Clinical presentation and impact of calcinosis in Systemic Sclerosis. An introduction of ultrasound as a valuable outcome measure

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

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Abstract

Introduction:
Calcinosis is a highly debilitating condition in Scleroderma causing pain and functional limitation often leading to ulceration with risk of infection and amputation. The pathogenesis of calcinosis remains unknown limiting the treatments available. With such high debilitation, patients highlight calcinosis as a priority area for research and clinical management.
This study explores the literature, describes the clinical characteristics and patient-reported impact of calcinosis, and initiates a framework of appropriate outcome measures to evaluate targeted treatments in future clinical trials.

Methodology:
Phase One: Postal survey of patient-reported prevalence and impact of calcinosis across six centres.
Phase Two: Expert consensus Delphi Agreement to define the characteristics of calcinosis using ultrasound.
Phase Three: Case/control observational study describing the clinical characteristics of calcinosis and patient-reported impact. Proof-of-concept study comparing the presence of US defined calcinosis at the hands with patient, physician and x-ray reported presence.

Results:
Patient-reported presence and hand function/disability of calcinosis is significantly higher in Scleroderma compared to SLE and DM.
A consensus-driven definition of calcinosis using US was agreed and identified important features which may impact severity. Reduced QoL is associated with calcinosis but negatively correlated with x-ray scores. Health and hand function/disability is not significantly different between matched case/control groups. US can detect calcinosis with minimal differences between x-ray reported regions. Imaging and patient reports

confirm subclinical calcinosis. Calcinosis is less prevalent outside of the hands but more painful.

Discussion:

Psychosocial factors are impacted more with calcinosis and early identification of subclinical calcinosis may be predictive of severe disease. Negative correlation of x-ray severity scores with patient-reported impact prioritises the need for alternative imaging, identified via ultrasound.

Summary:

No effective therapeutics exist to prevent or treat calcinosis and validated outcome measures are key for clinical trials exploring novel therapeutics. Ultrasound offers a valuable outcome measure for early diagnosis, severity, and impact to support future clinical trials in calcinosis.

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Abbreviations

(ACE) Angiotensin-converting enzyme
(ACR) American college of Rheumatology
(AHP) Allied Health Professional
(ANA) Antinuclear antibody
(ARA) American Rheumatism Association
(CCBs) Calcium Channel Blockers
(CCGs) Clinical Commissioning Groups
(CHFDS) Cochin Hand function and disability score
(CLCX4) chemokine (C-X-C motif) ligand 4
(CRP) C-reactive protein
(CT) computed tomography
(DC) Dystrophic calcinosis
(dcSSc) diffuse cutaneous systemic sclerosis
(DM) Dermatomyositis
(DiM) Diabetes Mellitus
(EDTA) ethylenediaminetetraacetic acid
(ESR) Erythrocyte sedimentation rate
(ESWL) Extracorporeal shock wave lithotripsy
(EULAR) European League Against Rheumatism
(GLA) y-carboxyglutamin acid
(GWAS) Genome-wide association study
(HFUS) high frequency ultrasound
(HLA) human leukocyte antigen
(HRCT) high resolution computed tomography
(IFN) Interferon
(ILD) Interstitial lung disease
(IISTS) Intra-lesional Sodium Thiosulfate
(LDI) Laser Doppler imaging
(lcSSc) limited cutaneous systemic sclerosis
(MCQ) Maudsley Calcinosis Questionnaire
(MRI) magnetic resonance imaging
(MRSS) Modified Rodnan Skin Score
(NHS) National Health Service
(NRS) Numerical Rating Scale
(NVC) Nail-fold Video-Capillaroscopy
(OCT) Optical Coherence Tomography

(OMERACT) Outcome Measures in Rheumatology

(OR) odds ratio

(PROMs) Patient Reported Outcome Measures

(PAH) Pulmonary Arterial Hypertension

(PF4) Platelet Factor 4

(PIS) Patient Information Sheet

(PS) Pitting Scars

(PTH) Parathyroid Hormone

(PRP) Primary Raynaud's Phenomenon

(PSVC) Plain-skin Video-Capillaroscopy

(QoL) Quality of Life

(RCS)The Raynaud's Condition Score

(RCTs) Randomised Controlled Trials

(ROI) Region of Interest

(ROI's) Regions of Interest

(RP) Raynaud's Phenomenon

(RA) Rheumatoid Arthritis

(SF-36v2) MOS 36-items Short-Form health survey, version 2

(SLE) Systemic Lupus Erythematous

(SLR) systematic literature review

(SSc) Systemic Sclerosis

(SSc QoL) Scleroderma Quality of Life Questionnaire

(TSTS) Topical Sodium Thiosulfate

(UAO) Ulnar Arterial Occlusion

(VEDOSS) very early diagnosis of SSc

(WBC) white blood cell

(WSF) World Scleroderma Foundation

Chapter 1

Calcinosis in Systemic Sclerosis

1.1 Systemic Sclerosis: a narrative overview

1.1.1 Overview

Systemic sclerosis (SSc), also called scleroderma, is an autoimmune connective tissue disease characterised by three main features: vasculopathy, immune activation and inflammation, and fibrosis of the skin and internal organs. The name “Scleroderma” is derived from the Greek words “sclero” meaning “thick” and “derma” meaning “skin”. Although fibrosis of the skin is characteristic of SSc, the same fibrosis occurs throughout the connective tissue of the body [1].

Owing to earlier diagnosis and intervention, survival rates of SSc have improved over the last few decades [2] with the 10 year survival rate being approximately 62.5% [3].

However, SSc still has the highest disease-related morbidity and mortality rates with a highly impaired quality of life when compared to diseases such as Rheumatoid Arthritis (RA) and Systemic Lupus erythematous (SLE) [2, 4-6]. In particular, hand function is a primary determinant of disability as the hands are one of the most affected sites. Several cross-sectional studies have demonstrated that Raynaud’s phenomenon (RP), inflammation and fibrosis of skin, digital ulcers (DU), inflammation/fibrosis of joints and peri-articular structures and calcinosis are all important drivers of poor hand function [7-12].

1.1.2 Pathogenesis

The pathogenesis of SSc is thought to begin with early micro-vascular and macro-vascular disease characterised by endothelial cell dysfunction and activation mechanisms promoting myofibroblast formation. There is a complex autoimmune response of both an innate and adaptive nature where specific autoantibodies are produced, and an inflammatory response initiated. The progression of the disease is worsened by circulating monocyte and fibrocyte cells, growth

factors and cytokines. The result is a highly variable degree of tissue and vascular fibrosis affecting both the skin and visceral organs often resulting in irreversible damage [13].

The initial vascular dysfunction is typically seen clinically as a worsening or new onset of RP. The pathogenesis of RP secondary to SSc is thought to relate to three distinct but interrelated abnormalities favouring vasoconstriction: vascular abnormalities, intravascular abnormalities and neural abnormalities [14].

1. Vascular abnormalities

This relates to several alterations to the vessels themselves. Structurally, the vessels undergo intimal thickening, breaks to the internal elastic lamina and narrowing of the lumen, sometimes leading to complete vessel occlusion. It is believed to be the result of endothelial cell apoptosis, up regulation of adhesion molecules and the interplay of cytokines, growth factors and pericyte activation [15-18]. Functionally, the endothelium reacts to regulate vasoactive responses by producing vasodilative/vasoconstrictive substances and promoting platelets activation, white blood cells adherence and diapedesis, ultimately promoting an inflammatory and immune response. In SSc, damage to the endothelium tissue disrupts the balance in favour of vasoconstriction in addition to becoming pro-coagulant and pro-inflammatory. Vasodilation is therefore impaired, either from a defected endothelium or a reduced number of endogenous vasodilators acting on the endothelium [15].
As part of the pathological activation, the endothelium also produces vasoconstrictors such as endothelin-1. Although the levels have been seen to be no higher in SSc compared to patients with Primary RP (PRP) or controls before and after a cold challenge [19], SSc skin biopsies do show an over-expression of endothelin-1 with increased endothelin–binding density [20, 21].

2. Intravascular abnormalities

Intravascular abnormalities have been attributed to platelet activation, fibrinolysis, white blood cell (WBC) activation, reduced

red blood cell deformability, increased viscosity, and oxidative stress [22].

In addition, chemokine (C-X-C motif) ligand 4/Platelet Factor (CXCL4/PF4) proteins induce expression of endothelin -1, and there is evidence of CXCL4 being expressed at higher levels in SSc, particularly in the early diffuse form [23, 24]. CXCL4 is considered a potential predictive biomarker for pulmonary fibrosis and hypertension in SSc [25]. It is relevant to the pathway of vasculopathy, fibrosis and RP and has a role in wound formation and inflammation [26], with studies showing higher CXCL4 serum levels in patients with SSc and DU [27].

3. Neural abnormalities

Neural abnormalities are considered, but less evidenced and understood in the pathogenesis of RP and SSc. It is recognised that the sympathetic nervous system aids in thermoregulation via small sensory nerve fibres and sympathetic vasoconstrictor/vasodilator nerves. Both autonomic [28, 29] and peripheral neuropathy [30-32] have been observed in SSc and may be comparable to the effect of vibration induced RP where neuropathy is clearly implicated [33].

In addition to neuropathy being the defining feature in vibration induced RP, processes lead to similar clinical presentations of the skin such as puffy fingers and sclerodactyly [34]. However, the same processes including activation of endothelin 1 during cold challenge do not occur in vibration-induced RP when compared to patients with SSc, neither do you see the typical structural capillary change seen with Nail-fold Video Capillaroscopy (NVC). This supports the concept that the vascular processes leading to vasculopathy in SSc are multifactorial.

As mentioned, these initial complex vascular processes termed vasculopathy, in turn promote the fibroblast formation, activation of autoantibodies and an inflammatory response. The clinical features and symptoms that these changes produce, and which define SSc, will be described later.

1.1.3 Epidemiology and demographics

Women are more commonly affected with SSc then men (4.7:1 ratio), with a peak age onset in the fifth decade of life [35-38]. Only a third of Randomised Controlled Trials (RCTs) identify race and ethnicity, but of those that do, participants with SSc are represented as White (79%), Asian (7%) and African American (6%) [39].

It is well recognised that risk factors for developing SSc do exist and are discussed in section 1.1.6.

A meta-cohort study of 17,838 patients from Australia, Canada, European and Singapore described the impact of gender, race on disease manifestations and survival rates [40]. The data from these four registers spanning across 225 centres support the age of onset and predominance of females being affected with SSc. The clinical manifestations between these groups and impact on quality of life, disability and survival rates are highly variable [41-44].

1.1.4 Prevalence and incidence

A recent systematic review and meta-analysis identified the prevalence of SSc globally to be 176/million and the incidence to be 14/million/year [37]. Data from over 10 million residents in the UK between the years 1998-2017, showed the overall estimated prevalence as 235.5/million with an incidence of 10.7/million/year [36].

Based on similar UK prevalence and incidence measures in 2018, it was estimated that there were approximately 20,000 people living with SSc in the UK at that time and 1180 new cases each year [38].

There have been many other worldwide regional studies with diversity in the range of cases/million. This can be related to differences in sampling methods and geographical variance in demographics.

The accuracy of this data is also uncertain given that the heterogeneous nature of the disease has historically made diagnosis and classification of the disease difficult.

Since the first classification of SSc was published in 1980 by the American Rheumatism Association (ARA) [45], further adopted

classifications have evolved. In 1988, LeRoy et al [46] provided a classification followed by an updated version jointly with Medsger in 2001 [47], and the latest version in 2013 was devised jointly by the American college of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) [48]. These classifications will be discussed in more detail later.

The relevance of the revised classifications shows recent prevalence and incidence data supporting an increase in trend over time owing to early and more accurate diagnosis [49]. This impact on prevalence and incidences rates has been exemplified in a Swedish study by Andreasson et al where 30–40% higher estimates of SSc prevalence and incidence were documented using the ACR-EULAR classification criteria compared to the 1980 ARA criteria [50]. This has been similarly supported in studies from Italy and Norway [51, 52].

In addition, following low sensitivity evidence that only 35% of 'very early SSc' patients satisfied the latest 2013 criteria [53], the EULAR Scleroderma Trial and Research group later adapted the ACR-EULAR criteria to then account for the very early diagnosis of SSc (VEDOSS) [54, 55]. This highlights the paramount importance of accurate classification and diagnostic criteria to enable early identification and intervention to prevent, or at least slow, the progression of disease.

1.1.5 Disease classification and diagnostic criteria

The first classification by the ARA in 1980 aimed to broadly distinguish SSc from non-SSc patients. It required the presence of proximal sclerodermatous changes or two or more of the following features: sclerodactyly; digital pitting scars on the fingertips or loss of distal finger fat pad; or bilateral pulmonary fibrosis [45]. This did not account for the level of heterogeneity of the disease, it was not sensitive to cases of early or mild SSc and did not consider the assessment of serum autoantibodies or vasculopathy.

The LeRoy classifications in both 1988 and 2001 aimed to distinguish two distinct subsets of the disease based predominantly on degree of skin involvement; limited cutaneous systemic sclerosis (lcSSc) where sclerosis of the skin is only

evident distally from the elbows, knees and at the clavicles; and diffuse cutaneous systemic sclerosis (dcSSc) where the whole skin is affected. They also proposed a limited version of SSc without skin involvement termed sine systemic sclerosis [46].

The updated 2001 version added an 'early' classification and included assessment of serum autoantibodies and vasculopathy evidenced by Nailfold Video-Capillaroscopy (NVC) changes [47]. The importance of these additional assessments is highlighted with recent multicentre data showing patients with SSc specific autoantibodies and either puffy fingers or NVC changes have >80% risk of progressing to definite SSc within 5 years, and around 50% risk at 30 months [56].

The 2013 ACR-EULAR criteria for classification of SSc re-focused on the main clinical manifestations of the disease and maintained the relevance of NVC changes and SSc related auto-antibody positivity. These items were derived from an initial 168 items as identified via 2 rounds of an expert Delphi agreement process. A third round reduced this to 23 items [57] which showed good discriminative validity when compared to database cases with diseases like SSc [48].

Sensitivity and specificity in the validation sample were 0.91 and 0.92, respectively, for the new classification criteria compared to 0.75 and 0.72 for the 1980 ACR classification criteria [48].

The classification uses a 9-point classification system where skin involvement proximal to the metacarpophalangeal joint alone would constitute a score of 9 and therefore immediate diagnosis. Without this, 7 other possible items with varying weighted scores would be considered and a total score of $\geq$9 would constitute a diagnosis. These 7 items include skin thickening of the fingers (puffy fingers/sclerodactyly), fingertip lesions (digital tip ulcers/fingertip pitting scars), telangiectasia, abnormal nailfold capillaries, pulmonary arterial hypertension and or interstitial lung disease, RP, and SSc-related autoantibodies.

The ACR-EULAR criteria had shown high accuracy and increased sensitivity in the classification of patients with early SSc with the highest scoring variables being proximal skin thickening, sclerodactyly, telangiectasia and SSc-related autoantibodies. Validation of the strongest values associated

with progression to definite SSc is the presence of serum antinuclear antibody (ANA) and puffy fingers [58, 59].
Raynaud's phenomenon is considered highly sensitive but with low specificity [60]. Understanding that the pathogenesis of SSc begins with very early micro-vascular changes, and as 96% of patients with SSc will have RP as the first symptom, patients at great risk of developing SSc may still not score high enough to fulfil the criteria. Therefore, a very early diagnosis of SSc (VEDOSS) and early initiation of possibly preventative therapy has the potential to be neglected.
It has therefore become crucially apparent that clinical diagnosis and early intervention is made without reliance on criteria score alone. A full clinical picture of other related risk factors and clinical manifestations should be explored and acknowledged to aid early diagnosis.

1.1.6 Risk factors

Serum auto-antibody risk factors

The pathogenic role of SSc-specific autoantibodies is not fully understood. However, recent in vitro studies have demonstrated the associated immunocomplexes activate endothelial cells and fibroblasts, suggesting a possible active role in inducing the known pro-inflammatory and profibrotic conditions pertinent in developing SSc [61, 62].
A link between anti-RNA polymerase III autoantibodies and cancer has also been found in patients with SSc, with a higher frequency (14.2%) associated with cancer compared to anti-Scl-70 (6.3%) and anti-centromere antibodies (6.8%). These cases suggest a paraneoplastic syndrome where the deregulation of the immune system in response to the cancer triggers the onset of SSc [63].
Table 1 listed in section 1.1.7 lists in more detail the known SSc related autoantibodies with the prevalence and likely associated disease outcomes.
Serum autoantibodies can be checked clinically via routine blood testing. It is not typical to request blood testing for gene studies, however, there are genetic risk factors to consider, and which give insight into the possible disease processes.

Genetic risk factors

A Genome-Wide Association Study (GWAS) has shown the most relevant genetic associations in SSc relate to involvement of human leukocyte antigen (HLA), Type 1 Interferon (IFN) and interleukin-12 as common signalling pathways, Others have identified CD247 as susceptibility locus, and a PPARG gene in the development of SSc [64-67]. This suggests a genetic risk factor related to deregulation of the immune system and an inflammatory response, as seen clinically in the disease.

Feghali-Bostwick et al (2003) found only a 4.7% concordance rate in developing SSc in the study of both monozygotic and dizygotic twins, but found a high concordance for the presence of autoantibodies concluding that inheritance may instead contribute to the development of serum antibodies associated with SSc [68].

Epigenetic factors that alter only the expression but not the DNA structure itself have been documented as instrumental in understanding the pathogenesis of SSc. A relationship has been found between a global hypomethylation pattern and clinical presentations of vasculopathy, lung, and gastrointestinal tract involvement [69, 70].

There is an increased risk of SSc in individuals that have a family history of Systemic Lupus Erythematous (SLE) and Sjogren's syndrome [71-74] with SLE being considered one of the closest in connective tissue disease similarity.

However, with SLE twin studies, the concordance rate is much higher, up to 69%, and specifically in monozygotic twins where the DNA shared is identical suggesting a much stronger genetic role in SLE than SSc [68, 75, 76].

One study identified the age of SSc onset of two members of the same family within four families differed significantly suggesting the condition is not merely encoded genetically but that an environmental trigger is likely needed to initiate the pathologic process of SSc [77].

This implies therefore a much stronger environmental role for the risk factors in developing SSc.

Environmental risk factors

Case-control studies and systemic literature reviews with meta-analysis have looked at the association of the clinical phenotypes of SSc plus the related autoantibodies with postulated exposure to environmental triggers [78]. Interestingly, the environmental risk factors are shared with scleroderma-like syndromes. The difference with scleroderma-like syndromes is the main presentation of skin fibrosis is void of the internal organ involvement and SSc diagnostic clues of RP, capillaroscopic abnormalities and SSc-related autoantibodies [79].

Foti et al, 2007, detailed 21 other scleroderma-like conditions that all present with the skin thickening characteristic of SSc, however with varying differences in distribution and associated features or causes [80]. For example, Eosinophilic Fasciitis is characterised by skin thickening of the limbs but sparing the hands, feet and face which is the predominant areas affected in SSc. In contrast, in Diabetes Mellitus (DiM), it is common for around 8-50% of people to present with bilateral thickening of the fingers resulting in contractures and the 'prayer sign' typical of SSc, yet DiM is considered void of other key clinical SSc features such as; telangiectasia, puffy fingers and RP [81].

Some patients presenting with SSc and Scleroderma-like conditions have a history of exposure to substances such as vinyl chloride, organic solvents, epoxy resins, silica dust, toxic oil and gadolinium [82-87] and there have also been reports of SSc and scleroderma-like conditions that have been drug induced [88].

The demographic and risk factors outlined are to be considered when assessing a new patient with possible SSc and should form a background to the assessment of other presenting clinical manifestations, which will now be discussed.

1.1.7 Clinical manifestations

The ACR-EULAR criterion refers to the most striking and prevalent clinical manifestations specifically seen in a confirmed and established case of SSc. These will be described briefly from the clinical symptom and clinical presentation perspective with a note on the general management plans available.

Raynaud's phenomenon

As highlighted, RP is usually the first clinical symptom presenting typically many years before the onset of SSc in the case of lcSSc, and a few years in the case of dcSSc [89]. It describes an episodic exaggerated vasospastic response of the blood vessels at the extremities in response to cold, emotional stress and reportedly hunger [90]. This results in a temporary reduction in blood flow, colour changes of the skin ranging from pallor (ischemia), cyanosis (deoxygenated) and rubor (reperfusion) with associated pain at the area affected [91]. Hands are typically the body area most effected, possibly because they are more distal to the core blood flow and more exposed to temperature change, although RP can also affect the toes, lips, ears nose and nipples [90]. Critical digital ischemia is reported to effect 68% of patients with SSc and DU, 18-23% presenting as gangrenous [92-94] and 1.2% per patient-year resulting in amputation [93]. RP and DU are known to create functional disability and are associated with poor quality of life [7, 95].

The first line management of RP typically starts with patient education on conservative measures to prevent where possible exposure to the cold and emotional stress and to avoid vasoconstrictive drugs, caffeine, and smoking [90]. In terms of medication, the following frequency of medications are used; calcium channel blockers (CCB) (71.6%), the prostanoid Iloprost (20.8%), endothelin receptor-1 antagonist Bosentan (20.4%) and PDE-5 inhibitors such as Sildenafil (16.5%) [96]. Calcium channel Blockers (CCGs) can help to reduce the frequency and severity of attacks [97].

Topical Glyceryl Tri-Nitrate (GTN) has shown effective vasodilatory responses in patients with lcSSc and primary RP with immediate and short term effect [98]. When applied routinely over the course of 6 weeks, a reduction in severity and frequency of attacks is observed [99]. A meta-analysis including 7 placebo-controlled trials of 346 patients observed similar effects but are limited for comparison due to differences in GTN preparation and outcome measures [100]. Topical GTN is prescribed less frequently due to contraindications with

commonly used drugs used for SSc, such as sildenafil and hydroxychloroquine.

Botulinum Toxin injections show increasing interest and promising evidence for effective management of RP with 28 case reports and five RCT's currently published [101]. The treatment options for dosages and location of administration are yet to be standardised with clinical guidelines. Further studies are required with consistency in standardisation, study populations and outcome measures.

The consequence of undermanagement of prolonged and/or repeated RP attacks is the risk of poor oxygenated and devitalised tissue at risk of ulceration.

Finger-tip lesions: Digital ulcers and pitting scars

It is general considered that DUs effect almost half of all SSc patients with some studies showing as high as 70% over a 10 year observation [102]. Digital ulcers usually present within the first five years of a SSc diagnosis with a third of patients experiencing recurrent ulceration [95]. Digital ulcers are considered a biomarker of internal organ involvement and mortality, even in early disease [103].

Many studies refer to both DU and pitting scars (PS) with a general acceptance that this relates to ischemic alterations at the finger tips, whereas ulcers presenting on the extensor surface of joints are considered to be due to mechanical and microtrauma confounded by increased skin tension [104].

There is however limited data on the association of RP associated ischemia and DUs which suggests the process of progression to skin ulceration is more complex [105].There are also discrepancies in the prevalence of ulcer locations with some studies favouring the fingertips and others the extensor aspect of the fingers more or equally, both with similar disability ratings [92, 106-108].

The treatments available for DU and PS vary considerably depending on geographical area, the services established and the health professionals skilled in ulcer care. Undertaking specific wound care techniques such as scalpel debridement is considered beneficial for optimising DU healing and prevention

of DU in the case of pitting scar management. Randomised controlled trials are currently underway to evidence this benefit in SSc and standardise the protocols for DU and PS management [109].

For medicines management, Bosentan, an endothelin receptor antagonist has been proven effective not only for the management of RP, but predominantly for the prevention of new DU in patients with SSc [110]. The effectiveness of this drug supports the role of endothelin -1 in the pathological activation of RP and skin inflammation as mentioned earlier in section 1.1.2.

The National Health Service (NHS) has now commissioned Sildenafil and Bosentan for patients requiring treatment of DU in SSc following the beneficial evidence and recommended guidelines from the British Society of Rheumatology and British Health Professionals in Rheumatology [111, 112].

These medications predominantly target the capillaries which are known in SSc to be altered functionally and structurally.

Abnormal nailfold capillaries

The structural vascular alterations of capillaries in SSc are seen via NVC with the presence of enlarged capillaries, mega capillaries, haemorrhages, loss/drop out, disorganisation and ramifications. The extent of each capillary feature present allows classification into an ‘early’, ‘active’, or ‘late’ pattern which corresponds to duration and/or severity of disease [113-117].

The association of clinical findings with NVC patterns in SSc show that capillaroscopy patterns also directly correlate with the extent of organ involvement and can be predictive of DU risk [118, 119].

There is no specific treatment for these alterations seen via NVC and they are used as means of aiding diagnosis only. NVC assessment technique and capillary descriptions are described and detailed in chapter 4.2.8.

Abnormal nailfold capillaries represent the capillary changes expected throughout the body and may be exemplified by telangiectasia seen clinically in the skin.

Telangiectasia

The presence of telangiectasia is clinically seen as dilated dermal capillary vessels located predominantly on the skin of the face, hands and arms and sometimes elsewhere on the body [120].

Telangiectasia have also been found internally within the gut, along with the fibrotic changes of SSc [121, 122].

Telangiectasia biopsies have shown thickened fibrotic vessels with thickened collagen fibres in the reticular or deep dermis and have been shown to require twice as many pulsed dye laser treatments to resolve [123]. Although only a small cohort was used, this concurs with other histopathology investigations which suggest that the surrounding infiltrates are non-inflammatory and relate to fibrotic changes [124].

This may indicate that the fibrotic processes, as we see with the skin in SSc, has more impact on the vascular alterations than the inflammatory response.

Skin fibrosis, puffy fingers and Sclerodactyly

The autoimmune response and fibroblast activation that leads to fibrosis of the skin usually occurs in three distinct stages [125]:

1) Early oedematous phase: This is usually referenced as ‘puffy fingers’ where there is painless oedema due to an increase in the amounts of interstitial fluid at the distal extremities, sometimes extending to the limbs. Patient may report morning stiffness and or arthralgia. This phase is usually short-lived.
2) Indurative phase: This is caused by dermal collagen deposition which leads to skin thickening and loss of elasticity describing the hallmark of “skin fibrosis”. The skin thickening and tightening starts to reduce the mobility of the skin and the mechanical structures (tendons and joints) underlying. Advanced skin fibrosis at the hands and feet can result in “Sclerodactyly” where the digits form a fixed claw-like shape.

   Patients may report pruritus and the skin appears shiny and tight with loss of dermal creases and sometimes erythema. This may take several years to progress in

lcSSc, and a fast progression may be indicative of dcSSc.

3) Atrophic phase: This is where the skin becomes thinner, sometimes tethered to underlying structures leading to further functional impairment.

The pathogenesis of skin fibrosis is still unclear but it is understood that excessive amount of extra cellular matrix (ECM) is produced and accumulates between the skin cells typically with an overproduction of Type I collagen [126].
The rate of skin fibrosis progression correlates with visceral involvement and mortality [127].
The assessment of skin fibrosis in SSc as an outcome measure will be discussed in detail in section 4.2.6
Autologous fat grafting has been shown to improve the overall tissue quality and slow the progression of SSc complication such as skin fibrosis, RP and chronic DU [128].
The management of skin fibrosis however, predominantly involves medications targeted at slowing disease progression via immunomodulation with mycophenolate mofetil as the first line choice [129].

Pulmonary arterial hypertension and interstitial lung disease

As mentioned, the vascular alterations and fibrosis are not limited to the skin. The internal organs undergo similar pathological changes with Pulmonary Arterial Hypertension (PAH) and interstitial lung disease (ILD) being the most significant in SSc.
Pulmonary hypertension (PH) in SSc is associated with three phenotypes: PAH resulting from pulmonary arterial vasculopathy, PH due to left heart disease and PH due to ILD [130]. PAH and ILD is the leading cause of death in SSc with a three year survival rate in only 52% [131, 132].
There are considered to be are up to 5 groups of differing pathophysiological mechanisms for the presence of PAH, of which a patient with SSc could be classified in multiple groups [133]. The vascular abnormalities mentioned earlier including an over expression of endothelin-1 are also seen in the pulmonary

arteries resulting in vascular narrowing and contribute to the PAH seen in SSc [134].

The effectiveness in managing PAH includes a similar approach as used for managing severe RP with endothelin receptors antagonists such as Bosentan, Ambrisentan and Macitentan [135-137].

Treatment of progressive SSc associated ILD focuses on immunosuppressants such as mycophenolate mofetil and cyclophosphamide with options of stem cell therapy and lung transplantation [138].

Related autoantibodies

Several SSc-related antibodies have been identified along with the typical associated clinical characteristics. Table 1 gives an overview of these.

Autoantibodies	Prevalence in SSc	Predictor/ clinical implications
Anti-nuclear antibodies (ANA)	Over 90%	Strongest for SSc diagnosis and digital microvascular damage when seen with RP and NVC changes [139]
ANA negativity and SSc	6.4%	Predominantly males, less vasculopathy but more frequent lower GI involvement [140]
Anti-centromere antibodies (ACA)	30%	More prevalent in Caucasians than African Americans or Asians. Diagnostic marker and predictive factor for lcSSc. Frequently associated with calcinosis and PAH [139].
Anti Th/To	1-13%	Higher prevalence in Caucasians, associated with lcSSc and ILD. Shorter disease onset for RP but with less severe disease than with ACA, more likely to be younger and male. Associated with pericarditis and higher frequency of ILD, PAH and myositis [139].

Anti-Topoisomerase I (anti Scl70)	9-15%	Risk of developing dcSSc, DU typical in the first three years and related to severe ILD [139].
Anti-RNA polymerase III	11%	Rapid skin thickness progression and joint contractures, renal crisis, gastric antral vascular ectasia (GAVE), the occurrence of synchronous cancers, and possible association with silicone breast implants rupture [139, 141, 142]
Anti-Ku	2-7%	Defines the PM/SSc overlap: synovitis, joint contractures, myositis, less vasculopathy [143]
Anti-U3RNP	7-11%	a well-defined clinical phenotype: Afro-Caribbean male patients, younger at diagnosis, and higher risk of PH and GI involvement [144, 145]
Anti-PM/Scl	2-5%	higher frequency of ILD, calcinosis, overlap conditions, dermatomyositis skin changes, and severe myositis [146, 147]
Anti-eIF2B, anti-RuvBL1/2 complex, anti-U11/U12 RNP, and anti-BICD2	10% of “seronegative” SSc patients	SSc subtypes with severe organ complications [139]

Table 1: Auto antibodies related to SSc: prevalence and predictive implications.

The above summarised clinical manifestations of SSc focuses only on the features detailed in the ACR-EULAR criteria. However, the criteria were only intended to be used for enrolment to research studies. In practice, diagnosis of SSc may be informed by other additional key clinical manifestations not included, such as dysphagia, tendon friction rubs and calcinosis.

Of these key manifestations, calcinosis was one of the ACR-EULAR criterion items which scored most highly for appropriateness in comparison to other items but did not meet the threshold to be included as part of the final classification of SSc.

Interestingly, the term ‘CREST disease’ was originally used to describe cases of SSc, and is an acronym of the key presenting clinical manifestations of: “Calcinosis, Raynaud’s, Esophageal dysfunction, Sclerodactyly and Telangiectasia” [148]. This implies that before the refinement and deeper understanding of the early and wider presentations of the SSc disease, calcinosis was a key clinical manifestation in established/severe disease which defined calcinosis as predominantly associated with more pronounced vascular (Raynaud’s, telangiectasia) and fibrotic (esophageal dysfunction, sclerodactyly) features.

In summary, there are a wide range risk factors and clinical manifestations to consider for the diagnosis and management of a patient with SSc. These include other highly appropriate clinical manifestations not detailed within the general disease classification, but which can aid diagnosis and early intervention to contribute to improved quality of life outcomes.

1.1.8 Quality of life in systemic sclerosis

Despite the relatively low prevalence of SSc in comparison to other rheumatic diseases, the effect and impact on patients’ quality of life has been reported to be more significant than conditions such as Rheumatoid Arthritis and Psoriatic Arthritis [4, 6, 149]

Quality of life in relation to the impact of calcinosis specifically, has been explored in SSc and shows worse outcomes using the Scleroderma Health Assessment Questionnaire (SHAQ) [150], but not within similar conditions in which it presents. Calcinosis is considered one of the key determinants of disability, poor hand function and a significant burden, which in turn, reduces reported quality of life. Understandably, calcinosis has therefore been identified by patients and practitioners as a key area in need of research and clinical support [151, 152].

1.1.9 Summary

SSc is an autoimmune condition with the highest disease-related morbidity and mortality rates with a highly impaired quality of life when compared to other similar autoimmune conditions.

The hallmark of the disease relates to both vascular and fibrotic alterations to the skin and internal organs with a heterogenous presentation of clinical manifestations. Revisions of the disease classification based on clinical manifestations has resulted in increased and early diagnosis and opportunity for earlier treatment interventions to reduce or slow the severity of disease.

In SSc, the hands are commonly and extensively affected by both the vascular and fibrotic tissue alterations, and hand disability and function are key determinants of overall quality of life and clinical outcomes.

Calcinosis is a key clinical manifestation and a driver of poor hand function which has been identified as a priority area for research and treatment options.

This disabling manifestation of calcinosis in SSc is the focus of this study and the literature will be explored in more detail in the next chapter with links to the underlying disease processes of SSc already discussed.

1.2 Calcinosis in Systemic Sclerosis: a narrative review

1.2.1 Introduction

Calcinosis is a clinically heterogeneous manifestation of SSc for both site and severity of involvement. It describes the presence of calcified material commonly reported as multiple, firm whitish papules, plaques or nodules deposited in the skin [153].
The treatments available are limited and based on a lack of understanding on the factors that drive the calcinosis formation in addition to those factors that impact the patient most. There are currently differing views regarding the formation of calcinosis. The clinical characteristics and disease features associated with calcinosis are to be explored in this study and presented with patient-reported impact.

1.2.2 Prevalence and incidence of calcinosis in systemic sclerosis

Calcinosis is reported to occur in around 25%-40% of people with SSc. Clinical reports usually place estimates at the low end of this range while studies using imaging report calcinosis in up 35-40% of patients, with radiographic analysis highlighting it to be the most common feature of the hands in SSc [153, 154].This emphasises the value of imaging to detect early formation that is otherwise not clinically evident.
No prospective longitudinal study has looked at the incidence rate of calcinosis, that is, the occurrence of new cases over time, either between or within subjects. However, a retrospective follow-up using a validated radiographical scoring system showed that 40% of subjects with calcinosis will show a worsening progression of the overall radiographical score of calcinosis over 12 months [155].

1.2.3 Classification and diagnosis of calcinosis

Calcinosis is generally accepted to subdivide in to five categories: dystrophic, metastatic, idiopathic, iatrogenic and calciphylaxis [156].
Classifications have been proposed and adapted throughout the literature and there continues to be some overlap and

inconsistency in the conditions associated with each classification and use of various descriptive terms.
There is a focus on linking a hypothesised pathogenesis and underlying primary condition to each classification, even though the pathogenesis of calcinosis remains unknown.
There is a generalised agreement that the term dystrophic calcification is a result of local tissue change and predominantly a change from healthy to devitalised tissue [157]. This opens broad potential causes for tissue damage; direct trauma, vascular insufficiency, local tissue inflammation or tissue change from an underlying condition, all factors that can be seen in SSc.
In addition to these categories, the literature identifies other commonly used descriptions and terms.
These include [158]:

- Calcinosis cutis: the umbrella term to describe all types of deposits affecting the two outermost layers of skin, the epidermis and dermis.
- Calcinosis (cutis) universalis: describes calcinosis cutis presenting in a more generalised, usually deeper, and widespread areas of the body.
- Calcinosis circumscripta: cases of smaller, localised forms of calcinosis cutis.
- Other terms (used in isolation or in addition to the above): Localised calcinosis, diffuse calcinosis, widespread calcinosis, systemic calcinosis, calcium deposition, soft tissue calcification, subepidermal calcified nodules or calcinosis in reference to body part.

The number of added descriptors and terms creates confusion with standardising the classification.
With this is mind, Fernandez-Flores, 2011, suggested a revised classification based on histological presentation of the calcium deposits [159]. This was based on the diagnosis of calcium deposition being the same, independently of the potential underlying cause. Instead, the classification is defined by the morphological appearance of the deposit.
This revised classification is divided into deposits with and without vascular involvement, the only type related purely to

vascular calcification being calciphylaxis, described as calcium deposition within the vessel walls. Figure 1 describes the proposed classification with associated description as cited and adapted from Fernandez-Flores (2011).

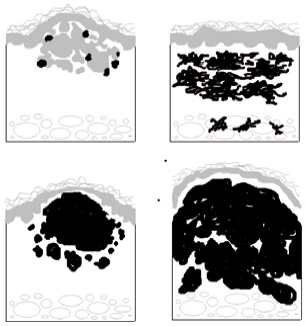
Date	Author	Classification	Description/terms
2011	Fernandez-Flores	Small scattered calcium deposits (Top left)	Calcinosis accompanying some tissue tumours/ cysts/ local traumas
		Widespread dermal calcium deposits (Top right)	'Calcinosis cutis' due to extravasation of calcium products
		Nodular and Granular calcium deposits (Bottom left)	Idiopathic calcinosis/ calcified subepidermal nodule/ millia-type calcinosis/ connective tissue diseases (early)
		Tumoral calcium deposits (Bottom right)	Calcinosis of metabolic disorders/ tumoral calcinosis/ connective tissue diseases (advanced)
		Calciphylaxis deposits	With vascular involvement (within the vessel walls)

Figure 1: Fernandez-Flores (2011), classification of calcinosis deposits according to histology presentation.

For clinical reporting it is suggested these deposit classifications are referenced to the location and condition in which the calcinosis presents [159]. For example, nodular calcium deposits (classification), of the hand (location), in SSc (condition).
This proposal is neatly structured; however, it relies on histological analysis which is considered impractical and invasive for the purpose of clinical classification, particularly in patients where the tissue viability is already compromised.
Despite attempts to reduce overlap of terms and descriptions, pre-existing use of historical terminology could affect the interpretation and adoption of such classification. For example, 'widespread deposits' based on the widespread infiltration

deposit pattern, can be localised to a small anatomical area. The term ‘widespread’ instinctively insinuates a presentation covering a wider anatomical area, (such as the previously reported ‘calcinosis universalis’ description or ‘tumoral/metastatic’ classification).

There is also no consideration that one patient may exhibit areas of small, scattered deposits alongside other areas of more established nodular calcinosis, and no references to what size the nodular calcinosis of early disease becomes classified as tumoral calcinosis indicative of advanced disease.

Referring to the classifications as with/without vascular involvement confuses the historical inclination to associate calcinosis with the underlying tissue state or condition. Particularly in SSc, it is considered that the hallmark of ‘vascular involvement’ is a likely cause of the tissue damage leading to dystrophic calcinosis.

The European League Against Rheumatism recognised a similar inconsistency with the diagnosis and classification of calcium pyrophosphate deposition and produced revised terminology and clinical recommendations for diagnosis [160]. Similarly, guidelines for the diagnosis of gout includes considered clinical risk factors and where crystals are unconfirmed [161]. For gout, it is recommended that imaging is used for diagnosis confirmation, particularly the use of ultrasound which unlike histology, is a non-invasive option.

Clinical recommendations for assessment and diagnosis of calcinosis do not exist. The use of ultrasound as an outcome measure to describe the deposits and diagnose calcinosis is not currently used or recognised despite its potential for diagnosis confirmation, and for assessment of any clinical associations or risk factors.

1.2.5 General risk factors and clinical associations of calcinosis in systemic sclerosis

An international multi-centre retrospective cohort study of 5218 subjects with SSc showed that in 1290 patients with calcinosis, the clinical associations included older age, longer disease duration, DU, telangiectasia, acro-osteolysis, cardiac disease,

pulmonary hypertension, gastrointestinal involvement, arthritis and osteoporosis [162].

In smaller studies, risk factors associated with having calcinosis in SSc have been associated with positive ACA and positive PM/Scl antibody [163, 164].

In contrast, a registry of Singapore subjects compared to subjects in Australia, Canada and Europe showed lower rates of calcinosis and DU despite a higher rate of dcSSc and the Scl70 antibody within the cohort [165]. This may be owing to the shorter disease duration or may relate to the higher percentage of subjects managed with immunosuppression and corticosteroids. The therapy may have had an unrecognised effect especially if dystrophic calcinosis develops due to the inflammatory and/or fibrotic alterations of skin in SSc.

Calcinosis has been said to be specifically associated with ILD which may correlate to some higher rates seen in dcSSc where ILD is more frequent and/or severe [166, 167]. Younger male subjects with dcSSc and shorter disease duration been found to have more calcinosis along with tendon friction rubs and synovitis [40].

In contrast, another study highlighted associations between the presence of calcinosis in the longer disease duration of lcSSc and with other autoantibody profiles suggesting the differences in the Singapore study may instead be influenced by ethnic background and geographic region [150].

Regarding clinical assessment, NVC shows an association with a ‘late’ pattern in subjects with calcinosis [168], but a ‘late’ pattern is also associated with DU with or without calcinosis [169]. Therefore, a late NVC pattern could be more indicative of DU risk in general and the underlying vasculopathy that leads to tissue breakdown. What differentiates the vasculopathy between those with DU and those with calcinosis may not relate solely to the changes seen on NVC. However, vasculopathy in general is considered to create the altered devitalised tissue environment associated with dystrophic calcinosis.

Similarly, an altered tissue state represented by a loss of digital pulp is predictive of progressive calcinosis and a higher prevalence of PS correlates with static and worsening calcinosis [155]. Again, this may represent a higher degree of altered tissue

state in patients with calcinosis, possibly more fibrotic than ischemic in nature.

These clinical associations and risk factors provide a picture of the type of patient who would present with calcinosis or be at risk of developing calcinosis. These factors however appear as heterogeneous as the disease of SSc as do the clinical presentations of calcinosis which will now be described.

1.2.6 Clinical presentation of calcinosis

There are varying forms of calcinosis ranging from solid stone like formation through to a consistency of grainy toothpaste, moose, or exudate [154]. The calcium deposits can be isolated, clustering, measure only a few millimetres in size or surround an entire anatomical area [159]. In SSc, deposits can develop as deep as bone and/or as superficial as the dermal layer occurring most commonly at the hands (65-83%), proximal upper extremity (27%), knee or proximal lower extremity (10-22%), and hip (6.7%) [153, 154].

Calcinosis of the spine has been reported in 17-83% of SSc cases with associated neurological symptoms including peripheral neuropathy due to nerve compression [30].

Calcinosis can be associated with extreme pain and complications such as joint contractures, tendon damage, ulceration of the skin, secondary infection, delayed healing and need for surgical intervention [92, 170, 171].

The mineral composition of calcinosis is predominantly hydroxyapatite and amorphous calcium phosphate crystals forming within the extracellular matrix of the dermis [172, 173].

Calcinosis is referenced throughout the literature as insoluble deposits. However, the clinical presentation varies from stone to fluid and can involve bidirectional change between these states, sometimes even demonstrating re-absorption. This indicates a possible biological solubility and solidification in certain tissue conditions.

Bartoli et al (2016), defined 316 radiographic calcinosis presentations in 52 SSc subjects as stone (91.4%), net (3.8%), plate (4.8%) mousse (31.8%) and some deposits being non-visible but clinically palpable (52.6%) [174].

The location of calcinosis related DUs at the hand shows higher prevalence for the fingertips compared to non-calcinosis DU's, however this was described when comparing only 18 calcinosis subjects versus 37 non-calcinosis [169].

It is suggested by the authors that the cause of the calcinosis formation at the fingertips could relate to the fingertips being subject to more repeat direct trauma creating an inflammatory response, or from the fingertips having reduced blood flow in general therefore prone to more frequent intermittent ischemia and subsequent tissue hypoxia associated with RP.

Ultimately, the pathogenesis of calcinosis is unclear.

1.2.7 Pathogenesis of calcinosis

Despite the inferences that the pathogenesis of calcinosis relates to inflammation and/or tissue hypoxia, the exact pathogenesis of calcinosis remains unknown. There is clear pathological evidence that calcium deposition occurs when there is an imbalance of tissue homeostasis in the metastatic form of calcinosis, where a blood serum test would show either hypercalcemia or hypophosphatemia. This is the most simplistic cause of calcium deposition and can be easily treated by correcting the tissue imbalance, however, this imbalance in blood serum is not evident in SSc [175].

Insights from other mineralisation disorders suggest that both local and systemic phosphate metabolism pathways involving certain genes and poly enzymes may play a critical role in calcinosis formation but this requires further study [176].

Currently, the evidence of risk factors and clinical associations of SSc has led to the main hypotheses that calcinosis formation occurs at the site of altered and devitalised tissue. This leads to a consensus in classifying calcinosis in SSc as dystrophic calcinosis.

There are several examples in which the skin in SSc is altered or compromised in a way which could lead to calcinosis formation and it is suggested that the skin in SSc is therefore a primed micro environment for calcinosis [177].

The key pathologies in SSc which may relate to priming the micro-environment for calcinosis formation will now be explored.

Vasculopathy

Vasculopathy is the hallmark of SSc and typically the first clinical presenting feature of the disease. The clinical features associated with both vasculopathy, and calcinosis are the presence of DU, an increase in telangiectasia, and a 'late' NVC pattern reflecting reduced capillary density and altered capillary architecture. Associations with macrovascular alterations have been made from 50% of patients with calcinosis exhibiting ulnar arterial occlusion [178, 179]. Interestingly, this is even in the absence of DU.

It is reasonable to link the capillary and arterial changes to the vasculopathy seen either in the longer disease duration of lcSSc, or the more severe disease of dcSSc. Although, this may equally represent the driving factor of increased fibrosis, resulting in vasculopathy specifically from vascular fibrosis with the increased skin fibrosis seen in dcSSc and longstanding lcSSc.

In addition to longer disease duration, older age in general is a risk factor associated with calcinosis and may relate to increased vasculopathy from natural aging. Vascular aging processes predominantly present as vascular calcification, but also brain and soft tissue calcification has been reported in the aging population [180, 181].

Undoubtedly, an environment that has reduced blood supply is at risk of tissue hypoxia and tissue death. However, vasculopathy being the causative factor of calcinosis would not explain why in a cohort of 100 subjects with SSc and a total of 1614 DUs observed, 49% of subjects in SSc presented with pure ischaemic ulcers and 0.8 % gangrene/necrotic digital ulcers without the presence of calcinosis. This compares to only 6.8% of ulcerations being derived from calcinosis [92]. The authors of this same study believed through wound observations that digital pitting scars, which are associated with both vasculopathy and calcinosis, also do not appear to be a mere ischaemic event as previously proposed.

Longer healing rates of calcinosis related DU are referenced throughout the literature [92]. Clinically however, it is evident that once the calcinosis has exuded or been removed, the wound

healing is much quicker than pure DU related to ischemia [182]. This suggests the overall vascular supply may be functionally better or compromised in different ways to those with pure DU not associated with calcinosis.

In support of the tissue hypoxia theory, tissue markers such as glucose transporter molecule-1, advanced glycation end (AGE) products and activation of the receptor for AGE products have been found to be increased in the skin of patients with lcSSc and calcinosis and in dcSSc, and freely circulating in patents with pure DU [183-185].

Valenzuela et al, (2020), concluded that calcinosis is associated with ischaemia, however ischaemia was measured as a surrogate of DU, loss of digital pulp and/or digital pitting scars [150]. Each of these clinical features can be challenged as not a true representation of ischemic severity. Instead, it could instead be indicative of more severe fibrotic changes with the related DU being a consequence of the physical presence of the calcinosis deposit causing ulceration. The severity of RP between the groups with and without calcinosis was not assessed. A comparison of RP severity between groups and vascular imaging would be considered a more representative assessment of ischemia than the presence of DU alone. In addition, finger pulp blood flow, although not standardised as a measure of ischemia, does not correlate with the presence of calcinosis [178].

Manning et al, (2020), conducted a within-patient control pilot study to assess blood perfusion using laser doppler. In contrast to the historical theory of ischemia being a driving factor of calcinosis, they showed no differences in oxygenation at sites with and without calcinosis. Thermography also showed no significant differences between all sites affected and unaffected with calcinosis. Perfusion as assessed by laser doppler was not significantly decreased. The size and depth of the calcinosis had no significant relationship with oxygenation or perfusion. Laser speckle contrast imaging did show a difference in perfusion in the very superficial layers of skin at the site of the calcinosis, however, this would be expected in the presence of superficial calcified material devoid of skin tissue and blood flow or may instead represent altered skin thickness at the site [186].

Unfortunately, there was no control group without calcinosis and

skin fibrosis, and surrounding areas of inflammation were not assessed to know if these, instead of vasculopathy, were more relevant contributing factors.

Inflammation

As highlighted, there are incidences where calcinosis presents in the absence of severe ischaemia or DU.

The prevalence of calcinosis at sites of mechanical stress and areas of repetitive pressure has been linked to inflammation at these sites contributing to the development of calcinosis. Similarly, inflammation being the cause or effect of calcinosis presence must be questioned. No studies have assessed the presence of inflammation systemically or at the site of the calcinosis deposits.

Increased inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are indicative of systemic inflammation.

Associations have been seen with the Scl70 antibody and calcinosis, and patients with the Scl70 antibody tend to express higher systemic inflammatory markers [187]. The presence of calcinosis does not appear to directly correlate with raised CRP or ESR [187, 188], despite these markers being associated with DU in general [189]. However, a lack of these markers in other SSc profiles does not exclude local inflammation.

There is evidence supporting local tissue inflammation in patients with SSc and the Scl70 antibody, represented by severe swelling of the soft tissue, inflammatory and sclerosing tenosynovitis and synovitis detected using ultrasound [190]. This has not yet been replicated in other patient profiles.

From a theoretical perspective, if tissue inflammation from pressure and mechanical stress were main contributing factors, we would expect to have more prevalence of calcinosis at the feet, particularly as we know that pressure and pain in SSc is higher in the feet than in the normal population namely due to structural changes from skin thickening, loading of bodyweight, a reduced plantar fat pad and external pressure from footwear [191]. Despite a high prevalence of self-reported foot problems, it has been identified that there lacks routine assessment of the

feet in clinical rheumatology practice which may explain this lack of reported evidence [192, 193]. Only one study found radiographical evidence of slightly more calcinosis at the feet (24%) than the hand (21%) of patients with the ACA [194]. In contrast, a longitudinal study of radiographic and clinical foot involvement in 100 patients with SSc found a significantly higher prevalence for calcinosis, acro-osteolysis and erosions at the hands compared to the feet [195]. These differences may be due to demographic and geographical variance which could influence factors such as activity levels and footwear choices.

Medicinal treatment targeting inflammation has successfully elevated symptoms associated with calcinosis by decreasing the inflammatory response [196]. The remission of calcinosis from targeting inflammation alone has not been adequately studied and will be presented in section 1.2.8.

Overall, skin inflammation is a presenting feature in SSc and in relation to calcinosis, however this could represent a cause and/or effect of calcinosis. In addition to inflammatory processes, there is further evidence of other complex cellular level changes occurring within the skin that should be considered.

Cellular level changes of the skin

Trace elements and minerals in the serum of subjects with dcSSc show higher levels of potassium, calcium, phosphorus and magnesium when compared to healthy controls suggesting an effect on cellular physiology [197]. However, there is no analysis into the correlation with calcinosis and no inclusion of subjects with lcSSc where calcinosis is considered more prevalent.

In addition, the uptake and release of calcium and phosphate takes place by closely interrelated processes at the subcutaneous level of isolated adipose tissue, which are dependent on mitochondrial energy production. Applicably, it is recognised that mitochondrial dysfunction and damage is a key cellular level process in SSc [198].

It has also been proposed that phosphate bound to denatured proteins of necrotic cells at sites of trauma or inflammation may induce calcium accumulation [199].

Normally, extracellular calcium distribution rises in concentration through the stratum granulosum towards the skin surface. Intracellular calcium is suggested as the main contributor to the epidermal calcium gradient, regulated by a calcium exchange between keratinocytes and extracellular fluid [200].
The clinical pattern of active calcinosis shows this very progression in migration of calcinosis deposits from the deeper subcutaneous tissue to the surface, eventually resulting in ulceration and expulsion out of the tissue.
In the case of SSc hand oedema, extracellular fluid is present and may trigger an osmotic effect of calcium exchange from the intracellular keratinocytes to the extracellular fluid.
A recent study looking at the presence of calcinosis in early SSc identified radiographic calcinosis in 50% of subjects in which calcinosis was not detected clinically, and showed a significant correlation with oedema at the onset of disease [188].

Lymphatic drainage

The lymphatic vessels are responsible for balancing the extracellular fluid and removing excessive particulate waste from the tissues via the extracellular matrix. Lymphatic vessels are rich in anchoring filaments that bind to the elastic and collagen fibres within the extracellular matrix where the exchange of calcium ions occurs between cells [201].
Biopsies of SSc skin have been shown to have a progressive 70% loss of lymphatic vessels beginning in the reticular dermis and later extending to include the papillary dermis, compared to only the 25% reduction seen in blood vessels [202].
In a cohort of 60 subjects with SSc and lower limb ulceration, 30% were seen to have related venous insufficiency and 20% were classified with lymphedema. Unfortunately, the macrovascular links of calcinosis related ulcers were not studied as the ulcers were considered as a potential consequence of calcinosis as opposed a pure result of macrovascular disease [203].

Bone related alterations

Studies have identified a lower bone density and osteoporosis in subjects with SSc and calcinosis, compared to SSc subjects without calcinosis suggesting a link with abnormal bone and mineral metabolism [204, 205].

An additional link with mineralisation imbalance has been proposed where mineralisation inhibitor inorganic pyrophosphate (PPi) has been shown to be increased in the serum of subjects with calcinosis [206].

The role of vitamins and the parathyroid hormone (PTH) have been studied in SSc due to their role in bone metabolism and reabsorption. Vitamin D deficiency and hyperparathyroidism is reportedly higher in SSc and shows a significant correlation between acro-osteolysis and PTH, calcinosis and disease duration and between PTH and Vitamin D levels [207].

Lower levels of vitamin D have been linked to the increased risk of osteoporosis in SSc and vitamin D may otherwise have an antifibrotic role in SSc [208].

The association of bone related acro-osteolysis with calcinosis has been inferred to relate to an ischemic process due to the location predominantly at the fingertips where RP creates repeated ischemic events in SSc. It is additionally possible that ischaemia is created because of increased tissue tension at the fingertips from skin fibrosis and sclerodactyly, and the skin tension and pressure at this site may have a direct effect of acro-osteolysis at the bone.

Bone has high levels of two main proteins which contain the vitamin K dependant calcium binding amino acid y-carboxyglutamin acid (GLA): osteocalcin and matrix GLA [209]. Both osteonectin, a bone-specific marker protein, and matrix GLA has been found to be expressed higher in lcSSc and more so in subjects with calcinosis [210].

Although not yet studied, there is a possible link with levels of vitamin K2 and the metabolism of bone-related proteins expressed within the skin. Vitamin K2 activates the calcium-binding actions of matrix GLA protein and osteocalcin. Osteocalcin within the bone is activated by vitamin K and low levels are associated with osteoporosis, as seen more commonly in SSc and calcinosis. Vitamin A and Vitamin D work in synergy

with vitamin K. Vitamin A treatment has improved the skin fibrosis in SSc [211] suggesting a possible deficiency of this and vitamin D with a subsequent possible impact on the role of vitamin K.

Gastrointestinal involvement

Gastrointestinal complications in SSc are commonly referenced as most impactful after skin involvement with suggestions of links between the two [212]. Notably, that early changes to gut microbiome alterations may contribute to scleroderma pathogenesis and skin fibrosis [213]. Vitamin K2 is produced by the gut bacteria and the gut bacteria is known to be deregulated in SSc, possibly leading to an imbalance of proteins and calcium binding actions in the skin that promote calcinosis formation.
The link between GI involvement and calcinosis has not yet been studied directly. A cross sectional study showed no differences in dietary intake or calcium and vit D supplementation between subjects with and without calcinosis [214]. However, the altered function and absorption within the gut may be the differentiating factor to consider as dietary intake is immaterial if the function and absorption is altered.

Collagen and skin fibrosis

The excessive deposition of collagen seen in the pathogenesis of skin fibrosis has not been directly linked to the pathogenesis of calcinosis, although there are observations and biological processes that are noteworthy and warrant further laboratory exploration.
Calcinosis has been significantly correlated with a higher modified Rodnan skin score in a cross sectional study of 36 patients with SSc [214].
Pitting scars have been strongly associated with calcinosis [166] and altered collagen fibres are seen within the deeper dermal layers of patients with PS [215].
Accumulation of myofibroblasts, resistant to apoptosis, enhance the extra cellular matrix stiffness, collagen secreted into the extra-cellular spaces undergoes crosslinking and maturation to produce a highly stable matrix and stiffness of skin. A “quarter-

staggered" structure creates a periodic gap and overlap regions and the mineral hydroxyapatite, the defining mineral of calcinosis, is deposited predominantly in the gap regions of the type I collagen fibrils [216].

The author of this thesis is first co-author of a published paper which identified that the microscopic crystal structure of calcinosis falls into two distinct shapes and clinical forms [217]. The paste/fluid form of calcinosis exhibits crystals of long thin needle-like structure, like that of the collagen fibres themselves or the thin extra-cellular spaces they create when layered. The hard stone form of calcinosis exhibits a rounded compact stone like structure which would appear to form easily within the more rounded gap regions of the fibrils. Collagen binds calcium ions [216], therefore it is reasonable to conclude that the increase of dermal collagen in SSc increases the binding of calcium ions. These, theoretically would accumulate in the gap regions available between and around the fibrils, remaining fluid like within the smaller spaces and compounding into more solid forms where space allows at the gaps.

Generally, collagen fibres are thicker in the deep reticular dermis and are more densely packed in the middle dermal zone [218]. Increased collagen in these areas in SSc would explain why subcutaneous calcinosis is seen to affect the sub dermal and dermal layers of skin and it is presumed this is where the calcinosis first forms.

Calcinosis in other connective tissue diseases

Similarities supporting inflammation and altered tissue states can be seen within other conditions in rheumatology, where calcinosis is seen in up 30% of adult Dermatomyositis (DM) and in 20-40% of Juvenile DM [219].

There is a rapid and extensive collagen turnover promoted during childhood to sustain tissue growth [220] which may perpetuate or be an additive factor to the production of calcinosis in this condition. It has also been suggested that activated macrophages and inflammatory cytokines contribute to calcinosis formation in Juvenile Dermatomyositis [219].

In SLE, calcinosis is described in up 40% of cases as ectopic and silent, only found incidentally on radiograph usually at the peripheral arteries, periarticular areas, and other soft tissues. It may take more than 20 years of disease duration before dystrophic calcinosis presents [221].

Powell proposed that calcification in patients with SLE may be secondary to local ischemia induced by a pressure effect of steroid induced hypertrophied fat cells [222].

In a similar theorised context, the pressure effect on the tissues due to oedema and skin fibrosis may lead to a physical local ischemia and the destruction of cell membranes leading to a release and accumulation of calcium concentration.

Occasional case reports have identified calcinosis in Rheumatoid arthritis, however there are other conditions complicating the presentation such as overlap CTD conditions and active skin ulceration [223-228].

Each of these autoimmune conditions present with alterations to the immune system, RP and inflammatory processes and it is not uncommon for an overlap of CTD conditions to give predominance of other CTD features.

Subcutaneous calcifications and associated ulceration in Werner syndrome have been shown to relate to a decreased drainage, narrower lumen and calcification of the lymphatic vessels with calcium content being significantly higher in the luminal structure when compared to the dermal tissue [229].

The anatomical location of the lymphatic vessels and predominant location of calcinosis has not yet been studied to know if these are correlated.

Calcinosis in the absence of connective tissue disease

Atherosclerosis is one condition seen in normal aging which can be complicated by calcium deposits forming within arterial vessel walls, without an associated CTD.

Instead of the previously considered result of purely lipid accumulation, the pathogenesis is has been identified as the result of osteoblast differentiation of vascular smooth muscle cell with mineralisation starting as octacalcium phosphate to later form hydroxyapatite [230].

Calcification frequently occurs in the intima or media layers of the vessel.

Calcification tends to occur after the development of advanced lesions within the vessel walls comprising of lipids and foam cells with interaction of T cells and macrophages, which later become necrotic. There is a layer of fibrous connective tissue associated with these advanced lesions, and B lymphocytes also contribute to chronic inflammation within the area. It is also proposed that the endothelial cells which line the vessel walls undergo injury from high blood pressure.

This process has similarities with the subcutaneous tissue of SSc where calcinosis forms with the involvement of deregulated macrophages, damaged endothelial cells, associated fibrosis, local inflammation and resulting in necrotic devitalised tissue.

In addition, the theory of devitalised tissue change is supported by calcinosis presenting in non-systemic disease at sites where tissue injury and subsequent inflammation has occurred [157, 231, 232].

For example, calcinosis is seen in devitalised tissue of traumatic wounds, particularly burns. This supports the possibility that inflammation and/or the remodelling/fibrotic phase of wound healing creates devitalised tissue and/or excessive fibrotic scar tissue, not unlike SSc related skin fibrosis, with subsequent calcinosis formation [231, 233-235].

Calcinosis is also be associated with benign neoplasms and it is proposed that this links to calcium-binding proteins found in poorly differentiated keratinocytes [236, 237].

Calcinosis is seen in 10% of patients with chronic venous insufficiency without CTD, and venous leg ulceration complicated with calcinosis accounts for 90% of non-healing or recurrent ulceration [238]. In these cases, it could be argued that the calcinosis is present due to the vasculopathy and/or the inflammation associated with the wound healing.

As this relates to venous insufficiency wounds, it could also be theorised that it is the accumulation of extra cellular fluid which contributes to the formation of calcinosis. Lipidermatosclerosis also features in chronic venous insufficiency presenting as skin thickness and fibrosis [239] showing similarities with the early oedematous stage and skin fibrosis in SSc. Again, this provides

a possible link to oedema as a contributing factor of calcinosis formation.

Case studies have highlighted another close association of fibrosis and calcinosis formation seen in localised linear scleroderma and morphea. Patients are described to present with calcinosis arising only from morphea and linear plaques [240-244].

The treatment of calcinosis in these non-CTD conditions focuses on treating the underlying related condition and any symptoms associated with the calcinosis presence.

1.2.8 Treatment of calcinosis in SSc

The treatments available for calcinosis in SSc are limited as they are based on a limited understanding on the factors that drive the calcinosis formation, in addition to limited knowledge on the factors that impact the patient most. The current treatment considerations are to manage the symptoms and maximise function, where possible removing the calcinosis to interrupt and slow the progression.

There are currently no guidelines explicating recommending a detailed staged approach encompassing all potential treatment options or treatment planning in general. Instead, it is at the discretion and knowledge of the clinician to instigate a treatment of choice. Unfortunately, this is usually the invasive surgical option at the point at which the deposit is already impacting on function and complete successful removal is limited with potential risk to nearby structures.

In general, the overriding theory that vasculopathy is implicated most in the pathogenesis of calcinosis directs treatment to improve circulation. This includes supporting smoking cessation, RP management, and control of RP triggers such as stress, cold exposure, and trauma. Supportive treatments to reduce related symptoms include anti-inflammatory and pain medications, including opioids [245, 246].

Targeted treatment for calcinosis in relation to systemic and local therapies have been attempted with varying effect.

Targeted systemic treatment

A systematic review described the current proposed systemic therapies, either as a monotherapy or in combination which include: calcium channel blockers, colchicine, bisphosphonates, warfarin, ceftriaxone, minocycline, probenecid, aluminium hydroxide, IVIG, anti-TNF and rituximab [245]. These results were based on the results only one small RCT and few small retrospective or case studies, emphasising the need for more robust research in the efficacy of systemic treatment options. An overview of this systematic review with medicinal treatments, the proposed effect and mode of action are summarised in Table 2, adapted from Valenzuela and Chung [247].

Drug group	**Commonly used/researched drugs**	**Proposed effect**	**Mode of action on calcinosis**
Calcium channel blockers (considered first line treatment by some authors)	Positive effect: *Diltiazem* 240 to 480mg/day Over 1-12 years in one case No/worsening effect: *Verapamil* (no effect in one case)	Alters the formation and crystallisation.	Reduces the intracellular calcium reflux in the affected tissues and local macrophages.
Bisphosphonates	Positive effect: *Etidronate* over 1 year in one case *Risedronate* over 6months in one case Intravenous *pamidronate* (several DM case reports) No/worsening effect: *Etidronate* (6 patients over 10 months, worsening in SSc)	Reversing the calcification process	Inhibiting macrophage pro-inflammatory cytokine production and reducing calcium turnover

Vitamin K antagonists	*Warfarin* <u>Positive effect:</u> 1mg/day for 18 months, 2/3 patients improved. <u>No/worsening effect:</u> *Warfarin*: As above, one case worsened. 5/6 patients had worsening calcinosis over 14 months. No positive response in 4 other patients in another study.	Reduces carboxyglutamic acid (known to be higher in patients with calcinosis)	Antagonization of vitamin K inhibits the production of carboxyglutamic acid which has calcium and phosphate binding properties
Tetracyclines	<u>Positive effect:</u> *Minocycline* 50-100mg per day over 3.5 years in one case. (Reduction in associated ulceration and inflammation) Alternative of cyclic long-term use (4-8 weeks, discontinued for 3-4 months)	Reduces inflammation and binds the calcium to prevent the calcium breakdown that potentially predisposes to ulceration	Anti-inflammatory and calcium binding properties
Anti-gout medications	<u>Positive effect:</u> *Colchicine* 1mg/day over 2-4 months. 3 /8 with complete resolution in one case.	Reduces inflammation associated with the calcinosis. Decreases serum	Disrupts leukocyte chemotaxis and phagocytosis by inhibiting microtubule polymerisation.

	500mg 3x a day for 7 months in one case	phosphate level, reducing the calcification process	Increases renal phosphate clearance
Cephalosporins	<u>Positive effect:</u> Ceftriaxone 2g/day intravenously for 20 days in one case	Regression of lesions and prevention of new deposits	Binds calcium ions to form insoluble calcium complexes
Aluminium Hydroxide	<u>Positive effect:</u> *Aluminium hydroxide* 30ml 4 times daily on one case	Decreases serum phosphate level, reducing the calcification process	Decreases intestinal absorption of serum phosphate levels
Intravenous Immunoglobulins	<u>Positive effect:</u> *Immunoglobulins* Over 5 months No control or outcome measures	Possibly reduction in inflammation	Possible suppression of activated macrophages
Biologic agents *Anti – TNF* *Chimeric anti-CD20 antibody*	<u>Positive effect:</u> *Infliximab* 3mg/kg infused at 0,2,6 and every 8 weeks thereafter over 41 months. *Rituximab* 2 courses 375mg/m2, 18 months apart in one case Complete resolution after 7 months of one cycle of above in one case	Reduces inflammation and associated TNF.	Presumed reduction of inflammation associated with calcinosis and general improvement of SSc disease

	3/6 on four weekly infusions had improvement by 6 months <u>No/worsening effect:</u> 2x (1 g) infusions every two weeks, then 6 months in one case		
Antidotes and chelators	*Sodium Thiosulphate* <u>No/worsening effect:</u> Intravenous 25g up 1-3 times weekly	Reduces presence of calcinosis	Chelates calcium ions, inhibits hydroxyapatite, has an antioxidant action, or dissolves calcifications

Table 2: Systemic management of calcinosis. Efficacy, proposed effect, and mode of action.

Targeted local treatments

Targeted local treatments of calcinosis appear to show great benefit despite them not being available as a standard of care. Surgical excision or debulking can reduce pain and improve functional outcomes, however, only 15% gain improved function and 44% will have complications related to delayed healing, infection and reoccurrence of calcinosis [248]. As mentioned, surgery is generally only initiated when the calcinosis is already severe, often with irreversible damage.

Therefore, the timing of treatments is crucial for the effectiveness at preventing significant progression and patient reported impact. Non-surgical proposals range from topical creams to minimally invasive procedures such as scalpel debridement at ulcerated or pre-ulcerated sites, micro-drilling, dry needling, and peri-lesion injections, with finally more invasive techniques such as laser therapy.

Unfortunately, the strength of the data to support these treatments is limited mainly to case reports, observational studies, and case series. This highlights the need for robust RCTs to evidence the effectiveness and gain support from Clinical Commissioning Groups (CCGs) to provide effective treatments as set standards of care.

Alternative topical treatment options studied will be summarised below.

Topical and intra-lesion sodium thiosulfate

Recent case studies report the benefit from sodium thiosulfate for calcinosis not only via intravenous administration but also via intradermal injection, or topical application [249-251].

A recent systematic review documented the studies of Topical Sodium Thiosulfate (TSTS) prescribed as a topical compound drug for the treatment of calcinosis. The formulation bases, concentrations, method, and duration of topical application differed between the studies highlighting a lack of standardisation for prescription and an inability to compare effectiveness.

However, the results are promising for TSTS, with an 81% response rate and reduction in calcinosis deposits over a longer duration of treatment when compared to other modalities. It may prove useful for early management of smaller deposits or for other larger extensive lesions where surgery would not be suitable, particularly at anatomically critical sites.

Similarly, the intra-lesion sodium thiosulfate (IISTS) applications studies differed in concentrations and dosages and were influenced by the patient tolerability of pain from the injections [252].

In comparison, complete response rates have been seen in 36% in IISTS, possibly owing to the ability to inject into deeper tissues surrounding the deposit where a higher concentration of the agent will remain without the need to be absorbed through skin first. This may be beneficial in areas of highly thickened scleroderma skin where absorption via topical application may be compromised. Twelve patients over two studies however showed no significant change in size of calcinosis with the author

suggesting that low dose and long interval time between treatments may have impacted this.

Intra-lesion steroid injection

This has shown some success in DM and suggests that reducing inflammation at the site will improve the outcome of calcinosis formation [253].

It has however been commented that the common side effects of steroidal injection include pain, telangiectasia and purpura, haemorrhage, ulceration, atrophy, iatrogenic calcification, secondary infection, pigment changes and granuloma [254]. It is suggested that subcutaneous steroid injection in SSc would not be ideal in atrophied and sclerodermatous skin.

Scalpel debridement

The appropriateness of certain treatments also depends on the size or depth of the calcinosis within the skin. Scalpel debridement for example, can only effectively occur at a time when the calcinosis is superficial enough to disrupt the integrity of the upper skin layers creating an already ulcerated or pre-ulcerative area of tissue. Once any overlying or surrounding devitalised tissue is removed, the calcinosis can be drained or removed using the scalpel blade. This is occasionally performed by an Allied Health Professional (AHP) skilled in scalpel debridement of skin lesions, predominantly Podiatrists in the UK, or specially trained nurses in Europe. The AHP treatment and cost effectiveness has not yet been published owing to the relatively limited services currently being offered.

However, it appears a cost-effective option as it does not require a surgeon or dermatologist and as healing rates can be improved with added support to address or prevent infection and promote wound healing with appropriate dressing selection.

Topical Neem oil and Hypericum Perforatum

An example of a calcinosis targeted dressing selection is topical neem oil and hypericum perforatum. A case control study found that the application of this preparation to calcinosis wounds post scalpel debridement improved healing times, reduced rate of infection and in some instances reduced the stone formation of calcinosis to a mouse formation for easier extraction via scalpel debridement. In line with improved removal of the calcinosis, pain levels were reduced overall compared to the control group using dry dressings and less removal of calcinosis [182].
Hypericum perforatum is used in treatment of mild inflammatory dermatological diseases and to treat skin wounds. Hyperforin is the most important active substance promoting wound healing, with a presumed action on fibroblasts, keratinocyte proliferation and differentiation. In addition, hyperforin has antimicrobial activity, particularly against Gram-positive bacteria [255, 256].
Neem Oil has been proposed to have anti-inflammatory properties by modulating macrophage migration and activity [257] all of which appear to address the tissue changes seen in calcinosis and SSc.
The use of other agents or basic carrier ointments as controls have not been compared to differentiate between the mode of action that is effective.
It could be suggested that any basic ointment that hydrates the calcinosis could result in a softening and ease of removal with subsequent reduced pain.

Ethylenediaminetetraacetic acid (EDTA)

EDTA is a chelating agent which has been shown in some early case studies to have minimal to no benefit in juvenile patients with calcinosis presumed secondary to dermatomyositis [258]. Whereas an earlier literature search suggested a positive effect in several small case reports [259] and later research has shown a positive effect for the management of calciphylaxis. [260, 261]
The author of this thesis and colleagues has have demonstrated that calcium deposits removed from the wounds of SSc patients can be dissolved in a small percentage of EDTA, and at a similar

percentage as used commonly and safely in most cosmetics, which holds promise for a potential chelator of calcinosis that could be applied topically [217]. This would potentially be a crucial final step to support other treatment options such as scalpel and surgical removal to cleanse the wound bed and remove any remaining crystals that would otherwise result in the reforming of deposits.

Micro-drilling and micro-needling

Micro-drilling using a high-speed burr to break up and remove larger, deeper calcium deposits following local anaesthesia has been used successfully by surgeons. It is a quick and minimally invasive procedure with quick healing rates and can be performed without the use of a digital torniquet, which is beneficial in SSc where the vascular supply can be compromised [262].

Caution in this technique relates to reported complications of weakness, numbness, decreased motion, and superficial wound infection. It has been suggested that treatment of discrete lesions using a microdrill results in better reported outcomes than those with extensive lesions on multiple fingers [263].

This could be used as an additional tool for AHPs where simple scalpel debridement does not allow full removal of the exposed calcinotic wound bed in calcinosis related DUs. This is not currently used in the wound care settings by AHP's despite the routine use of the same tool for enucleation of painful, deep, or neurovascular heloma.

A less invasive approach with the purposes of promoting an intrinsic wound-healing cascade has been identified in a case using essentially the same needle puncture wound and depth of micro-needling, via an 'inkless tattoo' in a patient with DM. It resulted in a reduction in pain and global regression of calcification despite no other intervention in this time and an otherwise relentless progression prior [264]. It is suggested that the 'inkless tattoo' promoted growth and repair factors as seen in micro-needling for scars [265].

New alternative candlelit microneedle technique for administrating an evenly distributed drug to the deeper dermal

layers shows improved outcomes and reduction in side effects [266].This has not yet been studied but may be a promising option with a dual effect of micro needling alongside drug distribution such as a calcium chelator mentioned previously to target calcinosis where deposits are located deeper within the tissue.

Laser therapy

Laser therapy can remove small calcification up to 2cm in a single session and is considered less invasive than surgery with minimal scar tissue [267, 268]. Laser therapy has the benefit of maintaining good haemostasis without the need for a tourniquet via duel cauterisation of capillaries allowing a clear operating field not afforded by scalpel debridement [268, 269].

A recent case report showed a 57% clearance of calcinosis at the pulp of a finger with low recurrence rate of 11% at 12 months [270].

A similar case report using three rounds of CO2 laser therapy has shown no reoccurrence at 6 months with improved function and resolution in symptoms [271].

Laser therapy is rarely utilised due to concerns of healing and expected reoccurrence, despite its observed potential. Larger longitudinal studies are needed to demonstrate its effectiveness.

Extracorporeal shock wave lithotripsy

Extracorporeal shock wave lithotripsy (ESWL) has shown some improvement to calcinosis symptoms with a reduction in calcinosis size.

Eight patients treated with ESWL showed a reduction in size of calcinosis and pain levels at 6 months following a 3 session treatment over 9 weeks [272]. The tolerability was good with no adverse events at 1.5 years follow-up. However, the sample size was very small, the related pathology was varied (4 chronic venous insufficiency, 3 with SSc and 1 with DM), the location varied and there was no comparative control group. Efficacy was better with smaller deposits but improved ergonomic designs are also needed for routine practice.

This study is supported by other case studies referenced and including one by Sparsa et al, (2005) where pain reduction has been a key finding along with rapid healing of chronic ulceration complicated by calcinosis [273-275]. This supports the theory that ulceration may not be due to ischemic tissue change and instead the physical presence of calcinosis creates tissue breakdown, which once removed, promotes fast healing.

Lymphatic drainage

Lymphatic drainage massage techniques have shown to improve hand oedema and hand function in SSc [276-278]. Hand oedema at SSc onset is associated with calcinosis and venous leg ulcers are known to be complicated with calcinosis deposits [203]. If oedema is a precursor or contributing factor to calcinosis formation, this could prove a simple targeted therapy to reduce the severity or occurrence of calcinosis but has not yet been studied directly.

Summary

In summary, the treatment options are limited and poorly evidenced with robust research, but all show some promise. The severity of calcinosis and delayed healing of calcinosis-related ulcerations may reflect geographical inconsistencies in treatment options undertaken. Targeted treatments may be effective if available and offered early in the diagnosis of calcinosis to reduce the severity of symptoms and improve the quality of life.

1.2.9 Calcinosis and quality of life

The presence and complications of calcinosis are commonly reported to be associated with high burden of pain, disability and in particular hand dysfunction [10, 279]. The burden of hand disability has been linked to increased social and economic costs and reduced ability to work having employability and financial implications [149, 280, 281].

The physical appearance of the hands with calcinosis and related functional limitations has been linked with emotional difficulty in social situations and this eventually leads to isolation [282].

The high prevalence of calcinosis at the fingertips and thumbs increases the difficulty in conducting everyday tasks and mechanical stress may worsen the development of calcinosis [283]. It is of no surprise that SSc patients with calcinosis report a higher degree of pain than patients without calcinosis [279] which will impact the physical capabilities and quality of life. Calcinosis related ulcerations show a significant increased time to heal compared to digital ulcers not derived from calcinosis [169]. Unfortunately, this delayed healing is more likely linked to a lack of treatment to aid quicker healing that could otherwise be possible once the deposit is removed.

Management of calcinosis related ulceration requires regular routine dressings which need to kept dry to prevent infection. Managing this with limited function of the hands poses another level of complexity and burden for maintaining adequate wound and self-care to reduce complication risk.

To exemplify this, a public and patient involvement exercise was conducted as part of the application for this study. These are two examples of separate patient quotes when asked specifically about the impact of calcinosis:

“..the worst thing is not knowing what or why it happens, and wondering how long it's going to take to build and break through again. Having to deal with that, the pain and redressing all the time, besides the fact it looks awful [pause] then you've got the infection and so on..” Patient 1.

“..just doing everyday stuff hurts, and it's awkward, like getting dressed or just picking up the kettle even...it’s so bad, I don't dare even hold my new great grandson in case I drop him.” Patient 2.

Despite the burden of calcinosis in SSc, the treatment options are currently limited and typically offered when severe calcinosis is already established. Understandably, calcinosis has therefore been identified by both patients and practitioners as a key area in need of research and clinical support [151, 152].

1.2.10 Summary and conclusion

Calcinosis in SSc has a high patient-reported impact and has been identified as a key area in need of research. Most importantly, the research needed should translate into improving clinical practice and the quality of life of patients burdened with calcinosis.

There are currently differing views that may contribute to the formation of calcinosis. The most generally accepted and less considered factors have been explored in this thesis with an understanding that although the pathogenesis of calcinosis currently remains unknown, there are key clinical presentations that could be targeted to aid in calcinosis management.

The evidence supporting effective treatments are currently lacking in number and quality. To measure the effectiveness of any intervention for calcinosis, there needs to be a reliable measure of both the impact and an appropriate outcome measure of the calcinosis itself.

The literature review has identified the need for a measure of calcinosis impact and to observe any key clinical characteristics of calcinosis in SSc which could be a target for management. Most importantly, the literature review highlights the opportunity and need to establish a suitable outcome measure of calcinosis severity to enable more robust clinical trials focussed on the targeted management of calcinosis in the future.

1.3 Why use ultrasound for calcinosis in SSc?

1.3.1 Introduction

To identify a new and reliable outcome measure of calcinosis in SSc, review of the literature and current clinical procedures in this area was explored. Ultrasound was easily identified with great potential as a highly reliable, safe, cost effective and easily accessible imaging modality for calcinosis. Ultrasound is utilised at the point-of-care and relies on non-destructive sound waves instead of ionizing radiation. It is increasingly used in the diagnosis and management of many rheumatic conditions. Although ultrasound imaging is a two-dimensional technique, real-time, dynamic ultrasound interrogation allows complete spatial visualization of soft tissue, vascular, and musculoskeletal structures including distinct lesions. Current US machine and probe technology allows for superior imaging resolution compared to X-ray, CT scan, and MRI, affording the ability to visualise structures down to the sub-millimetre level [284]. An overview of the use of US in SSc, other crystalline disease and the value and opportunity for this to be used for calcinosis will be presented here.

1.3.2 Ultrasound in SSc

Currently ultrasound has been shown to be an effective imaging modality in SSc for a range of clinical assessments, which have given insight into clinical features and predictive values pertinent in SSc [285]. For example, ultrasound has identified single organ observations such as a high prevalence of salivary gland abnormalities in SSc [286]. It has also been utilised for musculoskeletal observations commonly assessed within rheumatology with measures of enthesopathy [287-289], synovitis and tenosynovitis, bone erosions and cortical irregularities at the joints [290, 291] . In particular, synovitis and tendon friction rubs have been reported to be predictive of overall disease progression [292] and these features can be easily assessed with US.

Most importantly, US can identify articular pathologies in the absence of the typical clinical signs which is crucial for early

identification and intervention to prevent damage to underlying structures. It has been shown that synovial involvement is well established in SSc of both hands and feet and yet clinical examination is significantly underestimating the presence when compared to US confirmation [11, 293-295]. For example, one study including 17 patients with SSc reporting arthralgia symptoms but with no overt clinical signs of inflammation, tenosynovitis was found via US at baseline (46%) and at 6 months (47%) [295].

Aside from detection of inflammatory disease, US can also detect non-inflammatory musculoskeletal conditions such as neurological complications from nerve entrapment. In SSc, it is common to detect carpel tunnel syndrome and ulnar nerve entrapment at the elbow [296]. Related US findings show an increased size of the median nerve and reduced number of digital nerves [297-299]. There is also an association with median nerve entrapment and severity of vasculopathy. This has been exemplified with US guided carpel tunnel hydrodissection and corticosteroid injection which resulted in improvement of RP attacks, pain and healing of ulcers [300].

Ultrasound has been used to look at specific tissue, such as skeletal muscle mass. Reduced muscle mass at the gastrocnemius and rectus abdominus was correlated with dcSSc more than lcSSc, with a high sensitivity and negative predictive value [301].

Other specific tissue assessed by US includes the lung. As ILD is commonly related to mortality rates in SSc, the use of US to detect early lung pathology, to monitor changes and check response to treatment is extremely valuable [302]. Currently, the gold standard to diagnose ILD and aid in ascertaining prognosis of disease is the use of high-resolution computed tomography (HRCT). However, US offers a non-invasive alternative with the ability to conduct repeat measures without the high cost, ionising radiation and ethical issues associated with HRCT [303]. Several studies have demonstrated that US imaging of the lungs is able to diagnose even the earliest stages of SSc related ILD [304-318]. The OMERACT group conducted a systematic review and found that US for the assessment of lung pathology in SSc has both face and content validity and feasibility [319]. Although the

nine papers analysed lacked sufficient evidence to fulfil criterion validity, reliability and sensitivity to change, Hughes et al (2020), argue that lung US is both highly sensitive and with a negative predictive value and the variability in the current evidence can be explained by inconsistent standardisation of technique [285].

Similarly, given the nature of SSc primarily effecting the skin and the severity of skin involvement being predictive of overall disease severity and progression, US has also been researched extensively as a proposed effective outcome measure for skin involvement.

Skin US in SSc has been shown to identify differences in skin thickness with B mode US and skin stiffness using shear-wave or qualitative colour-scale US elastography, all of which correlate with clinical outcomes [320-323].

Compared to healthy controls, ultrasound assessment of dermal thickness is greater in SSc patients and in almost all the mRSS sites used clinically for measuring skin involvement [323-330]. In participants with dcSSc, skin is reportedly thicker on the hands, forearms, legs and chest compared to participants with lcSSc, yet interestingly, the finger sites did not show any significant difference [331]. This suggests that the fingers are equally and most significantly affected by skin thickening irrespective of disease subset.

As with other clinical manifestations, US has proven to identify subclinical alterations in very early disease reflecting the initial oedematous phase of skin involvement and at areas of ‘uninvolved’ skin scoring 0 using clinical mRSS measures [324, 331, 332]. This again, highlights the sensitivity of US as an early outcome measure in SSc and its advancement compared to clinical examination alone.

As part of the World Scleroderma Foundation (WSF) Skin Ultrasound Group, the author of this thesis has contributed to a published systemic review focusing on the validation and standardisation of US and elastography in the assessment of skin involvement in SSc [333]. This review followed the OMERACT filter of validity, reproducibility, responsiveness to change and feasibility [334]. After filtering on strength of evidence 30 articles were reviewed and highlighted significant gaps in feasibility, validity, and discrimination with the need to

standardise image acquisition and analysis. The author of this thesis is continuing to support this work via an international Delphi agreement process and prospective validation to determine these requirements and recommendations for future research, with the view to adopt this as a routine clinical outcome measure.

Related to skin, there have been several ultrasound-based studies which have demonstrated how US can detect the vasculopathy associated with SSc. Successful evaluations of the peripheral macro and microvasculature have been undertaken. Micro-vascular disease of the digital arteries has demonstrated the visualisation of sub-clinical vasculopathy in up to 67.4% of cases, where vasculopathy was not detected by capillaroscopy or laser speckle contrast analysis (LASCA) [335]. The importance of high frequency probes and assessment of distal arterioles has been highlighted, where more proximal assessment and lower doppler frequencies have missed abnormalities [336].

The use of colour and power doppler has been used to quantify finger vasculature and the volar aspect of the finger has shown high accuracy for detecting SSc-related RP compared to healthy controls [337]. The flexibility of US assessment is demonstrated by the ability to assess all levels of vasculopathy at various areas of the body from the carotid artery to the vertebral arteries, extending to the peripheral digital arteries [338] and even detecting vasculopathy within the bowel [339].

For macro-vascular disease Ulnar Arterial Occlusion (UAO) has been found bilaterally in 75% of cases and associated with ILD, late NVC and is predictive of DU [340-342].

In relation to DU specifically, ultrasound has been used to examine a range of DU presentations [343]. High frequency US has the benefit of detecting DU less than 1mm in size with adequate visualisation of the dermal layers which are interrupted in the formation of DU, but commonly replaced with hyperkeratosis and/or dried wound exudate. Clinically, the size and tissue replacement of DU is often mistakenly visualised as healed whereas the reality and underlying visualisation provided by US highlights deeper and wider tissue breakdown.

Ultrasound for the assessment of DU has demonstrated evidence for face, content, and criterion validity with responsiveness [343-346]. It is yet to demonstrate construct validity and discrimination for defining DU in SSc but offers great potential to be used in the assessment of underlying severity and pathology.

In summary, the use of US for the assessment of clinical outcomes in SSc is growing in interest. It shows great potential to be used as a non-invasive outcome measure for various SSc-related disease manifestations, with superior ability to detect pathology compared to clinical assessment alone.

1.3.3 Current outcome measures of calcinosis in SSc

Clinical assessment of calcinosis is lacking any formal clinical description to confirm diagnosis and how the clinical characteristics relate to patient reported symptoms is unknown. The clinical presence is usually confirmed at a point when the calcinosis is visibility breaking through the skin and the deposit itself or associated exudate is exposed. Outside of specialist centres where knowledge and experience of SSc is less common, calcinosis is typically misdiagnosed and treated as infection or gout.

The significance between the clinical presentation and related symptoms is not known, therefore assessing these is not considered an important part of routine clinical practice. At most, a measure of increased pain will be reported by the patient which may instigate a need to investigate, and usually only intervene if the calcinosis deposit is obvious to detect clinically.

Regarding specific patient reported outcome measures (PROMs) of calcinosis, The Maudsley Calcinosis Questionnaire is a calcinosis specific questionnaire currently in development which incorporates four domains: Quantity/Frequency, Pain/sensation, Physical Function and Psychological impact. These were developed via in-depth patient interviews and although it has not yet been fully validated, it offers insight in to the natural history of calcinosis, and the patient reported impact of calcinosis on self-management, quality of life and mental/physical function [347].

The only current clinical measure used for confirming the presence and monitoring of calcinosis is via x-ray. A scoring system has been validated for use in clinical trials but is specific to the hands only [348].

Although useful, using the validated x-ray scoring system as an outcome measure raises issues both clinically and for clinical trials as it limits assessment to the hands only and limits the number of repeat measures that can be undertaken due to radiation exposure.

A narrative review published by Mar et al, summarises other imaging modalities for the assessment of calcinosis including computed tomography (CT) scans and magnetic resonance imaging (MRI). They conclude that CT scans can prove helpful in instances of extensive calcinosis where minute detail is required to inform safety of surgery procedures. MRI is less sensitive and specific for detection of calcification and is more suitable when additional pathological information is required. CT is limited by radiation exposure and both CT and MRI are costly and time intensive [349].

Despite the low cost, and routine use of US for calcinosis-related tissue involvement of musculoskeletal structures, vasculature, skin and DU, the use of US for the assessment of calcinosis in SSc is not performed as part of routine clinical practice. Neither has it been validated as a potential outcome measure for clinical trials. This is also despite the validation and use of US for other similar crystalline conditions within rheumatology.

1.3.4 Ultrasound in other crystalline disease

In contrast, US is validated and used routinely for the assessment of crystalline disease within rheumatology [160, 161, 350-354].

Ultrasound is particularly useful in crystalline arthropathies such as gout and pseudogout where disease specific ultrasound findings such as tophi and the “double contour” sign can aid in clarifying a diagnosis and assessing disease severity. As a result, ACR/EULAR now incorporates ultrasound findings as part of their classification criteria for gout [161].

Through a similar process of exploration and validation, there is an opportunity to define calcinosis using ultrasound and determine the features that differentiate it from other crystalline diseases whilst measuring both severity and impact in SSc.

1.3.5 Ultrasound as an outcome measure for Calcinosis in SSc

Given the evidence to date, it is apparent that US has the potential to be an excellent outcome measure for calcinosis. The various compositions of calcinosis comprising of hydroxyapatite crystals, will create the difference in acoustic impedance from the surrounding soft tissues to produce a required image, as seen with the distinction of crystals in other crystalline conditions. Ultrasound irradicates the concerns related to the current gold standard of x-ay as it in non-invasive, can be utilised at various areas of the body over several short timepoints and more importantly can provide much more in-depth assessment of multiple tissue types simultaneously. This can occur at the point of care, all of which is not afforded by x-ray alone. Ultrasound has the added benefit of providing assessment of individual lesion microstructure, surrounding soft-tissue changes, and vascularity, and may provide important supplemental information related to severity and associated symptoms and potential predictive features of progression. This offers an excellent outcome measure for clinical trials with a vast range of detailed assessments that can be undertaken and the opportunity to understand how calcinosis may form and/or affect the nearby structures.

Ultrasound also provides more accurate visualisation of deposits to guide any proposed targeted intralesional injection therapy. Calcinosis has already been incidentally visualised in the assessment of DU, where calcinosis underlying the visible wound provided associated hyperechoic feedback [343] [344]. Calcinosis has also been an incidental finding during ultrasound assessment of musculoskeletal pathologies, easily detected and recognisable as hyperechoic masses consistent with clinical and radiographical appearance of calcinosis [293, 355]. However, it

remains that there lacks a validated definition of the appearance of calcinosis using US, preventing its formal use for clinical trials.

1.3.6 Summary

The use of US for the assessment of outcomes in SSc is growing in interest and showing great potential to be used as a non-invasive outcome measure for various SSc-related disease manifestations, with superior ability to detect abnormalities compared to clinical assessment alone.

Ultrasound has been successfully used in the assessment of other crystalline conditions and is an outcome measure recommended to confirm diagnosis in gout. Despite the numerous advantages over x-ray and the similarities between other crystalline conditions, ultrasound is not yet used for the diagnosis or assessment of calcinosis.

The current validated measure of calcinosis is x-ray, which provides important information regarding, bulk, density, and distribution of lesions. However, US imaging can provide complementary microstructural, soft tissue, and vascular details on a lesion level.

As seen with other clinical manifestations in SSc, ultrasound is a rapid, accurate, and inexpensive tool which will increase our understanding of the pathophysiology of calcinosis, aid in early detection and diagnosis, inform management strategies, and provide an outcome measure for clinical trials.

Despite all these benefits, a validated definition of calcinosis using US does not exist. In addition, there is no evidence of how the impact relates to the clinical characteristics of calcinosis. Details of the clinical presentation and associated PROMs are likely to compliment and direct the use of US to confirm early sub-clinical presence of calcinosis and assess the severity and progression in established cases.

Therefore the first key steps are to understand the relevance of the clinical presentations associated with patient-reported outcomes and to begin the validation process of US as the ideal tool to confirm calcinosis presence.

Both will serve to form a framework for improving the in-depth assessment of calcinosis severity, to inform targeted

management strategies and reduce the patient impact of this disabling condition.

1.4 Aims and objectives of the research project.

The governing hypothesis of this study is that the cause of calcinosis is multi-factorial and through exploring which characteristics are associated with calcinosis, and which impact the patient most, targeted treatments can be developed.

1.4.1 Aims

The aims of this study are:

- To describe the clinical characteristics of calcinosis and how these relate to patient-reported impact.
- To define calcinosis using ultrasound and measure the ability of ultrasound to detect the presence of calcinosis using this definition.

These aims are the initial steps in providing a framework and basis for appropriate outcome measures focussed on measuring the impact and severity of calcinosis in SSc. This initiates support for future clinical trials in the evaluation of calcinosis and appropriate targeted treatments, which could later extend to the management of calcinosis irrespective of related disease type.

1.4.2 Objectives

To fulfil the aims of this project I addressed three objectives in three successive phases:

Phase One. Objective One: Prevalence and impact of calcinosis in Systemic Sclerosis

An observational postal survey to determine the patient reported prevalence and impact of calcinosis in people attending specialist centres in the Yorkshire and Humber region.
This data includes patients reportedly with and without calcinosis. The observation includes patients with SSc, Dermatomyositis (DM) and Systemic Erythematosus Lupus (SLE). Function, pain, and quality of life outcomes are captured using patient-reported questionnaires.

Phase two. Objective two: Delphi Agreement: Expert consensus on defining calcinosis using ultrasound.

A Delphi expert group consensus to agree on a definition of calcinosis using ultrasound. This will inform the reporting of calcinosis presence for phase three and form the basis of work for future validation.

Phase three. Objective Three: Case-control study and sensitivity of ultrasound to confirm calcinosis.

A case-control and within-patient control sub-study to measure the impact of calcinosis on function, pain, and quality of life using patient-reported questionnaires. Clinical associations and clinical characteristics of calcinosis will be described in chapter 4 including clinical differences between most and least impactful lesions at the hands.

Phase three in chapter 5 will introduce US with a proof-of-concept study to assess the ability and feasibility to detect calcinosis using the agreed Delphi definition. Ultrasound reported presence will be compared to x-ray, clinical and patient-reported presence and determine the benefit of US as a complimentary imaging modality over x-ray alone for the assessment of calcinosis.

Chapter 2.

Phase One: Regional prevalence and impact of calcinosis in Systemic Sclerosis

2.1 Introduction

This chapter refers to phase and objective one of the study, the regional prevalence and impact of calcinosis in SSc. The aim was to gain a better understanding of the prevalence and impact of calcinosis in SSc across a local geographical area and how this compares to similar conditions of lupus and dermatomyositis which may also exhibit calcinosis. Each phase was constructed following expert consensus and review of the literature.
Section 2.2 describes the methodology employed for patient identification, recruitment, data collection and the analysis plan. The results for this first objective are presented and discussed in this chapter.

2.2 Methodology

2.2.1 Participant identification and recruitment

Ethical approval from the Health Research Authority was sought and obtained for all phases of the study ('CALSITE': IRAS 154667. See letter of approval, appendix 1). The schematic diagram of figure 2 summarises the methodology process for phase one.

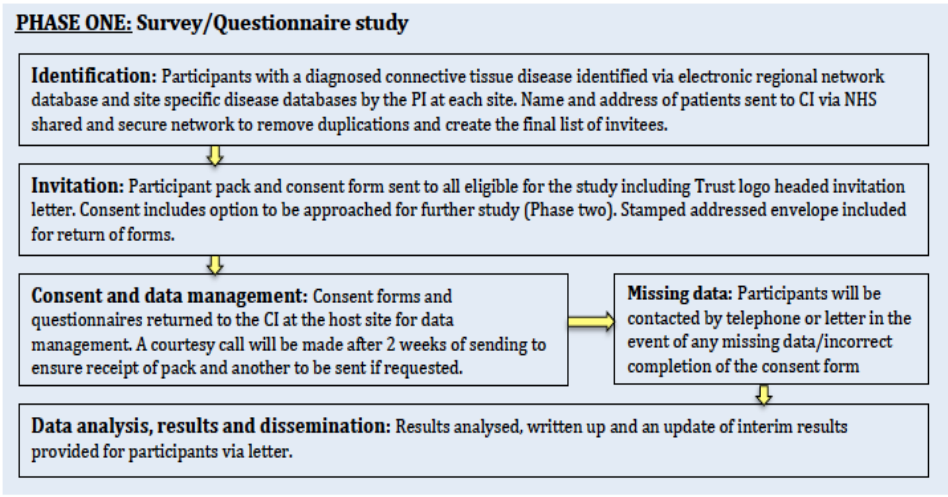


Figure 2: Phase One. Schematic methodology diagram.

2.2.3 Participant identification and recruitment

Ten hospital-based rheumatology centres in the Yorkshire and Humber region were approached for participation and at the proposal stage of the study, all ten sites agreed to take part supporting the application for funding.

At the point of study start up, 6 sites in total were able to participate. The reasons for the 4 sites declining were due to lack of research support staff and information technology systems that were not designed to retrieve the relevant data to ensure accurate identification of eligible patients.

For those sites that took part, diagnostic read codes within the electronic patient records were used to identify those eligible. The patient names and addresses were retrieved for postal contact. Research nurses were able to approach any patients opportunistically during routine clinics for newly diagnosed patients or those that may have missed the postal contact due to diagnostic read codes not being assigned.

Eligibility criteria:

- Aged 18 years or above.
- Primary diagnosis of SSc, SLE or DM
- With or without subcutaneous calcinosis

Exclusion criteria

- Diagnosis of an inflammatory arthritis, such as RA
- Diagnosis of any other crystal arthropathy, such as gout
- Cross over conditions such as mixed connective disease or overlap diseases.

2.2.4 Data Collection

Research packs were given or sent to those fulfilling the eligibility criteria. The research pack included a hospital headed letter explaining the nature and reason for the approach, two consent forms - one for the patient and one to return with the questionnaires, an Calcinosis Information Sheet (see appendix 2) and a series of questionnaires to complete including: a

diagrammatic picture map of the hands, dorsal and palmer views, a full body image to document the presence of calcinosis elsewhere on the body, the Cochin Hand Function and Disability Score (CHFDS) and the Scleroderma Quality of Life Questionnaire (SScQol) and the Short Form-36 v2 (SF36).
The questionnaire booklet included an initial example page instructing the participants on how to complete the different types of questionnaire measures. A pre-paid addressed envelope was included for ease of returning the questionnaires to the study site.
Prior to ethical approval, all documents were reviewed by patient representatives including those with SSc and calcinosis. All patient representatives were recruited via the Leeds Biomedical Research Centre (LBRC) and had undergone specific participant representative training with ongoing support from the LBRC staff where required.

2.2.5 Data analysis plan

Data would be presented descriptively and analysed using SPSS software v.21. Continuous data where normally distributed, would be reported as means +1 standard variation, or if not normally distributed, they would be reported as a median with reference to the interquartile range. Categorical data would be presented as frequencies. A complete case analysis would be undertaken for missing data supported by the supervisory statistician. Regression analysis would assess associations. Throughout, an exploratory approach was planned, without formal hypothesis testing.

2.3 Results

2.3.1 Overview

From the 6 sites 1328 patents were approached by mail. Two hundred and six participants replied with consent and 199 were recruited to the study. This showed a response and recruitment rate of 15.5% and 15% respectively.
Seven participants were excluded as they did not fulfil the eligibility criteria. The reasons for non-eligibility were overlap of

conditions and reporting of inflammatory and crystalline conditions such as RA and Gout.

2.3.2 Descriptive data

The demographic data of the 206 respondents showed an overall mean age of 58 years with no significant difference in age between those reporting no calcinosis, those confirming calcinosis presence and those who were unsure. Only four participants had DM and reported no calcinosis. The total number of participants with SLE was 54 (10 with calcinosis, 27 without calcinosis and 17 unsure) and 140 with SSc (76 with calcinosis, 34 without calcinosis and 30 unsure). The cohort gender balance was 7.5:1 females:males.

Table 3 details the main demographics and the outcomes from the questionnaires expressed in means with standard deviations. The sequential box plots visually present the CHFDS and SSc-QoL scores (figures 3 and 4).

There is a significant difference in the CHFDS between those with and without calcinosis, those with calcinosis reporting higher function and disability scores (mean 26.51 (SD 23.52) versus 10.49 (SD 13.12)). The interquartile range (IQR) for those with calcinosis range from 7 to 41.25 and from 0 to 15 for those without.

Quality of life scores were similar between those with and without calcinosis with a mean score of 14.86 (SD 7.93, IQR 10.5 – 20.5) with calcinosis versus 11.63 (SD 8.55 IQR 4-20) without calcinosis.

On a Linkert scale of 0-10, where 0=no pain, and 10=worst pain imaginable, participants with no calcinosis reported zero hand or body pain. Participants with calcinosis reported a mean average hand pain score of 4.9 (SD 2.94) and a mean body pain score of 6.93 (SD 4.03).

Patient characteristics	level	No calcinosis	Calcinosis present	Unsure	p-value
Number		69	88	49	
Age (mean (SD))		55.32 (13.92)	59.69 (12.34)	61.54 (11.09)	0.021
Age. Group (%)	(27,50)	21 (30.4)	16 (18.2)	7 (14.6)	
	(50,60)	19 (27.5)	27 (30.7)	12 (25.0)	
	(60,70)	19 (27.5)	26 (29.5)	18 (37.5)	0.338
	(70,87)	10 (14.5)	19 (21.6)	11 (22.9)	
Gender %	Female	64 (92.8)	78 (89.7)	39 (79.6)	0.079
	Male	5 (7.2)	9 (10.3)	10 (20.4)	
Main diagnosis	DM	4 (5.8)	0 (0.0)	0 (0.0)	
	SLE	27 (39.1)	10 (11.5)	17 (34.7)	<0.001
	SSc	34 (49.3)	77 (87.4)	30 (61.2)	
	Excluded	4 (5.8)	1 (1.1)	2 (4.1)	
Questionnaire Outcomes: (mean (SD))	CHDS	10.49 (13.12)	26.51 (23.52)	21.32 (20.62)	<0.001
	Hand. Pain	0.00 (0.00)	4.87 (2.94)	2.76 (2.92)	<0.001
	Body. Pain	0.00 (0.00)	6.93 (4.03)	7.71 (4.51)	<0.001
	SScQoL	11.63(8.55)	14.86 (7.93)	15.98 (8.44)	0.020

Table 3: Phase One. Participant demographics and patient-reported impact scores

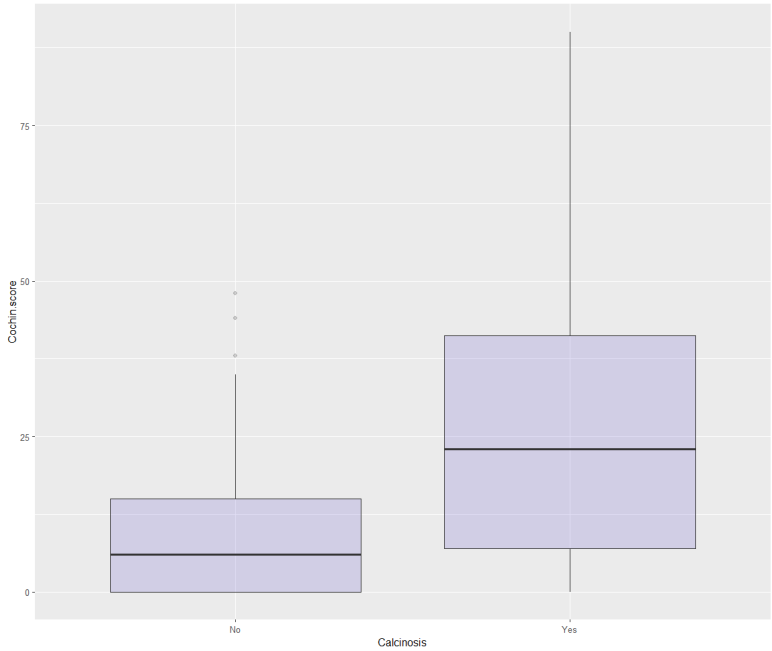


Figure 3: Phase One Box Plot. CHFDS outcome (without and with calcinosis)

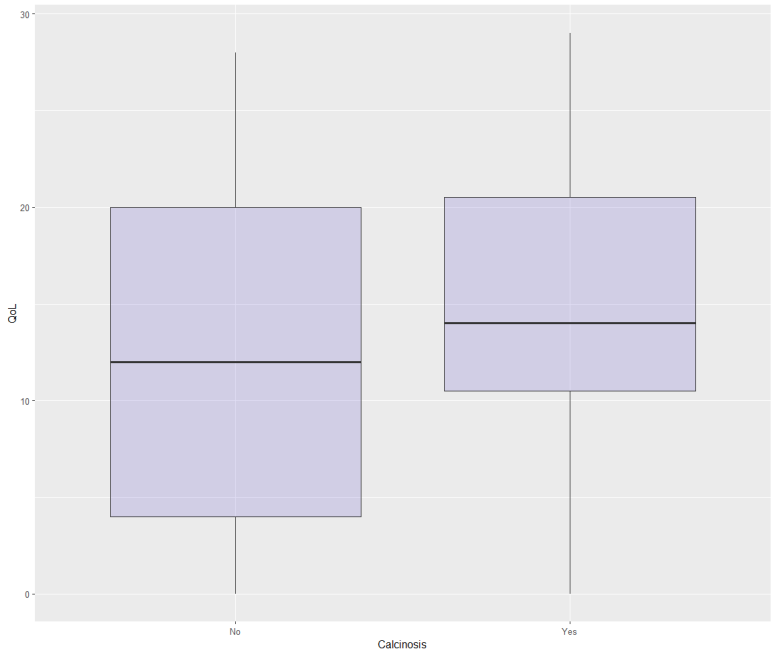


Figure 4: Phase One Box Plot. QoL outcome (without and with calcinosis)

2.3.3 Statistical analysis

To further analyse the odds ratio (OR) of which participants from the phase one cohort would most likely report calcinosis, a logistic regression analysis was performed for calcinosis (yes/no) on age, gender, and diagnosis. The unadjusted values are for the calcinosis outcome regressed only upon each single factor (age, gender, diagnosis) whereas the adjusted column is for OR's where all factors are entered into the logistic regression. Table 4 details the adjusted and unadjusted values which both show changes reflecting the correlation between the factors. Figure 5 shows the plot of adjusted OR's with confidence intervals.

Dependent: Calcinosis		OR (unadjusted)	OR (adjusted)
Age category	(27,50)	-	-
	(50,65)	1.66 (0.72-3.89, p=0.236)	1.24 (0.48-3.18, p=0.658)
	(65,83)	2.44 (1.02-6.00, p=0.047)	1.91 (0.72-5.14, p=0.194)
Gender	F	-	-
	M	3.06 (0.73-20.81, p=0.166)	3.56 (0.79-25.55, p=0.134)
Main Diagnosis	Lupus	-	-
	SSc	6.04 (2.70-14.42, p<0.001)	5.49 (2.39-13.49, p<0.001)

Table 4: Logistic regression odds ratio of calcinosis on age, gender, and diagnosis (adjusted and unadjusted)

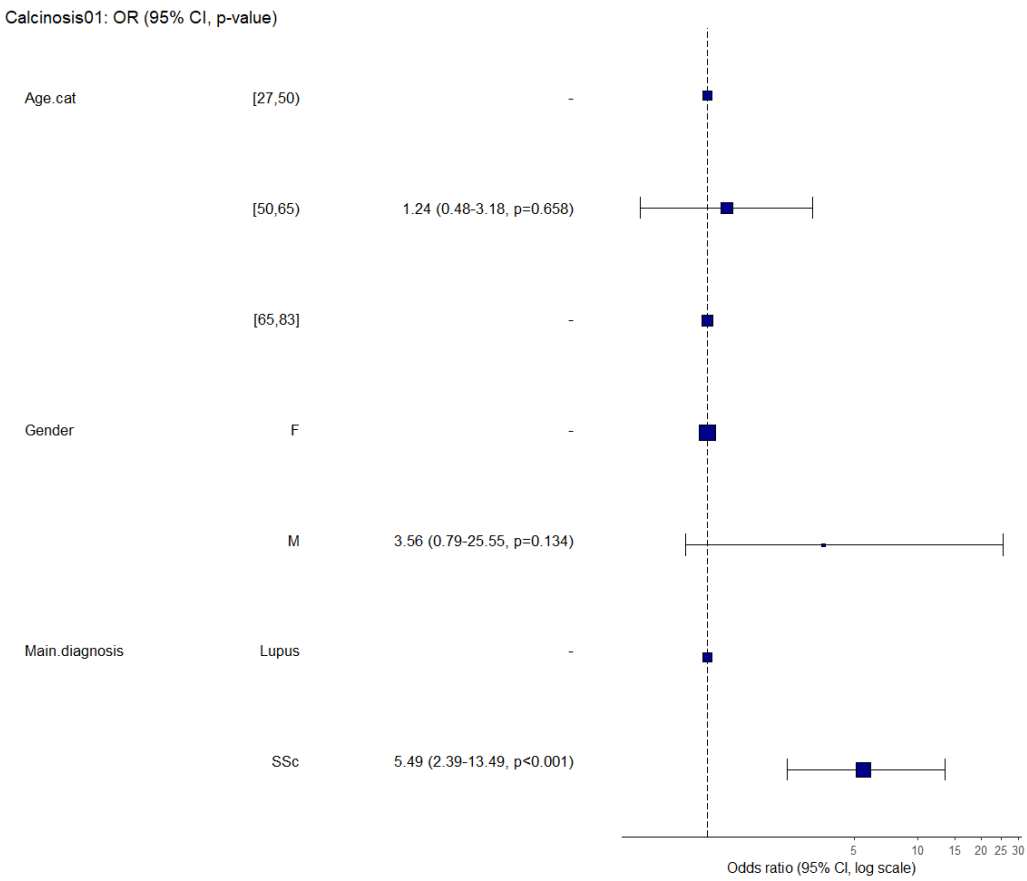


Figure 5: Phase One logistic regression odds ratio (95% CI, p-value)

The data suggests there is only a little evidence (p= >0.05) for calcinosis to be a greater risk in the age bracket of 50-65yrs, and for males, whereas there is strong evidence that calcinosis is a greater risk in those with a diagnosis of SSc (p= >0.01).
When looking at areas of the hands reportedly affected by calcinosis overall, the data shows a slightly higher percentage reported at the third finger (65.9%), with similar presence at the second finger (64.3%), reducing to the thumb (48.2%), the fourth finger (40%) and lastly the fifth finger (29.4%).

Digits affected	**Overall n= (%)**
Number	86
Thumb	41 (48.2)
2nd finger	54 (64.3)
3rd finger	56 (65.9)
4th finger	34 (40.0)
5th finger	25 (29.4)

Table 5: Phase One. Number and percentage of each digit reportedly affected by calcinosis.

Figure 6 highlights the reported prevalence of areas affected by calcinosis after stratifying by regions of the distal phalanx (DP), intermediate phalanx (IP), and proximal phalanx (PP). There is higher reported presence at the DP only compared to the other regions (17% versus 2-3%) and the digit with the highest reported DP presence of calcinosis is the 3rd finger (80%), followed by the second finger (50%) and thumb (30%).

Digit affected	DP only n=(%)	IP only n=(%)	PP only n=(%)
Number	20 (17.2)	4 (3.4)	3 (2.6)
Thumb	6 (30)	-	0 (0)
2nd finger	10 (50)	3 (75)	0 (0)
3rd finger	16 (80)	1 (25)	1 (33.3)
4th finger	2 (10)	1 (25)	2 (66.7)
5th finger	2 (10)	1 (25)	1 (33.3)

Figure 6: Phase One. Reported prevalence of calcinosis at the hands stratified by regions of the digits.

In this cohort, almost a third of participants reported that at least one whole digit of at least one hand to be affected by calcinosis (n=33), which included collectively the DP, IP and PP regions. The percentages are more evenly distributed across all fingers with the highest reported prevalence at the 2nd finger (90.9%).

Digit affected	DP, IP and PP n= (%)
Number	33 (28.4)
Thumb	26 (78.8)
2nd finger	30 (90.9)
3rd finger	25 (75.8)
4th finger	24 (72.7)
5th finger	18 (54.5)

Figure 7: Phase One. Reported whole digit prevalence of calcinosis at the hands.

Table 6 below shows the remaining patterns of presence when stratifying between DP, IP and PP regions per digit. Only 7% reported the calcinosis to be neither at the DP, IP or PP, therefore elsewhere on the hand. Similarly, only 7% or under of participants reported other pattern combinations of regions affected.

Digit affected	**DP and IP only**	**DP and PP only**	**IP and PP only**	**Neither DP, IP or PP**
n=	4 (3.44)	8 (6.88)	4 (3.44)	8 (6.88)
Thumb	0 (0)	6 (75)	0 (0)	0 (0)
2nd finger	4 (100)	4 (50)	3 (75)	0 (0)
3rd finger	4 (100)	6 (75)	3 (75)	0 (0)
4th finger	2 (50)	1 (12.5)	2 (50)	0 (0)
5th finger	2 (50)	0 (0)	1 (25)	0 (0)

Table 6: Phase One. Regions of the hands reportedly affected by calcinosis stratified by the distal phalangeal (DP), inter-phalangeal (IP) and proximal phalangeal (PP) regions

To see which regions affected by calcinosis impacted the Cochin and Qol score, modelling was performed on only the group reporting calcinosis. The results of the best fit final model, after dropping non-significant terms was as follows:

Cochin HADS = score of 14.9 +10.5 (for the 5th finger) and +15.6 (for the IP region of any finger)

QoL = score of 10.9 +5.0 (for the 4th finger) and +4.1 (for the IP region of any finger)

Both the cochin and QoL scores appear to be impacted most by the presence of calcinosis at the lesser fingers and region of the IP.

2.4 Discussion

Overall, phase one of this study highlighted that the quality of life of patients is worse and the hand function and disability is significantly worse in patients with calcinosis compared to those without calcinosis. It also highlighted that people with SSc are

significantly more likely to report the presence of calcinosis than those with SLE and DM.

The female to male ratio was expected given increased prevalence of females affected within the connective tissue diseases studied. The higher odds ratio for males reporting calcinosis may be reflective of the reduced number of males in the sample size and the likelihood of patients being more likely to respond to a questionnaire that has relevance to them, in this case the presence of calcinosis.

Overall, the study relied upon several factors, the patient identification was based on read codes from patient electronic records. This relied upon read codes being entered, entered correctly, and retrieved by clinical research staff at each site. Due to this reliance and the reduced number of sites being able to participate as planned, the prevalence is not fully representative of the Yorkshire and Humber region and the data is not suitable for further epidemiology analysis.

A review of response rates from patient populations across 321 surveys show an average response rate of 60% (SD 21%) [356]. The response rate in this study was therefore low at 15.5% and could have been improved.

Response rate to postal surveys from patents with RA have been shown to be 49% when a follow-up letter has been included, increasing to 56% response rate with a follow-up telephone call [357]. The response rate of mail alone was not measured however the response to telephone only contact rose to 64%. However, data collected this way is time exhaustive and has the potential to risk observer bias.

Postal survey response rates in dermatology conditions show much higher response rates of 74.1% but included a personalised cover letter with a $1 bill attached as a financial incentive and a final reminder and thankyou note in place of a phone call or reminder letter. A ‘more forceful’ third and fourth mailing was also actioned for all non-responders [358].

The methodology plan for this study included a follow up telephone call to non-responders, however the research support at sites was not available for this to occur.

According to a systematic review detailing factors that increase response rates to postal questionnaires, phase one fulfilled the

following key approaches [359]: the inclusion of a cover letter from the hospital detailing the university sponsoring the study, the use of coloured ink and an enclosed return stamped addressed envelope. Approaches that could have negatively affected the response rate for this study are that there was no monetary incentive, and the questionnaires were not sent recorded delivery or by first class post. However, the rationale for no monetary incentive and use of second class post related to the cost and funding available.

The questionnaire was potentially considered too long and of a sensitive nature which can also negatively affect the response rate, however, if the subject matter is of interest or importance to the patient, they are more likely to respond. With that in mind, there were more SSc patients who responded and more with reported calcinosis. Given the reported prevalence of calcinosis in SSc is up to 40%, 69% reporting SSc and calcinosis in this study likely highlights an increased interest of these participants to participate due to the measured burden calcinosis creates and the desire to report this important impact.

This is reinforced by examining the regions effected with calcinosis and the most prevalent areas reported as affected mirror the literature with a predominance for the thumb, second and third finger and at the region of the DP [290, 360]. However, this study identified the descending trend in the opposite direction starting with the middle finger as opposed thumb. In addition, it appears that those with calcinosis are more severely affected overall with a third reporting that at least one whole digit of at least one hand to be affected by calcinosis (n=33). The percentages are more evenly distributed across all fingers which also suggests that when one full digit is affected, it is likely that other full digits could be affected too.

Interestingly, the CHFDS and QoL scores were most impacted by the presence of calcinosis at the fourth and fifth fingers or at the region of the IP. This is contrary to the assumption that calcinosis presenting at areas of the thumb, second and third fingers and at the region of the DP would have most impact due to the predominant use of these areas for everyday tasks. However, this may instead suggest that when the regions of less prevalence are affected, the overall prevalence severity is higher

across all regions, which would explain the greatest impact on outcomes.

Surprisingly, all participants without calcinosis reported 0 for pain at the hands and body. It would be expected that there would be some reported pain both at the hands and generalised throughout the body as it is common in SLE and SSc regardless of calcinosis presence [279, 361, 362]. This finding may represent a cohort of very mild disease in the participants without calcinosis or it could be considered that the participants chose to answer this question in relation to pain from calcinosis only. We would then surmise that the calcinosis group answered the same way and interestingly, reported more body pain (presumably from calcinosis alone) than hand pain. This identifies that although calcinosis may be more prevalent at the hands, calcinosis elsewhere on the body is more painful. Differences in the characteristics of the deposits may offer the information to explain this trend.

There was a difference in the QoL outcomes with the calcinosis group demonstrating a stronger effect on reduced QoL, although this was not of high significance. This is comparing QoL with a group of combined diseases with the opportunity for QoL to be influenced by different disease characteristics.

One study has identified a significant difference in the Health Assessment Questionnaire disability index of the Scleroderma Health Assessment Questionnaire (SHAQ) in patients with SSc and calcinosis and also higher CHFDS measures, but after multivariant analysis were not statistically significant [150].

A QoL measure specific to calcinosis, such as the Maudsley Calcinosis Questionnaire would be best placed to measure this impact specifically, although this is not yet a validated measure [363].

The Short-Form 36 was used in this study to be applicable to other conditions in this study, however we encountered issues with licencing and time restrictions to analyse, so details have not been included.

For DM, the response rate was very low with no reported calcinosis despite calcinosis presenting in 30% of DM cases and usually more severely. This suggests that the methodology did not adequately target this patient group. It is most likely that DM

patients, especially those with skin complications such as calcinosis are managed predominantly in dermatology versus rheumatology departments. The study could have been improved to target these departments in addition and future studies comparing these conditions should consider the cross management of teams.

The percentage of patients with SLE and calcinosis is 40% according to the literature and only 11% in this cohort reported calcinosis but this may reflect the silent nature in this disease, usually detected only by radiology [219].

A limitation of the study was that the presence of calcinosis was based on patient-reported confirmation.

There were approximately a third (35%) of participants with SLE who were unsure if they had calcinosis and almost two thirds (62%) with SSc who were unsure. In this study, if calcinosis was confirmed, the sample size for those with SSc and calcinosis would have been considerably higher than those with SLE. This highlights the potential for undiagnosed cases of calcinosis and a possible higher prevalence that previously reported. Despite including a Calcinosis Patient Information Sheet detailing the presentation of calcinosis, the number of participants entering ‘unsure’ for having calcinosis, suggests that participants may be presenting with skin changes and/or symptoms resembling calcinosis, possibly at the early stages of calcinosis formation before diagnosis. It may also highlight a range of other clinical conditions that present in a similar way, posing a challenge for the accurate clinical diagnosis of calcinosis and a possible risk of misdiagnosis.

The potential for this should be considered within these results as the calcinosis group may not be a true representation of confirmed calcinosis but instead reflect the patient’s perceived presence of calcinosis.

This uncertainly highlights a need for further patient education, definition, and the need of an easily accessible, non-invasive diagnostic tool to confirm the presence of calcinosis.

2.5 Summary and conclusion

In summary, this phase of study highlighted that calcinosis is a significant manifestation of SSc with a significantly high impact on hand function and disability scores.

Despite other conditions such as SLE having similar prevalence to SSc of up 40% when detected via radiograph, the patient reported presence is much higher in SSc demonstrating a higher severity and symptomatic effect of calcinosis in SSc.

Interestingly, phase one demonstrated for the first time that if regions of the IP or fourth and fifth fingers are affected, then the overall severity of calcinosis is possibly worse and will have higher impact on hand function and disability and QoL measures.

It also reported for the first-time that calcinosis outside of the hands is potentially more painful and warrants further investigation of this and the clinical characteristics of these lesions.

Most importantly, this phase along with the literature review highlights the potential of sub-clinical and undiagnosed presence of calcinosis and the need for a non-invasive assessment tool to confirm the presence of calcinosis. This is especially significant in the case of SSc, where subclinical or early calcinosis is more likely to worsen in size and severity. It progresses to allow a clinical diagnosis, but unfortunately, this is usually at a size when invasive surgical intervention becomes necessary, with associated potential complications and without a guarantee of resolution.

This phase of the study highlighted and supported the need to define the presence of calcinosis as the most important outcome to confirm the patient-reported presence and uncertainty of diagnosis, whether established or in early formation.

This led to the first stages of using ultrasound to begin building the framework for ultrasound as an outcome measure for calcinosis as detailed in Chapter 3.

Chapter 3

Phase Two: Delphi Agreement-Expert consensus on defining calcinosis using Ultrasound.

3.1 Introduction

The popular Delphi Method in healthcare is an iterative process designed to combine the pooled knowledge of experts to reach a consensus agreement on a particular problem to guide decision making and ultimately improve health outcomes [364-367].

The power of the Delphi approach is exemplified in the literature review for the classification of SSc with improved sensitivity and accuracy of classification and validation of predictive values of disease [50-52]. This has resulted in an increase in prevalence and incidence data from improved and early diagnosis and a reduction in disease and mortality rates because of early intervention [2, 3].

The Delphi method is commonly used for confirming appropriateness of measurement tools or instruments and has been used to validate ultrasound as an outcome measure of musculoskeletal disease in rheumatology, including crystalline conditions similar to calcinosis such as gout and calcium pyrophosphate deposition [350, 354, 368].

This has led to early detection and clinical recommendations for these condition, using ultrasound as a non-invasive and effective diagnostic tool and outcome measure [160, 161, 351].

Calcinosis can be visualised using ultrasound however, a formal definition to confirm the presence of calcinosis using ultrasound does not exist. On this basis, the initial step to confirm the presence of calcinosis for the explorative stage of objective three was required via an agreed consensus for the working definition of calcinosis using ultrasound. This would form the basis of future research with the view to validate ultrasound as an outcome measure for calcinosis.

3.2 Methodology

Building on the methodology undertaken to validate ultrasound for the diagnosis of gout and other crystal arthropathies by the Outcome Measures in Rheumatology OMERACT working group [350, 353, 354], an expert Delphi group exercise using the OMERACT stepwise approach was conducted [368].
Three components are identified in the Delphi method [369]:

1) The issue: a question that cannot be solved with other research methods and required knowledge and opinion of experts.
2) A co-ordinating team: responsible for the design and co-ordination of the process usually 2-5 people.
3) The expert panel: expertise should be appropriate for the question, have capacity to participate for a potential several rounds and the group number should range between 7-30.

At the time of the study, there had been no reports of a formal definition of calcinosis using ultrasound despite it being clinically acknowledged and observed. Therefore, agreement from expert opinion and knowledge was required.
The co-ordinating team comprised of the author with support from two rheumatology experts well published in the Delphi agreement methodology who supported the process and identification of the appropriate expert panel.
Experts from the OMERACT working group were approached initially by one of the rheumatologists from the co-ordinating team to inform them of a pending request for support, and to obtain verbal consent to be approached for participation. Contact details were obtained for 126 experts and the thesis author led the group invitation via email, detailing the purpose and requirements of participation. From the 126 experts, 63 agreed in principle to participate. From these, 32 confirmed they had expertise in performing and reporting on ultrasound evaluations of crystalline deposits, with experience of clinical assessment and understanding of SSc. This final group most eligible for the

study purpose proceeded with Round 1 of the Delphi exercise with the primary objective to agree upon a working definition of calcinosis using US.

First, the proposed definition was created by the thesis author based on the descriptive features observed from calcinosis in 10 routine hand scans obtained for clinical purposes. These were discussed with two experts of the coordinating group who had experience in defining crystalline conditions.

The proposed definition included the appearance of the deposit(s), the border appearance, the size, the shape, the depth of tissue and the structures affected. The ultrasound features including the echogenicity of the deposit, the presence of an acoustic shadow, Power Doppler, and the appearance of the surrounding tissue.

Figure 8 shows some examples of ultrasound images with and without calcinosis. The first image [A] shows a normal B mode US appearance of the DP and IP at the volar aspect of a finger. The second and third images [B,C] show the same region but in a finger with varying amounts of single and multiple hyperechoic areas, with smooth and irregular borders, with and without acoustic shadow, with or without associated Power Doppler and some involvement of periarticular structures. The last image [D] shows calcinosis presenting as a single deposit with a smooth hyperechoic border, involvement of tendon and a hypoechoic centre with particulate material/hyperechoic foci within which was observed as compressible material during US assessment.

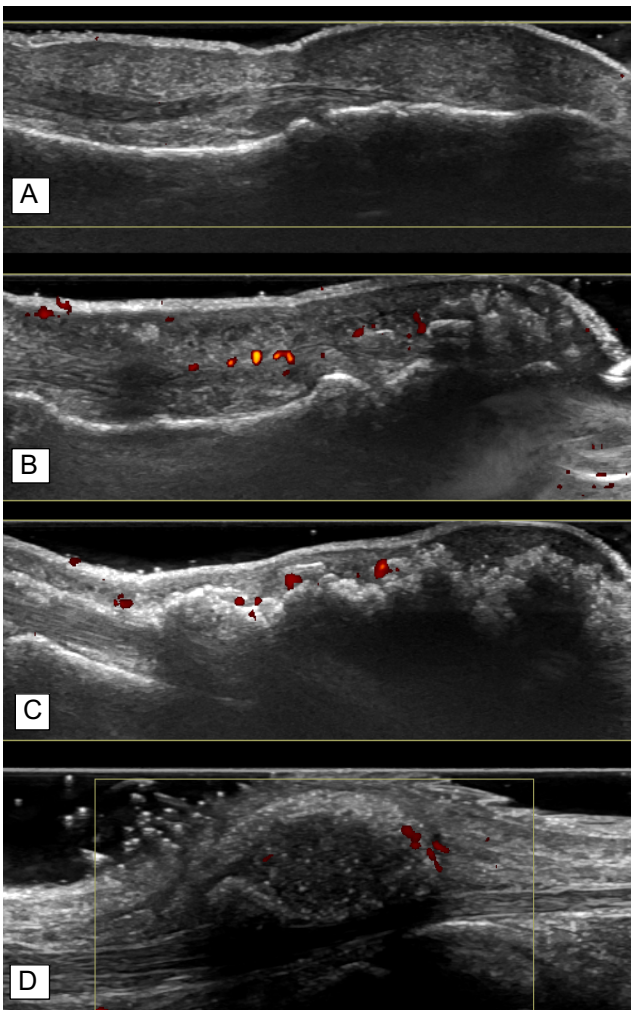


Figure 8 Examples of ultrasound images without and with calcinosis in various forms.

The initial definition proposed by the author of the thesis in consultation with the co-ordinating team was described as follows:

"Calcinosis can present as either single or multiple deposits with a smooth or irregular hyperechoic border varying in shape, size, and depth of tissue with or without an acoustic shadow and with or without Doppler signal. The deposits can be isolated within the subcutaneous tissue or involve articular and periarticular structures. Surrounding tissue may exhibit a normal structure or show compressible hypoechoic areas with/without particulate material/hyperechoic foci"

The author of this thesis continued the lead in the conduct of this study, designing the online survey and communicating with the panel of experts. A link to the online survey was sent to the expert panel via email with a 3-week deadline to complete.

The survey was carefully designed to balance simplicity with depth, ensuring it did not overwhelm the experts while still providing meaningful, unbiased insights. The survey length was intentionally kept short and developed using a professional institutional survey platform, which supported accessibility across various devices for ease of use. Once completed, the survey was piloted by the author and the coordinating team. The wording of the introduction, questions, and closing statements was thoroughly reviewed and revised by the team to eliminate ambiguity and bias. Adjustments were made as needed, and all settings were configured to prevent missing data from skipped responses.

Given the voluntary nature of the Delphi process and the fact that the experts involved were busy professionals, it was standard practice to maintain regular communication and use empowering language to emphasise the significance of their contributions to the study. Consequently, deadlines were extended, and communication with individuals was initiated to provide adequate support and ensure full group participation. Providing feedback on the results after each round of the iterative process was essential to sustain their engagement and ensure continued support for subsequent rounds.

The author was responsible for the wording and sending of these reminder and update emails. The author was also responsible for collecting the data and conducting the initial analysis of results for the co-ordinating team to review.

Survey One presented the proposed definition of calcinosis using ultrasound and asked the expert panel if they agreed with the statement (yes/no). They were then asked if they agreed that other tissue types can be affected by calcinosis in SSc (yes/no). As standard for the OMERACT stepwise approach, consensus is reached when the definition achieves 75% or more agreement of scores. Open ended questions with a free text answer option explored which tissue types are affected by calcinosis and which US features are considered important in the assessment and

reporting of calcinosis using ultrasound. Free text option was available for any further comments and considerations to the proposed definition, the questions presented or the study in general.

3.3 Results

3.3.1 Round One: Delphi Agreement on the proposed definition, tissue types affected, and the ultrasound features associated with the presence of calcinosis

Twenty eight of the 32 experts completed the first round of the Delphi resulting in good agreement (75%) and confirmed consensus for the content of the proposed definition. Despite the confirmed consensus, the definition was advised to be split into two following feedback from Round One: 1) a definition to confirm the presence of calcinosis and 2) an operational description of ultrasound detection:

1) *Calcinosis can present as either single or multiple deposits with a smooth or irregular hyperechoic border varying in shape, size, and depth of tissue with or without an acoustic shadow and with or without Doppler signal.*

2) *The deposits can be isolated within the subcutaneous tissue or involve articular and periarticular structures. Surrounding tissue may exhibit a normal structure or show compressible hypoechoic areas with/without particulate material/hyperechoic foci.*

Free text feedback from Round One on the tissue types and the ultrasound appearances that should be evaluated in US assessment of calcinosis were grouped into common components. The author was responsible to collecting these free text responses and they were grouped into the following themes:

- Skin: *hyperechoic or fibrotic (hyperechoic) increased dermal thickness, changes in elastography, skin inflammation (increased Doppler signal), dermal fistula, tethered skin, skin*

ulceration, presence of 'soft' calcinosis appearing like abscesses (egg shaped, mild hyper-echoic with more hyperechoic spots inside, vascularised).

- Blood flow: *Ulnar artery occlusion, finger pulp blood flow, digital artery vasculopathy.*
- Inflammation: *signs of inflammation due to depositions, hypertrophy of surrounding tissue, power doppler signal, inflammation of skin.*
- Tendon: *tenosynovitis, tendonitis*
- Bone/joint: *synovitis, acro-osteolysis, arthritis*

The grouping of the descriptions was agreed upon with the co-ordinating team. The components and their importance in the assessment and reporting of calcinosis was detailed in Round Two for consensus/ratification by the expert panel.

3.3.2 Round Two: Delphi agreement on the revised definition and item generation for proposed components associated with calcinosis for assessment and reporting purposes

In Round Two, 24 of 27 experts agreed on the revised split definition to confirm the presence of calcinosis. This resulted in excellent agreement of >80% for both the definition to confirm presence of calcinosis using ultrasound (1) and for the operational description of ultrasound detection for calcinosis (2).

Regarding the components to be evaluated on US assessment of calcinosis, the presence of inflammation, and the relationship of the lesions with the skin, tendons, and bone/joints achieved expert agreement of at least 66%.

A stronger agreement was attributed to assessment of the skin (74.1%) almost but not quite reaching consensus. The most striking agreement and consensus, however, was the relevance to report the presence of inflammation (92.6%), attributed to hypertrophy of surrounding tissue, power doppler signal and inflammation of skin surrounding the deposits.

Table 7 summarises the results of both Round One and Round Two.

Items	Agreement n (%)
Round 1	**n = 28**
Do you agree that this statement is comprehensive and clear enough to define calcinosis in SSc?	21 (75%)
Do you believe that different tissue types can be affected with calcinosis in SSc?	28 (100%)
Round 2	**n = 27**
Do you agree that that the first section of the definition would be appropriate to confirm the presence of calcinosis?	24 (88.9%)
Do you agree that the second half appropriately describes the operational description of calcinosis?	22 (81.5%)
Please select the following categories localized to calcinosis that you believe are important to assess and describe in the overall US report (descriptions taken from round 1)	
SKIN: *(Hypoechoic) or fibrotic (hyperechoic) increased dermal thickness, changes in elastography, skin inflammation (increased doppler signal), dermal fistula, tethered skin, skin ulceration, presence of 'soft' calcinosis appearing similar to abscesses (egg-shaped, mild hyperechoic with more hyperechoic spots inside, vascularized)*	20 (74.1%)
BLOOD FLOW: *Ulnar artery occlusion, finger pulp blood flow, digital artery vasculopathy*	9 (33.3%)
INFLAMMATION: *Signs of inflammation due to the depositions: hypertrophy of surrounding tissue, power doppler signal. Inflammation of skin*	25 (92.6%)
TENDONS: *Tenosynovitis, tendonitis*	18 (66.7%)
BONE/JOINT: *Synovitis, acro-osteolysis, arthritis*	18 (66.7%)
None of the above	1 (3.7%)

Table 7: Phase Two. Items and agreement of Delphi Rounds 1 and 2 for US definition of calcinosis

Of the few experts who did not agree with the revised definition, individual reasoning was obtained either from the free-text components of the survey or after additional contact via email for further clarification. Collecting these responses highlighted minor wording requests without major alteration to the contextual information. These edits were honoured after subsequent full agreement from the expert panel.
This resulted in the following final proposed definition of calcinosis in SSc using ultrasound:

“Calcinotic deposits present as distinct collections of mixed echogenic material usually surrounded by a clearly defined hyperechoic border. Deposits may vary in size, shape and depth of tissue with or without an acoustic shadow and with or without Doppler signal associated with the deposit.”

3.4 Discussion

The inclusion of the experts from the OMERACT group was appropriate given the vast expertise in using ultrasound for outcome measures in rheumatology. Narrowing the group of experts down to those experienced in using ultrasound to assess crystalline conditions with clinical experience in SSc focussed the expertise further but still provided a large group of 28 participants for round one and retaining 27 participants for round two. The level of experience was not detailed or measured but relied upon confirmation of the expert.

The methodology followed the iterative process of the Delphi design until adequate consensus was reached and went further to strengthen this agreed consensus from 75% to 89% for the working definition.

This provided the definition for the initial steps required to compare the prevalence of US reported calcinosis with calcinosis observed via clinical examination, reported by participants, and as confirmed via x-ray in phase three of this study.

The future steps require continuation of the Delphi agreement to validate the definition with the group of experts and from this data, develop a severity score of calcinosis using US.

As highlighted in the narrative of chapter one, ultrasound has the added benefit of providing assessment of individual lesion microstructure, surrounding soft-tissue changes, and vascularity, and may provide important supplemental information related to severity, associated symptoms and potential predictive features of progression. Exploration of the ultrasound features associated with expert-reported severity as compared to PROMs will give insight into the US features that impact severity most.

The accessibility and non-invasive quality of ultrasound provides an ideal imaging tool to be incorporated in the routine clinical assessment and management of calcinosis once a validated definition and severity measure has been developed.

3.5 Summary and conclusion

In summary, the first two rounds of the Delphi process produced the first strong consensus-driven working definition to confirm the presence of calcinosis using US. Additionally, the process identified ultrasound features that are important for calcinosis assessment and reporting and may be associated with calcinosis severity. Ultrasound has the potential to increase our understanding of the pathophysiology of calcinosis, aid in early detection and diagnosis, inform management strategies, and provide an outcome measure for clinical trials.

The definition agreed from this phase of study was used to confirm the presence of calcinosis in SSc using ultrasound for the next explorative phase of the study.

This is the first preliminary step before future validation of the definition and development of an US-based scoring system for calcinosis for use as an effective and non-invasive imaging tool for clinical trials and routine clinical monitoring.

Chapter 4

Phase Three: Case-control and within-patient control study observing the clinical characteristics of calcinosis.

4.1 Introduction

This objective was to measure the patient reported impact of calcinosis and describe the SSc disease features associated with calcinosis presence. The clinical characteristics of calcinosis would be detailed and presented with participant-reported outcomes. The focus of this study at the hands was reflective of a higher prevalence of calcinosis at the hands according to the literature, and to allow a comparison with the current validated x-ray severity scoring system for calcinosis at the hands. The importance of documenting the clinical characteristics and associated impact of participant-reported calcinosis elsewhere on the body was directed from the phase one results.

4.2: Methodology

4.2.1 Participant identification and recruitment

Potential participants were identified from one hospital site from an existing longitudinal SSc observational study named CONVAS (Connective Tissue Disease and Vasculitis Cohort Cross-sectional and Longitudinal Clinical & Basic Science Evaluation). Participants were approached via telephone if already consented for approach to additional studies and/or via clinical contact at their usual hospital appointment.
Participants had to fulfil the following criteria:

Inclusion criteria

- aged 18 years or more with a consultant diagnosis of SSc.
- with or without a diagnosis of calcinosis
- able and willing to give informed written consent.

Exclusion criteria

- Patients with other CTDs as their primary condition, such as dermatomyositis, Systemic Lupus Erythematous, rheumatoid arthritis or any undifferentiated/mixed CTD
- Participants unable or unwilling to give informed written consent.
- Clinical assessments are contraindicated or not consented by the patient.

All protocol assessments were non-invasive except for the x-ray at the hands. However, in regards to radiation exposure it is considered very minimal (0.004mSv) [370] and was undertaken as part of the subjects usual clinical care.
A Patient Information Sheet (PIS) was given detailing the study information explained verbally. Patients were able to take this home to consider further.
Participants were recruited via methods of convenience sampling and new patients were approached until the sample size was reached or when no further eligible patients were available to recruit at the study site.
Participants were withdrawn if they declined consent or participation, or if the assessments were contraindicated due to safety reasons.

4.2.2 Initial data Collection and group allocation

Due to the time required to collect data and allowing an opportunity to fully read and consider the study information, patients were scheduled to return to the hospital for a single 1-hour study visit. Witten informed consent was obtained at this appointment prior to any data collection.
All participants undertook a posteroanterior x-ray view of the hands as part of their usual clinical care prior to study involvement. Cases were defined with calcinosis from confirmation of an independent radiographer reporting the x-ray. The observer responsible for US and clinical data collection at the study visit was blinded to the x-ray images. Group allocation was only fully confirmed to the observer after data collection and verification of the x-ray report. Participants were allocated to the

case group if calcinosis was present at the hands and were allocated to the control in the absence of calcinosis at the hands, or elsewhere on the body.

The cases were matched to controls by age (+/- 3 years), gender and disease type. Matching between groups was checked halfway through the recruitment process to ensure appropriate matching overall between the groups and to highlight any differences that needed to be prioritised.

4.2.3 Clinical systemic features and general demographics

Clinical systemic features were collected to re-confirm eligibility and included the subset of SSc diagnosed, i.e. lcSSc or dcSSc. A list of medication was documented, and reports of the most recent blood serum markers as collected routinely for clinical assessment.

SSc-related internal organ involvement was confirmed via participant medical records and included: Scleroderma Renal Crisis, Cardiac disease (including pericarditis, myocarditis, conduction system abnormalities), Interstitial lung disease, Pulmonary arterial hypertension, Pulmonary Fibrosis, and any GI involvement (gastroesophageal reflux (GERD), Esophageal or small bowel dysmotility, small bowel bacterial overgrowth, Malabsorption syndrome or use of parenteral nutrition, Gastric antral vascular ectasia).

General demographics collected for each patient included gender, age, and ethnicity.

4.2.4 Function and disability health assessment questionnaires

The following questionnaires were completed for objective three: The Cochin Hand Function and Disability score (CHFDS), the SSc Health Assessment Questionnaire (SHAQ) and the Scleroderma Quality of Life (SScQol) questionnaire. In addition, the case group completed the Maudsley Calcinosis Questionnaire (MCQ) after group confirmation.

Handedness was recorded to detail which hand would likely be subject to most direct trauma from everyday tasks as it has been suggested to have links with the formation and impact of calcinosis [150, 283].

4.2.5 Assessment of vasculopathy

General

General vasculopathy related observations were recorded and included date of first RP and non-RP symptom with reference to the disease duration of SSc from these symptoms. The presence of telangiectasia, smoking habit and BMI was recorded. Participants confirmed refraining from alcohol and/or caffeine consumption for at least 24hrs prior to the assessment.

Nail fold video-capillaroscopy

Nailfold video-capillaroscopy (NVC) is a simple, non-invasive tool to assess the density and structure of the capillaries at the nail-fold. It has been used to assess for significant micro-circulation changes in systemic disease [371], predominantly with in the fields of rheumatology and dermatology [372-375] and more recently with conditions such as diabetes [376].
It is well documented that NVC is a useful tool to differentiate between primary RP and secondary RP in connective tissue disease, particularly in SSc.
Most notably, is the work undertaken in systemic sclerosis and the further identification of patterns relative to the stage of the condition [377].
Appropriate standardisation was followed as detailed in a more recent consensus report by Smith et al, 2020, utilising the gold-standard 200x magnification video-capillaroscopy assessment tool and appropriate contact oil [378]. The gold standard NVC technique was employed for data collection and analysis, capturing at least two 1mm adjacent images in the middle of the nailfold and assessing both qualitatively.
Qualitative assessment provided confirmed presence of an overall ‘scleroderma pattern’ representing the severity of small vessel vasculopathy and described using the patterns of early, active or late SSc NVC patterns.

4.2.6 Assessment of fibrosis

Modified Rodnan Skin Score

The mRSS is a fully validated measure of skin thickness in SSc. All subjects had all 17 skin sites evaluated which included regions of the face, upper arms, forearms, dorsum of the hands, fingers, chest, abdomen, thighs, lower leg, and feet with a maximum score of 51 graded from 0 – normal skin, 1 – thickened skin 2, thickened and unable to pinch and 3- thickened and unable to move. The mRSS is a subjective assessment with interobserver reliability and within-patient standard deviation scores largely affected only by lack of appropriate and repeat training. [379] Structured in-house training led by two experienced Rheumatology consultants was undertaken which required agreement between raters for a minimum of 10 full body scores to ensure adequate skill of the author to perform skin assessment for the study.

4.2.7 Clinical features at the hands

Clinical features at the hands were recorded and included the presence of DU, pitting scars, puffy fingers, sclerodactyly and any loss of the digital pulp. The presence of other ulceration proximal to the PIPs and at the extensor surfaces of the digits were also recorded including the date of the first digital ulcer present. The definitions of each feature are described below as described in the ACR/EULAR classification of SSc.

Clinical feature	Definition
Digital ulcers	'*Denuded areas with a defined border, loss of epithelialization and loss of epidermis and dermis on the VOLAR aspect distal to PIPs'.*
Other digital ulcers	*'Denuded areas with a defined border, loss of epithelialization and loss of epidermis and dermis* proximal to the PIPs and at the extensor surfaces of digits'
Pitting scars	'*Depressed areas at digital tips as a result of ischemia, rather than trauma or exogenous causes'*

Puffy fingers	*'Swollen digits or a diffuse, usually nonpitting increase in soft tissue mass of the digits extending beyond the normal confines of the joint capsule. Normal digits are tapered distally with the tissues following the contours of the digital bone and joint structures. Swelling of the digits obliterates these contours. Not due to other causes such as inflammatory dactylitis'*
Sclerodactyly	'*Skin thickening distal to the metacarpophalangeal joints but proximal to the proximal interphalangeal joints'*

Table 8: Phase Three. Clinical feature definitions for DU, Pitting scars, Puffy fingers and Sclerodactyly

Neuropathy testing to assess patient sensitivity to light touch at the hands was assessed using the Von Frey monofilament technique with has shown validity and reproducibility as a quantitative sensory measure of mechanical hyperalgesia [380].

4.2.8 Clinical assessment of calcinosis

The presence of calcinosis at the hands was assessed clinically. Participants detailed presence of calcinosis at the hands by drawing/shading areas affected on the hands on a diagram as similarly represented in Figure 8.

The presence of calcinosis anywhere on the body was assessed clinically and with support from participant-reported history of symptoms. It was confirmed if there was a palpable mass with the clinical appearance and symptomatic history typical of calcinosis.

The date of first apparent presence of calcinosis was recorded and this would be compared to the demographic dates of first RP symptom, the first non-RP symptom and the diagnosis of SSc.

The body locations were divided into the following regions: Face and neck, trunk, hands, upper arms/forearms, elbows, thighs/lower leg, knees, and feet.

For each body region the characteristics of the calcinosis present were described. The consistency of calcinosis via palpation was described as either hard or soft, and if the actual calcinosis was breaking through the skin and able to palpate. Ulceration could

be present or absent in cases of the calcinosis breaking through the skin. This is due to some variants of presentation where anhidrotic stone-like calcinosis can protrude through the skin whilst the skin remains healed around the deposit sealing in the borders. Ulceration, by contrast, described an unhealed opening to the skin, weeping exudate and/or calcinosis and at risk of infection.

Signs of clinical inflammation was recorded if the following signs were present: redness, swelling and heat surrounding the deposit with possible associated loss of function depending on the location.

Clinically apparent infection was suspected and recorded if signs of inflammation mentioned above were more severe including increased pain, spreading erythema, increased viscosity of exudate, unusual colour of exudate, any related odour or if the participant had recently commenced antibiotic therapy ideally with some reduction in symptoms to confirm likely infection present.

The size of deposit was subjectively measured via palpation and described as either >1cm or <1cm.

Tenderness related to any reported tenderness of the tissue surrounding the deposit which could be present with or without clinical signs of inflammation.

A 0-10 Linkert scale participant-reported global assessment of calcinosis was recorded and worded as follows:

Overall, considering how much pain, discomfort, limitations in your daily life, and other changes in your body and life, how severe would you rate your calcinosis? 0= no symptoms/impact 10= Extremely active or severe calcinosis.

Similarly, a clinician-reported global assessment of calcinosis was scored for comparison. The rater scoring the global assessment of calcinosis was blinded to the participant score and any imaging, therefore, the clinician score was conducted based on clinical assessment only.

Where applicable, regions of interest (ROI's) were defined and chosen by the patient according to the 'most impactful' calcinosis deposit and the 'least impactful' deposit. A Visual Analogue Scale (VAS) score, ranging from 0-10 with 0 representing no pain and 10 representing worst pain imaginable was collected for

these ROIs. Data on the same calcinosis characteristics as mentioned above were recorded for each.

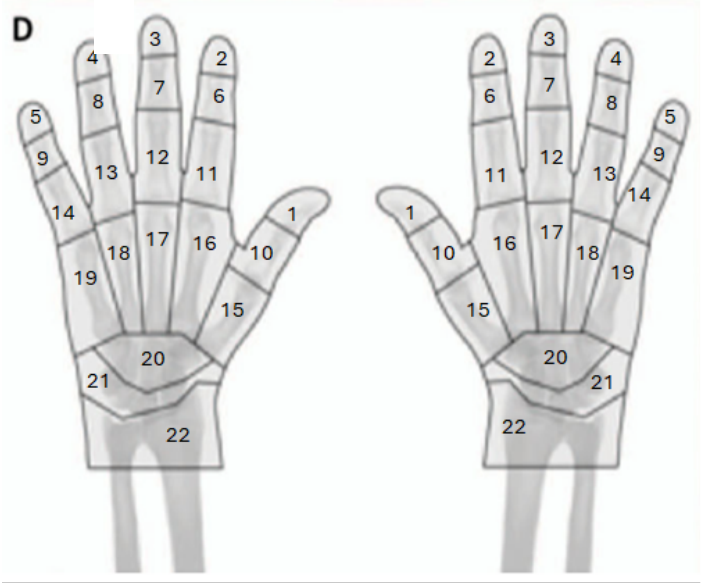


Figure 9: Phase Three. Diagrammatic representation of regions measured for calcinosis at the hands.

4.2.9 Data Analysis plan

Data would be presented descriptively and analysed using SPSS software v.21. Exploratory statistics would check assumptions of normality, homogeneity of variance for each variable and to confirm the most appropriate test for further analysis ie. parametric or non-parametric.

Assessment of normal distributions and outliners in the data would be undertaken. Continuous data where normally distributed, would be reported as means +1 standard variation, or if not normally distributed, they would be reported as a median with reference to the range and interquartile ranges.

Categorical data would be presented as frequencies.

To measure differences in variables between the participants with and without calcinosis, a t-test would be used for interval variables that met the assumptions of normality and homogeneity of variance. The Mann-Whitney test would be used for any interval variables that did not meet the assumptions of normality and homogeneity of variance. Categorical variables would be compared using the Pearson's Chi-squared test and *p*

values of <0.05 would be considered significant and values <0.001 would be considered highly significant.

To support statistical significance of data, a power calculation was undertaken and identified that a total of 60 subjects was required for each of the case-control group to allow for 6 other variables to be assessed once adjusted for gender, age and disease subset. Given the exploratory plan for phase three, the restriction of applying matched group methodology, the rarity of SSc as a condition and the manifestation of calcinosis being at most 40% of this condition, it was agreed reasonable to obtain at approximately 40 subjects per group as was also reflective of the average number of subjects accepted for study publication at the time.

4.4 Results

4.4.1 Overview

A total of 107 participants were recruited. A total of 11 participants were withdrawn from the study for the following reasons: death prior to data collection (1), too poorly (6), recent close bereavement (2), too busy (1), work and childcare commitments (1). A further 11 patients were lost to follow-up after non-attendance to the arranged data collection appointments. Four participants were excluded for the following reasons: Not SSc (1), overlap conditions with RA/SLE/Mixed CTD (3).

Unexpectedly, two participants were recruited but data not used for the main analysis as they did not fit into either the case or control group. They were found to have calcinosis elsewhere on the body, but not at the hands.

This resulted in a total of 79 participants being recruited and retained for data collection divided by 37 cases and 42 controls.

Table 9 provided the overview of the demographic, clinical and impact outcomes and comparing the control (no calcinosis) and case (with calcinosis) group with significance differences referenced with a *p*-value where applicable.

Participant outcomes	Ref	CONTROL	CASE	*p*-value
	n =	42	37	
Age-yrs.	Mean (SD)	59.9 (10.6)	57.7 (12.21)	0.4
Gender	n= (%)			
Female		36 (85.7)	35 (94.6)	
Male		6 (14.2)	2 (5.4)	0.19
Ethnicity	n= (%)			
White		38 (90.5)	33 (89.2)	
Non-white (Asian, Black)		3,1 (7.1, 2.4)	4,0 (10.8,0)	0.85
SSc subset	n= (%)			
Limited		31 (73.8)	27 (73)	
Diffuse		11 (26.2)	10 (27)	0.9
SSc disease duration-yrs.	mean (SD)	11.25 (8.9)	14.72 (8.1)	0.07
RP duration-yrs.	mean (SD)	22.48 (18.4)	26.75 (15.5)	0.27
BMI	mean (SD)	24.8 (8)	25.32 (4.6)	0.72
Smoking status	n= (%)			
Never		28 (66.7)	24 (64.9)	0.87
Past		12 (28.6)	12 (32.4)	0.71
Current		0(0)	1 (2.7)	
unknown		2 (4.8)	0 (0)	
Serology	n= (%)			
Scl70		10 (23.8)	9 (24.3)	0.67
Anti-centromere		21(50)	23 (62.2)	0.28
PMScl70		3 (7.1)	1(2.7)	0.37
RNAPol_III		1 (2.4)	0 (0)	
Anti-Ro		8 (19)	2 (5.4)	0.07
Anti La		1(2.4)	1(2.7)	0.93
ANCA		1(2.4)	0 (0)	
Total mRSS	mean (SD)	1.5 (3.3)	4 (4)	**0.0032****
Telangiectasia	n= (%)	28 (66.6)	33 (89.2)	**0.017***

Scleroderma Renal Crisis	n= (%)	0 (0)	1 (2.7)	
Cardiac Disease	n= (%)	11 (26.2)	7 (18.9)	0.44
Pulmonary hypertension	n= (%)	4 (8.5)	9 (24.3)	0.77
Ischaemic lung disease	n= (%)	10 (23.8)	8 (21.6)	0.82
GI involvement	n= (%)	37 (88)	35 (94.6)	0.31
Medication management:	n= (%)			
Calcinosis specific		0 (0)	4 (10.8)	
Immunosuppression /DMARD		25 (59.5)	21 (56.75)	0.8
Vasculopathy		34 (80.9)	31 (83.8)	0.74
GI		37 (88)	32 (86.5)	0.83
Cardiac		20 (47.6)	22 (59.45)	0.29
Pain		22 (46.8)	28 (75.7)	**0.03***
SSRI		10 (2.4)	7 (18.9)	0.59
Questionnaire Outcomes:	mean (SD)			
Cochin HFDS (0-90)		18 (18.7)	24 (21.8)	0.19
SSc QoI (0-29)		8 (14.4)	14.9 (8.8)	**0.01****
SHAQ (0-100)		25.1 (36.3)	30 (44.75)	0.59
Maudsley calcinosis		-	58 (52.3)	-

*Table 9: Phase three. Patient demographics, clinical presentation and patient reported impact. *Statistical significance p=<.05 **Statistical significance p=<.01*

4.4.2 Differences in demographic and systemic features

The data shows that the groups were appropriately matched by gender, age, ethnicity, SSc subset, disease duration, RP duration, BMI, smoking status, and serology profiles. There were no significant differences between the groups in these variables. Regarding clinical features, there were significant differences in presence of telangiectasia (p=0.071) and a higher use of pain medication (p=0.03) in the calcinosis group. The total mRSS showed the most dramatic difference between groups with the higher scores seen in the calcinosis group (p=0.0032).

4.4.3 Differences in function, disability, health, and quality of life assessment scores

There was a highly significant difference (p=0.01) in the QoL outcome as measured by the SScQoL questionnaire with worst outcomes associated with calcinosis. There was a difference in the CHFDS between the case and control groups, but this was not quite significant (p= 0.19). The SHAQ showed very little difference between groups (p=0.59).

4.4.4 Differences in the clinical characteristics at the hands

When comparing the differences of clinical characteristics at the hands between the calcinosis and control group, there were highly significant differences on almost all measured characteristics. Only the presence of puffy fingers and the skin sensitivity was reported with no significant differences between groups. The mean monofilament number detected in healthy controls at the hands is reported in the literature to be 4.68 (SD 1.14 n=39) using the von Frey monofilament testing [381]. The mean scores in this cohort show almost a statistically significant difference when compared to healthy controls p= 0.07 for the calcinosis group, but no significant difference p= 0.29 compared to controls in this study.

The NVC SSc patterns identified significant differences in the number of ‘normal’ and ‘late’ patterns between groups. The calcinosis group had significantly more ‘late’ NVC patterns, and the control group has significantly more ‘normal’ NVC patterns. The number of early, active, and non-specific patterns between groups were similar with no significant differences.

Hand Characteristics	reference	CONTROL No calcinosis	CASE With calcinosis	*p*-value
	n=	**42**	**37**	
History of DU	n= (%)	5 (11.9)	22 (59.45)	**0.00001***
Ulcers proximal to DP	n= (%)	2 (4.8)	14 (37.8)	**0.0002***
Digital pitting scars	n= (%)	12 (28.6)	28 (75.7)	**0.00002***
Loss of digital pulp	n= (%)	6 (14.2)	23 (62.2)	**0.00001***
Puffy fingers	n= (%)	36 (85.7)	28 (75.7)	0.26
Sclerodactyly	n= (%)	12 (28.6)	26 (70.27)	**0.0002***
NVC SSc pattern	n= (%)	37 (88)	32 (86.5)	
Normal		10 (27)	1 (3.1)	**0.007***
Early		6 (16.2)	5 (15.6)	0.95
Active		4 (10.8)	3 (9.4)	0.84
Late		7 (18.9)	18 (56.2)	**0.001***
Non-specific		10 (27)	5 (15.6)	0.25
Monofilament scores	Mean (SD)	5.08 (1.93)	5.51 (2.12)	0.34
RP impact score 0-10	Mean (SD)	3.15 (0)	4.5 (2.7)	**0.002***

*Table 10. Phase Three. Statistical significance of clinical hand characteristics (case vs control). *Statistical significance p=<.01*

4.4.5 Calcinosis history, status, and patient perceived progression

The history of calcinosis in the case group highlighted a mean duration of 10 years (SD 9), averaging approximately 17 years (SD 14) from the first RP symptom, 7 years (SD 7) from the first non-RP SSc symptom, and 4 years (SD 6) from the SSc diagnosis.

The calcinosis global assessment showed no significant difference between practitioner and participant perceived scores and averaged approximately 3-4/10.

Participants perceived progression of the calcinosis since their last clinic review, highlighted that almost half the participants (n=16) felt the calcinosis was the same and almost the same

number of participants (n=13) felt it had worsened, or worsened significantly (n=2). Only 5 patients felt the calcinosis had improved. This was attributed to resolution of a recent flare where expulsion of calcinosis fragments via ulceration resulted in a calming of symptoms and subsequent healing.

History of calcinosis presentation	**n= 37** Mean (SD)
Duration of calcinosis- yrs.	10 (9)
Duration of calcinosis from first RP symptom- yrs.	16.8 (14.13)
Duration of calcinosis from first non-RP symptom- yrs.	7.38 (7.08)
Duration of calcinosis from SSc diagnosis- yrs.	4.22 (6.21)
Perceived global score of calcinosis (0-10)	
Physician score	3.24 (2.8)
Patient score	4.03 (3.56)
p value	0.29

Table 11: Phase Three. Patient-reported history and progression of calcinosis

Calcinosis presenting at the hands and elsewhere of the body was recorded. Figure 9 provides a graphical representation of the number and percentage of areas effected with calcinosis from the group of 37 cases with calcinosis. The body parts affected most, start at the hands (n=37, 100%), followed by the elbows (n=13, 35.1%), knees (n=9, 24.3%), upper arms and forearms (n=8, 21.6%). The thighs/lower legs were affected the same as the arms (n=8, 21.6%) and at a slightly higher measure than the feet (n=6, 16.2%). The least affected areas were the face and neck (n=5,13.5%) and lastly the trunk (n=4, 10.8%).

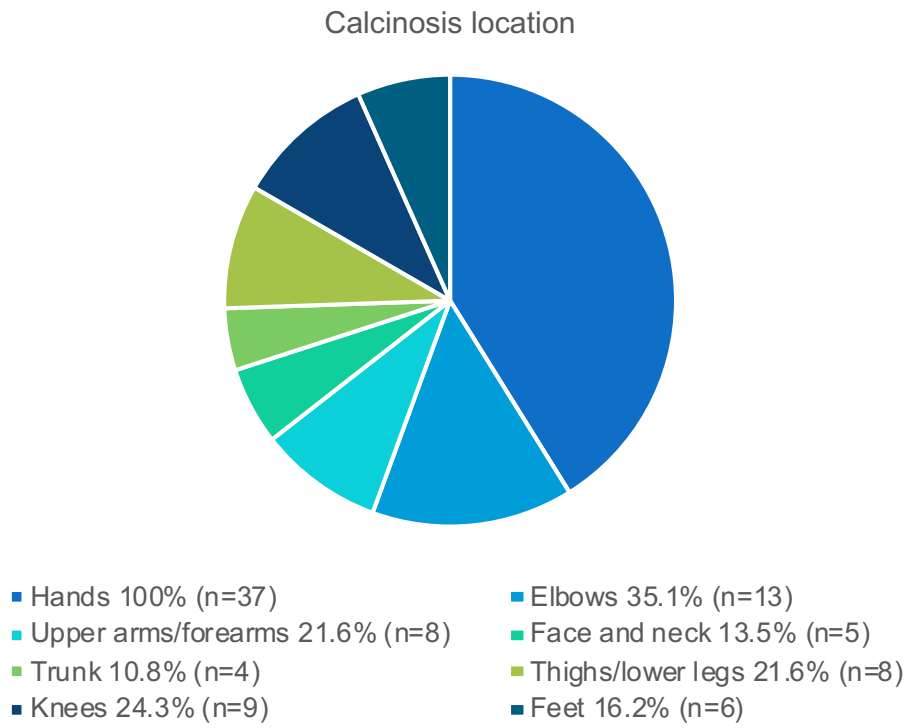


Figure 10: Phase Three. Percentage of body regions affected with calcinosis.

The characteristics of the calcinosis deposits in other regions of the body compared to the hands are documented within a complete table in appendix 4 and described below.

Most deposits were palpable and hard (60-100%) but with a higher percentage of deposits outside of the hands being palpable and soft in presentation (23-62.5% vs 19% at the hands).

The highest percentages of tenderness surrounding the deposits were at deposits of the upper extremity and at the joints of the extremities; knee (77.8%), elbow (77%), hand (67.7%), arms/forearms (62.5%), followed closely by the feet (66.7%).

Every region apart from the face had deposits observed as breaking through the skin (16.7% - 38.5%). Ulceration was present at the arms/forearms and thigh/legs equally (12.5%), at the knee (11.1%) and at the elbow (7.7%) but mostly at the hands (16.2%).

Only four clinically infected deposits were observed in this cohort, 1 at the leg (12.5%), 1 at the knee (11.15%) and 2 at the hands (5.4%).

Clinical inflammation as defined by redness, swelling and heat surrounding the deposit was observed similarly at deposits of the

trunk, legs and knees (25%) with slightly higher percentages at the hands (27%) and elbows (30.8%).

Multiple deposits were present in all regions (38.5%-87.5%) except the face with the highest presence of multiple deposits at the arms (87.5%). The size of deposits varied between >1cm to <1cm for each body region sometimes presenting with both sizes where multiple deposits existed. Deposits of <1cm were observed more commonly than deposits of >1cm at the hands (86.5% vs 35.1%), feet (66.7%, 33.3%), elbows (84.6% vs 23%), face (100% vs 0%) and trunk (75% vs 50%). Larger deposits were observed more commonly at the arms (75% at >1cm vs 62.5% at <1cm) and both smaller and larger deposits equally at the legs (62.5%).

Pains scores (Linkert scale of 0-10) of lesions at each site varied from a mean average of 1.8 – 4.9. The highest mean pain scores were reported at the upper arms/forearms (4.9 SD 1.74), closely followed by the knees (4.1 SD 2.4) and then hands (3.92 SD 3.3).

4.4.6 Differences in pain scores between phase one and phase three

Comparisons of the pain scores between phase one and phase three cohorts show higher pain scores reported in phase one for both hands and body from only participant-reported calcinosis. However, the difference in pain scores at the hands with calcinosis between the cohorts of phase one and three is not statistically significant. There is instead a highly significant difference in pain elsewhere in the body between phase one and three for patients with calcinosis (p= 0.01).

Highest pain scores	**Phase One**	**Phase Three**	***p* =value**
Hands	4.9 (2.94)	3.92 (3.3)	0.10
Body	6.93 (4.03)	4.9 (1.74)	*0.0039

Table 12: Differences in pain scores at hand and body from phase one and phase three

4.4.7 Differences in clinical characteristics of calcinosis between the most impactful and least impactful deposit at the hand

Table 13 highlights the characteristics of the calcinosis deposit which were reportedly most impactful compared with the reportedly least impactful deposit within the same participant. The least impactful deposits total 31 because 6 participants only had one deposit to measure.

Highly significant differences (p= 0.01) were found between the pain scores and whether the deposit was present on the dominant hand (p=0.02). The highly significant deposit characteristics that differentiated the most impactful deposit from the least impactful deposit also included deposits being palpable and hard (p=0.007), tenderness surrounding the deposit (p=0.002), the calcinosis breaking through the skin (p=0.014) and the presence of inflammation (p=0.015). There were no significant differences of impact related to the size of deposits, the palpable soft deposits, or whether multiple deposits were present. There were only instances of ulceration (n=6) and infection (n=2) associated with the most impactful deposits.

Calcinosis characteristics	Most impactful deposit (n=37)	Least impactful deposit (n=31)	*p*-value
Pain scores mean (SD)	3.91 (3.3)	0.19 (0.5)	**0.0001***
On dominant hand	28 (75.7)	15 (48.4)	**0.02***
Only on x-ray/US	15 (40.5)	17 (54.8)	0.24
Palpable hard	24 (64.9)	10 (32.2)	**0.007***
Palpable soft	8 (21.6)	2 (6.4)	0.078
Tenderness	23 (62.2)	8 (25.8)	**0.002***
Breaking through skin	9 (24.3)	1 (3.2)	**0.014***
Ulceration	6 (16.2)	0 (0)	
Clinical inflammation	11 (29.7)	2 (6.4)	**0.015***
Clinical infection	2 (5.4)	0 (0)	
Size: <1cm	19 (51.3)	21 (67.7)	0.17
Size >1cm	11 (29.7)	6 (19.3)	0.32
Multiple deposits	12 (32.4)	7 (22.6)	0.22

Table 13: Phase Three. Clinical characteristics and significant differences of the patient-reported most and least impactful region of calcinosis

4.4.8 Statistical analysis

Missing data from questionnaires was encountered in only one participant per group and the descriptive and statistical calculations were altered to account for this using imputed data methods. All data were checked for normality, analysed, and presented in accordance with the statistical plan.

4.5 Discussion

The objectives of phase three were to describe the clinical presentation of patients with SSc and calcinosis from the perspective of general disease features to the clinical features at the hands and the clinical characteristics of the presenting calcinosis. Measures of participant-reported impact of calcinosis on health, disability and function, and quality of life were described and compared in relation to calcinosis and for the first time with the SScQol measure.

The working definition to confirm presence of calcinosis using ultrasound from objective two was used to conduct a proof-of-concept study comparing US reported presence with the clinical presence reported by participant, clinician and confirmed by x-ray. The aim was to determine the impact and characteristics of calcinosis from the perspective of current clinical assessment and participant-reported outcomes to inform appropriate targets for clinical care and ultimately evaluation of targeted management strategies.

The study outcomes will be discussed in relation to overall methodology, findings, and limitations in respect of the existing literature.

4.5.1 General

As observer, the thesis author had appropriate training and experience for all clinical assessments including formal qualification or/and continued in-house training in mRSS, NVC and US advanced imaging. The thesis author who was also responsible for assessing clinical characteristics of the calcinosis had 6 years of clinical experience at the time of data collection focussed primarily on the assessment and management of DU and in particular, DU derived from calcinosis. Independent

observers for the scoring of x-rays were trained as per protocol and experienced in the development and use of the x-ray severity scoring system for calcinosis.

4.5.2 Recruitment

The site for recruitment for phase three was fortunate to have an existing cohort of SSc patients already identified and research active.

The sample size for this study did not reach the ideal size of 60/60 for case/control but reached the expected approximate number of 40 per group. The reasons for withdrawal and decline emphasises the burden of illness that patients with SSc suffer and how it impacts on their ability or desire to undertake additional activities.

Most reasons for decline/withdrawal were reportedly due to illness or not attending the study appointment (without a known reason). This affected both groups similarly for those approached (control n=12, cases =10).

A possible negative effect on recruitment could be from approaching research active patients who may feel that they do not want to participant in additional studies. The time already given to ongoing research projects should be accounted for and acknowledged during the approach.

For some patients, the travel into hospital was considered too exhausting and highlights the need for flexibility of study participation, and if not too time onerous, should aim to be incorporated into an existing hospital visit. The time taken for data collection in this study may have influenced the participation rate and decline to extend an expectedly long routine hospital visit. Anecdotally, it is reported that patients have anxiety concerning longer appointment times, sometimes due to the complications of SSc such as bowel incontinence, general discomfort, or travel restrictions.

Although there has been research into the reasons that patients with SSc do not attend support groups [382], there is no research into the implications of patients with SSc taking part in research, even though some of these reasons may be similar.

Despite the sample size being representative of published case-control observational studies the results must be viewed with the smaller sample size limitation in mind.

One study patient died prior to data collection, and it is worth noting that since data collection, subsequent patients of the cohort have since passed away. This gives insight into the potential limitations of longitudinal studies in SSc, and particularly for RCT's where effects on attrition rates can introduce bias [383].

The rate at which calcinosis can show significant clinical worsening or natural remission of symptoms can be as little as 2 days. Interviews with patients have informed the MCQ to measure symptoms in respect of 2 weeks.

This is useful to know in respect of the delivery of future RCT's evaluating treatments for calcinosis over shorter timeframes but also highlights the variability in presenting symptoms and reported impact at any given time.

4.5.3 Case-control group matching

The focus on patients with SSc for phase three was imperative given the results of phase one which highlighted a significant difference in reported calcinosis and related impact compared to SLE and DM. The exploration of calcinosis in SSc was more robust in phase three through effective matching of the case and control groups. There were no differences between age, gender, ethnicity, disease subset, disease duration for RP and SSc diagnosis.

General clinical variables that may have affected differences in PROMs between groups or general clinical variables associated with calcinosis were also not significantly different between groups. The serology finding is not entirely unexpected and validated the variability in studies regarding the association of autoantibody markers such as ACA and Scl-70 in patients with calcinosis [163-165]. The presence of calcinosis has been associated with the longer duration of lcSSc and the dcSSc subset, however, this sample did not show a difference between groups in these trends. The proportion of lcSSc to dcSSc is balanced between groups but the ratio does not reflect the

general population (2.8:1/2.7:1 (controls/cases) versus 4.7:1 (population)) [384]. This shows a higher representation of dcSSc within the sample which may have impacted the clinical features and PROMs overall but not between groups. The strength in matching removed any bias between groups and allowed for stronger differentiating of the clinical variables that would be most strongly associated with calcinosis irrespective of disease profile and duration.

4.5.4 Statistical significance of general clinical variables

The highly significant differences of general clinical variables associated with calcinosis were a higher mRSS and the presence of telangiectasia, validating associations reported within the literature [123, 205, 385].

There was no difference in the other SSc related disease outcomes, contrary to the literature which suggests calcinosis is associated with higher rates of ILD and GI involvement [122, 167]. This again, may be owing to the strong matching between groups and higher ratio of dcSSc in which severe disease features are most prevalent.

An important finding is the significant difference in prescription of pain management being greater the calcinosis group, which in addition to an overall reduced quality of life measure, may be contributing to an increased socio-economic burden in this group [386, 387].

Schieir et al, show only a minimal difference in pain between lcSSc and dcSSc but associated increased pain with more frequent episodes of RP, active DU, worse synovitis and GI symptoms [279]. The reason for the prescribed pain medication was not recorded in this study, therefore, it could be argued that the need for pain medication in this group was for the management of these factors shown to be significantly associated with calcinosis, as opposed management of direct pain from calcinosis. Either way, it highlights the need to explore effective pain response and recommendations for management in patients with calcinosis, especially when despite therapy being administered, the pain levels remain reportedly higher in this group.

4.5.5 Differences in clinical characteristics at the hands

Despite not all differences associated with general SSc disease profile and calcinosis were observed, exploration of the clinical characteristics at the hands did fully validate the literature for the following features. This study validated highly significant differences (p= <0.01) in the presence of DU and proximal ulcerations, digital pitting scars, loss of digital pulp, RP impact scores and NVC late pattern associated with the calcinosis group [150, 155, 166, 168].

The highly significant association of calcinosis with sclerodactyly was reported for the first time in this study. Interestingly, this association supports recent literature which has postulated that increased A1 pulley thickness with peritendinous and soft tissue calcifications, identified via US, contribute to the formation of hand contractures [388, 389].

The duration of RP was not significantly different between groups, but the higher impact score was suggestive of more severe RP in the calcinosis group. The RP impact score was taken from a 0-10 Linkert scale of the SHAQ questionnaire which both groups completed. This asks, 'IN THE PAST WEEK, how much has RAYNUAD's interfered with your daily activities?". Although this relates to impact specifically, it does not measure the severity of RP. The Raynaud's Condition Score (RCS) is a validated measure of patient reported severity of RP with minimally important differences established for interpretation in studies [390]. The RCS was not used in this study however it has been identified to have significant limitations with potential barriers to drug initiation [391]. This has led to more recent exploration of the 'patient experience' to better inform the development of a new outcome measure for assessing the severity of RP in SSc [392] which is both reliable and validated [393]. Although this outcome measure was not available at the time of this study, it is worth considering for the assessment of RP associated with calcinosis in the future. Particularly as Manning et al, 2020 highlighted the possibility that blood perfusion and oxygenation in patients with SSc is not compromised as we would expect in more supposedly severe

RP [186] and wound healing is much quicker than pure DU once the offending calcinosis is removed. A patient-reported severity score of RP compared to objective measures and controls would be valuable to fully appreciate the nature of RP in calcinosis compared to severe RP cases without calcinosis.

The Von Frey monofilament assessment has identified significant reduced sensation at the feet in SSc [394] and has been reported in the literature at the hands in healthy controls but with no comparisons with SSc at the hands [395].

This is the first time a difference has been observed in people with calcinosis compared to healthy controls, with thresholds of measures as published in the literature [381], but showed no significant difference within this cohort between case and control. Although the technique for testing was the same as with healthy controls, the location of testing at the hands was different. In this study, the apex of each distal phalanx was tested, whereas the thenar of the hand was tested for healthy controls rendering the findings not fully comparable.

The presence of neuropathy has been associated with worse RP symptoms [31,32,33], however a difference in skin sensitivity was not significantly worse in the calcinosis group in line with the worsened RP scores. Similarly, monofilament outcome did not mirror the effect of skin change from higher mRSS and loss of digital pulp suggesting that local tissue alteration has minimal effect on sensation at the distal phalanx. This can be accounted for in the case of the mRSS, as fibrosis is measured and most valid at the dorsal PP as opposed the volar DP.

This apparent preserved sensation could be considered fortunate to enable protective sense to detect injury/pathology, including calcinosis, particularly at the DP where it is considered most prevalent [169, 360, 396] Equally it suggests that if the area is sensitive to light touch despite skin changes, that pain levels could also be considered within normal ranges or even heightened in respect of tissue alterations that should theoretically reduce sensation.

A limitation of this assessment is that the monofilament only assesses the mechanical threshold for light touch via the large diameter myelinated nerve fibres and does not include potential effects on the small diameter myelinated or unmyelinated nerve

fibres which are responsible for temperature, pain sensation and automimic function [397, 398]. This may explain the non-significance with the presence of calcinosis otherwise related to increased pain, and why the outcome did not mirror that difference between groups for RP impact.

The presence of puffy fingers showed no significant difference between groups, even though the literature has shown a correlation with calcinosis and the presence of oedema in the early stage of disease [331]. A quantitative measure of the severity of oedema was not taken to know if this differed between groups.

Although it cannot be strongly inferred which characteristics could be driving the pathogenesis of calcinosis, it is apparent that calcinosis presents within a group of patients that exhibits more severe tissue alterations and therefore possibly a worse disease state overall.

4.5.6 Patient-reported history and progression of calcinosis

The clinical reported history of calcinosis in the case group was defined by a mean diagnosis duration of 10 years with a standard deviation of 9 years. Eight participants did not have a diagnosis of calcinosis until the study date and were either asymptomatic or symptomatic but without knowledge of the underlying reason being attributed to early calcinosis formation. The literature suggests calcinosis is associated with a longer disease duration, particularly in lcSSc [10, 162]. Therefore, the average mean of 4 years duration of calcinosis from SSc diagnosis was relatively short compared to the expectation from the literature review, however this could be due to the inclusion of more patients with dcSSc, and the added number of patients identified at data collection with subclinical/early calcinosis formation. The cohort may be less representative of typically diagnosed calcinosis cases which tend to be diagnosed at the point of overt clinical symptoms and clinical presentation reflective of more severe established cases.

Dates of symptoms and calcinosis duration was participant reported and/or retrieved from medical records therefore subject to recall bias.

The calcinosis global assessment showed no significant difference between clinician and participant perceived scores and averaged mildly at approximately 3-4/10. This highlights a contrast between study phases. It was concluded that phase one represented more severe cases of calcinosis, yet phase three in contrast, appears to report less severe calcinosis with global assessment scores being relatively low when comparing to other conditions impacting health. This highlights the cohort to have other highly rated disease factors related to SSc overall and the calcinosis group to have more range in the symptomatic reporting of calcinosis severity.

Fifteen/thirty-seven participants (40.5%) perceived a worsening progression of the calcinosis since their last clinic review. This validates a retrospective study measuring increased calcinosis progression via x-ray over 12 months to be 40% [155]. The study in the literature did not include reference to PROMs, but this thesis validates the prevalence of patient-reported progression to be the same. The duration however is not entirely comparable as the last clinic review may have been sooner than 12 months.

The few patients who felt the calcinosis had improved, stated this was due to having a previous flare which resulted in expulsion of calcinosis fragments and a calming of symptoms. This highlights the importance of repeat measures over a short time to acutely capture the average impact and progressive pattern of a calcinosis flare. It also reinforces the possible variability of results at different time points and the need to monitor the change in clinical characteristics in close succession over time with reference to PROMs. There has been no prospective longitudinal assessment of calcinosis to date looking at these trends.

4.5.7 Differences in participant-reported outcome measures

Surprisingly, despite the differences in the clinical manifestations at the hands in the calcinosis group, the CHFDS and SHAQ in phase three showed no significant difference between case and control group, yet the SScQol did show a significant difference. The SScQol has demonstrated validity, reliability and statistical sufficiency [399] and was developed specifically from the

patients perspective of SSc and the impact it has on their QoL. It covers four themes of emotion, physical adaptation, impact on self and impact on others and due to its robust validity, it has since been translated into seven different languages [400].

The CHFDS shows good construct validity and correlates well with the SHAQ, which should explain the similarities in outcomes between these PROMs [401]. However, this study outcome in phase three is contrary to the results of phase one. It can be surmised that the inclusion of other diseases may have contributed to this difference in the case of the QoL measure. It certainly validates that the SScQol is, as intended, most applicable to people with SSc alone. The difference in scores is significantly higher representing a significant negative impact on QoL for people with calcinosis in SSc. It may also indicate that the psychosocial impact of people with calcinosis is much greater than the functional impact.

The possible explanation of the CHFDS and SHAQ being non-significant in calcinosis is insightful. It could simply be argued that the study is underpowered, and a larger sample size would present the accurate difference. However, as co-author of recent published data from 396 patients at the same study site, we demonstrated that the CHFDS showed no significant difference with the presence of calcinosis over time [402]. It therefore appears that calcinosis alone is not adequately measured by the CHFDS due to the nature of SSc and the compounding pathologies at the hands which all impact hand function.

The key factors related to worsening hand involvement as measured by the CHFDS are identified as the male gender, disease subset, RCS, tenosynovitis, and pain. It is worthy to note that the presence of calcinosis and tenosynovitis for this study was measured from clinical palpation or confirmed clinical reports only.

Pain, as identified in this study, is also associated with calcinosis and therefore although CHFDs did not discriminate with an overt clinical diagnosis of calcinosis, the pain of may be representative of early, subclinical, and undiagnosed calcinosis.

Considering the worse disease factors and worse clinical features associated with calcinosis, Figure 11, from Del Galdo et al [403], has been adapted to include a theoretical placement (in

red) where calcinosis predominantly sits within the progression of SSc demonstrating how it is affected equally by other factors driving poor hand function and disability.

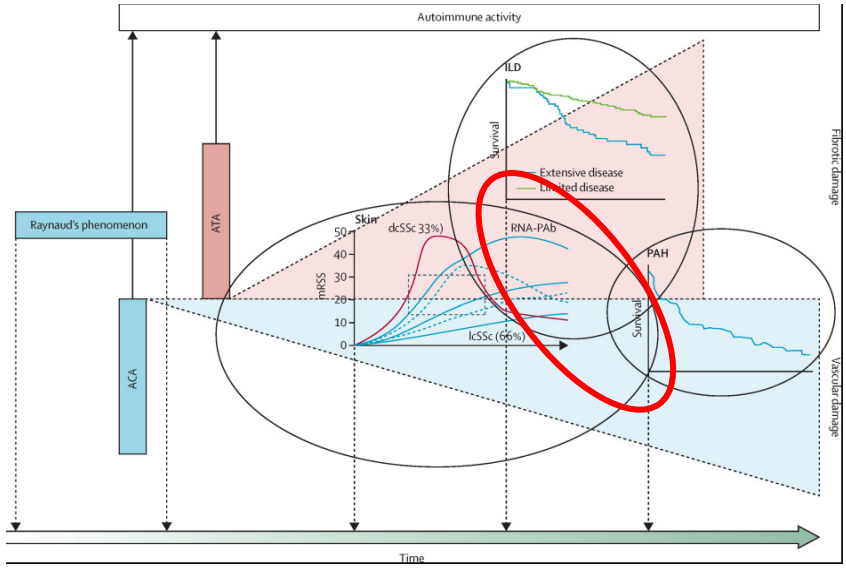


Figure 11: Theoretical placement of calcinosis in the progression of SSc disease. Adapted from Del Galdo et al.

This explanation supported by the findings of this study highlights the need to have a specific measure of the severity and impact of calcinosis alone with adequate discrimination validity to differentiate from other disease factors, particularly at the hands.

The Maudsley Calcinosis Questionnaire is beginning to address the unmet need to assess the patient reported severity of calcinosis specifically, however, the questionnaire cannot be applied in the study of case/control to evaluate the differences between people with and without calcinosis.

The difference in CHFDS results between phase one and phase three further strengthens the theory that the postal survey recruitment of phase one appealed to participants with much more severe impact of calcinosis. This is compounded further with phase three including participants with subclinical and/or asymptomatic calcinosis detected only via imaging. This alone may explain the effect on the insignificance of the CHFDS result and the possibility that calcinosis has a threshold of severity to reach before the CHFDS can detect the impact.

Furthermore, the current severity scoring of calcinosis using x-ray in this study did not correlate with the CHFDS, the SHAQ, the MCQ or pain scores at the hands. It did however have a negative correlation with the SScQol, the only statistically significant participant-reported impact measure of this study. There is no current literature comparing the x-ray severity score with PROMs. The lack of correlation suggests that other factors associated with SSc can influence the PROMs or it is the result of a smaller sample size. Interestingly, it could imply that size, bulk, or extent of calcinosis does not directly influence the patient reported impact. Instead, very early or active calcinosis may be more impactful than larger longstanding deposits.

This theory seems counter-intuitive, but it could be reasonable to equate larger established deposits with fixed hand deformity, in which case function is reduced and in turn less likely to actively flare and cause impact and pain. This in contrast to early smaller formations where the skin is still undergoing tensile and pressure forces from everyday movement.

This is supported by data in this this study where the difference in clinical size had no statistical difference between the most or least impactful lesion.

As the SScQol focusses on psychosocial impact, the negative correlation with x-ray scores could represent a better understanding and acceptance of calcinosis and their disease in general in the more established cases of calcinosis at the hands.

4.5.8 Clinical characteristics of calcinosis according to body location

As the literature shows, calcinosis appears most prevalent at the hands compared to other regions of the body. The deposits tend to feel hard when palpated, whereas calcinosis presenting more proximally exhibits a higher prevalence and combination of softer paste-like forms.

The arms presented most with deposits that were soft, with surrounding tenderness and multiple deposits. This could represent more actively growing calcinosis. Equally this could be representative of active breakdown and attempt of reabsorption. This study collected data at only one timepoint. The actual

clinical characteristics and related participant-reported symptoms throughout calcinosis progression has not yet been explored.
Tenderness of the surrounding tissue is more prevalent at joints and extremities suggesting a mechanical influence that may be introducing inflammation or may be linked to proximity of active tenosynovitis or synovitis. The presence of tenosynovitis and synovitis in SSc has been documented in SSc but not shown to be associated with calcinosis per se.
Interestingly, pain was reportedly higher in the arms/forearms and knees than at the hands.
This mirrors and supports the patient-reported pain scores seen in phase one being higher outside the body than at the hands and highlights the importance of clinical and research focus to extend further from areas most prevalent to areas most impactful from the patient perspective.
The calcinosis presence in this study at other body locations represents data limited to only clinical presentation without confirmed imaging. This is compared to lesions confirmed by imaging at the hands which includes identification of subclinical lesions. Therefore, the extent of calcinosis at other body regions is likely to be underestimated with no appreciation of deeper calcinosis formation in tissue which is more subjective to palpate compared to the hands. This is reflected in the literature with calcinosis of the spine has been reported via imaging in up to 83% [30], However, it is not typically visible clinically, nor do patients recognise calcinosis as the cause of their symptoms.
It has been reported that patients with calcinosis at the hands do not have more complications than patients with calcinosis elsewhere, but no data was available to clarify this statement further and whether calcinosis elsewhere actually had more complications (related to ulcerations, infection, tenderness, erythema or extruding thorough the skin)[150].
Importantly, two patients had calcinosis elsewhere on the body but not at the hands. This presents a distinct group of patients contrary to expectation and where if management of calcinosis relied only on baseline x-rays of hands, the progressive development and impact from calcinosis elsewhere would be neglected.

4.5.9 Clinical characteristics and participant-reported differences between most and least impactful calcinosis deposits at the hands

The characteristics of the least and most impactful calcinosis deposits at the hands gives insights for the first time into the significant impact relating to pain, hand dominance, stone-like formation of calcinosis, tenderness of surrounding tissue, calcinosis breaking through the skin and associated inflammation. The presence of ulceration and infection only presenting with deposits reported as most impactful, highlights these as important factors too.

The size of deposit was not significant however this was limited by a subjective measure of size with only one cut off point of 1cm. This was chosen after consultation with patients who felt that at the hand, most deposits they would refer to as small were under 1cm and any larger would begin to occupy most of an anatomical region, such as the pulp of the DP and likely extend beyond one region if bigger than 1cm. This measure lacks any range and relies on vision and palpation usually with restriction from skin fibrosis, oedema, and associated tenderness/pain. Interestingly the overall hand volume/bulk as scored by x-ray did not correlate significantly with pain scores or PROMs, further emphasising that size or overall bulk of calcinosis deposition is not necessarily suggestive of most impact. Although the scores of two independent observers in this study correlated, there was still an observed difference in the measures and the scoring system has been reported to have poor interrater reliability [348]. This highlights that even with imaging, the subjective measure between individuals can vary considerably, especially where a small difference in size is to be measured. For this study, the subjective clinical assessment of size was only measured by one observer once and on one occasion so does not account for intra or inter-rater reliability in the clinical assessment of calcinosis characteristics.

What is most interesting is that 40% of the most impactful deposits were only visible following imaging and 54.8 % for the least impactful deposits. This shows that subclinical calcinosis can represent most impact compared to clinically overt

calcinosis, and that less impactful lesions still exist with potential to progress.

This also highlights that the extent of participant-reported calcinosis in other areas of the body in this study, and in general, could be highly underestimated and related symptoms disregarded until progression is established. Imaging however can detect these early and offers an opportunity for early directed management.

4.6 Summary and conclusion

In summary, health and hand function/disability is not significantly different between matched calcinosis/control groups. The impact of calcinosis at the hands, as measured by the CHFDS, does not discriminate between other disease features of SSc that affect the hand, such as skin fibrosis and vasculopathy, even though these clinical features are worse in patients with calcinosis. This highlights that individual clinical factors that contribute to poor hand function and disability are variable, and causality cannot be inferred from the outcomes of current PROMs or for the pathogenesis of calcinosis.

The study showed for the first time, a reduced sensitivity of light touch at the fingertips associated with calcinosis compared to the hands of healthy controls but this difference is not quite significant and no different between matched controls with SSc. This study also identified that although calcinosis elsewhere in the body is reportedly more painful than at the hands.

Clinical characteristics of calcinosis that reflect patient reported impact include pain, hand dominance, stone-like formation of calcinosis, tenderness of surrounding tissue, calcinosis breaking through the skin, associated inflammation, ulceration, and infection. Size and amount of calcinosis did not differentiate between patient-reported ‘most’ and ‘least’ impactful regions.

Chapter 5

Phase three: Proof of concept study: reliability of US to detect calcinosis compared to x-ray, practitioner and patient reported presence and the association with patient-reported impact.

5.1 Introduction

The working definition to confirm presence of calcinosis using ultrasound from phase two was used to conduct a proof-of-concept study comparing US reported presence with patient, clinician, and x-ray reports.

5.2: Methodology

5.2.1 Assessment of calcinosis via imaging

X-ray

A posterior-anterior view x-ray of both hands was undertaken as part of routine clinical care and results were reported by an independent radiographer to verify the presence of calcinosis. The x-ray images were anonymised and shared with Stanford University in line with an agreed institutional data sharing contract arranged for by the author. Two independent observers provided scoring on the severity of calcinosis.

Ultrasound

A scan of the hands and wrists, dorsal and volar aspects, was performed using a Supersonic Imagine Ultrasound machine and a 6-15MHz matrix linear probe transducer and sufficient coupling gel. The machine was standardised using a machine pre-set setting for musculoskeletal superficial assessment and at a frequency of 15MHz. Two focal zones were used and positioned at the ROIs. In the presence of ulceration, infection control measures were in place which included the use of sterile coupling gel and sterile probe sheath (’Probetection’). The wound was cleansed and re-dressed post assessment.

The presence of calcinosis using ultrasound was confirmed using the definition as agreed by the Delphi agreement undertaken in phase two.

A detailed scan of the hands was undertaken to identify any calcinosis at the 22 anatomical regions per hand, mirroring the regions used for scoring calcinosis via x-ray. Each region was scored as either '1' calcinosis present or '0' no calcinosis. The observer was blinded to the regions effected by calcinosis as confirmed by x-ray.

5.2.2 Data analysis Plan

Using the x-ray data, a calcinosis score would be calculated by two independent observers using the validated scoring system by Chung et al. [348]. This involves dividing the hands into 22 anatomical regions each with a predefined scoring weight and subjectively scoring the percentage area covered (0-100%) x average density score (1-3) x weight for each anatomical area to achieve a final score of calcinosis at the hands. Percentage area coverage score is an estimated percentage area covered with calcinosis for that region. The average density score is defined as 1 = less dense than trabecular bone, 2 = like trabecular bone, 3 = like or denser than bone cortex.

The scoring was undertaken by two experienced independent scorers who were involved in the development of the scoring system. An average of the scores would be taken and analysed for correlations with PROMs and the regions of confirmed presence would be compared to regions of reported presence from US, participant, and clinician assessment.

5.3 Results

5.3.1 X-ray severity scoring and the association with patient-reported impact.

X-ray scores were obtained from the two independent scorers for 18 of the calcinosis cases. The two scores were averaged to one score and correlated with the PROMs. The only significant correlation was between the x-ray and SScQol questionnaire which is presented graphically in Figure 10 and demonstrates a

negative correlation. Table 14 shows the correlation using Spearman r with 95% confidence intervals

	AV xray scores vs. cochin	AV xray scores vs. SScQol	AV xray scores vs. SHAQ	AV xray scores vs. Maudsley	AV xray scores vs. Pain scores at hands
Spearman r					
r	-0.3874	-0.5584	-0.3870	-0.03618	0.2857
95% confidence interval	-0.7305 to 0.1118	-0.8183 to -0.1091	-0.7303 to 0.1123	-0.5059 to 0.4501	-0.2233 to 0.6723
P value					
P (two-tailed)	0.1122	0.0160	0.1126	0.8867	0.2504
P value summary	ns	*	ns	ns	ns
Exact or approximate P value?	Approximate	Approximate	Approximate	Approximate	Approximate
Significant? (alpha = 0.05)	No	Yes	No	No	No
Number of XY Pairs	18	18	18	18	18

Table 14: Phase Three. Correlations and significance of x-ray scores vs. CHFDS, SScQol, SHAQ, MCQ and pain scores at the hands

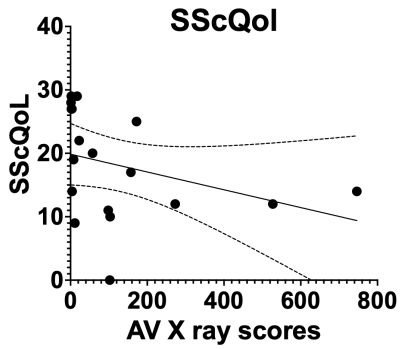


Figure 12: Phase Three. Scatter graph demonstrating significant negative correlation of SScQol with average hand x-ray scores.

5.3.2 Sensitivity of ultrasound, clinician and participant to detect calcinosis compared to x-ray

Ultrasound assessment using the Delphi agreed definition confirmed the presence of calcinosis in all 37 cases (100%) of the calcinosis group.

Comparisons were made with the 22 regions of the hands scoring 1 for presence of calcinosis and 0 for no presence. This was reported separately for the x-ray images, the ultrasound

assessment, the clinical assessment and for participant-reported presence. The number of times that scores differed per region when compared to x-ray are presented in Table 15 including the total number of different scores and percentage difference. Zero differences occurred most between x-ray and US (n=23) with most differences occurring at only one region (n=10) and in up to 5 regions on one occasion. The number of times differences occurred between clinically assessed presence and x-ray was greatest (n=99) and in as high as 13 regions on one occasion. Participant-reported presence was better than clinical assessment with less difference in regions detected (n=43 vs n=99 respectively) and the variability of difference was mostly between only one region (n=13) and as high as 7 regions on one occasion. Overall, there was only a 1.29% difference between US and x-ray scores per region and the difference between participant-reported presence and x-ray scores was lower (2.64%) than between clinical reported presence and x-ray (6.08%).

Scores per region (n=37)	Ultrasound vs Xray	Clinical Presence vs Xray	Participant reported presence vs Xray
0 different	23	13	13
1 different	10	2	13
2 different	3	6	6
3 different	0	3	3
4 different	0	4	1
5 different	1	2	0
6 different	0	1	0
7 different	0	1	1
8 different	0	2	0
9 different	0	0	0
10 different	0	0	0
11 different	0	0	0
12 different	0	2	0
13 different	0	1	0
Total different	21	99	43
Percentage	1.29%	6.08%	2.64%

Table 15: Phase Three. Percentage difference of scores confirming the presence of calcinosis at regions of the hands comparing x-ray with US, clinical presence and patient reported presence.

5.3.3 Statistical analysis

Although the interrater reliability of the x-ray scoring system has shown to be poor [348], the two independent scores for this study were analysed and showed positive correlation to enable a representative average to be taken. The number of x-ray scores completed from the two independent scorers was 18/37. This lower sample size led to skewed data when testing for normality, so scores were adjusted for correlating with PROMs using the non-parametric Spearman's rank correlation coefficient.

5.4 Discussion

The Delphi agreement definition was able to verify the presence of calcinosis in 100% of the confirmed cases. Regarding regions detected, the differences in regions confirmed on x-ray were marginal compared to US. This supports early studies which have suggested no significant difference in the detection of calcinosis by US compared to x-ray [290, 293]. A more recent study which mirrors the thesis in regarding to anatomical regions observed found US to have a sensitivity of 61-76% and a specificity of 96-100% compared to x-ray [178]. The lower sensitivity reported and of that compared to this study, can be attributed to the shorter and more feasible scanning times reflective of routine clinical assessment. In this study, adequate time was allocated for focussed scanning of each region.

In addition, the definition used to confirm calcinosis in the literature study was simply 'hyperechoic foci with or without posterior acoustic shadowing' which may not have captured the mixed or soft formations of calcinosis as defined by the Delphi agreement definition used in this study. In comparison, on x-ray this may have a much fainter amorphous, cloud-like appearance in contrast to the dense nodular formations [404], which would more closely compare to hyperechoic deposits on US.

Importantly, the difference between patient-reported presence and regions detected by clinical assessment was the greatest, with patient reported presence being more accurate for calcinotic sites confirmed by x-ray.

This is of key significance to inform the approach of calcinosis in SSc. Exploration into the potential early symptoms associated with subclinical calcinosis is warranted to direct patient education on early reporting of symptoms to ensure assessment and diagnosis is timely. The voice of the patient is instrumental in early diagnosis as patients are acutely aware of subclinical calcinosis and more accurate at confirming its presence than even experienced practitioners.

Clinicians should perform routine baseline imaging at the point of diagnosis with the expectation that calcinosis may form as early as an average of 4 years as seen with the data from this study. This, with the inclusion of regular routine US assessment, is likely to shift the theoretical placement of calcinosis within the progression of SSc to be much sooner and before symptomatic and/or clinically apparent.

It is also worth highlighting that the assessment for clinical presence of calcinosis was by a clinician experienced in the subtle visual and physical changes of skin associated with calcinosis and the assessment of clinical presence was more thorough than would be expected in routine practice, guided by assessing each of the 22 study regions individually. Therefore, the detection of this is likely to be overestimated compared to general clinical practise.

It is also worthy to note that assumptions on differences comparing US to x-ray should not be judged on the assumption that error was from US. A posteroanterior x-ray view of the hands is 2 dimensional in nature and calcinosis deposits on the palmer aspect of the hand can be hidden from the overlying bone [405, 406]. Also, where fingers were amputated a score of 0 was applied, which although reasonable, has the potential for bias should the decision to amputate have been influenced by calcinosis.

The study was limited to the use of US to only confirm the presence of calcinosis as the first step to confirming the definition of calcinosis using ultrasound. However, the narrative of ultrasound presented in this study highlights its value as an outcome measure of severity.

Unlike x-rays, which expose patients to radiation, ultrasound is non-invasive and can be safely performed multiple times over

short intervals, facilitating continuous monitoring for both clinical and research applications.

Ultrasound also provides a wider range of benefits particularly useful for the assessment of calcinosis. It can be used in real-time, making it a practical tool for immediate diagnosis and monitoring, and it offers wider coverage of assessment at multiple body sites, not just the hands, as is the case with the current x-ray scoring system. This is particularly significant, as this thesis has demonstrated that calcinosis occurring outside the hands is often overlooked, despite having a greater impact on patients' quality of life. Ultrasound can be easily adapted to assess all areas of subcutaneous tissue that it commonly affects. Although ultrasound has a limited field of view, it has the added benefit of enhanced and highly sensitive detail, evaluating microstructural characteristics of individual lesions: those of much smaller size and in the very early stages of formation, identifying subtle, subclinical deposits early, with the potential of improving prognosis through earlier detection and intervention. Incidental findings of calcinosis during assessments for other pathologies and results from this study suggest its utility in detecting asymptomatic cases and confirming the presence of symptomatic but not yet clinically apparent lesions. In real time, targeted treatments like intralesional injections could simultaneously be administered with precise visualisation, guided more accurately by ultrasound at the point of diagnosis. In addition, ultrasound has the capability to analyse skin changes, such as elasticity, which is often compromised to varying degrees in SSc. This was highlighted by an expert during the Delphi agreement process as an important feature to consider in the assessment and reporting of calcinosis using ultrasound.

As discussed in the literature review, there may be a connection between fibrotic skin changes and the presence or severity of calcinosis. This relationship suggests that assessing skin elasticity could be a critical aspect of evaluating calcinosis, of which ultrasound is well-equipped to provide.

Ultrasound also allows dynamic assessment of surrounding soft-tissue structures such as tendons and vascular flow involvement. As this thesis highlights, these details are important features to

assess and could reveal severity, symptoms, and progression indicators not detectable via x-ray.

Ultrasound offers a more adaptable field of view, conforming to the body's contours and allowing access to areas that X-rays cannot effectively assess. This is especially beneficial for individuals with scleroderma, where hand contractures are common and often unsuitable for evaluation through X-rays.

In general terms, US is cost effective, compared to MRI and CT, is more affordable and less time-consuming and with superior accessibility, it is practical for routine use, even outside specialist centres. The successful integration of US in the diagnosis and monitoring of gout (e.g., detecting tophi and the “double contour” sign) underscores its feasibility for calcinosis. By adapting and validating similar criteria for calcinosis, ultrasound could become a cornerstone for its assessment. This would serve as a valuable complement to existing imaging modalities such as X-ray and CT, which provide a broader field of view and a more comprehensive assessment of less common, deeper, and larger deposits. These more invasive, costly and time-consuming imaging modalities could instead be resigned to cases where internal organ involvement or complex surgical planning is required.

5.5 Summary and conclusion

This study was the first to identify that current validated outcome measures focussed on severity from the perspective of measuring most prevalence areas and characteristics of size/bulk is not representative of patient impact of calcinosis. X-ray severity scores of calcinosis do not correlate with current PROMs. QoL measures focussed on psychosocial factors are worse in patients with calcinosis and are negatively correlated with higher x-ray severity scores.

This highlights the need for new calcinosis specific outcome measures and work is underway from specialist working groups to develop and validate these further [363].

Most importantly, the results have provided the first proof-of-concept study for the confirmation of calcinosis using ultrasound as informed by an international expert group Delphi Agreement.

This study supports that ultrasound can detect calcinosis with minimal differences between x-ray reported regions.

It confirms that imaging and patient reports confirm subclinical calcinosis and early identification of subclinical calcinosis may be predictive of severe disease.

In conclusion, the lack of and negative correlation of x-ray severity scores with patient-reported impact prioritises the need for alternative imaging and calcinosis specific PROMs.

Ultrasound is an ideal imaging modality to offer this, it is non-invasive, feasible, can be applied to all areas of the body and provide additional information that may better reflect severity and impact from the perspective of the patient.

Overall, ultrasound provides robust imaging data that can complement clinical outcomes and patient-reported outcomes. It could evaluate how calcinosis forms and progresses, offering insights for new management strategies and allow precise visualisation to guide targeted treatments like intralesional injections.

While x-rays provide valuable information about the size, density, and distribution of calcinosis deposits, they fall short in offering dynamic and multidimensional assessments. Ultrasound not only overcomes this limitation but also opens new avenues for understanding the pathogenesis and progression of calcinosis in systemic sclerosis (SSc).

The ability to correlate US findings with patient-reported outcomes (e.g., pain, functional limitations, psychological impact) enriches its utility as a tool for both clinical management and research.

Ultrasound offers unparalleled advantages over x-ray in the assessment of calcinosis in SSc, including safety, versatility, and diagnostic depth. Its integration into routine clinical practice and clinical trials has the potential to transform the understanding and management of this debilitating condition. The next phase of development requires validation of the US-specific agreed calcinosis definition from this study and the establishment of its relevance to patient outcomes.

Chapter 6

Discussion

6.1 Introduction

The thesis concerns the extremely debilitating condition of calcinosis in SSc, the impact to patients, and how the treatment options are lacking due to the unmet advancement of outcome measures to effectively evaluate novel therapeutics in clinical trials. The clinical observations and clinical characteristics of calcinosis are described with associated impact in relation to the only current gold standard of x-ray to measure calcinosis severity and current PROMs. The thesis pushes current thinking and shifts the focus to what clinical outcomes impact the patient most whilst conducting the very first Delphi agreement to begin validation of ultrasound as the new potential gold standard for calcinosis assessment.

The remainder of this chapter summarises the whole thesis with chapter specific synopses. It provides further discussion on the impact of the thesis with respect of the thesis limitations and the need for further research. Personal research plans of the author are presented which progress this unmet need of calcinosis management in SSc.

6.2 Thesis synopsis

Chapter One: Calcinosis in SSc. A narrative review

Chapter One explores the literature and provides a narrative review on the background of SSc. It explores the theories of calcinosis formation, the current evidence on the associated factors and how the disease factors of SSc could be implicated in the formation of calcinosis. It reinforces that the pathogenesis of calcinosis remains unknown and therefore targeted treatment options are limited. The systemic and local treatment options that have been attempted are explored, and new potential treatments are suggested for possible future evaluation.

The narrative reviews conclude that without a measure of patient-reported impact and severity of calcinosis, clinical trials

cannot effectively evaluate novel therapeutics. This leads to an explorative narrative of ultrasound in SSc and other crystalline diseases and highlights the value of ultrasound as a novel outcome measure for calcinosis.

Chapter Two: Phase One. Prevalence and impact of calcinosis in Systemic Sclerosis

Chapter two covers the first of three stages of study in this thesis. It describes the patient-reported prevalence and impact of calcinosis via a postal survey targeting eligible patients across six centres. It includes comparisons of participant-reported presence and impact of calcinosis in SSc compared to SLE and DM. This phase identifies participants with calcinosis to have worse hand function and disability scores as measured by the CHFDS questionnaire and indicates that scores of the lesser fingers and IP regions are associated with more severe cases. Participants detail the regions at the hands most affected by calcinosis and suggest that calcinosis related pain is worse in other areas of the body. Phase one demonstrates that participant-reported calcinosis is significantly higher in SSc and although the prevalence and focus of calcinosis is at the hands, other regions of the body should not be neglected.

Chapter Three: Phase Two. Delphi Agreement: Expert consensus on defining calcinosis using ultrasound

Chapter three covers a preparatory phase of study to support the use of ultrasound in confirming the presence of calcinosis in SSc. It details the first Delphi expert consensus agreement conducted to define the presence of subcutaneous calcinosis using ultrasound. It resulted in the first ever definition and working definition of calcinosis and provides the foundations for further validation and future clinical recommendations in the assessment and management of calcinosis.

Phase two provides a consensus-driven definition for confirming the presence of calcinosis using ultrasound for the phase three observational case/control study of the thesis.

Chapter Four: Phase Three: Case-control and within-patient control study with ultrasound

Chapter four explores the impact and disease features of people with and without calcinosis in SSc. The strong matching of groups provides clear distinctions of the clinical associations of calcinosis at the hands which validates current literature and provides insight into the potential related pathogenesis of calcinosis formation discussed in chapter one. It identifies that other associated disease features of SSc at the hands compound true evaluation of the impact of calcinosis using current patient-reported outcome measures. Chapter four demonstrates that, regardless of disease profile, the psychosocial factors impacting quality of life are significantly worse in patients with calcinosis, and particularly in patients with early and subclinical calcinosis formation. It is highlighted for the first time that the current gold standard of hand x-ray does not reflect participant-reported impact. It identifies through comparison of clinical characteristics at most and least impactful lesion, that features other than size/amount of calcinosis are significant factors of impact. Chapter four also provides evidence that current research focussed on anatomical prevalence and scoring systems at the hand is neglecting calcinosis formation elsewhere on the body which has greater participant-reported impact on measures of pain.

Chapter Five: Proof of concept study: reliability of US to detect calcinosis compared to x-ray, practitioner and patient reported presence and the association with patient-reported impact

Chapter five explores the imaging data in relation to patient reported presence and impact, It confirms that ultrasound is able to identify subclinical and early asymptomatic presence of calcinosis using the first consensus-driven definition created in chapter three. Reflections are made from the narrative of chapter one for the value of ultrasound to validate the relevant factors that influence patient-reported impact of calcinosis. It highlights the benefits of ultrasound over plain x-ray for the assessment and monitoring of calcinosis and concludes how ultrasound could be the future complementary imaging modality in both the clinical

and research setting, particularly as an outcome measure for clinical trials targeting novel therapeutics.

Chapter 6: Discussion

Chapter six introduces and provides an overview of the thesis including a chapter synopsis followed by a general discussion on how the thesis has contributed to the field of calcinosis and any recent advances in relevant research since the study started. The impact of the thesis is highlighted, the general limitations are discussed and linked to the priorities of a future research agenda including a personal research plan of the author.

Chapter Seven: Summary and close

Chapter Seven summarises the thesis findings and its contribution to the field of calcinosis with focus on how this work builds the foundation and framework for the future effective evaluation of targeted treatments in the management of calcinosis, ultimately with the goal to improve the quality of life of patients suffering with this debilitating condition.

6.3 Discussion

This thesis has provided a vital contribution to the field of calcinosis through observation of the clinical characteristic of calcinosis and insight into the importance of patient-reported feedback. It has highlighted for the first time that the physician focus of extensive calcinosis formation, usually when treatment options are limited further and less effective is, in addition, too late to address the greater impact encountered in earlier and active calcinosis formation. The thesis is the first to identify that the severity scores of x-rays, focusing on size, bulk, and density of calcinosis, do not correlate with patient-reported impact on health, function, and disability and in fact have a negative correlation with psychosocial quality of life measures.
It has also been the first to highlight that although the hands are most affected with calcinosis, the patient-reported lesions at other areas of the body such as the larger joints and upper extremity of the arms are associated with more pain.

Pain management is utilised most in patients with calcinosis and yet pain levels remain higher in this group suggesting that the pain management is an area yet to be managed successfully.

This new understanding should shift the focus of SSc assessment to have stronger consideration for the potential of calcinosis at the point of SSc diagnosis and not limited to the hands. It reinforces the need for appropriate education of patients to express new relevant symptoms and to promote regular assessment from clinicians to detect symptoms and subclinical calcinosis early.

This thesis has also evidenced for the first time that patient-reports of calcinosis are accurate in early and sub-clinical calcinosis formation and are more accurate at confirming calcinosis than an experienced clinician assessment.

This provided new understanding that patients are acutely aware of symptoms associated with early and subclinical calcinosis formation, even before clinical diagnosis is made.

This is particularly important as the general profile of SSc disease does not necessarily differentiate from patients with/without calcinosis, but a worse presentation of specific clinical tissue characteristics related to skin fibrosis and vasculopathy is strongly associated with confirmed calcinosis presence. Early sub-clinical calcinosis detection may therefore be a potential predictive marker of more severe fibrotic and vascular changes.

This thesis has highlighted that baseline x-rays may be useful as an initial baseline to confirm the presence of calcinosis, however, this would usually only be utilised at the hands and repeated annually due to risk of repeat radiation exposure.

What this thesis offers, is the introduction of ultrasound as a formal alternative non-invasive and accurate tool to confirm subclinical, early, and established calcinosis presence. It benefits from easy and reliable assessment not just at the hands, but for calcinosis elsewhere on the body which has been highlighted to have more patient-reported impact.

The thesis provides the first Delphi expert consensus on using ultrasound to define the presence of calcinosis and has shown that it is highly accurate at confirming this compared to the gold standard of x-ray. This is the first step to providing an accurate

and more appropriate tool for clinical trials where non-invasive repeat measures can be made and features of patient-reported impact, which are not afforded by x-ray, can be explored, and measured.

Since this work was undertaken, US to assess skin wounds in SSc has defined the presence of calcinosis simply as 'hyperechoic foci with or without posterior acoustic shadowing' [344] which partially describes the prosed definition agreed by the experts. Another recent study by Gamissans et al (2022), compared the ultrasound appearance of dystrophic calcinosis (n=50), tumoral calcinosis (n=3) and calciphalaxis (n=5). A more detailed distinction was made between the appearance of the acoustic shadow, being narrow in dystrophic calcinosis compared to a wider acoustic shadow in tumour calcinosis. This is accounted for by the deposits being more singular and oval shaped in DC, and more widespread in a thin band parallel to the epidermis in tumoral calcinosis. Calciphylaxis has been described with a combination of the two; thin bands of deposit with a narrow acoustic shadow. The key differences highlighted by the authors to differentiate between these forms of calcium deposition however was the size and location as opposed ultrasound features. [407]. The balance of sample size per group may not give a true representation of these differences but is worthy of further exploration and may contribute to the refinement of the current definition in time.

6.4 Limitations

This study provides key insights into the clinical presentation of calcinosis using a case/control design that is considered stronger in evidence than observations through alternative cross-sectional, case series and case reports.

It provides a representation of the clinical presentation and impact of patients with calcinosis compared to those without at one time point. Given the observed clinical nature of calcinosis, the severity of clinical characteristics and related impact tends to fluctuate whilst on an increased trajectory of worsening severity overall. Therefore, a longitudinal study of frequent repeat

measures would be more representative of overall average impact over time.

The thesis highlights the main limitation in general calcinosis research which is the lack of an adequate outcome measure of calcinosis severity which aligns with patient-reported impact. Although correlations are reported, correlations do not imply causation without an assessment of potential bias. This study highlights an example of confounding bias where the CHFDS outcome measure for hand function and disability in phase three showed no significant difference between patients with and without calcinosis. It could be inferred that calcinosis has no impact on hand function and disability however, given our clinical knowledge about calcinosis, it is more likely that the cofounding disease features in SSc introduce cofounding bias, indicating that calcinosis is not identified and measured accurately within that particular outcome measure.

Similarly, clinical associations made throughout the literature review and as supported in this study, do not infer a causal link in the pathogenesis of calcinosis. That is, we cannot conclude that the degree of skin fibrosis and vasculopathy is the cause of calcinosis formation, or that calcinosis is the driving force of skin thickening and vasculopathy, merely that they are associated and commonly present together. What we can also acknowledge from patient reported information and respective studies, is that calcinosis overall tends to progress and worsen, just as the disease factors of SSc progress and worsen. There are, however, instances where calcinosis seemingly and spontaneously improves without intentional direct management. These anecdotal reports appear in line with improvement of other confounding disease features suggesting systemic management of a factor responsible for driving all disease features results in improvement/resolution in some cases. Identifying these direct factors is key for understanding the pathogenesis of calcinosis and to inform targeted treatments. Evaluation of systemic disease flares/remission may also align with calcinosis flare/remission and offer insight into the factors that promote impact and severity.

The differences between phase one and phase three demonstrate the potential for unintentional selection bias

reflective of the study method and recruitment process chosen. The very nature of postal surveys attracts respondents to which the subject matter appeals to most and for phase one a much larger pool of patients was indirectly approached resulting in cases surmised as being more severe. Whereas the direct selection of participants for phase three was reflective of a single routine clinical cohort and contained a more representative range of cases including asymptomatic and sub-clinical calcinosis. As a result, the outcome measure results were different.

The largest limitation to phase one is the focus being patient-reported presence of calcinosis without clinical confirmation, hence the need and objective to overcome this limitation in phase three.

In addition, all participant-reported outcomes have the potential for recall bias including history of calcinosis and data completed for questionnaires which typically ask for measures pertaining to the previous 2 weeks.

Phase two included important description of calcinosis presence outside of the hands, however this was based on clinical and patient-reported presence. Given the flexibility of US, confirmation using the Delphi agreed definition could have been incorporated. However, comparative imaging was limited to the hands due to the current limitation of only a validated x-ray scoring system available for the hands.

Similarly, clinical characteristics of infection were not confirmed via microbiology and relied upon clinical appearance alone.

The independent measures of phase two were from the author and could have benefited from a second observer to verify outcomes, however as a purely observational study, the inter-rater reliability at this stage was not available or considered essential for reporting of clinical characteristics, or for performing the first proof-of-concept study. It is however essential for the next stage of the validation process of the definition and development of a scoring system of calcinosis which will be discussed in section 5.5.

6.5 Future research agenda

The main areas of focus for future research into calcinosis remain with the end goal of providing effective targeted treatments.

Attempts have already been made to present promising treatment options, however without the strength of clinical trial methodology to validate the effectiveness. For this, an effective outcome measure of severity and impact is required.

This thesis has highlighted that adequate impact measures via questionnaires do not correlate with the current gold standard measure of calcinosis severity using x-ray and has introduced ultrasound as an alternative valuable outcome measure for calcinosis.

The definition of calcinosis using ultrasound has been agreed via an international Delphi consensus agreement supported by a positive proof-of-concept study within this thesis.

The future research agenda should now focus on the validation of this definition and the development of a scoring system for calcinosis using US. This, once validated, would support the future evaluation of targeted therapeutics in clinical trials.

Continued validation of the Maudsley Calcinosis Questionnaire is invaluable as a patient-reported impact measure.

In addition, early and longitudinal exploration into the ultrasound features of calcinosis throughout its fluctuations of patient-reported impact and in its progression overall could give key insights into the pathogenesis of calcinosis and identify the US features that impact the patient most.

This will provide structure and recommendations of appropriate timeframes for monitoring using ultrasound and appropriate time to intervene with the future effective treatments identified through clinical trials.

This body of work will produce publications detailing focussed standard operating procedures and clinical guidelines on the management of calcinosis and address improved patient and physician education to promote early diagnosis. This is with the aim to reduce patient-reported impact and slow or prevent progression to extensive calcinosis formation.

6.6 Personal research plans

The author of this thesis has demonstrated a strong commitment to improving the quality of life of patients burdened with calcinosis with a focus on imaging outcome measures to improve future care. The author has contributed to publications relating to the overview of calcinosis burden [385], the ultrasound standardisation of ultrasound to measure skin in SSc [333] and is joint-first author to a publication describing calcinosis on a molecular level with evidence of an ability to dissolve calcinosis crystals in vitro with clinically safe concentrations of EDTA [217]. This last study identifies that the soft and hard formations of calcinosis differ in molecular morphology of hydroxyapatite crystals. It also demonstrated that hydroxyapatite can be dissolved using a dosage of EDTA considered safe for clinical application.

The position of the author in a clinical-academic role is already focussed heavily on instigating systemic management options and providing local targeted treatments of calcinosis via scalpel debridement and wound management in the clinical setting. This role bridges research and practice, fostering a translational approach to both systemic and targeted treatments for calcinosis.

The author intends to advance the clinical assessment criteria and management strategies for calcinosis further at the specialist SSc centre, leveraging ultrasound as the key investigative tool. This will improve diagnostic precision and treatment monitoring. Structured clinical longitudinal audits will be developed and reported through feasibility and case report studies. This will provide valuable insights into the natural progression of calcinosis, informing management strategies and aiding in the development of tailored RCTs using ultrasound as an outcome measure of severity.

The author intends to continue exploring EDTA as a potential in-patient topical treatment, which could offer a non-invasive and effective therapy for both soft and hard calcinosis formations. Following the agreed procurement of the topical ointment, a pilot study will be conducted to assess its feasibility and tolerability, with case reports published to document initial findings. If the outcomes are promising, the process could advance to the

development of a randomized controlled trial (RCT) for further validation

To use ultrasound as an outcome measure for the proposed future work, the author has already secured funding from the Scleroderma Clinical Trial Consortium (SCTC) Working Group in calcinosis. The author is principal investigator and will be first author for the current ‘STONE Study’ (Ultrasound Based Outcome Measure of Soft Tissue Calcinosis in Scleroderma) findings. This study leads in the foundational stages of the proposed research agenda detailed in section 6.5. focusing on advancing the Delphi consensus process with the international panel of experts. This collaborative effort aims to refine and achieve agreement on key definitions and methodologies related to the ultrasound assessment of calcinosis.

To date, the author has played a pivotal role and led in several critical components of this research as detailed below.

- *Development of Standardized Protocols:* devised a comprehensive Standard Operating Procedure (SOP) to standardise the collection of ultrasound (US) images of calcinosis, ensuring consistency and reproducibility across study sites (Leeds in the UK and a counterpart site in the USA).
- *Patient Engagement:* Conducted semi-structured interviews within patient focus groups to explore the lived experiences of calcinosis, using these insights to create a patient-reported impact score of calcinosis. This measure seeks to capture the subjective impact of the condition on patients' daily lives, complementing clinical imaging data.
- *Multisite Recruitment:* Facilitated participant recruitment ensuring diverse and representative data collection. A database of 120 images have been collected of calcinosis lesions and some matched control areas unaffected with calcinosis from 60 patients. Data includes patient reported scores of severity and clinical assessment per lesion.

- *Delphi expert survey continuation:* Two further surveys have been conducted using the images obtained. Survey One: to confirm/deny the presence of calcinosis and to describe the ultrasound features per lesion to validate the agreed definition from phase two of this thesis. Survey Two: to provide a severity score of the lesion and a perceived patient impact score which will be compared to actual patient reported severity scores and descriptive features of the lesions both clinically and via ultrasound.

The over-riding goal of the STONE study is to:

- *Validate the definition of calcinosis using ultrasound:* Achieve consensus on the sonographic features that define calcinosis, ensuring clarity and reliability in its identification.
- *Develop and validate a severity scoring system:* Design a dual-system scoring framework that combines expert sonographer assessments with patient-reported severity ratings to provide a comprehensive evaluation of calcinosis severity
- *Conduct a prospective validation study:* Implement a forward-looking study to rigorously test the definition and scoring system across diverse clinical settings.

Once the definition and a severity score are validated, the next steps will focus on integrating ultrasound as a formal outcome measure in the assessment and management of calcinosis. This includes:

- *Incorporation into Randomized Controlled Trials (RCTs):* Ensuring ultrasound metrics are utilised to evaluate treatment efficacy in future interventional studies.
- *Clinical Guidelines Development:* Producing evidence-based guidelines to standardize the use of ultrasound in routine clinical care for calcinosis, enhancing diagnostic accuracy, patient monitoring, and treatment planning.

By addressing these objectives, the author's research will not only fill significant gaps in the understanding and management of calcinosis but also pave the way for more patient-centred and precise approaches in both clinical and research settings.

6.7 Conclusion:

This thesis has highlighted an unmet need in the management of calcinosis by identifying that current outcome measures of calcinosis severity are not adequate for measuring patient-reported impact. The author has identified further gaps and areas of research and clinical care that are neglected due to the lack of adequate outcome measures of severity and patient-reported impact

The author has been the first conduct a Delphi agreement to define calcinosis using ultrasound and evidenced it a suitable imaging tool for the confirming the presence of calcinosis and for measuring clinical factors that are more reflective of patient reported impact.

The author has also demonstrated a continued commitment to expanding the research in the field and is ideally placed to begin implementing and translating the developments into clinical practice.

Chapter 7

Summary and close

Calcinosis is a highly debilitating condition in SSc causing pain and functional limitation often leading to ulceration with risk of infection and amputation. The pathogenesis of calcinosis remains unknown limiting the treatments available. With such high debilitation, patients highlight calcinosis as a priority area for research and clinical management.

This thesis explores the literature of SSc disease and how the clinical manifestations link with the theorised pathogenesis of calcinosis, the clinical associations of calcinosis in SSc, and in other disease.

The study describes patient-reported impact of calcinosis in SSc compared to other CTDs. It validates the current literature mirroring the clinical features most associated with calcinosis in SSc but goes further to explore clinical characteristics of calcinosis that have greatest impact for patients on a lesion level. It establishes that psychosocial impact is significantly associated with calcinosis and adequately measured using the SScQol, whereas current measures of health, hand function and disability (measured by SHAQ and CHFDS respectively) do not differentiate calcinosis from the coexisting clinical features in SSc. The voice of the patient is instrumental in early diagnosis as patients are acutely aware of subclinical calcinosis.

Calcinosis is confirmed to be more prevalent at the hands, however, calcinosis elsewhere can be more painful, and risks being neglected and underestimated with the current routine clinical assessment methods and focus of research.

The focus of clinical assessment and research at the hands is influenced by prevalence rates and the only validated severity score of calcinosis confined to the hands. This measures features only afforded by x-ray such as bulk and density of calcinosis.

However, this study has been the first to demonstrate no correlation of x-ray severity scores with patient-reported impact which reinforces the priority need for alternative imaging.

This work has produced the first ever definition and working definition of calcinosis using the alternative imaging modality of ultrasound. The ability to detect calcinosis using ultrasound has been shown in this study to be as effective as x-ray with the ability for future assessment of the features identified as most impactful to the patient and not afforded by x-ray.

The first definition of calcinosis using ultrasound provides the foundations for further validation as a measure of severity in respect of patient reported impact. Importantly, it will provide validation of using an imaging tool that is cost effective, easily accessible, non-invasive and can support repeat measures pertinent to monitoring the fluctuating nature and quick progression of calcinosis. This work, led by the author, is already progressing, and will provide the first validation of ultrasound as an appropriate outcome measure for use in the much-needed clinical trials evaluating novel and targeted treatments for calcinosis. This will also aid in further exploration, giving insight to the potential pathogenesis of calcinosis and shape the future of clinical management to improve the quality of life of our patients.

Appendices

Appendix 2: Health Research Approval. Study approval cover letter.

NHS
Health Research Authority

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12 October 2016

Dear Dr DelGaldo

Letter of HRA Approval for a study processed through pre-HRA Approval systems

Study title:	**Factors contributing to the impact of CALcinoSis In connecTivE tissue disease.(CALSITE Study)**
IRAS project ID:	**154667**
Sponsor	**University of Leeds**

Thank you for your request for HRA Approval to be issued for the above referenced study.

I am pleased to confirm that the study has been given **HRA Approval.** This has been issued on the basis that the study is compliant with the UK wide standards for research in the NHS.

The extension of HRA Approval to this study on this basis allows the sponsor and participating NHS organisations in England to set-up the study in accordance with HRA Approval processes, with decisions on study set-up being taken on the basis of capacity and capability alone.

This approval includes a Substantial amendment submitted to the HRA on 30 June for which regulatory approval and HRA Approval has been given.

Participation of NHS Organisations in England

Please note that full information to enable set up of participating NHS organisations in England is not provided in this letter, on the basis that activities to set up these NHS organisations is likely to be underway already.

Appendix 2: Phase One: Calcinosis Patient Information Sheet

Calcinosis Information Sheet

What is it?

Calcinosis is the name given to lumps, plaques or nodules of calcium, which develop under the skin and around the joints.

What does it look like?

Calcinosis can look different from one person to the next. You may have just one small lump close to the surface of the skin or you may have lots of little or large lumps in different areas that may also extend much deeper. When close to the skin surface, the lumps can appear white and even chalky.

Sometimes the calcinosis looks like an arthritic joint. More commonly however, the skin will periodically open up (ulcerate) with small fragments of calcium breaking out of the skin. The calcium deposit may look like a small white stone or more like a white paste, either with or without fluid. The surrounding tissue may look normal, may have rough, dry skin over the top or it may be red and swollen, particularly if it is beginning to ulcerate or an ulcer has already formed. This is also very similar to how gout can appear. The difference is that the lumps/paste in gout are made of uric acid crystals rather than calcium crystals.

Is it painful?

Calcinosis can be extremely painful, or it may not be painful at all. It may only become painful at times when it is knocked, under pressure or when it is starting to ulcerate. Pain is usually eased or cured when the calcinosis deposit leaves the body. More often than not, a new deposit will develop again.

Who is affected?

Calcinosis is commonly seen in Scleroderma but can also be seen in other conditions that affect the connective tissue of the body. Therefore people with conditions such as lupus and dermatomyositis can also suffer with calcinosis.

Where does it affect?

Calcinosis can affect any part of the body and we do not yet understand why some areas are more commonly affected than others and why this differs between different connective tissue diseases.

Appendix 3: Phase Three. Clinical characteristics of calcinosis at different body regions

Phase Three. Clinical characteristics of calcinosis at different body regions

	Face and neck	Trunk	Upper arms/ forearms	Elbows	Thighs/ lower legs	Feet	Knees	Hands
Number (%)	5 (13.5)	4 (10.8)	8 (21.6)	13 (35.1)	8 (21.6)	6 (16.25)	9 (24.3)	37 (100)
Palpable hard	3 (60)	3 (75)	7 (87.5)	13 (100)	8 (100)	4 (66.7)	9 (100)	26 (70.3)
Palpable soft	3 (60)	0 (0)	5 (62.5)	3 (23)	0 (0)	0 (0)	1 (11.1)	7 (19)
Tender	2 (40)	1 (25)	5 (62.5)	10 (77)	2 (25)	4 (66.7)	7 (77.8)	25 (67.6)
Breaking through skin	0 (0)	1 (25)	2 (25)	5 (38.5)	3 (37.5)	1 (16.7)	3 (33.3)	9 (24.3)
Ulceration	0 (0)	0 (0)	1 (12.5)	1 (7.7)	1 (12.5)	0 (0)	1 (11.1)	6 (16.2)
Inflammation - not infected	0 (0)	1 (25)	0 (0)	4 (30.8)	2 (25)	0 (0)	2 (22.2)	10 (27)
Clinically infected	0 (0)	0 (0)	0 (0)	0 (0)	1 (12.5)	0 (0)	1 (11.1)	2 (5.4)
Size: <1cm	5 (100)	3 (75)	5 (62.5)	11 (84.6)	5 (62.5)	4 (66.7)	4 (44.4)	32 (86.5)
Size >1cm	0 (0)	2 (50)	6 (75)	3 (23)	5 (62.5)	2 (33.3)	5 (55.5)	13 (35.1)
Multiple deposits	3 (0)	3 (75)	7 (87.5)	10 (38.5)	4 (50)	4 (66.7)	4 (44.4)	18 (48.6)
Pain score 0-10 (mean (SD))	1.8 (1.64)	2.25 (2.62)	4.9 (1.74)	3.3 (2.3)	2.5 (2.67)	2.7 (1.2)	4.1 (2.4)	3.92 (3.3)

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