pain in patients with SSc and contains eight variables. Four variables have a direct effect on foot pain: general function (R_{gwt}=0.28), general pain (R_{gwt}= 0.28), TP arterial waveform (R_{gwt}=-0.20), and anxiety (R_{gwt}=0.13); with general function and general pain being the best predictors of foot pain. The other four variables i.e breathing interference, Raynaud's interference, anxiety and depression, have an indirect effect and contribute to foot pain in an indirect manner through mediator variables.

While not directly affecting foot pain, breathing problems has a direct impact on general function (R_{gwt}=0.53). Patients with worse Raynaud's interference reported poorer general function (R_{gwt}=0.24) and worse general pain (R_{gwt}=0.24), both of which had a direct effect on foot pain. Not surprisingly, patients with poorer general function also tended to report worse general pain (R_{gwt}=0.53) and higher levels of depression (R_{gwt}=0.62). In addition, depression has a large effect on anxiety (R_{gwt}=0.67), while increase anxiety has both an indirect effect, through Raynaud's interference (R_{gwt}=0.26), and a direct effect (R_{gwt}=0.13) on foot pain.

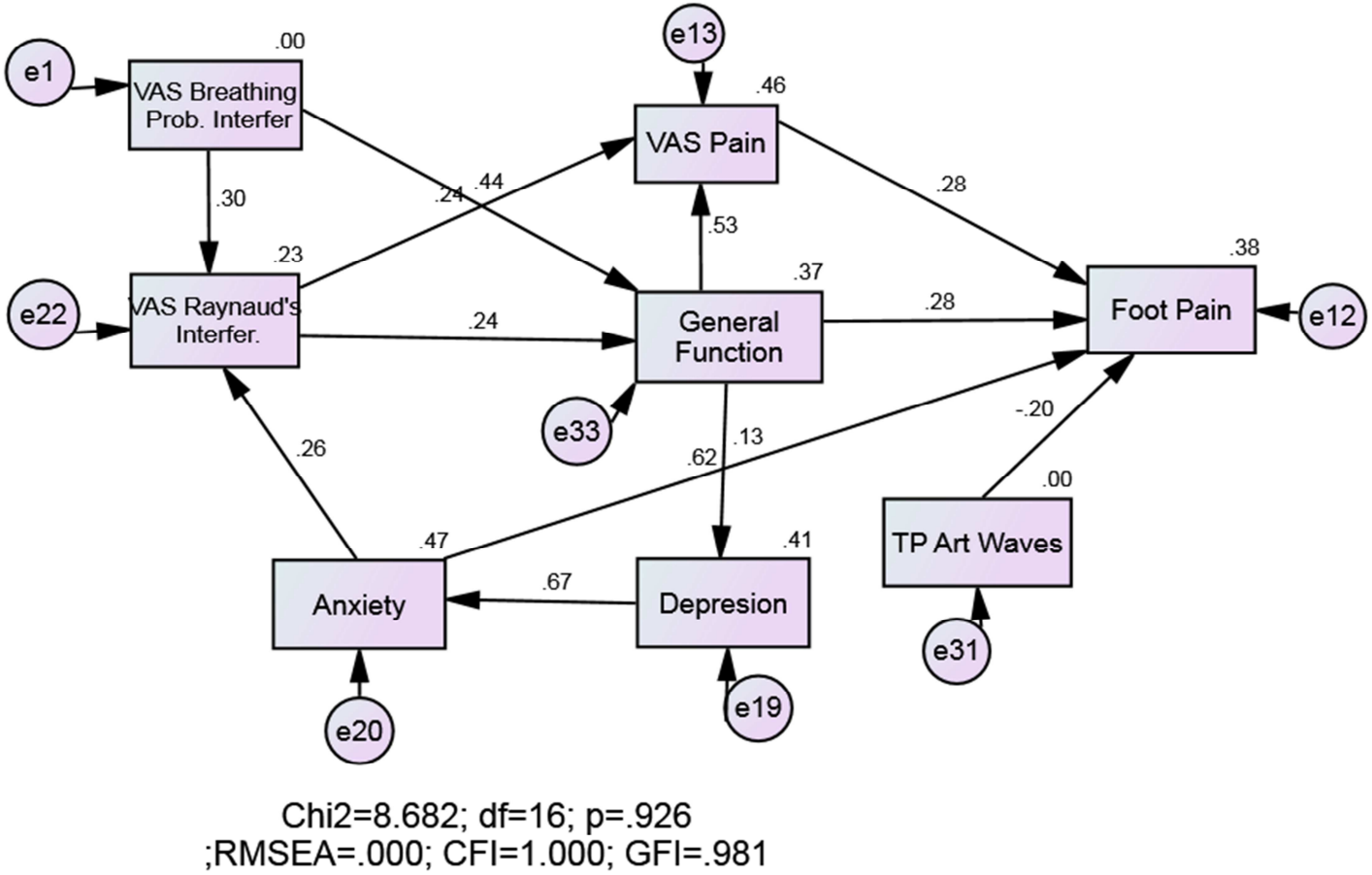

Figure 4.6 Best fitting structural equation model for the factors contributing to foot pain in patients with SSc. The numbers at the top right corner of each variable represents the R^2 value, or percentage of variance of the variable explained by the model. The arrows represent the direction in which the relationship is and the values on top of each line are the regression weights. The larger the regression weights the better predictor the variable is of the variable that the arrow is feeding into. The variables disease duration, number of comorbidities, age, gender and diagnosis subtype when entered in the model they did not contribute to the model.

Once again, poorer general function was the largest single predictor of foot pain in this model (0.51), having both a direct and indirect effect. General function (0.28), together with general pain (0.28) also had the highest direct effects. A summary of the direct, indirect and total standardised effects for the variables of this model is provided in Table 4.12

Variable	Direct effect	Indirect effect	Total effect
General function	0.282	0.224	0.506
VAS pain	0.283	0.000	0.283
VAS Breathing prob. interference	0.000	0.282	0.282
TP arterial wave	-0.197	0.000	-0.196
VAS Raynaud's interference	0.000	0.189	0.189
Anxiety	0.127	0.049	0.176
Depression	0.000	0.117	0.117

Table 4.12 Standardised direct, indirect and total effect for the variables as they relate to foot pain

4.4.6 **Model Three: exploration of multifactorial pathways that contribute to impact on quality of life**

The initial model developed to identifying the impact of foot problems on the QoL is presented in Figure 4.7. Initial analysis showed a significant χ^2 statistics (χ^2= 57.31, df = 38, p= 0.023) and the fit statistics indicated a poor model fit (RMSEA= 0.065, GFI= 0.979;CFI= 0.933). The initial model diagnostics information was again assessed and modifications to the model were undertaken until the best fitting model was obtained, following the same iterative process explained in model one. The iterations of the model indicating which relationships were removed or added are summarised in Table 4.13

The Mardia's coefficient for the best fitting model for impact of foot problems in QoL was 5.10 indicating a moderate multivariate non-normality. Therefore, bootstrapping was used to obtain more robust results [281, 372, 378]

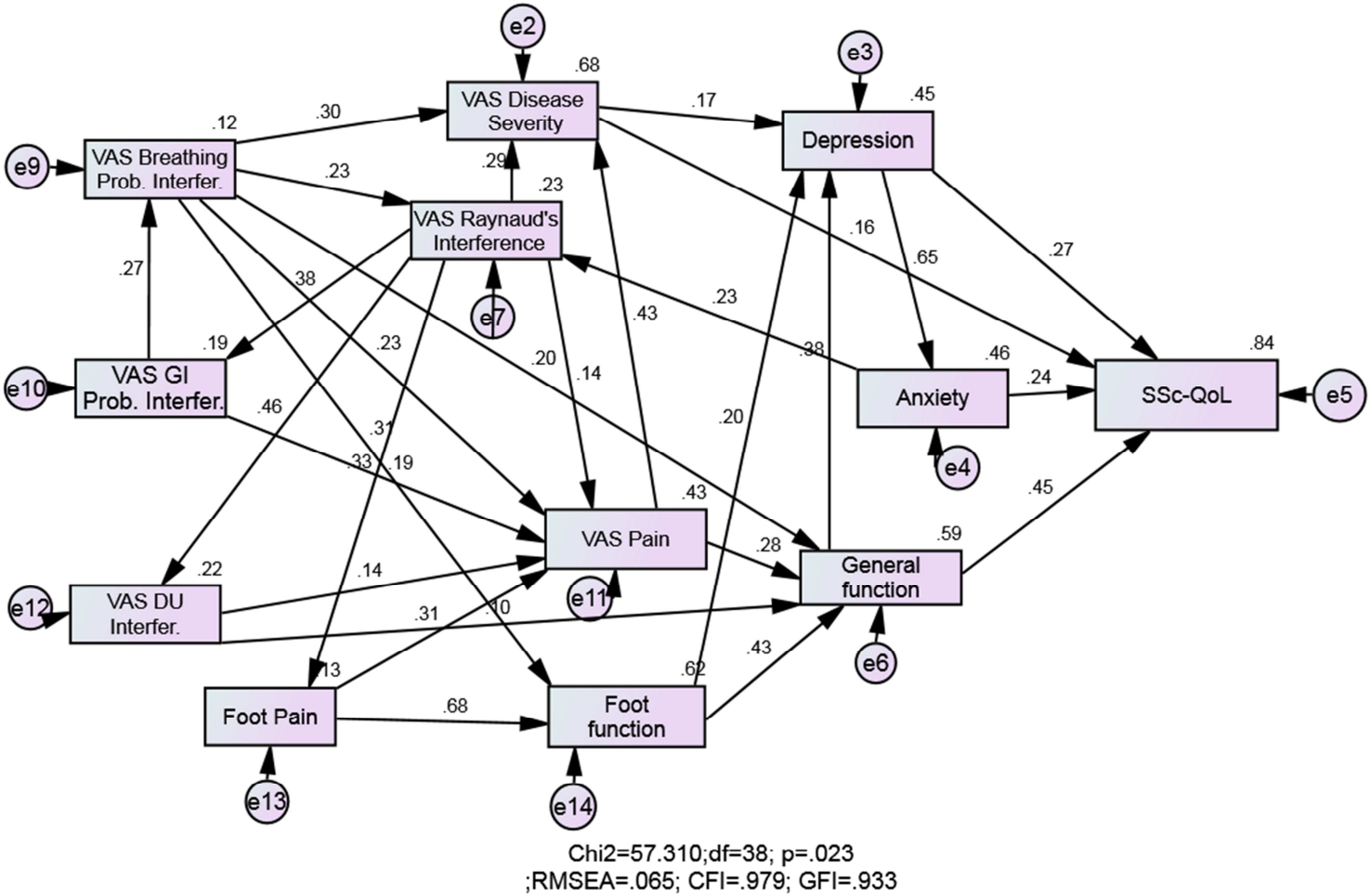

Figure 4.7 Structural equation model testing the effects of impact of foot problems on the QoL of patients with SSc. The numbers at the top right corner of each variable represents the R^2 value, or percentage of variance of the variable explained by the model. The arrows represent the direction in which the relationship is and the values on top of each line are the regression weights. The larger the regression weights the better predictor the variable is of the variable that the arrow is feeding into.

Model	N	Relationships Modifications	χ^2	df	*P* Ideal: >0.05	RMSEA Ideal: <0.05	CFI Ideal: >0.95	GFI Ideal: >0.95
1	121	Initial model	57.31	38	0.023	0.065	0.979	0.933
2	121	Remove relationship VAS Raynaud's ➔ VAS pain	59.80	39	0.018	0.067	0.977	0.929
3	121	Remove relationship VAS DU ➔general function	81.48	41	0.000	0.091	0.956	0.904
4	121	Add relationship VAS pain ➔ general function	62.30	40	0.014	0.068	0.976	0.926
5	121	Add anxiaty ➔VAS DU interfearence	48.87	39	0.133	0.046	0.989	0.939
6	121	Add relationship VAS DU interference ➔ general function	44.22	38	0.225	0.037	0.993	0.943
7	121	Add VAS GI problems interference ➔ VAS DU interference	37.02	37	0.468	0.002	1.000	0.951

Table 4.13 Summary table of iterations of structural equation model for the impact of foot problems on QoL

The best fitting model obtained for the impact of foot problems on the QoL (χ^2=44.22, df= 38, p= 0.225, RMSEA= 0.037, GFI= 0.993, CFI= 0.943) demonstrates good fit (Figure 4.8). This model explains nearly 84% of the variance (R^2 = 0.84) that affects QoL in patients with SSc. This variance is explained by the influence of 11 variables, three of which have a direct effect on QoL: general function (R_{gwt}=0.45), depression (R_{gwt}= 0.27) and anxiety (R_{gwt}=0.24); with general function being the strongest predictor of QoL. The other eight variables i.e patient's perceived disease severity, breathing interference, Raynaud's interference, GI problems interference, DU interference, general pain, foot pain and foot function, have an indirect effect and contribute to QoL through mediator variables.

While not directly affecting QoL, breathing problems have a direct effect on Raynaud's interference (R_{gwt}= 0.32), foot function (R_{gwt}= 0.31), patient's perceived disease severity (R_{gwt}=0.31), general pain (R_{gwt}= 0.26), gastrointestinal problems interference (R_{gwt}= 0.23) and general function (R_{gwt}= 0.20). Patients that report worse GI problems interference if they have DUs are more likely to report worse DU interference (R_{gwt}= 0.23), and worse general pain (R_{gwt}= 0.20). Both GI and DU interference are both directly affected by Raynaud's interference in daily activities; R_{gwt}= 0.35 and 0.52 respectively. In addition Raynaud's interference is affected by anxiety (R_{gwt}= 0.21), and an increase in Raynaud's interference leads to an increase in patient perceived disease severity (R_{gwt}= 0.29) and in foot pain (R_{gwt}= 0.33).

When patients report increase in foot pain they are more likely to report worse general pain (R_{gwt}= 0.33) and worse foot function (R_{gwt}= 0.68); both of which variables have a direct effect to general function; R_{gwt}= 0.28 and 0.43

respectively; the latter having a direct effect to QoL (R_{gwt}= 0.45). Therefore, foot pain and foot function have an indirect effect on the QoL of patients with SSc.

General function is worse in those patients that have general pain (R_{gwt}= 0.28), and general pain also worsens in patients with DU that report DU interference (R_{gwt}= 0.23). While general pain has a direct effect on patients perceived disease severity (R_{gwt}= 0.44). Patients reporting worse perceived disease severity are more likely to report worse QoL (R_{gwt}= 0.16) and worse depression (R_{gwt}= 0.18).

Depression has also a direct effect on QoL (R_{gwt}= 0.27) and a large effect on anxiety (R_{gwt}= 0.68). Perhaps surprisingly however, those patients that are more anxious are less likely to report worsening of the DU interference (R_{gwt}= -0.40). Anxiety is also one of the variables having a direct effect on QoL (R_{gwt}= 0.24).

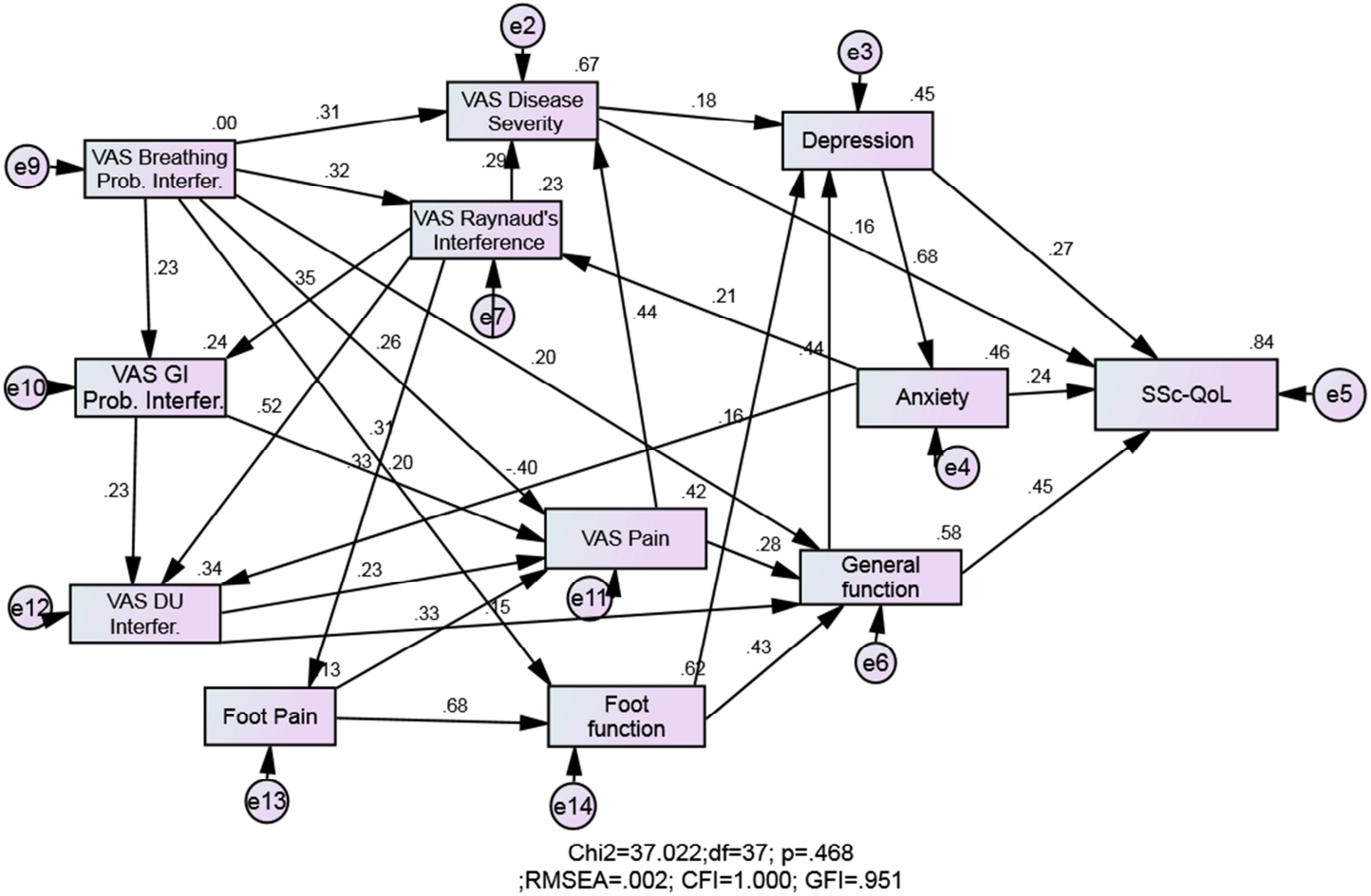

Figure 4. 8 Best fitting structural equation model for the impact of foot problems of the QoL of patients with SSc. The numbers at the top right corner of each variable represents the R^2 value, or percentage of variance of the variable explained by the model. The arrows represent the direction in which the relationship is and the values on top of each line are the regression weights. The larger the regression weights the better predictor the variable is of the variable that the arrow is feeding into. . The variables disease duration, number of comorbidities, age, gender and diagnosis subtype when entered in the model they did not contribute to the model,

Breathing problems are associated with the largest number of variables. General function is the highest single predictor of quality of life (0.64), followed by impact of breathing problems (0.49) and depression (0.43). Foot function and foot pain are the variables having the fourth and fifth largest effect on QoL (0.34 and 0.33 respectively), and interestingly they are more influential on QoL than six other variables including GI and Raynaud's interference. A summary of the direct, indirect and total standardised effects for the variables of this model is provided in Table 4.14

Variable	Direct effect	Indirect effect	Total effect
General function	0.454	0.186	0.640
VAS Breathing problems interference	0.000	0.493	0.493
Depression	0.270	0.158	0.428
Foot function	0.000	0.343	0.343
Foot pain	0.000	0.327	0.327
VAS Raynaud's interference	0.000	0.294	0.294
VAS pain	0.000	0.284	0.284
Vas Disease severity	0.160	0.076	0.236
Anxiety	0.236	-0.003	0.233
VAS DU interference	0.000	0.162	0.162
VAS GI problems interference	0.000	0.095	0.095

Table 4.14 Standardised direct, indirect and total effect of the variables as they relate to QoL, including foot problems

Summary

The results from this modelling have highlighted that SSc has both a physical and psychological impact. This impact is reflected in the feet as substantial foot problems, which are affected by complex interrelations between multiple factors. Foot problems are a significant contributor to the impact on the quality of life of patients with SSc.

Chapter 5
Discussion and Summary

5.1 Overview

The studies undertaken in this thesis aimed to identify the factors that contribute to the development and impact of foot problems in patients with SSc. A multi-methodological approach was used to achieve this aim.

First, a literature review and expert clinician consultation was developed to identify the candidate factors that influence foot pathology in SSc; second a case-control cross-sectional study was undertaken to establish the prevalence and impact of foot pathology in this disease; and last, SEM was used to explore the multifactorial pathways in foot pathology and its impact on the QoL of patients with SSc.

The hypothesis underpinning this thesis was that the development and impact of foot problems on the QoL of patients with SSc is multifactorial; involving a complex inter-relationship between disease, functional impairment, personal factors, environmental factors and psychosocial factors.

The results from the SEM support this hypothesis. From our literature review and clinician consultation, we identified factors that were considered important in the development of foot problems, including skin, vascular, musculoskeletal and neurological factors. By exploring these factors in patients with SSc and healthy volunteers, we were able to determine that foot pathology was substantial in patients with SSc. Patients reported poorer general health, poorer

health-related quality of life and high levels of anxiety and depression. Patients also had increased odds of having absent or monophasic vascular waveforms, indicating PAD, and loss of protective sensory acuity increasing the risk of ulceration, poor healing and infection. There were also significant gait changes in patients with SSc: they had reduced forces going through the foot during gait, reduced plantar pressures, and reduced joint range of motion in the foot. These together with a slower gait, indicates that SSc patients have impaired biomechanics of the foot.

As reported in Chapter 4, the initial theoretical model of the impact of foot problems on quality of life was not supported by the data. Modification of the initial model caused the models to lose its confirmatory properties of the theoretical model as initially stated, and the approach switched therefore to a model generation process [282].

Three models were built using the SEM. Two models explored the multifactorial pathway in foot pathology, focusing on foot pain and foot function, and the third model explored the impact of foot pathology in the patients QoL. The factors included in the foot pain model predicted 38% of variance of foot pain, while those included in the foot function model predicted 51% of the variance of foot function. The third model identified the factors that predicted a substantial 84% of the impact on the Qol of patients with SSc, of which foot function and foot pain were highly associated with poorer quality of life. All three models represented well-defined models.

The models of foot pain and foot function indicated that each are predicted by multiple, inter-related factors. Furthermore, foot pain and foot function had an indirect effect contributing to QoL through mediator variables. Foot function and

foot pain became the fourth and fifth variable with the biggest contribution to the impact on QoL after general function, patient perceived breathing problems interference and depression.

5.2 Major findings

This programme of work has produced several important findings. These findings will be discussed in this chapter under the following four themes.

I. Patients with SSc have substantial foot problems
II. Systemic sclerosis has both physical and psychosocial impact
III. Foot pathology in patients with SSc is multifactorial
IV. Foot pathology is a significant contributor to the impact on the Qol of patients with SSc.

5.2.1 **Patients with SSc have substantial foot problems.**

As described in Chapter Two, SSc is a complex heterogeneous disorder, which involves several systems. The multisystem nature of the disease manifests in the patient as different signs and symptoms affecting many body organs and structures. Some of these manifestations are physical and others psychological, and together they have an effect at the psychosocial level. The different systems involved in the disease systemically include integumentary, vascular, nervous and musculoskeletal, cardio-pulmonary, renal and gastrointestinal systems. However the systems that have a direct effect on the foot are the integumentary, vascular, nervous and musculoskeletal systems, and the results have indicated a significant role of the last three in foot pathology.

Whilst previous literature has reported the effects of the disease in the lower limb, ours is the first study to investigate the impact and interactions of different systems contributing to foot problems.

Skin involvement in foot pathology

While skin pathology is a defining, central feature in SSc [89, 187], it is also known that the clinical presentation of skin involvement can vary. In our group of patients with SSc, the overall skin involvement as measured by the mRSS can be considered mild. When compared to other cohorts from published literature the mRSS scores are relatively low [276, 379-381]. The lower scores in our cohort reflect a predominantly lcSSc cohort although additional factors including milder involvement (especially in the dcSSc patients) and longer disease duration may also be relevant,(although the latter was similar to the other studies). However, we know that the mRSS has better intra-observer reproducibility than inter-observer reproducibility [305, 382], which introduces difficulties in comparing scores between studies and limits the legitimacy of drawing firm conclusions about the scores in different studies.

It has also been identified that in addition to the skin involvement, there are other soft-tissue subcutaneous structures that are affected by the disease pathology, such as subcutaneous-fat atrophy [25, 374]. This was partially supported in this study as evidenced by the measurable soft-tissue changes on the plantar aspect of the foot quantified with ultrasound. The decrease in soft-tissue thickness in patients with SSc could be as a result of plantar fat-pad atrophy through the same pathological process that affects subcutaneous fat atrophy in other areas of the body [149], or it could be as a result of muscle atrophy as shown in other cohorts of people with toe deformities [383, 384];

muscle atrophy is also known to occur in patients with SSc [257, 263]. These findings should be interpreted with caution however as the difference in soft-tissue thickness under the 3rd MTPJ and heel, while statistically significantly different compared to healthy participants, was very small. Particularly at the heel, the measured difference in means between groups was smaller than the measurement error identified in the intra-rater reliability testing (Appendix 1).

Vascular involvement on foot pathology

In SSc, although both microvascular and macrovascular pathology has been reported, microvascular abnormality is a primary process underpinning the disease [15, 96].

In this study, measuring microvascular involvement with clinically accessible assessments proved to be difficult. It had been intended to measure toe brachial pressures (TBP) and photoplethysmography (PPG) of the digits to detect blood flow changes in the microvascular bed of the toes. After taking these measurements in the first five patients however, it became clear that the measures were unrealistically time consuming. In addition, the SSc disease process confounded obtaining reliable readings. These two tests were excluded from the battery of vascular tests for the remaining 116 cases. An alternative to this test and a more reliable way of assessing the microvascular pathology in patients with SSc may have been to use capillaroscopy of the nail fold of the toes. This test has been used in the toes although is not formally validated, and is also a lengthy test to conduct, requiring extensive training and experience [385] . It is therefore accepted that the lack of any microvascular measurement is a limitation in this study.

The tests used in this study to assess macrovascular disease in the lower limb have a good validity and reliability [308-310]. In SSc patients the ABPI analysis suggested that the macrovascular system in patients with SSc remains relatively normal. While the current results concur with some published studies [386], they also differ from others [15, 387]. In most of the previous literature, with the exception of a study by Bichile et al [387], patients were selected without any specifications regarding the presence or absence of lower limb vascular symptoms or disease. Bichile et al [387], however, recruited from patients with symptomatic peripheral vascular disease (PVD), and 50% of their sample had abnormal ABPI. Consequently, the presentation of abnormal ABPI in patients with SSc remains unclear and it cannot be determined whether PAD of large vessels is more prevalent in patients with SSc. On the other hand, the arterial wave analysis, which also assesses PAD, showed that there was a significant association between the presence of PAD and whether the participant had SSc or not. The odds ratio of having PAD as defined by absent or monophasic arterial waveforms were five times higher in the participants with SSc than in the healthy participants. This could indicate that patients with SSc are more likely to develop occlusive peripheral arterial disease than healthy individuals or they could have vascular calcification, which has been previously reported in patients with SSc [8]; although if the latter option was the case then one would have expected to obtain ABPI results above 1.30, as they are indicative of calcified arteries, and this was not the case. The basis for PAD in SSc is an area that could be expanded with future research but would require populations carefully controlled for usual risk factors associated with large vessel atherosclerotic disease.

Neurological involvement in foot pathology

Unlike the vascular involvement in SSc, neurological involvement in the disease has been considered less common; although acknowledged as being present in some cases. Amongst other neurological abnormalities (described in Chapter 2) there is some evidence for the presence of peripheral neuropathy involving the feet [169-171]. The results from the current study agree with the published literature, as patients with SSc when compared to healthy individuals showed sensory deficiency in their feet.

The results from the neurological tests conducted in the current study to determine the sensory deficiency were different in some ways from those published in the literature. For instance, Schady et all [237] reported that there was no difference between patients with SSc and healthy participants in terms of vibration and pain thresholds, while the results from this thesis found that vibration and heat induced pain threshold from the dorsum of the foot was poorer in patients with SSc compared to healthy volunteers with reported higher pain and vibration thresholds in the cases. An explanation for this discrepancy could be due to difference in the anatomical area being tested. Schady et al [237] carried out the test under the lateral malleolus while in this thesis the test for vibration was carried out on the dorsum the foot. Testing a distal part of the foot might be more likely to detect sensory abnormalities as peripheral neuropathy in SSc usually has a distal pattern.

There were some quantitative sensory tests (QST) results from this thesis, which were similar to those previously published. The results for the tests on light touch, cold perception threshold on the dorsum of the foot and heat perception threshold on the dorsum and plantar of the foot indicated that

patients with SSc had a significantly reduced sensation in perceiving touch on the foot and heat on the dorsum and plantar aspect of the foot, and a highly significant reduction in cold sensation on the dorsum of the foot. Schady et al [237] also identified empirically that some patients with SSc had a reduced touch and heat sensation. These results must be interpreted with caution however, as the authors reported the percentage of patient having 'abnormal readings' but did not quantify the difference between groups.

Interestingly, even though patients with SSc also had reduced sensation when perceiving heat induced pain or cold on the plantar aspect of the foot, the difference between groups did not reach statistical significance. Conversely the heat perception threshold test on the plantar surface, which did reach significance, was based on a difference between groups smaller than that obtained from the dorsum. As this difference was less than one degree and had a small effect size this poses the question of how big the difference needs to be for it to be considered pathological/clinically meaningful.

It has been proposed that sensory deficit is more likely to be significant in the dorsum of the foot than on the plantar aspect as a result of skin fibrosis damaging the sensory skin receptors via incarceration of the nerves in collagen[226]. It is known that involvement of the dorsum of the foot, although less frequent than the dorsum of the hands, can result in fibrosis, while fibrotic changes on the plantar of the foot are not well documented. Alternatively, the neuropathy could be associated with a vasculopathy or inflammatory process but with these two mechanisms one would expect to observe similar affects in both areas of the foot.

Even though there are limited published neurological data on the difference between patients with SSc and controls with which to specifically compare the results from the current study, there are published normative data [388-391] which is relatively consistent with the data obtained from the healthy participants in this study, It is important to note however, that for some tests a statistically significant difference between groups and a moderate statistical effect size, might not be clinically significant because of a very small difference. For example, the light touch test yielded a systematic difference of a median of one site per participant (i.e patients with SSc had slightly reduced sensation because they did not feel the monofilament in one out of eight sites on average). This raises the question of what thresholds from this tests can determine what is pathological and how should such tests be used in clinical assessment.

Overall, the significant results from the neurological QST investigations indicate that patients with SSc have some large and small fibre neuropathy. The vibration and light pressure perception results imply large fibre neuropathy, affecting the myelinated (A-alpha and A-beta) fibres. The heat, cold and pain perception results indicate small fibre neuropathy, affecting the non-myelinated (C) fibres and the thinly myelinated (A-delta) fibres. Impaired sensations related to the skin, numbness, increased sensibility and loss of sensory functions all were identified in a Central–European qualitative study, as some of the factors perceived by the patients with SSc to be impairment of body function and structures, as classified by the ICF criteria [4].

Musculoskeletal involvement in foot pathology

The disease pathology has an effect on the musculoskeletal system involving intra-articular, periarticular and muscular structures [169, 241, 243]. This musculoskeletal involvement has also been observed in the feet [189, 254] but its effects on spatiotemporal gait parameters and foot function-related impairment has not been described in depth in the literature. The results from this thesis provide some insight into foot function and impairment in SSc.

Foot function and impairment was measured by capturing different spatiotemporal gait parameters and patient reported outcomes measures, which has not previously been reported in the literature. A significant reduction ankle ROM in the sagittal plane (plantarflexion-dorsiflexion) and reduce ROM of the forefoot with regards to hindfoot in the coronal plane movement (inversion-eversion) was observed. During gait, patients with SSc had a significantly reduced ROM in the ankle and reduce ROM of the forefoot relative to the hindfoot in terms of inversion-eversion. This reduction in ROM could be as a result of skin fibrosis, intra-articular and periarticular joint involvement as observed in previous studies [189] and/or as a result of an altered gait because of foot pain; as patient reported significant foot pain in this thesis. The hindfoot and forefoot reduce ROM can have an effect on other gait parameters, which together may explain the high levels of impairment in foot function reported by the patients with SSc in this study. In addition, the patients also had a reduced mean force under the heel and the 3rd MTPJ and a reduction in the ground reaction force at heel strike and push-off. In healthy participants a reduction in these forces during gait are indicative of slower gait [392]. In patients with SSc therefore, reduction in mean force and ground reaction force together with an increase in time spent in the stance phase suggests that patients with SSc walk

more slowly than healthy participants and possibly spend more time in double stance. This would also corroborate the findings from a previous study carried out by the candidate, in which walking speed and stride length was noted to be reduced and the double support period was increased in patients with SSc [10]. Interestingly, the earlier study also found that patients with SSc had increased plantar pressures at the 1st MPT joint while the current study found no difference in plantar pressure at the 3rd MTP joint between groups. It is relevant however that the pressure measurement used in this thesis was maximum mean pressure as opposed to pressure time integral; although even though these two measurement were different it does not account for the large difference in the results. Of note however, the regions of interest were different; the current study looked at the plantar pressures at pre-specified sties under the 3rd MTPJ and heel because of wanting to explore the relationship between plantar soft-tissues thickness in this areas and plantar pressures. Furthermore, the 3rd MTP joint was selected over the 1st because the latter is commonly affected by other pathologies that are not specific to the SSc disease e.g. hallux abductor valgus, therefore acting as a confounders. Further analysis of the current data looking at the average plantar pressure across the MTP joints would be possible after completion of the main study and would be necessary to determine whether patients with SSc have increased pressures across the forefoot.

The impairment of foot function during gait reflects the presence of foot problems and/or foot pain. Through the MFPDI, the patients have also reported both of these factors to be significant.

The direct or indirect interrelations of these gait parameters are further described in model one, section 4.4.4

For some of the neurological and musculoskeletal variables, which yielded differences between cases and controls that were significant and had at least a small effect size, the difference between means and medians was small. In part at least this could have been due to the heterogeneous nature of the disease, which introduces greater variance within the SSc sample, thereby masking some of the cases with larger differences. Nevertheless, the small magnitude of the overall between-group differences does raise the question as to whether these differences are clinically meaningful. The current study was not constructed to provide that answer but it provides the basis for future research, to build upon the results and explore further the factors identified to contribute to foot pathology in patients with SSc.

5.2.2 Systemic sclerosis has both physical and psychosocial involvement

It is well established in the literature that SSc is a disease that affects many systems of the body. The complex and multisystem nature of the disease can have a profound affect on both the structures and functions of the body. Together with the personal and environmental factors this combination can have a consequence on the patient activity and cause participation restriction leading to perception of disability [367]. Physical disability does not always reflect the full impact of the disease however, as psychological involvement is also relevant [272-274] and together with the physical impairment has a psychosocial impact. One of the objectives of this study was to capture the

patients' perception of their physical, psychological and psychosocial impact caused by the disease.

To explore the impact of SSc relative to other conditions the responses from the current SSc cohort were compared with another six conditions using the SF-36. Patients with SSc scored on average lower (or poorer scores) than many other diseases; with scoring ranging from 29 to 65 across the different domains (Figure 5.0). Patients with SSc reported the worse physical function, bodily pain, general health, vitality, social interference and mental health amongst the six conditions. They reported having similar role physical and role emotional problem than patients with angina pectoris, who score the lowest on these two domains. In addition, our patients with SSc reported a highly significant physical and mental health disability when compared to healthy participants. Both MCS and PCS SF-36 summary scores for patients with SSc obtained in the current study are similar to those reported in the literature [393]. Furthermore, the scores obtained for the SF-36 summary of physical function in this study were particularly low. As a reference for the meaning of the scores, in a U.S. general population has been reported that scores between 8-34 relate to higher rates of job disability, job loss within a year, subsequent hospitalisations, greater disease burden, and greater likelihood of death within five years, when compared to those with higher scores. In relation to the five-year mortality, scores between 25-34 were associated to have an increase in mortality of 15.1%. While for the SF-36 summary of mental health scores lower than 54 were more likely to screen positive for depression and more likely to report lower satisfaction with their life in comparison to those scoring higher [394].

However, this data has not been published for patients with SSc therefore in this population the scores could yield different results.

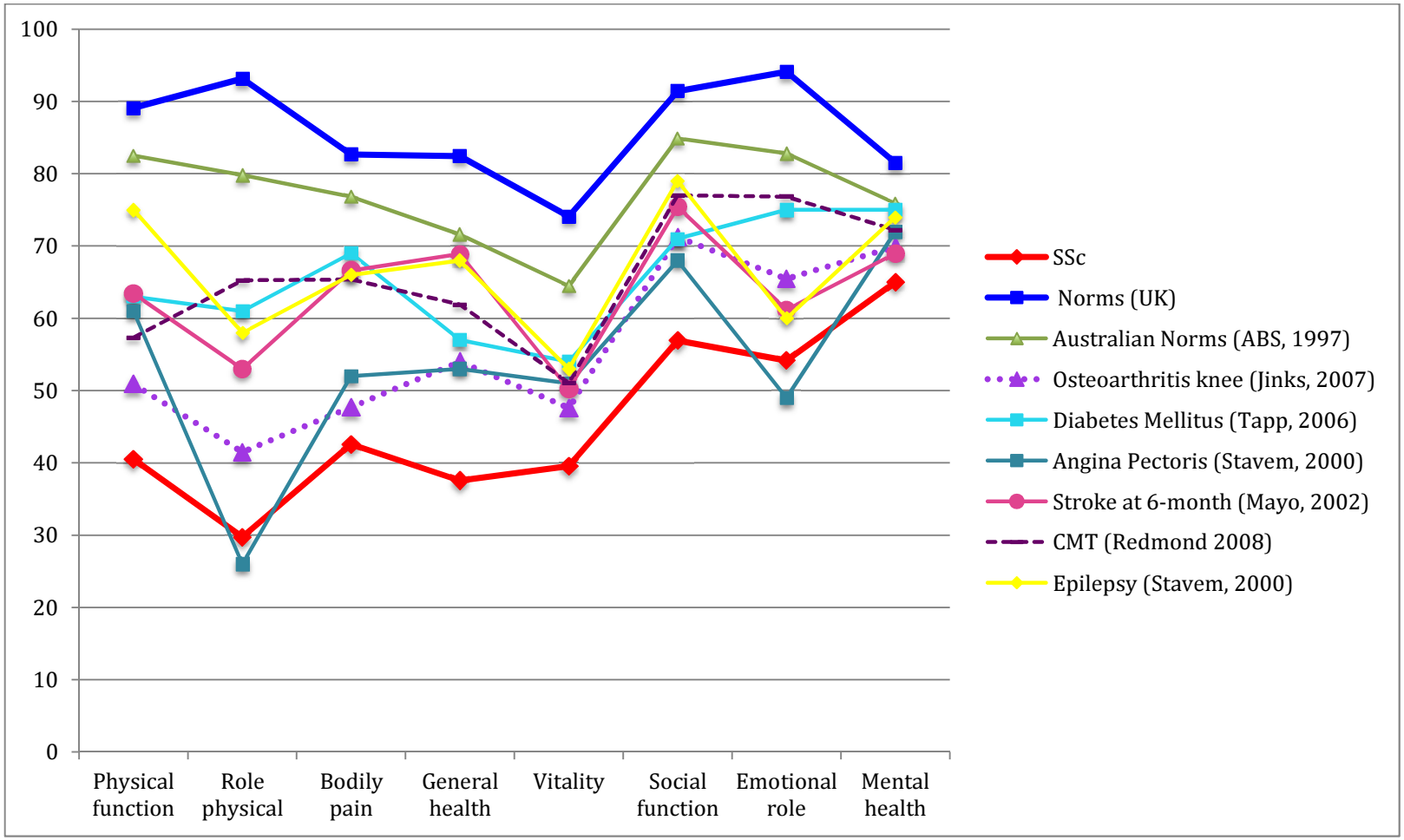

Figure 5.0 SF-36 scores for a range of conditions, including systemic sclerosis (SSc). Graph modified from Redmond et al[395], CMT: Charcot-Marie-Tooth.

The SF-36 as a generic outcome provided information to compare the generic impact of the disease [344, 345], while the disease specific and symptom specific tools captured a more specific impact, which could have been missed by the generic tools. These results are consistent with those in the published literature [197, 396]. When exploring the impact of specific organ symptoms of activities of daily–living the results varied according to the related symptoms. Impact ranged from very low interference with ADLs associated with the digital ulcers to an increase in interference in daily activities in ADLs associated with GI problems, breathing problems, pain and Raynaud's' symptoms, with the highest interference caused by Raynaud's' symptoms. For the overall disease interference that represents part of the patients' perceived impact of the disease in their life, the median score was 5/10 which compared to the severity implied by the scores of the SF-36 summary physical function, may have been expected to result in higher scores. The overall rating of the disease interference might be influenced however, by aspects of personality, psychosocial adjustment to illness and/or social support, and might not therefore reflect the indices of illness severity and more a complex interaction between different factors, as it is the case for depressive symptoms [272].

As a note, it is important to highlight that the VAS scales in the SSc-HAQ do not provide an option for the response to be non-applicable, and if the patients did not suffer from the specific symptom therefore their answer was zero. This response pattern can be misinterpreted as it is not reflecting the interference of this symptom/disease with the daily activities, but indicates that they did not suffer from it. A good example for this is the VAS for digital ulcers as only 49%

of the participants have ever had an ulcer therefore the 51% of participants scored zero.

In addition to the physical impairment noted previously, SSc also leads to a psycological impact. In the current study the SSc patients had significantly higher levels of anxiety and depression than the healthy individuals, and many reached the threshold required to be considered clinically anxious; with a score ≥ 8 being identified as diagnostic of anxiety or depression [346, 352]. These results are in agreement with the published literature, as it is well documented that patients with SSc suffer from higher rates of depression and anxiety; both of which conditions have been reported to be the most predominant psychological symptoms [150, 354].

Any form of disability from a physical or psychological level tends to have an impact on the person's QoL. The results from the PROMs in this study showed the considerable impact of this disease on the patients' daily life and to their QoL. The disease therefore was shown to considerably affect the patients' QoL, as the mean scores were higher than half of the maximum score. The factors contributing to the impact in the patients' QoL have been identified and described in model three in section 4.4.6

5.2.3 **Foot pathology in patients with SSc is multifactorial**

Usually, when pathology is present, it is because the homeostasis of the body has been disrupted causing an imbalance between the complex interactions of different body structures, at a physical and/or psychological level. This can be observed in the complex interactions of the multiple factors that have been identified in this study to contribute to foot pathology.

In this study regional foot pathology was conceptualised theoretically as influencing foot function and one of the major symptoms of pathology, foot pain.

The factors identified to predict patient-perceived foot function, predicted 51% of the variance in foot function; meaning that in this model over half of what is considered to affect foot function is determined by these factors. General functional ability (as measured by HAQ-SSc) was the major contributor to variance in foot-specific function, followed by the mean force at the heel during gait. The fact that general function was the major predictor of foot function was not a surprise, because in a person with impaired general function caused by the disease which might also affects the feet in similar ways, then the general function is likely to affect foot function. This theoretical link was also supported by the fact that general function also had a direct effect on ground reaction forces at push-off indicating that if there is impairment in general function, this function also has an affected foot function, specifically relating to a variable that is indicative of the level of dynamic propulsion during walking. On the other hand, it is more difficult to explain all the interactions that occur during gait between some other factors. During walking forces acting on the foot are affected by the external ground reaction force occurring from when the heel

strikes the ground to when the forefoot pushes-off, and affected by the dynamic accelerations and decelerations of the body. This biomechanical process explains why the force going through the heel during gait and the ground reaction forces are predictors of foot function as any pathology that affects acceleration or deceleration of the body such a myopathy, intra-articular or periarticular pathology will have an effect on reducing the forces, which in turn will have an effect on the other gait parameters. This is reflected in plantar foot pressures which have been reported to be correlated with gait velocity in healthy participants, with lower velocities leading to lower forces and hence lower pressures [334]. In addition, the foot function model from this current study identified that plantar foot pressures of the heel and 3rdMTPJ also were affected by plantar soft-tissue thickness, and even though soft-tissue thickness had only a small indirect effect on foot function, heel plantar pressure was the third most influential predictor of impact on foot function. Soft-tissue thickness has been previously reported to affect plantar pressures in healthy participants [383, 384], and the provision of a cushioning insole has been shown to be effective in modifying plantar pressures[397], indicating that tailored interventions can improve foot function.

Interestingly, from the three variables capturing ROM of the foot joints, only ankle ROM contributed to the model although its effect on foot function was indirect and very small.

The results from the foot function model have to be interpreted with some caution as the multivariate normality assumption in this model was violated. The bootstrapping technique was used to deal with the multivariate non-normality but in small samples (i.e. N=100) bootstrapped estimates might not be accurate

[282]. The sample size from this thesis was N=121 and so although is larger than the required minimum, it is acknowledged that the sample is close to the minimum N=100.

Foot pathology is often accompanied with symptoms and in particular, foot pain. The SEM identified eight factors that with their interrelated interactions explained nearly 40% of the foot pain pathway.

Foot pain was predicted by factors resulting from physical pathology such as the TP arterial waves representing PAD, psychological factors such as depression and anxiety, patient-perceived general function and patient-reported symptom interference such as breathing problems, RP and general pain. Interestingly, a major predictor of foot pain was general function, followed by pain and breathing problems. The complex nature of the interaction between factors can be observed in the following example from the model: when general function is impaired it has an effect on depression which in turn worsens anxiety, and an increase in anxiety causes an increase in perception of foot pain. General pain was also affected by general function in this model. Conversely, Benrud-Larson et al reported depressive symptoms and pain to be determinants of physical functioning but not the other way around [22]. In the current model, general pain was also affected by Raynaud's interference and this relationship has been described as correlational previously [398]. The current data indicate that Raynaud's interference in daily activities is worsened in the presence of anxiety. This interaction agrees with previous results from the literature [94], and seems to support the fact that worsening of the Raynaud's interference is as a result of a physiological involvement triggered by anxiety, as opposed to anxious patients simply perceiving worse Raynaud's interference in

daily activities, if this were not the case it could be expected that breathing problems might also be worsened by anxiety.

Two interesting relationships found in this model were the large effect of breathing problems and Raynaud's on general function.

Pain in general was identified by patients with SSc as an impairment and is classified on the ICF under disability of a body function and structures[4], implying that the presence of pain can cause disability. In patients with SSc disability in body function as a result of pain has been previously reported, as pain has been identified as a predictor of physical functioning [22].

The fact that pain is represented by an interaction between physical, psychological and patient-perceived impairments indicates that foot pain is a complex interaction of many factors. When treating foot pain therefore, for the treatment to achieve a satisfactory outcome, the clinician has to consider and possibly address other factors that can have an indirect effect on pain.

5.2.4 Foot pathology is one of the major contributors to the impact on the QoL of patients with SSc

One of the key findings of this thesis is that QoL in SSc is associated with a complex interaction between physical and psychosocial factors; and that foot pathology, represented in this study by foot function and foot pain, is one of the major predictors of overall QoL.

It is well established in the literature that general function is affected in SSc [399], and that poor general function affects psychosocial adjustment to illness[273] and worsens psychological symptoms [197]. The results from this

current study are the first to suggest that in SSc general function is possibly the main contributor to impaired QoL.

General function was also affected by a number of other factors, among which were foot-specific function and foot pain. Through their effects on general function both of these factors made a substantial contribution to reduced overall QoL. Interestingly, worse foot function impairment and increased foot pain had a bigger impact on QoL than Raynaud's, general pain, patient reported disease interference in daily activities, anxiety, DU interference in daily activities and GI problems interference, in this order. This highlights that foot pathology needs to be assessed in the clinical setting on a regular basis, as addressing foot problems when present, can contribute to the improvement of the patients QoL. Furthermore, patents with SSc identified mobility as an impairment of body function and structures, as classified by the ICF criteria [4]. This in turn reinforces the fact that special attention needs to be given to the prevention and/or improvement of function in this patient group.

Breathing problems also affected general function and due to the significance of indirect effects on other factors, was the second biggest predictor of overall QoL, followed by depression.

Of the psychological factors, anxiety and depression both had a direct and indirect contribution to QoL, this also is in line with published literature, which reports that depression and anxiety in SSc has an impact on patients' lives [197]. Of the two factors, depression had greater influence on QoL than anxiety, which was the opposite of what was observed in the foot pain model, where anxiety was more influential. These results might indicate that anxiety worsens somatic symptoms but depression is associated with overall poor quality of life.

In general anxious people are more likely to suffer from disability[400] and depression as a comorbidity incrementally worsens health [401], and consequently both of these conditions have a big impact on the patients health and QoL. The current model indicates that foot function and general function both have an effect on depression. Impaired function has previously been reported to contribute to depression in SSc [197, 278, 396], and in other rheumatic disease such as RA depression has been associated with worse function [22, 402, 403]. It has also been reported however that HAQ-DI was not associated with depression in SSc [404]. Amongst other factors, depressive symptoms have been identified as having a psychosocial impact, as they are associated with lower satisfaction with social support, poorer emotion-focus coping, helplessness and higher fear of disease progression[404]. This may in part explain why depression is one of the main predictors affecting QoL.

Throughout the development and analysis of the models, general function has been a factor that has recurred as a principal predictor in all three models. Thus especial attention needs to be paid clinically to improve patients' general function and those factors that affect it, as general function appears to be a key factor for the improvement of foot-specific function, foot pain and overall QoL.

The final model exploring predictors of overall QoL had a very good fit to the data and the interactions between the physical and psychosocial variables estimated a high proportion of the variance of QoL in SSc. Eighty-four per cent of the variance in QoL was explained by the model, which can be considered a high proportion of prediction, as there are published models that only explain 25% of the variance in the outcome. Furthermore, to the candidate's knowledge

this is the first model produced that explains the impact of foot-specific problems on the overall QoL of patients with SSc.

It is important to note that while all of the final models tested fitted the data well, there is always the possibility that other models would have also fitted the data. When interpreting the results from the model one has to bear in mind two things. First, SEM and path analysis model probabilistic causality as opposed to deterministic causality, which means that SEM allows for change to occur in affected variables, as opposed to the same consequence being observed in all cases for the affected variable; which is the case in deterministic causality. Second, that it must be appreciated that the initial models were modified and therefore the modelling approach became exploratory as opposed to confirmatory[282].

5.3 Limitations of the study

The findings from this study have to be interpreted in light of the limitations of the study described.

For the cross-sectional study comparing patients with SSc to healthy volunteers, the ratio of patients with SSc to healthy participants was two to one. While other studies have also used this ratio, one to one, or one patient with SSc to 2 healthy volunteers are arguably more robust because of the related increase in sample size. This might have led to this study reporting absence of a significant difference were there was one in truth, as a result of not having enough power to detect a real difference (i.e type II error).

The factors identified as potential candidate factors for the SEM were selected in a systematic manner, but it is acknowledged that they might not have captured the full burden of the pathology especially with regard to foot specific factors, where selection might not have been as comprehensive. For example, the data obtained for the vascular assessment relates mainly to macrovascular disease, and therefore microvascular involvement and associated vasculopathy, which is a major underpinning pathogenic process in SSc is less well represented in these models. Due to the excessive time burden on participants and poor quality of resulting data, a conscious decision was taken to abandon the two tests that captured microvascular pathology, and it may be that these would need to be explored in future stand-alone studies of microvasculopathy.

Even though the potential candidate factors were selected in a systematic manner through expert consultation, the factors selected may have been different if a Delphi approach using a larger expert panel was used.

While we tried to recruit a sample with ethnic diversity, it is a limitation that the majority of the participants were British Caucasians nonetheless, as a consequence the results from the case-control cross-sectional study and from the models might not be representative of a population with different ethnic composition.

Interpretation of the he SEM/path analysis needs to consider a number of limitations. First, the sample size was smaller than recommended (N=200), which often results in models, which do not work. Due to SSc being a rare disease however, it can be acceptable to use smaller sample sizes. Consequently, although the sample size was smaller than recommended, the models have shown to demonstrate a very good fit across a range of different fit

statistics. The sample size also affected the validation of the model as the sample size was not big enough to retest and validate the final model, however the model can be validated by another sample any other time.

Second, some of the factors included in the models were ordinal or categorical variables, which is not optimal for the modelling. Ideally the ordinal data should have been Rasch transformed into continuous data, although transformations are not yet available for the variables explored in this study.

Finally, foot function, foot pain or QoL might be affected by un-model factors, i.e. factors that contribute to these outcomes but were not included in the model, such as fatigue, microvascular vasculopathy, fear of disease progression, or social support.

5.4 Directions for future research

This study has provided a platform for further research into foot pathology and quality of life in SSc.

i. Development of target interventions

The results from the model provided an initial insight into the factors that affect foot function, foot pain and QoL. Future research should be aiming to define which factors from the models are modifiable and non-modifiable, through general consensus using a panel of experts. Once the modifiable factors are identified they should be assessed for suitability for targeted intervention and a range of therapeutic options should be defined. From the defined therapeutic intervention, one or more proof of concept studies should be undertaken to explore the feasibility of the targeted approaches and investigate the

mechanism of action for any observed effect. Depending on the result of the feasibility study, randomised control trials should be developed to test the interventions to inform evidence based clinical practice and improve foot pathology and patients' quality of life. This structured future research plan can be applied to any of the results of the three models.

ii. Development of composites outcomes measure

The results from the clinical testing protocols also highlighted that for some neurological and musculoskeletal tests there are inadequate criteria to determine the clinically meaningful thresholds for each test that determine cut-points for normal versus pathology. Future research could focus therefore on determining relevant thresholds and developing composite outcome measures that capture and determine the severity of neuropathy, vasculopathy, and musculoskeletal involvement in the feet, and to aid clinical and research assessments.

iii. Exploration of foot pathology in patients reporting foot problems.

The heterogeneity of the disease across the sample in the study might have caused some of the difference in foot pathology between SSc patients and healthy participants to be missed. Future studies could be carried out exploring in depth the pathology of the disease in those patients with SSc that specifically report foot problems.

5.5 Summary

The major findings of this thesis can be summarised as follow:

i. SSc has an impact on the individual at many levels. Some people with SSc have significant foot disability, which is associated with significant detrimental physical and psychological consequences.

ii. Foot pathology in SSc involves inter-related features affecting the integumentary, vascular, neurological and musculoskeletal systems.

iii. Foot pathology in patients with SSc has multifactorial drivers, with physical and psychological factors contributing to the foot pathology.

V. Foot pathology is one of the major contributors to the impact on the overall QoL of patients with SSc, influencing QoL at a higher level than other systemic symptoms.

VI. General function is the major predictor of foot function, foot pain and QoL, and especial attention should be paid in the clinic to address the relevant factors.

Appendix

Intra-Rater Reliability of Ultrasound Measurement of Plantar Soft-Tissue Thickness

5.1 Introduction

To ensure that the candidate was able to obtain reliable ultrasound measurements of the plantar soft-tissue thickness under the 3rd MTP joint and heel, an intra-rater reliability study was undertaken. The ultrasound measurements were considered necessary to assess whether the plantar soft-tissues are affected by the same pathophysiological process of fibrosis that affects other subcutaneous tissues in patients with SSc. The reliability with which the candidate measures the thickness of the plantar soft-tissue in the Regions Of Interest (ROI) is important to ensure that the measurements represent features of the disease rather than measurement artefact. Reliability is an important consideration when using ultrasound because the technique is known to be operator dependent.

It is useful to quantify the repeatability or reproducibility of the observations, as repeatability is a measure of consistency. Measurements of repeatability are variously referred as reliability, reproducibility, consistency, test-retest variability or stability [405, 406]. For the purposes of the current study the concept of repeatability is being explored only through reliability analysis and in particular focuses on the intra-rater reliability. The candidate's intra-rater reliability in obtaining measures of the soft tissue thickness in the plantar soft tissues is

quantified by taking measurements from relevant areas in the same group of participants at two different time points.

5.2 Participants and methods

5.2.1 Participants

i. Sample size

This study aimed to recruit 35 participants as this sample size is recommended as an adequate sample size for intra-rater reliability studies on validated tests [407]. From these 35 participants the target was to recruit 30 healthy participants, as the purpose was to investigate the technique of the operator rather than the clinical use, and also 5 patients with SSc to confirm the technique in patients but causing them minimum inconvenience.

ii. Recruitment

Recruitment was undertaken at Chapel Allerton Hospital (Leeds Teaching Hospitals NHS Trust). The healthy participants were members of the staff from the Leeds Teaching Hospitals NHS Trust approached directly by the candidate regarding taking part in the study. Those that indicated willingness to participate were given a brief verbal explanation of the study and if they wished to participate consent was obtained. The patients with SSc were approached by the candidate when they were attending their routine care at the connective tissue disease clinics. Patients indicating a willingness to participate were given a brief verbal explanation of the study, and consent was obtained.

iii. Inclusion/ exclusion criteria

All participants had to be aged ≥18 years old, and assent freely to participate in the study. The healthy participants had to have general good health, i.e free of any chronic disease; while the only requirement for the patients was to have a physician diagnosis of SSc (ARA/ACR 1980 criteria).

5.2.2 Methods

All measurements were carried out using high-resolution diagnostic ultrasound (GE Voluson, GE Healthcare, Chalfont, UK) with an 18 Mhz probe.

The regions of interest (ROI) from the plantar aspect of the foot were the 3rd metatarsophalangeal (MTP) joint and heel. The soft-tissue thickness in both regions were measured by the candidate at two different time points, at least two weeks apart.

To measure the plantar soft-tissues thickness at the 3rd MTP joint, measurements were taken from the closest point of the metatarsal head to the ultrasound transducer, to the most superficial point of the epidermis as illustrated in Figure A.1. When taking the measurements, the longitudinal axis of the ultrasound transducer was aligned to the longitudinal axis of the metatarsal head.

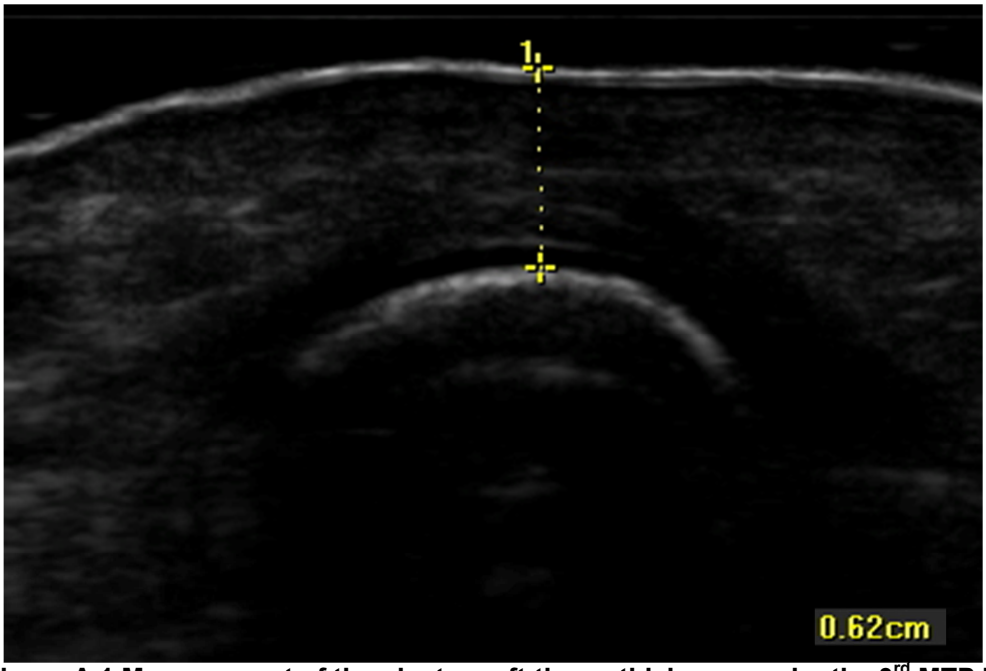

Figure A.1 Measurement of the plantar soft-tissue thickness under the 3rd MTP joint

To measure the plantar soft-tissues thickness at the heel, measurements were taken from the closest point of the calcaneus to the ultrasound transducer, to the most superficial point of the epidermis as illustrated in Figure A.2. When taking the measurements the longitudinal axis of the ultrasound transducer was aligned to the longitudinal axis of the calcaneus.

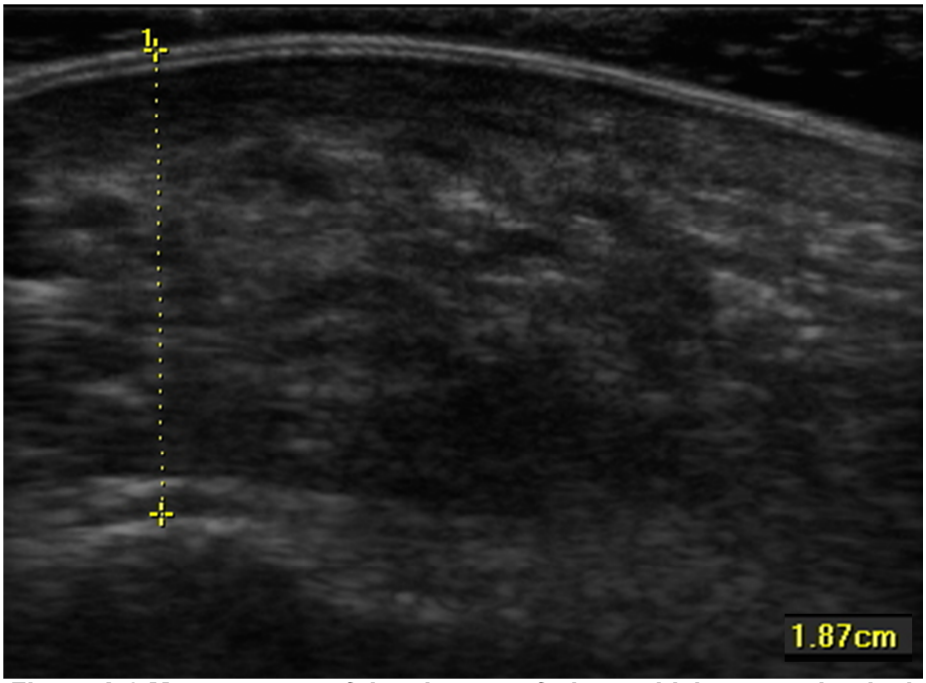

Figure A.2 Measurement of the plantar soft-tissue thickness under the heel

To obtain the most accurate possible reading, compression with the ultrasound probe of the skin was avoided, as the slightest compression would cause reduction of the soft-tissues thickness being measured. Compression was avoided by ensuring a visible layer of gel remained between the transducer head and the out layer of the epidermis on the B mode image.

5.3 Statistical Analysis

Three statistical approaches were used to assess the intra-rater reliability: Intraclass Correlation Coefficient (ICC) to examine reliability; Standard Error of the Measure to obtain an absolute index of reliability[405, 406, 408]; Bland–Altman plot to examine agreement and stability of the measure over the range of values [409, 410].

The ICC is a relative measure of repeatability that provides a reliability index, which is indicative of the overall measurement error. In this reliability study the ICC was used to quantify the relative consistency between measurements [411] by estimating the proportion of the total variance that is accounted for by the variation between subjects, while the remaining variance was attributed to the variation obtain from the repeated measurements within subjects [406, 408].

To assess the intra-rater, between-day repeatability the ICC model used was the one-way, single measure model [412, 413]. This is recommended for use in determining the intra-rater reliability of repeated measurements and is the most conservative of all the ICC models [411-415]. The ICC provides a reliability coefficient, but it cannot easily be interpreted clinically because it does not indicate the magnitude of disagreement between measurements. Thus, it has

been suggested that ICCs should be complemented by also calculating the Standard Error of the Measure which assesses the within subject test-retest variation and offers a value for the absolute range in which a subject's 'true' measurement is likely to lie [405, 406, 411]. The Standard Error of the Measure was calculated using the ICC and as indicated by the following formula:

$$Standard\ Error\ of\ the\ Measure\ = \frac{SD\ of\ difference}{\sqrt{ICC\ (1 - ICC)}}$$

The Bland–Altman plot, also known as the Mean-vs-difference plot, was plotted with the 95% limits of agreement. The Bland–Altman plot was used to assess agreement between the two measurements, and to identify whether there was any systematic variation in the difference as the magnitude of the measurement (skin thickness) varied across the measures within the cohort. Agreement is an important component of the intra-rater reliability. For agreement to occur the bias and variation has to be uniform throughout the range of measurements.

The 95% limits of agreement provided the range within 95% of the difference between measurements were expected to lie [409, 410].

5.4 Results

Of the 35 recruits 30 participants completed both sessions of the reliability study. The final sample was composed of 27 healthy participants and 3 participants with SSc. Three healthy participants and two patients with SSc were lost at follow-up because of inability or unwillingness to attend.

Of the 30 participants, 22 were female and 8 were male. The mean age of the participants was 42 (SD ±12.) and the mean time between measurements was 13.5 days (SD 15.9).

i. Intra-rater reliability results for the 3rd MTPJ

The results from the ICC of the 3rd MTPJ was 0.58 (95%CI 0.28 to 0.77) indicating a moderate reliability in the measurement of the 3rd MTPJ [416]. The Standard Error of the Measure, yielded a measurement error for the 3rd MTPJ of 0.30cm indicating that the candidate's measurement error for this ROI was 3mm.

The Bland–Altman plot consisted of the mean of the two groups of measurements plotted against the difference between the two measurements (Figure A.3). For the 3rd MTPJ soft-tissue thickness, the Bland-Altman plots showed that the mean of the difference was very close to 0, at -0.031cm, indicating that there was little systematic variability between the two measurements. The 95% limits of agreement, ± 0.303cm supported the Standard Error of the Measure results indicating that the candidate was able to re-measure skin thickness at the two sites within 3mm [410]. The differences from the mean were also consistent over the range of skin thicknesses measured indicating stability in this degree of error.

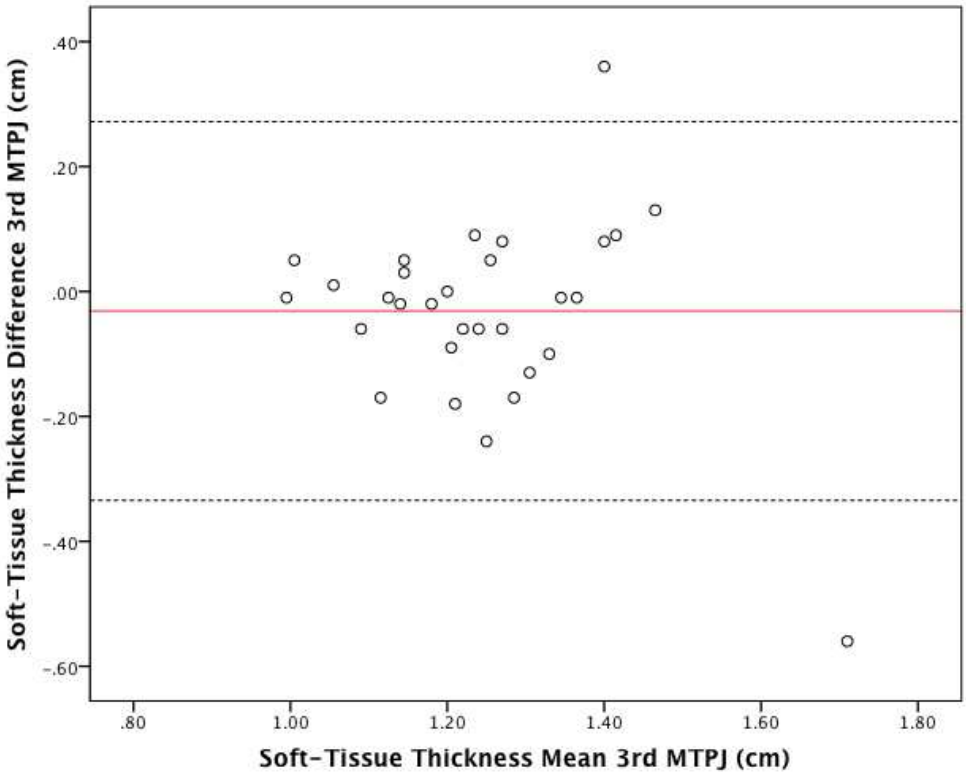

Figure A.3 Bland-Altman plot 3rd MTPJ. The constant line represents the difference of the means at -0.031cm, the two discontinued lines represent the 95% limits of agreement (mean±2SD) at ± 0.303cm

ii. Intra-rater reliability results for the heel

The ICC value for intra-rater testing at heel was 0.79 (95%CI 0.60 to 0.89) indicating that the reliability in the measurement of the heel thickness is substantial [416]. The Standard Error of the Measure, yielded a measurement error for the heel of 0.34cm, indicating that the candidate's measurement error for the heel was again approximately 3mm.

The Bland–Altman plot for the heel soft-tissues thickness (Figure A.4) showed similar results to those from the 3rd MTPJ. The mean of the difference was also very close to 0 (-0.033cm), again indicating that there was little systematic variability between the two measurements. The difference between the two measurements was relatively close to the mean of the difference, indicating good agreement. The 95% limits of agreement, ±0.289cm for the heel plot,

again supported the Standard Error of the Measure results indicating that the candidate was able to re-measure skin thickness at the heel to within approximately 3mm [410]. Again the differences from the mean were consistent over the range of values indicating stability in the magnitude of error.

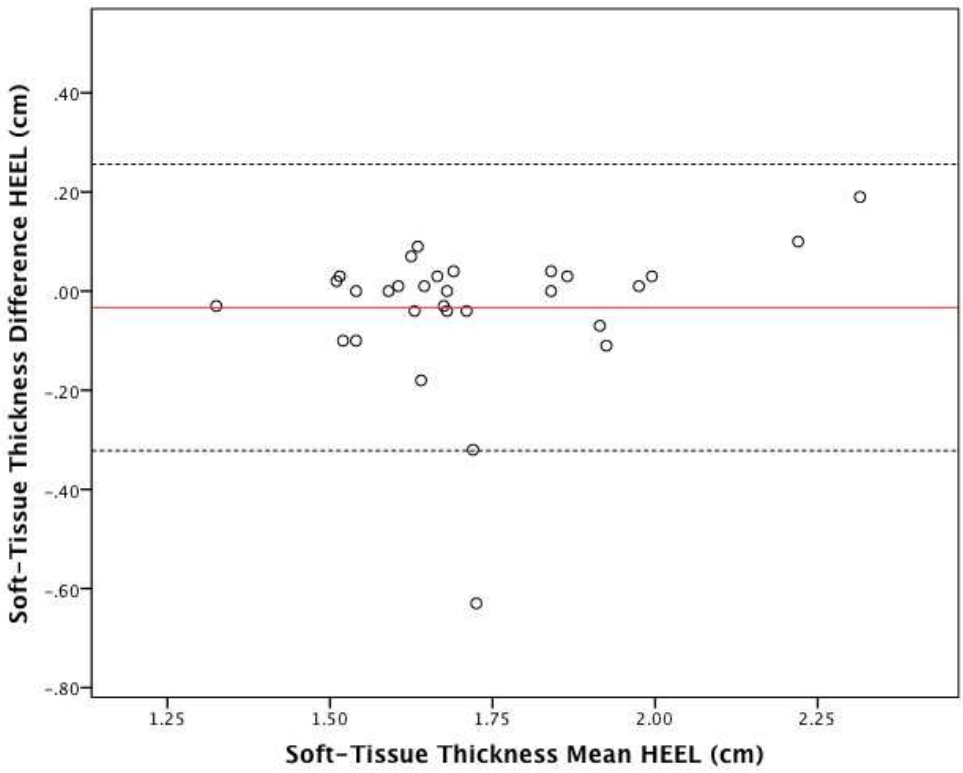

Figure A.4 Bland-Altman plot heel: The constant line represents the difference of the means at -0.033cm, the two discontinued lines represent the 95% limits of agreement (mean±2SD) at ± 0.289cm.

Both graphs showed the variability being constant throughout the range of measurements and the absence of any trend indicating systematic bias.

5.5 Discussion

The test-retest results indicate that US readings of plantar soft-tissue thickness at the 3rd MTPJ and at the heel, taken by the candidate two weeks apart, were

adequately reliable and yielded a measurement error of approximately 3mm. Clinically, a 3mm measurement error is considered small compared to the magnitude of the values obtained from the soft-tissues thickness of the ROI which ranged from 0.9cm to 2.3cm. The implications of the measurement error for the thesis are that any between-group difference in soft-tissue thickness identified in the main study, would need to exceed 3mm to be considered a 'true' difference and not simply to represent measurement error.

In addition, the Bland-Altman plots indicated that the variability and bias are consistent throughout the range of measurements and that the candidate is able to measure a range of different soft-tissues thickness within the ROI reliably and independent of the thickness of the soft-tissues.

When interpreting this reliability study, the limitations of this study have to be considered. The candidate used only a single measurement of the soft-tissues from the ROI at two-time points, as opposed to an average of multiple repeat measurements. Although using single measurements is acceptable for reliability studies [413, 417], the use of averaged measures would have increased the consistency of the measurements and strengthened the technical reliability overall. This would not however have reflected the approach used in the main study, which employed a single measure due to the time constraints introduced by the collection of data from multiple body systems. It was considered more appropriate in the current study therefore to explore the reliability of the protocol to be used in the main study.

It is acknowledged that there was a small representation of participants with SSc in the sample, with only 3 out of 30 participants having SSc. As there was a small representation of the population that was to be evaluated in the main

study the possibility remains that the measurements of the plantar soft–tissues thickness may not to be as reliable in patients with SSc as it is for healthy participants. This is not likely however, as the ultrasound measure is technical, rather than disease –specific and the results from the Bland-Altman plot showed that the candidate is able to measure a range of different soft-tissues thickness on the ROI in a reliable way, independently of the thickness of the soft-tissues. This indicating that if the plantar soft-tissue thickness varies systematically in patients with SSc compared to controls, the candidate should be able to measure the thickness with the same reliability as per the healthy participants.

5.6 Conclusion

The results obtained from the tests undertaken to assess the different components of the intra-rater reliability have shown that the candidate's ultrasound measurements of the plantar soft-tissues of the 3rd MTPJ and heel are adequately reliable as shown by moderate and substantial reliability indices indicated by the ICC, good agreement demonstrated by the Band-Altman plots and the small Standard Error of the Measure and reliability coefficients. This indicates that the candidate was able undertake the ultrasound measurements in a repeatable enough manner to warrant their inclusion in the main study.

List of references

1. Johnson, S.R., et al., *Validation of potential classification criteria for systemic sclerosis.* Arthritis Care & Research, 2012.
2. Abraham, D.J. and J. Varga, *Scleroderma: from cell and molecular mechanisms to disease models.* Trends in Immunology, 2005. 26(11): p. 587-595.
3. Geyer, M. and U. Müller-Ladner, *The Pathogenesis of Systemic Sclerosis Revisited* Clinic Rev Allerg Immuno, 2011. 40: p. 92-103.
4. Stamm, T., et al., *Concepts of functioning and health important to people with systemic sclerosis: a qualitative study in four European countries.* Annals of the rheumatic diseases, 2011. 70(6): p. 1074-1079.
5. Bérezné, A., et al., *Impact of systemic sclerosis on occupational and professional activity with attention to patients with digital ulcers.* Arthritis care & research, 2011. 63(2): p. 277-285.
6. Cossutta, R., et al., *[Evaluation of quality of life in patients with systemic sclerosis by administering the SF-36 questionnaire].* Reumatismo, 2002. 54(2): p. 122-127.
7. Khanna, D., et al., *Correlation of the degree of dyspnea with health-related quality of life, functional abilities, and diffusing capacity for carbon monoxide in patients with systemic sclerosis and active alveolitis: results from the Scleroderma Lung Study.* Arthritis and rheumatism, 2005. 52(2): p. 592-600.
8. Sari-Kouzel, H., et al., *Foot problems in patients with systemic sclerosis.* Rheumatology (Oxford, England), 2001. 40(4): p. 410-413.
9. La Montagna, G., et al., *Foot involvement in systemic sclerosis: A longitudinal study of 100 patients* Seminars in Arthritis and Rheumatism, 2002. 31(4): p. 248-55.
10. Alcacer-Pitarch, B., et al., *Foot Problems In Ssc, A Pilot Study Investigating Foot Health Status And Gait [Ab0374]* Annals of Rheumatic Disease 2009. 68(3): p. 748.
11. Della Rossa A. and G. Valentini.. *European multicentre study to define disease activity criteria for systemic sclerosis. I Clinical and epidemiological features of 290 patients for 19 centres.* Annals of Rheumatic Diseases, 2001. 60 p. 585-591.
12. Balbir-Gurman A., et al., *Non-invasive measurement of biomechanical skin properties in systemic sclerosis.* Annals of Rheumatic Diseases 2002. 61: p. 237-241.
13. Veale, D., T. Collidge, and J. Belch, *Increased prevalence of symptomatic macrovascular disease in systemic sclerosis.* Annals ofthe Rheumatic Diseases 1995. 54: p. 853-855.
14. Rosato, E., et al., *In systemic sclerosis macrovascular damage of hands digital arteries correlates with microvascular damage.* Microvascular Research, 2011. In Press, Corrected Proof.
15. Ho M., et al., *Macrovascular disease and systemic sclerosis.* Annals of Rheumatic Diseases, 2000. 59 (39-43).

16. Allcock R.J., et al., *A study of the prevalence of systemic sclerosis in northeast England.* Rheumatology, 2004. 43 p. 596-602
17. Mayes, M., *Scleroderma epidemiology.* Rheumatic Disease Clinics North America 2003. 29(2): p. 239-254.
18. Robert-Thompson P.J., et al., *Scleroderma in south Australia: further epidemiological observations supporting a stochastic explanation.* International Medicine Journal 2006. 36: p. 489-497
19. Wilson, L., *Cost-of-illness of scleroderma: The case for rare diseases.* Seminars in arthritis and Rheumatism, 1997. 27 (2): p. 73-84.
20. Sari-Kouzel, H., Hutchinson,C.E. Middleton, A. Webb, F. Moore,T. Griffin, K.and Herrick, A.L. , *Foot problems in patients with systemic sclerosis.* British Society for Rheumatology 2001. 40(410-413).
21. Schady, W., et al., *Peripheral nerve dysfunction in scleroderma.* QJM, 1991.
22. Benrud-Larson, L.M., et al., *The impact of pain and symptoms of depression in scleroderma.* Pain, 2002. 95(3): p. 267-275.
23. Silman, A.J., *Scleroderma.* Bailliere's Clinical Rheumatology 1995. 9(3): p. 471-473.
24. LeRoy, E.C., et al., *Scleroderma (systemic sclerosis): classification, subsets and pathogenesis.* The Journal of Rheumatology, 1988. 15(2): p. 202-205.
25. Krieg, T. and K. Takehara, *Skin disease: a cardinal feature of systemic sclerosis.* Rheumatology, 2009. 48 Suppl 3: p. iii14-8.
26. Goetz, R. and M. Berne, *The pathophysiology of progressive systemic sclerosis (generalised scleroderma) with especial reference to changes in the viscera.* Clin Proc 1945. 4: p. 337-92.
27. Tuffanelli, D.L. and R.K. Winkelmann, *Diffuse systemic scleroderma. A comparison with acrosclerosis.* Annals of internal medicine, 1962. 57: p. 198-203.
28. Winterbauer, R., *Multiple telangiectasia, Raynaud's phenomenon,sclerodactyly, and subcutaneous calcinosis: A syndrome mimickinghereditary hemorrhagic telangiectasia. .* Bull Johns Hopkins Hospital 1964. 114: p. 361-83.
29. Barnett, A.J. and D.A. Coventry, *Scleroderma. 1. Clinical features, course of illness and response to treatment in 61 cases.* The Medical journal of Australia, 1969. 1(19): p. 992-1001.
30. Rodnan, G., S. Jablonska, and T. Medsger, *Classification and nomenclature of progressive systemic sclerosis.* Clinical Rheumatic Dissease 1979. 5: p. 5-13.
31. Giordano, M., et al., *Different antibody patterns and different prognoses in patients with scleroderma with various extent of skin sclerosis.* The Journal of Rheumatology, 1986. 13(5): p. 911-6.
32. Holzmann, H., S. Sollberg, and P. Altmeyer, *[Classification of progressive systemic scleroderma]. Einteilung der progressiven systemischen Sklerodermie.* Der Hautarzt; Zeitschrift fur Dermatologie, Venerologie, und verwandte Gebiete, 1987. 38(5): p. 253-7.
33. Masi, A.T., *Classification of systemic sclerosis (scleroderma): relationship of cutaneous subgroups in early disease to outcome and serologic reactivity.* The Journal of Rheumatology, 1988. 15(6): p. 894-8.

34. LeRoy, E.C. and T.A. Medsger, Jr., *Criteria for the classification of early systemic sclerosis.* The Journal of Rheumatology, 2001. 28(7): p. 1573-6.
35. Scussel-Lonzetti, L., et al., *Predicting mortality in systemic sclerosis: analysis of a cohort of 309 French Canadian patients with emphasis on features at diagnosis as predictive factors for survival.* Medicine, 2002. 81(2): p. 154-67.
36. Ferri, C., G. Valentini, and F. Gozzi, *Systemic sclerosis: demographic, clinical, and serologic features and survival in 1,012 Italian patients. .* Medicine Baltimore, 2002. 81: p. 139-53.
37. Maricq, H.R. and I. Valter, *A working classification of scleroderma spectrum disorders: a proposal and the results of testing on a sample of patients.* Clinical and Experimental Rheumatology, 2004. 22(3 Suppl 33): p. S5-13.
38. Johnson, S.R., B.M. Feldman, and G.A. Hawker, *Classification criteria for systemic sclerosis subsets.* The Journal of Rheumatology 2007. 39(9): p. 1855-1863.
39. Matucci-Cerinic, M., et al., *The challenge of early systemic sclerosis for the EULAR Scleroderma Trial and Research group (EUSTAR) community. It is time to cut the Gordian knot and develop a prevention or rescue strategy.* Annals of the Rheumatic Diseases, 2009. 68(9): p. 1377.
40. Zulian, F., *Systemic sclerosis and localized scleroderma in childhood.* Rheumatic Diseases Clinics of North America, 2008. 34(1): p. 239-55; ix.
41. Masi, A.T., D. Subcommittee For Scleroderma Criteria of the American Rheumatism Association, and C. Therapeutic Criteria, *Preliminary criteria for the classification of systemic sclerosis (scleroderma).* Arthritis & Rheumatism, 1980. 23(5): p. 581-590.
42. Hachulla, E. and D. Launay, *Diagnosis and classification of systemic sclerosis.* Clinical Reviews in Allergy and Immunology, 2011. 40(2): p. 78-83.
43. LeRoy, E.C. and T.A. Medsger Jr, *Criteria for the classification of early systemic sclerosis.* The Journal of Rheumatology, 2001. 28(7): p. 1573.
44. Wigley, F., *When is scleroderma really scleroderma?* The Journal of Rheumatology, 2001. 28(7): p. 1471-1473.
45. Poormoghim, H., et al., *Systemic sclerosis sine scleroderma: Demographic, clinical, and serologic features and survival in forty-eight patients.* Arthritis and Rheumatism, 2000. 43(2): p. 444-451.
46. den Hoogen, F., et al., *2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative.* Arthritis & Rheumatism, 2013.
47. Avouac, J., et al., *Preliminary criteria for the very early diagnosis of systemic sclerosis: results of a Delphi Consensus Study from EULAR Scleroderma Trials and Research Group.* Annals of the Rheumatic Diseases, 2011. 70(3): p. 476.
48. Pope, J., et al., *Report from the EULAR ACR scleroderma classification criteria committee.* Rheumatology 2012. 51: p. ii1-ii26.
49. Avouac, J., et al., *Preliminary criteria for the very early diagnosis of systemic sclerosis: results of a Delphi Consensus Study from EULAR*

Scleroderma Trials and Research Group. Annals of the rheumatic diseases, 2011. 70(3): p. 476-481.
50. Bijlsma, J.W., *Eular Compedium on Rheumatic Diseases*, ed. J.W. Bijlsma. 2009, Canada: BMJ and European Leage against rheumatism. 824.
51. Ranque, B. and L. Mouthon, *Geoepidemiology of systemic sclerosis.* Autoimmunity Reviews 2010. 9 p. A311–A318.
52. Chifflot, H., et al. *Incidence and prevalence of systemic sclerosis: a systematic literature review*. 2008. Elsevier.
53. Walker, U.A., et al., *Geographical variation of disease manifestations in systemic sclerosis: a report from the EULAR Scleroderma Trials and Research (EUSTAR) group database.* Ann Rheum Dis, 2009. 68(6): p. 856-62.
54. Silman, A., et al., *Geographical clustering of scleroderma in south and west London.* British Journal of Rheumatology, 1990. 29(2): p. 93-6.
55. Silman, A., et al., *An epidemiological study of scleroderma in the West Midlands.* British Journal of Rheumatology, 1988. 27(4): p. 286-290.
56. Allcock, R.J., et al., *A study of the prevalence of systemic sclerosis in northeast England. .* Rheumatology, 2004. 43 p. 596-602.
57. Bijlsma, J.W., *EULAR Compendium on rheumatic diseases*. 2009: BMJ Publishing Group Limited.
58. Granel, B., F. Bernard, and C. Chevillard, *Genetic susceptibility to systemic sclerosis: From clinical aspect to genetic factor analyses.* European Journal of Internal Medicine, 2009. 20: p. 242–252
59. Arnett, F.C., et al., *Familial occurrence frequencies and relative risks for systemic sclerosis (scleroderma) in three United States cohorts.* Arthritis Rheum, 2001. 44(6): p. 1359-62.
60. Feghali-Bostwick, C., T. Medsger, and T. Wright, *Analysis of systemic sclerosis in twins reveals low concordance for disease and high concordance for the presence of antinuclear antibodies.* Arthritis and rheumatism, 2003. 48(7): p. 1956-1963.
61. Broen, J., M. Coenen, and T. Radstake, *Deciphering the genetic background of systemic sclerosis.* Expert review of clinical immunology, 2011. 7(4): p. 449-462.
62. Broen, J., M. Coenen, and T. Radstake, *Genetics of systemic sclerosis: an update.* Current rheumatology reports, 2012. 14(1): p. 11-21.
63. Ahmed, S.S. and T. F.K., *Identification of novel targets in scleroderma: update on population studies, cDNA arrays, SNP analysis, and mutations.* Current Opinion Rheumatology 2003. 15: p. 766–771.
64. Johnson, R., M. Tew, and F. Arnett, *The genetics of systemic sclerosis.* Current rheumatology reports, 2002. 4(2): p. 99-107.
65. Reveille, J., *Ethnicity and race and systemic sclerosis: how it affects susceptibility, severity, antibody genetics, and clinical manifestations.* Current rheumatology reports, 2003. 5(2): p. 160-167.
66. Laing, J., et al., *Racial differences in scleroderma among women in michigan.* Arthritis & Rheumatism, 1997. 40(4): p. 734-742.
67. Mayes, M., et al., *Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population.* Arthritis and rheumatism, 2003. 48(8): p. 2246-2255.

68. Le Guern, V., et al., *Prevalence of systemic sclerosis in a French multi-ethnic county.* Rheumatology (Oxford, England), 2004. 43(9): p. 1129-1137.
69. Fernada-Mora, G., *Systemic Sclerosis: Environmental Factors.* Journal of Rheumatology, 2009. 36: p. 2383-96.
70. Gourley, M. and F. Miller, *Mechanisms of disease: Environmental factors in the pathogenesis of rheumatic disease.* Nature clinical practice. Rheumatology, 2007. 3(3): p. 172-180.
71. Haustein, U., et al., *Silica-induced scleroderma.* Journal of the American Academy of Dermatology, 1990. 22(3): p. 444-448.
72. Makol, A., M. Reilly, and K. Rosenman, *Prevalence of connective tissue disease in silicosis (1985-2006)-a report from the state of Michigan surveillance system for silicosis.* American journal of industrial medicine, 2011. 54(4): p. 255-262.
73. Rodnan, G., et al., *The association of progressive systemic sclerosis (scleroderma) with coal miners' pneumoconiosis and other forms of silicosis.* Annals of internal medicine, 1967. 66(2): p. 323-334.
74. Erasmus, L., *Scleroderma in goldminers on the Witwatersrand with particular reference to pulmonary manifestations.* South African journal of laboratory and clinical medicine. Suid-Afrikaanse tydskrif vir laboratorium- en kliniekwerk, 1957. 3(3): p. 209-231.
75. Garabrant, D. and C. Dumas, *Epidemiology of organic solvents and connective tissue disease.* Arthritis research, 2000. 2(1): p. 5-15.
76. Mayes, M., *Epidemiologic studies of environmental agents and systemic autoimmune diseases.* Environmental health perspectives, 1999. 107 Suppl 5: p. 743-748.
77. Diot, E., et al., *Systemic sclerosis and occupational risk factors: a case-control study.* Occupational and environmental medicine, 2002. 59(8): p. 545-549.
78. Bovenzi, M., et al., *A case-control study of occupational exposures and systemic sclerosis.* International archives of occupational and environmental health, 2004. 77(1): p. 10-16.
79. Nietert, P., et al., *Is occupational organic solvent exposure a risk factor for scleroderma?* Arthritis and rheumatism, 1998. 41(6): p. 1111-1118.
80. Hamamdzic, D., L. Kasman, and E. LeRoy, *The role of infectious agents in the pathogenesis of systemic sclerosis.* Current opinion in rheumatology, 2002. 14(6): p. 694-698.
81. Radić, M., D. Martinović Kaliterna, and J. Radić, *Infectious disease as aetiological factor in the pathogenesis of systemic sclerosis.* The Netherlands journal of medicine, 2010. 68(11): p. 348-353.
82. Arnson, Y., et al., *The role of infections in the immunopathogensis of systemic sclerosis--evidence from serological studies.* Annals of the New York Academy of Sciences, 2009. 1173: p. 627-632.
83. Neidhart, M., et al., *Increased serum levels of antibodies against human cytomegalovirus and prevalence of autoantibodies in systemic sclerosis.* Arthritis and rheumatism, 1999. 42(2): p. 389-392.
84. Mora, G., *Systemic sclerosis: environmental factors.* The Journal of rheumatology, 2009. 36(11): p. 2383-2396.

85. Silman, A. and M. Hochberg, *Occupational and environmental influences on scleroderma.* Rheumatic diseases clinics of North America, 1996. 22(4): p. 737-749.
86. Sternberg, E., et al., *Development of a scleroderma-like illness during therapy with L-5-hydroxytryptophan and carbidopa.* The New England journal of medicine, 1980. 303(14): p. 782-787.
87. Umezawa, H., et al., *New antibiotics, bleomycin A and B.* The Journal of antibiotics, 1966. 19(5): p. 200-209.
88. Poisson, J., A. Low, and Y. Park, *The treatment of nephrogenic systemic fibrosis with therapeutic plasma exchange.* Journal of clinical apheresis, 2013.
89. Katsumoto, T.R., M.L. Whitfield, and M.K. Connolly, *The Pathogenesis of Systemic Sclerosis.* The Annual Review of Pathology: Mechanisms of Disease, 2011. 6: p. 509–37.
90. Abraham, D.J., et al., *Overview of pathogenesis of systemic sclerosis.* Rheumatology (Oxford), 2009. 48 Suppl 3: p. iii3-7.
91. Denton, C.P., C.M. Black, and D.J. Abraham, *Mechanisms and consequences of fibrosis in systemic sclerosis.* Nature Clinical Practice Rheumatology, 2006. 2(3): p. 134-144.
92. Kallenberg, C., *Connective tissue disease in patients presenting with Raynaud's phenomenon alone.* Annals of the rheumatic diseases, 1991. 50(10): p. 666-667.
93. Herrick, A., *Pathogenesis of Raynaud's phenomenon.* Rheumatology (Oxford, England), 2005. 44(5): p. 587-596.
94. Freedman, R.R. and P. Ianni, *Role of cold and emotional stress in Raynaud's disease and scleroderma.* British medical journal (Clinical research ed.), 1983. 287(6404): p. 1499.
95. Cutolo, M., W. Grassi, and M. Matucci Cerinic, *Raynaud's phenomenon and the role of capillaroscopy.* Arthritis & rheumatism, 2003. 48(11): p. 3023-3030.
96. Guiducci, S., R. Giacomelli, and M. Cerinic, *Vascular complications of scleroderma.* Autoimmunity reviews, 2007. 6(8): p. 520-523.
97. Guiducci, S., R. Giacomelli, and M.M. Cerinic, *Vascular complications of scleroderma.* Autoimmunity Reviews, 2007. 6(8): p. 520-523.
98. Kahaleh, B., *The microvascular endothelium in scleroderma.* Rheumatology (Oxford), 2008. 47 Suppl 5: p. v14-5.
99. Shi□wen, X., et al., *Endothelin is a downstream mediator of profibrotic responses to transforming growth factor β in human lung fibroblasts.* Arthritis & Rheumatism, 2007. 56(12): p. 4189-4194.
100. Cambrey, A.D., et al., *Increased levels of endothelin-1 in bronchoalveolar lavage fluid from patients with systemic sclerosis contribute to fibroblast mitogenic activity in vitro.* Am. J. Respir.Cell Mol. Biol., 1994. 11: p. 439–445.
101. Denton, C.P. and D.J. Abraham, *Transforming growth factor-[beta] and connective tissue growth factor: key cytokines in scleroderma pathogenesis.* Current opinion in rheumatology, 2001. 13(6): p. 505-511.
102. Denton, C.P., et al., *Scleroderma fibroblasts show increased responsiveness to endothelial cell-derived IL-1 and bFGF.* Journal of investigative dermatology, 1997. 108(3).

103. Vancheeswaran, R., et al., *Localization of endothelin-1 and its binding sites in scleroderma skin.* J. Rheumatol., 1994: p. 1268–1276.
104. Humbert, M., et al., *Cellular and molecular pathobiology of pulmonary arterial hypertension.* Journal of the American College of Cardiology, 2004. 43(12 SUPPL.): p. 13S-24S.
105. Cerinic, M.M., et al., *Blood coagulation, fibrinolysis, and markers of endothelial dysfunction in systemic sclerosis.* Seminars in Arthritis and Rheumatism, 2003. 32(5): p. 285-295.
106. Matucci-Cerinic, M., U. Pietrini, and S. Marabini, *Local venomotor response to intravenous infusion of substance P and glyceryl trinitrate in systemic sclerosis.* Clinical and experimental rheumatology, 1990. 8(6): p. 561-565.
107. Tucker, A., et al., *Effect of nitric-oxide-generating system on microcirculatory blood flow in skin of patients with severe Raynaud's syndrome: a randomised trial.* Lancet, 1999. 354(9191): p. 1670-1675.
108. Anderson, M., et al., *Digital vascular response to topical glyceryl trinitrate, as measured by laser Doppler imaging, in primary Raynaud's phenomenon and systemic sclerosis.* Rheumatology (Oxford, England), 2002. 41(3): p. 324-328.
109. Knock, G., et al., *Characterization of endothelin-binding sites in human skin and their regulation in primary Raynaud's phenomenon and systemic sclerosis.* The Journal of investigative dermatology, 1993. 101(1): p. 73-78.
110. Vancheeswaran, R., et al., *Localization of endothelin-1 and its binding sites in scleroderma skin.* The Journal of rheumatology, 1994. 21(7): p. 1268-1276.
111. LeRoy, E.C., *Systemic sclerosis. A vascular perspective.* Rheumatic Disease Clinics of North America, 1996. 22(4): p. 675-694.
112. Steen, V., et al., *Digital ulcers: overt vascular disease in systemic sclerosis.* Rheumatology, 2009. 48 Suppl 3: p. iii19-24.
113. Sari-Kouzel, H., et al., *Foot problems in patients with systemic sclerosis.* British Society for Rheumatology 2001. 40: p. 410-413.
114. Dorevitch, M., L. Clemens, and J. Webb, *Lower limb amputation secondary to large vessel involvement in scleroderma.* British journal of rheumatology, 1988. 27(5): p. 403-406.
115. Distler, O., et al., *Uncontrolled expression of vascular endothelial growth factor and its receptors leads to insufficient skin angiogenesis in patients with systemic sclerosis.* Circulation Research, 2004. 95(1): p. 109-116.
116. Muller-Ladner, U., et al., *Mechanisms of vascular damage in systemic sclerosis.* Autoimmunity, 2009. 42(7): p. 587-95.
117. Guiducci, S., R. Giacomelli, and M. Matucci Cerinic, *Vascular complications of scleroderma.* Autoimmunity Reviews, 2007. 6: p. 520-523.
118. Kuwana, M., et al., *Defective vasculogenesis in systemic sclerosis.* The Lancet, 2004. 364(9434): p. 603-610.
119. Del Papa, N., et al., *Circulating Endothelial Cells as a Marker of Ongoing Vascular Disease in Systemic Sclerosis.* Arthritis and Rheumatism, 2004. 50(4): p. 1296-1304.

120. Distler, J.H.W., et al., *Angiogenic and angiostatic factors in the molecular control of angiogenesis.* Quarterly Journal of Nuclear Medicine, 2003. 47(3): p. 149-161.
121. Nitsche, A., *Raynaud, Digital Ulcers and Calcinosis in Scleroderma.* Reumatología Clínica (English Edition), 2012. 8(5): p. 270-277.
122. Kahaleh, B. and M. Matucci-Cerinic, *Raynaud's phenomenon and scleroderma: Dysregulated neuroendothelial control of vascular tone.* Arthritis and Rheumatism, 1995. 38(1): p. 1-4.
123. Bunker, C., et al., *Deficiency of calcitonin gene-related peptide in Raynaud's phenomenon.* Lancet, 1990. 336(8730): p. 1530-1533.
124. Ekenvall, L., et al., *alpha-Adrenoceptors and cold-induced vasoconstriction in human finger skin.* American Journal of Physiology-Heart and Circulatory Physiology, 1988. 255(5): p. H1000-H1003.
125. Flavahan, N., et al., *Increased alpha2-adrenergic constriction of isolated arterioles in diffuse scleroderma.* Arthritis and rheumatism, 2000. 43(8): p. 1886-1890.
126. Jeyaraj, S.C., et al., *Cooling evokes redistribution of α2C-adrenoceptors from Golgi to plasma membrane in transfected human embryonic kidney 293 cells.* Molecular pharmacology, 2001. 60(6): p. 1195-1200.
127. Kahaleh, M. and E. LeRoy, *Autoimmunity and vascular involvement in systemic sclerosis (SSc).* Autoimmunity, 1999. 31(3): p. 195-214.
128. Ngian, G., J. Sahhar, and I. Wicks…, *Cardiovascular disease in systemic sclerosis-an emerging association?* Arthritis Res …, 2011.
129. Au, K., et al., *Atherosclerosis in systemic sclerosis: a systematic review and meta-analysis.* Arthritis and rheumatism, 2011. 63(7): p. 2078-2090.
130. Kao, L. and C. Weyand, *Vasculitis in systemic sclerosis.* International journal of rheumatology, 2010. 2010: p. 385938.
131. Oddis, C., et al., *Vasculitis in systemic sclerosis: association with Sjögren's syndrome and the CREST syndrome variant.* The Journal of rheumatology, 1987. 14(5): p. 942-948.
132. D'Angelo, W., et al., *Pathologic observations in systemic sclerosis (scleroderma). A study of fifty-eight autopsy cases and fifty-eight matched controls.* The American journal of medicine, 1969. 46(3): p. 428-440.
133. Herrick, A., et al., *Vasculitis in patients with systemic sclerosis and severe digital ischaemia requiring amputation.* Annals of the rheumatic diseases, 1994. 53(5): p. 323-326.
134. Dyck, P., et al., *Nonsystemic vasculitic neuropathy.* Brain : a journal of neurology, 1987. 110 (Pt 4): p. 843-853.
135. Nihtyanova, S. and C. Denton, *Autoantibodies as predictive tools in systemic sclerosis.* Nature reviews. Rheumatology, 2010. 6(2): p. 112-116.
136. Steen, V., *Autoantibodies in systemic sclerosis.* Seminars in arthritis and rheumatism, 2005. 35(1): p. 35-42.
137. Koening, M., M. Dieude, and J. Senecal, *Predictive value of antinuclear autoantibodies: the lesson of the systemic sclerosis autoantibodies. .* Autoimmun Rev 2008. 7: p. 588–93.
138. Bizzaro, N., et al., *Sensitivity and specificity of immunological methods for the detection of anti-topoisomerase I (Scl70) autoantibodies: results of a multicenter study.* Clinical chemistry, 2000. 46(10): p. 1681-1685.

139. Czömpöly, T., et al., *Anti-topoisomerase I autoantibodies in systemic sclerosis.* Autoimmunity reviews, 2009. 8(8): p. 692-696.
140. Hamaguchi, Y., *Autoantibody profiles in systemic sclerosis: predictive value for clinical evaluation and prognosis.* The Journal of dermatology, 2010. 37(1): p. 42-53.
141. Walker, J. and M. Fritzler, *Update on autoantibodies in systemic sclerosis.* Current opinion in rheumatology, 2007. 19(6): p. 580-591.
142. Koenig, M., et al., *Heterogeneity of autoantibodies in 100 patients with autoimmune myositis: insights into clinical features and outcomes.* Arthritis research & therapy, 2007. 9(4).
143. Ulanet, D., et al., *Autoantibodies against B23, a nucleolar phosphoprotein, occur in scleroderma and are associated with pulmonary hypertension.* Arthritis and rheumatism, 2003. 49(1): p. 85-92.
144. Gabrielli, A., E. Avvedimento, and T. Krieg, *Scleroderma.* The New England journal of medicine, 2009. 360(19): p. 1989-2003.
145. Koenig, M., M. Dieudé, and J.-L. Senécal, *Predictive value of antinuclear autoantibodies: the lessons of the systemic sclerosis autoantibodies.* Autoimmunity reviews, 2008. 7(8): p. 588-593.
146. Okano, Y., V. Steen, and T. Medsger, *Autoantibody reactive with RNA polymerase III in systemic sclerosis.* Annals of internal medicine, 1993. 119(10): p. 1005-1013.
147. Gu, Y.S., et al., *The Immunobiology of Systemic Sclerosis.* Seminars in Arthritis and Rheumatism, 2008. 38(2): p. 132-160.
148. Prescott, R.J., et al., *Sequential dermal microvascular and perivascular changes in the development of scleroderma.* The Journal of pathology, 1992. 166(3): p. 255-263.
149. Fleischmajer, R., J. Perlish, and J. Reeves, *Cellular infiltrates in scleroderma skin.* Arthritis and rheumatism, 1977. 20(4): p. 975-984.
150. Amaral, T.N., et al., *Neurologic involvement in scleroderma: A systematic review.* Seminars in Arthritis and Rheumatism, 2013(0).
151. Dyck, P.J. and G.G. Hunder, *A case-control and nerve biopsy study of CREST multiple mononeuropathy.* NEUROLOGY, 1997. 49(6): p. 1641-5.
152. Schumacher, J., *Joint involvement in progressive systemic sclerosis (scleroderma): a light and electron microscopic study of synovial membrane and fluid.* Plastic and Reconstructive Surgery, 1974.
153. Barry, M., L. Katz, and L. Cooney, *An unusual articular presentation of progressive systemic sclerosis.* Arthritis Rheum, 1983. 26(8): p. 1041-3.
154. Rodnan, G., *The nature of joint involvement in progressive systemic sclerosis (diffuse scleroderma).* Annals of internal medicine, 1962. 56: p. 422-439.
155. Sato, S., et al., *Altered blood B lymphocyte homeostasis in systemic sclerosis: Expanded naive B cells and diminished but activated memory B cells.* Arthritis and Rheumatism, 2004. 50(6): p. 1918-1927.
156. Harris, M.L. and A. Rosen, *Autoimmunity in scleroderma: The origin, pathogenetic role, and clinical significance of autoantibodies.* Current Opinion in Rheumatology, 2003. 15(6): p. 778-784.
157. Varga, J. and D. Abraham, *Systemic sclerosis: a prototypic multisystem fibrotic disorder.* The Journal of clinical investigation, 2007. 117(3): p. 557-567.

158. Ferri, C., et al., *Systemic sclerosis: Demographic, clinical, and serologic features and survival in 1,012 Italian patients.* Medicine, 2002. 81(2): p. 139-153.
159. Ferri, C., et al., *Heart involvement and systemic sclerosis.* Lupus, 2005. 14(9): p. 702-707.
160. Poanta, L., et al., *Systolic and diastolic function in patients with systemic sclerosis.* European journal of internal medicine, 2009. 20(4): p. 378-382.
161. Clements, P.P. and D.E. Furst, *Heart involvement in sytemic sclerosis* Clinics in Dermatology 1994. 12: p. 267-275.
162. Allanore, Y., J. Avouac, and A. Kahan, *Systemic sclerosis: an update in 2008.* Joint, bone, spine : revue du rhumatisme, 2008. 75(6): p. 650-655.
163. Trad, S., et al., *Pulmonary arterial hypertension is a major mortality factor in diffuse systemic sclerosis, independent of interstitial lung disease.* Arthritis & Rheumatism, 2006. 54(1): p. 184-191.
164. Denton, C. and C. Black, *Scleroderma--clinical and pathological advances.* Best practice & research. Clinical rheumatology, 2004. 18(3): p. 271-290.
165. Varga, J., *Systemic sclerosis: an update.* Bull NYU Hosp Jt Dis, 2008. 66(3): p. 198-202.
166. Rodnan, G., G. Schreiner, and R. Black, *Renal involvement in progressive systemic sclerosis (generalized scleroderma).* The American journal of medicine, 1957. 23(3): p. 445-462.
167. O'Hare, A. and K. Johansen, *Lower-extremity peripheral arterial disease among patients with end-stage renal disease.* Journal of the American Society of Nephrology : JASN, 2001. 12(12): p. 2838-2847.
168. Margolis, D., O. Hofstad, and H. Feldman, *Association between renal failure and foot ulcer or lower-extremity amputation in patients with diabetes.* Diabetes care, 2008. 31(7): p. 1331-1336.
169. Hietaharju, A., et al., *Peripheral neuromuscular manifestations in systemic sclerosis (scleroderma).* Muscle & nerve, 1993. 16(11): p. 1204-1212.
170. Mondelli, M., et al., *Electrophysiological evidence of "nerve entrapment syndromes" and subclinical peripheral neuropathy in progressive systemic sclerosis (scleroderma).* Journal of neurology, 1995. 242(4): p. 185-194.
171. Schady, W., et al., *Peripheral Nerve Dysfunction in Scleroderma.* Quarterly Journal of Medicine, 1991. New Series 80(No. 292,): p. pp 661-675.
172. Brouns, R. and P.P. De Deyn, *Neurological complications in renal failure: a review.* Clinical Neurology and Neurosurgery, 2004. 107(1): p. 1-16.
173. Di Trapani, G., et al., *Peripheral neuropathy in course of progressive systemic sclerosis. Light and ultrastructural study.* Acta neuropathologica, 1986. 72(2): p. 103-110.
174. Rose, S., M. Young, and J. Reynolds, *Gastrointestinal manifestations of scleroderma.* Gastroenterology clinics of North America, 1998. 27(3): p. 563-594.
175. Wipff, J., et al., *Prevalence of Barrett's esophagus in systemic sclerosis.* Arthritis and rheumatism, 2005. 52(9): p. 2882-2888.
176. Sallam, H., T. McNearney, and J. Chen, *Systematic review: pathophysiology and management of gastrointestinal dysmotility in*

systemic sclerosis (scleroderma). Alimentary pharmacology & therapeutics, 2006. 23(6): p. 691-712.
177. Baron, M., et al., *Malnutrition is common in systemic sclerosis: results from the Canadian scleroderma research group database.* The Journal of rheumatology, 2009. 36(12): p. 2737-2743.
178. Omair, M. and P. Lee, *Effect of gastrointestinal manifestations on quality of life in 87 consecutive patients with systemic sclerosis.* The Journal of rheumatology, 2012. 39(5): p. 992-996.
179. Denton, C., et al., *Demographic, clinical and antibody characteristics of patients with digital ulcers in systemic sclerosis: data from the DUO Registry.* Annals of the rheumatic diseases, 2012. 71(5): p. 718-721.
180. Alivernini, S., et al., *Skin ulcers in systemic sclerosis: Determinants of presence and predictive factors of healing.* Journal of the American Academy of Dermatology, 2009. 60(3): p. 426-435.
181. Tandara, A. and T. Mustoe, *Oxygen in wound healing--more than a nutrient.* World journal of surgery, 2004. 28(3): p. 294-300.
182. Davidson, J. and T. Mustoe, *Oxygen in wound healing: more than a nutrient.* Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society, 2001. 9(3): p. 175-177.
183. Stadelmann, W., A. Digenis, and G. Tobin, *Impediments to wound healing.* American journal of surgery, 1998. 176(2A Suppl).
184. Edwards, C. and R. Marks, *Evaluation of biomechanical properties of human skin.* Clinics in Dermatology. 13(4): p. 375-380.
185. Edwards, C. and R. Marks, *Evaluation of biomechanical properties of human skin.* Clinics in dermatology, 1995. 13(4): p. 375-380.
186. Denton, C.P. and C. M. Black, *Scleroderma--clinical and pathological advances.* Best Practice & Research Clinical Rheumatology, 2004. 18(3): p. 271-290.
187. Claman, H.N., R.C. Giorno, and J.R. Seibold, *Endothelial and fibroblastic activation in scleroderma: the myth of the "uninvolved skin".* Arthritis & Rheumatism, 1991. 34(12): p. 1495-1501.
188. Pierard, G.E. and M. Lapiere Ch, *Physiopathological variations in the mechanical properties of skin.* Archives of Dermatological Research, 1977. 260(3): p. 231-239.
189. La Montagna, G., et al., *The arthropathy of systemic sclerosis: a 12 month prospective clinical and imaging study.* Skeletal Radiology 2005. 34: p. 35–41.
190. Poole, J., V. Watzlaf, and F. D'Amico, *A five-year followup of hand function and activities of daily living in systemic sclerosis (scleroderma).* Journal of hand therapy : official journal of the American Society of Hand Therapists, 2004. 17(4): p. 407-411.
191. Chung, L. and D. Fiorentino, *Digital ulcers in patients with systemic sclerosis.* Autoimmunity reviews, 2006. 5(2): p. 125-128.
192. Amanzi, L., et al., *Digital ulcers in scleroderma: staging, characteristics and sub-setting through observation of 1614 digital lesions.* Rheumatology (Oxford), 2010. 49(7): p. 1374-82.
193. Reidy, M.E., V. Steen, and J.J. Nicholas, *Lower extremity amputation in scleroderma.* Arch Phys Med Rehabil, 1992. 73(9): p. 811-813.

194. Czirjak, L., I. Foeldvari, and U. Muller-Ladner, *Skin involvement in systemic sclerosis.* Rheumatology, 2009. 47(SUPPL. 5): p. v44-v45.
195. Otter, S.J., C. Bowen, and A. Young, *Forefoot Plantar Pressures in Rheumatoid Arthritis.* Journal of the American Podiatric Medical Association, 2004. 94(3): p. 255-260.
196. Van der Leeden M., et al., *Forefoot joint damage, pain and disability in rheumatoid arthritis patients with foot complaints: the role of plantar pressure and gait characteristics.* Rheumatology 2006. 45: p. 465-469.
197. Richards, H.L., et al., *Psychological adjustment to systemic sclerosis - Exploring the association of disease factors, functional ability, body related attitudes and fear of negative evaluation.* Psychology, Health and Medicine, 2004. 9(1): p. 29-39.
198. van Lankveld, W.G.J.M., et al., *Appearance self-esteem in systemic sclerosis—subjective experience of skin deformity and its relationship with physician-assessed skin involvement, disease status and psychological variables.* Rheumatology, 2007. 46: p. 872–876.
199. Belch, J., *Raynaud's phenomenon: its relevance to scleroderma.* Annals of the rheumatic diseases, 1991.
200. Tuffanelli, D.L. and R. Winkelmann, *Systemic scleroderma: a clinical study of 727 cases.* Archives of Dermatology, 1961. 84(3): p. 359.
201. Cutolo, M., A. Sulli, and V. Smith, *Assessing microvascular changes in systemic sclerosis diagnosis and management.* Nature Reviews Rheumatology, 2010. 6(10): p. 578-587.
202. Cutolo, M., et al., *Capillaroscopy as an Outcome Measure for Clinical Trials on the Peripheral Vasculopathy in SSc—Is It Useful?* International journal of rheumatology, 2010. 2010.
203. Herrick, A., *Diagnosis and Management of Scleroderma Peripheral Vascular Disease.* Rheumatic Disease Clinics of North America, 2008. 34(1): p. 89-114.
204. Smith V, et al., *Nailfold capillaroscopy for prediction of novel future severe organ involvement in systemic sclerosis.* Journal of Rheumatology, 2013. 40(12): p. 2023-8.
205. Herrick, A.L., et al., *Nail-fold capillary abnormalities are associated with anti-centromere antibody and severity of digital ischaemia.* Rheumatology, 2010. 49(9): p. 1776-1782.
206. Bredemeier, M., et al., *Nailfold capillary microscopy can suggest pulmonary disease activity in systemic sclerosis.* The Journal of rheumatology, 2004. 31(2): p. 286-294.
207. Maricq, H.R., G. Spencer-Green, and E.C. LeRoy, *Skin capillary abnormalities as indicators of organ involvement in scleroderma (systemic sclerosis), Raynaud's syndrome and dermatomyositis.* The American journal of medicine, 1976. 61(6): p. 862-870.
208. de Takats, G. and E.F. Fowler, *Raynaud's phenomenon.* JAMA: The Journal of the American Medical Association, 1962. 179(1): p. 1-8.
209. Koenig, M., et al., *Autoantibodies and microvascular damage are independent predictive factors for the progression of Raynaud's phenomenon to systemic sclerosis: a twenty-year prospective study of 586 patients, with validation of proposed criteria for early systemic sclerosis.* Arthritis and rheumatism, 2008. 58(12): p. 3902-3912.

210. Kahaleh, M., *Vascular disease in scleroderma. Endothelial T lymphocyte-fibroblast interactions.* Rheumatic diseases clinics of North America, 1990. 16(1): p. 53-73.
211. Herrick, A., *Contemporary management of Raynaud's phenomenon and digital ischaemic complications.* Current opinion in rheumatology, 2011. 23(6): p. 555-561.
212. Wigley, F., *Clinical practice. Raynaud's Phenomenon.* The New England journal of medicine, 2002. 347(13): p. 1001-1008.
213. Galluccio, F. and M. Matucci-Cerinic, *Two faces of the same coin: Raynaud phenomenon and digital ulcers in systemic sclerosis.* Autoimmunity Reviews, 2011. 10(5): p. 241-243.
214. Mouthon, L., et al., *Impact of digital ulcers on disability and health-related quality of life in systemic sclerosis.* Annals of the rheumatic diseases, 2010. 69(1): p. 214-217.
215. Ferri, C., et al., *Systemic sclerosis: demographic, clinical, and serologic features and survival in 1,012 Italian patients.* Medicine, 2002. 81(2): p. 139-153.
216. Hafner, J., et al., *Management of leg ulcers in patients with rheumatoid arthritis or systemic sclerosis. .* Journal of Vascular Surgery 2000. 32 p. 322-329
217. Sant, S. and G. Murphy, *Neurotropic ulceration in systemic sclerosis.* Clin Exp Dermatol, 1994. 19(1): p. 65-6.
218. Korn, J.H., et al., *Digital ulcers in systemic sclerosis: Prevention by treatment with bosentan, an oral endothelin receptor antagonist.* Arthritis and Rheumatism, 2004. 50 (12): p. 3985-3993.
219. Bogoch, E. and D. Gross, *Surgery of the hand in patients with systemic sclerosis: outcomes and considerations.* The Journal of rheumatology, 2005. 32(4): p. 642-648.
220. Mouthon, L., et al., *Impact of digital ulcers on disability and health-related quality of life in systemic sclerosis.* Ann Rheum Dis, 2010. 69(1): p. 214-7.
221. Herrick, A., *Neurological involvement in systemic sclerosis.* British journal of rheumatology, 1995. 34(11): p. 1007-1008.
222. Herrick, A., et al., *Nervous system involvement in association with vasculitis and anticardiolipin antibodies in a patient with systemic sclerosis.* Annals of the rheumatic diseases, 1994. 53(5): p. 349-350.
223. Kibler, R. and F. Rose, *Peripheral neuropathy in the "collagen diseases": a case of scleroderma neuropathy.* British medical journal, 1960. 1(5188): p. 1781-1784.
224. Dyck, P., D. Conn, and H. Okazaki, *Necrotizing angiopathic neuropathy. Three-dimensional morphology of fiber degeneration related to sites of occluded vessels.* Mayo Clinic proceedings. Mayo Clinic, 1972. 47(7): p. 461-475.
225. Olney, R., *AAEM minimonograph# 38: neuropathies in connective tissue disease.* Muscle & nerve, 1992.
226. Richter, R., *Peripheral neuropathy and connective tissue disease.* Journal of neuropathology and experimental neurology, 1954. 13(1): p. 168-180.

227. Wilhelm, K., *Die mucoide Degeneration der peripheren Nerven.* Virchows Archiv für Pathologische Anatomie und Physiologie und für Klinische Medizin, 1939. 304.
228. Corbo, M., et al., *Peripheral neuropathy in scleroderma.* Clinical neuropathology, 1993. 12(2): p. 63-67.
229. Tagliafico, A., et al., *The role of ultrasound imaging in the evaluation of peripheral nerve in systemic sclerosis (scleroderma).* European Journal of Radiology, 2011. 77(3): p. 377-382.
230. Bajocchi, G., et al., *Evidence of a selective nociceptive impairment in systemic sclerosis.* Clinical and Experimental Rheumatology, 2009. 27(3 SUPPL. 54): p. S9-S14.
231. Bertinotti, L., et al., *The autonomic nervous system in systemic sclerosis. A review.* Clinical rheumatology, 2004. 23(1): p. 1-5.
232. Klimiuk, P., et al., *Autonomic neuropathy in systemic sclerosis.* Annals of the rheumatic diseases, 1988. 47(7): p. 542-545.
233. Poncelet, A. and M. Connolly, *Peripheral neuropathy in scleroderma.* Muscle & nerve, 2003. 28(3): p. 330-335.
234. Kostis, J.B., et al., *Prognostic importance of cardiac arrhythmias in systemic sclerosis.* The American journal of medicine, 1988. 84(6): p. 1007-1015.
235. Lock, G., et al., *Association of autonomic nervous dysfunction and esophageal dysmotility in systemic sclerosis.* Journal of rheumatology, 1998. 25(7): p. 1330-1335.
236. Lee, P., J. Bruni, and S. Sukenik, *Neurological manifestations in systemic sclerosis (scleroderma).* The Journal of rheumatology, 1984. 11(4): p. 480-483.
237. Schady, W., et al., *Peripheral nerve dysfunction in scleroderma.* QJM, 1991.
238. Frech, T., et al., *Peripheral neuropathy: a complication of systemic sclerosis.* Clinical rheumatology, 2013.
239. Baubet, T., et al., *Mood and anxiety disorders in systemic sclerosis patients.* Presse médicale (Paris, France : 1983), 2011. 40(2): p. 9.
240. Hyphantis, T., et al., *The impact of psychological functioning upon systemic sclerosis patients' quality of life.* Seminars in arthritis and rheumatism, 2007. 37(2): p. 81-92.
241. Blocka, K., et al., *The arthropathy of advanced progressive systemic sclerosis. A radiographic survey.* Arthritis and rheumatism, 1981. 24(7): p. 874-884.
242. Armstrong, R. and T. Gibson, *Scleroderma and erosive polyarthritis: a disease entity?* Annals of the rheumatic diseases, 1982. 41(2): p. 141-146.
243. Baron, M., P. Lee, and E. Keystone, *The articular manifestations of progressive systemic sclerosis (scleroderma).* Annals of the rheumatic diseases, 1982. 41(2): p. 147-152.
244. Avouac, J., et al., *Characteristics of joint involvement and relationships with systemic inflammation in systemic sclerosis: Results from the EULAR Scleroderma Trial and Research Group (EUSTAR) database.* Journal of Rheumatology, 2010. 37(7): p. 1488-1501.

245. Barry, M., L. Katz, and L. Cooney, *An unusual articular presentation of progressive systemic sclerosis.* Arthritis and rheumatism, 1983. 26(8): p. 1041-1043.
246. Clark, J., et al., *Synovial tissue changes and rheumatoid factor in scleroderma.* Mayo Clinic proceedings. Mayo Clinic, 1971. 46(2): p. 97-103.
247. Schmeiser, T., J. Pons-Kühnemann, and F. Özden…, *Arthritis in patients with systemic sclerosis.* European journal of …, 2012.
248. Misra, R., et al., *Arthritis in scleroderma.* British journal of rheumatology, 1995. 34(9): p. 831-837.
249. Chitale, S., et al., *Magnetic resonance imaging and musculoskeletal ultrasonography detect and characterize covert inflammatory arthropathy in systemic sclerosis patients with arthralgia.* Rheumatology, 2010. 49(12): p. 2357-61.
250. Wild, W. and W. Beetham, *Erosive arthropathy in systemic scleroderma.* JAMA : the journal of the American Medical Association, 1975. 232(5): p. 511-512.
251. Wilde, A., H. Mankin, and G. Rodman, *Avascular necrosis of the femoral head in scleroderma.* Arthritis and rheumatism, 1970. 13(4): p. 445-447.
252. Karten, I., *CRST syndrome and "neuropathic" arthropathy.* Arthritis and rheumatism, 1969. 12(6): p. 636-638.
253. Randone, S.B., S. Guiducci, and M.M. Cerinic, *Musculoskeletal involvement in systemic sclerosis.* Best Practice & Research Clinical Rheumatology, 2008. 22(2): p. 339-350.
254. Allali, F., et al., *Erosive arthropathy in systemic sclerosis.* BMC public health, 2007. 7: p. 260.
255. Tuna, H., et al., *Pedobarography and its relation to radiologic erosion scores in rheumatoid arthritis.* Rheumatology international, 2005. 26(1): p. 42-47.
256. Bassett, L., et al., *Skeletal findings in progressive systemic sclerosis (scleroderma).* American Journal of Roentgenology, 1981. 136(6): p. 1121-1126.
257. Medsger, T., et al., *Skeletal muscle involvement in progressive systemic sclerosis (scleroderma).* Arthritis and rheumatism, 1968. 11(4): p. 554-568.
258. Brower, L. and J. Poole, *Reliability and validity of the Duruoz Hand Index in persons with systemic sclerosis (scleroderma).* Arthritis and rheumatism, 2004. 51(5): p. 805-809.
259. Schumacher, J. and H. Ralph, *Joint and periarticular involvement in systemic sclerosis.* Clinics in Dermatology, 1994. 12(2): p. 277-282.
260. Minns, R. and A. Craxford, *Pressure under the forefoot in rheumatoid arthritis. A comparison of static and dynamic methods of assessment.* Clinical orthopaedics and related research, 1984(187): p. 235-242.
261. Johnson, S., et al., *Quality of life and functional status in systemic sclerosis compared to other rheumatic diseases.* The Journal of rheumatology, 2006. 33(6): p. 1117-1122.
262. Richards, H., et al., *Systemic sclerosis: patients' perceptions of their condition.* Arthritis and rheumatism, 2003. 49(5): p. 689-696.

263. Clements, P., et al., *Muscle disease in progressive systemic sclerosis: diagnostic and therapeutic considerations.* Arthritis and rheumatism, 1978. 21(1): p. 62-71.
264. Drachman, D.B., et al., *" Myopathic" changes in chronically denervated muscle.* Archives of neurology, 1967. 16(1): p. 14.
265. Follansbee, W., T. Zerbe, and T. Medsger, *Cardiac and skeletal muscle disease in systemic sclerosis (scleroderma): a high risk association.* American heart journal, 1993. 125(1): p. 194-203.
266. Mimura, Y., et al., *Clinical and laboratory features of scleroderma patients developing skeletal myopathy.* Clinical rheumatology, 2005. 24(2): p. 99-102.
267. Vayssairat, M., et al., *Clinical significance of subcutaneous calcinosis in patients with systemic sclerosis. Does diltiazem induce its regression?* Annals of the rheumatic diseases, 1998. 57(4): p. 252-254.
268. Harper, R. and D. Jackson, *Progressive systemic sclerosis.* The British journal of radiology, 1965. 38(455): p. 825-834.
269. Boulman, N., et al., *Calcinosis in rheumatic diseases.* Seminars in arthritis and rheumatism, 2005. 34(6): p. 805-812.
270. Rabens, S. and J. Bethune, *Disodium etidronate therapy for dystrophic cutaneous calcification.* Archives of dermatology, 1975. 111(3): p. 357-361.
271. Pope, J., *Musculoskeletal involvement in scleroderma.* Rheumatic Diseases Clinics of North America 2003. 29(2): p. 391-408.
272. Roca, R., F. Wigley, and B. White, *Depressive symptoms associated with scleroderma.* Arthritis and rheumatism, 1996. 39(6): p. 1035-1040.
273. Moser, D., et al., *Predictors of psychosocial adjustment in systemic sclerosis. The influence of formal education level, functional ability, hardiness, uncertainty, and social support.* Arthritis and rheumatism, 1993. 36(10): p. 1398-1405.
274. Georges, C., et al., *Impact of pain in health related quality of life of patients with systemic sclerosis.* Rheumatology (Oxford, England), 2006. 45(10): p. 1298-1302.
275. Hyphantis, T., et al., *Clinical features and personality traits associated with psychological distress in systemic sclerosis patients.* Journal of psychosomatic research, 2007. 62(1): p. 47-56.
276. Malcarne, V., et al., *Medical signs and symptoms associated with disability, pain, and psychosocial adjustment in systemic sclerosis.* The Journal of rheumatology, 2007. 34(2): p. 359-367.
277. Kwakkenbos, C.M.C., *Psychological well-being in systemic sclerosis: moving forward in assessment and treatment.* 2013.
278. Malcarne, V. and H. Greenbergs, *Psychological adjustment to systemic sclerosis.* Arthritis care and research : the official journal of the Arthritis Health Professions Association, 1996. 9(1): p. 51-59.
279. Reay, N., *The Quality of Life in Patients with Diffuse and Limited Systemic Sclerosis*, in *Faculty of Medicine and Health*2009, University of Leeds: Leeds.
280. WHO, *Towards a Common Language for Functioning, Disability and Health: ICF*, W.H. Organisation, Editor 2002: Geneva.
281. Pallant, J. and A. Tennant, *Introduction to structural equation modeling with AMOS* 2011, University Of Leeds: Leeds, UK

282. Kline, R.B., *Principles and practice of structural equation modeling*. 2011: Guilford press.
283. Cohen, J., *Statistical Power Analysis for the Behavioral Sciences* Second ed. 1988: L. Erlbaum Associates.
284. Poormoghim, H., et al., *Systemic Sclerosis Sine Scleroderma.* Arthritis & Rheumatism, 2000. 43(2): p. 444-451.
285. Committee., S.f.s.c.o.t.A.R.A.D.a.T.C., *Preliminary criteria for the classification of systemic sclerosis (scleroderma). .* Arthritis & Rheumatism, 1980. 23(5):581-90(5): p. 581-90.
286. Watson, H., et al., *Seasonal variation of Raynaud's phenomenon secondary to systemic sclerosis.* The Journal of rheumatology, 1999. 26(8): p. 1734-1737.
287. Block, J.A. and W. Sequeira, *Raynaud's phenomenon.* The Lancet, 2001. 357(9273): p. 2042-2048.
288. Clements, P.J., et al., *Skin thickness score as a predictor and correlate of outcome in systemic sclerosis: High-dose versus low-dose penicillamine trial.* Arthritis and Rheumatism, 2000. 43(11): p. 2445-2454.
289. Merkel, P.A., et al., *Current status of outcome measure development for clinical trials in systemic sclerosis. Report from OMERACT 6.* Journal of Rheumatology, 2003. 30(7): p. 1630-1647.
290. Allison, M.A., et al., *The relevance of different methods of calculating the ankle-brachial index.* American Journal of Epidemiology, 2010. 171(3): p. 368-376.
291. Aboyans, V., et al., *The intra- and interobserver variability of ankle-arm blood pressure index according to its mode of calculation.* Journal of Clinical Epidemiology, 2003. 56(3): p. 215-220.
292. McDermott, M.M., et al., *Lower ankle/brachial index, as calculated by averaging the dorsalis pedis and posterior tibial arterial pressures, and association with leg functioning in peripheral arterial disease.* Journal of Vascular Surgery. 32(6): p. 1164-71.
293. Leng, G.C., et al., *Use of ankle brachial pressure index to predict cardiovascular events and death: A cohort study*. 1996: British Medical Journal. 313(7070)(pp 1440-1444), 1996. Date of Publication: 1996.
294. Baumgartner, U., et al., *Neurogenic hyperalgesia versus painful hypoalgesia: two distinct mechanisms of neuropathic pain.* Pain, 2002. 96(1-2): p. 141-151.
295. Rolke, R., et al., *Quantitative sensory testing: A comprehensive protocol for clinical trials.* European Journal of Pain, 2006. 10(1): p. 77-88.
296. Goldberg, J.M. and U. Lindblom, *Standardised method of determining vibratory perception thresholds for diagnosis and screening in neurological investigation.* Journal of Neurology Neurosurgery and Psychiatry, 1979. 42(9): p. 793-803.
297. Geber, C., et al., *Test-retest and interobserver reliability of quantitative sensory testing according to the protocol of the German Research Network on Neuropathic Pain (DFNS): A multi-centre study.* Pain, 2011. 152(3): p. 548-556.
298. Shukla, G., M. Bhatia, and M. Behari, *Quantitative thermal sensory testing -- value of testing for both cold and warm sensation detection in evaluation of small fiber neuropathy.* Clinical Neurology and Neurosurgery, 2005. 107(6): p. 486-490.

299. Stebbins, J., et al., *Repeatability of a model for measuring multi-segment foot kinematics in children.* Gait & Posture, 2006. 23(4): p. 401-410.
300. Carson, M.C., et al., *Kinematic analysis of a multi-segment foot model for research and clinical applications: a repeatability analysis.* Journal of Biomechanics, 2001. 34(10): p. 1299-1307.
301. Meyers-Rice, B., et al., *Comparison of three methods for obtaining plantar pressures in nonpathologic subjects.* Journal - American Podiatric Medical Association, 1994. 84(10): p. 499-504.
302. Hesselstrand, R., et al., *High-frequency ultrasound of skin involvement in systemic sclerosis reflects oedema, extension and severity in early disease.* Rheumatology, 2008. 47(1): p. 84-87.
303. Clements, P.J., et al., *High-dose versus low-dose D-penicillamine in early diffuse systemic sclerosis: Analysis of a two-year, double-blind, randomized, controlled clinical trial.* Arthritis and Rheumatism, 1999. 42(6): p. 1194-1203.
304. Pope, J.E., et al., *A randomized, controlled trial of methotrexate versus placebo in early diffuse scleroderma.* Arthritis and Rheumatism, 2001. 44(6): p. 1351-1358.
305. Clements, P., et al., *Inter and intraobserver variability of total skin thickness score (Modified Rodnan TSS) in systemic sclerosis.* Journal of Rheumatology, 1995. 22(7): p. 1281-1285.
306. Furst, D., et al., *Systemic sclerosis - Continuing progress in developing clinical measures of response.* Journal of Rheumatology, 2007. 34(5): p. 1194-1200.
307. Sanada, H., et al., *Vascular function in patients with lower extremity peripheral arterial disease: a comparison of functions in upper and lower extremities.* Atherosclerosis, 2005. 178(1): p. 179-185.
308. Lijmer, J.G., et al., *ROC analysis of noninvasive tests for peripheral arterial disease.* Ultrasound in Medicine and Biology, 1996. 22(4): p. 391-398.
309. Feigelson, H.S., et al., *Screening for peripheral arterial disease: the sensitivity, specificity, and predictive value of noninvasive tests in a defined population.* American Journal of Epidemiology, 1994. 140(6): p. 526-34.
310. Fowkes, F., *The measurement of atherosclerotic peripheral arterial disease in epidemiological surveys.* International Journal of Epidemiology, 1988. 17(2): p. 248-254.
311. Caruana, M.F., A.W. Bradbury, and D.J. Adam, *The Validity, Reliability, Reproducibility and Extended Utility of Ankle to Brachial Pressure Index in Current Vascular Surgical Practice.* European Journal of Vascular and Endovascular Surgery, 2005. 29(5): p. 443-451.
312. Allen, J., et al., *A prospective comparison of bilateral photoplethysmography versus the ankle-brachial pressure index for detecting and quantifying lower limb peripheral arterial disease*. 2008: Journal of Vascular Surgery. 47(4)(pp 794-802), 2008. Date of Publication: Apr 2008.
313. Schroder, F., et al., *A modified calculation of ankle-brachial pressure index is far more sensitive in the detection of peripheral arterial disease.* Journal of Vascular Surgery. 44(3): p. 531-6.

314. Niazi, K., T.H. Khan, and K.A. Easley, *Diagnostic utility of the two methods of ankle brachial index in the detection of peripheral arterial disease of lower extremities.* Catheterization and Cardiovascular Interventions, 2006. 68(5): p. 788-792.
315. Hirsch, A.T., et al., *ACC/AHA 2005 Practice Guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation.* Circulation, 2005. 113(11): p. e463-654.
316. Walton, L., T.R.P. Martin, and M. Collins, *Prospective assessment of the aorto-iliac segment by visual interpretation of frequency analysed doppler waveforms--A comparison with arteriography.* Ultrasound in Medicine & Biology, 1984. 10(1): p. 27-32.
317. Sensier, Y., P.R.F. Bell, and N.J.M. London, *The ability of qualitative assessment of the common femoral doppler waveform to screen for significant aortoiliac disease.* European Journal of Vascular and Endovascular Surgery, 1998. 15(4): p. 357-364.
318. Eiberg, J.P., et al., *Screening for Aortoiliac Lesions by Visual Interpretation of the Common Femoral Doppler Waveform.* European Journal of Vascular and Endovascular Surgery, 2001. 22(4): p. 331-336.
319. Williams, D.T., K.G. Harding, and P. Price, *An evaluation of the efficacy of methods used in screening for lower-limb arterial disease in diabetes.* Diabetes Care, 2005. 28(9): p. 2206-2210.
320. Jager, K.A., et al., *Noninvasive mapping of lower limb arterial lesions.* Ultrasound in Medicine & Biology, 1985. 11(3): p. 515-521.
321. Hartshorne, T.C., *Lower limb vascular assessment by ultrasound* Imaging 2001. 13: p. 399-405.
322. Verim, S. and I. Taşçı, *Doppler ultrasonography in lower extremity peripheral arterial disease.* Arch Turk Soc Cardiol- Türk Kardiyol Dern Arş -, 2013. 41:: p. 248-255.
323. Hoitsma, E., et al., *Small fiber neuropathy: a common and important clinical disorder.* Journal of the Neurological Sciences, 2004. 227(1): p. 119-130.
324. Meier, P.M., et al., *Quantitative assessment of cutaneous thermal and vibration sensation and thermal pain detection thresholds in healthy children and adolescents*. 2001: Muscle and Nerve. 24(10)(pp 1339-1345), 2001. Date of Publication: 2001.
325. Andrews, K., *The effect of changes in temperature and humidity on the accuracy of von Frey hairs.* Journal of Neuroscience Methods, 1993. 50(1): p. 91-93.

326. Rolke, R., et al., *Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): Standardized protocol and reference values.* Pain, 2006. 123(3): p. 231-243.
327. Whitton, T.L., R.W. Johnson, and A.T. Lovell, *Use of the Rydel-Seiffer graduated tuning fork in the assessment of vibration threshold in postherpetic neuralgia patients and healthy controls.* European Journal of Pain, 2005. 9(2): p. 167-171.
328. Heldestad, V., et al., *Reproducibility and influence of test modality order on thermal perception and thermal pain thresholds in quantitative sensory testing.* Clinical Neurophysiology, 2010. 121(11): p. 1878-1885.
329. Shy, M.E., et al., *Quantitative sensory testing. Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology.* Neurology, 2003. 60: p. 898–904.
330. Selim, M.M., et al., *Variation in quantitative sensory testing and epidermal nerve fiber density in repeated measurements.* Pain, 2010. 151(3): p. 575-581.
331. Cheliout-Heraut, F., et al., *Exploration of small fibers for testing diabetic neuropathies.* Joint Bone Spine, 2005. 72(5): p. 412-415.
332. Wright, C.J., et al., *Repeatability of the modified Oxford foot model during gait in healthy adults.* Gait & Posture, 2011. 33(1): p. 108-112.
333. Redmond, A., J. Crosbie, and R. Ouvrier, *Development and validation of a novel rating system for scoring standing foot posture: The Foot Posture Index.* Clinical Biomechanics 2006. 21: p. 89–98.
334. Taylor, A.J., H.B. Menz, and A.-M. Keenan, *The influence of walking speed on plantar pressure measurements using the two-step gait initiation protocol.* The Foot, 2004. 14(1): p. 49-55.
335. Rao, S., J.F. Baumhauer, and D.A. Nawoczenski, *Is barefoot regional plantar loading related to self-reported foot pain in patients with midfoot osteoarthritis.* Osteoarthritis and Cartilage, 2011. 19(8): p. 1019-1025.
336. Putti, A.B., et al., *Normal pressure values and repeatability of the EmedÆ ST4 system.* Gait & Posture, 2008. 27(3): p. 501-505.
337. Maetzler, M., T. Bochdansky, and R.J. Abboud, *Normal pressure values and repeatability of the EmedÆ ST2 system.* Gait & Posture, 2010. 32(3): p. 391-394.
338. Turner, D., et al., *Pes planovalgus in RA: A descriptive and analytical study of foot function determined by gait analysis.* Musculoskeletal Care, 2003. 1(1): p. 21-33.
339. Akesson, A., et al., *Longitudinal development of skin involvement and reliability of high frequency ultrasound in systemic sclerosis.* Annals of the Rheumatic Diseases, 2004. 63(7): p. 791-796.
340. Low, A.H.L., et al., *Magnetic resonance imaging of the hand in systemic sclerosis.* Journal of Rheumatology, 2009. 36(5): p. 961-964.
341. Keen, H.I., et al., *MRI and musculoskeletal ultrasonography as diagnostic tools in early arthritis.* Rheumatic Disease Clinics of North America, 2005. 31(4): p. 699-714.
342. Tan, A.L., et al., *Imaging of the musculoskeletal system: magnetic resonance imaging, ultrasonography and computed tomography.* Best Practice & Research Clinical Rheumatology, 2003. 17(3): p. 513-528.
343. Ostergaard, M., B. Ejbjerg, and M. Szkudlarek, *Imaging in early rheumatoid arthritis: roles of magnetic resonance imaging,*

ultrasonography, conventional radiography and computed tomography. Best Practice & Research Clinical Rheumatology, 2005. 19(1): p. 91-116.
344. McHorney, C., J.E. Ware, and R. A., *The MOS 36-Item Short-Form Health Survey (SF-36): II. Psychometric and clinical tests of validity in measuring physical and mental health constructs* Medical Care, 1993. 31(3): p. 247-263.
345. Ware, J.E. and C.D. Sherbourne, *The MOS 36-Item Short-FOrm Health SUrvey (SF-36): I. Conceptual Framework and Item Selection.* Medical Care, 1992. 30(6): p. 473-83.
346. Zigmond, A.S. and R.P. Snaith, *The Hospital Anxiety and Depression Scale.* Acta Psychiatrica Scandinavica, 1983. 67(6): p. 361-370.
347. Steen, V. and T. Medsger, *The Value Of The Health Assessment Questionnaire And Special Patient-Generated Scales To Demonstrate Change In Systemic Sclerosis Patients Over Time.* Arthritis & Rheumatism, 1997. 40(11): p. 1984-1991.
348. Merkel, P.A., et al., *Measuring disease activity and functional status in patients with scleroderma and Raynaud's phenomenon.* Arthritis & Rheumatism, 2002. 46(9): p. 2410-2420.
349. Garrow, A.P., et al., *Development and validation of a questionnaire to assess disabling foot pain.* Pain, 2000. 85(1-2): p. 107-113.
350. Jenkinson, C., A. Coulter, and W. Wright, *Short form 36 (SF36) health survey questionnaire: normative data for adults of working age.* British Medical Journal 1993. 360: p. 1437-39.
351. Garratt, A., et al., *The SF36 health survey questionnaire: an outcome measure suitable for routine use within theNHS?* British Medical Journal, 1993. 306: p. 1440-43.
352. Bjelland, I., et al., *The validity of the Hospital Anxiety and Depression Scale An updated literature review.* Journal of Psychosomatic Research, 2002. 52 p. 69–77.
353. Herrmann, C., *International experiences with the hospital anxiety and depression scale- a review of validation data and clinical results.* Journal of Psychosomatic Research, 1996. 42(1): p. 17-41.
354. Baubet, T., et al., *Mood and anxiety disorders in systemic sclerosis patients.* Presse Medicale, 2011. 40(2): p. e111-9.
355. Bruce, B. and J. Fries, *The Stanford Health Assessment Questionnaire: A Review of Its History, Issues, Progress, and Documentation.* The Journal of Rheumatology, 2003. 30(1): p. 167-178.
356. Fries, J., et al., *Measurement of patient outcome in arthritis.* Arthritis and Rheumatism 1980. 23(2): p. 137-145.
357. Merkel, P.A., *Measurement of functional status, self-assessment, and psychological well-being in scleroderma.* Current Opinion in Rheumatology, 1998. 10(6): p. 589-594.
358. Poole, J., et al., *Concurrent validity of the health assessment questionnaire disability index in scleroderma. .* Arthritis care and research, 1995. 8: p. 189-193.
359. Johnson, S., G. Hawker, and A. Davis, *The Health Assessment Questionnaire Disability Index and Scleroderma Health Assessment Questionnaire in Scleroderma Trials: An Evaluation of Their Measurement Properties.* Arthritis & Rheumatism (Arthritis Care & Research), 2005. 53(2): p. 256-262.

360. Smyth, A., et al., *A cross-sectional comparison of three self-reported functional indices in scleroderma.* Rhematology 2003. 42: p. 732-738.
361. McKenna, S.P. and L.C. Doward, *The Needs Based Approach to Quality of Life Assessment.* Value in Health, 2004. 7(s1): p. S1-S3.
362. Maslow, A.H., et al., *Motivation and personality*. Vol. 2. 1970: Harper & Row New York.
363. Gill, T.M. and A.R. Feinstein, *A critical appraisal of the quality of quality-of-life measurements.* Jama, 1994. 272(8): p. 619-626.
364. Alcacer-Pitarch, B., et al., *Pressure and pain in systemic sclerosis/scleroderma--an evaluation of a simple intervention (PISCES): randomised controlled trial protocol.* BMC musculoskeletal disorders, 2012. 13: p. 11.
365. Cook, C., et al., *Calibration of an item pool for assessing the disability associated with foot pain: an application of item response theory to the Manchester Foot Pain and Disability Index.* Physiotherapy (London), 2007. 93(2): p. 89-95.
366. Roddy, E., S. Muller, and E. Thomas, *Defining disabling foot pain in older adults: further examination of the Manchester Foot Pain and Disability Index.* Rheumatology (Oxford, England), 2009. 48(8): p. 992-996.
367. WHO, *International Classification of Function, Disability and Health: Introduction*, W.H. Organisation, Editor 2001: Geneva.
368. Field, A., *Discovering statistics using IBM SPSS statistics*. 2013: Sage.
369. Heavey, E., *Statistics for Nursing: a Practical Approach*. Second ed. 2015, Burlington MA: Jones & Bartlett Learning.
370. Hays, R., D. Revicki, and K. Coyne, *Application of structural equation modeling to health outcomes research.* Evaluation & the health professions, 2005. 28(3): p. 295-309.
371. Schumacker, R.E. and R.G. Lomax, *A beginner's guide to structural equation modeling.* Psychology Press, 2004.
372. Byrne, B.M., *Structural Equation Modelling with AMOS:Basic Concepts, Applications, and Programing. Multivariate Applications.* 2001, Mahwah, New Jersey: Lawrence Erlbaum Associates, Inc.
373. Fornell, C., *A second generation of multivariate analysis.* Vol. 1: Methods. 1982, New York Praeger.
374. Schumacker RE., L.R., *A beginners guide to structural Equation Modeling.* Second ed. 2004, New Jersey: Lawrence Erlbaum Associates.
375. Browne, M.W. and R. Cudeck, *Alternative Ways of Assessing Model Fit.* Sociological Methods & Research, 1992. 21.
376. Cohen, J., *A power primer.* Psychol Bull, 1992. 112(1): p. 155-9.
377. Boulton, A.J., *Pressure and the diabetic foot: clinical science and offloading techniques.* The American Journal of Surgery, 2004. 187(5): p. S17-S24.
378. Schumacker, R.E. and R.G. Lomax, *A beginner's guide to structural equation modeling*. Third ed. 2010: Routledge.
379. Caramaschi, P., et al., *Scleroderma patients nailfold videocapillaroscopic patterns are associated with disease subset and disease severity.* Rheumatology, 2007. 46(10): p. 1566-1569.
380. Roustit, M., et al., *Abnormal digital neurovascular response to local heating in systemic sclerosis.* Rheumatology, 2008. 47(6): p. 860-864.

381. Scheja, A., et al., *Circulating collagen metabolites in systemic sclerosis. Differences between limited and diffuse form and relationship with pulmonary involvement.* Rheumatology, 2000. 39(10): p. 1110-1113.
382. Silman, A., M. Harrison, and P. Brennan, *Is it possible to reduce observer variability in skin score assessment of scleroderma? The ad hoc International Group on the Assessment of Disease Outcome in Scleroderma.* The Journal of rheumatology, 1995. 22(7): p. 1277-1280.
383. Mickle, K., et al., *Sof-Tissue thickness under teh metatarsals: Is it reduced in those with toe deformities?*, in *International Foot and Ankle Biomechanics (i-FAB)*2011: Seattle.
384. Mueller, M.J., et al., *Forefoot structural predictors of plantar pressures during walking in people with diabetes and peripheral neuropathy.* Journal of biomechanics, 2003. 36(7): p. 1009-1017.
385. Lambova, S., W. Hermann, and U. Müller-Ladner, *Capillaroscopic pattern at the toes of systemic sclerosis patients: does it" tell" more than those of fingers?* JCR: Journal of Clinical Rheumatology, 2011. 17(6): p. 311-314.
386. Wan, M., et al., *Ankle brachial pressure index in systemic sclerosis: influence of disease subtype and anticentromere antibody.* Rheumatology, 2001. 40(10): p. 1102-1105.
387. Bichile, L.S., et al., *Ankle Brachial Pressure Index Measurement And Its Correlation To Claudication In Patients With Scleroderma.* Internet Journal of Rheumatology, 2008. 5(2).
388. Yarnitsky, D. and E. Sprecher, *Thermal testing: normative data and repeatability for various test algorithms.* Journal of the Neurological Sciences, 1994. 125(1): p. 39-45.
389. Yarnitsky, D., et al., *Heat pain thresholds: normative data and repeatability.* Pain, 1995. 60(3): p. 329-332.
390. Wasner, G.L. and J.A. Brock, *Determinants of thermal pain thresholds in normal subjects.* Clinical Neurophysiology, 2008. 119(10): p. 2389-2395.
391. Ziegler, D., et al., *Assessment of small and large fiber function in long-term type 1 (insulin-dependent) diabetic patients with and without painful neuropathy.* Pain, 1988. 34(1): p. 1-10.
392. Chiu, M.-C. and M.-J. Wang, *The effect of gait speed and gender on perceived exertion, muscle activity, joint motion of lower extremity, ground reaction force and heart rate during normal walking.* Gait & Posture, 2007. 25(3): p. 385-392.
393. Hudson, M., et al., *Health-related quality of life in systemic sclerosis: a systematic review.* Arthritis Rheum, 2009. 61(8): p. 1112-20.
394. Ware, J.E. and M. Kosinski, *Interpreting SF&-36 summary health measures: A response.* Quality of life research, 2001. 10(5): p. 405-413.
395. Redmond, A.C., J. Burns, and R.A. Ouvrier, *Factors that influence health-related quality of life in Australian adults with Charcot–Marie–Tooth disease.* Neuromuscular disorders, 2008. 18(8): p. 619-625.
396. Moser, D.K., et al., *Predictors of psychosocial adjustment in systemic sclerosis.* Arthritis & Rheumatism, 1993. 36(10): p. 1398-1405.
397. Redmond, R., et al., *EULAR13-4126 Effects of pressure relieving insoles for foot problems in people with SSc: The PISCES randomised controled trial*, in *EULAR* 2013: Madrid.

398. Schieir, O., et al., *Prevalence, severity, and clinical correlates of pain in patients with systemic sclerosis.* Arthritis Care & Research, 2010. 62(3): p. 409-17.
399. Kallen, M.A., et al., *The Symptom Burden Index: Development and initial findings from use with patients with systemic sclerosis.* The Journal of rheumatology, 2010. 37(8): p. 1692-1698.
400. Hendriks, S.M., et al., *Disability in Anxiety Disorders.* Journal of Affective Disorders, 2014.
401. Moussavi, S., et al., *Depression, chronic diseases, and decrements in health: results from the World Health Surveys.* The Lancet, 2007. 370(9590): p. 851-858.
402. Shin, S.Y., L. Julian, and P. Katz, *The relationship between cognitive function and physical function in rheumatoid arthritis.* The Journal of rheumatology, 2013. 40(3): p. 236-243.
403. Morris, A., et al., *Long-term patterns of depression and associations with health and function in a panel study of rheumatoid arthritis.* Journal of health psychology, 2011. 16(4): p. 667-677.
404. Kwakkenbos, L., et al., *Disease-related and psychosocial factors associated with depressive symptoms in patients with systemic sclerosis, including fear of progression and appearance self-esteem.* Journal of psychosomatic research, 2012. 72(3): p. 199-204.
405. Bowers, D., A. House, and D. Owens, *Understanding Clinical Papers*. Second Edition ed. 2006, Chichester, England: John Wiley & Sons Ltd.
406. Peat, J., *Health science research. A handbook of quantitative methods*. 2001, Australia: Allen & Unwin. 313.
407. Julious, S.A., *Sample size of 12 per group rule of thumb for a pilot study.* Pharmaceutical Statistics, 2005. 4(4): p. 287-291.
408. Weir, J., *Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM.* Journal of strength and conditioning research / National Strength & Conditioning Association, 2005. 19(1): p. 231-240.
409. Bland, J. and D. Altman, *Measuring agreement in method comparison studies.* Statistical methods in medical research, 1999. 8(2): p. 135-160.
410. Bland, J. and D. Altman, *Statistical methods for assessing agreement between two methods of clinical measurement.* Lancet, 1986. 1(8476): p. 307-310.
411. Rankin, G. and M. Stokes, *Reliability of assessment tools in rehabilitation: an illustration of appropriate statistical analyses.* Clinical rehabilitation, 1998. 12(3): p. 187-199.
412. Shrout, P.E. and J.L. Fleiss, *Intraclass correlations: uses in assessing rater reliability.* Psychol Bull, 1979. 86(2): p. 420-428.
413. Fleiss, J.L., *Reliability of Measurement*, in *The Design and Analysis of Clinical Experiments*. 1999, John Wiley & Sons, Inc. p. 1-32.
414. Bartko, J.J., *The intraclass correlation coefficient as a measure of reliability.* Psychological reports, 1966. 19(1): p. 3-11.
415. Bartko, J.J., *On various intraclass correlation reliability coefficients.* Psychological bulletin, 1976. 83(5): p. 762-765.
416. Landis, J.R. and G.G. Koch, *The measurement of observer agreement for categorical data.* biometrics, 1977: p. 159-174.
417. McGraw, K.O. and S. Wong, *Forming inferences about some intraclass correlation coefficients.* Psychological methods, 1996. 1(1): p. 30.