Investigation and risk stratification of systemic sclerosis heart involvement

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Intellectual Property and Publication Statements

The candidate confirms that the work submitted is her own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Chapter 4 is based on work from a jointly authored publication by Dumitru RB, Bissell LA (joint first authors) and Erhayiem B, Abignano G, Fent G, Kidambi A, Donica H, Burska A, Del Galdo F, Biglands J, Buckley DL, Greenwood JP, Plein S, Graham L, Buch MH entitled "Incidental significant arrhythmias in Scleroderma associate with cardiac magnetic resonance measure of fibrosis and hs-TnI and NT-proBNP", published in Rheumatology (Oxford) Journal, 2018.

The initial concept and the design of the study were set up by Professor Buch, Dr L Graham and Dr LA Bissell. RB Dumitru was responsible for the study visits between June 2015 and January 2018, that were carried on a 3-monthly basis (unless screening or baseline visit), for the perfusion and LGE CMR quantitative analysis for all patients included in the study and data entry between June 2015 and 2018. RB Dumitru was also responsible for the statistical analysis, interpretation of data and led the manuscript writing with the help of Dr LA Bissell, while Professor Buch and other co-authors revised the draft for important intellectual content and approved the final manuscript for submission.

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List of publications and presentations arising from the thesis

Original articles

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Bissell L-A*, **Dumitru RB***, Erhayiem B, Abignano G, Fent G, Kidambi A, Donica H, Burska A, Del Galdo F, Biglands J, Buckley DL, Greenwood JP, Plein S, Graham L, Buch MH Incidental significant arrhythmias in Scleroderma associate with cardiac magnetic resonance measure of fibrosis and hs-TnI and NT-proBNP, Rheumatology (Oxford) Journal, 2019.

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Goodall AF, Broadbent DB, **Dumitru RB**, Buckley DL, Tan AL, Buch MH, Biglands JD. A Feasibility Study for MRI Based Skeletal Muscle Extracellular Volume Measurement. Radiology, submitted

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Review articles

Mavrogeni SI, Buch HM, Markousis-Mavrogenis G, **Dumitru RB,** Pugliese NR, Gargani L. The perpetual sword of Damocles: Cardiac involvement in systemic sclerosis and the role of non-invasive modalities in medical decision making, European Journal of Rheumatology, submitted

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Dumitru RB et al Implantable loop recorder in systemic sclerosis over three years confirms incidental significant arrhythmia and suggests cardiac MRI and cardiac biomarker association. Presented at:

- The British Society of Rheumatology Congress (BSR), Liverpool, May 2018
- Romanian Congress of Rheumatology, Bucharest, October 2017

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Abstract

Primary systemic sclerosis primary heart involvement (pSSc-HI) is one of the leading causes of death in SSc. Sensitive testing suggests that the majority of patients have subclinical involvement; however, there is little understanding of its pathogenesis, dynamics and importantly, the optimal approach to clinical detection and management. Identifying the at-risk patient for more intensive monitoring and management is thus essential.

By applying sensitive tools, including implantable loop recorder (ILR) and cardiovascular magnetic resonance (CMR) the thesis proposes to provide a comprehensive assessment of pSSc-HI and to identify clinical and cardiac serum markers that could aid early identification of patient at risk of pSSc-HI. The thesis also evaluates the predictive value of subclinical CMR abnormalities for the development of cardiovascular (CV) outcomes and assesses for any interval change of CMR detected pSSc-HI and whether disease-modifying anti-rheumatic (DMARD) or vasodilator treatment can alter the course of pSSc-HI.

The thesis demonstrates significant ILR findings especially in a poor prognosis group, supporting therefore the use of ILR in SSc patients. CMR-extracellular volume (ECV), marker of diffuse fibrosis and cardiac biomarkers high-sensitivity troponin I (hs-TnI) and N-terminal pro-brain natriuretic peptide (NT-proBNP) associate with ILR abnormalities, suggesting their potential value in detecting pSSc-HI arrythmias. Three distinctive CMR findings are identified: diffuse and focal fibrosis and reduced myocardial perfusion. Skin score, digital ulcers and cardiac biomarkers are associated with CMR abnormalities, whilst CMR-ECV and NT-proBNP predict the development of future CV outcomes. Finally, no significant interval change in CMR parameters is observed, albeit a higher likelihood of CMR deterioration is noted in a poor prognosis group, whilst DMARD and vasodilator treatment do not seem to alter the course of CMR pSSc-HI.

The results of the thesis are thus providing a first step towards risk stratification of pSSc-HI, that will support future larger studies.

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List of abbreviations

ACA	Anticentromere antibodies
ACR	American College of Rheumatology
AECA	Anti-endothelial cell antibodies
ANA	Antinuclear antibodies
ATS	Atherosclerosis
AUC	Area under curve
AV	Atrioventricular
BMI	Body mass index
BP	Blood pressure
BSA	Body surface area
CAD	Coronary artery disease
CHB	Complete heart block
CK	Creatine kinase
CMR	Cardiac MRI
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CTD	Connective tissue disease
CVD	Cardiovascular disease
dcSSc	Diffuse cutaneous systemic sclerosis
DLCO	Carbon monoxide diffusing capacity
DLCO/VA	DLCO/alveolar volume
DMARD	Disease-modifying antirheumatic drugs
DU	Digital ulcers
ECV	Extracellular volume
EMB	Endomyocardial biopsy
EP	Electrophysiological
ET-1	Endothelin -1
EULAR	European League against Rheumatism
FVC	Forced vital capacity
GAVE	Gastric antral vascular ectasia

GORD	Gastro-oesophageal reflux disease
GI	Gastro-intestinal
HI	Heart involvement
HRCT	High resolution computed tomography
Hs-TnI	High sensitivity cardiac troponin I
HV	Healthy volunteers
ICD	Implantable cardiac device
IHD	Ischemic heart disease
IL-4	Interleukin 1
IL-5	Interleukin 5
IL-13	Interleukin 13
ILD	Interstitial lung disease
IMID	Immune mediated inflammatory diseases
IVC	Inferior vena cava
lcSSc	Limited cutaneous systemic sclerosis
LIRMM	Leeds Institute of Rheumatic and Musculoskeletal Medicine
LV	Left ventricle
LVEDV	Left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end-systolic volume
LVSV	Left ventricular stroke volume
MCP1	Monocyte chemo-attractant protein 1
MMT	Manual muscle testing
MOLLI	Modified Look-Locker inversion
MRI	Magnetic resonance imaging
MRSS	Modified Rodnan skin score
MBF	Myocardial blood flow
MPR	Myocardial perfusion reserve
NFC	Nailfold capillaroscopy
NSIP	Non-specific interstitial pneumonia
NT-proBNP	N-terminal pro-brain natriuretic peptide
NYHA	New York Heart Association

PAH	Pulmonary arterial hypertension
PASP	Pulmonary arterial systolic pressure
PCR	Polymerase chain reaction
PDGF	Platelet derived growth factor
PFT	Pulmonary function tests
PWV	Pulse wave velocity
PRO	Patient reported outcomes
RBB	Right bundle branch block
RHC	Right heart catheterisation
ROC	Receiver operating curve
SD	Standard deviation
Scl70	Anti-topoisomerase antibody
S-HAQ	Sclerdoerma-Health Assessment Questionnaire
SPAMM	Spatial modulation of magnetization
SSc	Systemic sclerosis
SIBO	Small intestine bacterial overgrowth
SJC	Swollen joint count
SSFP	single-shot steady-state free precision
SVE	Supraventricular ectopic
SVEB	Supraventricular ectopic beat
SVT	Supraventricular tachycardia
TJC	Total joint count
TGF-B	Transforming growth factor
TLC	Total lung capacity
UIP	Usual interstitial pneumonia
VA	Valve area
VAS	Visual analogue scale
VE	Ventricular ectopic
VEB	Ventricular ectopic beat
VT	Ventricular tachycardia

Chapter 1. Introduction

Systemic sclerosis (SSc, scleroderma) is a heterogeneous autoimmune disease characterised by vasculopathy and progressive fibrosis of the skin and internal organs.

Cardiac involvement in SSc is a significant cause of mortality, with up to twenty-five percent of the deaths being attributed to cardiac causes (1). In contrast to other autoimmune disease such as rheumatoid arthritis and systemic lupus erythematosus, where atherosclerosis is commonly described, cardiac involvement in SSc is mainly primary, developing as a direct consequence of the disease. The pathological hallmark is myocardial fibrosis, with or without myocardial inflammation (2-4). The manifestations of primary SSc-heart involvement (pSSc-HI) are broad and may include myocarditis, heart failure (systolic or diastolic dysfunction), arrhythmias with associated sudden cardiac death and less commonly pericardial and valvular involvement (5, 6).

The prevalence of pSSc-HI varies greatly between studies, depending on the methods of assessment and definition, ranging from 10-20% when clinically manifest to over 70% when multiparametric testing such as cardiovascular magnetic resonance (CMR) are used (7, 8).

When clinically overt, pSSc-HI has a poor prognosis, with a 5-year mortality rate of 70%(6), being more commonly described in SSc patients with poor prognostic factors including diffuse cutaneous SSc (dcSSc), musculoskeletal involvement, tendon friction rubs, male gender, interstitial lung disease and presence of Scl-70 and anti-U3RNP antibodies (9-11). Whilst these patients carry an overall poor prognosis, owing to multiple organ involvement, especially in the first 5 years of disease, cardiac-related mortality remains one of the main causes of death in SSc. Early identification of the at-risk patient for clinically overt pSSc-HI is therefore essential.

CMR is a well-established multi-parametric tool to identify cardiac pathology, providing anatomical, myocardial function as well as tissue characterisation(12, 13). Myocardial focal and diffuse fibrosis and myocardial perfusion abnormalities have all been described in SSc patients (14-16). However, there is little understanding of when CMR

or other such sensitive cardiac investigative methods be used in the management of patients with SSc. Risk stratification and early detection of cardiac involvement in a general, asymptomatic SSc cohort are lacking. Identifying effective clinical and/or serum surrogate markers to aid risk stratification for pSSc-HI, thus enabling early diagnosis and intervention is an unmet need.

Whilst cardiac pathology has been documented in a majority of SSc patients in autopsy studies and with the use of sensitive testing such as CMR (3, 14, 17, 18), the prognostic implications of these subclinical findings remains unclear though. Identifying those abnormalities that would require more intensive monitoring and establishing whether subclinical abnormalities progress to overt clinical findings needs to be further established.

1.1 Thesis hypothesis and aims

The overall hypothesis of the thesis is that pSSc-HI is prevalent when sensitive tools are used, and a composite of clinical and serum cardiac markers can risk stratify for CMR-detected pSSc-HI and thus clinically relevant pSSc-HI.

1.1.1 Aims

The individual aims of the thesis are described below:

<u>With the use of the implantable loop recorder (ILR):</u>

1. To describe the prevalence of electrophysiological abnormalities in SSc patients with no cardiovascular disease (CVD), as determined by ILR
2. To explore the association between ILR-arrythmias and CMR findings
3. To explore the association between ILR-arrythmias and serum cardiac biomarkers

<u>With the use of the CMR:</u>

1. To describe the prevalence of pSSc-HI in a regional SSc cohort
2. To evaluate the association between CMR abnormalities and disease characteristics
3. To assess the association between CMR abnormalities and cardiac serum biomarkers

4. To explore for predictive capacity of clinical phenotype and serum cardiac biomarkers and CMR detected pSSc-HI
5. To evaluate whether CMR together with cardiac serum biomarkers can predict the development of pSSc-HI CV outcomes
6. To evaluate the course of pSSc-HI by CMR and understand:
    a. Whether CMR abnormalities change over time
    b. Whether early subclinical diffuse fibrosis (ECV) progresses to areas of more focal established myocardial fibrosis (LGE)
    c. Whether CMR detected pSSc-HI associates with progression to clinically overt pSSc-HI
    d. To evaluate for an association of change in CMR abnormalities with disease phenotype and serum cardiac biomarkers
    e. To evaluate whether DMARD and/or vasodilator treatment alters the course of CMR findings
7. To assess the association between pSSc-HI and peripheral muscle involvement
    - To evaluate the feasibility of T1 mapping in the peripheral muscle of SSc patients
    - To evaluate for T1 mapping detected muscle fibrosis in SSc patients
    - To assess for any association of T1 mapping detected muscle fibrosis with clinical and biological parameters in SSc
    - To assess for an association between cardiac and peripheral muscle T1 mapping detected diffuse fibrosis in SSc

1.2 Thesis outline

The outline of the thesis is summarised below:

Chapter 2: Literature review

A review of the literature was undertaken on the most employed investigative tools used for the identification of pSSc-HI, with a focus on CMR, the most accurate method for assessing pSSc-HI. The review also discusses the prevalence of pSSc-HI as assessed

by different investigative tools and association with disease phenotype. Finally, the review seeks to highlight the drawbacks and unmet needs of pSSc-HI literature.

Chapter 3: Methodology

This chapter describes the core methodologies employed for the several studies included in this thesis.

Chapter 4: Identifying implantable loop recorder detected abnormalities and investigating the association with cardiovascular magnetic resonance and cardiac serum biomarkers (ELCASA study)

This chapter aims to evaluate the feasibility of the ILR in SSc and describe the prevalence of ILR arrythmias and conduction abnormalities. The study also seeks to investigate the association between ILR-abnormalities and CMR and cardiac biomarkers.

Chapter 5: Phenotyping subclinical systemic sclerosis heart involvement with cardiovascular magnetic resonance and predicting cardiovascular outcomes

This chapter aims to provide a comprehensive assessment of CMR abnormalities in SSc, including assessment of focal and diffuse fibrosis and quantitative perfusion and find clinical and serum cardiac markers that associate with CMR abnormalities and could aid risk stratification for CMR pSSc-HI. Secondly, the study aims to find CMR predictors of pSSc-HI CV outcomes.

Chapter 6: Describing the course and natural history of cardiovascular magnetic resonance-detected heart involvement in systemic sclerosis

This chapter assesses the course of subclinical CMR findings, evaluating whether CMR changes over time and whether DMARD or vasodilator treatment can alter the course of pSSc-HI

Chapter 7: Extracellular volume measurement in peripheral muscle and the association with heart involvement in systemic sclerosis

This chapter aims to assess the feasibility of T1 mapping in the peripheral muscle of SSc patients and the association of T1 mapping with clinical and serum biomarkers as well as to investigate the association between cardiac and peripheral muscle involvement in SSc.

Chapter 8: Discussion

This chapter brings together the findings of the entire thesis and discusses their contribution to the published literature and future research directions.

Chapter 2. Review of the literature

This review details the background of SSc, including prevalence, aetiopathogenesis, classification criteria; with a focus on the evidence base for investigative tools used for the diagnosis and monitoring of pSSc-HI and association with disease phenotype. A comprehensive review on the use of CMR in SSc is also discussed and finally, based on the current literature, the review will highlight the unmet needs in pSSc-HI.

2.1 Systemic sclerosis

Systemic sclerosis (SSc, scleroderma) is a heterogeneous autoimmune disease characterised by vasculopathy and progressive fibrosis of the skin and internal organs. The disease is classified into a limited cutaneous (lcSSc) and a diffuse cutaneous (dcSSc) form, according to LeRoy classification (19). LcSSc is characterised by skin sclerosis confined to face and extremities, with possible extension to forearms and typically, anticentromere antibody (ACA) positive status. Generally, a more indolent course is seen; Raynaud's phenomenon usually precedes the disease onset by years, patients have less internal organ involvement but higher prevalence of pulmonary arterial hypertension (PAH). DcSSc is characterised by a more dynamic course; Raynaud's phenomenon usually develops at the same time or within one year of the skin changes onset, skin sclerosis extends proximally to involve the upper limbs and/or chest and/or abdominal wall, with more frequent internal organ complications such as lung fibrosis and renal crisis, and usually positive anti-topoisomerase antibody (Scl-70) status.

2.1.1 Epidemiology

Systemic sclerosis is a rare disease, with varying incidence and prevalence between countries and continents. The reported prevalence of SSc is consistently higher in USA and Australia compared to Japan and Europe (20-23). A large multicentre US study documented a prevalence of SSc ranging from 13.5/100.000 in 2003 to 18.4/100.000 in 2008 with an incidence rate of 5.6 cases per 100.000 (24). In Europe, the prevalence ranges between 31 and 154 cases per million with lower rates reported in northern European countries, suggesting a north-south gradient and clustering in certain regions (20). Highest prevalence of SSc was reported among a group of Choctaw Indians living

in Oklahoma, USA, with a prevalence of 469 cases per 100.000 in full-blooded Choctaws (25).

The reported incidence also varies depending on the definition, classification of SSc and time period, the incidence ranging from 4 per million per year in 1988 (26) to 20 per million in 2014 (27).

Overall, there is a substantial female predominance with a female/male ratio of 3.8:1 (28, 29). The age of onset varies according to gender and ethnic background but has a peak incidence between the 4th and 5th decade, with an earlier onset age for females than males (20, 30).

2.1.2 Diagnosis and classification criteria

Classification criteria for systemic sclerosis were initially developed by the American College of Rheumatology (ACR) in 1980 (31) (Table 2-1).

Table 2-1 Table 1980 ACR Scleroderma Classification Criteria

Major criterion	Proximal cutaneous sclerosis/skin thickening (non-pitting) proximal to the MCPs (metacarpophalangeal joints), affecting other parts of the body, usually bilateral, symmetrical, and almost always including sclerodactyly
Minor criteria	Sclerodactyly
	Digital pitting scars of fingertips or loss of substance of the distal finger pad
	Bibasal pulmonary fibrosis
The major criterion is sufficient or 2/3 minor criterion are needed to fulfil the classification criteria	

ACR, American College of Rheumatology

The shortcomings of the criteria were that they can miss limited or early SSc. These criteria also do not include specific antibodies such as Scl-70 or ACA or nailfold capillaroscopy changes that can help differentiate between primary and secondary Raynaud's phenomenon.

To overcome these deficiencies, the new ACR/EULAR SSc criteria were developed in 2013 (32). These show good discriminant, face, and construct validity as well as better sensitivity and specificity compared to the 1980 ACR criteria (Table 2-2).

Table 2-2 ACR-EULAR Criteria for the classification of Systemic Sclerosis

Items	Sub-items	Weight/score
Skin thickening of the fingers of both hands extending proximal to the metacarpophalangeal joints *(sufficient criterion)*		9
Skin thickening of the fingers *(only count the highest score)*	-Puffy fingers - Sclerodactyly of the fingers (distal to MCP but proximal to the PIPs)	2 4
Fingertip lesions *(only count the highest score)*	Digital Tip Ulcers Fingertip Pitting Scars	2 3
Telangiectasia		2
Abnormal nailfold capillaries		2
Pulmonary arterial hypertension and/or Interstitial lung Disease* (*Maximum score is 2)		2
Raynaud's phenomenon		3
Scleroderma related antibodies** (any of anti-centromere, anti-Scl 70], anti-RNA polymerase III) (**Maximum score is 3)	Anti-centromere Anti-topoisomerase Anti-RNA polymerase III	3
A total score of 9 or more is needed to fulfil the classification criteria		

ACR-EULAR, American College of Rheumatology- European League against Rheumatism.

Le Roy classification criteria is used to diferentiate between lcSSc and dcSSc (Table 2-3) (19).

Table 2-3 LeRoy's classification of systemic sclerosis

Diffuse cutaneous systemic sclerosis	Limited cutaneous systemic sclerosis
Short interval (<1 year) between onset of Raynaud's phenomenon and the development of skin changes	Long history of Raynaud's phenomenon
Truncal and peripheral skin involvement	Limited skin involvement
Tendon friction rubs	
Lung, renal, diffuse gastrointestinal and myocardial involvement	Late onset pulmonary hypertension, calcinosis, telangiectasies
Scl-70 positive	Anticentromere antibody positive
Capillary drop out visible in nail folds	Dilated nail fold capillaries visible in nail folds

2.1.3 Pathogenesis

The aetiopathogenesis of SSc is not yet fully understood, but is known to comprise the triad of microvascular damage, activation of the immune system and fibrosis (33, 34) (Figure 2-1). There is growing evidence that the initiating process is endothelial cell damage and vascular dysfunction, which further initiates the production of cytokines and the fibrotic process (34).

The hypothesis of vascular dysfunction as a primary pathogenetic process in SSc is supported by the early development of Raynaud's, which precedes by years the skin fibrosis, but also by histopathological findings of endothelial cell apoptosis (33, 35). Several biomarkers have been identified to play important roles in SSc vasculopathy.

Endothelial cell adhesion molecules and anti-endothelial cell antibodies (AECA) are known to play substantial roles in SSc vasculopathy by inducing endothelial cell apoptosis (33, 36, 37). Endothelin-1 (ET-1) is a vasoconstrictor released by the vascular endothelium but also implicated in fibroblast activation. Elevated endothelin-1 (ET-1) levels have been detected in SSc patients with pulmonary hypertension and with advanced microangiopathy on capillaroscopy. Studies have also showed that ET-1 is expressed early in the affected skin and in the SSc-associated fibrotic lung tissue (38, 39). An abnormal endothelial nitric oxide synthase has been also suggested in SSc (40). To some extent, infectious agents, such as cytomegalovirus, may also be involved in SSc pathogenesis and endothelial cell damage(41).

Both innate and adaptive immunity are involved in the pathogenetic process of SSc, which is thought to precede the development of fibrosis. T and B lymphocytes, natural killer cells, dendritic cells, monocytes, mast cells, macrophages and eosinophils are involved in tissue damage and fibrosis in SSc (33). An important role in SSc pathogenesis have T cells, in particular CD4+ cells (42). Activated T cells are present from the very early stages of disease in the skin and peripheral blood of SSc patients, express signs of antigen driven proliferation, and are responsible for producing interleukin (IL)-4, IL-5 and IL-13, which have been associated with the development of fibrosis (43, 44). There is increasing evidence suggesting also the presence of CD8+T cells in the skin of SSc patients, being directly involved in modulating dermal fibrosis (45, 46).

The fibroblast is normally activated in the skin in the context of wound healing. However, in SSc, the fibroblast remains activated, in the form of myofibroblast, and is responsible for the production of extracellular matrix and for sustaining the fibrotic process (47). Various potent mediators of tissue fibrosis have been identified, but one of the most important is transforming growth factor (TGF-β)(48). Multiple studies show that its signalling pathway is hyperactivated in the skin and lungs of patients with dcSSc, being thought to play an important role in the transition of fibroblasts in myofibroblasts(48, 49). Other key factors that are implicated in the pathogenesis of scleroderma and are important determinants in the initiation and the development of

fibrosis are platelet derived growth factor (PDGF) and chemokines such as monocyte chemo-attractant protein 1 (MCP1) or chemokine ligand 16 (CXCL16) (50, 51).

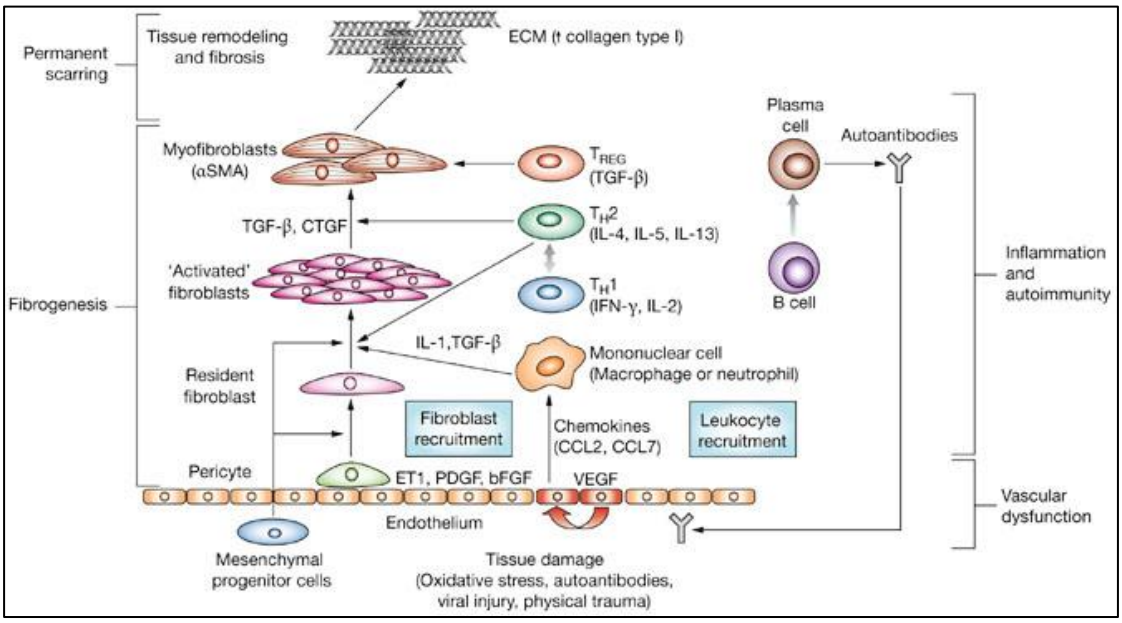


Figure 2-1 Systemic sclerosis pathogenesis (Denton CP et al, Mechanism and consequences of fibrosis in systemic sclerosis, Nature Clinical Practice Rheumatology, 2006).

2.1.4 Clinical findings of SSc

The skin is almost always involved in scleroderma (otherwise termed scleroderma sine scleroderma). Le Roy et al (19) was the first to classify SSc into a limited and diffuse subset according to skin involvement. LcSSc is characterized by skin thickening of the distal extremities and face while dcSSc denotes widespread skin involvement involving the proximal extremities and trunk. Puffiness and swelling may represent early findings of skin involvement and are important red flags signs for the future development of SSc. Indeed, puffy fingers along with the presence of Raynaud's, ANA positivity and SSc capillaroscopy pattern constitute preliminary criteria for a very early diagnosis of SSc (VEDOSS) (52). Subsequent findings of skin thickening and then atrophy are following the oedematous phase of the skin. Modified Rodnan skin score (mRSS) is a validated, widely used clinical measure to assess the skin (53, 54). The thickness of the skin is assessed over 17 sites of the body and scored semi-quantitatively (0-3). Histopathological studies of the skin show alteration of both

dermis and epidermis: atrophy and increased pigmentation of the epidermis, epidermal papillae loss, the presence of hyalinised collagen and myofibroblasts, microvascular and perivascular alteration, and disappearance and entrapment of dermal adnexae and calcification (55-57). Other skin features are represented by hyperpigmentation, generalised or giving a salt and paper appearance, telangiectasia and digital ulcers, the hallmarks of vasculopathy, and subcutaneous calcinosis.

Raynaud's phenomenon is a predominant clinical feature that occurs in over 90% of SSc patients. It is characterised by pallor, cyanosis and erythema of the fingers in relation to cold and occasionally stress exposure. Secondary Raynaud's is distinguished from primary Raynaud's as it develops in the context of a connective tissue disease (CTD) and can include severe ischaemic lesions. The anatomical features of the underlying vasculopathy can be assessed by capillaroscopy, which can detect specific hallmarks in patients with SSc such as haemorrhages, megacapillarries and loss of capillaries (58-60).

Musculoskeletal involvement in SSc is not uncommon and can involve the joints, muscles and tendons. Arthralgia and less often arthritis can be part of the scleroderma spectrum (61, 62). Muscles can also be affected with features of mild muscle weakness to severe myositis, with high CK levels, especially in relation to myocarditis (63). Other musculoskeletal features are tendinitis, joint contractures and periarticular calcifications (64). Often, synovitis and tendon friction rubs have been associated with worse outcome and disease progression (65).

Lung manifestations in SSc have more recently superseded renal disease as the main cause of mortality in SSc (66). Typical manifestations include interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH). PAH has been described in both lcSSc and dcSSc (secondary to parenchymal lung disease) with a prevalence of around 10% (67). It is considered, along with ILD and cardiac involvement to be one of the leading causes of death in SSc (1). The gold standard for the diagnosis of PAH is right heart catheterisation (RHC) (68).

ILD accounts for 33% of SSc related deaths (66, 69). Risk factors associated with ILD are dcSSc, higher mRSS, positive Scl-70 antibodies, cardiac disease and higher creatine kinase (CK) and creatinine levels (70, 71). The most frequent histopathological subset is non-specific interstitial pneumonia (NSIP) but usual interstitial pneumonia (UIP) can also affect patients with SSc (72). Pulmonary function tests and high-resolution computer tomography (HRCT) are the preferred methods for diagnosing and monitoring ILD (73-75).

Renal involvement with the so called "scleroderma renal crisis" usually affects dcSSc, being characterised by malignant high blood pressure, acute renal failure and the presence of microangiopathic haemolysis (76). Risk factors for developing renal crisis include anti RNA polymerase III antibodies and the use of steroids (77).

Gastro-intestinal involvement in SSc is common and affects both limited and diffuse subtypes (78). Gastro-oesophageal manifestations, such as gastro-oesophageal reflux, dysphagia, oesophagitis and dysmotility of the distal oesophagus have been reported in up to 70-90% of SSc patients (79, 80). Less commonly, patients with SSc can develop gastric antral vascular ectasia (GAVE), the so called "watermelon stomach" which can cause gastrointestinal (GI) haemorrhage (81). The intestine can also be affected and can lead to pseudo-obstruction, malabsorption, the development of small intestine bacterial overgrowth (SIBO) and anorectal dysfunction (82, 83).

The heart is one of the major organs affected in SSc and a significant cause of morbidity and mortality in patients with SSc (84, 85). Cardiac involvement accounts for 14% of the deaths in the EUSTAR database, the main causes being arrhythmias (6%) and heart failure (3%) (1). Its prevalence varies between studies, depending on the methods of assessment and definition(7, 8), with a prevalence of 15-30% when clinically evident (10, 84). Heart involvement in SSc can be underpinned by macrovascular disease, which leads to atherosclerosis (ATS) and peripheral vascular disease; or microvascular abnormalities and fibrosis of primary SSc heart involvement (pSSc-HI).

In contrast with other autoimmune/CTD disease such as rheumatoid arthritis and systemic lupus erythematosus, where data suggest high prevalence of ATS, in SSc, the literature is discordant. According to some studies, the prevalence of coronary artery

disease (CAD) is similar to that observed in the general population(86). However, more recent studies report higher prevalence of ATS in SSc, which correlates with poorer outcomes (87-89).

2.2 Systemic sclerosis primary heart involvement (pSSc-HI)

pSSc-HI develops as a direct consequence of SSc and may affect the myocardium, pericardium, and less often, the endocardium. The prevalence of pSSc-HI varies greatly, depending on the investigative methods, ranging from 20-30% (10, 84), to 70-90% when more sensitive testing is used (3, 14, 17) and the definition used; with the majority of studies not fully excluding secondary CVD. pSSc-HI has a poor prognosis when clinically evident, with a 5-year mortality rate of 70% (6).

Factors associated with clinically relevant pSSc-HI are dcSSc, male gender, musculoskeletal involvement, digital ulcers, tendon friction rubs and ILD (11, 84, 90). Patients with pSSc-HI are more likely to have Scl-70 or anti U3RNP positive antibodies (9, 10), and some evidence report also an association of pSSc-HI with anti Ku(91), anti-Histone(92) and anti RNA polymerase antibodies(93).

The principal features/pathogenetic mechanisms of pSSc-HI is fibrosis with/without inflammation.

2.2.1 Aetiopathogenesis of pSSc-HI

The pathogenetic mechanism of pSSc-HI is not well established, the most accredited hypothesis being the vascular mechanism, which has been postulated to underlie the fibrotic process (3, 94). The process is presumed to first include vasospastic episodes followed by microvascular damage and subsequent development of myocardial fibrosis. Cardiac Raynaud's in SSc patients was first documented by Alexander et al (95), which demonstrated the development of cold induced transient myocardial ischemia on thallium-scintigraphy. Other evidence supporting this hypothesis is the presence of contraction band necrosis and arteriolar concentric intimal hypertrophy on histopathology (2, 96, 97). An autopsy study of eight cases with pSSc-HI reported widespread involvement of the small coronary arteries, including mural and intimal

fibrosis, endothelial proliferation, medial hyperplasia, fibrinoid necrosis and platelet-fibrin clots affecting the ventricular myocardium, the sinus node and AV node artery, in the absence of macrovascular disease (4).

Moreover, recent data on the murine models showed that the transgenic overexpression of the activator protein 1 transcription factor-Fra 2, which induces apoptosis of endothelial cells and microvascular alteration, might have an important role in the aetiopathogenesis of pSSc-HI (98).

Histopathological findings of myocardial fibrosis are reported in the majority of SSc patients (99). A study performing endomyocardial biopsy in 25 SSc patient with pSSc-HI reported a degree of myocardial fibrosis (8-32%) in all, but also inflammatory cells, either CD3T lymphocytes or activated MCH II positive CD 68 macrophages in 24/25 patients (17). There is increasing evidence demonstrating the presence of myocardial inflammation in the early stages of the disease, especially in those with a dcSSc and poor prognosis (100, 101). CMR and histopathological studies document the presence of early extensive inflammation in the myocardium, suggesting perhaps a different pathological predominance in a subgroup of patients, where inflammation precedes the fibrotic process and might occur as a result of myocarditis (17, 100).

More recent work of Whitfield and Hinchcliff, who performed genome expression analysis of the skin identified four different gene expression SSc subsets: normal-like, limited, inflammatory and fibroproliferative subset (102). Furthermore, work of the same group demonstrated an association between different gene SSc subsets and distinct molecular signatures, including IFNα and TGFβ in various organs (103, 104). Whether the heart in SSc expresses different gene expression subsets and whether conventional imaging modalities (e.g. CMR) capture these molecular subsets needs to be further investigated. Similar histopathological changes to SSc and SSc heart are also described in inflammatory myopathies (IM). Muscle biopsy in IM describe microvascular injury, including microinfarction, as well as inflammatory infiltrates with the predominance of CD4 (dermatomyositis) or CD8 T lymphocytes (polymyositis) but also macrophages, myeloid dendritic cells and fibrosis (105, 106). Nevertheless, gene expression studies have similarly described interferon alpha and interferon-beta

inducible expression genes in IM (107). These similarities are worth further exploration as they would aid our understanding of the 2 conditions.

2.2.2 Manifestations of pSSc-HI

The array of SSc cardiac manifestations is broad and include cardiac failure (systolic or diastolic dysfunction), myocarditis, arrhythmias, pericardial and occasionally valvular involvement (5).

Diastolic dysfunction is frequently recorded in patients with SSc (108, 109), but it leads uncommonly to diastolic heart failure (110). When present, systolic dysfunction in SSc (111, 112) is associated with a poor prognosis, being either a consequence of coronary artery disease (CAD) or myocarditis and/or myocardial fibrosis (111, 113, 114). Less commonly, a restrictive cardiomyopathy with left or right ventricular involvement, increased ventricular mass and hypokinesia has been reported in SSc (115).

Arrythmias and conduction abnormalities are not uncommon findings, the literature reporting high burden of ventricular ectopics, supraventricular arrythmias but also more serious findings such as atrio-ventricular (AV) block or ventricular tachyarrhythmias, which are associated with increased mortality (116-118).

Pericarditis is a common feature of inflammatory conditions and in SSc can manifest as fibrous pericarditis, pericardial effusion, and rarely as pericardial tamponade or constrictive pericarditis (119, 120). The evidence of an increased prevalence in SSc is debated though, studies reporting similar prevalence when compared to controls (121, 122).

Valvular involvement can also represent a feature of pSSc-HI. Endocarditis, thickening of the valves and valve prolapse has been uncommonly described in SSc patients (5, 101).

2.2.3 Presentation of pSSc-HI

Patients with pSSc-HI may present with exertional or at rest dyspnoea, orthopnoea, paroxysmal nocturnal dyspnoea, fatigue, peripheral oedema, palpitations, dizziness, syncope or chest pain.

Clinical findings suggestive of pSSc-HI include signs of left or right cardiac failure, such as peripheral oedema, evidence of hepato-jugular reflux, pulmonary rales, cardiac murmurs or arrhythmias.

However, distinguishing between pSSc-HI and other more common complications of SSc such as PAH and ILD can be difficult, thus a thorough examination should be undertaken in all patients presenting with any of the above symptoms or signs.

2.2.4 Association between peripheral muscle and cardiac involvement

The nature of peripheral muscle involvement in SSc remains to be fully characterised. There is a lack of new studies investigating peripheral muscle involvement in SSc, however, the existing data describes either an inflammatory pattern, characterised by acute clinical presentation and high level of muscle enzymes, and a non-inflammatory pattern, characterised by mild clinical and biological findings (123).

The process underlying the peripheral myopathy in SSc is complex, with histopathological studies describing stigmata of microangiopathy, fibrosis and inflammation (124).

There are increasing data suggesting an association of myocarditis with myositis (63, 125); these patients commonly present with increased CK and TnI levels, with regression of the inflammatory process once immunosuppressive treatment is commenced (63, 101).

The evidence for an association between the non-inflammatory myopathy and pSSc-HI however is more limited. Follansbee et al. described that 21% of 183 SSc patients with peripheral myopathy had cardiac involvement and there was no difference in the prevalence of pSSc-HI between patients with myositis and non-inflammatory myopathy (126). Using new CMR T1 mapping technique with extracellular volume

(ECV) quantification, one study showed interstitial remodelling of both the skeletal and cardiac muscle (127). Further evidence is needed to evaluate the association between the heart and muscle involvement in SSc.

2.3 Cardiac investigative tools in pSSc-HI

2.3.1 Electrophysiological studies in SSc

Arrhythmias and conduction abnormalities including supraventricular, ventricular and atrioventricular blocks (AVB) are commonly described in SSc and have been shown to be an independent predictor of mortality (116). In the EUSTAR database, half of the SSc-cardiac related deaths were caused by malignant arrhythmias (1). The mechanism underlying arrhythmias in SSc is likely to be multifactorial, thought to include direct effects of microvascular impairment, myocardial fibrosis as well as autonomic dysfunction (4, 128).

Studies employing short term monitoring electrophysiological tools (e.g. electrocardiography (ECG) /24-hour Holter) describe supraventricular/ventricular ectopics (SVE/VE) in up to 75% of the SSc patients (116, 129-131). However, the significance of these findings remains unknown, as unless of high burden, supraventricular and ventricular ectopics represent benign findings. Nevertheless, a higher frequency of autonomic function abnormalities manifested by impaired heart rate variability or heart rate turbulence have been described in SSc patients compared to healthy controls (132, 133). Atrial arrhythmias such as supraventricular arrhythmias, atrial flutter or atrial fibrillation have been also described in up to 40% of the SSc patients(134, 135).

More significant ventricular arrhythmias and AVB such as ventricular tachycardia (VT) and complete heart block (CHB) are less often described in studies using ECG/24-hour Holter (130, 135-138). By using 24-hour Holter ECG, Kostis et al documented SVT in 21% of 183 SSc patients, VT in 7%, CHB in 1% as opposed to SVE and VE which were present in 61% and 67% of the SSc patients (116).

Other reported electrophysiological findings in SSc are ST wave depression, T wave flattening, Q waves and prolonged QTc intervals, that are indicative of myocarditis and/or myocardial fibrosis (118, 139).

Signal average ECG records delayed depolarization of myocardial areas with slow conduction, that can represent the substrate for re-entrant arrythmias. Higher prevalence of late ventricular potentials have been documented in SSc patients compared to HC, indicating the presence of myocardial scar tissue, which can further initiate the development of VT (140, 141).

A small cohort study also documented the benefit of automatic implantable cardioverter-defibrillator (AICD) use in patients with SSc; 3 out of ten patients included in the study developed VT which was successfully reverted with the AICD (142). However, criteria for ICD use in SSc remain to be clearly defined.

The relationship between electrophysiological findings and disease phenotype is not well-established. No clear association with disease subtype, manifestations, disease duration or antibody profile has been found (117, 139). However, the majority of the studies do not attempt to distinguish atrial from ventricular or AV block (e.g. VT, CHB); or to exclude patients with traditional cardiovascular disease (CVD) or pulmonary arterial hypertension (PAH), which can also lead to development of arrhythmias. With electrophysiological studies relying on short term monitoring, the true prevalence of ventricular arrhythmia and AV block may therefore be underestimated.

2.3.2 Echocardiography in SSc

Echocardiography is the most widely used method for assessing cardiac pathology including involvement in SSc. The advantages of echocardiography are that it provides real time evaluation of the anatomy, structure and function of the heart, fast acquisition of the images, being a safe investigative method.

Two dimensional and Doppler echocardiography have been commonly employed to assess cardiac involvement in SSc. The most common echocardiography finding in SSc is a non-restrictive diastolic dysfunction (110, 122, 143). A mild impaired left

ventricular (LV) diastolic dysfunction with an E/A (early to late filling peak velocity ratio of the mitral valve) ratio ≤1 is frequently reported on Doppler echocardiography, being described in up to half of the SSc patients, affecting both limited and diffuse subsets (109, 122, 144). A restrictive mitral inflow pattern has been also reported although on a lower scale in SSc patients. Groote et al performed Doppler echocardiography on 570 SSc patients and detected an impaired systolic function in 1.4%, LV hypertrophy in 22.5%, diastolic dysfunction in 17.7% of which 8.4% had a restrictive mitral inflow pattern in the absence of any cardiovascular pathology. Only female gender but not blood pressure (BP), age, heart rate or SSc subtype was identified to associate with a restrictive mitral inflow pattern when compared to those with normal mitral flow pattern, suggesting the presence of a true restrictive cardiomyopathic process in a subgroup of SSc patients (145). The study did not allow patients with significant cardiac disease such as systolic or diastolic heart failure but did not exclude patients with secondary heart involvement such as IHD or PAH, thus the prevalence of these findings might be overestimated.

Less commonly, an impaired LV systolic function has been also described on Doppler echocardiography, in association with dcSSc, myocarditis and poor prognostic factors of SSc (145). According to the EUSTAR data, 5.4% of the 7,073 SSc patients included in the study had a reduced ejection fraction (LVEF<55%), which associated with age, male gender, history of myositis, digital ulcers, lung involvement and absence of treatment with calcium channel blocker (11).

Right ventricle (RV) impairment has been also reported in SSc, although the majority of the echocardiographic studies do not attempt to exclude patients with PAH, making it difficult to differentiate between pSSc-HI and right sided cardiac abnormalities developed as a consequence of PAH. Few studies have documented the occurrence of RV dysfunction in SSc patients in the absence of PAH or other aetiologies of cardiac pathology (146, 147).

To a lesser extent, pericardial effusion and pericarditis is also described on echocardiography, studies reporting small to moderate pericardial effusions which usually do not associate with a haemodynamic compromise (144, 148).

The more recently developed Tissue Doppler imaging (TDI) provides a more comprehensive assessment of ventricular function, allowing measurement of the peak systolic as well as diastolic velocity (149). By using pulsed-TDI, Meune et al reported decreased systolic mitral annular velocity in 14/100 patients (Sm <7.5/cm), although only 7 had an impaired EF%, and an abnormal LV filling in 30/100 patients (E_A of <10 cm/second). The study also gives information in regards to the RV function, demonstrating an impaired systolic tricuspid annular velocity in 15 patients (ST <11.5 cm/second) (121). Neither age, disease duration, disease subtype, PAH or lung involvement associated with abnormal TDI measurements.

More recently, speckle-tracking echocardiography has emerged as a sensitive tool in the early identification of myocardial systolic dysfunction, providing important information in regards to myocardial deformation in longitudinal, radial and circumferential axes (150, 151). There are increasing data with regards to the use of speckle tracking echocardiography in SSc (152, 153). One study performed speckle tracking echocardiography on 104 SSc patients and showed impaired global longitudinal (GLS) and circumferential strain (GCS) compared to healthy controls (154). An impaired GLS and GCS associated with dcSSc and both GLS and GCS were found to be independent predictors of ventricular arrhythmias. Another study showed left and right ventricular global longitudinal strain impairment in SSc compared to HC, but failed to demonstrate an association with disease subtype, ANA antibodies, gender, age or traditional cardiovascular risk factors (152). Further larger longitudinal studies are required to determine the value of speckle-tracking echocardiography in SSc.

Regional wall motion abnormalities (RWMA) typically occur in the context of CAD but they have also been described in non-ischaemic cardiomyopathies (155). Dobutamine stress echocardiography is one of the methods of choice for assessing hypokinesia. One study has assessed the value of dobutamine stress echocardiography also in SSc; RWMA were detected in 13 of the 30 SSc patients included in the study, having a patchy pattern, not following the coronary artery distribution and more frequently in association with a dcSSc (9/13), suggesting thus the presence of microvascular perfusion abnormalities in SSc (7).

Echocardiography is thus a reliable and accessible investigative method for screening pSSc-HI. One of the main drawbacks of echocardiography is the inability to provide pathophysiological insights but also less reliability and sensitivity.

2.3.3 Cardiovascular magnetic resonance (CMR) in SSc

Cardiovascular magnetic resonance imaging (CMR) is a well-established investigative tool, providing a comprehensive assessment of cardiac abnormalities, including evaluation of morphology, function and tissue characterisation. CMR is widely used in cardiological practice to identify and characterise ischaemic and non-ischaemic cardiomyopathies, to detect oedema and focal processes (e.g. sarcoidosis, amyloidosis) and assess myocardial perfusion (156, 157).

There is a growing body of evidence showing the value of CMR in pSSc-HI (158-160). The morphological and functional CMR module permits good characterisation of the left and right ventricular dimensions, structure and function. Both diastolic and systolic dysfunction as well as RWMA have been described in SSc (111, 161).

CMR has also the ability to provide details in regards to myocardial tissue, in particular myocardial fibrosis, the hallmark of pSSc-HI (156) (Figure 2-4, Table 2-5). Contrast-CMR studies in SSc suggest a specific pattern of myocardial fibrosis, with the identification of a focal/patchy midwall and/or subepicardial distribution (15, 158) distinct from CAD where fibrosis follows the coronary artery distribution and involves the subendocardium extending to the epicardial regions (Figure 2-2). The basal and mid segments are predominantly involved in SSc, with a predilection for the inferoseptal, inferior and inferolateral walls (162-164). Hachulla et al found LGE in 11/52 SSc patients but none in the CMR of healthy controls. The distribution was linear and included mainly the mid-wall (8/11) and less frequently the subendocardium (3/11). No association between CMR-LGE findings and other disease characteristics such as disease subtype, pulmonary fibrosis, pulmonary function tests (PFT) or skin score was found (165) whilst Tzelepis et al documented an association of LGE with longer history of Raynaud's (15).

CMR also has the ability to detect oedema with the use of T2 weighted imaging (166). Myocarditis has been described in up to 10% of the SSc patients, being usually associated with poor prognostic factors (165) (Figure 2-4). Patients with evidence of myocarditis are likely to have early dcSSc, Scl 70+ and concomitant myositis (100, 125, 167). The current CMR criteria for diagnosing myocarditis (Lake Louise criteria) are detailed in Table 2-4 (166).

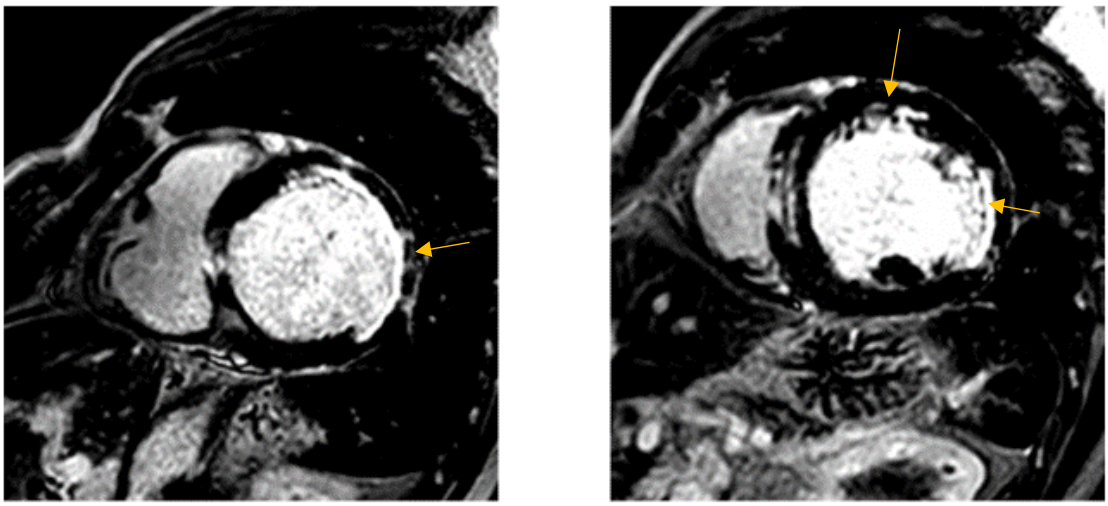


Figure 2-2 Coronary artery disease – Basal and mid short axis view showing 2 vessels myocardial infarction in the same patient affecting the left circumflex (lateral LV wall) and left anterior descending artery territory (anterior, anteroseptal segments)

Table 2-4 Lake Louise consensus criteria for diagnosing myocarditis on CMR

• Regional or global myocardial signal intensity increase in T2-weighted images
• Increased global myocardial early gadolinium enhancement ratio between myocardium and skeletal muscle in gadolinium-enhanced T1-weighted images
• At least one focal lesion with non-ischaemic regional distribution of gadolinium-enhanced T1-weighted images (late gadolinium enhancement)
CMR findings are consistent with myocardial inflammation, if at least two of the above criteria are present

New mapping techniques with extracellular volume (ECV) quantification have emerged as promising tools in detecting diffuse myocardial fibrosis, and evidence suggests good correlation with histological findings of myocardial interstitial fibrosis in various clinical contexts (168, 169). Moreover, data from non-ischaemic cardiomyopathies demonstrate that ECV is a poor prognostic marker for CV outcomes including systolic/diastolic dysfunction and arrhythmic events (170). There are increasing data looking at the prognostic value of T1 mapping in SSc, with studies demonstrating higher ECV and T1 native values compared to HC, independent of the presence of focal LGE fibrosis (127, 159, 163, 171) (Figure 2-4). Ntusi et al performed T1 mapping CMR in 19 SSc patients and showed significantly higher ECV values in SSc compared to HC (35.4 ± 4.8 vs. 27.6 ± 2.5%, $p < 0.001$) and in those with DcSSc compared to lcSSc (37 ± 4 vs. 33 ± 5%, $p = 0.002$). Higher ECV also showed association with mRSS ($R=0.60$, $p = 0.03$) and disease activity index ($R=0.60$, $p = 0.04$) (159). Another CMR study that performed T1 mapping on 33 SSc patients demonstrated strong association of ECV with left atrial volumes ($r=0.52$; $p=0.002$) the degree of diastolic dysfunction, and GCS, implying diffuse fibrosis as a mechanistic basis for the development of these findings (172).

Myocardial microvascular dysfunction has been described in several entities, including diabetes, hypertrophic cardiomyopathy and vasospastic angina (Figure 2-3) (173-175). The aetiology of microvascular dysfunction in diabetes is complex, thought to include hyperglycaemia, insulin resistance, chronic inflammation, alteration in the peroxisome proliferator-activated receptor-y signalling pathway, increased oxidative stress, with the subsequent development of endothelial dysfunction (176). The presence of myocardial microvascular dysfunction also in SSc has been documented in several CMR studies (14, 177). The perfusion defects do not follow the coronary distribution, are diffuse, affecting the whole subendocardium, reflecting thus microvascular perfusion impairment. Reyna et al showed that out of 62 SSc patients, 79% had low signal subendocardial perfusion defects on CMR, with no relation with disease subtype, atherosclerotic disease or calcium scores (14). Schicchi et al reported CMR perfusion defects in 14/24 patients, of which 10 had subendocardial lesions and 4 had the whole myocardium affected; 6 out of the 14 patients had also LGE in the same areas. There

was no association with disease subtype, however, patients with perfusion defects had a disease duration longer than 2 years (177).

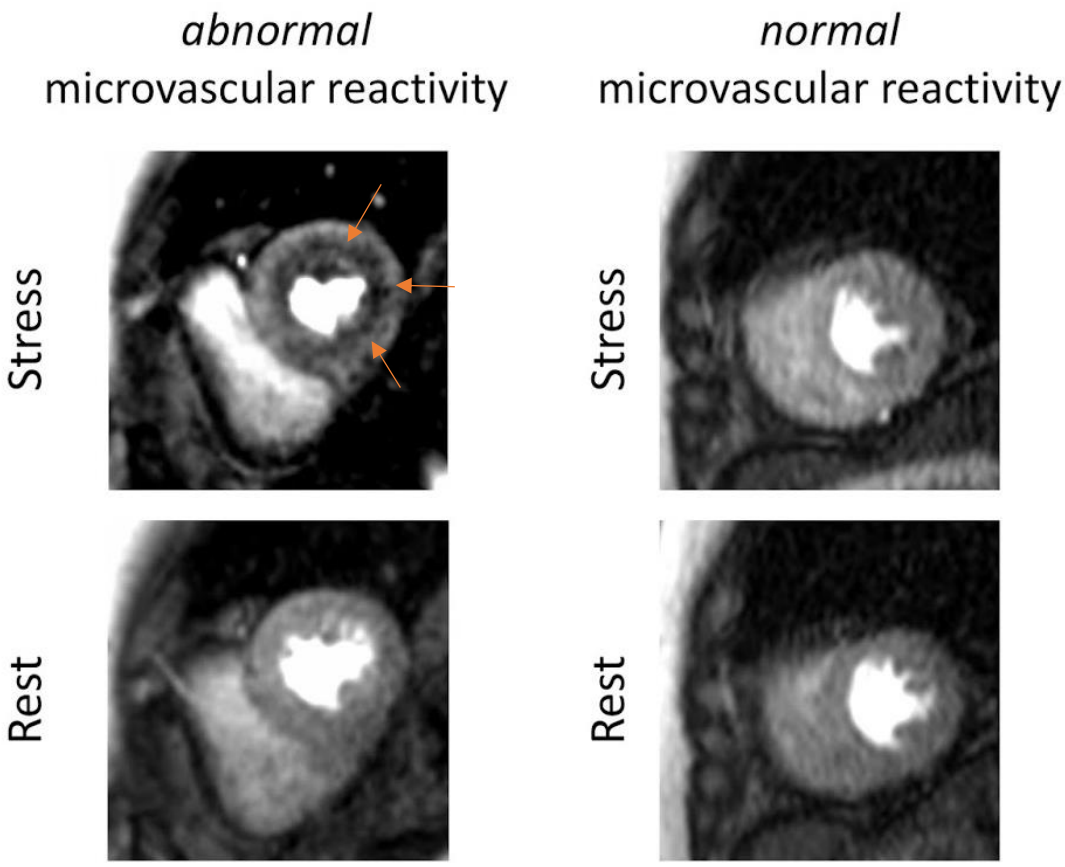


Figure 2-3 Microvascular impairment on CMR: diffuse, subendocardial perfusion defect in the left ventricle (upper left image, arrows) with normal rest perfusion scan (lower left image) and normal scan on the right (Laboratory for Translational Imaging of Microcirculation)

Several CMR studies in SSc also included quantitative perfusion analysis (178, 179). Myocardial blood flow (MBF) at rest and stress are determined, which allows calculation of myocardial perfusion reserve/index (MPR), a ratio of maximal stress/resting MBF. In a small study of nineteen SSc patients, Gyllenhammar et al demonstrated global reduction in MBF at stress compared to HC (3.1 ± 0.9 vs. 4.2 ± 1.3 ml/min/g, p= 0.008), which in the absence of epicardial coronary artery disease is indicative of myocardial microvascular impairment (179). Stress MBF did not differ between the limited and diffuse subtype nor did it associate with nailfold capillaroscopy density (179). Two additional studies of the same group found

significant increase in MPR on CMR and tissue Doppler echocardiography following Nifedipine, respectively Bosentan administration (16, 180).

CMR thus represents a gold standard imaging technique, for the detection of pSSc-HI whether subclinical or in the context of SSc patients with clinical presentation. The limitations of CMR include it's cost and contraindication for patients with allergy to contrast agents, those with retained metal and renal impairment.

A description of the CMR studies to date in SSc is presented in Table 2-5.

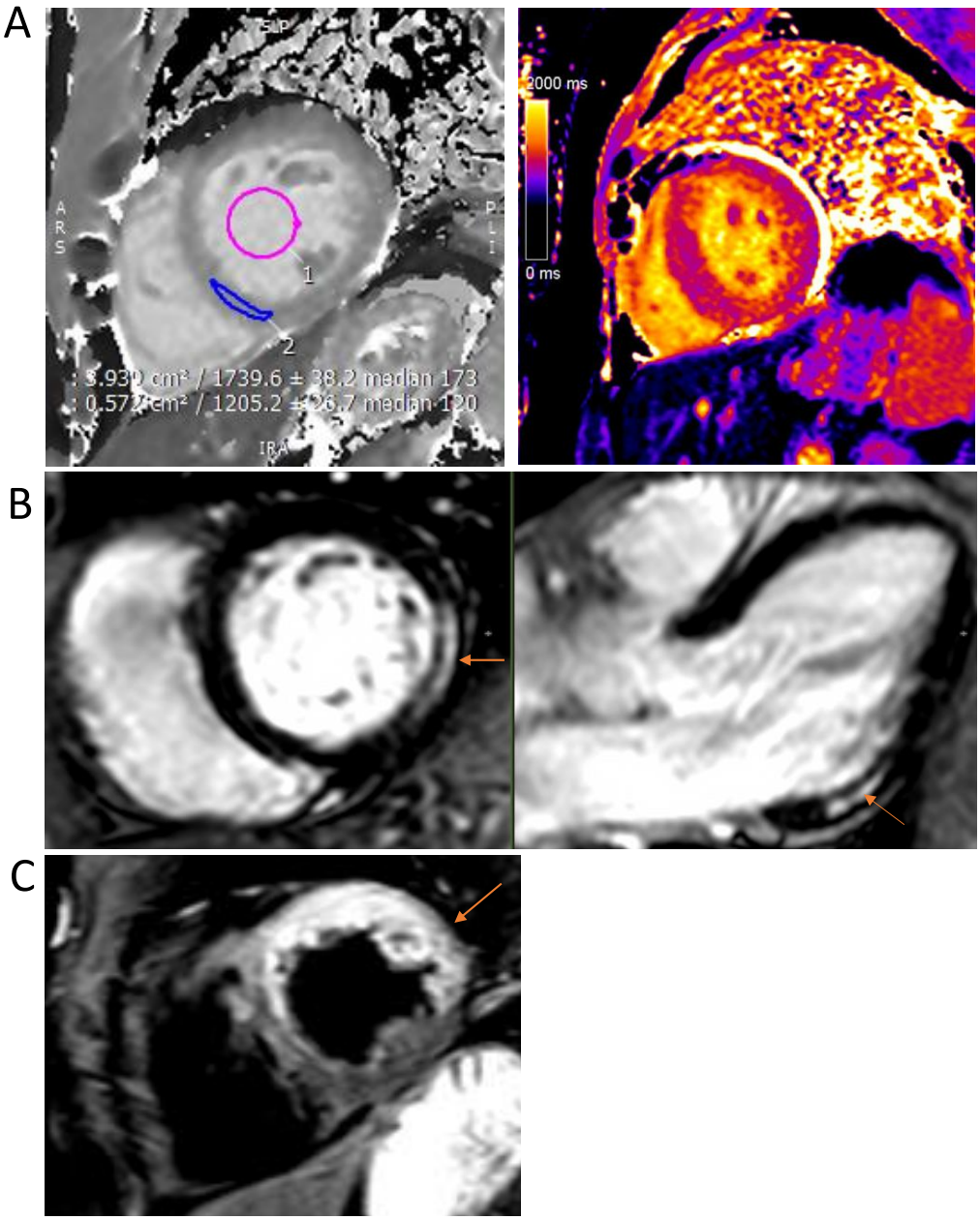


Figure 2-4 Different CMR sequences and abnormalities utilised for evaluating pSSc-HI A: T1 mapping with ECV measurement - left image : black and white map where 1 represents ROI in the blood pool and 2 represents ROI in the inferospetal LV wall, right image: coloured map; B. Focal fibrosis in a SSc patient. Basal-mid midwall LGE (arrows) of the inferolateral segment: short axis view (left image) and crosscuts in the 4-chamber view to confirm LGE (right image) C. T2 weighted image showing florid oedema (arrow) in a SSc patient.

Table 2-5 CMR studies using LGE, T1 mapping and ECV quantification and T2 weighted (excluding case reports, studies with CMR <10 participants)

Author, year	**N patients scanned** **SSc subtype**	**Symptomatic/** **asymptomatic patients, other comments**	**Perfusion CMR** N patients Distribution	**LGE** N patients Pattern/Distribution	**ECV %** Mean (SD)	**T2 weighted** N patients Distribution
Mavrogeni, 2015 (18)	n=44 dcSSc=44	Asymptomatic, but only dcSSc	-	0/44, but in 40/44 using quantitative LGE assessment	-	2/44 Distribution not specified
Rodriguez-Reyna TS, 2015(14)	n=62 lcSSc=33; dcSSc=29	NA High number of patients with perfusion defects ? ischemic	49/62 subendocardial	28/62, intramural (36% linear, 18% patchy), mixed - 26%, subedocardial- 11%, transmural - 7%	-	-
Moroncini G, 2014 (177)	n=24 lcSSc=16; dcSSc=8	Both	14/24 10 subendocardial	6/24	-	-

Author, year	**N patients scanned** **SSc subtype**	**Symptomatic/** **asymptomatic patients, other comments**	**Perfusion CMR** N patients Distribution	**LGE** N patients Pattern/Distribution	**ECV %** Mean (SD)	**T2 weighted** N patients Distribution
		But also, secondary causes: IHD, PAH	4 transmural			
Kobayashi H, 2009 (181)	n=10 lcSSc=5; dcSSc=5	Asymptomatic	5/10 subendocardial	3/10 2/3: nonsegmental, subendocardial 1/3: segmental, subendocardial	-	-
Hachulla, 2009 (165)	n=52 lcSSc=32; dcSSc=20	Excluded IHD, but not PAH, other CVD	-	11/52 8/11 midwall, linear pattern 3/11 subendocardial	-	6/52 LV myocardium, transmural

Author, year	**N patients scanned** **SSc subtype**	**Symptomatic/** **asymptomatic patients, other comments**	**Perfusion CMR** N patients Distribution	**LGE** N patients Pattern/Distribution	**ECV %** Mean (SD)	**T2 weighted** N patients Distribution
Tzelepis G, 2007 (15)	n=36 lcSSc=13, dcSSc=23	NA Included PH on echocardiography	-	24/36, midwall, linear pattern	-	-
Krumm P, 2016 (94)	n=20 lcSSc=1, dcSSc=19	Both, not excluded secondary CVD	-	13/20 3/20 - linear patchy pattern 10/20 - diffuse pattern	-	-
Sano M, 2014 (182)	n=40 lcSSc=14; dcSSc=26	Both, excluded IHD and severe CVD, PAH not excluded	-	7/40 patchy and linear pattern: basal to mid IVS and the RV insertion points	-	-

Author, year	**N patients scanned** **SSc subtype**	**Symptomatic/** **asymptomatic patients, other comments**	**Perfusion CMR** N patients Distribution	**LGE** N patients Pattern/Distribution	**ECV %** Mean (SD)	**T2 weighted** N patients Distribution
Di Cesare E, 2013 (164)	n=58 lcSSc=33; dcSSc=25	Asymptomatic	-	25/58 16/25: linear pattern LV mid myocardial layer 9/25: patchy, midwall	-	-
Barison A, 2015 (127)	n=30 lcSSc=28, dcSSc=2	Asymptomatic, PAH not specified, majority lcSSc	-	7/30: patchy (inter-ventricular septal insertion) (n=3) subepicardial (n=2), midwall (n=1), subendocardial/transmural (n=1)	30±4 (vs 18 ± 4 HC)	-

Author, year	**N patients scanned** **SSc subtype**	**Symptomatic/** **asymptomatic patients, other comments**	**Perfusion CMR** N patients Distribution	**LGE** N patients Pattern/Distribution	**ECV %** Mean (SD)	**T2 weighted** N patients Distribution
Ntusi NA, 2014 (159)	n=19 lcSSc=10, dcSSc=9	Asymptomatic, PAH not specified	-	10/19: patchy, mid-wall, septum	35.4 ± 4.8 (vs 27.6 ± 2.5 HC)	N not specified Focal myocardial oedema within the LV
Hromadka M, 2017 (163)	n=33 lcSSc=7; dcSSc=26	Asymptomatic, mainly dcSSc	-	14/33 Focal, septal: midwall (2/3 patients) freewall (1/3)	27.5 ± 2.8, (vs 22.8 ± 1.9 HC)	1/33, small area of edema
Thunny F, 2014 (172)	n=33 lcSSc=26; dcSSc=7	Asymptomatic, mainly dcSSc	-	-	30.0 (28.0–31.9)	-

Author, year	**N patients scanned** **SSc subtype**	**Symptomatic/** **asymptomatic patients, other comments**	**Perfusion CMR** N patients Distribution	**LGE** N patients Pattern/Distribution	**ECV %** Mean (SD)	**T2 weighted** N patients Distribution
					vs 29.2 (26.8- 29.7) in HC	
Sugiyama K, 2019 (183)	n=49 lcSSc=24; dcSSc=25	Asymptomatic	-	27/49, 16 linear pattern, 7 nodular	-	-
Gargani L, 2018 (162)	n=201 SSc subtype- not specified	Both, IHD not excluded		56/201 14/53- interventricular septum 10/53- Inferior/lateral/ inf-lateral		5/201 Distribution not specified

Author, year	**N patients scanned** **SSc subtype**	**Symptomatic/** **asymptomatic patients, other comments**	**Perfusion CMR** N patients Distribution	**LGE** N patients Pattern/Distribution	**ECV %** Mean (SD)	**T2 weighted** N patients Distribution
				15/53- insertion points Other/overlapping- 14/53 3- Ischemic		
Lee DC, 2018 (171)	n=24 lcSSc=11; dcSSc=13	Symptomatic, IHD not excluded, PAH		8/24 LGE scar mass: mean (SD) 1.6 ± 3.5	30.0 ± 4.2 (vs 24.1 ± 3.5 in HC)	
Gyllenhamar T, et al, 2018 (179)	n=19 lcSSc=12; dcSSc=7	NA PAH, other CVD not excluded	None with visual perfusion defects Lower MP during adenosine stress for SSc	3/19, RV insertion point fibrosis		

Author, year	**N patients scanned** **SSc subtype**	**Symptomatic/** **asymptomatic patients, other comments**	**Perfusion CMR** N patients Distribution	**LGE** N patients Pattern/Distribution	**ECV %** Mean (SD)	**T2 weighted** N patients Distribution
			vs HC (3.1 ± 0.9 vs. 4.2 ± 1.3 ml/min/g, p = 0.008)			
Muresan L, 2018 (131)	n=30	Both PAH/CVD not excluded		25/30		
Meduri A, 2017 (160)	n=50 lcSSc=19; dcSSc=31	Both, Secondary causes not excluded	2/50	17/50 7-linear subendocardium; 4-difusse subendocardium; 3- subepicardial; 2 -intramural, subepicardial		5/50 Distribution not specified

Author, year	**N patients scanned** **SSc subtype**	**Symptomatic/** **asymptomatic patients, other comments**	**Perfusion CMR** N patients Distribution	**LGE** N patients Pattern/Distribution	**ECV %** Mean (SD)	**T2 weighted** N patients Distribution
				LGE scar mass mean=3.6		
Giacomelli R, 2017 (178)	n=16 Early SSc	Asymptomatic, but all VEDOSS	6/16 visual perfusion defects mean (SD) MPI in SSc with visual perfusion defects 2.45 ± 0.83 vs 2.43 ± 0.39 in those with no perfusion defects	1/16	-	-
Vignaux, 2005 (16)	n=18 LcSSc=8; dcSSc=10	Asymptomatic, but IHD not excluded	13/18 Nifedipine treatment increased MPI from median (range) of 0.19 (0.05) to 0.26 (0.07), p=0.0003			

Author, year	**N patients scanned** **SSc subtype**	**Symptomatic/** **asymptomatic patients, other comments**	**Perfusion CMR** N patients Distribution	**LGE** N patients Pattern/Distribution	**ECV %** Mean (SD)	**T2 weighted** N patients Distribution
Allanore, 2006 (180)	n=18 LcSSc=8; dcSSc=10	Asymptomatic, but not excluded IHD	SSc had lower median (range) MPI compared to HC: 0.17 (0.09–0.23) vs 0.23 (0.21–0.24); p = 0.0023). Bosentan increased MPI from a median (range) of 0.17 (0.09–0.23) to 0.22 (0.13–0.30), p= 0.0004			

CMR, cardiovascular magnetic resonance imaging; CVD, cardiovascular disease; dcSSc, diffuse cutaneous SSc; ECV, extracellular volume; IHD, ischemic heart disease; IVS, interventricular septum; lcSSc, limited cutaneous SSc; MP, myocardial perfusion; MPI, myocardial perfusion index; N, number of patients; NA, not available; SSc, systemic sclerosis; RV, right ventricle.

Specific standardised protocols for clinical application of CMR in SSc are lacking. However, the current CMR protocol recommended by the Society of for Cardiovascular Magnetic Resonance (SCMR) for the assessment of non-ischaemic LV cardiomyopathies can be reasonably applied. This includes the LV structure and function module, LGE module with the addition of more advanced tissue characterisation modules (T2 weighted, T1 mapping) in the case of suspected myocarditis and perfusion module if IHD needs to be ruled out (13) (Table 2-6).

Table 2-6 Recommended core clinical CMR protocol, following the Society for Cardiovascular Magnetic Resonance (SCMR)

Non-ischaemic LV cardiomyopathies, including myocarditis	• LV structure and function • ±Advanced tissue characterization (for myocarditis) including T2 weighted, T2 mapping, T1 mapping, Early gadolinium module-T1 weighted imaging before and after gadolinium • Late gadolinium module • ±Adenosine stress-rest perfusion imaging/ high dose dobutamine stress functional imaging to exclude IHD

IHD, ischemic heart disease; LV, left ventricle

2.3.4 Serum cardiovascular biomarkers in SSc

Cardiac troponin I (TnI) and cardiac troponin T (TnT) are expressed in the cardiac muscle and are released into blood following myocardial cell death. These are established biomarkers used towards the diagnosis of acute coronary syndromes (184). In SSc, both have been shown to be significantly elevated compared to HC, in the absence of IHD or cardiovascular risk factors (185). Whilst TnI increases only in the context of myocardial injury, TnT is also expressed in skeletal muscle, and can increase in various muscular conditions (184, 186). TnI may thus be more specific for detecting myocardial involvement.

In addition, the high sensitivity (Hs) troponin assay has emerged as sensitive tool in the detection of acute ischaemic events and several studies have demonstrated its prognostic value in SSc (185, 187). One study employing different cardiac investigative tools for investigating subclinical cardiac involvement in SSc showed strong correlation of Hs-TnT with QT interval, suggesting the potential role of the high sensitivity assay in detecting subclinical myocardial injury/fibrosis (188).

N-terminal pro-brain natriuretic peptide (NT-proBNP) is one of the natriuretic peptides that is released by ventricular myocytes in response to increased wall tension. In SSc, NT-proBNP has been found to have prognostic value in detecting PAH and increased levels have been associated with the presence of precapillary pulmonary hypertension (187, 189) and RV dysfunction (190, 191). NT-proBNP has also showed to be a useful marker for the detection of left systolic dysfunction and elevated levels have been associated with the presence of myocarditis (192-194). More recently, NT-proBNP increase has also been observed in the context of arrythmias and high arrhythmogenic burden in SSc (195, 196).

Both NT-proBNP and hs-TnT were found to associate with mRSS and to inversely correlate with disease duration (188). Bosello et al measured hs-TnT and NT-proBNP in 245 SSc patients showing elevated levels in those with dcSSc and higher mRSS, lower EF% on echocardiography and right bundle branch block on ECG. Both these biomarkers have also been observed to predict cardiac related death in SSc (197).

Increased CK levels have also been demonstrated in SSc patients in the context of myocarditis, typically in association with peripheral myositis (114). However, it's sensitivity and specificity is lower given that CK is released also by skeletal muscle (198, 199). CK-MB, found primarily in the heart could be thus more specific for assessing cardiac involvement in SSc, along with TnI and NT-proBNP.

Serum cardiac biomarkers may therefore be of use for detecting pSSc-HI, when CAD (in the context of highly increased and dynamic changes in Tn levels) and PAH (in the case of raised NT-proBNP) have been ruled out. TnI may be more sensitive compared to TnT in detecting true primary myocardial involvement in SSc.

2.3.5 Endomyocardial biopsy in SSc

Endomyocardial biopsy (EMB) offers definitive tissue diagnosis of myocarditis and possibility to determine aetiopathogenesis in various cardiomyopathies. EMB is obtained with the use of a bioptome and is usually conducted under fluoroscopic guidance. Right ventricular biopsy via the jugular or femoral vein is the usual preferred method although the left ventricular approach is also considered in specific cases. The European Society of Cardiology Working Group recommends performing EMB early, within 2 weeks from when myocarditis is suspected. At least 3 specimens, each 1-2 mm in size are recommended to be obtained in order to avoid sampling error, followed by histology, immunohistochemistry and viral polymerase chain reaction (PCR) testing (200).

Two different types of fibrosis are described in ischaemic and non-ischaemic cardiomyopathies: replacement fibrosis, which is detected by LGE-CMR and represents myocardial scarring and necrotic tissue, and interstitial (or reactive) fibrosis (201, 202). The latter develops as a results of increased collagen synthesis from myofibroblast in the extracellular matrix, has a diffuse distribution, is commonly perivascular, being described in more 'miscellaneous' cardiomyopathies, including diabetes, hypertension, dilated cardiomyopathy and remote peri-infarct zone (202-204). T1 mapping CMR with ECV quantification is the imaging method of choice for detecting interstitial fibrosis (205, 206).

Various types of inflammatory infiltrates including eosinophilic, lymphocytic, polymorphic, giant cell and sarcoidosis have been described in myocarditis. Both lymphocytic and giant cell infiltrates are described in autoimmune myocarditis, with the predominance of anti-CD3 (T lymphocyte marker) respectively anti-CD68 antibodies (macrophage marker) (200).

Although EMB studies in SSc are scarce and usually reported as case series (17, 93, 100) the existent data provide important information with regards to the mechanistic basis of pSSc-HI.

Mueller et al performed EMB from the right ventricle septum of 25 SSc patients with suspected cardiac involvement which was diagnosed based on clinical findings with/without the presence of elevated cardiac biomarkers (TnI elevation levels > 0.03µg/l and/or elevated B-type natriuretic peptide (BNP) levels >100ng/l) and/or arrythmias. All patients had between 8-32% fibrosis and 24/25 patients had varying degrees of inflammation (represented by CD3+ T lymphocytes and MHC II+ macrophages) on EMB. Only 4/16 patients with available CMR data had evidence of LGE, of which 3 had moderate fibrosis and one had grade 3 fibrosis on EMB. The study also demonstrated that the degree of EMB fibrosis and inflammation associated with cardiovascular events, including cardiovascular death, implantable cardioverter-defibrillator shock/arrhythmic event and heart failure (17).

Pieroni et al performed EMB, CMR, scintigraphy and angiography in 7 patients with evidence of heart involvement (chest pain, heart failure and palpitations). Six of the 7 patients had evidence of active myocarditis and variable degrees of interstitial and perivascular fibrosis, 1 patient had chronic/borderline myocarditis, and two patients had evidence of replacement fibrosis on histology. All seven patients had raised cardiac enzymes, 6/7 had focal fibrosis on LGE-CMR, 2/7 had increased signal intensity on T2-weighted images and none had evidence of IHD on angiography (100).

Autopsy studies in SSc also provide information with regards to pSSc-HI, confirming a high incidence of fibrosis in the myocardium but also the presence of microvascular obstruction (2-4). Follansbee et al reported on autopsy examinations of 54 SSc patients and documented the presence of myocardial fibrosis in 70% of the SSc patients compared to 17 % controls. SSc patients with greater LV dysfunction had more advanced myocardial fibrosis and contraction band necrosis compared to controls, implying microvascular dysfunction as the main pathogenetic substrate in pSSc-HI (3).

EMB can therefore provide important information with regards to the histopathological substrate of pSSc-HI. However, EMB remains an invasive technique with attendant risks, which should only be considered in selected cases and specialised centres where non-invasive testing prove suboptimal. Furthermore, one of the main drawbacks of biopsy is sampling error caused by missing the affected myocardium,

studies reporting a diagnostic performance as low as 25% (207). This is of particular importance in pSSc-HI as the pattern of fibrosis is usually focal/diffuse, affecting more commonly the left ventricle. Biventricular biopsy (208) and the development of real-time CMR-guided EMB (209) could minimise these impediments and increase the rate of detecting myocardial abnormalities in SSc.

2.4 Discussion

The remit of this comprehensive literature review was to provide an overview of pSSc-HI and the most meaningful investigative tools employed for detecting pSSc-HI, describe the prevalence, and find clinical corelates of pSSc-HI. The review also aimed to provide a comprehensive overview on the use of CMR in SSc.

The review showed that the prevalence of pSSc-HI ranges widely between studies, from 5% up to 90%, depending on the methods of assessment and definition. Clinically overt pSS-HI including systolic and diastolic dysfunction, arrythmias and conduction abnormalities or myocarditis as well as more subtle cardiac abnormalities such as CMR and autopsy findings of myocardial fibrosis or microvascular impairment, early findings of myocardial systolic dysfunction and supraventricular or ventricular ectopics are all described in SSc.

The investigative tools that are most commonly employed for detecting pSSc-HI are an integration of short-term electrophysiological studies (ECG, 24-hour Holter), echocardiography, with CMR and more recently also cardiac serum biomarkers being increasingly applied. Whilst EMB is a very important tool for confirming the diagnosis of myocarditis and for informing on pathophysiological substrate in pSSc-HI that could be also valuable for guiding treatment, the risks of an invasive method mean this is reserved whenever the diagnosis is uncertain, particularly for excluding infectious myocarditis.

An association of pSSc-HI with poor prognostic markers of SSc and disease activity has been also highlighted in this review, although clinical surrogate markers for identifying

subclinical pSSc-HI are not yet established. This underscores the need for future studies to aid risk stratification of pSSc-HI.

2.4.1 Drawbacks of pSSc-HI literature

Whilst there is a sizeable literature in SSc-HI, a key limitation of many studies is the lack of differentiation between primary and secondary SSc cardiac disease, with studies frequently including patients with secondary heart involvement such as pulmonary hypertension and IHD (145, 162). Whilst real-life patient cohorts will include combination of both primary and secondary HI, and standard prognostic pharmacotherapy is implemented in primary pSSc-HI; distinguishing between primary and secondary cardiac involvement is invaluable to inform on specific pathogenetic drivers, optimal detection and management strategies (including role of immunosuppressive therapies).

Another drawback of the literature is represented by the heterogeneity in the definition of cardiac disease and the lack of differentiation between clinical and subclinical findings (7, 8).

Also, the SSc population included in the studies is not always homogenous, some studies having a predilection for including dcSSc (18, 94) or lcSSc (127). Thus, the prevalence of pSSc-HI may be either over/or under-estimated.

The discrepancy in the prevalence of pSSc-HI is also a result of the use of different protocols/techniques as well as interpretation of findings with over-reporting of abnormalities. Currently, there are no specific protocols for use in echocardiography or CMR to assess pSSc-HI.

2.4.2 Drawbacks of CMR pSSc-HI literature

CMR is the most accurate tool for the identification of pSSc-HI pathology and this review highlights the current literature on CMR findings in SSc. Myocardial focal and diffuse fibrosis, perfusion abnormalities, and less often myocarditis are all described in SSc.

Whilst myocarditis is likely to occur in SSc patients with poor prognosis (63, 114), no clear association of subclinical CMR findings and disease phenotype has been reported (14, 158, 177). Standard cardiac biomarkers TnI and NT-proBNP are widely used in cardiological practice and their value in pSSc-HI has been recently documented (185, 192). However, no studies to date have assessed the value of cardiac biomarkers in detecting CMR defined pSSc-HI. Identifying surrogate markers and development of risk models comprising clinical and/or biological markers to aid risk stratification and thus early identification of patients with subclinical and clinically overt pSSc-HI is an unmet need.

Moreover, the prognostic value of subclinical CMR findings in the development of CV events remains poorly understood. Myocarditis is associated with a poor prognosis, leading to systolic or diastolic heart failure, arrythmias and cardiovascular death in the general population but also in SSc patients (63, 200). Myocardial fibrosis has been associated with major arrhythmic events and the need for ICD implantation in both ischaemic and non-ischaemic cardiomyopathies (210, 211) but the prognostic implications of myocardial fibrosis in SSc hasn't been yet established.

In addition, there is little understanding of the course of subclinical cardiac findings, whether and which abnormalities progress to and associate with clinically overt pathology and require more intensive monitoring and treatment.

Another important matter that needs to be explored is whether treatment can alter the course of pSSc-HI. The use of immunosuppression therapy is documented although not clearly proven in non-viral myocarditis, including SSc myocarditis (100, 200, 212, 213). There is also no evidence on the use of DMARD and/or vasodilator treatment in CMR defined pSSc-HI, where myocardial fibrosis (with/without inflammation) and/or microvascular impairment are the main features. This is extremely important for preventing SSc-cardiac mortality. This would also have implications for other cardiac pathologies where fibrosis represents the main feature.

Thus, this thesis intends to fill these knowledge gaps to provide a better understanding of the pathophysiology, nature and course of pSSc-HI; as well as identify surrogate markers that could aid risk stratification in pSSc-HI.

2.5 Summary

This review highlights the current literature on the most common investigative tools employed for the detection of pSSc-HI. The prevalence of pSSc-HI varies greatly between studies, depending on the definition and methods of assessment. With more sophisticated tools, pSSc-HI is detected in the majority of SSc patients. Electrophysiological studies, echocardiography, cardiac serum biomarkers and CMR are valuable tools in detecting both clinical and subclinical pSSc-HI. EMB can also provide important information in regards to the pathophysiological substrate of pSSc-HI but its use is limited due to its invasive nature. CMR can accurately detect myocardial focal and diffuse fibrosis, microvascular impairment and myocarditis in SSc. Although there are increasing data with regards to CMR defined pSSc-HI, the prognostic value of these findings is poorly understood.

2.6 Key messages

1. pSSc-HI is prevalent but varies depending on the definition and method of assessment
2. Electrophysiological studies, echocardiography cardiac serum biomarkers and CMR are useful for detecting both clinical and subclinical pSSc-HI
3. CMR is one of the most accurate tools for informing on pSSc-HI
4. Further studies to inform on the significance of subclinical pSSc-HI, it's prognostic value and course are needed to help risk stratification in pSSc-HI

Chapter 3. Methodology

This chapter describes the core methods employed for the studies of this thesis. Specific methods are described in more detail in each chapter.

3.1 Design and target population

Prospective observational cohorts were used for all the studies included in this thesis. The studies were undertaken between February 2013 and January 2019.

SSc patients with no past history of CVD, minimal (see eligibility criteria later) traditional cardiovascular risk factors, and no known pulmonary hypertension attending the specialist SSc clinic at the Leeds Teaching Hospitals NHS Trust, Chapel Allerton Hospital were approached. All patients fulfilled the 2013 American College of Rheumatology (ACR)/European League of Rheumatism (EULAR) criteria for SSc (32) and were classified as lcSSc or dcSSc according to LeRoy classification criteria (19).

3.2 Ethical approval

All participants provided informed consent and the research was undertaken in compliance with the Declaration of Helsinki. SSc patients were consented to one (or more) of the following studies:

- ELCASA (The ELectrophysiology and CArdiac Imaging in SclerodermA) study (REC 12/YH/0298)
- CONVAS (Leeds Teaching Hospitals Connective Tissue Disease and Vasculitis Cohort Cross-sectional and Longitudinal Clinical and Basic Science Evaluation) main and cardiovascular sub-study (REC Ref: RR10/9608, RR10/9608 respectively)
- MUSCLE II study (novel Magnetic resonance imaging and UltraSound CLinical Evaluation of muscle pathology): with Contrast-Enhanced MRI (REC ref: 17/EM/0079)

3.3 Withdrawal

Patients were free to withdraw from the study at any time and were asked at each visit whether they wished to continue to be part of the study. All data and blood samples that were collected prior to the withdrawal were included in the final statistical analysis (unless patients also withdrew consent for this).

3.4 Eligibility criteria

The following inclusion and exclusion criteria applied for all participants in the study.

3.4.1 Inclusion Criteria

1. Between 18 and 80 years old
2. Capable of understanding and signing an informed consent
3. Meet the 1980 American College of Rheumatology (ACR) criteria and ACR/European League of Rheumatism (EULAR) criteria 2013 (32)

3.4.2 Exclusion Criteria

1. Prior diagnosis of cardiovascular disease (cardiac, peripheral or cerebral)
2. Diagnosis of pulmonary hypertension
3. Diagnosis of Diabetes mellitus
4. More than one (or two for CONVAS study) of 5 traditional cardiovascular risk factors, including: high blood pressure, dyslipidaemia, family history of premature cardiovascular disease (before 55 years of age for men and 60 year for women), and current smoker
5. Exclusion criteria for the CMR: pregnancy, breastfeeding, pacemakers, surgical clips within the head, certain inner ear implants, neuro-electrical stimulators or metal fragments within the eye or head, asthma requiring previous hospital

admission or oral steroids, an eGFR less than 45 ml/min/1.73m², or any allergy to contrast administration.

3.5 Informed consent

All participants provided informed consent. Each patient received a patient information sheet to read with at least 24 hours for the patient to consider the study. The patients were then contacted to determine whether they were interested to participate in the study and to discuss further enquiries with regards to the study. RBD or one of the study investigators took consent. The original consent was kept in the site file whilst one copy was given to the patient and another copy was filed in the notes.

3.6 Data collection and storage

Clinical data were stored pseudo-anonymously for all participants, in an electronic database. Patients were identifiable by date of birth and study number only. Serum samples that were collected were stored anonymously in the Leeds Institute of Rheumatic and Musculoskeletal Medicine (LIRMM) Laboratory.

Data obtained were stored on a University of Leeds computer on a secure drive, provided by the University of Leeds. Access to the study-specific database was restricted to investigators and study staff involved in data entry and analysis.

3.6.1 Clinical and demographic data collection

All SSc patients had clinical and demographic data, SSc disease assessment and CV risk assessment collected. A general and a SSc specific physical examination was undertaken. No longer than 4 weeks was allowed between the clinical visit and the CMR.

The following clinical data was collected:

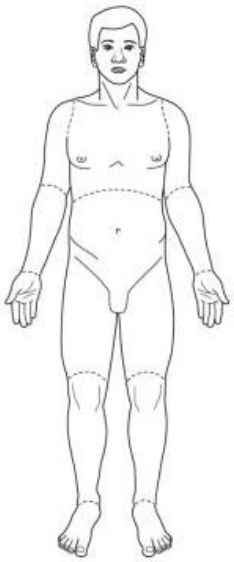
- Age, gender, ethnicity
- Concomitant diseases

- Cardiovascular risk assessment:
  - History of hypertension, dyslipidaemia, smoking habit, family history of premature cardiovascular disease
- SSc disease assessment
  - Disease subtype: limited or diffuse
  - Disease onset: Raynaud's onset and time since non-Raynaud's syndrome onset
  - Internal organ involvement
- Current and previous medication
  - Disease modifying antirheumatic drugs (DMARD), including prednisolone
  - Biological DMARD
  - Vasodilator treatment
  - ACE inhibitor treatment
  - Any other treatment including lipid lowering treatment and any other cardiovascular treatment
- Physical examination, including:
  - Modified Rodnan skin score (mRSS) (214), assessed in 17 different regions by a trained clinician
  - Presence of digital ulcers, tendon friction rubs, calcinosis, digital pits
  - Cardiac, chest, abdominal, neurological, skin findings
  - Swollen joint count (SJC) and tender joint count (TJC)
  - Muscle tenderness

3.6.1.1 mRSS

The mRSS were performed by a rheumatologist, trained in performing mRSS (RBD or LAB, MHB, FDG or SE) or were obtained from one of the LIRMM scleroderma studies (Stratification for risk of progression in scleroderma, STRIKE), providing that the mRSS were performed within 3 weeks of the thesis study visits. Intra and inter-score reliability was thus inferred, from what is a pragmatic, real life cohort study.

MRSS was assessed in 17 different areas including fingers, hands, forearms, arms, upper arms, face, chest, abdomen, thighs, legs and feet. A 0-3 scale was used, 0 indicating no thickening, 1-mild thickening, 2-moderate thickening and 3 severe thickening of the skin. The mRSS ranges between 0 and 51 (Figure 3-1) (53, 215).



	Right				Left			
Fingers	0☐	1☐	2☐	3☐	0☐	1☐	2☐	3☐
Hands	0☐	1☐	2☐	3☐	0☐	1☐	2☐	3☐
Forearms	0☐	1☐	2☐	3☐	0☐	1☐	2☐	3☐
Upper Arms	0☐	1☐	2☐	3☐	0☐	1☐	2☐	3☐
Face			0☐	1☐	2☐	3☐		
Anterior Chest			0☐	1☐	2☐	3☐		
Abdomen			0☐	1☐	2☐	3☐		
Thighs	0☐	1☐	2☐	3☐	0☐	1☐	2☐	3☐
Legs	0☐	1☐	2☐	3☐	0☐	1☐	2☐	3☐
Feet	0☐	1☐	2☐	3☐	0☐	1☐	2☐	3☐
Column Totals								
Total:								
Key: *0 – No Thickening*	*1 – Mild Thickening*		*2 – Moderate Thickening*		*3 – Severe Thickening*			
Notes:								

Figure 3-1 Modified Rodnan skin score assessment in 17 different areas (Khanna D, Standardization of the modified Rodnan skin score for use in clinical trials of systemic sclerosis, Journal of scleroderma and related disorders, 2017)

Swollen joint count (SJC), tender joint count (TJC)

The 28 SJC and TJC was used to assess for any swollen or tender joints of SSc patients (216). This was performed during the clinical visit by RBD or LAB (ELCASA study).

3.6.2 Sample collection

Patients were asked to fast for 8 hours before coming for the study visit and having their blood drawn. Bloods were taken during the clinical visit.

3.6.2.1 Cardiac serum biomarkers

All patients had creatine kinase (CK), high sensitivity- troponin I (hs-TnI) and NT-pro brain natriuretic peptide (NT-proBNP) measured when coming for the study visit (no

more than 4 weeks before having the CMR). CK and hs-TnI were tested on a Siemens Advia XPT system (Advia Chemistry XPT and Advia Centaur XPT Immunoassay respectively) at the Leeds Teaching Hospital NHS Trust, UK. NT-proBNP was tested at the Department of Biochemical Diagnostics, Clinical Hospital No. 1, Medical University of Lublin, Poland. NT-proBNP concentration was measured using Cobas 6000 (immunochemistry module Cobas e601) with the appropriate kits supplied by Roche Diagnostics. According to the manufacturer recommendations, a concentration above 37 ng/ml for Hs-TnI and above 125 ng/ml for NT-proBNP was considered abnormal.

3.6.2.2 Additional blood tests

In addition to serum cardiac biomarkers, all SSc participants had routine clinical blood tests, including full blood count (FBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), urea and creatinine (U&E) and liver function tests (LFT). U&E was checked for all SSc patients prior to having the CMR. Patients were required to have an eGFR >45 ml/min/m^2 in order to have the CMR.

All patients had an ANA screening and/or ANA immunoblot performed. All the above tests were processed at the Leeds Teaching Hospital NHS Trust, UK.

3.6.3 Cardiovascular Magnetic Resonance imaging

All SSc participants underwent one or two CMRs (depending on the study). The CMR scans were performed at the Leeds General Infirmary, Leeds Institute of Cardiovascular and Metabolic Medicine (LICAMM), CMR Department. CMR scans were accessed only by the CMR team and the CMR readers were blinded to the patient's clinical data.

The scan usually took place on a separate day than the study visit. CMR studies were performed on a 3T Philips Achieva MR system equipped with a 32-channel receiver coil. The scan lasted approximately one hour.

Participants were asked not to drink any caffeinated drinks for 24 hours and to avoid having any meals 2 hours prior to the CMR, in order to permit adenosine administration and mitigate against adenosine-associated side effects.

At arrival for the CMR, patients were handed a CMR safety questionnaire. If no safety issues were identified, the weight and height of the patient were recorded. Prior to the scan, patients had 2 cannulas inserted: one for adenosine and the other for gadolinium administration. The cannula used for adenosine administration was connected to a dual head power injector and a 3-way stopcock was used to allow dual bolus administration. A blood pressure (BP) cuff was placed to allow BP measurement during the scan and ECG electrodes were positioned on the chest. The scans were performed by trained radiographers.

3.6.3.1 CMR Protocol

The CMR protocol (Figure 3-2) included:

Cine images

Scout images were used to plan cine images in the vertical long axis, pseudo short axis and horizontal long axis (balanced steady state free precession [bSSFP] acquisition) (Table 3-1). Cine image stack were acquired covering the entire heart. Image acquisition parameters for bSSFP are as follows: TR 2.6 ms, TE 1.3 ms, flip angle 40°, spatial resolution 2.0 × 1.63 × 8 mm^3, 30 cardiac phases (217, 218).

Tissue tagging

Tissue tagging were generated from the basal, mid and apical LV using the'3-of-5' approach (219). The spatial modulation of magnetization (SPAMM) pulse sequence was used to acquire more than 18 phases (Table 3-1). The following parameters were used: spatial resolution 1.51×1.57×10 mm^3, tag separation of 7 mm, typical TR/TE 5.8/3.5 ms, flip angle of 10°.

Aortic distensibility

Sagital-oblique and transverse cines were acquired to measure the diameter and area of the ascending aorta, descending aorta and aortic arch (220) (Table 3-1). The blood pressure and heart rate (at rest) were obtained prior to image acquisition for aortic stiffness measurement.

T1 maps acquisition

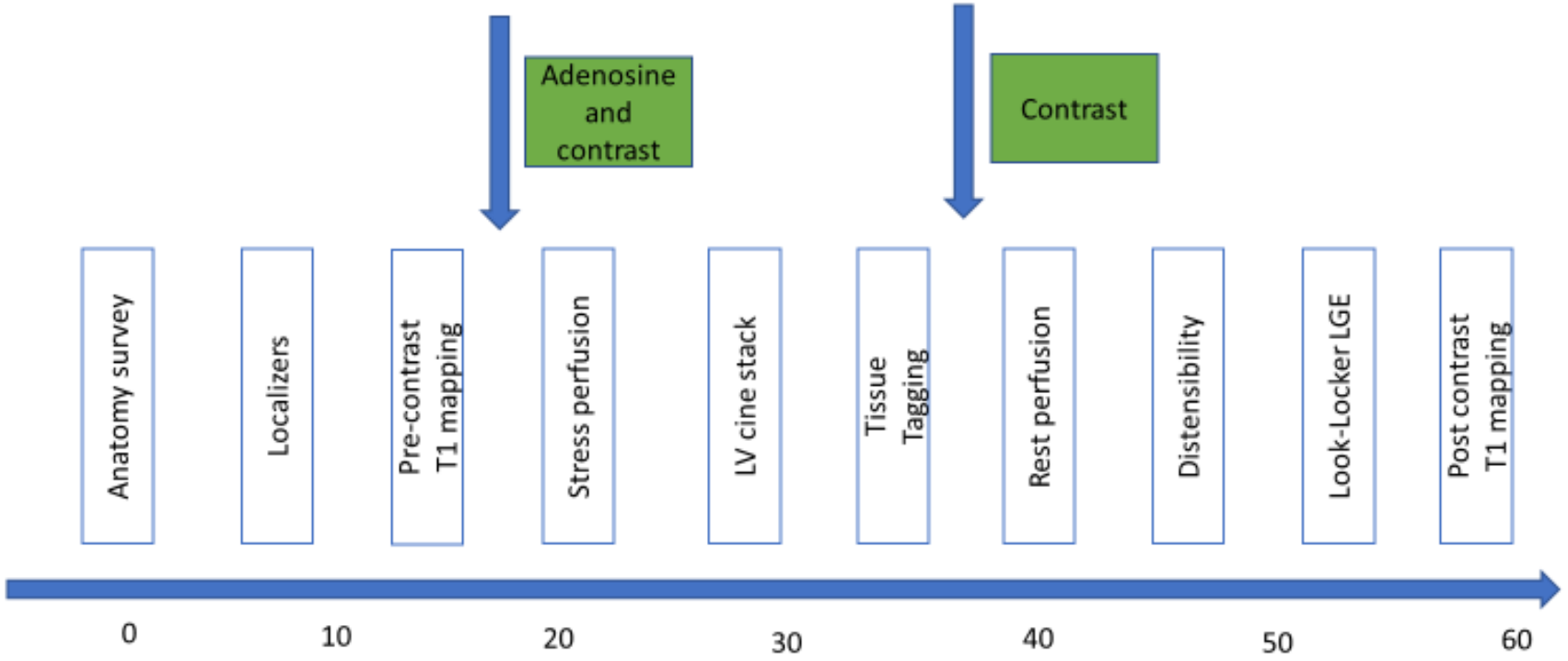
Native and post contrast T1 mapping were planned in a single short axis slice at mid LV level using an ECG-triggered modified Look-Locker inversion (MOLLI) using the '3 of 5' approach, with 3x R-R interval recovery epochs, voxel size 1.7 x 2.14 x 10 mm^3 Trigger delay at end-diastole, flip angle 35°, FOV 320 – 420 mm (221, 222); Post-contrast T1 mapping was acquired 15 minutes after contrast administration (Table 3-1).

First pass myocardial perfusion

Stress first-pass myocardial perfusion was acquired after intravenous adenosine administration (140 mcg/kg/min for three minutes) under continuous ECG monitoring and assessment for adequate haemodynamic response. A Gadolinium contrast agent was used (Magnevist®, 'gadopentane', and from 2018 onwards Gadavist®, 'gadobutrol' at 0.075 mmol/kg, bolus) for both stress and rest first-pass myocardial perfusion. A spoiled turbo gradient echo with a 5 × k-t Broad-use Linear Acquisition Speed-up Technique, 11 training profiles, 1.31 × 1.32 × 10 mm^3 acquired resolution, pre-pulse delay of 100 ms, acquisition shot of 123 ms/slice, flip angle 20°, TR/TE 3.0/1.42 ms and three short axis slices was used (223) (Table 3-1).

Late gadolinium enhancement

Late gadolinium enhancement (LGE) images were acquired between 10 and 15 minutes following contrast administration using inversion recovery-prepared T1-weighted gradient echo. The optimal inversion time to null signal from normal myocardium was determined using a Look-Locker approach (Table 3-1). 10 to 12 short axis slices were acquired, with further slices in the vertical and horizontal long axis orientations, or phase-swapped if indicated. Typical parameters for LGE images were as follows: TE 2.0 ms, TR 3.5 ms, flip angle 25°, acquired spatial resolution 1.54 × 1.76 × 10 mm^3. Alternate heartbeat acquisitions by navigator was used for poor breath holders.



LGE, late gadolinium enhancement; LV, left ventricle; MOLLI, Modified Look-Locker inversion method; SPAMM, spatial modulation of magnetization.

Figure 3-2 Complete cardiovascular magnetic resonance protocol used for the studies

Table 3-1 CMR sequence used and sequence usefulness

Sequence name	Sequence usefulness
Cine SSFP	Myocardial function and volume
SPAMM	Tagging - myocardial strain assessment
ECG-triggered modified Look-Locker inversion	T1 mapping – diffuse, interstitial fibrosis
Cine SSFP of the aorta	Aortic distensibility
Spoiled turbo gradient echo	Stress/rest first-pass perfusion
Inversion recovery-prepared T1-weighted gradient echo	Late gadolinium enhancement - myocardial focal fibrosis

SPAMM, spatial modulation of magnetization; SSFP, single-shot steady-state free precision.

3.6.3.2 CMR image analysis

Image analysis was performed using cvi42 (v4.1.3, Circle Cardiovascular Imaging Inc., Calgary, Canada) and InTag (v1.0, CREATIS lab, Lyon, France) software, in accordance to recognised reporting standards (224) and using established and validated protocols (225). CMR analysis was performed by CMR readers (RBD or cardiology fellows BE or GF). A second CMR expert reader, with more than 5-year experience, assessed the LGE and perfusion images (AK). The CMR's were performed under the supervision of a senior cardiology investigator and CMR expert (SP). Any unexpected CMR findings were discussed with the senior investigator and reports were released and GP informed. John Biglands (Physicist) analysed the perfusion CMR data.

For LV volume measurements, LV contours were drawn manually at both end diastole and end systole on the LV short axis SSFP cine stack to determine LV volumes and function (224). Papillary muscles were considered as part of the LV cavity.

For myocardial strain assessment, tissue tagging data were analysed using a semi-automated method (226). The peak LV circumferential strain was measured at apex, mid-ventricle and base. LV twist was calculated by subtracting the basal from apical rotation. Basal and apical radius were determined from cine images in diastole (227).

$$Torsion = \frac{Peak\ twist\ \times (Apical\ Radius + Basal\ Radius)}{2 \times Apex\ to\ Base\ length}$$

For aortic distensibility, aortic cross-sectional measurements were made by manual planimetry of the endovascular-blood pool interface, at maximal and minimal distension of the aorta. Strain was calculated by dividing the difference between the maximal and minimal distension to the baseline area. The following equation was used to calculate aortic distensibility ($mmHg^{-1}$) (228).

$$Aortic\ distensibility = \frac{Strain}{Pulse\ pressure} \times\ 1000$$

Native and post-contrast myocardial T1 was measured by delineating a region of interest in the mid interventricular septum (229) and in the LV blood pool. Care was taken to avoid partial-volume effects from neighbouring tissue or blood pool when delineating the region of interest (ROI). The following formula was used to calculate extracellular volume (ECV), where R1 is 1/T1 and myo pre and myo post are the pre and post-contrast myocardial T1 values and blood pre and blood post are the pre-contrast and post-contrast blood pool T1 values (230).

$$ECV\ = (1 - hematocrit) \times \frac{R1\ myo\ post - R1\ myo\ pre}{R1\ blood\ post - R1\ blood\ pre}$$

LGE was reported according to the 16 segment American Heart Association (AHA) model (231). Quantitative LGE scar mass assessment was performed using the five-standard deviation (5SD) method (232), a semi-automated method. A ROIs representing remote myocardium with no LGE was delineated on the LGE short axis images with scar. Automated calculations of the scar mass was then obtained.

Perfusion was firstly assessed visually by comparing the rest and stress perfusion images using the 16 segment AHA model (231). Quantitative analysis of the perfusion data was performed to generate estimates of myocardial blood flow (MBF) at stress and rest. Myocardial perfusion reserve (MPR) values were calculated by dividing the stress by the rest MBF. Breathing motion in the dynamic series was corrected using an automated registration algorithm (Circle Cardiovascular Imaging, Calgary, AB, Canada). Regions of interest defining the myocardium and a region within the left

ventricular blood pool were then used to generate signal versus time curves. Signal values were converted to contrast agent concentrations using the pre-contrast T_1 measurements and the equation for the imaging sequence derived from the Bloch equations (233, 234). The calibration factor S_0 was derived from the pre-contrast image signal (SI_{pre}) and pre-contrast T_1 (T_{1_pre}) as follows:

$$S_0 = \frac{SI_{pre}}{f(T_{1\_pre})}$$

Where *f(…)* denotes the equation describing the imaging pulse sequence(233). The T_1 at each time point $T_1(t)$ was then obtained from the signal intensity *SI(t)*:

$$T_1(t) = min\{(S_0.f(T_1(t)) - SI(t)^2)\}$$

The contrast agent concentration, *C(t),* was then obtained as follows:

$$C(t) = \frac{\frac{1}{T_{1(t)}} - \frac{1}{T_{1\_pre}}}{r_1}$$

Where r_1 is the contrast agent relaxivity.

The concentration versus time curves for the blood pool and myocardium were then used to estimate myocardial blood flow using model independent deconvolution(234, 235). The following equation was minimized to find the flow weighted response function $R_f(t)$:

$$min\left\{\left\|A.R_f(t) - C_m(t)\right\|^2 - \lambda^2\left\|LR_f(t)\right\|^2\right\}$$

A is the convolution matrix operator calculated form the arterial concentration curve (236) (4), C_m is the myocardial concentration versus time curve, *L* is the identity matrix and λ, is coefficient that determines the degree to which the solution is forced to be smooth by the side constraint $\left\|LR_f\right\|^2$. The optimal value for λ was determined using the L-curve method (234, 237). The MBF was taken as the maximum value of the flow weighted response function *Rf(t)*.

According to the departmental reference ranges, an ECV >29% and T1 native > 1240 ms were considered abnormal (238, 239).

3.6.4 Additional Cardiac Testing

3.6.4.1 Electrocardiography (ECG)

A 12 lead ECG was performed for all SSc patients participating in the studies. This was performed by the study investigator (RBD) or one of the research nurses (for ELCASA study) when patients attended for the clinical study visit, in Chapel Allerton Hospital. The following data were recorded (Table 3-2).

Table 3-2 Twelve lead ECG data collected

Heart rate (bpm)	Rhythm: Sinus/AF/Atrial flutter/other (specify)
Axis	AV-block: No/First degree/Second degree/Third degree
PR interval (ms)	QRS duration (ms)
QTc (ms)	P pulmonale: No/Yes
P mitrale: No/Yes	Signs of RV hypertrophy: No/Yes
Strain: No/Yes	Signs of LV hypertrophy: No/Yes
Right bundle branch block: No/Incomplete/Complete	Left bundle branch block: No/Incomplete/Complete
Left anterior fascicular block: No/Yes	Left posterior fascicular block: No/Yes
ST elevation No/Yes	ST depression No/Yes

AV, atrioventricular; LV, left ventricle; RV right ventricle.

3.6.4.2 Doppler Transthoracic Echocardiogram (TTE)

All SSc participants have yearly Doppler TTE, as part of the routine SSc assessment. The TTE is undertaken at the Leeds General Infirmary, Leeds Teaching Hospital NHS Trust by trained echocardiographers. The echocardiography results that were closest to the study visit were collected for all SSc patients (Table 3-3). For the longitudinal CONVAS study, a minimum interval of 12 months was allowed between the CMR study visit and Doppler TTE.

Table 3-3 Doppler TTE data recorded

LA: Normal/Dilated (mm)	RA: Normal/Dilated (mm)
LVEF %	Diastolic dysfunction
Valvular stenosis/regurgitation (>grade 1)	Tricuspid valve annular motion (mm)
Aortic stenosis: grade, gradient mean/max, VA	Aortic regurgitation: grade
Mitral stenosis: grade, gradient mean/max, VA (cm2)	Mitral regurgitation: grade
Tricuspid stenosis: grade, gradient mean/max, VA (cm2)	Tricuspid regurgitation: grade
IVC: Not dilated/Dilated – variable with respiration/Dilated – not variable with respiration	RV: Normal/Dilated (mild/ moderate/ severe)
RV function: Normal/Depressed (mild/moderate/severe)	RV-RA gradient (mmHg)
Pericardial effusion Absent/ present (mild/moderate/ severe)	

IVC, inferior vena cava; LA; left atrium; LVEF, left ventricle ejection fraction; RA, right atrium; RV, right ventricle; VA, valve area.

3.6.5 Non-cardiac tests

3.6.5.1 Nailfold capillaroscopy

Nailfold capillaroscopy is used to evaluate the microvascular abnormalities at the nailbed (60). A video-capillaroscope was used to assess capillary morphology and architecture of the fingers for all SS patients included in the PhD studies. Participants were instructed to avoid removing their nail cuticles and to remove any nail polish prior to have the nailfold capillaroscopy. A drop of vegetable oil was placed on the nailfold in order to improve the image resolution. A 200x magnification was used to assess the capillaries. The capillaroscopy was undertaken by experienced rheumatologists (RBD, LAB or LG).

The following parameters were recorded:

- Capillary density - a density of above 9 capillaries/ mm was considered normal
- Capillary dimensions: width and length
- Avascular areas
- Capillary distribution
- Capillary shape
- Subpapillary venous plexus visibility

Based on the findings, patients were classified as having either non-specific/normal findings either an early, active or late SSc pattern (240).

A normal pattern is characterised by a normal distribution and density of the capillaries, with the absence of haemorrhages and giant capillaries.

An early pattern is defined as few enlarged/giant capillaries, few capillary haemorrhages, no evidence of loss of capillaries and a good capillary distribution.

An active pattern is defined as frequent giant capillaries, frequent haemorrhages, moderate loss of capillaries, absent/mild capillary ramification and mild disorganisation of the capillary architecture.

Late pattern is defined as few or absent giant capillaries, severe loss of capillaries, few/absent giant capillaries or haemorrhages, disorganisation of the normal capillary array with the development of ramified or bushy capillaries.

3.6.5.2 Pulmonary function tests (PFT)

Spirometry, lung volumes and carbon monoxide diffusing capacity (DLCO) are measured on a yearly basis for all SSc patients attending the SSc clinic at the Leeds Teaching Hospital NHS Trust. PFT are undertaken at Saint James Hospital, Leeds, in the PFT Lab. The PFT results that were closest to the study visit were collected for all the study participants and included:

- Total lung capacity (TLC), the maximum volume of the lungs after maximum inspiration
- Forced vital capacity (FVC), the volume of air following forced inspiration
- DLCO, the extent of the carbon oxide transfer from the lung to the blood stream
- DLCO/alveolar volume (VA)

3.7 Statistical analysis

The statistical analysis was performed using SPSS (IBM SPSS Statistics 22), Graph Pad Prism 8 and R (version 3.5.2). Complete details on statistical analysis used are described in each relevant chapter.

Chapter 4. Identifying implantable loop recorder - detected abnormalities and investigating the association with cardiovascular magnetic resonance imaging and cardiac serum biomarkers (ELCASA study)

4.1 Introduction

Primary scleroderma-heart involvement (pSSc-HI) is associated with significant mortality (241, 242). The European Scleroderma Trials and Research (EUSTAR) study reported that 14% of deaths were secondary to myocardial disease, with 6% of all deaths due to arrhythmias (243). Whilst increased cardiovascular disease (CVD) of autoimmune diseases occurs mostly in the context of atherosclerosis, the hallmark of primary pSSc-HI is primary myocardial fibrosis, with or without myocardial inflammation (244). The underlying pathophysiology of pSSc-HI and associated arrhythmias however remains unclear. An interplay of the processes of myocardial fibrosis, microvascular injury and autonomic dysfunction with/without left ventricular dysfunction likely drives arrhythmogenic potential. Furthermore, myocardial fibrosis appears to occur both independently, and/or as a consequence of microvascular perfusion abnormalities (244-246).

Several studies have investigated pSSc-HI, mainly employing imaging and/or electrophysiological (EP) studies to identify functional, structural and/or conduction abnormalities(139, 165). Most of these however have not attempted to distinguish ischaemic heart disease (IHD) from primary pSSc-HI, potentially missing specific disease insights and tailored management. EP studies to date (116, 247) have usually included supraventricular (SVE) and ventricular ectopics (VE) as abnormal, which unless of high burden, are considered benign; in contrast to serious ventricular arrhythmia and AV block including ventricular tachycardia (VT) and complete heart block (CHB) (116, 139, 247). In addition, the EP monitoring methods (electrocardiogram (ECG), 24-72 hour ECG monitoring) employed only cover a limited period. The implantable loop recorder (ILR), used routinely in the investigation of

(unexplained) syncope (248), can record EP data for up to three years. The value of ILR to screen for significant arrhythmias such as VT and CHB in asymptomatic but high-risk individuals, including SSc has not been evaluated to date. Such early detection in patients with SSc could ensure timely intervention with appropriate drug therapy and/or device (implantable cardiac defibrillator (ICD)), preventing their associated morbidity or mortality. Cardiovascular magnetic resonance (CMR) imaging, which has the ability to interrogate functional parameters and also provide tissue characterisation (100, 245, 246) is increasingly used to investigate pSSc-HI. Late gadolinium enhancement (LGE) that reflects focal fibrosis has been typically described. More recently, extracellular volume (ECV), an indicator of diffuse fibrosis that has shown prognostic relevance in the general population can also be measured (249). An association between CMR findings and conduction abnormalities in SSc however is not clear.

Cardiac serum biomarkers have also emerged as useful tools in SSc (187). Both troponin I and NT-proBNP have been shown to be elevated in SSc patients compared to healthy controls and appear to associate with SSc cardiac involvement(185, 197). These serum cardiac bioamarkers could thus be of use for identifying patients at risk of pSSc-HI arrhythmias.

4.1.1 Hypothesis and aims

4.1.1.1 Hypothesis

- ILR can accurately detect arrhythmia and/or conduction abnormalities in SSc
- ILR-arrhythmia and/or conduction abnormalities are prevalent in SSc patients free of CVD
- ILR arrhythmia and/or conduction abnormalities in SSc associate with cardiac serum biomarkers and CMR findings; providing a means for risk stratification and early detection

4.1.1.2 Aims

1. To describe the prevalence of EP abnormalities in SSc patients with no CVD, as determined by ILR
2. To assess the association between ILR-arrythmias and CMR findings
3. To assess the association between ILR-arrythmias and serum cardiac biomarkers

4.2 Methods

4.2.1 Candidate's role in the project

The concept and the design of the study were set up by Professor Buch, Dr Lee Graham and Dr Lesley-Anne Bissell. I was responsible for the study visits between June 2015 and January 2018. Lesley-Anne Bissell and other study co-investigators performed the clinical visits between January 2014 and May 2015. The study visits were carried on a 3-monthly basis (in addition to the screening and baseline visits) and included clinical assessment, serum sample collection, nailfold capillaroscopy and ECG (the latter 2 performed on a 6-monthly basis). The study visits were performed by myself with the help of one of the research nurses. I was also responsible for the perfusion and LGE CMR quantitative analysis for all patients included in the study and for sending the serum blood samples for processing. I was also responsible for data entry and statistical analysis. I drafted the manuscript along with Dr. Lesley-Anne Bissell, and Professor Buch and other co-authors revised the manuscript for important intellectual content for submission.

4.2.2 Study design

The ELectrophysiology and CArdiac Imaging in SclerodermA (ELCASA) study (REC 12/YH/0298) was a single centre observational pilot study of patients receiving standard of care treatment assessing the feasibility of ILR in SSc. Patients with SSc with no cardiovascular disease were recruited from the weekly SSc clinic in Chapel Allerton Hospital, Leeds Teaching Hospital. Patients had an ILR (Medtronic Reveal® XT) implanted at baseline and were followed-up on a 3-monthly basis, for 144 weeks,

when the ILR was explanted and the study ended. Clinical data and various cardiovascular assessments were performed at baseline and yearly thereafter, including ECG, Signal Average ECG, autonomic testing, Holter ECG and echocardiography. Contrast and perfusion CMR were also performed at baseline.

ILR significant EP abnormality was defined as any arrhythmia and/or AV block potentially requiring pharmacotherapy and/or insertion of anti-arrhythmic device.

4.2.3 Eligibility criteria

4.2.3.1 Inclusion Criteria

The same exclusion and inclusion criteria as described in the methodology chapter were applied. Patients had to meet the 1980 American College of Rheumatology (ACR) criteria and ACR/European League of Rheumatism (EULAR) criteria 2013 (32).

Patients were excluded if they had any history of CVD (cardiac, peripheral or cerebral), pulmonary hypertension (PH), diabetes mellitus, more than one of 5 traditional cardiovascular risk factors, including high blood pressure, hypercholesterolaemia /hypertriglycerideamia, family history of premature CVD (before 55 years of age for men and 60 year for women), and current smoker.

The contrast-CMR exclusion criteria (detailed in the methodology chapter) applied to all SSc participants.

4.2.4 Target population

The patients were enrolled from the weekly SSc clinic attending the LTHT. Patients were classified as lcSSc or dcSSc according to LeRoy classification criteria (19).

4.2.5 Ethical approval

All participants provided written informed consent as described in the methodology section. For the purpose of this study, patients were consented to the ELectrophysiology and CArdiac Imaging in SclerodermA (ELCASA) study (REC 12/YH/0298).

4.2.6 Study schedule

Patients were followed up in the study for 144 weeks. Patients firstly attended for the screening visit, followed by the baseline visit within four weeks and then followed up every 3 months, at weeks 12, 24, 48, 60, 72, 84, 96, 108, 120, 132 and 144. The visits consisted of clinical assessment and additional investigations, depending on the study visit. Every 6 months, patients had blood samples and patient reported outcomes (PRO) collected. At baseline and yearly thereafter, patients had cardiovascular assessments and nailfold capillaroscopy performed.

4.2.7 Study visits

4.2.7.1 Screening visit

Patients were screened and if eligible, consented to the study. A patient number was assigned. Clinical data, including physical examination, current medication and past and present medical history was collected at this visit as well as urinalysis and fasting blood samples. Patients also had a 12 lead ECG and chest x-ray performed if this wasn't performed in the last 24 weeks.

4.2.7.2 Baseline visit

The baseline visit took place within 4 weeks from screening. The inclusion/exclusion criteria and the results of the laboratory tests collected at screening were checked to determine if the patient was still eligible for the study. Clinical data, physical examination, including height, weight, waist and hip circumference measurement, vital signs (blood pressure, heart rate, body temperature) and PRO were collected. All participants had a general and SSc specific clinical assessment. Patients also had fasting blood samples and a nailfold capillaroscopy performed.

The following cardiac investigative procedures were carried out at baseline (within 4 weeks from the date of baseline visit):

- 12-lead ECG
- Doppler echocardiography

- 3T Cardiac MRI, performed prior to the ILR insertion
- Insertion of Reveal® implantable loop recorder

4.2.7.3 Follow-up visit (Weeks 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144)

Clinical data, including physical examination and SSc evaluation were collected at each visit. ILR data were downloaded remotely every 3 months and/or triggered by the patient in the event of symptoms suggestive of EP event. Blood samples, HAQ and VAS were also collected every 6 months.

In addition, at visit 6, 10 and 14 (Weeks 48, 96 and 144) additional investigations were carried out including 12 lead ECG, signal average ECG, 24-hour Holter, Doppler echocardiography, autonomic testing and nailfold capillaroscopy.

4.2.8 Data collection

4.2.8.1 Clinical data and demographics collection

- Age, gender, ethnicity
- Concomitant diseases
- Cardiovascular risk assessment:
  - History of hypertension, dyslipidaemia, smoking habit, family history of premature cardiovascular disease
- SSc disease assessment
  - Disease subtype: limited or diffuse
  - Disease onset: Raynaud's onset and time since non-Raynaud's syndrome onset
  - Internal organ involvement
- Current and previous medication
- Physical examination, including:
  - mRSS (214) was assessed in 17 different regions by a trained clinician
  - Presence of digital ulcers, tendon friction rubs, calcinosis, digital pits
  - Cardiac, chest, abdominal, neurological and musculoskeletal examination
  - Swollen joint count (SJC) and tender joint count (TJC)

4.2.8.2 Patient reported outcomes (PRO)

Scleroderma Health Assessment Questionnaire (S-HAQ) (250) was completed by each SSc patient at baseline. SHAQ comprises HAQ and Visual analogue scale. HAQ is an index that measures the quality of life, and covers 8 different activity domains addressing the following questions:

- Dressing and grooming – Are you able to:
    - Dress yourself, including tying shoelaces and doing buttons
    - Shampoo your hair?
- Arising - Are you able to:
    - Stand up from an armless straight chair?
    - Get in and out of bed?
- Eating - Are you able to:
    - Cut your meat?
    - Lift a full glass to your mouth?
    - Open a new milk carton?
- Walking - Are you able to:
    - Walk outdoors on flat ground?
    - Climb up five stairs?
- Hygiene - Are you able to:
    - Wash and dry your entire body?
    - Take a tub bath?
    - Get on and off the toilet
- Reach - Are you able to:
    - Reach and get down a 5 pound object (such as a bag of sugar) from just over your head?
    - Bend down and pick up clothing off the floor?
- Grip - Are you able to:
    - Open car doors?
    - Open jars that have been previously opened?
    - Turn faucets on and off?
- Activities - Are you able to:

- Run errands and shop?
- Get in and out of a car?
- Do chores such as vacuuming or yardwork?

Any aids and devices need to be confirmed for each domain. A HAQ score between 0 and 1 indicates mild to moderate disability, a score between 1 to 2 indicates moderate to severe disability and a score between 2 and 3 indicates severe to very severe disability.

The SSc VAS covers six domains including pain, gastro-intestinal and lung involvement, Raynaud's, digital ulcers, and overall disease severity. A scale from o to 10 is used with 0 indicating no limitation and 10 very severe limitation.

4.2.8.3 Sample collection

Patients were asked to fast for 8 hours before attending the relevant study visit and having their blood drawn. Fasting blood samples were collected at screening, baseline and every 6 months thereafter. At screening, if the plasma glucose test result was ≥7.1mmol/L the test was repeated and if again ≥7.1mmol/L, diabetes mellitus was confirmed, and patient was excluded from the study and the patient's GP informed. If at screening, the total cholesterol was ≥5mmol/L, LDL cholesterol ≥3mmol/L or triglycerides ≥2.3mmol/L this was recorded as a cardiovascular risk factor and the GP was informed.

The following blood tests were collected every 6 months: full blood count (FBC), CRP, ESR, U&E, LFT, plasma glucose and lipid profile including HDL and LDL cholesterol and triglycerides. At baseline and yearly thereafter, patients also had immunological tests, including ANA, ACA, Scl-70 performed. All the above tests were processed at the Leeds Teaching Hospital NHS Trust, UK.

In addition to these routine bloods, patients had additional serum samples collected at baseline, week 24, 48, 96 and 144 that were stored (-30^{0}C) and later used for biomarker analysis. Creatine kinase (CK), high sensitivity-troponin I (hs-TnI) which were further processed at Leeds Teaching Hospital NHS Trust, UK and NT-proBNP, processed at the Department of Biochemical Diagnostics, Clinical Hospital No. 1,

Medical University of Lublin, Poland were tested. Full details on processing the samples are described in the methodology chapter.

4.2.8.4 Non-cardiac testing

Nailfold capillaroscopy using light microscopy or videocapillaroscopy method was used to assess capillary morphology and architecture. Based on the findings, patients were classified as having either non-specific/normal findings either an early, active or late SSc pattern (240).

4.2.8.5 Routine Cardiac Testing

4.2.8.5.1 ECG

ECG was performed at baseline and yearly thereafter. Table 4-1 shows the recorded data.

Table 4-1 ECG data collected

Heart rate (bpm)	Rhythm: Sinus/AF/Atrial flutter/other (specify)
Axis	AV-block: No/First degree/Second degree/Third degree
PR interval (ms)	QRS duration (ms)
QTc (ms)	P-pulmonale: No/Yes
P-mitrale: No/Yes	Signs of RV hypertrophy: No/Yes
Strain: No/Yes	Signs of LV hypertrophy: No/Yes
Right bundle branch block: No/Incomplete/Complete	Left bundle branch block: No/Incomplete/Complete
Left anterior fascicular block: No/Yes	Left posterior fascicular block: No/Yes
ST elevation No/Yes	ST depression No/Yes
T wave flattening No/Yes	T wave inversion No/Yes
T wave alternans No/Yes	Biphasic T wave No/Yes

4.2.8.5.2 Doppler Transthoracic Echocardiogram (TTE)

Doppler TTE was performed at baseline, year 1, 2 and 3. The measurements detailed in table 4-2 were recorded.

Table 4-2 Doppler TDE data recorded

LA: Normal/Dilated (mm)	RA: Normal/Dilated (mm)
LVEF %	
Valvular stenosis/regurgitation? (>grade 1)	Tricuspid valve annular motion (mm)
RV function: Normal/Depressed: mild, moderate, severe	RV: Normal/Dilated: mild, moderate, severe
RV-RA gradient (mmHg)	IVC: Not dilated/Dilated – variable with respiration/Dilated – not variable with respiration

IVC, inferior vena cava; LA, Left atrium; RA, right atrium; RV, right ventricle.

4.2.8.6 CMR

The CMR were performed at Leeds General Infirmary, in the CMR Department. The scan usually took place on a separate day than the study visit and prior to the ILR insertion. CMR studies were performed on a 3T Philips Achieva MR system equipped with a 32-channel receiver coil by trained radiographers and cardiologists at Leeds Institute of Cardiovascular and Metabolic Medicine.

The CMR protocol included LV function and volume, late gadolinium enhancement (LGE), myocardial perfusion, T1 mapping for T1 native and extracellular volume (ECV) quantification, tissue tagging and aortic distensibility.

The following CMR parameters were collected for the purpose of this study:

- LV volumes and function of the heart: LV end-diastolic volume (LVEDV) indexed to body surface area(BSA) (ml/m2), LV end-systolic volume

(LVESV)/BSA (ml/m2), LV mass indexed to body surface area(g/m2), LV ejection fraction (LVEF), LV stroke volume/BSA (LVSV) (ml/m2)

- Torsion
- Presence and extent of LGE
- ECV %
- Native T1 (ms)
- Aortic distensibility
- Myocardial blood flow (MBF) at stress and rest and myocardial perfusion reserve (MPR)

Image analysis was performed using cvi42 (v4.1.3, Circle Cardiovascular Imaging Inc., Calgary, Canada) and InTag (v1.0, CREATIS lab, Lyon, France) software, in accordance to recognised reporting standards (224) and using established and validated protocols (225). CMR analysis was performed by 2 blinded expert readers (BE and GF, CMR research fellows) under the supervision of the senior CMR expert, SP. Dr. John Biglands (Physicist) analysed the perfusion CMR data.

Complete details on CMR protocol and analysis are described in the methodology chapter.

4.2.8.7 ILR

The ILR (Medtronic Reveal® XT) was implanted subcutaneously in a left pectoral position using local anaesthesia by LG +/- one of his clinical team. The ILR device was interrogated every 3 months and/or if the patient was symptomatic. The events were either auto-triggered or patient-triggered.

The patient was required to hold a receiver over the Care Link device for the data to be transferred remotely to a secure databank. Data were then reviewed and analysed at the pacemaker clinic, within the Leeds Teaching Hospital NHS Trust, UK. The ILR was removed after 3 years, when the study ended.

4.2.9 Missing data

All efforts were made to avoid missing data. This was a small cohort exploratory study, the analysis included the whole existent data and no imputation was used. The ILR data were missing for one patient due to patient death. CMR data were missing due to poor intravenous access, claustrophobia and/or adenosine administration refusal. Reason for missing sample collection was poor venous access or failure in processing the samples (Figure 4-1).

4.2.10 Statistical Analysis

As a first pilot study, formal power calculation for determining sample size was not appropriate however 20 subjects was considered sufficient for this novel feasibility study.

The statistical package SPSS (IBM SPSS Statistics 22) was used for analysis. The primary outcome was development of significant EP abnormality (defined as potentially requiring pharmacotherapy and/or insertion of anti-arrhythmic device). The distribution of each variable and outcome measure was determined and checked for extreme outliers. A descriptive analysis described the cohort, including differences in those with and without significant EP abnormality, with the use of the independent t-test to indicate significant differences where appropriate. Correlations between significant EP abnormality and serum cardiovascular markers and continuous CMR measures were assessed by Spearman's test. Kaplan Meyer survival curves were also built to evaluate the time to ILR-detected EP abnormality.

As this was a small sample study, descriptive statistics was usually applied and p-values, if reported, were used to only ascribe extent of descriptive differences rather than assign definitive significance, in line with good practice (251).

4.3 Results

4.3.1 Patient disposition

Twenty patients were included in the study and had the ILR implanted, however ILR

data were available for 19 patients as one patient died abroad of unknown cause 6 weeks after baseline visit and the ILR data were not retrievable (Figure 4-1).

One patient of the 19 with available ILR data did not continue further on the study 4 weeks after the baseline visit because of ILR detected complete heart block. This patient had a permanent pacemaker inserted. The patient's data were included in the analysis as per intention to treat analysis.

Only one patient felt discomfort at the ILR site reported at week 48/60 when the ILR was explanted. The patient continued to be followed-up in the study and to have all other investigations. One patient died of unrelated SSc causes and had available data up to and including week 96 (visit 10).

Of the 19 patients included in the study, 15 patients had a CMR performed. All had available LV function and volume, 14/15 had contrast CMR and 12/15 had perfusion CMR data. Figure 1 shows the patient flow pathway (Figure 4-1).

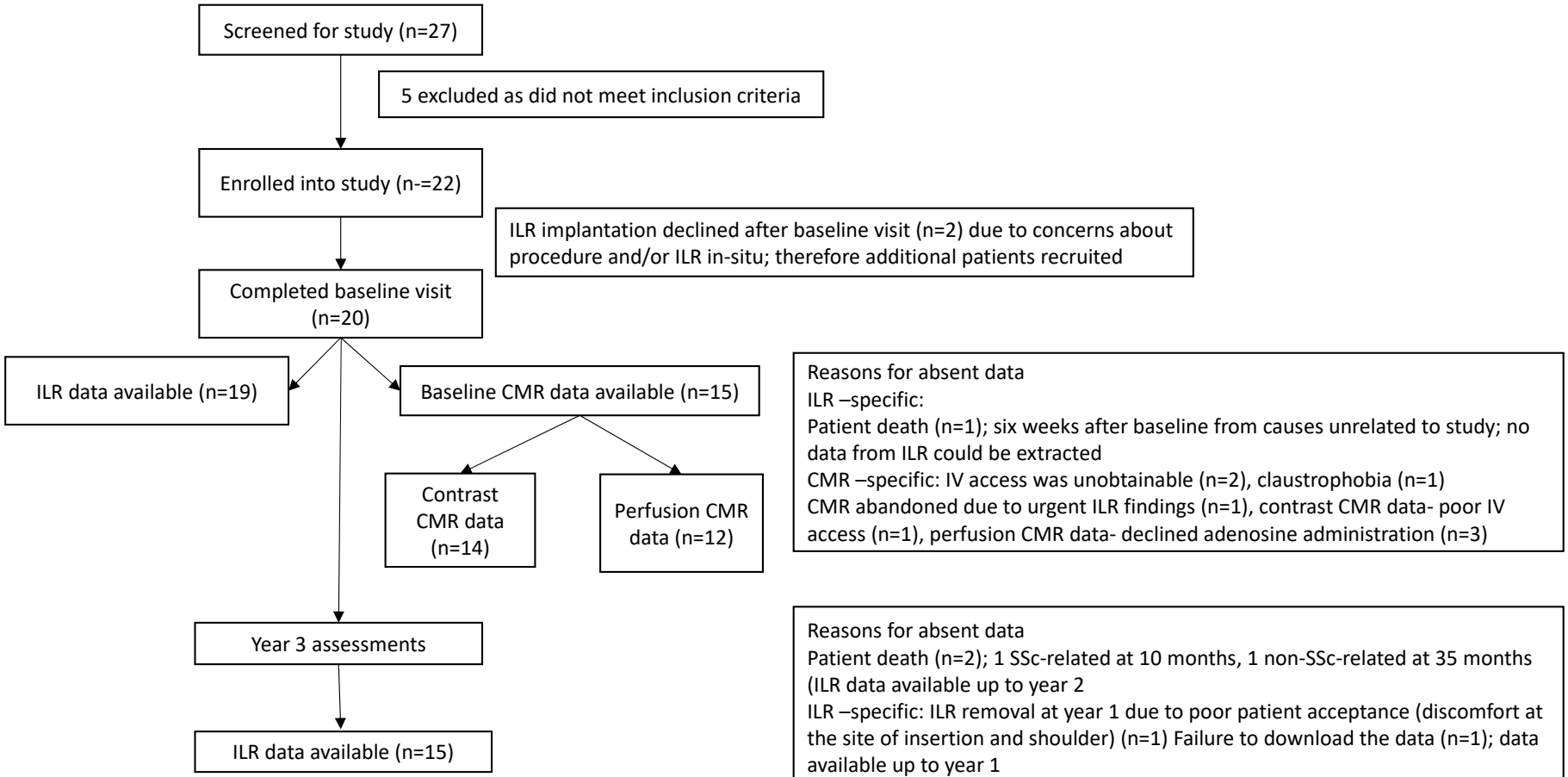


Figure 4-1 Patient selection, recruitment, feasibility and withdrawals throughout the study

CMR, cardiovascular magnetic resonance; ILR, implantable loop recorder; SSc, systemic sclerosis

4.3.2 Baseline characteristics

The baseline patient characteristics are reported in Table 4-3, 4-4 and 4-5.

Eleven (63%) were female with a mean [standard deviation (SD)] age of 53 (12) years, and median (interquartile range (IQR)) disease duration (defined as time from first non-Raynaud's phenomenon) of 7.5 (1.8, 19.5) years. Seven (37%) had dcSSc, 6 (32%) had previous digital ulceration (DU) and 8 (42%) had known interstitial lung disease (ILD). No patient had a history of pulmonary hypertension (PH). Six and 5 patients were ACA and Scl70 positive respectively. Eleven (58%) patients were receiving immunosuppressive treatment at the time of diagnosis and 7 (37%) had previous treatment with cyclophosphamide (Table 4-3).

On direct questioning, six participants offered a previous history of palpitations at baseline (although limited by recall bias). On baseline questioning, 1 patient had regular palpitations 1 to 3 times per month, 1 had regular palpitations 1 to 5 times per day, 1 patient had regular palpitations more than 5 times a day, 1 patient irregular palpitations 1 to 5 times per day, 1 irregular palpitations more than 5 times per day and 1 irregular palpitations 1-6 times per week. One patient had left axis deviation with complete left bundle branch block (LBBB) and another patient had left anterior fascicular block on ECG. Left ventricular ejection fraction (LVEF) on baseline echocardiogram was described as 'good' for 1 patient, 40% for another patient, and more than 50% for the remaining patients. The mean (SD) right ventricular-right atrial (RV-RA) gradient was 22(6) mmHg (Table 4-4).

Two patients each had one traditional CV risk factor. None had a history of hypertension or dyslipidaemia and 2 had a history of premature cardiovascular disease (Table 4-5).

Table 4-3 Disease specific characteristics of patients with systemic sclerosis

SSc phenotype	**SSc patients, n=19**
Demographics and disease history	
Female, n (%)	12 (63)
Age	53 (12)
Ethnicity, n (%) White Asian Afro-Caribbean	 16 (84) 2 (11) 1 (5)
Disease subtype, n (%) LcSSc DcSSc	 12 (63) 7 (37)
Presence of RP, n (%)	19 (100)
Time since onset of RP, years, median (IQR)	10.1 (2.4, 21.8)
Time since onset of first non-RP symptom, years, median (IQR)	7.5 (1.8, 19.5)
History of, n (%) Digital ulceration GORD Non-GORD GI involvement Interstitial lung disease	 6 (32) 17 (90) 3 (16) 8 (42)

Palpitations	9 (47)
Medication history	
Current use of DMARD, n (%)	11 (58)
Name of current DMARD, n (%) Mycophenolate Methotrexate Hydroxychloroquine Cyclophosphamide Sulfasalazine	 6 (32) 2 (11) 1 (5) 2 (11) 1 (5)
Previous use of cyclophosphamide, n (%) Of these, number of previous cyclophosphamide infusions, median (IQR)	7 (37) 6 (6, 12)
Current use of prednisolone, n (%)	2 (11) on 5mg 1 (5) on 8mg 2 (11) on 10mg 1 (5) on 15mg
Current treatment with, n (%) Iloprost Sildenafil Bosentan	 5 (26) 2 (11) 1 (5)

ACE inhibitor	11 (58)
Calcium channel blocker	15 (79)

Mean (SD) values are presented unless stated otherwise

ACE, angiotensin converting enzyme; dcSSc, diffuse cutaneous Systemic Sclerosis; DMARD, disease modifying antirheumatic drug; lcSSc, limited cutaneous Systemic Sclerosis; GI, gastrointestinal involvement; GORD, gastro-oesophageal reflux disease; RP, Raynaud's phenomenon.

Table 4-4 Clinical profile, PRO, serology and standard of care cardio-pulmonary tests of patients with systemic sclerosis

Clinical profile	
Total modified Rodnan skin score, median (IQR)	2 (2, 6)
Presence of, n (%)	
Digital pits	9 (47)
Digital ulceration	2 (11)
Tendon friction rubs	1 (5)
Calcinosis	5 (26)
TJC28, median (IQR)	1 (0.0, 4.0)
SJC28, median (IQR)	0 (0.0, 0.0)
NFC vasculopathy pattern, n (%)	
Non-specific	1 (5)
Early	13 (68)
Active	3 (16)
Late	2 (11)

Patient Reported Outcomes	
HAQ-DI	1.39 (0.87)
Visual Assessment scores, median (IQR) Pain Gastrointestinal Breathing RP Digital ulceration Overall	 5.9 (3.8, 6.8) 2.1 (0.1, 6.2) 3.4 (0.4, 6.8) 6.8 (2.2, 7.8) 1.1 (0, 5.5) 5.9 (2.8, 7.2)
Serology & acute phase, n (%)	
ANA ACA Scl70	18 (95) 6 (32) 5 (26)
Baseline CRP (mg/L) (normal range <5), median (IQR)	0 (0.0, 1.3)
Baseline ESR (mm/hr) (normal range 1-15), median (IQR)	9 (4.3, 32.3)
Standard of care tested cardio-pulmonary profile	
Forced vital capacity, %	92 (25)
Total lung capacity, %	92 (20)
DLCO, %	63 (16)
DLCO/VA, %	78 (17)
Electrocardiogram, n (%) Rhythm Axis	 19 (100) sinus rhythm

Block	2 (11) LAD 1 (5) incomplete RBBB 1 (5) LBBB 1 (5) left anterior fascicular block
LV ejection fraction % on echocardiogram, n (%)	56 (9)%, n=14 2 (11) >55% 1 (5) >50% 5 (26) 'good/preserved' or 'normal'
RV-RA gradient on echocardiogram, mmHg*	22 (6), n=12

Mean (SD) values are presented unless stated otherwise

ACA, anti-centromere antibody; CRP, ANA, antinuclear antibodies; C-reactive protein; DLCO, diffusing capacity of the lungs for carbon monoxide; DLCO/VA, DLCO adjusted for volume; ESR, erythrocyte sedimentation rate; HAQ-DI, health assessment questionnaire-disability index; LAD, left axis deviation; LBBB, left bundle branch block; LV, left ventricular; NFC, nail-fold capillaroscopy; RBBB, right bundle branch block; RV-RA, right ventricular-right atrial; Scl70, anti-topoisomerase antibody; SJC, swollen joint count; TJC, tender joint count

*many values missing due to poor tricuspid regurgitation/image quality

Table 4-5 Patient baseline cardiovascular risk profile

Variable	**SSc patients, n=19**
CV risk profile	
Smoking status: Never Ex Current	 8 (42) 11 (58) 0 (0)
Hx hypertension, n (%)	0 (0)
Hx Hypercholesterolaemia, n (%)	0 (0)
FH premature CVD*, n (%)	2 (11)
Waist/Hip Ratio	0.82 (0.09)
BMI	25.1 (5.5)
Systolic BP, mmHg	115 (13)
Diastolic BP, mmHg	73 (8.7)
Five or more fruit/vegetables daily intake, days/week, median (IQR)	5 (3, 7)
Moderate exercise, mins/week, median (IQR)	0 (0, 0) (n=18)
Alcohol intake, units/week, median (IQR)	3 (0, 6)
Current use of statin, n (%)	0 (0)
Fasting blood	
Glucose, mmol/L	4.8 (0.5)
Total cholesterol, mmol/L	4.9 (1.0)
HDL-C, mmol/L	1.5 (0.4)
LDL-C, mmol/L	2.8 (0.8)
TC/HDL-C ratio	3.7 (1.3)
Triglycerides, mmol/L	1.6 (0.8)
CK (IU/L)	141 (148)
Hs-TnI (ng/L)	76 (137)
NT-proBNP (ng/L)	145 (130)

Mean (SD) values are presented unless stated otherwise

BMI, body mass index; BP, blood pressure; CK, creatine kinase; CMR, cardiovascular magnetic resonance; CVD, cardiovascular disease; FH, family history of; HDL-C, high density lipoprotein cholesterol; Hx, history; IQR, interquartile range; LDL-C, low density lipoprotein cholesterol; NT-proBNP, N-terminal pro brain natriuretic peptide; SD, standard deviation; SSc, Systemic Sclerosis; TC, total cholesterol.

*defined as first degree relative with a history of CVD when 60 years old or younger if female, and 55 years old or younger if male

4.3.3 Implantable loop recorder analysis

Over the three years, the ILR detected findings in 13 (68%) participants, ranging from benign to significant EP abnormality (Table 4-6). Ten (53%) patients had SVE, 9 (47%) had VE, 3 (16%) had bigeminy, 1 couplets, 1 salvos, and 8 (42%) had significant findings. Of these eight, one had CHB, 2 had non-sustained VT (NSVT) and 5 had atrial arrhythmias of which 1 had atrial flutter, 1 AF, 1 SVT followed by AF, 1 SVT, and 1 patient with atrial flutter followed by AF and SVT. Only one patient (with NSVT) was symptomatic with palpitations at the time of the arrhythmia.

The median (IQR) time from ILR implantation to significant EP abnormality detection was 12 (5, 24) months. The mean (95% CI) survival time (to an event) was 26 (21,32) months (Figure 4-2).

Table 4-6 Type of ILR abnormalities over 36-month period

ILR abnormality	**Number detected in study participants, n (%) (n=19)**			
	Year 1	**Year 2**	**Year 3**	**Total over 3 years**
Any abnormality	11 (58)	2 (11)	0	13 (68)
Significant EP abnormality requiring pharmacotherapy/device	4 (21)	1 (5)	1 (5)	6 (32)
Significant EP abnormality (requiring monitoring for pharmacotherapy/ device)	5 (26)	1 (5)	2 (11)	8 (42)
Atrial fibrillation	1 (5)	0	2 (11)	3 (16)
Atrial flutter	2 (11)	0	0	2 (11)
Supraventricular tachycardia	1 (5)	0	2 (11)	3 (16)
Ventricular tachycardia	1 (5)	1 (5)	0	2 (11)
Complete heart block	1 (5)	0	0	1 (5)
Benign findings	8 (42)	4 (21)	0	12 (63)
Supraventricular ectopics	7 (37)	3 (16)	0	10 (53)
Ventricular ectopics	2 (11)	4 (21)	3 (16)	9 (47)
Bigeminy	3 (16)	0	0	3 (16)
Couplets	1 (5)	0	0	1 (5)
Triplets	0	0	0	0
Salvos	1 (5)	0	0	1 (5)

ILR, implantable loop recorder

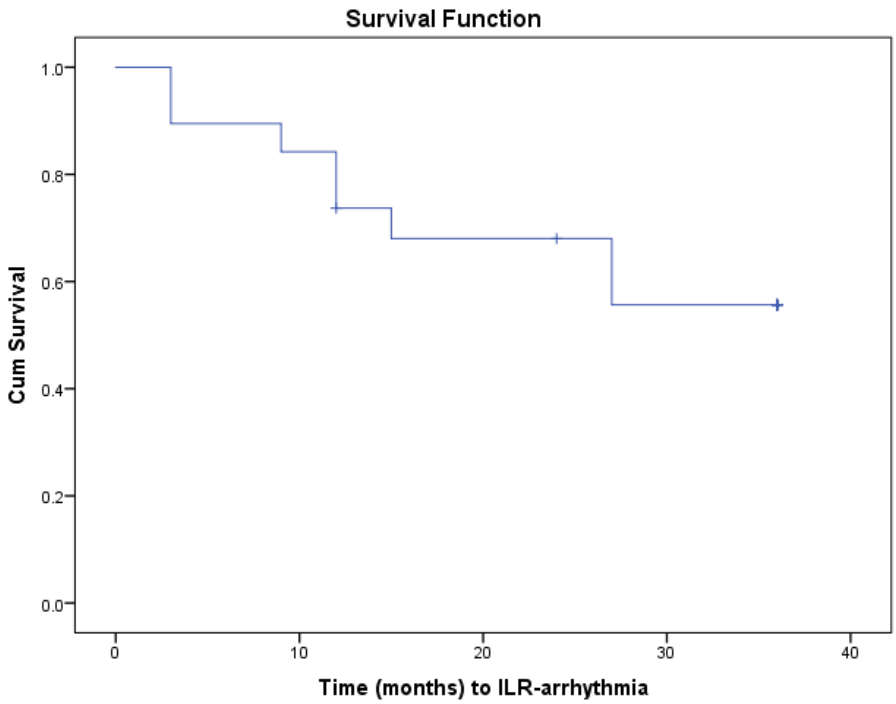


Figure 4-2 Kaplan-Mayer survival curve showing time to ILR-detected event (months)

+ Censored data: ILR explanted for 1 patient due to discomfort, 1 patient died at 24 months

ILR, implantable loop recorder

Table 4-7 describes the characteristics of the eight SSc patients with significant EP abnormality. Six patients were female with a mean (SD) age of 55 (10) years and a median (IQR) time from first non-RP symptom of 3.1 (20.1) years. Seven patients had a SSc-pattern of vasculopathy on NFC. All 3 patients with serious EP abnormality (NSVT/CHB) comprised a diffuse subset and known ILD, 2 with DU, male and Scl70 positive (the third ANA negative), with a mean (SD) disease duration of 2 (1.3) years. The other 5 patients with atrial arrhythmia included only one dcSSc, 1 with history of DU, all ACA positive except 1 with a nucleolar ANA pattern on immunofluorescence. Four patients were receiving treatment with a disease-modifying anti-rheumatic (DMARD) drug on recruitment and two patients had received treatment with cyclophosphamide in the past. Three were on treatment with sildenafil or bosentan.

Table 4-7 Baseline characteristics of scleroderma patients with significant EP abnormalities over the three years

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6*	Patient 7	Patient 8
EP abnormality detected	**Atrial flutter to AF, SVT**	**Atrial flutter**	**SVT, AF**	**AF**	**SVT**	**Non-sustained VT**	**Non-sustained VT**	**Complete heart block**
Timepoint significant arrhythmia detected	9 months, 21 months and 36 months	12 months	3 and 30 months respectively	27 months	27 months	12 months	15 months	3 months
ILR method of detection	ILR device triggered	ILR device triggered	ILR device triggered	ILR device triggered	ILR device triggered	Patient triggered	ILR device triggered	ILR device triggered
Age, Sex	49, female	55, female	54, female	73, female	62, female	50, male	52, female	41, male
CV risk factors	Family history	Ex-smoker	Family history	Family history	Smoker	Ex-smoker	Family history	None
DcSSc	X	x	ü	x	x	ü	✓	ü
Time from 1st non-RP, years	2.4	21.8	1.4	21.7	23.3	2.3	1.3	3.9

ILD	X	x	x	x	✓	ü	✓	ü
Previous DU	X	ü	x	x	x	x	✓	ü
History palpitations	X	x	ü	✓	✓	ü	✓	ü
DMARD	X	x	MMF	x	x	MMF, previous CYC	CYC	MMF, previous CYC
ANA	ACA	ACA	ANA nucleolar pattern	ACA	ACA	Scl70	Scl70	Negative
NFC pattern	Active	Early	Early	Early	Active	Non-specific	Early	Early
ECG baseline	Left anterior fascicular block	Normal	Normal	Normal	Normal	Normal	Normal	Complete LBBB with LAD
Echocardiography: LVEF RV-RA gradient	Good 18mmHg	>55% 20mmHg	70% 27mmHg	60% Not measured	53% 28mmHg	60% Not measured	>50% Not measured	40% 21mmHg
CK(BL) IU/L	81	83	544	101	57	68	112	168
Hs-TnI (BL) ng/L	5.8	30.6	349	2.5	5.3	2.6	313	418
NT-proBNP(BL) ng/L	32	141	297	126	288	38	328	336

*****Patient symptomatic at time of arrhythmia

ACA, anti-centromere antibody; AF, atrial fibrillation; ANA, anti-nuclear antibody; CK, creatine kinase; CV, cardiovascular; CYC, cyclophosphamide; dcSSc, diffuse cutaneous Systemic Sclerosis; DMARD, disease modifying anti-rheumatic drug; DU, digital ulceration; ECG, electrocardiogram; ILD, interstitial lung disease; LAD, left axis deviation; LBBB, left bundle branch block; lcSSc, limited cutaneous Systemic Sclerosis; LVEF; left ventricular ejection fraction; MMF, mycophenolate mofetil; NFC, nail-fold capillaroscopy; NSVT, non-sustained ventricular tachycardia; NT-proBNP, N-terminal pro brain natriuretic peptide; RA, right atrial; RP, Raynaud's Phenomenon; RV, right ventricular; Scl70, anti-topoisomerase antibody; SVEs, supraventricular ectopics; SVT, supraventricular tachycardia

4.3.3.1 Further analysis of the 8 patients with significant ILR-detected abnormality

The participant with CHB (detected within the first 6 weeks of the study) was a 41-year old white male who had never smoked, with no history of hypertension or family history of CVD. He had ANA negative, dcSSc, with a time since first non-Raynaud's phenomenon symptom of 3.9 years, with ILD, a history of DU and irregular palpitations. He was managed on mycophenolate mofetil (MMF) with previous cyclophosphamide use (12 pulses in total). His 24-hour ECG monitor six weeks earlier had revealed sinus rhythm with a few SVE/VE and three couplets only. However, he had complete LBBB with left axis deviation on baseline ECG and a LVEF of 40% on baseline echocardiogram (RV-RA gradient 21mmHg). The patient had noticed increasing fatigue in the weeks preceding the CHB detection, but no other new symptoms.

The patient with AF, atrial flutter and SVT developed a short run of ventricular tachycardia (VT) during right heart catheterisation (RHC) procedure for assessment of pulmonary arterial pressure. As this was a complication of RHC, the ILR finding was considered iatrogenic and was excluded from the analysis.

There was no difference in the mean (SD) disease duration between those with and without significant EP abnormality detected on ILR. However, those who developed serious ILR-detected event (NSVT/CHB) had a trend to lower mean (SD) disease duration compared to the rest of those with significant EP abnormality [mean difference (CI) -12 (-28, 5)] or those with no EP abnormality on ILR [mean difference (CI) -8 (-18, 3) years].

4.3.4 CMR and development of ILR-detected significant EP abnormality

ECV, indicative of diffuse myocardial fibrosis was higher in those with significant ILR-detected event compared to those without [mean difference (95% CI) 2(-2,6)]. Although within normal range, a trend for greater left ventricular ejection fraction (LVEF) and LV mass also appeared in those with significant EP abnormality [mean difference (95% CI) 2(-3,8), respectively 2(-10,15)].

Five of the 14 patients with contrast enhanced CMR images had evidence of LGE consistent with a focal myocardial fibrosis. Only one of these patients however developed a significant arrhythmia (NSVT) with LGE present in the basal and mid inferolateral midwall. Of the remaining four, two had focal LGE (one in the basal inferolateral wall, one in right ventricular insertion point (RVIP)), one had diffuse LGE, and one had transmural LGE in the basal inferolateral wall (ILR data available to year 1 only).

Twelve patients had perfusion CMR data available. None of the patients had visual perfusion defects on first pass myocardial perfusion imaging. There were no differences in myocardial blood flow (MBF) at rest or stress and in myocardial perfusion reserve (MPR) between those with and without significant EP abnormalities (quantitative perfusion data available for 3/8 with significant EP abnormalities). Differences in CMR outcome measures between patients with significant EP abnormalities detected on ILR (n=8) to those without are described in Table 4-8.

Table 4-8 Difference in key baseline CMR measures in ELCASA participants with significant EP abnormalities compared to those without significant arrhythmias

CMR baseline	**Significant arrhythmia on ILR (n=6)**	**No significant arrhythmia on ILR (n=9)**	**Mean difference [95% CI]**
ECV (%), n=14	32 (2)	29 (4)	2 [-2,6]
T1 native (ms)	1172 (94)	1213 (56)	-42 [-125, 41]
LVEDV/BSA (ml/m^2)	84 (16)	83 (20)	1 [-21, 22]
LVEF (%)	62 (4)	59 (5)	2 [-3, 8]
LV mass/BSA (g/m^2)	46 (7)	44 (14)	2 [-10, 15]
LVESV/BSA (ml/m^2)	33 (9)	34 (10)	-2 [-13, 9]
LVSV /BSA (ml/m^2)	51 (8)	49 (11)	2 [-9, 13]
Distensibility (10^{-3}mmHg^{-1})	5 (4)	4 (2)	0.4 [-3, 4]
Torsion (°)	12 (6)	14 (5)	-2 [-8, 5]
LGE, n=14	n = 1/5	n =4/9	
Scar mass (g)	2.8, n=1	3.7 (1.8), n=4	-0.9 [-7.3, 5.4]
Myocardial perfusion, n=12	n=3	n=9	
Stress MBF (ml/g/min)	2.4 (0.4)	2 (0.9)	0.4 [-0.8, 1.6]
Rest MBF (ml/g/min)	1.3 (0.3)	1 (0.2)	0.3 [0.0, 0.6]
MPR	1.9 (0.4)	2.1 (1.2)	-0.2[-1.3, 0.8]

Mean (SD) unless stated otherwise

BSA, body surface area; CI, confidence interval; CMR, cardiovascular magnetic resonance; ECV, extracellular-volume fraction; EDV, end-diastolic volume; ESV, end-systolic volume; ILR, implantable loop recorder LGE, late gadolinium enhancement; LV, left ventricular; LVEF, left ventricular ejection fraction; MBF, myocardial blood flow; MPR, myocardial perfusion reserve.

4.3.5 Baseline serum cardiovascular biomarkers and ILR-detected significant EP abnormalities

Baseline Hs-TnI was higher in the 8 patients with significant EP abnormalities [mean (SD) 141 (184) vs 23(51), mean difference (95% CI) 117 (-11, 245) ng/L], especially in those who developed serious EP abnormality (NSVT/CHB) (n=3) [mean difference (95% CI) 202 (46, 359) ng/L] when compared to those with no significant EP abnormalities. Baseline NT-proBNP also appeared greater in those with significant EP abnormalities [mean (SD) 198 (128) vs 106 (122), mean difference (95% CI) 92 (-30, 215)ng/L]. There was no difference in CK levels [(mean difference (95% CI) 19 (-134, 172) IU/L] in those with and without significant EP abnormalities at baseline (Figure 4-3).

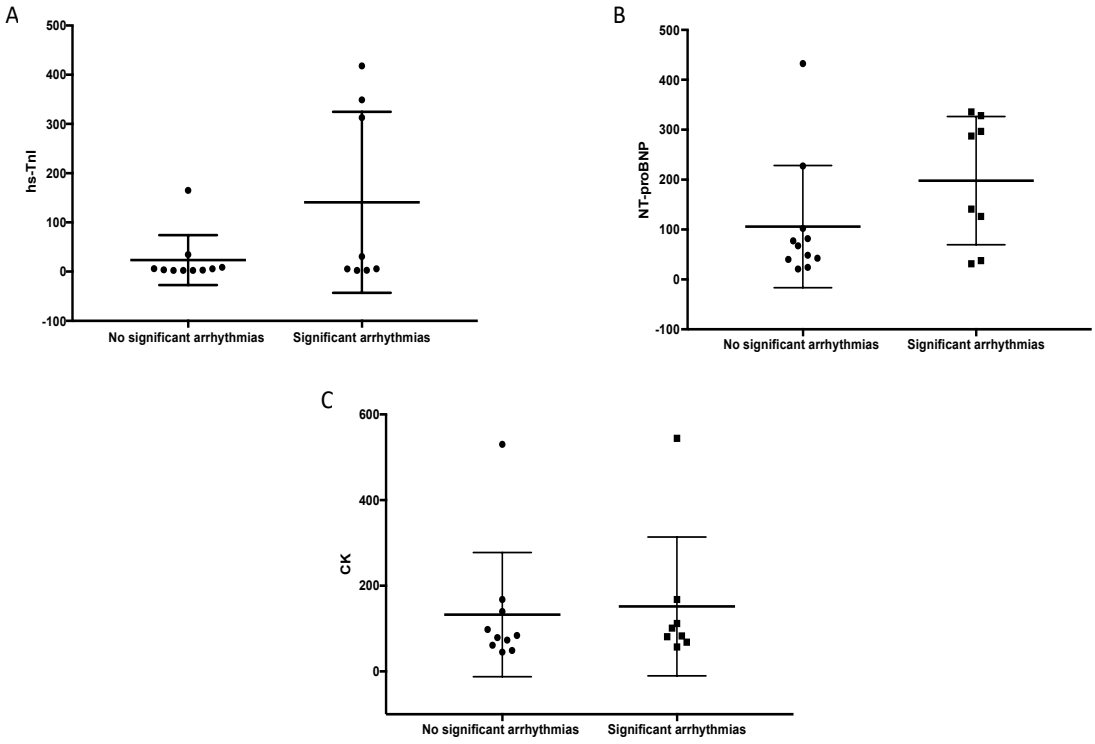


Figure 4-3 Baseline soluble cardiovascular biomarkers: (A) hs-TnI (ng/l), (B) NT-proBNP (ng/l) and (C) CK (iu/l) in those with and without ILR-detected significant EP abnormalities

CK, creatine kinase; Hs-TnI, high-sensitivity troponin I; NT-proBNP, N-terminal pro brain natriuretic peptide

4.3.6 Serum cardiac biomarker change throughout the study period

A decrease in Hs-TnI at year 1 was noted for those with significant arrhythmia. There were no differences in the rest of the serum cardiac biomarkers between those who had and did not have significant EP abnormalities throughout the remaining study period (Figure 4-4).

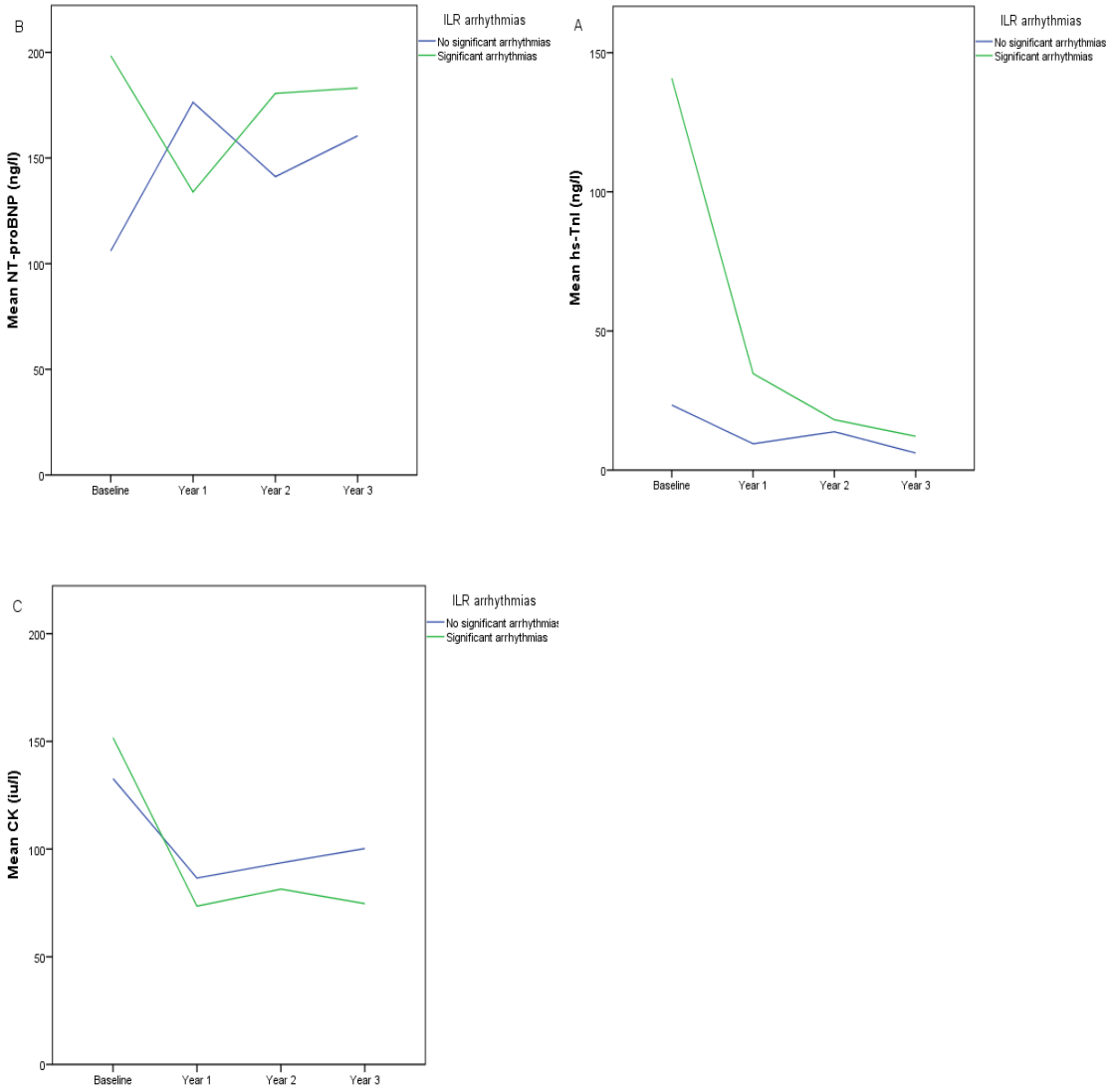


Figure 4-4 The change in soluble cardiovascular biomarkers over the three years between the patients with significant EP abnormalities and those without. (A) change in hs-TnI over study period. (B) change in NT-proBNP over study period. (C) change in CK over study period.

CK, creatine kinase; Hs-TnI, high-sensitivity troponin I; ILR, implantable loop recorder; NT-proBNP, N-terminal pro brain natriuretic peptide.

4.3.7 Baseline soluble cardiovascular biomarkers and CMR

Baseline Hs-TnI and NT-proBNP were higher in those patients with evidence of LGE on CMR [mean difference (95% CI) 91(-14, 196) ng/L, 95(-49, 239) ng/L respectively] and had a negative correlation with MPR (r=-0.59, p=0.053 and r=-0.74, p=0.006). There was a trend towards a positive correlation between Hs-TnI and ECV (r=0.45, p=0.125) and a negative correlation between hs-TnI and LVEF (r=-0.39, p=0.162) (Table 4-9).

Table 4-9 Baseline soluble cardiovascular measures and association with CMR measures

	Baseline soluble cardiovascular biomarker		
CMR measures, n=15	**Hs-TnI (ng/l)**	**Nt-proBNP (ng/l)**	**CK (iu/l)**
LGE present, n=5	*98 (139)	*186 (184)	*99 (46)
LGE not present, n=9	*7 (10)	*91 (64)	*132 (151)
Mean difference [95%, CI]	91 [-14, 196]	95 [-49, 239]	-33 [-187, 120]
ECV (%)	r=0.45, p=0.125	r=-0.04, p=0.887	r=-0.04, p=0.876
T1 native (ms)	r=-0.23, p=0.438	r=0.22, p=0.427	r=-0.3, p=0.277
LVEDV/BSA (ml/m^2)	r=0.19, p=0.521	r=-0.20, p=0.467	r=-0.06, p=0.820
LVEF (%)	r=-0.39, p=0.162	r=0.08, p=0.791	r=0.13, p=0.639
LV mass/BSA (g/m^2)	r=0.18, p=0.531	r=-0.12, p=0.666	r=0.03, p=0.930
LVESV/BSA (ml/m^2)	r=0.29, p=0.316	r=-0.20, p=0.475	r=-0.03 , p=0.930
Myocardial perfusion			
Rest MBF (ml/g/min)	r=0.16, p=0.647	r=0.60, p=0.036^	r=-0.11, p=0.729
Stress MBF (ml/g/min)	r=-0.59, p=0.058	r=-0.27, p=0.391	r=0.09, p=0.779
MPR	r=-0.59, p=0.053	r=-0.74, p=0.006^^	r=0.22, p=0.484

Spearman rho correlation unless stated otherwise

*Mean (SD) values; ^p<0.05; ^^p<0.01

BSA, body surface area; CK, creatine kinase; CI, confidence interval; CMR, cardiovascular magnetic resonance; ECV, extracellular-volume fraction; EDV, end-diastolic volume; ESV, end-systolic volume; Hs-TnI, high-sensitivity troponin I; ILR, implantable loop recorder; LGE, late gadolinium enhancement; LV, left ventricular; LVEF, left ventricular ejection fraction; MBF, myocardial blood flow; MPR, myocardial perfusion reserve; NT-proBNP, N-terminal pro brain natriuretic peptide

4.3.8 Medical outcomes for participants

Six participants required medical treatment as a direct result of the study.

4.3.8.1 Device

The patient with CHB was admitted the same day the AV block was detected for insertion of a permanent pacemaker. With both active ILD and presumed myocardial involvement, the patient went on to receive cyclophosphamide followed by Rituximab but died because of ILD progression 10 months after recruitment to this study.

4.3.8.2 Pharmacotherapy

Both patients with NSVT were commenced on beta-blockers, with on-going monitoring to consider ablation as needed; and calcium channel blocker started for the patient with SVT and AF. The patient with atrial flutter followed by AF and SVT received treatment with a beta-blocker (later switched to a calcium channel blocker due to severe Raynaud's). Anticoagulation was commenced in one patient with AF.

After clinical review by the cardiologists, the remaining patients received no change in their treatment given that they were asymptomatic at the time and had a low CV risk.

Over the study period, no patients were diagnosed with PH or CVD. Two additional patients received DMARD treatment during the study period due to active disease. Five SSc patients that received Cyclophosphamide during the study period appeared unrelated to development of arrhythmia; with one patient developing NSVT one year following cyclophosphamide treatment and another with SVT before.

4.4 Discussion

This is the first study to evaluate the use of ILR for the screening of incidental primary pSSc-HI associated EP abnormality. Almost half the patients had a clinically meaningful event, significant in three patients. Secondly, ILR-detected abnormalities appeared to be associated with cardiac serum biomarkers and the measure of diffuse fibrosis (CMR-ECV) rather than focal fibrosis; although finally, focal fibrosis (LGE) also correlated with hs-TnI and NT-proBNP. Together with known SSc poor prognostic factors, these data collectively provide an opportunity for risk stratification to guide ILR use for the screening of significant arrhythmia in the at-risk patient and inform pathophysiological understanding of pSSc-HI.

Patients with SSc may exhibit both traditional IHD and/or primary pSSc-HI. Within both SSc-related and non-SSc related deaths, a third are attributable to cardiac causes (243), highlighting the importance to identify IHD before determining pSSc-HI (252). Pathways to risk stratify and manage IHD are well-established. In contrast, detection and management of primary pSSc-HI remains unclear, and study designs to date have rarely focused on primary pSSc-HI. In addition, EP studies have relied on short period monitoring methods; and most including benign findings runs the risk of miscalling prevalence of clinically meaningful abnormalities.

This study minimised the role of IHD by excluding patients with prior history of traditional CVD, its key risk factor (diabetes), and only permitting individuals with one traditional CV risk factor. In addition, there were no patients with PH in this study; its presence could otherwise have explained atrial arrhythmias.

Just under half the patients (8/19) were found to have incidental significant abnormality. The changes were detected over the course of the three years, highlighting the limited value of routine short-term EP monitoring methods to detect clinically relevant abnormalities in at-risk patients. Nevertheless, the EP abnormaltities that were picked up by the ILR haven't been detected by routine ECG. Whlist the majority of patients had a history of palpitations, only one patient reported palpitations at the time of the arrhythmia, suggesting such history as of also limited

value. All three patients with more significant findings (CHB/NSVT) had early dcSSc, known ILD, 2 were Scl70 positive and 2 males. These findings reinforce the already existing data on the association of pSSc-HI/arrhythmias with SSc poor prognostic factors (253, 254).

In individuals with IHD, fibrosis is identified as the principal underlying pathophysiological process of sudden cardiac death (255). Few studies have assessed the association between myocardial fibrosis and EP outcomes in SSc (14, 131). Muresan et al also found no association between focal fibrosis (CMR-LGE) and ventricular arrhythmia burden/conduction abnormalities as assessed by 24-hour Holter (n=30) (131), whereas another study identified abnormal Holter monitoring with greater burden of myocardial LGE (15). In our study, five patients had evidence of LGE focal fibrosis, of which only one (with mid-wall LGE) had significant arrhythmia (NSVT). One patient had transmural LGE (albeit with only one year of ILR data available) and the remaining three patients had focal or patchy/diffuse LGE distribution. The location of myocardial fibrosis might play an important role in this context. CMR and EP studies in non-ischaemic cardiomyopathies such as aortic stenosis and dilated cardiomyopathy suggest a strong association between mid-wall fibrosis and major arrhythmic events and sudden cardiac death (210, 211). The extent of LGE is also recognised as an important determinant in the development of arrhythmias and a predictor of ICD implantation in both ischaemic and non-ischaemic cardiomyopathies (256, 257). Our study included quantitative LGE, and revealed low scar mass values, possibly explaining the lack of an association between arrhythmias and LGE. Further studies on larger cohorts are warranted to establish whether the distribution and extent of LGE fibrosis on CMR can predict the development of arrhythmias in SSc.

This study was a first for evaluating association of CMR-ECV, with arrhythmia, and here, an association with significant EP abnormalities was suggested. ECV quantification is a promising tool in the detection of early subclinical, diffuse myocardial fibrosis. Data suggest good correlation with histological findings of myocardial fibrosis in various clinical contexts (168, 169) and ECV values are higher in patients with SSc (159). ECV may therefore provide a sensitive and quantitative

measure of diffuse fibrosis, which could be used to identify those at higher risk of EP abnormalities in SSc.

Five arrhythmias were supraventricular in origin, occurring in females; two with lcSSc, none with ILD. None had common causes such as IHD, hypertension or mitral valve disease, implying pSSc-HI as the underlying cause. Further studies are required to confirm whether there is an association of lcSSc with atrial arrhythmias, and determining the pathophysiological basis for this; being mindful that if fibrosis is indeed relevant, CMR studies report fibrosis more commonly affecting the ventricles and not atria (258), possibly as focal fibrosis in atria is challenging to detect on CMR.

Serum cardiac biomarkers, NT-proBNP and troponin are sensitive tools in the detection of cardiac disease in the general population, but also in SSc (185, 259). High sensitivity troponin T (hs-TnT) has been shown to be significantly elevated in SSc patients with no CVD or CV risk factors (187). This is the first study to show an association of presumed primary SSc-related non-ischaemic arrhythmias with hs-TnI and NT-proBNP, pinpointing myocardial injury as a substrate in the development of conduction abnormalities in SSc. The mechanism of myocardial damage is not clear but may reflect microvascular myocardial impairment as a consequence of endothelial dysfunction and subsequent scarring.

Finally, this study also showed an association of hs-TnI and NT-proBNP with the CMR measures of fibrosis and MPR, suggesting the potential utility of these biomarkers for identifying patients with subclinical cardiomyopathy. MPR had a good correlation with cardiac biomarkers, indicating that abnormal myocardial perfusion, which is likely the result of the small vessel disease, could lead to myocardial injury and dysfunction in SSc patients.

In summary, this pilot study, assessing the value of ILR in SSc demonstrated incidental significant arrhythmias and AV block in asymptomatic patients, with the more severe findings detected in patients with poor prognostic factors. The study supports therefore the need for more aggressive cardiac monitoring, including the use of ILR in SSc patients with poor prognostic features. CMR-ECV and cardiac serum biomarkers,

together with poor prognostic factors of SSccould provide a basis for the development of screening algorithms for those at higher risk of EP abnormalities requiring more intensive cardiac monitoring and intervention, such as ICD or permanent pacemaker. Nevertheless, the study also provides valuable information with regards to pSSc-HI pathogenesis, suggesting an association between EP abnormalities and microvascular alteration and fibrosis. Larger studies are warranted to determine the clinical utility of serum cardiac biomarkers and CMR measures in providing risk stratification for EP abnormalities and thus refine patient selection for pre-emptive ILR insertion.

4.4.1 Limitations of study

The main limitation is the size of the study; however, this was a first, pilot study in a rare disease population to demonstrate the feasibility of the ILR in asymptomatic SSc and test the hypothesis that significant EP abnormalities would be detected.

Fully excluding atherosclerosis as the basis of an ILR event is challenging, however, the risk was minimised with the exclusion of those with diabetes, traditional CVD and more than one traditional CV risk factor. Moreover, none of the patients had perfusion CMR defects, making the diagnosis of IHD improbable. The next step is to undertake a larger study to validate the initial findings of prevalent EP abnormalities and association with CMR and biochemical abnormalities and establish a risk model to inform ILR use in clinical practice.

4.4.2 Key messages

- ILR detected EP abnormalities in asymptomatic SSc patients, free of CVD are common
- ILR detected serious arrhythmia and/or AV block in SSc patients with poor prognosis
- CMR-ECV and serum cardiac biomarkers Hs-TnI and NT-proBNP associate with ILR EP abnormalities, thus providing a potential basis for identifying at risk SSc patients
- Hs-TnI and NT-proBNP also associated with CMR measures of fibrosis and MPR, suggesting the potential utility of these biomarkers for identifying patients with

subclinical pSSc-HI

Chapter 5. Phenotyping subclinical systemic sclerosis heart involvement with cardiovascular magnetic resonance and predicting cardiovascular outcomes

5.1 Introduction

Whilst clinically overt primary systemic sclerosis heart involvement (pSSc-HI) is described in up to 20-30% of SSc patients (10, 84) cardiac pathology has been documented in a majority of SSc patients in historical autopsy studies and with the use of more sensitive and multiparametric methods such as CMR (3, 14, 17, 18). As detailed in the review chapter, cardiovascular magnetic resonance (CMR) provides a comprehensive assessment of cardiac morphology, function and tissue characterisation and can thus detect subclinical SSc-HI (3, 14, 17, 18). Focal and diffuse fibrosis, as well as myocardial microvascular impairment have been all described in SSc (14, 15, 158, 159, 179).

Poor prognostic factors of SSc including diffuse SSc (dcSSc) subtype, anti-topoisomerase antibody (Scl-70), male gender, high skin score and major internal organ involvement (interstitial lung disease) have been associated with clinically overt pSSc-HI and cardiac associated mortality (9-11). However, means for risk stratificantion of CMR defined pSSc-HI are lacking.

No studies to date provided a comprehensive assesment of CMR detected pSSc-HI or looked to find clincal and serum correlates of CMR pSSc-HI that would aid early identification of patients at risk of pSSc-HI.

Whilst presence of pSSc-HI is thus increasingly recognised with the use of sensitive imaging such as CMR, it remains unclear how best to utilise and interpret within a general SSc cohort. The prognostic implications of CMR detected pSSc-HI has not been yet established. Only a subgroup of SSc patients develop clinically overt pSSc-HI, including myocarditis, conduction abnormalities, arrhythmia and/or heart failure. Such cardiac involvement is associated with poor prognosis, with a mortality rate as

high as 70% (6). In addition, use of serum cardiac biomarkers in diagnosis and monitoring of pSSc-HI has not been well-delineated.

Identifying patients at risk of pSSc-HI and the indices associated with progression to clinically overt pSSc-HI is an unmet need. Clinical phenotyping together with serum biomarkers and CMR evaluation in particular offer a clear opportunity for initial step in risk stratification and early detection of cardiac involvement in a general, asymptomatic SSc cohort.

5.1.1 Hypothesis and aims

5.1.1.1 Hypothesis

- SSc patients have a high prevalence of CMR focal and diffuse fibrosis (as assessed by LGE and ECV respectively) and microvascular impairment in comparison to healthy controls (HC)
- Clinical phenotype and serum cardiac markers can predict those with CMR abnormalities including fibrosis and myocardial perfusion; thus form a first step in risk stratification of pSSc-HI
- CMR abnormalities and/or cardiac biomarkers can predict the development of pSSc-HI CV outcomes providing a second step in risk stratification of pSSc-HI

5.1.1.2 Aims

1. To describe the prevalence of pSSc-HI in a regional SSc cohort using CMR
2. To evaluate the association between CMR abnormalities and disease characteristics
3. To assess the association between CMR abnormalities and cardiac serum biomarkers
4. To explore for predictive capacity of clinical phenotype and serum cardiac biomarkers and CMR detected pSSc-HI
5. To evaluate whether CMR together with cardiac serum biomarkers can predict the development of pSSc-HI CV outcomes

5.2 Methods

5.2.1 Candidate's role in the project

The concept and the design of the study were set by myself, Professor Buch and Professor Sven Plein, with input into analysis also provided by Dr Del Galdo. My role was to approach and consent the participants, arrange and perform the clinical visit including nailfold capillaroscopy, collect the blood for cardiac biomarkers processing and perform the ECG. The serum samples were stored in the department and were further processed at the NHS Leeds Teaching Hospital (Hs-TnI and CK). I arranged the transport of the NT-proBNP samples that were further processed in Poland, at the Medical University of Lublin. I was responsible for the preparation of the SSc patients for the CMR, cannulation and CMR scanning along with the radiographers. I was also responsible for checking the CMR for any urgent findings (cardiac and extracardiac) and releasing CMR reports as required. These were further discussed with Professor S. Plein or in his absence, one of the other CMR expert consultants. I performed a complete CMR analysis for 48 scans, and LGE and myocardial perfusion quantitative analysis for the whole study cohort (n=83). Dr. Ananth Kidambi, Consultant Cardiologist was the second expert reader for LGE images. The first 35 CMR scans were partially analysed by Bara Erhayiem and Graham Fent, CMR cardiology fellows.

Data entry, data analysis and chapter writing were carried out by myself. I was also responsible for drafting the manuscript while Professor Buch together with the other co-authors revised it. I have also been responsible for submitting the manuscript for publication.

5.2.2 Ethical approval

All patients included in the study provided written informed consent. For the purpose of this study, patients were consented to the CONVAS (Leeds Teaching Hospitals Connective Tissue Disease and Vasculitis Cohort Cross-sectional and Longitudinal Clinical and Basic Science Evaluation) main and cardiovascular sub-study (REC Ref: RR10/9608, RR10/9608 respectively) and ELCASA study (REC:10/H1306/88).

The CONVAS study is a Leeds Teaching Hospital single centre observational study following patients with connective tissue diseases (CTD). The CONVAS cardiovascular sub-study allows various cardiovascular assessments including specific cardiac biomarkers testing and CMR.

The ELCASA study (described in Chapter 3) is a single centre observational longitudinal study of patients receiving standard of care treatment assessing the feasibility of implantable loop recorder (ILR) in SSc. As part of the study, all patients had and ILR implanted and CMR performed.

5.2.3 Study design

A longitudinal observational study was undertaken in consecutive SSc patients attending the SSc clinic at the Leeds Teaching Hospital, NHS Trust; that had no known cardiovascular disease (CVD) and minimal risk factors.

All participants had clinical and demographic data and serum cardiac biomarkers collected and contrast enhanced and stress perfusion CMR performed at baseline. Patients were followed up for minimum one year (the majority for 3 years) and had clinical and echocardiography data collected. CV outcomes judged to be pSSc-HI were recorded for all patients.

5.2.4 Eligibility criteria

5.2.4.1 Inclusion criteria

- Between 18 years and 80 years old.
- Capable of understanding and signing an informed consent form.
- Meet the 2013 ACR/European League of Rheumatism (EULAR) criteria for SSc(32).

5.2.4.2 Exclusion criteria

- Prior diagnosis of CVD (cardiac, peripheral or cerebral)

- Any other inflammatory autoimmune conditions
- Diagnosis of pulmonary hypertension (PH)
- Diagnosis of diabetes mellitus
- More than two of remaining 5 traditional cardiovascular risk factors, including: high blood pressure, hypercholesterolaemia/hypertriglycerideamia, family history of early cardiovascular disease (before 55 years of age for men and 60 year for women) and current smoker.
- Exclusion criteria for having a CMR: pacemakers, surgical clips within the head, certain inner ear implants, neuro-electrical stimulators or metal fragments within the eye or head, asthma requiring previous hospital admission or oral steroids, an eGFR less than 45 ml/min/1.73m2, pregnancy, breastfeeding, and any allergy to contrast.

5.2.5 Target population

Patients were enrolled from the weekly SSc clinic attending the LTHT, Chapel Allerton Hospital. Healthy controls (HC) served as control group and had the same CMR protocol as the SSc patients. They were frequency matched with the SSc patients for gender and age. HC were recruited by the CMR team at the Leeds General Infirmary and consented to one of the CMR department studies (CE-MARC 2, Optimization, of Image Acquisition and Analysisis Method', REC: 12/YH/0551). Age, gender as well as CV risk factors were recorded for all HC. HC were excluded if they had any CVD history and/or prior and/or ongoing cardiovascular treatment.

5.2.6 Study visits

5.2.6.1 Baseline visit

Al participants had clinical and demographic data collected and contrast enhanced and stress perfusion CMR performed at baseline. Serum samples for Hs-TnI and NT-proBNP testing were also collected.

5.2.6.2 Follow up visit

Clinical and echocardiography follow-up data were recorded. When clinically indicated, patients had routine electrophysiological investigation [including electrocardiogram (ECG), 24 Holter ECG]. A subgroup of patients (n=19) had an implantable loop recorder as part of the ELCASA study (as described in Chapter 4) that also provided clinical outcome data for this study. The primary endpoint of clinically overt pSSc-HI was defined as any episode of myocarditis, heart failure, rhythm disturbances and/or clinically relevant echocardiographic abnormalities including systolic dysfunction and/or a diastolic dysfunction > grade 1.

5.2.7 Data collection

5.2.7.1 Baseline data

5.2.7.1.1 Demographic and clinical baseline data

- Including age, gender, ethnicity
- Concomitant diseases
- Cardiovascular risk assessment
    - History of hypertension, dyslipidaemia, smoking habit, family history of premature cardiovascular disease
- Disease characteristics: disease subtype and onset, internal organ involvement
- Current and previous treatment
- Physical examination, including:
    - mRSS (214) was assessed in 17 different regions by a trained clinician
    - Presence of digital ulcers, tendon friction rubs, calcinosis, digital pits

5.2.7.1.2 Sample collection

Cardiac serum biomarkers, including CK, Hs-TnI, NT-proBNP were collected. Hs-TnI and CK were tested on a Siemens Advia XPT system (Advia Chemistry XPT and Advia Centaur XPT Immunoassay respectively) and NT-proBNP on Cobas 6000 (immunochemistry module Cobas e601) using appropriate kits supplied by Roche Diagnostics.

According to the manufacturer recommendations, a concentration above 37 ng/ml for Hs-TnI and above 125 ng/ml for NT-proBNP were considered abnormal.

All patients had routine blood tests including full blood count, urea and electrolytes and C reactive protein (CRP).

5.2.7.1.3 CMR imaging

The same CMR protocol described in the methodology section was used for the purpose of this study and included LV function and volume, late gadolinium enhancement (LGE), myocardial perfusion and T1 mapping for T1 native and extracellular volume (ECV) quantification. The CMR was performed by the same research team at Leeds General Infirmary, in the CMR Department, using a 3T Philips Achieva MR system equipped with a 32-channel receiver coil. According to the departmental reference ranges, an ECV>29% and T1 native > 1240 ms were considered abnormal (238, 239).

5.2.7.1.4 Other investigative methods

Nailfold videocapillaroscopy

All patients underwent nailfold videocapillaroscopy to assess capillary morphology and architecture. Based on the findings, patients were classified as having either non-specific/normal findings either an early, active or late SSc pattern.

Pulmonary function tests

All patients had annual pulmonary function tests (PFT) performed. The more recent PFT results were collected for all participants, including predicted total lung capacity (TLC), forced vital capacity (FVC), diffusing capacity of the lungs for carbon monoxide (DLCO) and DLCO adjusted for volume (DLCO/VA).

ECG

Participants had an ECG performed when attending for the study visit. The following data was recorded:

- Rhythm: Sinus /atrial fibrillation (AF) /Atrial flutter/other
- Heart rate (bpm)
- Axis: normal/left axis deviation (LAD) /right axis deviation (RAD)
- P wave abnormalities: P mitral, P pulmonale
- Signs of right ventricle (RV) /left ventricle (LV) hypertrophy
- Atrioventricular block: No/First degree/Second degree/Third degree
- Right/ left bundle branch block
- ST elevation/ depression
- T wave abnormalities: flattening, inversion, biphasic, alternans
- Pathological Q wave

5.2.7.2 Follow-up data

Participants had clinical and echocardiography data collected at follow-up. A minimum of 12 months was required between the CMR and follow up data.

Any clinically significant event presumed to be secondary to pSSc-HI was recorded. These comprised:

- Episode of myocarditis, manifested with clinical symptoms, raised cardiac biomarkers, and/or new heart failure, confirmed by CMR
- Heart failure
- Rhythm disturbances, including conduction abnormalities (left, right bundle branch block or AV blocks) and supraventricular or ventricular arrhythmia
- Any of the following echo abnormalities:
    - systolic dysfunction
    - diastolic dysfunction > grade 1

All patients with follow up data had Doppler echocardiography performed. The following data was recorded (Table 5-1).

Table 5-1 Echocardiography data collected at follow-up

LVEF% • Presence/absence systolic dysfunction	Diastolic function • Presence/absence diastolic dysfunction
Tricuspid annular plane systolic excursion (TAPSE)	Any regional wall motion abnormalities (present/absent)
Left atrium dilatation: absent/mild/moderate/severe	Right atrium (RA): Normal/Dilated (mm)
Right ventricle (RV): Normal/Dilated: mild, moderate, severe	RV function: Normal/Depressed: mild, moderate, severe
RV-RA gradient (mmHg)	Right atrial pressure (RAP)

5.2.7.3 Missing data

Reasons for missing data capture were recorded. Participants who did not have echocardiography data at follow-up were excluded from the longitudinal study analysis. Principal components analysis was used to account for the missing data in the predictive model (in particular CMR perfusion data) which evaluated the value of CMR measures and cardiac biomarkers for the development of CV outcomes.

5.2.8 Statistical analysis

Data from ELCASA study documents an event rate (focal myocardial fibrosis) of around 30%. Thus, having a SSc population size of ~ 200 patients, a confidence level of 95% with a margin of error of 8%, a minimum of 78 patients was considered adequate for the purpose of this exploratory, pilot study.

The statistical analysis was performed using SPSS (IBM SPSS Statistics 22), Graph Pad Prism 8 and R (version 3.5.2). Descriptive summary statistics was provided for all variables. Continuous variables were reported as mean (SD) or median (IQR) and categorical data was reported as percentage. Student's t-test, Mann-Whitney U test or chi square test where appropriate were used to assess for significant between

group differences with a 2-tailed p value of 0.05 indicating statistical significance. Correlation between CMR measures and clinical and serum biomarkers were assessed by Spearman's rho test.

Linear univariate and multivariate logistic analysis (for continuous variables) and logistic regression analysis (for binary variables) was used to assess the association between the CMR measures of interest and disease characteristics. Log transformation was used for variables with skewed distribution prior to entering the linear model. The independent variables that entered the model were chosen based on the results at univariate logistic regression. A p value of less than 10% was the cut off value based on which the variable entered the model. The presence of CV risk factors was added into all models as this was thought to be an important factor for the prediction of CMR fibrosis and myocardial perfusion impairment. Receiver-operating characteristic (ROC) curves were built to assess the ability of cardiac biomarkers for identifying abnormal CMR measures.

For the longitudinal dataset, Kaplan-Meier survival curves and log rank test were performed to plot cumulative survival time between groups. Univariate logistic regression was used to find association between CV outcomes and CMR and cardiac serum biomarkers.

If a small number of events in the sample, estimates from logistic regression can be biased. The penalized maximum likelihood estimation method was thus employed to evaluate the predictive value of CMR measures and cardiac biomarkers for future CV outcomes. The method was chosen to account for likely small number of events and modest sample size. Principal components analysis was used to account for the missing values.

5.3 Results

5.3.1 Patient disposition

One hundred and twenty-three SSc patients were screened for the study, out of whom twenty-nine were excluded as they did not meet the inclusion criteria or refused the

CMR. Ninety-three patients were recruited but only eighty-three had CMR data available. Reasons for absent data is illustrated in Figure 5-1. Reason for missing CMR data was refusal to have adenosine administration for CMR perfusion mainly due to anxiety, and image artefacts in which cases CMR data was not interpretable. Cardiac serum biomarkers data was missing due to difficulties in drawing the blood and failure in processing the samples. Of the eighty-three patients with CMR data, function/volume analysis was available for all patients, LGE-CMR and T1 native data was available for 80 patients, ECV% for 78 patients, perfusion data was available for 61 patients, and aortic distensibility for 75 patients.

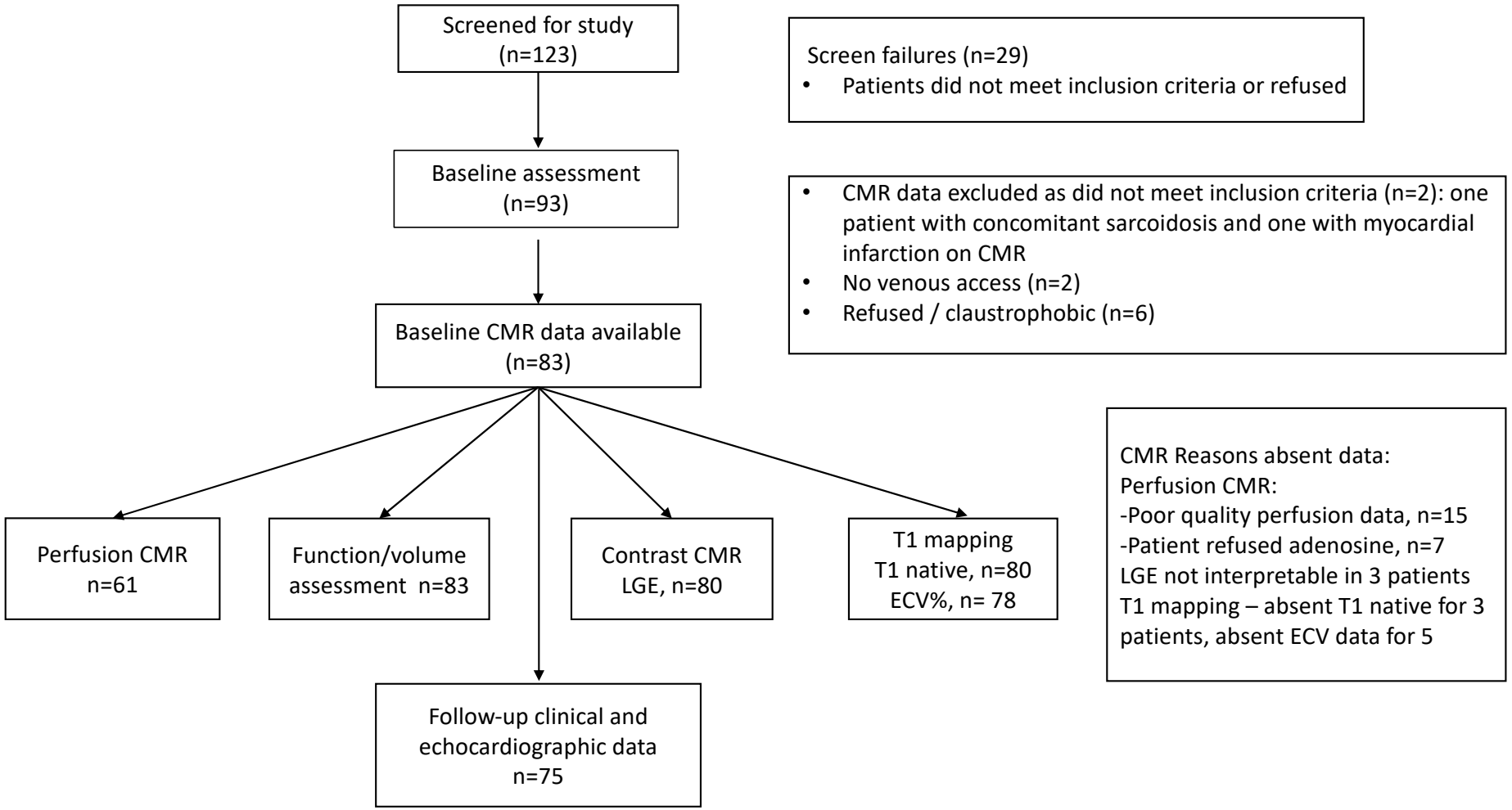


Figure 5-1 Flowchart showing patient selection, recruitment and feasibility

5.3.2 Baseline characteristics

Participants had a median (IQR) age of 54 (49, 54), disease duration (defined as time from non-Raynaud's phenomenon) of 7 (2,7) years, 84% female and 34% had dcSSc. Forty percent had known ILD, 4% myositis, 24% and 25% a history of digital ulceration (DU) and calcinosis respectively. No patients had a diagnosis of pulmonary hypertension. Seventy-eight (94%) SSc patients were ANA positive of whom 28 (34%) and 24 (29%) were ACA and Scl70 positive respectively (Table 5-2, Table 5-3).

Clinical examination of the SSc participants revealed a median (IQR) modified Rodnan skin score (mRSS) of 2 (1,6), 3/83 had tendon friction rubs (TFR) and 14 (17%) had DU. Fifty-seven patients had a SSc specific nailfold capillaroscopy pattern: 21% had an early pattern, 27% an active pattern and 22% a late pattern. All patients were in sinus rhythm (SR), 6 had LAD, 2 incomplete RBB and 1 complete RBB, 5 had unspecific T wave abnormalities (flattened T wave) (Table 4).

Table 5-2 Disease characteristics of SSc patients

SSc phenotype	**SSc patients, n=83**
Demographics	
Age, median (IQR)	54 (49,63)
Female, n (%)	70 (84)
Ethnicity, n (%) Caucasian Asian African	 69 (83) 11 (13) 3 (4)
Disease history	

Disease subtype, n (%)	
LcSSc	55 (66)
DcSSc	28 (34)
Presence of RP, n (%)	83 (100)
Disease duration (years), median (IQR)	7(2,7)
History of, n (%)	
Digital ulceration (DU)	20 (24)
Calcinosis	21 (25)
Myositis	3 (4)
GORD	73 (88)
Non-GORD GI involvement	11 (13)
SIBO	6 (7)
GAVE	4 (5)
Esophagitis	1(1)
Interstitial lung disease	33 (40)
NSIP	31 (37)
UIP	2 (2)
Palpitations	22 (27)

DcSSc, diffuse cutaneous systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis; GORD, gastro-oesophageal reflux disease; GAVE, gastric antral ventricular arrhythmia; NSIP, non-specific interstitial pneumonia; RP, Raynaud's Phenomenon; SIBO, small bacterial overgrowth syndrome; UIP, usual interstitial pneumonia

Table 5-3 Clinical, serological and standard of care cardio-pulmonary profile

Clinical profile	
Total modified Rodnan skin score, median (IQR)	2 (1,6)
Presence of, n (%) Digital pits Digital ulceration Tendon friction rubs Calcinosis Joint contractures	 27 (33) 14 (17) 3 (4) 17 (21) 13 (16)
Any TJC	18 (22)
Any SJC	4 (5)
NFC vasculopathy pattern, n (%) Non-specific Early Active Late	 23 (28) 17 (21) 22 (27) 18 (22)
Serology & acute phase	
Antibody positive, n (%) ANA ACA	 78 (94) 28 (34)

Scl70	24 (29)
CRP (mg/L), median (IQR)	5 (5,5.1)
Standard of care cardio-pulmonary profile	
FVC, mean (SD)	101 (21)
TLC, mean (SD)	92 (15)
DLCO, mean (SD)	62 (14)
DLCO/VA, mean (SD)	80 (15)
Electrocardiogram, n (%) Rhythm Axis Block Other	 83 (100) sinus rhythm 6 (7) LAD 2 (2) incomplete RBBB 1 (1) RBBB 1 (5) left anterior fascicular block 2 (2) SVE 1 (1) VE 5 (6) Unspecific T wave abnormality (T wave flattened)

ACA, anti-centromere antibody; ANA, antinuclear antibodies; CRP, C-reactive protein; DLCO, diffusing capacity of the lungs for carbon monoxide; DLCO/VA, DLCO adjusted for volume; FVC, forced vital capacity; GORD, gastro-oesophageal reflux disease; LAD, left axis deviation; NFC, nailfold capillaroscopy; RBB, right bundle branch block; Scl70,

anti-topoisomerase antibody; SD, standard deviation; SVE, supraventricular ectopics; TLC, total lung capacity; VE, ventricular ectopics.

Twenty-two of 83 patients had at least one traditional cardiovascular risk factor, 17/22 one CV risk factor and 5 patients had 2 CV risk factors (Table 5-4).

Table 5-4 Cardiovascular profile of SSc participants

Dyslipidaemia, n %	3 (4)
Hypertension, n%	8 (10)
Smoking, n%	7 (8)
Family history of CVD, n%	8 (10)
Patients with any CV risk factors, n% 1 cv risk factor, n% 2 cv risk factors, n%	22 (27) 17 (21%) 5 (6%)
Systolic BP, mean (SD)	116 (16)
Diastolic BP, mean (SD)	67 (12)
BMI, mean (SD)	25 (5)
Cardiac serum biomarkers NT-proBNP, median (IQR) Hs-TnI, median (IQR) CK, median (IQR)	 92 (48,143) 4 (3,8) 73 (63,105)

N % presented unless stated otherwise

BMI, body mass index; BP, blood pressure; CK, creatine kinase; CV, cardiovascular; CVD, cardiovascular disease; CRP, C-reactive protein; Hs-TnI, high-sensitivity troponin I; IQR, interquartile range; NT-proBNP, N-terminal pro brain natriuretic peptide; SD, standard deviation.

5.3.2.1 Concurrent treatment

Regarding the treatment, 41 patients (49%) were receiving treatment with a disease-modifying anti-rheumatic drug (DMARD) at the time of recruitment, the majority with mycophenolate mofetil (35%) and methotrexate (10%). Four percent were receiving treatment with cyclophosphamide and 1% with rituximab. Nineteen (23%) participants were receiving iloprost vasodilator treatment, 17 (21%) sildenafil and 4 (5%) bosentan (Table 5-5).

Table 5-5 Current treatment and medication history of patients with SSc

Current use of DMARD, n (%)	49 (59)
Name of current DMARD, n (%)	
Mycophenolate	29 (35)
Methotrexate	8 (10)
Hydroxychloroquine	1 (5)
Sulfasalazine	1 (1)
Cyclophosphamide	3(4)
Rituximab	1(1)
Previous use of cyclophosphamide, n (%)	23 (28)
Current use of prednisolone, n (%)	18 (22)
Current vasodilator treatment with, n (%)	
Iloprost	19 (23)
Sildenafil	17 (21)
Bosentan	4 (5)
ACE inhibitor	31 (37)
Calcium channel blocker	48 (58)
Statin use	5(6)

ACE, angiotensin converting enzyme inhibitor; DMARD, disease modifying antirheumatic drugs.

5.3.3 CMR findings in SSc versus HC

Of the 83 patients recruited, complete CMR function/volume assessment were available in all subjects, LGE and native T1 in 80, ECV in 78 and perfusion CMR in 61 patients (Figure 5-1). Of the 44 HC, LV function/volumes, LGE, T1 native and ECV were available in all and perfusion CMR in 36. HC were well matched to patients with a median (IQR) age of 55 (37, 63), 37 (84%) being females.

None of the HC had LGE on CMR, whilst 17 (21%) of the SSc patients had focal LGE in a non-ischaemic pattern, with a mean (SD) LGE scar mass of 2.08 (1.74). Sixteen patients had evidence of right ventricular insertion point (RVIP) LGE, which is a non-specific finding and was not included in the LGE analysis (260).

Native T1 and ECV, markers of diffuse fibrosis, were significantly higher in SSc compared to HC [Native T1: mean (SD) 1241ms (76) vs 1209ms (51), p=0.008; ECV mean (SD) 31% (4) vs 25% (2), p<0.001] (Table 5-6). Both native T1 and ECV were above the normal reference value (native T1 >1240, ECV>29%). Forty-three (52%) and 51 (61%) SSc patients respectively had native T1 and ECV above normal values.

None of the HC or SSc patients with perfusion CMR data had visual perfusion defects on first pass myocardial perfusion imaging. Quantitative analysis showed significantly lower MBF at stress and lower MPR in SSc patients compared to HC [median (IQR) 1.9 (1.4, 2.6) vs 2.6 (2.2,3.3), p<0.001; median (IQR) 1.9 (1.6, 2.4) vs 3 (2,3.6), p<0.001 respectively] (Table 5-6). There was no difference in MBF at rest between the two groups.

LV volumes and function, including LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), left ventricular stroke volume (LVSV), LV ejection fraction (LVEF) and LV mass were comparable between the HC and SSc participants and the means within normal limits (Table 5-6).

Table 5-6 CMR parameters in SSc patients versus HC

CMR variable	**HC (n=44)**	**SSc patients (n=83)**	**p value**
Fibrosis			
ECV (%)	25 (2)	31 (3)	<0.001**
T1 native (ms)	1209 (51)	1241 (76)	0.008*
LGE, n% LGE scar mass (g)	0 0	17/80 (21%) 2.08 (1.74)	
Myocardial perfusion, median (IQR)			
Stress MBF (ml/g/min) Rest MBF (ml/g/min) MPR	2.6 (2.2,3.3) 0.9 (0.7, 1.2) 3 (2,3.6)	1.9 (1.4, 2.6) 0.9 (0.6, 1.2) 1.9 (1.6, 2.4)	<0.001** 0.734 <0.001**
LV Function/volume			
LVEDV/BSA (ml/m^2)	77 (14)	77 (15)	0.513
LVESV/BSA (ml/m^2)	30 (9)	30 (9)	0.898
LVSV/BSA (ml/m^2)	47 (9)	48 (7)	0.553
LVEF (%)	61 (7)	61 (7)	0.565
LV mass/BSA (g/m^2)	45 (10)	43 (10)	0.415

* p<0.05; ** p<0.01. Mean (SD) unless stated otherwise

BSA, body surface area; CMR, cardiovascular magnetic resonance; ECV, extracellular volume; EDV, end-diastolic volume; ESV, end-systolic volume LGE, late gadolinium enhancement; LV, left ventricular; EF, ejection fraction; MBF, myocardial blood flow; MPR, myocardial perfusion reserve; SV, stroke volume

5.3.4 LGE pattern and distribution

Seventeen (21%) of the 80 patients with available late enhancement images had evidence of LGE in a non-coronary distribution.

A linear LGE pattern was noted in 9 participants, a focal distribution in 6 and a diffuse distribution in 2 (1 participant had both a focal and linear distribution) (Figure 5-2). The LGE was distributed at the midwall for 7 patients, subepicardial for 6 patients, 2 had midwall-subepicardial distribution and 2 had a diffuse distribution. Eleven of 17 had LGE localised at the base, 5 at the basal and mid slices and 1 patient had LGE at the mid slice (Table 5-7). The distribution of the LGE is detailed in Figure 5-3. The most involved segment was the inferolateral segment (7 patients) followed by the anteroseptal and inferoseptal segments. Eleven of 17 patients had more than 1 segment involved.

Table 5-7 Pattern and distribution of LGE in SSc patients

	LGE location (slice), n=17		
	Basal, n %	Basal and mid, n %	Mid, n%
LGE distribution			
Diffuse/transmural	2 (12)	0	0
Midwall	4 (24)	3 (18)	0
Midwall-subepicardial	1 (6)	1	0
Subepicardial	4(24)	1	1 (6)
LGE pattern			
Focal	4 (24) (1- both linear and focal)	2	1 (6)
Linear	6(35) (1- both linear and focal)	3 (18)	0
Diffuse	2 (12)	0	0

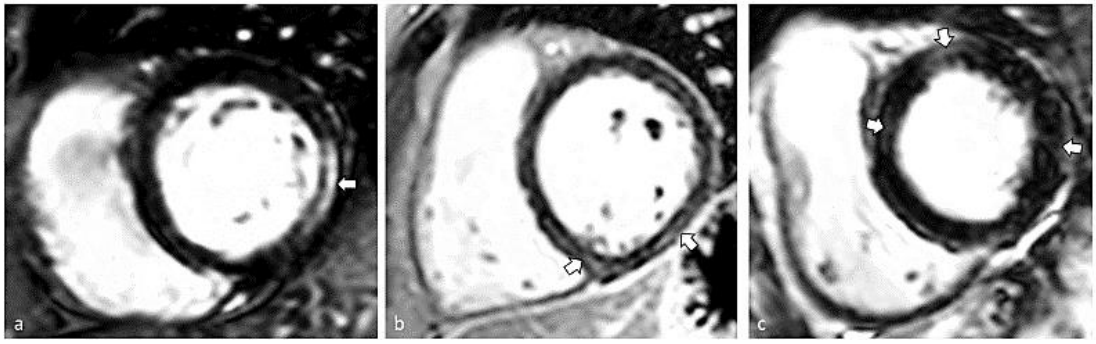


Figure 5-2 LGE patterns: a- focal; b-linear; c-diffuse

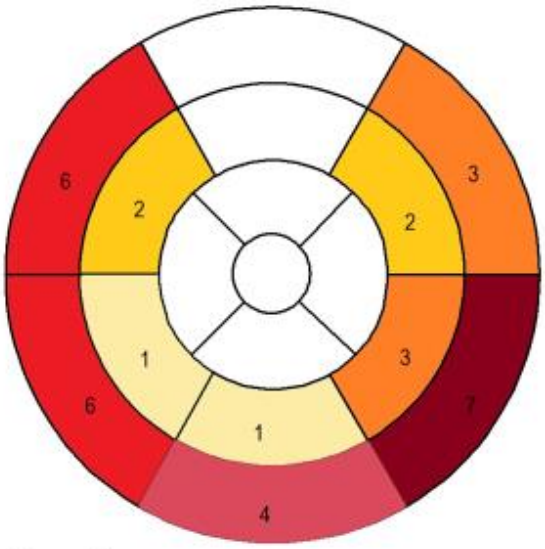


Figure 3a

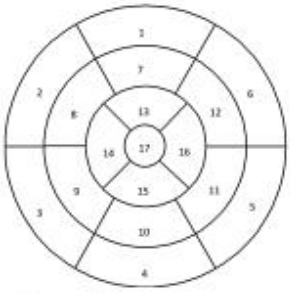


Figure 3b

Figure 5-3a. Number of patients with LGE fibrosis as per each cardiac segment.

Figure 5-3b. 17 segment model 1:basal anterior; 2: basal anteroseptal; 3: basal inferoseptal; 4: basal inferior; 5: basal inferolateral; 6: basal anterolateral; 7: mid anterior; 8: mid anteroseptal; 9: mid inferoseptal; 10: mid inferior; 11: mid inferolateral; 12: mid anterolateral; 13: apical anterior; 14: apical septal; 15: apical inferior; 16: apical lateral; 17: apex.

*11/17 patients had more than 1 segment involved

5.3.5 Association of CMR measures of fibrosis and myocardial perfusion with clinical phenotype

Patients with LGE focal fibrosis had significantly higher modified Rodnan skin score (mRSS) [median (IQR) 4 (2, 9) vs 2 (1,5), p=0.038] (Figure 5-4) and higher CRP levels compared to those without LGE [median (IQR) 5 (5,18) vs (5, 5), p=0.038]. Logistic regression confirmed mRSS to be associated with LGE (OR=1.107, p=0.048) (Table 5-8). LGE scar mass also correlated with mRSS (rho=0.231, p=0.039).

ECV was higher in those patients with DU compared to those without [mean (SD) 34 (4) vs 29 (3), p<0.001] (Figure 5-4). Univariate analysis indicated associations with DU (R^2=0.156, p<0.001), mRSS [(R^2= 0.136, p=0.00) and DLCO/VA (R^2= 0.057, p=0.040). Association of mRSS and the presence of DU with ECV was confirmed on multivariate analysis (R^2=0.335, p=0.003 respectively p<0.001) (Table 5-8).

Significantly higher MPR values were noted in SSc patients with DU compared to those without [median (IQR) 3 (2,3) vs 2 (2.2), p=0.001] (Figure 5-4). The presence of DU associated with MPR at both univariate (R^2= 0.1, p=0.013) and multivariate analysis (R^2=0.146, p=0.016) (Table 5-8).

Patients with LGE fibrosis had significantly higher MRSS compared to those without [median (IQR) 4(2, 9) versus 2 (1,5), p=0.038]. Higher CRP levels were also noted in patients with LGE fibrosis [median (IQR) 5(5,18) vs (5, 5), p=0.038]. Further logistic regression showed that MRSS was the only clinical variable associated with LGE (OR=1.107, p=0.048) (Table 5-8).

ECV was higher in those patients with DU compared to those without [mean (SD) 34 (4) vs 29(3), p=0.000] (Figure 5-4). Among SSc patients with DU, there was no significant difference between lcSSc or dcSSc subsets (p=0.373). Both MRSS and the presence of DU associated with ECV at univariate [(R^2= 0.136, p=0.001), (R^2=0.156, p<0.001) respectively] and multivariate analysis (R^2=0.335, p=0.003, p<0.001 respectively) (Table 5-8).

Significantly higher MPR values were noted in SSc patients with DU compared to those without [median (IQR) 3(2,3) versus 2 (2.2), p=0.001](Figure 5-4). No significant difference between SSc patients with DU and lcSSc and those with DU and dcSSc was noted (p>0.05). Further analysis showed that the presence of DU associated with MPR at both univariate (R^2= 0.1, p=0.013) and multivariate analysis (R^2=0.163, p=0.009) (Table 5-8).

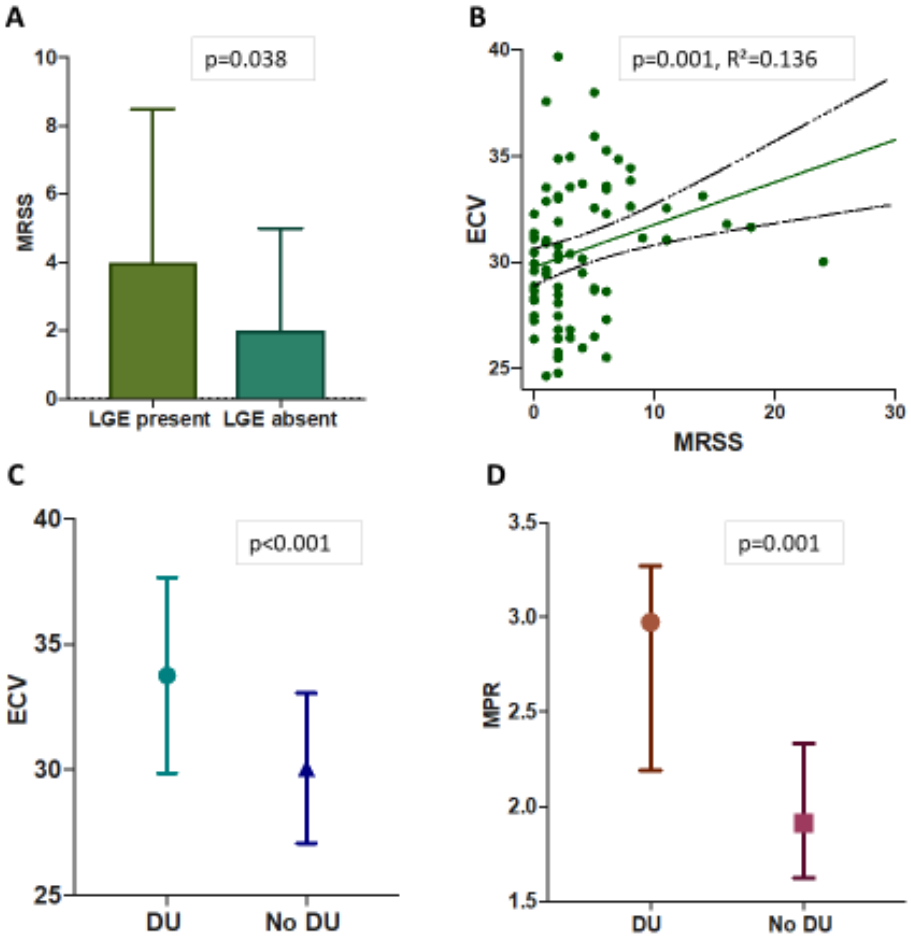


Figure 5-4 Disease phenotype and CMR parameters: (A) Presence or absence of LGE fibrosis and median (IQR) mRSS. (B) Association between ECV and mRSS. (C) Presence or absence of DU and ECV. (D) Presence or absence of DU and MPR.

DU digital ulcers; ECV extracellular volume; LGE late gadolinium enhancement; MPR myocardial perfusion reserve; mRSS modified Rodnan skin score.

Table 5-8 Logistic and linear regression looking at the association between LGE, ECV, MPR and disease phenotype

	LGE presence (n=17)/absence (n=63)		**ECV**				**MPR**			
Variable	Logistic regression-Univariate analysis		Linear univariate analysis		Multivariate analysis, R^2 =0.335		Linear univariate analysis		Multivariate analysis, R^2 =0.163	
	OR	p value	Beta	p value	Beta	P value	Beta	p value	Beta	P value
Male gender	2.11	0.275	-0.05	0.966			-0.195	0.133		
Age	0.986	0.541	0.019	0.869			-0.231	0.073	-0.204	0.062
Disease duration	0.969	0.411	-0.216	0.059	-0.161	0.128	0.028	0.831		
Presence of CV risk factors	0.904	0.875	0.217	0.056	0.138	0.175	-0.139	0.287	-0.027	0.890
Presence of ILD	1.444	0.504	-0.12	0.296			0.031	0.810		

	LGE presence (n=17)/absence (n=63)		**ECV**				**MPR**			
DLCO/VA	0.893	1.003	-0.238	0.040*	-0.102	0.343	-0.112	0.356		
MRSS	1.107	0.048*	0.369	0.001**	0.250	0.030*	-0.145	0.264		
DcSSc	1.621	0.392	0.185	0.105			0.009	0.945		
Digital ulcers	0.222	0.154	0.395	<0.001**	0.379	<0.001**	0.299	0.013*	0.307	0.009**
NFC Early pattern Active pattern Late pattern	 0.421 0.267 0.444	 0.265 0.156 0.297	 -0.185 0.186 0.131	 0.107 0.105 0.257			 -0.029 0.006 -0.025	 0.827 0.966 0.849		
DMARD treatment	0.402	0.148	0.151	0.186			-0.003	0.984		

	LGE presence (n=17)/absence (n=63)		ECV				MPR			
ACE inhibitor	1.428	0.447	-0.087	0.451			-0.04	0.978		
CRP	1.064	0.067	-0.056	0.624			-0.075	0.567		

* p<0.05; ** p<0.001

ACE, angiotensin converting enzyme; CV, cardiovascular; CRP, C-reactive protein; dcSSc, diffuse cutaneous systemic sclerosis; DLCO/VA, DLCO adjusted for volume; DMARD, disease modifying antirheumatic drugs; ECV, extracellular volume; ILD, interstitial lung disease; LGE, late gadolinium enhancement; MPR, myocardial perfusion reserve; MRSS, modified Rodnan skin score; NFC, nailfold capillaroscopy; OR odds ratio.

5.3.6 Association of CMR measures of fibrosis and myocardial perfusion with cardiovascular biomarkers

Hs-TnI was significantly higher in those patients with LGE focal fibrosis compared to those without [median (IQR) 43 (83) vs 17 (50), p=0.012] (Figure 5-5) and had a significant correlation with LGE scar mass (rho=0.283; p=0.014). Patients with a focal LGE pattern (also with greater scar mass) had higher Hs-TnI levels compared to those with a linear or diffuse pattern [median (IQR) 53 (6, 165) vs 4 (3,11) vs 6 (5,6)]. A positive correlation was observed between NT-proBNP and ECV (rho=224; p=0.048) (Figure 5-5) and higher levels associated with the presence of LGE fibrosis although this did not reach statistical significance [mean (SD) 260 (357) vs 106 (96), p=0.083]. ROC curves were plotted to assess the ability of these serum cardiac biomarkers to identify CMR fibrosis. AUC (95%CI) of Hs-TnI for identifying LGE was significant at 0.695 (0.55, 0.837), p=0.015. A Hs-TnI≥ 5.5 ng/l had a sensitivity of 65% and a specificity of 70%. AUC (95%CI) of NT-proBNP for identifying ECV (ECV<29%; ECV≥29%) was 0.586 (0.447, 0.726), p=0.213 (Figure 5-6).

There was no significant correlation between CK and any of the CMR parameters or between MPR and cardiac biomarkers (p>0.05).

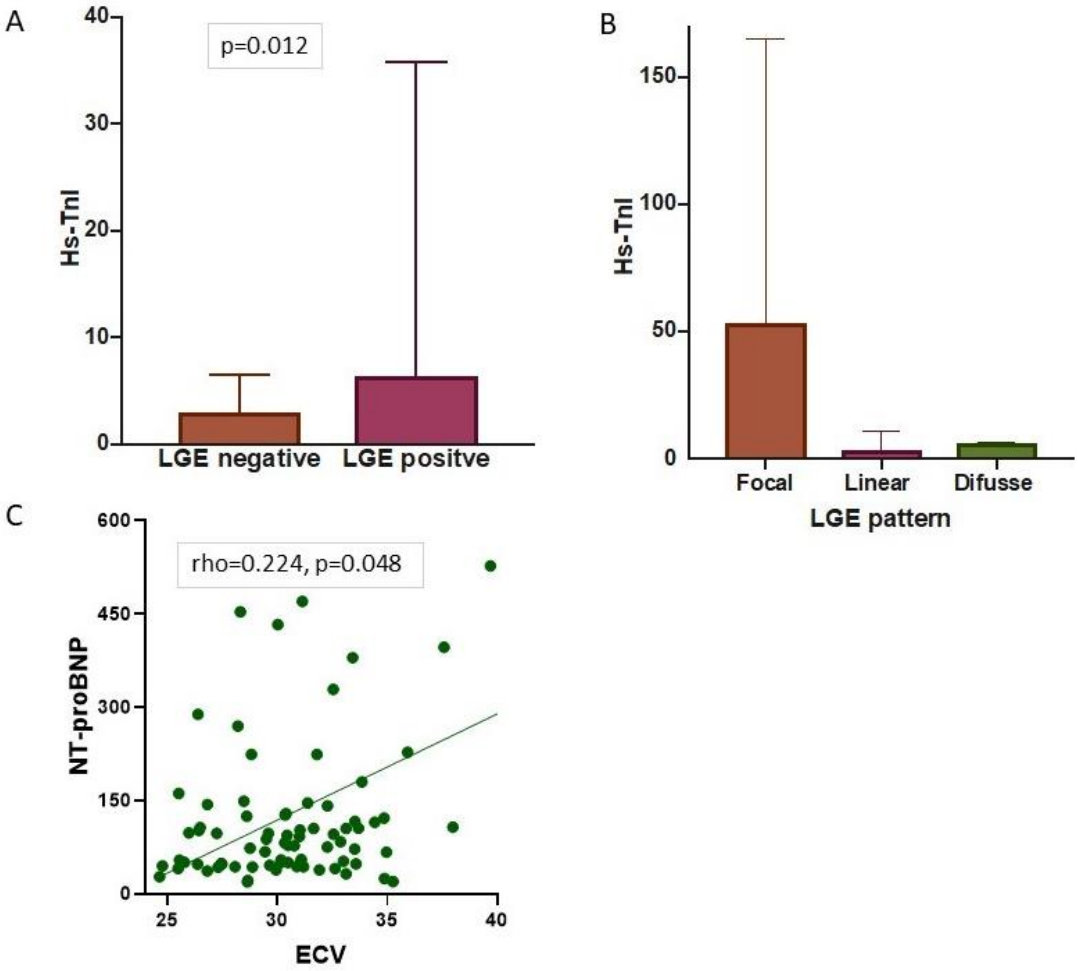


Figure 5-5 Association between cardiac biomarkers and CMR variables: (A) Presence or absence of LGE and Hs-TnI. (B) Hs-TnI in focal, linear or diffuse LGE pattern. (C) Association between ECV and NT-proBNP.

ECV extracellular volume; Hs-TnI high sensitivity troponin I; LGE late gadolinium enhancement; NT-proBNP N-terminal pro-brain natriuretic peptide.

Figure 5-6 A

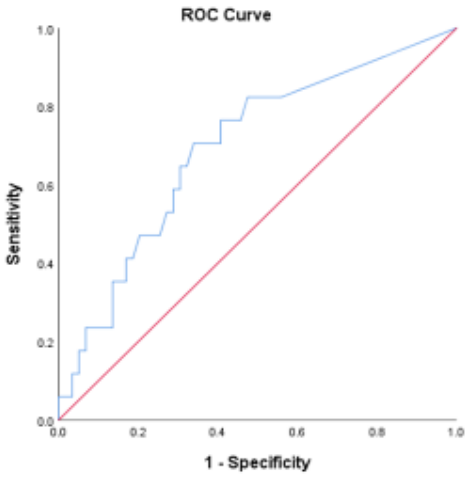


Figure 5-6 B

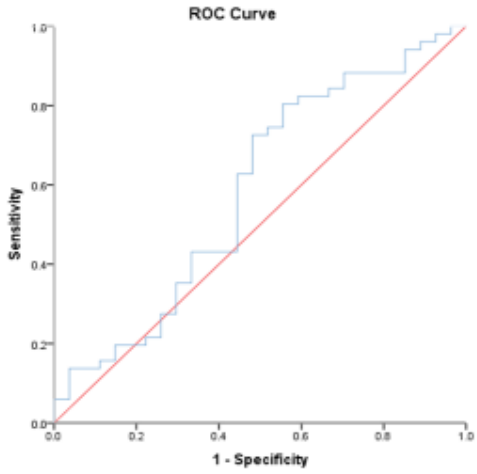


Figure 5-6 A. ROC curve of Hs-TnI for predicting LGE. 5-6 B.ROC curve of NT-proBNP for predicting ECV

5.3.7 CMR and cardiac biomarkers predictors of CV events

5.3.7.1 Patient disposition and baseline characteristics

Seventy-five SSc patients (of a total of 83 patients originally recruited) had available baseline and follow up clinical and echocardiographic data. Participants had a median (IQR) age of 57 (49, 64), 25 (33%) had dcSSc and 29 (39%) had a history of ILD. Twenty-seven (36%) were ACA positive and 22 (29%) were Scl70 positive (Table 5-9). Forty-three (57%) were receiving a disease modifying antirheumatic drug (DMARD) at the time of recruitment. Twenty-one (28%) patients had CV risk factors, 16 had one CV risk factor and 5 had 2 CV risk factors. Of the 75 patients, all had functional and volume CMR data, 73 had LGE and T1 native data, 71 had ECV, 68 had aortic distensibility and 56 myocardial perfusion CMR data.

Table 5-9 Disease characteristics of SSc patients

SSc phenotype	**SSc patients, n=75**
Age, median (IQR)	57 (49,64)
Female, n (%)	64 (85)
Disease subtype, n (%) lcSSc dcSSc	 50 (67) 25 (33)
Disease duration (years), median (IQR)	8 (2,16)
History of, n (%) Digital ulceration Calcinosis Myositis GORD Interstitial lung disease	 17 (23) 18 (24) 3 (4) 66 (88) 29 (39)
Current use of DMARD, n (%)	43 (57)
Clinical profile	
Total modified Rodnan skin score, median (IQR)	2 (1,6)
Presence of, n (%) Digital pits Digital ulceration	 24 (32) 11 (15)

Tendon friction rubs Calcinosis Joint contractures	3 (4) 14 (19) 10 (13)
Serology & acute phase	
Antibody positive, n (%) ANA ACA Scl70	 70 (93) 27 (36) 22 (29)
Any CV risk factors • 1 cv risk factor • 2 cv risk factors	21 (28) 16 (21) 5 (7)

N % presented unless stated otherwise

ACA, anti-centromere antibody; ANA, antinuclear antibodies; CRP, C-reactive protein; dcSSc, diffuse cutaneous systemic sclerosis; DMARD, disease modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; GORD, gastro-oesophageal reflux disease; IQR, interquartile range; lcSSc, limited cutaneous systemic sclerosis; RP, Raynaud's Phenomenon; Scl70, anti-topoisomerase antibody; SD, standard deviation; SSc, Systemic Sclerosis.

5.3.7.2 Cardiovascular outcomes

Patients were followed up for a median (IQR) 22 (15, 54) months. All participants had more than 12 months follow up. A total of eleven CV outcomes in 10 patients were recorded, comprising one diagnosis of myocarditis confirmed on CMR, triggered by echocardiograph- reported systolic dysfunction and 9 patients with arrhythmias (Table 5-10 and 5-11). The arrhythmia included 3 non-sustained ventricular tachycardia (NSVT) and 6 supraventricular arrhythmias; of which one patient had atrial tachycardia, three atrial fibrillation (AF) of which one also with atrial flutter, 1 patient with atrial flutter and another one with supraventricular tachycardia. None of the 75 patients had a diastolic dysfunction>grade 1. Their median (IQR) time to CV outcome was 14 (8,27) months.

Their median (IQR) age was 58 (52,64), 9 were females, 5 had a DcSSc, 4 a diagnosis of ILD, 3 were Anti Scl70 and 5 were ACA positive (Table 5-11).

Table 5-10 CV outcomes in SSc patients

Echocardiography findings	N
Systolic dysfunction	1 also with myocarditis
Diastolic dysfunction> grade 1	0
Myocarditis	1
Arrhythmia	
Ventricular arrhythmias, NSVT	3
Atrial arrhythmias	6

Table 5-11 Baseline characteristics of patients with CV outcomes

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
CV outcome	SVT	NSVT on stress ECG	Myocarditis Systolic dysfunction	NSVT	AF	AF	SVT	Atrial flutter	NSVT	AF and flutter
Baseline ECG	-	SR, VE	SR	SR, flattened T wave	SR	SR	SR	SR	SR	SR
Age	74	59	60	50	57	73	62	55	52	49
Gender	F	F	F	M	F	F	F	F	F	F
SSc subset	lcSSc	dcSSc	dcSSc	dcSSc	dcSSc	lcSSc	lcSSc	lcSSc	dcSSc	lcSSc
ILD	0	1	1	1	0	0	0	0	1	0

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
MRSS	6	18	9	0	14	2	4	6	11	2
ANA	ACA	Anti Ro	Scl70	Scl70	-	ACA	ACA	ACA	Scl70	ACA
HxDU	1	0	0	0	0	0	0	1	0	0
NT-proBNP	161	105	470	38	105	127	288	141	329	32
Hs-TnI	3	5	225	3	3	-	5	31	313	6

AF, atrial fibrillation, ACA, anti-centromere antibody; ANA, antinuclear antibodies; CRP, C-reactive protein; dcSSc, diffuse cutaneous systemic sclerosis; ECG, electrocardiogram; Hs-TnI, high sensitivity troponin I; DU, history of digital ulcers; ILD, interstitial lung disease; MRSS, modified Rodnan skin score; NT-proBNP, N-terminal pro-brain natriuretic peptide; SR, sinus rhythm, SVT, supraventricular tachycardia.

5.3.7.3 CMR and cardiac biomarker findings in SSc patients with and without CV outcomes

No CMR parameters appeared to associate with the development of CV outcomes and the means of LV volume and function were within normal range. However, higher LV mass was noted in those with CV outcomes [mean (SD) 48 (10) vs 43 (11), p=0.127] (Table 5-12) and an ECV above the normal threshold (>29%) was found in 8/9 patients presenting with CV outcomes versus 40/62 patients with no CV outcomes (p=0.144).

Patients with CV events had higher Hs-TnI [median (IQR) 5 (3, 127) vs 3 (3, 7), p= 0.133] and NT-proBNP levels [median (IQR) 134 (88, 298) vs 83 (48, 127), p= 0.139] compared to those with no CV outcomes, although these did not reach statistical significance. Higher NT-proBNP levels, above the normal range (>125 pg/ml) were found in 6/10 patients with CV outcomes versus 16/65 patients with no CV outcomes (p=0.022) (Table 5-12).

Table 5-12 CMR parameters and cardiac biomarkers in SSc patients with CV events versus SSc patients with no CV events

	SSc patients with CV outcomes	**SSc patients with no CV outcomes**	**P value**
CMR variable			
LVEDV/BSA (ml/m^2)	80 (14)	77 (16)	0.501
LVESV/BSA (ml/m^2)	30 (8)	30 (10)	0.941
LVSV/BSA (ml/m^2)	50 (7)	47 (7)	0.208
LV mass/BSA (g/m^2)	48 (10)	43 (11)	0.127
LVEF %	63 (5)	62 (5)	0.439
ECV%	31 (2)	30 (3)	0.610
ECV (£29, >29 %), n%	8/9	40/62	X^2, 0.144
T1 native (ms)	1216 (88)	1237 (72)	0.434
T1 native (£1240, >1240), n%	5/9	33/64	X^2, 0.822
LGE Scar mass	1/9 0.42 (1.27)	14/64 0.46 (1.2)	X^2, 0.454 0.931
MPR, median (IQR)	1.86 (1.5,2.4), n=51	1.96 (1.6,2.5)	0.696

Aortic distensibility	4.2 (3)	3.8 (2)	0.605
Cardiac biomarkers			
Hs-TnI, median (IQR)	5 (3,128)	3 (3, 7)	0.133
Hs-TnI (£37, >37 ng/l), n%	2/9	5/62	X^2, 0.183
NT-proBNP, median (IQR)	134 (88, 298)	83 (48, 127)	0.139
NT-proBNP (£125, >125 pg/ml), n%	6/10	16/65	X^2, 0.022*

* p<0.05

Mean (SD) unless stated otherwise

BSA, body surface area; CMR, cardiovascular magnetic resonance; ECV, extracellular-volume fraction; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; Hs-TnI high sensitivity troponin I; LGE, late gadolinium enhancement; LV, left ventricular; MBF, myocardial blood flow; MPR, myocardial perfusion reserve; NT-proBNP, N-terminal pro-brain natriuretic peptide.

5.3.7.4 CMR and cardiac serum biomarkers and probability of CV outcomes

The probability of CV outcomes was significantly higher in those with NT-proBNP above the normal threshold compared to those with normal NT-proBNP levels (X^2= 4.19, p=0.045) and poorer survival course was also noted in those with higher ECV compared to those with normal ECV values, although this did not reach statistical significance (X^2= 2.659, p = 0.103) (Figure 5-7).

No difference in survival time was observed between patients with Hs-TnI above the normal threshold (≥37 ng/l) compared to those with normal Hs-TnI levels (X^2-0.044,

p=0.834) or between those with LGE compared to those with no LGE (X^2 = 0.290, p=0.590) (Figure 5-7).

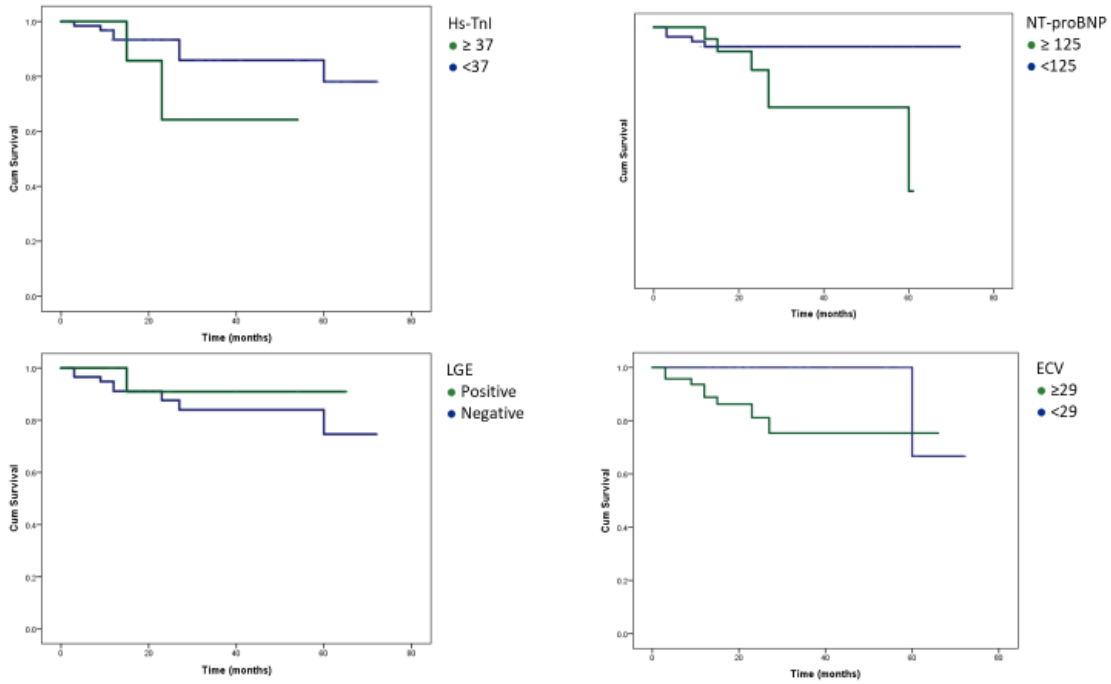


Figure 5-7 Survival plots for CV outcomes in patients with normal and abnormal Hs-TnI, NT-proBNP, ECV and presence or absence of LGE

ECV extracellular volume; Hs-TnI high sensitivity troponin I; LGE late gadolinium enhancement; NT-proBNP N-terminal pro-brain natriuretic peptide.

5.3.7.5 CMR and cardiac biomarkers as predictors of CV outcomes

Univariate logistic analysis showed an association of Hs-TnI with the presence of CV outcomes (OR=1.009, p=0.044). NT-proBNP above the normal threshold (> 125 pg/ml) also associated with the presence of CV outcomes (OR=4.594, p=0.031). Using penalised likelihood estimation, a predictive model that included CMR variables and cardiac biomarkers was built. NT-proBNP above the normal threshold (>125 pg/ml) was the only variable associated with the development of CV outcomes (OR=5.918, p=0.028) albeit an ECV above 29% almost reached statistical significance (OR=5.838, p=0.075). No other CMR measures predicted the development of CV outcomes (Table 5-13).

Table 5-13 Penalized logistic regression analysis for predicting CV events including CMR parameters and cardiac biomarkers

Variable	Estimate	p	OR
NT-proBNP>125 pg/ml	1.778	0.028*	5.919
Hs-TnI>37 ng/l	0.807	0.462	2.241
LVEDV/BSA	-0.032	0.552	0.968
LVESV/BSA	0.127	0.316	1.136
LVEF	0.168	0.252	1.183
LV mass/BSA	0.041	0.378	1.042
LGE	-0.854	0.391	0.426
T1 native	-0.007	0.168	0.993
ECV % (>29)	1.764	0.075	5.838
MPR %	-0.264	0.465	0.768

*p<0.05

BSA, body surface area; CMR, cardiovascular magnetic resonance; ECV, extracellular-volume fraction; EDV, end-diastolic volume; ESV, end-systolic volume; Hs-TnI, high sensitivity troponin I; LGE, late gadolinium enhancement; LV, left ventricular; EF, ejection fraction; MPR, myocardial perfusion reserve; NT-proBNP, N-terminal pro-brain natriuretic peptide; OR, odds ratio.

A separate model including also clinical variables: ACR subset, mRSS, disease duration, gender and presence of DU was performed. NT-proBNP was the only variable to show association with CV outcomes (p=0.061, OR=5.120) but none of the clinical variables predicted CV outcomes (p>0.05).

5.4 Discussion

This report is the largest study to date to identify pSSc-HI using comprehensive CMR indices of both fibrosis and quantitative perfusion with clinical and serum correlates. The results confirm that both subclinical (focal and diffuse) fibrosis and reduced myocardial perfusion form the pathophysiological basis for pSSc-HI. These subclinical processes associate with Hs-TnI and NT-proBNP, and markers of SSc disease activity as well as DU. A combination of poor prognosis clinical factors and Hs-TnI and NT-proBNP could therefore stratify an unaffected SSc cohort for CMR-detected pSSc-HI, thus providing the opportunity for more tailored monitoring and intervention of those liable to develop clinically overt pSSc-HI. Moreover, the longitudinal dataset was the first to assess the prognostic value of CMR and serum cardiac biomarkers for future development of pSSc-HI. Just under 15% (10/75) developed CV outcomes including myocarditis, systolic dysfunction and arrythmias. NT-proBNP was the only variable that predicted future CV outcomes, and higher ECV also seemed to associate with CV outcomes although it did not reach statistical significance. These data suggest the utility of routine serum cardiac biomarkers and CMR-ECV for identifying and thus more closely monitoring patients at risk of clinically overt pSSc-HI and provide a first step in understanding the relevance of CMR abnormalities in a SSc cohort.

Myocardial fibrosis, the hallmark of pSSc-HI [6, 27] is commonly described in SSc [10, 13]. Our study showed that one quarter of the SSc patients (and no HC) had a non-coronary LGE pattern, in keeping with a primary cardiac pathology. Our study did not include patients with IHD, PAH, diabetes, other inflammatory musculoskeletal diseases, with minimum presence of traditional CV risk factors, thus reducing the risk of including cardiac fibrosis associated with IHD or other conditions. Moreover, the study excluded LGE localised at the inferior RVIP that is considered non-specific and may represent a normal variant [26]. Three distinctive patterns of fibrosis were found; linear, diffuse and focal, the latter being associated with higher LGE scar mass. LGE was localised predominantly at the basal anteroseptal and inferoseptal segments and had a midwall or subepicardial distribution, similar to previous studies [28, 29]. A lateral subepicardial LGE distribution which is more commonly described in

myocarditis [30] was present in nearly half of the SSc patients. This would imply that at least in a subgroup of SSc patients, a silent inflammatory process is the substrate for the development of myocardial fibrosis. Whether different LGE patterns have distinctive underlying pathogenetic mechanism in SSc, for example, inflammation and/or microvascular dysfunction needs to be further explored.

Increased ECV has been previously recorded in SSc cohorts [15, 31]. Our study confirmed higher ECV in SSc compared to HC, indicating a diffuse interstitial process in the myocardium of SSc. Although the prognostic implication of this finding in SSc has not yet been determined, in non-ischaemic cardiomyopathies, ECV is a poor prognostic marker for CV outcomes [32] with pathophysiological studies suggesting that interstitial fibrosis can progress to replacement/scarring fibrosis (seen as LGE on CMR) [33, 34]. Furthermore, our recently reported association between ECV and implantable loop recorder detected arrhythmia in asymptomatic SSc patients [25] underscores the potential clinical importance of detecting raised ECV.

The present study showed significantly lower global MPR in SSc patients compared to HC with no localised perfusion defects, indicating microvascular disease. One previous small study (n=19) also performed quantitative perfusion CMR and demonstrated lower stress MBF in SSc compared to HC [mean (SD) 3.1 ± 0.9 vs. 4.2 ± 1.3 p = 0.008)] [17].

Our data demonstrate that the clinical phenotypes of SSc associated with CMR findings. Two previous studies reported an association of ECV with mRSS [15, 35] and longer history of Raynaud's in those with focal fibrosis on LGE [13] with another study failing to reveal an association between the CMR measures of fibrosis and SSc disease phenotype [29]. Our study showed that both LGE and ECV associated with mRSS in a predictive model. Patients with LGE also had higher CRP levels. Collectively, these findings support the association of myocardial fibrosis with disease activity and suggest a common, likely concurrent fibrotic process affecting both the skin and myocardium.

Both ECV and MPR associated with the presence of DU (indicative of severe peripheral vasculopathy). The positive association between ECV and DU could indicate a pathogenetic process of microvascular impairment, which leads to diffuse fibrosis and remodelling of the heart. We also observed an increase in MPR (a ratio of maximal stress: resting myocardial blood flow) with the presence of DU. This may appear counter-intuitive in the face of vasodilatory treatments that would be expected to improve coronary microvasculature (increasing myocardial resting blood flow) and thus depress the MPR. One could postulate that the coronary microvasculature of SSc patients, and in particular, the subgroup with DU may be less responsive to such therapies. Similar observations of improved myocardial perfusion index following vasodilatory therapies have been reported [36, 37] although without the above consideration of underlying coronary physiology. Mechanistic investigation could clarify this further.

Significantly increased TnI is observed in acute coronary syndrome, but the relevance of modest TnI elevation in the general population is not clear, with renal pathology, inflammation and/or infection possible contributors [38]. Our results showed that the moderate Hs-TnI levels observed associated with the presence of LGE and LGE scar mass indicating that this biomarker is sensitive in detecting myocardial injury in SSc. Moreover, Hs-TnI was higher in patients demonstrating a focal pattern of fibrosis, implying a direct relationship between myocardial scar mass (that we observed is increased in focal pattern of fibrosis) and Hs-TnI. This is of particular interest as LGE extent has been associated with worse CV outcomes in both ischaemic and non-ischaemic cardiomyopathies [39, 40]. A recent CMR study in systemic lupus erythematosus (SLE) reported a strong association of T1 and T2 native, CMR markers of interstitial remodelling and inflammation with hs-TnT [41]; as opposed to fibrosis observed in our study of SSc, suggesting different pathophysiological processes may predominate between immune-mediated diseases that are associated with primary myocardial involvement. NT-proBNP, released by ventricular myocytes in response to increased wall tension associated with ECV, which might be explained by interstitial remodelling and expansion of the myocardium. Higher levels of NT-proBNP also associated with LGE, although the results did not reach statistical significance. Both

Hs-TnI and NT-proBNP thus appear to be sensitive tools for detecting (focal and diffuse) fibrosis. Whilst ROC curve showed a moderate diagnostic performance of Hs-TnI for identifying LGE, the diagnostic performance of ECV for identifying NT-proBNP was poor. This would likely be improved upon by evaluation of a still larger sample size despite this study being the largest such CMR study in SSc.

The current longitudinal study is a first to evaluate the prognostic value of CMR and serum cardiac biomarkers for future development of pSSc-HI CV outcomes. A significant proportion of unselected SSc patients have subclinical cardiac involvement when sensitive methods are used (154, 164) however, the prognostic implications of these findings is not well established. Several cross-sectional studies have reported an association between myocardial fibrosis and electrophysiological studies; whilst two studies showed a relationship between LGE burden and ventricular arrhythmia as assessed by 24-Holter ECG (15, 162), other studies did not confirm these findings (15, 131).To our knowledge, we report the largest CMR study in SSc with follow up data that looked to evaluate the prognostic role of CMR abnormalities and serum cardiac biomarkers. Thirteen percent of the patients developed CV outcomes consistent with pSSc-HI at follow up including myocarditis, arrhythmias and systolic dysfunction on echocardiography. Patients with CV outcomes had higher Hs-TnI and NT-proBNP levels compared to those with no CV outcomes, whilst NT-proBNP was the only variable to predict the development of CV outcomes. Hs-TnI and NT-proBNP increase indicate the presence of initial silent myocardial pathology that may progress to clinically overt pSSc-HI. These data suggest that routinely available serum cardiac biomarkers, in particular NT-proBNP could be of further use for risk stratification in pSSc-HI. Similar findings have been reported in one previous study, where both hs-TnT and NT-proBNP associated with the development of CV events in SSc patients (197).

A trend suggesting an association between ECV and the development of CV outcomes, with a worse event-free course in those with higher ECV was observed. Indeed, the majority of SSc patients with CV outcomes (8/9) had an ECV above the normal threshold, albeit higher ECV levels were present also in those with no pSSc-HI outcomes. This may still suggest that a normal range ECV is associated with a lower risk for developing CV events. Nonetheless, an ECV above the normal threshold (>29)

almost reached statistical significance for predicting CV outcomes. We and others have reported the association of higher ECV with arrhythmias and poor CV outcomes (261, 262). LV mass and left ventricular hypertrophy are also recognised as predictors of sudden cardiac death and arrhythmia (263). In our study, higher LV mass was noted in patients with confirmed pSSc-HI, although this did not reach statistical significance. Larger studies are likely required to confirm a prognostic value of raised ECV and LV mass in SSc.

Only one of the fifteen patients with fibrosis on LGE had a CV outcome. The relatively small sample size and event ratio likely account for this finding. However, the extent and location of LGE need to be taken into consideration as these are important determinants for the development of arrhythmias and CV events in both ischemic and nonischaemic-cardiomyopathies (210, 257). The low scar mass values in our study patients probably contribute to the lack of association with the outcome observed.

In conclusion, this study is the largest CMR study in SSc to include quantitative perfusion, quantitative scar mass and T1 mapping, providing a comprehensive assessment of CMR-detected pSSc-HI. The study demonstrates that in a well-phenotyped cohort of unaffected SSc patients, subclinical (focal and diffuse) fibrosis and reduced myocardial perfusion form the predominant pathological basis of CMR pSSc-HI. Markers of SSc disease activity and complicated peripheral vasculopathy, together with Hs-TnI and NT-proBNP provide a tangible basis for a first step of risk stratification to identify CMR-defined pSSc-HI; a proportion of which will develop clinically overt pSSc-HI.

Nonetheless, the longitudinal dataset was the first to show that serum biomarkers, in particular NT-proBNP and CMR-ECV could be of further use in risk stratification for pSSc-HI CV outcomes. Larger longitudinal studies are required to investigate the prognostic implications of cardiac biomarkers and CMR detected pSSc-HI (in particular, ECV, LGE, and LV mass) to better inform risk stratification for clinically overt pSSc-HI.

5.4.1 Limitations

Time of occurrence of pSSc-HI and whether DMARD treatment can alter the course of pSSc-HI are important clinical questions that would ideally require recruitment of an inception cohort. Such studies in a rare disease are challenging.

Atherosclerotic disease is also difficult to fully exclude, however, the current study minimised this risk by excluding patients with CVD, diabetes and more than 2 CV risk factors. None of the patients had evidence of myocardial perfusion defects indicative of IHD on CMR, making inadvertent inclusion of secondary IHD in our cohort unlikely.

Whilst this is the first good-sized longitudinal follow-up study to date, larger cohort size is needed to bring out prognostic value and development of a risk model of abnormal CMR indices. In addition, the minimum follow-up of one year means CV events that might have occurred later for the patients with shorter follow up could have been missed. Larger, longitudinal studies with longer and equal length of follow up are thus warranted.

5.4.2 Key messages

- CMR abnormalities in SSc have a high prevalence compared to HC
- Myocardial focal and diffuse fibrosis and microvascular impairment form the pathophysiological basis for pSSc-HI
- Three distinct LGE patterns have been identified: linear, diffuse and focal which might have distinctive underlying pathogenetic mechanism
- Markers of disease activity and complicated peripheral vasculopathy together with cardiac biomarkers associate with CMR-defined pSSc-HI; these indices may provide a basis for risk stratification of pSSc-HI
- pSSc-HI CV outcomes were identified in 13% of the SSc patients, and cardiac biomarkers, in particular NT-proBNP and CMR-ECV could be of further use in risk stratification for pSSc-HI CV outcomes

Chapter 6. Describing the course and natural history of cardiovascular magnetic resonance-detected heart involvement in systemic sclerosis

6.1 Introduction

With the use of the more advanced imaging techniques such as cardiovascular magnetic resonance (CMR), speckle tracking imaging or myocardial perfusion scintigraphy, subclinical pSSc-HI is described in a significant proportion of SSc patients (152, 158, 264). However, there is little understanding of the significance of such subclinical findings, whether these are sensitive to change over time and can progress to clinically overt pSSc-HI.

Change in subclinical pSSc-HI has been documented in several echocardiographic studies (265). Using speckle-tracking strain echocardiography and excluding SSc patients with prior myocardial infarction, cardiomyopathy and moderate to severe valvular disease, one study showed deterioration of the global longitudinal strain (GLS), left ventricular diastolic function and right ventricular systolic function in 39 out of 234 patients (266). SSc patients with a GLS reduction >15% had higher mRSS [median [IQR] 2 (0.0–5.0) 4 (1.5–9.5) p=0.177], proximal muscle weakness [n (%) 13 (8) vs 10 (26) p=0.001] and lower DLCO [n (%) 106 (64) vs 34 (87), p=0.009] compared to the ones with no change in GLS. Another study that performed standard and tissue doppler echocardiography in 74 SSc patients showed deterioration of both systolic and diastolic function at follow up compared to controls, which associated with age, New York Heart Association (NYHA) functional class >=2 and poorer Medsger score at baseline (267).

CMR is one of the most accurate tools for investigating pSSc-HI, providing information in regards to the function, volume, perfusion abnormalities and tissue characterisation (14, 171, 179). Change in CMR has been assessed only in one previous small sized study of 11 patients (out of the 44 patients included in the study) that had a follow-up CMR;

a deterioration in the myocardial perfusion index and LGE fibrosis was documented (18).

Whether disease modifying anti-rheumatic (DMARD) treatment can alter the course and stop the progression of pSSc-HI is not clear. Case report studies document the value of immunosuppressive treatment in myocarditis (100, 125), but the effect of DMARDs in CMR detected pSSc-HI, where myocardial fibrosis represents the predominant feature, is yet to be explored. The effect of vasodilator treatment in pSSc-HI has been assessed in several small cohort studies with short follow-up, which have shown an increase in myocardial perfusion index following vasodilator treatment with Nifedipine respectively Bosentan (16, 180).

Further studies are therefore required to investigate the CMR change in SSc and whether treatment can alter the course of pSSc-HI.

6.1.1 Hypothesis and aims

6.1.1.1 Hypothesis

- The natural history and course of pSSc-HI can be accurately assessed with CMR
- Clinical and serum cardiac markers aid the identification of CMR progression
- Investigating CMR change is of use for risk stratification for the development of clinical pSSc-HI

6.1.1.2 Aims

1. To evaluate the course of pSSc-HI using CMR and understand:
    a. Whether CMR abnormalities change over time
    b. Whether early subclinical diffuse fibrosis (ECV) progresses to areas of more focal established myocardial fibrosis (LGE)
    c. Whether CMR detected pSSc-HI associates with progression to clinically overt pSSc-HI
2. To evaluate for an association of change in CMR abnormalities with disease phenotype and serum cardiac biomarkers

3. To evaluate whether DMARD and/or vasodilator treatment alters the course of CMR findings

6.2 Methods

6.2.1 Candidate's role in the project

The concept and the design of the study were set-up by myself, Professor Buch and Professor Sven Plein. Twenty patients were approached by Dr Lesley-Ann Bissell and had the first CMR as part of the ELCASA or CONVAS study. I approached and consented 11 more patients to have the first CMR and planned the second CMR for the initial 20 and the remaining 11 patients. I arranged and performed the clinical visit including nailfold capillaroscopy and collected serum for cardiac biomarkers processing, which were stored in the department and were further processed at the NHS Leeds Teaching Hospital (for hs-TnI and CK). I arranged the transport of the NT-proBNP samples that were further processed in Poland at the Medical University of Lublin. I was responsible with the preparation of the patient for the CMR, cannulation and CMR scanning along with the radiographers. I was also responsible for checking the CMR for any urgent findings (cardiac and extracardiac) and releasing CMR reports if required. These were further discussed with Professor S Plein or in his absence, one of the other CMR expert cardiology consultants. I performed the CMR analysis of 41 scans (the first 20 scans were analysed by Graham Fent, CMR cardiology fellow). Ananth Kidambi was the second reader of the LGE. Data entry, data analysis and chapter writing were performed by myself.

6.2.2 Ethical approval

All SSc patients included in the study provided written informed consent. For the purpose of this study, patients were consented to the CONVAS (Leeds Teaching Hospitals Connective Tissue Disease and Vasculitis Cohort Cross-sectional and Longitudinal Clinical and Basic Science Evaluation) main and cardiovascular sub-study (REC Ref: RR10/9608, RR10/9608 respectively).

6.2.3 Study design

A longitudinal observational study was undertaken in consecutive SSc patients with no CVD attending the SSc clinic at the Leeds Teaching Hospital NHS Trust. Patients had two CMRs performed that were at least one year apart.

6.2.4 Eligibility criteria

The same exclusion/inclusion criteria as described in the methodology chapter were applied. All patients fulfilled the 2013 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for SSc (32) and were classified as limited or diffuse cutaneous SSc (lcSSc or dcSSc respectively) according to LeRoy classification (19). Patients were excluded if they had any prior diagnosis of ischemic heart disease (IHD), pSSc-HI, diabetes or more than two traditional cardiovascular (CV) risk factors which were defined as current smoker, hypertension, hypercholesterolaemia/hypertriglyceridemia and family history of premature CV disease (CVD). Patients with any other immune mediated inflammatory diseases (IMID) were also excluded. The contrast-CMR exclusion criteria (detailed in the methodology chapter) applied to all participants.

6.2.5 Study visits

All participants had a first CMR visit (V1) and a second CMR visit (V2) which was undertaken between 12 and 36 months from the first visit.

6.2.6 Data collection

6.2.6.1 Clinical and demographics data collection

Clinical and demographic data was collected for all patients, including age, gender, CV risk assessment, SSc disease assessment, current and previous medication and general and SSc specific physical examination (as detailed in the methodology chapter).

6.2.6.2 Sample collection

Cardiac serum biomarkers, including CK, hs-TnI, NT-proBNP were collected at both visits. Creatine kinase (CK) and high-sensitivity troponin I (hs-TnI) were measured at the Leeds Teaching Hospital NHS Trust, UK and N-terminal pro-brain natriuretic peptide (NT-proBNP) was measured at the Medical University of Lublin, Poland, as described in the methodology section.

All patients had routine blood tests including full blood count, liver function test, urea and electrolytes, C-reactive protein (CRP) and antinuclear antibody (ANA) screening and/or ANA immunoblot whenever the ANA screen was negative.

6.2.6.3 CMR

The same 3T CMR protocol and analysis as described in the methodology chapter was applied for both CMR visits.

CMR protocol included LV function and volume, late gadolinium enhancement (LGE), myocardial perfusion, T1 mapping for T1 native and extracellular volume (ECV) quantification, tissue tagging and aortic distensibility.

Cvi42 software (v4.1.3, Circle Cardiovascular Imaging Inc., Calgary, Canada) was used for CMR image analysis. According to the departmental reference ranges, an ECV >29% and T1 native > 1240 ms were considered abnormal (238, 239).

6.2.6.4 Other investigations

All patients had nailfold capillaroscopy when attending for the clinical visit. Echocardiography and pulmonary function tests (PFT) were also recoded for all participants.

6.2.6.5 Missing data

All efforts were made to avoid missing data. This study was a pure exploratory small sample size study, thus the analysis was performed including all data and no imputation analysis was used.

6.2.6.6 Statistical analysis

No formal power calculation was carried out for this exploratory longitudinal study. Thirty SSc patients was considered acceptable for the purpose of this initial evaluation.

SPSS (IBM SPSS Statistics 22) and Graph Pad Prism (V.8) were used for statistical analysis. Descriptive summary statistics were provided for all variables. Continuous variables were reported as mean (SD) or median (IQR) and categorical data were reported as percentage. Paired sample t test or Wilcoxon test were used to assess the differences between CMR measures at V1 compared to V2 as indicated.

Student t-test, chi square test, Mann-Whitney U test or Fisher's exact test when appropriate were used to assess for significant differences between groups. Correlation between CMR measures and clinical and serum biomarkers were assessed by Spearman's or Pearson rho test when appropriate. Linear univariate analysis was used to assess the association between cardiac biomarkers and change in CMR measures.

As this was a small sample size study, p values, if reported were used mainly to guide on strength of findings rather than significance, in line with good practice (251).

6.3 Results

6.3.1 Patient disposition

Thirty-one SSc patients participated in the study and had available baseline and follow-up CMR data. Forty-two patients that participated in the cross-sectional study were expected to participate in the longitudinal study and have a follow-up CMR. Reason for not having the CMR was either refusal (9 patients) or death (2 patients).

All patients had at least one year in between the first and second CMR visit, with the majority-21 (68%) patients having 3 years in between the two CMR visits. Patients were followed-up for a median (IQR) of 33 (17, 37) months.

6.3.2 Disease characteristics of SSc patients

Table 6-1 details baseline characteristics of study subjects. Participants had a median (IQR) age of 52 (47,60) and a disease duration of 9 (2, 16) years, 74% were female, 32% had dcSSc. Sixteen (52%) had a diagnosis of ILD, 8 (26%) a history of DU, 6 (19%) a history of calcinosis, 27 (87%) a history of GORD. Twenty-eight (90%) had a positive ANA of which 9 (29%) were Scl70 and 11 (36%) were ACA positive.

Twenty (65%) of 31 SSc patients received DMARD treatment between the first and second visit, of which 8 patients had treatment with 2 DMARDs. Thirteen (42%) had treatment with mycophenolate (MMF), 2 (7%) with methotrexate, 3 (10%) with cyclophosphamide. Twenty-eight received vasodilator treatment: 22 (71%) with calcium channel blockers (CCB), ten (32%) with iloprost, 14 (45%) with sildenafil and 4 (13%) with bosentan (Table 6-2).

Table 6-1 Disease characteristic of SSc patients at V1

SSc phenotype	SSc patients, n=31
Demographics and disease history	
Age, median (IQR)	52 (47, 60)
Female, n (%)	23 (74%)
Disease subtype, n (%)	
LcSSc	21 (68%)
DcSSc	10 (32%)
Disease duration (years), median (IQR)	9 (2,16)
History of, n (%)	
Digital ulceration	8 (26%)
Calcinosis	6 (19)
Myositis	1 (3)
GORD	27 (87%)
Interstitial lung disease	16 (52%)
NSIP	13 (42%)
UIP	1 (3%)
Clinical profile	
Total modified Rodnan skin score, median (IQR)	2 (1,6)
Presence of, n (%)	
Digital pits	9 (29%)
Digital ulceration	3 (10%)
Tendon friction rubs	1 (3%)

Calcinosis	3 (10%)
Joint contractures	6 (19%)
Any TJC	14 (45%)
Any SJC	3 (10%)
NFC vasculopathy pattern, n (%)	
Non-specific	9 (29%)
Early	5 (16%)
Active	11 (36%)
Late	5 (16%)
Serology and acute phase	
Antibody positive, n (%)	
ANA	28 (90)
ACA	9 (29%)
Scl70	11 (36%)
CRP (mg/L), median (IQR)	5 (5, 7.3)
Pulmonary function tests and CV risk profile	
Forced vital capacity, mean (SD)	100 (20)
Total lung capacity, mean (SD)	90 (15)
DLCO, mean (SD)	63 (11)
DLCO/VA, mean (SD)	81 (14)
Dyslipidaemia, n %	1 (3%)
Hypertension, n%	2 (7%)
Smoking, n%	3 (10%)

Family history of CVD, n%	5 (16%)
Patients with any CV risk factors, n% 1 CV risk factor, n% 2 CV risk factors, n%	8 (26%) 5 (16%) 3 (10%)
Systolic BP	114 (13)
Diastolic BP	70 (10)
BMI	25 (5)

ACA, anti-centromere antibody; ANA, antinuclear antibodies; DcSSc, diffuse cutaneous systemic sclerosis; DLCO, diffusing capacity of the lungs for carbon monoxide; DLCO/VA, DLCO adjusted for volume; FVC, forced vital capacity; GORD, gastro-oesophageal reflux disease; lcSSc, limited cutaneous systemic sclerosis; NFC, nailfold capillaroscopy; NSIP, non-specific interstitial pneumonia; RP, Raynaud's Phenomenon; Scl70, anti-topoisomerase antibody; SD, standard deviation; total lung capacity; UIP, usual interstitial pneumonia.

Table 6-2 DMARD, vasodilator and ACEi treatment between the first and second CMR visit

Use of DMARD, n(%)	20 (65)
Type of DMARD, n (%)	
Mycophenolate	13 (42)
Methotrexate	2 (7)
Hydroxychloroquine	5 (16)
Sulfasalazine	1(3)
Azathioprine	1 (3)
Cyclophosphamide	3 (10)
Rituximab	1 (3)
Vasodilator treatment, n (%)	
CCB	22 (71%)
Iloprost	10 (32%)
Sildenafil	14 (45%)
Bosentan	4 (13%)
ACEi	13 (42%)

ACEi, Angiotensin converting enzyme inhibitor; CCB, calcium channel blockers; DMARD, disease modifying antirheumatic drug.

6.3.3 Change in CMR measures at V1 compared to V2

Of the 31 SSc patients, complete LV function and LGE CMR assessment was available for all, ECV for 30 SSc patients, T1 native for 29, aortic distensibility for 26, MPR and strain analysis for 23 patients.

6.3.3.1 Change in CMR measures of function, perfusion and aortic distensibility

A mild decrease in LVSV was noted at V2, although the means remained within normal limits with little clinical relevance [mean (SD) 47 (8) vs 45 (7), p=0.009]. No other notable change in LV function or volume parameters was noted and the means remained within normal limits (Table 6-3, Figure 6-1).

No change in LV torsion [mean (SD) 13 (4) vs 14 (5), mean diff 0.2 (-2.2, 2.7), p=0.846] or LV peak twist [mean (SD) 14 (4) vs 14 (5) mean diff 0.74 (-1.6, 3.1), p=0.528] was noted over the follow up period.

No change in MPR (median (IQR) of 2.06 (1.61, 2.4) vs 2.17 (2.17, 2.87), p=0.375) or aortic distensibility between the 2 visits was noted [mean (SD) 4.6 (2), vs 4.5 (2), p=0.844] (Table 6-3, Figure 6-1).

6.3.3.2 Change in CMR markers of fibrosis

No significant change in ECV was noted at V2 [mean difference (95 % CI) -0.8 (- 2, 0.4), p=0.192]. Eleven of 15 patients with an ECV above the normal range at V1 (> 29%) had an ECV above the normal range at V2 and for 4/15 patients, ECV decreased and reached normal values at V2(<29%).

A trend increase in T1 native was noted at V2 [mean difference (95 % CI) 30 (-5, 65), p=0.090] (Figure 6-1).

Four of 31 patients had a non-ischaemic LGE pattern at V1. The LGE was distributed at the basal septal segments in 2 patients and basal inferolateral respectively basal-mid lateral segments for the other 2. Two patients had subepicardial distribution, one had midwall distribution and one had diffuse distribution. There was no change in the pattern and distribution of LGE between V1 and V2. Overall, there was no significant change in LGE scar mass between V1 and V2 [median (IQR) of 1.88 (1.01, 6.34) vs 1.70

(1.21, 4.18)]. Two patients had minimal decrease in LGE scar mass, whilst one patient had a more notable reduction in LGE scar mass at V2 compared to V1 (from 7.59 to 4.99 g) (Figure 6-1). One of the 4 patients had an increase in LGE scar mass (from 0.92 to 1.66 g). Two patients with no fibrosis at V1 developed new fibrosis at V2 of which one was in the context of myocarditis (Table 6-4, Figure 6-2).

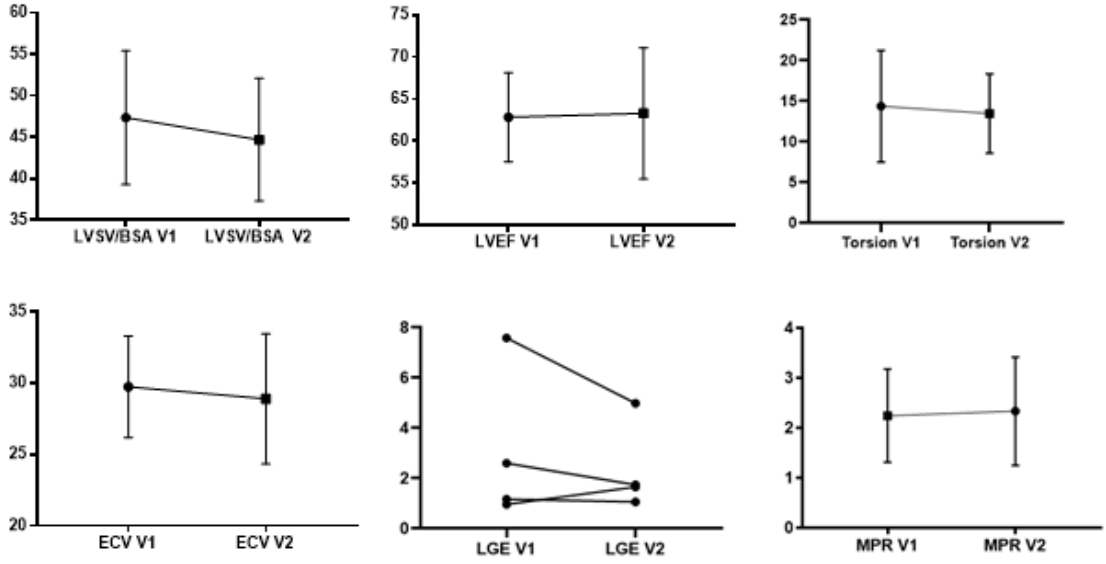


Figure 6-1 Change in CMR measures between V1 and V2

ECV, extracellular-volume fraction; SV, stroke volume; LGE, late gadolinium enhancement; LV, left ventricular; EF, ejection fraction; MPR, myocardial perfusion reserve.

Table 6-3 Change in CMR measures between V1 and V2

CMR variable	CMR V1	CMR V2	Mean difference (95% CI)	P value
LGE, n	4/31	6/31		<0.001** (X^2test)
LGE scar mass (g) median (IQR)	1.88 (1.01, 6.34)	1.70 (1.21, 4.18)	0.484 (-0.69, 2.59)	0.1667
ECV%	29.6 (4)	28.8 (5)	-0.8 (-2, 0.4)	0.192
T1 native	1218 (65)	1248 (60)	30 (-5, 65)	0.090
MPR, n=23 median (IQR)	2.06 (1.61, 2.43)	2.17 (2.17, 2.87)	0.0009 (-1.23, 0.675)	0.375
LVEDV/BSA (ml/m^2)	75 (17)	72 (15)	-3 (-7, 1)	0.136
LVESV/BSA (ml/m^2)	29 (9)	27 (11)	-2 (-5,1)	0.158
LVSV/BSA (ml/m^2)	47 (8)	45 (7)	-3 (-5,1)	0.009*
LV mass/BSA (g/m^2)	43 (13)	44 (11)	1 (-1,3)	0.352
LVEF (%)	63 (5)	63 (8)	0.4 (-2,3)	0.701
Torsion^o	13 (4)	14 (5)	0.2 (-2.2, 2.7)	0.846
Peak twist	14 (4)	14 (5)	0.7(-1.6, 3.1)	0.528
Aortic distensibility (10^{-3}mmHg^{-1})	4.6 (2)	4.5 (2)	-0.1 (-1, 1)	0.844

* p<0.05; ** p<0.001

Mean (SD) unless stated otherwise

BSA, body surface area; CMR, cardiovascular magnetic resonance; ECV, extracellular-volume fraction; EDV, end-diastolic volume; ESV, end-systolic volume LGE, late gadolinium enhancement; LV, left ventricular; EF, ejection fraction; MBF, myocardial blood flow; MPR, myocardial perfusion reserve.

Table 6-4 Baseline disease characteristics of patients with LGE at V1 and V2

Patients with LGE at V1 and V2					**Patients with new LGE at V2**	
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Disease subset	dcSSc	lcSSc	lcSSc	lcSSc	dcSSc	dcSSc
ANA antibodies	Anti-RNA, ACA	ANA homogenous	Sm/RNP	Ro52	Scl70, Sm/RNP	Scl70
Age	51	43	44	57	38	60
Gender	F	F	M	M	M	F
Disease duration (years)	1	1.7	15.8	8.9	6	2
mRSS	6	0	1	2	4	9
DU	0	0	0	0	0	0
ILD	1	1	1	0	1	1
DMARD between V1 and V2	MMF	MMF	MTX	SSZ, HCQ	MMF	CF, MMF
LGE change between V1 and V2 (increase↑/decrease↓) *	↑	↓(minimal)	↓↓ (more significant)	↓(minimal)	↑ (new)	↑ (new LGE and myocarditis)

*Increase in LGE (↑): Increase LGE scar mass or new scar. Decrease LGE (↓): Reduction in scar mass

ACA, anticentromere antibodies; ANA, antinuclear antibodies; CF, cyclophosphamide; DMARD, disease modifying antirheumatic drugs; DU, digital ulcers; F, female; HCQ, hydroxychloroquine; ILD, interstitial lung disease; MMF, mycophenolate mofetil; MRSS, modified Rodnan skin score; Scl70, anti-topoisomerase antibody; Sm/RNP, Smith/Ribonucleoprotein nuclear antibodies; SSZ, sulfasalazine.

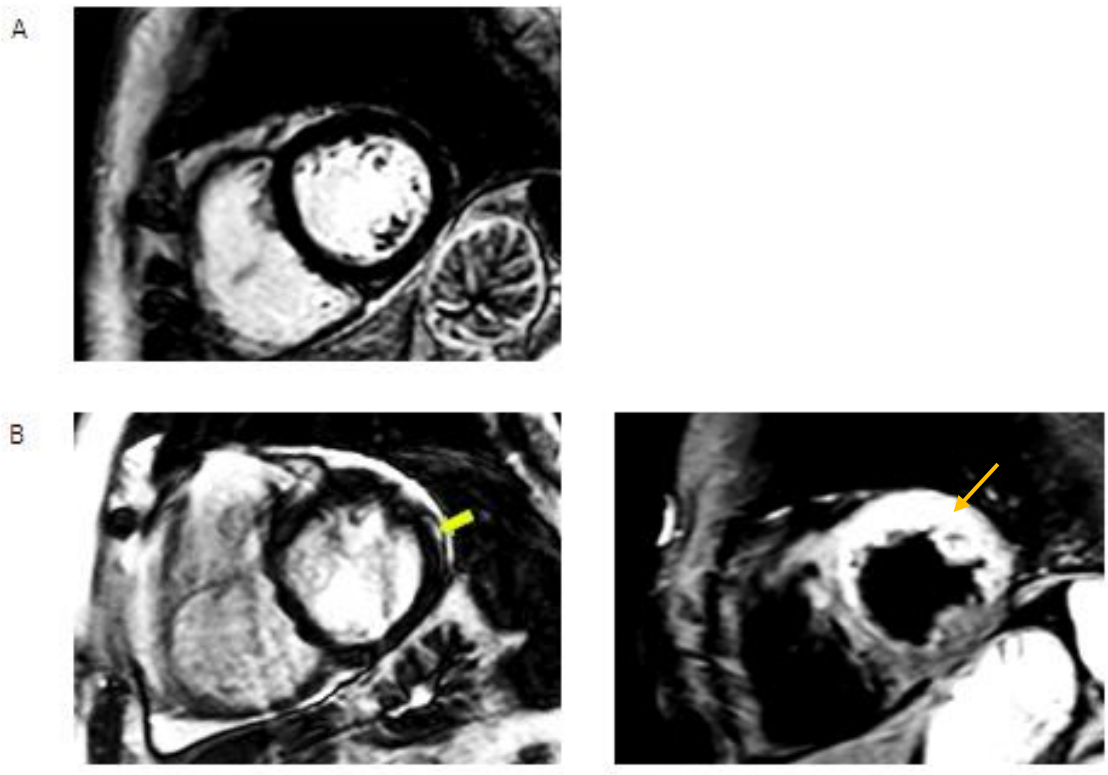


Figure 6-2 CMR showing patient who developed myocarditis at follow-up. A. LGE-CMR at V1 showing no fibrosis. B. LGE CMR at V2 showing subepicardial LGE in the anterolateral wall, typical of myocarditis (left image). T2 weighted sequence showing oedema especially in the anterior, anterolateral, anteroseptal segments (right image). CMR, cardiovascular magnetic resonance; LGE, late gadolinium enhancement.

6.3.4 Association of disease characteristics with CMR change

SSc patients with increase in LGE; either increase in pre-existing LGE scar mass (n=1) or new LGE (n=2, of which one with myocarditis) were all dcSSc, with a diagnosis of ILD, 2 with Scl70, 1 Anti RNA positive and their mean (SD) mRSS was 6 (3) (Table 6-3). The SSc patient with myocarditis also had a diagnosis of myositis and inflammatory arthritis and the one with an increase in LGE scar mass also had an increase in mRSS at V2 (from 6 to 13). Two of the 3 patients had a worsening in their pulmonary function tests between V1 and V2, with a total forced vital capacity drop of 5% (from 97% to 90%) and 12% respectively (from 108% to 95%), the latter also with a total drop in DLCO of 11% (from 55% to 49%). The patient with a more significant decrease in LGE

scar mass at follow-up was a Sm/RNP positive male, receiving treatment with methotrexate, with mild ILD and a history of arthritis.

Patients with ILD showed an increase in LVEDV (rho=0.527; p=0.02), LVESV (rho=0.455, p=0.01) and a decrease in MPR (rho=-0.457, p=0.029) (Table 6-5).

An association between mRSS and increase in LV mass was also noted (rho=356, p=0.049).

A trend for an association of a history of DU with an increase in ECV (rho=0.348, p=0.059) and a decrease in MPR (rho=-0.381, p=0.073) was suggested (Table 6-5).

The change in pulmonary function tests and mRSS in relation to CMR change was further tested. A reduction in FVC% and TLC% associated with a reduction in LVEF (rho=0.413, p=0.021; rho-0.335, p=0.07) and MPR (rho=0.543, p=0.007; rho=0.627, p=0.002). An association between a decrease in DLCO% and LVEF decline (rho=0.307, p=0.093) was also suggested. mRSS increase showed a trend for an association with MPR and ECV decline (rho=-0.356, p=0.095; rho=-0.316, p=0.062).

Table 6-5 Correlation between change in CMR measures and disease characteristic

	Change LVESV/ BSA	**Change LVSV/ BSA**	**Change LVEDV/ BSA**	**Change LVEF**	**Change LVmass/ BSA**	**Change ECV**	**Change T1 native**	**Change MPR**	**Change Torsion**	**Change Peak twist**	**Change distensibility**
Rho correlation, p value											
Disease duration	-0.056 0.765	0.005 0.979	-0.164 0.379	0.069 0.712	0.245 0.184	0.297 0.112	-0.05 0.798	0.058 0.792	-0.257 0.226	-0.139 0.507	0.063 0.761
History DU	0.049 0.792	-0.041 0.826	-0.082 0.659	-0.074 0.692	0.288 0.116	0.348 0.059	0.138 0.474	-0.381 0.073	0.007 0.973	0 1	0.377 0.057
ILD	0.455* 0.01	0.13 0.486	0.527** 0.002	-0.31 0.089	-0.159 0.394	-0.204 0.279	-0.198 0.303	-0.457* 0.029	0.103 0.633	0.278 0.179	-0.046 0.823
MRSS	-0.146 0.432	-0.097 0.602	0.004 0.981	0.141 0.45	0.356* 0.049	0.272 0.147	0.044 0.822	-0.021 0.923	0.365 0.08	0.228 0.274	0.197 0.335
DcSSc subset	0.069 0.711	-0.093 0.62	0.147 0.431	-0.201 0.279	0.023 0.902	-0.033 0.864	0.241 0.209	-0.299 0.166	0.298 0.157	0.148 0.479	0.067 0.746
ACA positive	-0.191 0.304	-0.032 0.865	-0.111 0.551	0.04 0.832	0.159 0.393	0.13 0.493	0.027 0.891	0.138 0.529	-0.348 0.096	-0.259 0.21	-0.286 0.157

	Change LVESV/ BSA	Change LVSV/ BSA	Change LVEDV/ BSA	Change LVEF	Change LVmass/ BSA	Change ECV	Change T1 native	Change MPR	Change Torsion	Change Peak twist	Change distensibility
Scl70 positive	0.226 0.221	0.06 0.747	0.264 0.152	-0.106 0.572	0.158 0.395	0.02 0.917	0.234 0.222	-0.161 0.463	0.171 0.425	0.011 0.957	0.295 0.143

**p<0.05, * p<0.01

ACA, anticentromere antibody; BSA, body surface area; CMR, cardiovascular magnetic resonance; DMARD, disease modifying antirheumatic drugs; DU, digital ulcers; ECV, extracellular-volume fraction; EDV, end-diastolic volume; ESV, end-systolic volume; LV, left ventricular; EF, ejection fraction; ILD, interstitial lung disease; MPR, myocardial perfusion reserve; MRSS, modified Rodnan skin score; SV, stroke volume. Scl70, anti-topoisomerase antibody

6.3.5 CMR change and cardiac biomarkers

6.3.5.1 Baseline serum cardiac biomarkers and CMR change

Baseline hs-TnI and NT-proBNP associated with a decrease in LVEF (rho=-0.710, p<0.001 vs rho=-0.483, p=0.006) and LVSV (rho=-0.361, p=0.050; rho=-0.406, p=0.024 respectively) and an increase in LVESV at V2 (rho=0.689, p<0.001; rho=0.518, p=0.03).

6.3.5.2 Change in serum cardiac biomarkers and CMR parameters

An increase in NT-proBNP showed a strong association with a reduction in LVEF (rho=-0.664, R^2=0.441, p<0.001). There was also a positive association between the increase in NT-proBNP and LVESV (rho=0.714, R^2 =0.510, p<001). No association between an increase in hs-TnI and change in CMR parameters was found.

6.3.6 Treatment and change in CMR measures

6.3.6.1 DMARD treatment and change in CMR measures

Patients receiving DMARD treatment had higher baseline LVEDV/BSA [mean (SD) 78 (19) vs 69(10), p=0.167] LVESV/BSA [mean(SD) 24(5) vs 31(10), p=0.47] and lower LVEF [mean(SD) 62(5) vs 65(5), p=0.159] compared to those with no DMARD treatment. However, a greater decrease in LVSV [mean (SD) 49 (8) vs 46 (8), p =0.023] and an increase in T1 native [mean (SD) 1208 (65) vs 1265 (56), p=0.008] was noted in those receiving DMARD treatment at V1 vs V2. As mentioned earlier, all 3 patients with increase in LGE scar mass or new LGE received DMARD treatment between the 2 visits, two with MMF, one with cyclophosphamide, followed by MMF.

SSc patients on DMARD treatment were more likely to have an increase in mRSS (rho=0.279, p=0.128) and a decline in FVC (rho=-0.333, p=0.67) over the follow up period.

6.3.6.2 ACEI treatment and change in CMR measures

There was no significant difference in baseline CMR indices between those receiving ACEi treatment compared to those on no ACEi treatment. SSc patients receiving ACEi had no significant change in CMR variables between V1 and V2 (Table 6-6).

Table 6-6 Change in CMR measures in those with and without DMARD and ACEi treatment

CMR parameters	No DMARD treatment n=11		DMARD treatment n=20		p value	No ACEi treatment n=18		ACEi treatment n=13		p value
	Change Median (IQR)	Baseline value	Change Median (IQR)	Baseline value		Change Median (IQR)	Baseline	Change Median (IQR)	Baseline	
LVEDV/BSA (ml/m^2)	-6.2 (-11.1, -2.0)	69 (10)	-0.3 (-10.7, 2.1)	78 (19)	0.066	-3.1 (-11, -1)	74 (13)	-0.1 (-11, 5)	76 (22)	0.135
LVESV/BSA (ml/m^2)	-4.6 (-8.1, -2.9)	24 (5)	-0.3 (-5.4, 1.5)	31 (3)	0.060	-2.9 (-6, 1)	28 (8)	-2.7 (-8, 3)	31 (10)	1
LVSV/BSA (ml/m^2)	-0.8 (-3.5-0.2)	44 (7)	-2.5 (-8.6, 2.6)	49 (8)	0.528	-1.7 (-6, 0.2)	47 (6)	-1.6 (-8, 3)	48 (10)	0.594
LV mass/BSA (g/m^2)	1.4 (-4.1, 5.6)	40 (9)	1.6 (-2.7, 5.8)	44 (14)	0.792	0.2 (-8, 6)	43 (13)	1.9 (1, 6)	43 (13)	0.125
LVEF %	2.5 (0.1, 7.9)	65 (5)	0.9 (-2.8, 1.5)	62 (5)	0.020	1.3 (-2, 3)	63 (6)	1.2 (-2, 5)	62 (5)	0.984

CMR parameters	No DMARD treatment n=11		DMARD treatment n=20		p value	No ACEi treatment n=18		ACEi treatment n=13		p value
	Change Median (IQR)	Baseline value	Change Median (IQR)	Baseline value		Change Median (IQR)	Baseline	Change Median (IQR)	Baseline	
ECV %	-0.1 (-1.5, 3)	29 (5)	-0.7 (-3, 1)	30 (3)	0.328	0.05 (-3, 2)	30 (4)	-1.3 (-3, 1)	30 (3)	0.368
T1 native (ms)	-4 (-50, 49)	1233 (64)	60 (-6,110)	1211 (65)	0.049	27 (-25, 75)	1220 (6)	69 (-37, 102)	1218 (73)	0.611
MPR	0.4 (-0.2, 1.8)	2.3 (0.8)	-0.1 (-0.9, 1.1)	2.1 (1)	0.201	0.4 (-0.5, 1.3)	2.2 (0.8)	-0.1 (-0.9, 0.5)	2.3 (1.1)	0.316
Aortic distensibility	-0.4 (-1.6, 0.4)	3.9 (2)	0.1 (-1.5, 1.5)	4.6 (2)	0.458	-0.1 (-1.4, 0.9)	4 (2)	-0.2 (-0.2,1.1)	5 (2)	0.980
Torsion	- 2.2 (-5.2, 0.4), n=6	14 (4)	2.1 (-0.4, 5.2), n=17	13 (5)	0.410ˆ	1.7 (-1.3, 0.9)	13 (4)	-1 (-10, 4.1)	14 (5)	0.208
Peak twist	-0.28 (-7.2, 2.2)	14 (5)	1.58 (-0.4, 4.9)	13 (4)	0.640ˆ	2.5 (-0.5, 4)	13 (4)	1.1 (-8.3, 2)	14 (5)	0.303

ˆ Fisher's exact test

Mean (SD) unless stated otherwise; ACEi, angiotensin converting enzyme inhibitor; BSA, body surface area; CMR, cardiovascular magnetic resonance; ECV, extracellular-volume fraction; EDV, end-diastolic volume; ESV, end-systolic volume; LV, left ventricular; EF, ejection fraction; SV, stroke volume; MPR, myocardial perfusion rese

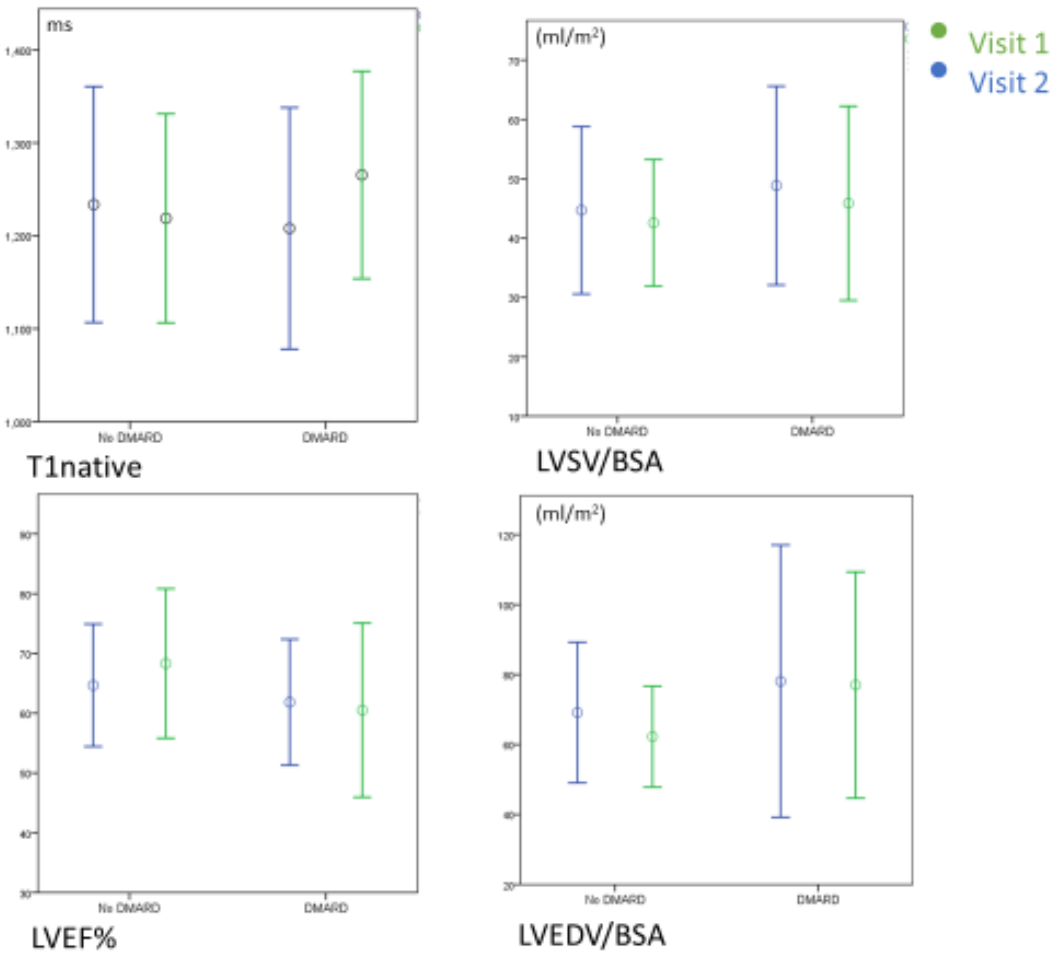


Figure 6-3 Mean (2SD) of T1 native, LVSV/BSA, LVEF, and LVEDV/BSA at V1 compared to V2 in those with and without DMARD treatment.

BSA, body surface area; DMARD, disease modifying antirheumatic drugs; EDV, end-diastolic volume; SV, stroke volume; LV left ventricular; EF, ejection fraction.

6.3.6.3 Vasodilator treatment and change in myocardial perfusion

The majority of patients (n=28, 90%) were receiving vasodilator treatment, including with CCB. Further dividing patients in those receiving more targeted vasodilator treatment (in the form of iloprost, sildenafil or bosentan) and those without this treatment showed no significant difference in baseline myocardial perfusion CMR variables between the 2 groups ($p>0.05$). There was no significant change in MBF at rest, stress and MPR for those receiving vasodilator treatment between V1 and V2 ($p>0.05$).

6.4 Discussion

This is a first study to assess the interval change of CMR detected pSSc-HI and its association with clinical and biochemical markers, and to explore for any effect of DMARD and vasodilator treatment on CMR detected pSSc-HI. In a SSc cohort, free of CVD and clinical pSSc-HI, overall, there was no significant change in CMR indices. However, poor prognostic factors, dcSSc, presence of ILD and DU associated with new or increased fibrosis and greater deterioration of LV function, whilst ILD progression showed association with LVEF and MPR reduction. A greater decline in systolic function and increase in T1 native was noted in the DMARD group, likely reflecting a poor prognostic group rather than effect of treatment. Only one patient developed clinically overt pSSc-HI detected on CMR at follow up (myocarditis), thus the prognostic value of CMR detected pSSc-HI remains unclear and needs to be further explored in larger longitudinal studies.

There is little evidence with regards to the natural course and change in CMR detected pSSc-HI over time. Only one previous study explored the CMR change; 44 SSc patients had baseline CMR which showed a low MPR in all and evidence of fibrosis as assessed by quantitative analysis in 40 patients. A follow-up CMR at 2 years, available for 11/44 patients showed further deterioration in MPR (0.5±0.1 *vs* 0.9±0.2) and evidence of LGE established fibrosis in 8/11 patients (18). Our study demonstrated that the pattern, distribution and LGE scar mass, remained stable for 3/4 patients with LGE fibrosis and one had a reduction in LGE scar mass at V2. The ECV also remained stable and T1 native showed a trend increase, albeit not statistically significant. Half of the SSc patients had an ECV above the normal value, but higher ECV did not progress into established LGE fibrosis over the follow-up period. Moreover, no significant deterioration in MPR was noted and the LV function and mass remained within normal limits over the follow up period.

The association between CMR interval change and disease characteristics hasn't been previously explored. All 3 patients with an increase in LGE, either increase in pre-existing scar mass or new LGE at follow up, were dcSSc, with ILD, 2 Scl 70 positive, 1 Anti-RNA positive, 1 also with myocarditis, demonstrating that poor prognostic factors

of SSc are associated with pSSc-HI CMR and fibrosis, as previously documented in the former chapter. Nonetheless, a decline in EF and MPR associated with a diagnosis of ILD and a decline in FVC, TLC and DLCO, suggesting that LV function and myocardial perfusion deterioration is associated not only with poor prognostic factors of SSc but also with active ILD and pulmonary deterioration.

An increase in ECV associated with a history of DU; these results are confirming the findings of the previous chapter, suggesting a common pathogenetic process - microvascular impairment of the myocardium with subsequent development of diffuse fibrosis.

An association between a history of DU and a negative change in MPR was also noted, suggesting concurrent peripheric and myocardial micro vasculopathy. The results are inconsistent with the findings of chapter 5, where a positive association of DU and MPR was noted. The endothelin modulating vasodilator therapy (in the form of sildenafil and/or bosentan) which was commenced for a proportion of SSc patients over the follow-up period, resulting in higher rest MBF and thus a decrease in MPR (ratio of MBF at stress/MBF rest) as well as the small sample size might account for these findings.

Hs-TnI is observed in acute coronary syndrome and it's dynamic changes have prognostic value in diagnosing myocardial infarction and cardiovascular events (268-270). The prognostic implications of serial changes in NT-proBNP in heart failure has also been recently emphasized (271, 272). The results of the current study showed an association of hs-TnI and NT-proBNP with a reduction in LVEF and increase in LVEDV and LVESV. Moreover, both biomarkers were sensitive to change and associated with systolic function deterioration. These findings confirm the potential utility of dynamic changes in both biomarkers for identifying patients at risk of CMR pSSc-HI progression. No association between cardiac biomarkers and fibrosis (focal or diffuse) was found, but this is likely to be related with the small sample size.

Finally, the study explored the effect of DMARD and vasodilator treatment on CMR detected pSSc-HI. The benefit of immunosuppressive treatment has been

documented in autoimmune myocarditis and case studies also report it's benefit in SSc myocarditis (100, 125, 200, 212). However, there is little evidence on the potential effect of DMARD treatment in subclinical pSSc-HI, where myocardial fibrosis represents the main feature. Evidence from acute myocardial infarction and myocarditis, where there is an important oedematous component, suggest that LGE does not always imply irreversible injury and can regress over time (167, 273). Diffuse interstitial fibrosis can also be a reversible process with appropriate treatment (274, 275) but can also lead to replacement fibrosis (LGE on CMR) (276, 277). One study using standard echocardiography and tissue Doppler imaging (TDI) in 74 SSc patients showed deterioration of the diastolic and systolic function at follow up [16/19 SSc patients with basal $E_m/A_m > 1$ (84%) showed mitral $E_m/A_m < 1$ vs 1/36 controls, $p < 0.0001$); 9/62 SSc patients with basal $E_t/A_t > 1$ (15%) showed $E_t/A_t < 1$ vs 2/46 controls, $p = 0.11$)]. However, DMARD treatment did not associate with the change in TDI findings at multiple regression analysis (267). The current work showed no improvement in CMR measures for those receiving DMARD treatment. On the contrary, this group had a deterioration in LVEF, and an increase in T1 native. Moreover, all 3 patients with new or increased LGE scar mass received immunosuppressive treatment during the follow-up period. These findings are likely reflecting the poor prognosis group rather than effect of DMARD treatment. However, one of the 4 patients with LGE, that was receiving treatment with methotrexate did have a notable reduction in the LGE scar mass over the follow up period. Further studies are required to test whether myocardial fibrosis in SSc can recede over time and whether and which DMARD treatment can alter this process. Interestingly, whilst LVEF decreased in the DMARD group, an increase in LV torsion was noted in these patients. LV torsion might serve as a compensatory mechanism for preserving LV systolic function which might explain this finding (278).

The benefit of vasodilator treatment on myocardial perfusion has been assessed in several SSc studies. An improvement in the MP index on CMR and myocardial strain rates on TDI echocardiography following 14 days of Nifedipine respectively 4 weeks of Bosentan administration was noted in SSc patients (16, 180). No significant change in myocardial perfusion indices over the follow up period was noted in those receiving

vasodilator treatment in our study. The beneficial role of angiotensin-converting enzyme inhibition (ACEi) on LV remodelling in heart failure, LV hypertrophy and MI is well known (279-281) however, the value of ACEi treatment in pSSc-HI hasn't been explored to date (200). No considerable change in any of the CMR parameters was noted in those receiving ACEi treatment compared to those with no treatment. Larger, preferentially inception cohort studies could further clarify whether treatment (either DMARD, vasodilator or ACEi) can alter the course of CMR in SSc.

In conclusion, this study demonstrated that CMR does not significantly change over time and measures of fibrosis remain stable. However, an association of poor prognostic factors of SSc and active lung involvement with an increase in focal fibrosis and a reduction in systolic function and MPR was noted. DMARD and vasodilator treatment did not seem to have a beneficial effect on CMR indices, likely reflecting the poor prognosis group rather than effect of treatment. Only one patient developed clinically overt pSSc-HI detected on CMR (myocarditis), thus the prognostic value of CMR defined pSSc-HI needs to be further explored. Larger longitudinal studies with longer follow up are warranted to confirm these findings.

6.4.1 Limitations

One of the main limitations of the study is the small sample size. However, this was a first exploratory study to investigate for interval change of CMR detected pSSc-HI and its association with disease phenotype and cardiac biomarkers; as well as the effect of DMARD or vasodilator treatment on CMR course. The study thus provides valuable information for the development of larger longitudinal studies that will aid risk stratification in pSSc-HI.

Another limitation of the study is the relatively short period of time that patients were followed up for. However, all patients had more than 12 months, with the majority having 36 months in between the 2 CMR visits.

Time of occurrence of pSSc-HI remains an important question, which needs to be further addressed. Evidence suggest that patients with clinically overt pSSc-HI such as myocarditis are more likely to have early dcSSc (100), but no clear association between

subclinical pSSc-HI findings and disease duration has been found (117, 139, 159). Ideally, inception cohorts with longer follow are warranted to address this issue, although recruitment of an inception cohort in a rare disease group remains challenging.

Myocardial measures of function (e.g. EF, LVEDV, LVESV, etc) which showed interval change in the current study could have also been determined with echocardiography, however, CMR remains the most accurate investigating tool for assessing myocardial function, structure and tissue characterisation.

Atherosclerotic disease is difficult to fully exclude, however the study minimised this risk by excluding patients with CVD, diabetes and more than 2 CV risk factors. None of the patients had evidence of myocardial perfusion defects indicative of ischemic heart disease (IHD) on CMR, thus inclusion of secondary IHD in our cohort is unlikely.

6.4.2 Key messages

- Overall, no significant interval change in CMR measures was noted
- However, poor prognostic factors of SSc and ILD progression associated with new or increased fibrosis and greater deterioration of LV function
- No beneficial effect of DMARD, vasodilator or ACEi treatment on CMR abnormalities was noted
- Only one patient developed clinically overt CMR abnormality (myocarditis) at follow-up, thus the prognostic value of CMR defined pSSc-HI needs to be further explored

Chapter 7. Extracellular volume measurement in peripheral muscle and the association with heart involvement in systemic sclerosis

7.1 Introduction

Peripheral muscle involvement in systemic sclerosis (SSc) represents a significant cause of disability and is associated with worse survival when cardiopulmonary involvement is also present (126, 282, 283). Its prevalence ranges widely, depending on the definition and diagnostic modalities used, with estimates of between 10% to 90% (123, 124, 126, 284).

The pathogenesis of SSc myopathy is complex and in line with SSc pathology, stigmata of microangiopathy, fibrosis and inflammation are all described at histopathological level (63, 124, 285).

There is a lack of new evidence investigating SSc myopathy and the nature of peripheral muscle involvement in SSc. However, former studies commonly describe an inflammatory pattern, characterised by acute clinical presentation of myositis with markedly increased muscle enzymes, usually in association with poor prognostic factors of SSc including diffuse cutaneous SSc (dcSSc), interstitial lung disease (ILD), male gender and higher modified Rodnan skin score (mRSS) (123, 125, 283). This so called “complicated myopathy” as Clements et al defined it (123) resembles that of dermatomyositis/polymyositis and usually subsides with immunosuppressive treatment. However, a non-inflammatory pattern, characterised by mild clinical and biological findings including proximal weakness and myasthenia and mildly elevated creatine kinase (CK) levels, the so called “simple myopathy” is described in a significant proportion of patients. As opposed to the more inflammatory pattern, no clear association between the non-inflammatory myopathy and disease phenotype has been found (123, 286).

The association of cardiac disease with skeletal muscle involvement in patients with SSc has been commonly documented, and it is now generally accepted that SSc

patients with myositis are at higher risk of myocarditis (63, 125). The evidence for an association between the non-inflammatory peripheral myopathy pattern and primary SSc-heart involvement (pSSc-HI) however is more limited. Follansbee et al. reported that 21% of the 183 SSc patients with peripheral myopathy also had cardiac involvement and there was no difference in the prevalence of SSc-HI between patients with myositis and non-inflammatory myopathy (126).

T1 mapping, is now widely used in cardiovascular magnetic resonance (CMR) to assess interstitial, diffuse processes, showing good reproducibility and correlation with biopsy histological findings (171, 204, 205). More recently, T1 mapping with ECV measurement has also been tested in skeletal muscle (62, 287). Several studies have successfully applied T1 mapping in amyloidosis, showing significantly higher ECV in the heart, liver, spleen as well as skeletal muscle of patients with amyloidosis compared to healthy controls ($P<0.001$) (287, 288). Another two studies demonstrated increased T1 and T2 mapping parameters in the myocardium and thoracic skeletal muscle of patients with idiopathic inflammatory myopathy compared to patients with viral myocarditis (289) and healthy controls (290).

In SSc, only one study that performed CMR T1 mapping using a cohort of 30 SSc patients showed interstitial remodelling of both thoracic skeletal and cardiac muscle as determined by ECV, in comparison to HV [mean (SD) 23 (6) vs 18 (4), $p< 0.01$] (127). The study also showed an association of myocardial and skeletal muscle ECV (rho=0.58) suggesting concurrent diffuse fibrosis in both the heart and skeletal muscle.

All of the above studies used the thoracic muscle that was visible at the edge of the CMR image field for measuring native T1 and ECV, where image artefacts are likely to appear. Moreover, the studies assumed that the time delay between measurements necessary to achieve contrast equilibrium is the same in the skeletal muscle as it is in the heart, however, a longer time for achieving contrast equilibrium might be needed as a result of reduced resting perfusion rate of skeletal muscle (291). In addition, the peripheral muscle as opposed to axial muscles evaluated in these studies are predominantly involved in SSc and other immune mediated inflammatory disease (IMID) (292, 293).

No studies to date have assessed the feasibility of T1 mapping in the peripheral muscle in the general population or in SSc, and the association with clinical and biological parameters as well as CMR detected interstitial fibrosis in SSc.

7.1.1 Hypothesis and aims

The following hypothesis and aims will be addressed in this study

7.1.1.1 Hypothesis

- T1 mapping can be implemented in peripheral muscle and can evaluate diffuse fibrosis
- SSc patients have evidence of peripheral muscle diffuse fibrosis in comparison to healthy volunteers (HV)
- SSc patients with minimal clinical and biological muscle findings (non-inflammatory myopathy) have evidence of diffuse fibrosis on T1 mapping MRI
- CMR detected diffuse fibrosis is associated with the presence of MRI peripheral muscle fibrosis

7.1.1.2 Aims

1. To evaluate the feasibility of T1 mapping in the peripheral muscle of SSc patients
2. To evaluate for T1 mapping detected muscle fibrosis in SSc patients
3. To assess for any association of T1 mapping detected muscle fibrosis with clinical and biological parameters in SSc
4. To assess for an association between cardiac and peripheral muscle T1 mapping detected diffuse fibrosis in SSc

7.2 Methods

7.2.1 Candidate's role in the project

The concept and the design of the study were set up by Professor Buch and myself. Physicist John Biglands and Alex Goodall developed the peripheral muscle T1 mapping

sequence that was first tested in HV, then applied in SSc patients. I approached and consented the SSc patients and HV. HV were recruited by sending an email to the Leeds Institute of Rheumatic and Musculoskeletal Medicine (LIRMM) Department explaining the study and providing my contact details for further information. I arranged the clinical and MRI visits and arranged serum sample testing for the HV and SSc patients. I was responsible with the preparation of the patient for the CMR, cannulation and CMR scanning along with the radiographers. I was also present with physicists JB and AG for the peripheral muscle MRI. AG and JB performed the skeletal muscle MRI analysis and I performed the CMR analysis. Data entry, statistical analysis and chapter writing was performed by myself.

7.2.2 Ethical approval

All participants read and provided written informed consent. HV were consented to the MUSCLE II study (novel Magnetic resonance imaging and UltraSound CLinical Evaluation of muscle pathology): Contrast-enhanced MRI Scan (RR17/92933, REC ref: 17/EM/0079) which allowed contrast administration.

SSc patients were consented to CONVAS (Leeds Teaching Hospitals Connective Tissue Disease and Vasculitis Cohort Cross-sectional and Longitudinal Clinical and Basic Science Evaluation) main and cardiovascular sub-study (REC Ref: RR10/9608, RR10/9608 respectively) and MUSCLE II study: with Contrast-Enhanced MRI (RR17/92933, REC ref: 17/EM/0079).

7.2.3 Study design

A cross-sectional study was undertaken to implement T1 mapping in the peripheral muscle and to test the differences between SSc patients and HV and association between cardiac and muscle diffuse fibrosis in SSc. SSc patients attending the weekly Leeds Teaching Hospital NHS Trust SSc clinic were approached to have contrast cardiac and peripheral muscle MRI performed. No more than 3 weeks was allowed between the 2 scans. Clinical data and serum samples were collected for all participants. Patients with a non-inflammatory myopathy were identified from the LTHT SSc clinic. Non-inflammatory myopathy was defined as either presence/history of repeated

minimally raised CK (>320 IU/l, <600 IU/l), presence of clinical symptoms/signs including proximal myasthenia and/or myalgia or a Manual Muscle Testing (MMT) score <5 in the thighs.

HV were recruited by sending an email to the LIRMM Department with details about the study and providing my contact details for further information. HV willing to participate were contacted and the study explained in more detail.

All participants, including HV and SSc patients were consented and had serum sample testing for U&E prior to having the MRI. A GFR >70 was required in order for the participants to have the contrast MRI scan.

7.2.4 Eligibility criteria

The same exclusion/inclusion criteria described in the methodology section applied to the SSc patients. Moreover, SSc patients and HV were excluded if they had a confirmed diagnosis of myositis, any known degenerative muscle disease and any other IMID other than SSc. Low dose corticosteroid treatment (prednisolone ≤5 mg) was allowed in the study. Two SSc groups were included in the study: participants with no muscle involvement and participants with minimal muscle symptoms including muscle weakness, myalgia or low level CK levels (non-inflammatory myopathy, see above). The general inclusion/exclusion criteria for contrast MRI detailed in the Methods chapter was applied. SSc participants or HV were excluded if they had any know allergy to contrast, renal insufficiency or any retained metal in the body.

7.2.5 Data collection

7.2.5.1 Clinical and demographics data collection

Clinical and demographic data were collected for all patients, including age, gender, CV risk assessment; and details on SSc disease assessment, current medication and general and SSc specific physical examination were obtained.

The standardised MMT 8 score (294, 295) was used to assess shoulder abduction, elbow flexion, ankle extension, hip abduction, hip extension, knee extension, wrist

extension and neck flexion for all SSc patients participating in the study. The following scale and grading system were used:

0 No visible or palpable contraction

1 Trace, evidence of muscle contraction with no movement

2 Poor, full range of motion, gravity eliminated

3 Fair, full range of motion against gravity

4 Good, complete range of motion against gravity, moderate resistance

5 Normal, complete range of motion against gravity, maximum resistance

MMT grading system:

0

- No visible or palpable contraction

I

- Trace Evidence of contraction, with no range of motion (ROM)

II

- Poor- 2- Partial ROM, gravity eliminated
- Poor 2 Full ROM, gravity eliminated
- Poor+ 2+ Gravity eliminated/slight resistance or less than half ROM against gravity

III

- Fair- 3- > more than half, but less than full ROM against gravity
- Fair 3 Full ROM against gravity
- Fair+ 3+ Full ROM against gravity, slight resistance

IV

- Good- 4- Full ROM against gravity, mild resistance
- Good 4 Full ROM against gravity, moderate resistance
- Good+ 4+ Full ROM against gravity, almost full resistance

V

- Normal Normal, maximal resistance

7.2.5.2 Sample collection

Serum biomarkers, including CK, high sensitivity troponin I (hs-TnI) and N-terminal pro brain natriuretic peptide (NT -proBNP) were tested for all SSc patients. CK and hs-TnI was measured at the Leeds Teaching Hospital NHS Trust. NT-proBNP were tested at the University of Lublin, Poland. According to the manufacturer, a CK value above 320 IU/l was considered abnormal.

All patients had routine blood tests including full blood count, liver function test, C-reactive protein (CRP) and antinuclear antibodies (ANA) screening /ANA immunoblot. All HV had U&E performed up to one month prior to performing the muscle MRI for assessing the glomerular filtration rate.

7.2.5.3 CMR

The CMR protocol included LV function and volume, late gadolinium enhancement (LGE), myocardial perfusion, T1 mapping for native T1 and extracellular volume (ECV) quantification, tissue tagging and aortic distensibility. Full details on CMR protocol and analysis are described in the methodology chapter. For the purpose of the study, T1 mapping, including T1 native and ECV measurement were the CMR indices of interest (Figure 7-1). Cvi42 software (v4.1.3, Circle Cardiovascular Imaging Inc., Calgary, Canada) was used for CMR image analysis.

7.2.5.4 Peripheral muscle MRI

7.2.5.4.1 Muscle protocol

SSc participants and HV were imaged on a Siemens Verio 3 T scanner (Siemens HealtHVare, Erlangen, Germany), feet-first supine with two small 4-channel flexible-coils placed 4 cm above the patella, and a body-matrix coil placed on the chest. The contralateral leg was shielded using an RF blanket. The chest image was acquired at an oblique sagittal angle imaging from the top the aortic arch following the track of the aorta. T1 measurements were performed using an inversion recovery steady-state free precession sequence. A non-selective inversion pulse was used to minimise in-

flow effects. An image acquired without an inversion pulse was used to obtain the initial estimate for the signal at equilibrium (S_0).

Pre-contrast acquisitions were acquired for the thigh and the aorta before 0.1 mmol kg^{-1} gadoterate meglumine (Dotarem, Guerbet LLC, USA) was administered intravenously as a single bolus by hand. Alternate aorta and thigh post-contrast scans were then acquired sequentially for at least 25 minutes post-injection.

The following formula was used to calculate extracellular volume (ECV), where R1 is 1/T1 and muscle pre and muscle post are the pre and post-contrast skeletal muscle T1 values and blood pre and blood post are the pre-contrast and post-contrast blood pool T1 values (230).

$$ECV = (1 - hematocrit) \times \frac{R1\ muscle\ post - R1\ muscle\ pre}{R1\ blood\ post - R1\ blood\ pre}$$

7.2.5.4.2 Muscle MRI analysis

Image analysis was performed using MATLAB (MATLAB R2015a, The MathWorks Inc., Natick, MA, 2015) and ImageJ (ImageJ 1.51k, National Institute of Health, Maryland, USA). A region of Interest (ROI) within the vastus intermedius and the aorta were drawn for each participant (Figure 7-1). The distal aorta was used in order to minimise in-flow effects. ROIs were propagated for each slice and corrected manually or re-drawn for gross deformation if necessary.

T1 values were obtained by fitting a mono-exponential recovery equation to the mean signal intensity values from each ROI at each time point using a non-linear least squares fitting algorithm (lsqcurvefit, Matlab):

$$S = S_0 \cdot \left(1 - \left([1 - \cos(\theta)] \cdot e^{-TI/T_1}\right)\right)$$

Where θ is the inversion flip angle and S_0, q, and T1 are all free parameters in the fit. Constraints were applied so that S_0 and T1 were positive values, and θ was between 0 and π radians. The imaging sequence generated magnitude data and this was accounted for in the fitting using the method described by Messroghli et al (296).

ROI within the biceps femuris were also calculated but given that there were no significant differences of T1 measurement in the two proximal muscles, the vastus intermedius T1 mapping measurements were further used in the study.

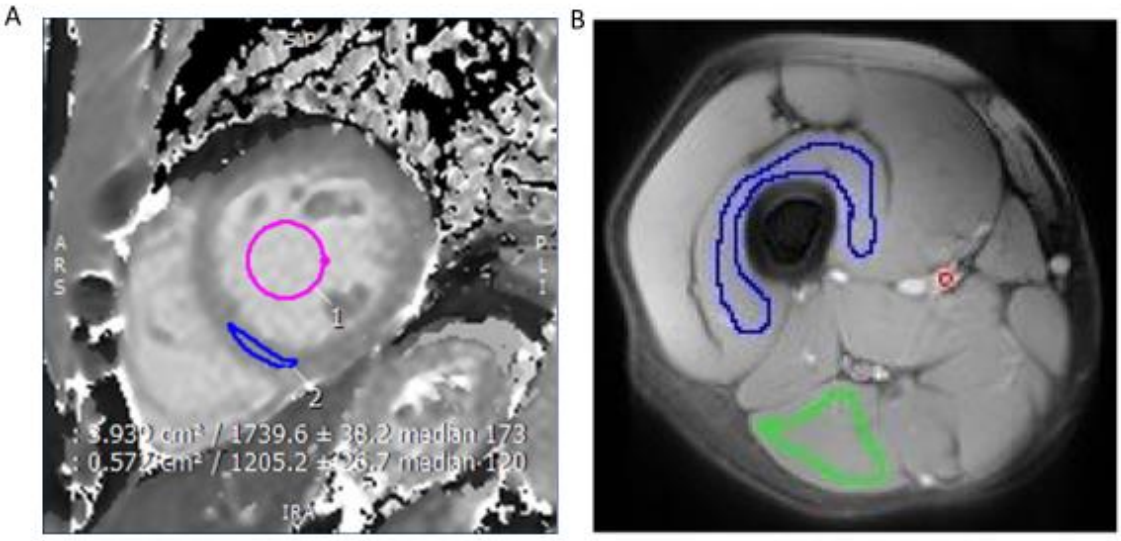


Figure 7-1 T1 mapping and ECV calculation in the: A. myocardium (blue- ROI in the myocardium, pink- ROI in the myocardial cavity, blood pool). B. thigh (blue - ROI in the vastus intermedius, green -biceps femoris, red - femoral artery)

7.2.5.5 Other investigations

All patients had nailfold capillaroscopy performed when attending for the clinical visit. The most recent pulmonary function tests (PFT) results were recoded for all participants.

7.2.6 Missing data

All efforts were made to avoid missing data. This study was a pure exploratory small sample study, thus the analysis was performed including all available data.

7.2.7 Statistical analysis

This was an exploratory study thus no formal power calculation was carried out. At least 10 SSc patients and 10 HV was considered sufficient to address the hypothesis-generating pilot study. An equal number of SSc patients with/without muscle

involvement (at least 5 in each group) was desired to test the differences between these 2 groups.

The statistical analysis was performed using SPSS (IBM SPSS Statistics 22) and GraphPad Prism V8. Descriptive summary statistics are provided for all variables. Continuous variables are reported as mean (SD) or median (IQR) and categorical data reported as percentage. Student's t-test, chi square test, Fischer exact test or Mann-Whitney U test when appropriate were used to assess for significant differences between groups. Correlation of clinical and serum biomarkers and MRI measures were assessed by Spearman's or Pearson rho test. For exploratory purposes, Receiver-operating characteristic (ROC) curves were built to assess the ability of muscle ECV for identifying myopathy and Youden Test was applied to find a cut-off value for ECV by maximizing both sensitivity and specificity.

As this was a small sample study, p-values were reported to only ascribe extent of descriptive differences rather than assign definitive significance, in line with good practice (251).

7.3 Results

7.3.1 Patient disposition

Twelve SSc patient and 10 HV participated in the study. 18 SSc patients and 14 HV were initially screened for the study. Five SSc patients were screened but either did not meet the inclusion criteria or refused to take part in the study and another patient consented but did not attend the peripheral muscle MRI. Two HV did not meet the inclusion criteria and other 2 HV were consented to the study but had U&E tested more than 1 months, thus couldn't have the peripheral muscle contrast-MRI.

Patient and HV selection and recruitment is illustrated in Figure 7-2.

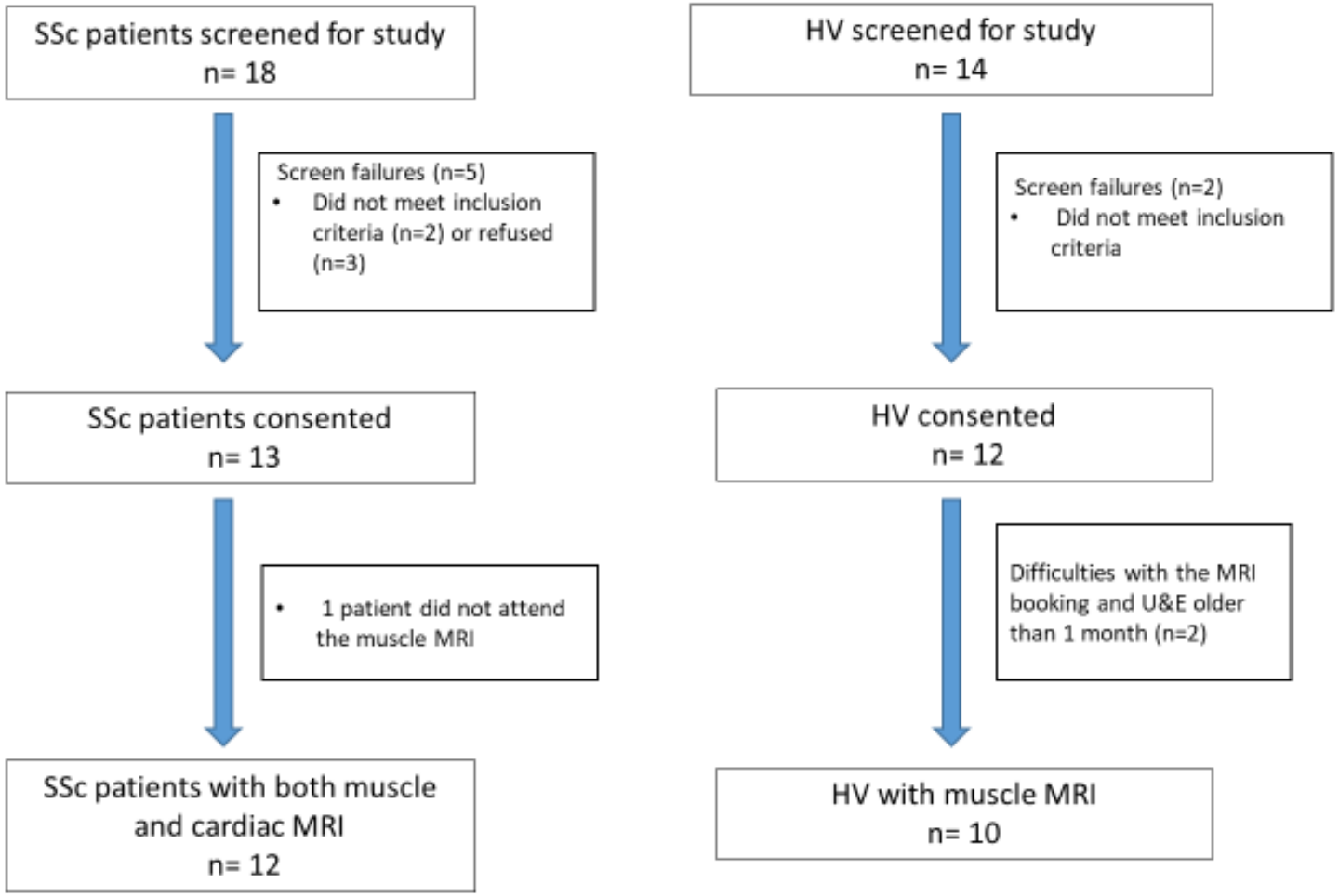


Figure 7-2 Flowchart showing patient and HV selection and recruitment

HV, healthy volunteers, MRI, magnetic resonance imaging.

7.3.2 Muscle MRI finding in HV compared to SSc patients

The 10 HV had a median (IQR) age of 36 (33, 40) and 6 (60%) were female. None had any muscle pathology, and none were receiving any medication. The SSc patients had a median (IQR) age of 52 (41,65), 10 being female (83%).

Significantly greater skeletal muscle ECV was noted in SSc patients compared to HV [mean (SD) 23 (11) vs 11(4), mean diff (95%CI) -12 (-19,-40), p=0.04]. Skeletal muscle native T1 values were comparable between the 2 groups although modestly higher in SSc patients compared to HV [mean (SD) 1396 (56) vs 1387 (42), mean diff (95%CI) -8 (-53, 37)] (Figure 7-3).

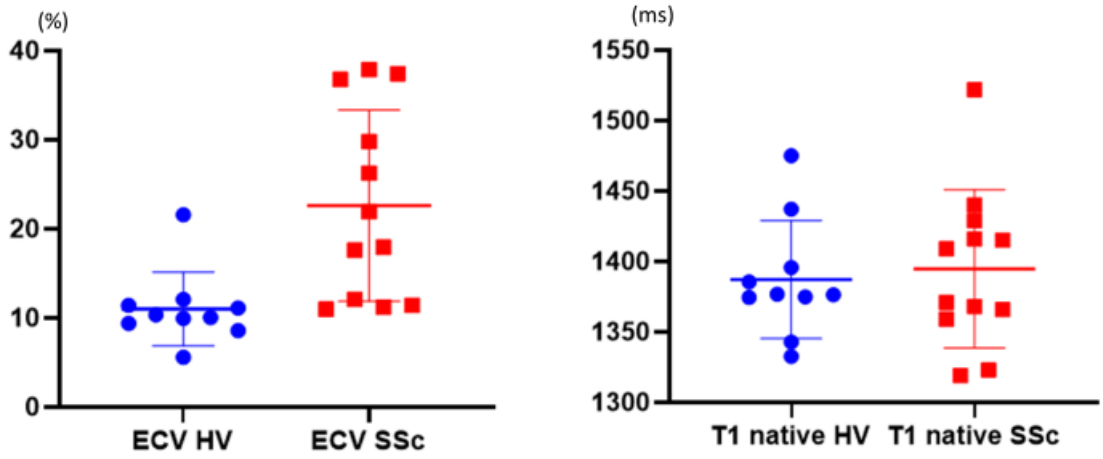


Figure 7-3 Peripheral muscle T1 native and ECV in HV compared to SSc patients

ECV, extracellular volume; SSc, systemic sclerosis

7.3.3 Disease characteristics of SSc patients with peripheral muscle and cardiac MRI

Of the 12 patients included in the study, 9 were limited cutaneous SSc (lcSSc), with a median (IQR) disease duration of 7 (2,17) years and a median (IQR) modified Rodnan skin score (MRSS) of 3 (1,4), 1 with arthritis and 4 with a history of digital ulcers (DU) and interstitial lung disease (ILD). All patient had antinuclear antibodies (ANA) positive: 5 anti-topoisomerase antibody (Scl70), 4 anticentromere antibody (ACA), 2 Pm/Scl70 positive antibodies and 1 Anti Ro antibodies (Table 7-1).

None of the SSc patients were receiving lipid lowering treatment with statins. Two patients were on minimal steroidal treatment: one patient receiving 2.5 mg and one 5 mg of prednisolone, and 7/12 were on disease modifying antirheumatic drugs (DMARD) treatment: 5 on mycophenolate mofetil and 2 on methotrexate (Table 7-1).

Table 7-1 Disease characteristics of patients with peripheral and cardiac MRI

SSc phenotype	**SSc patients, n=12**
Demographics and disease history	
Age, median (IQR)	52 (41, 65)
Female, n (%)	10 (83%)
Disease subtype, n (%) LcSSc DcSSc	 9 (75%) 3 (25%)
Disease duration (years), median (IQR) RP duration	7 (2,17) 14 (3, 29)
History of, n (%) Digital ulceration Calcinosis GORD Interstitial lung disease	 4 (33%) 2 (17%) 10 (83%) 4 (33%)
Clinical profile	
Total modified Rodnan skin score, median (IQR)	3 (1,4)
Presence of, n (%) Digital ulceration Tendon friction rubs	 4 (33%) 0

Calcinosis	3 (25%)
Joint contractures	2 (17%)
Any TJC	3 (25%)
Any SJC	1 (8%)
NFC vasculopathy pattern, n (%)	
Non-specific	3 (25%)
Early	3 (25%)
Active	4 (33%)
Late	2 (17%)
Antibody profile and serology	
Antibody positive, n (%)	
ANA	12 (100%)
ACA	4 (33%)
Scl70	5 (42%)
Pm-Scl70	2 (17%)
Ro antibodies	1 (8%)
CRP (mg/L) (normal range <5), median (IQR)	5 (5, 6)
CK, median (IQR)	83 (53, 190)
Hs-TnI, median (IQR)	6 (3,11)
NT-proBNP, median (IQR)	81 (56, 119)

Pulmonary function tests	
FVC, mean (SD)	107 (20)
TLC, mean (SD)	95 (16)
DLCO, mean (SD)	63 (19)
DLCO/VA, mean (SD)	82 (21)
Cardiovascular profile	
Any cardiovascular factors, n (%)	1 (8%) – family history of CVD
Systolic BP	114 (17)
Diastolic BP	62 (12)
BMI	26 (4)

ACA, anti-centromere antibody; ANA, antinuclear antibodies; CK, creatine kinase; DcSSc, diffuse cutaneous systemic sclerosis; DLCO, diffusing capacity of the lungs for carbon monoxide; DLCO/VA, DLCO adjusted for volume; FVC, forced vital capacity; Hs-TnI, high-sensitivity troponin I; GORD, gastro-oesophageal reflux disease; lcSSc, limited cutaneous systemic sclerosis; NFC, nailfold capillaroscopy; NSIP, non-specific interstitial pneumonia; NT-proBNP, N-terminal pro brain natriuretic peptide; Scl70, anti-topoisomerase antibody; SD, standard deviation; TLC, total lung capacity.

Seven of 12 SSc patients had a diagnosis of non-inflammatory myopathy and none had a confirmed diagnosis of myositis. Four patients had a MMT score of 4/5 involving the hip adductors and 5 reported proximal myasthenia of the lower limbs. CK was raised at the time of the visit (400 IU/l) for one patient and 3 more patients had a history of minimally raised CK levels (>320 IU/l, <600 IU/l) (Table 7-2).

Table 7-2 Muscle involvement in SSc patients

Muscle involvement, n (%)	
Presence/history minimally raised CK	4/12 (33%)
Proximal myasthenia	5/12 (42%)
Proximal myalgia	0
MMT score	MMT score of 4 – 4/12 (33%)

CK, creatine-kinase; MMT, manual muscle testing.

7.3.4 Peripheral muscle MRI and association with disease phenotype

Except for a negative association between T1 native and a DcSSc subset (rho=-0.623, p=0.03), no other association with disease characteristic, including a diagnosis of ILD, history of DU, disease duration, arthritis, mRSS, or antibodies was found (p>0.05).

7.3.5 Association between peripheral muscle MRI findings and CMR detected pSSc-HI

SSc patients had a myocardial ECV mean (SD) of 31(3) and a myocardial native T1 mean (SD) of 1287 (54), both higher than the normal reference range (ECV >29%, native T1 >1240).

An association between peripheral muscle native T1 and myocardial ECV was suggested (rho=0.470, p=0.123) and a negative correlation between the cardiac and muscle ECV (rho=-0.485, p=0.110). No association between the native T1 in the myocardium and muscle was found (rho=0.224, p=0.484) (Figure 7-4). No association of hs-TnI with muscle ECV (rho=0.041, p=0.899), muscle T1 native (rho=-0.285, p= 0.370) or CK (rho=0.095, p=0.768) was found.

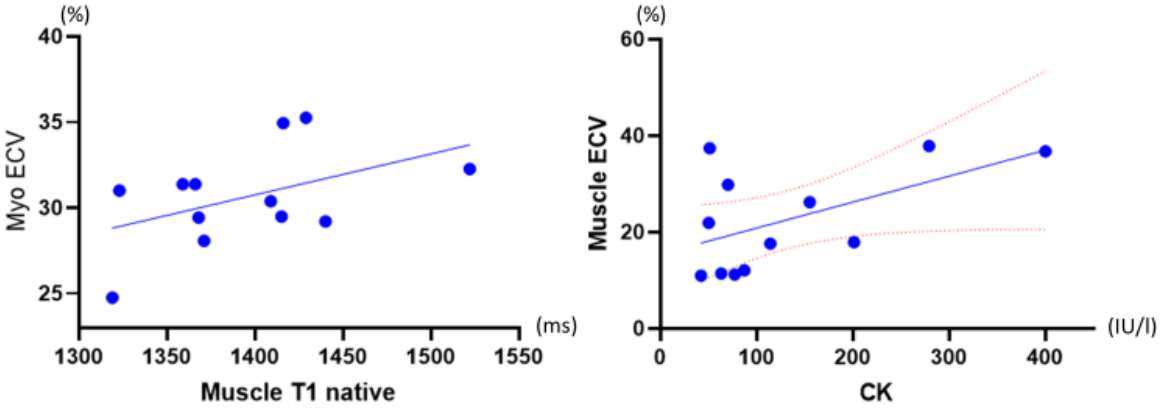


Figure 7-4 Correlation between myocardial ECV and muscle native T1 and between CK and muscle ECV

CK, creatine-kinase; ECV, extracellular volume, Myo-myocardial.

7.3.6 Muscle MRI findings in SSc patients with myopathy

Peripheral muscle ECV associated with CK (R^2 =0.307, rho=0.554, p=0.0615) (Figure 7-4) and significantly higher muscle ECV values were found in those with evidence of myopathy compared to those with no muscle involvement [28 (10) vs 15 (5), mean diff (95%CI)-13 (-24, -3), p=0.023] (Table 7-3, Figure 7-5).

Table 7-3 Differences in muscle T1 native and ECV in SSc patients with and without myopathy

	Myopathy	**No myopathy**	**Mean difference (95% CI)**	**p**
Muscle T1 native	1395 (73)	1395 (26)	0.08 (-77, 77)	0.998
Muscle ECV	28 (10)	15 (5)	-13 (-24, -3)	0.023*

*p<0.01

ECV, extracellular volume

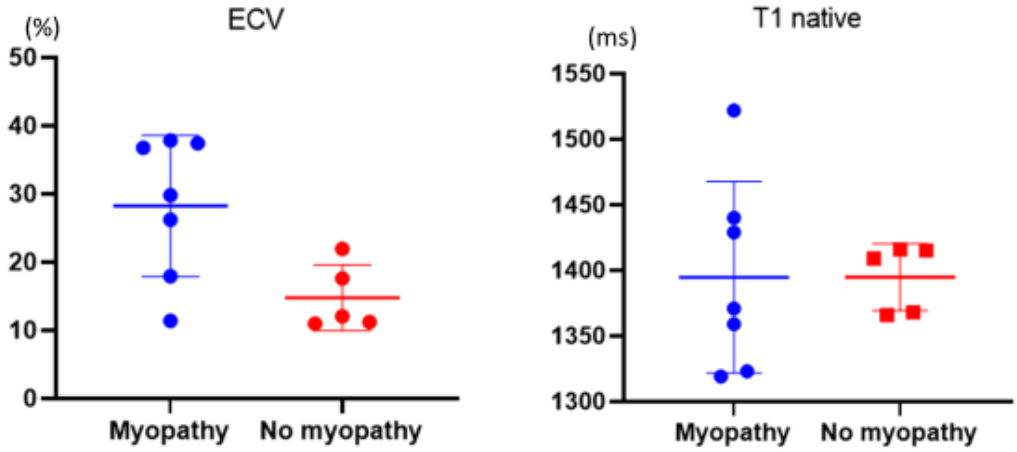


Figure 7-5 Mean (SD) ECV and native T1 in those with and without myopathy

ECV, extracellular volume

For exploratory purposes, a ROC curve was plotted to find potential cut-off values of muscle ECV for identifying myopathy. AUC (95%CI) was significant at 0.886 (-0.690, 1), p=0.028. Using Youden test, an ECV value of 22% was found to best identify myopathy with a sensitivity of 71.4 % and a specificity of 80% (Figure 7-6, Table 7-4).

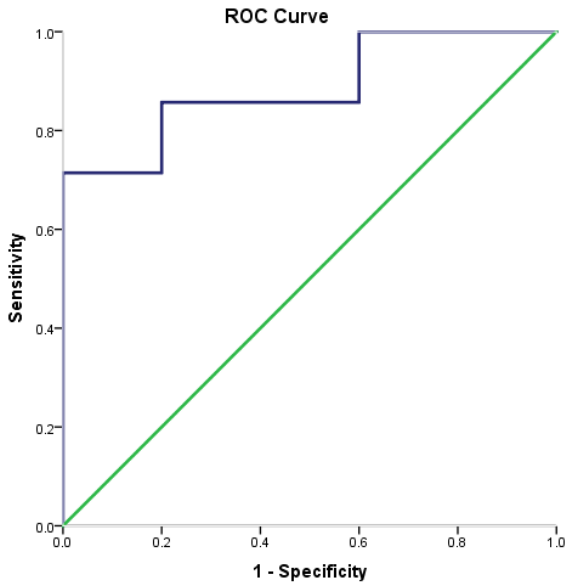


Figure 7-6 ROC curve of ECV for myopathy AUC=0.886. p=0.028, CI (-0.690, 1)
AUC, area under curve; ECV, extracellular volume.

Table 7-4 Sensitivity and specificity of ECV for identifying myopathy

ECV (positive if greater than or equal to)	**Sensitivity**	**1 - Specificity**
9.9900	1.000	1.000
11.1050	1.000	0.800
11.3100	1.000	0.600
11.7350	0.857	0.600
14.8400	0.857	0.400
17.7800	0.857	0.200
19.9350	0.714	0.200
24.0800	0.714	0.000
28.0250	0.571	0.000
33.2950	0.429	0.000
37.1000	0.286	0.000
37.6450	0.143	0.000
38.8700	0.000	0.000

ECV, extracellular volume.

7.4 Discussion

This pilot study demonstrates the feasibility of T1 mapping MRI in the peripheral muscle of SSc. Higher ECV was noted in SSc patients compared to HV, indicating the presence of diffuse fibrosis in the peripheral muscle of SSc patients which may represent the substrate for the non-inflammatory myopathy. An association of myocardial ECV and peripheral muscle native T1 was found, indicating the presence of interstitial remodelling in both the heart and peripheral muscle.

This study was a first to test T1 mapping in the peripheral muscle. Significantly higher ECV was found in the peripheral muscle of SSc patients compared to HV, indicating that a diffuse interstitial process is present in the peripheral muscle of SSc patients. Several studies have previously measured skeletal muscle ECV, using the thoracic muscle visible at the edge of the CMR field (127, 288, 290). Our study applied T1 mapping in the thigh, the predominant muscles affected in SSc and other IMID, as opposed to axial muscle, minimising thus cardiac and breathing artefacts and providing large, axial muscle volume for T1 measurement.

Muscle ECV values of HV were comparable to those reported in the literature, albeit with significant discrepancies between the muscle T1 native values of both HV and SSc patients and ECV values of SSc patients compared to other studies (127, 289, 290). These discrepancies might be related to the above-mentioned limitations of using CMR for measuring T1 mapping indices in skeletal muscle.

Except for an association of lcSSc and ECV, no other association with clinical characteristics was found. This is in accordance with other studies which found no association between disease characteristics, in particular poor prognostic factors of SSc and the non-inflammatory myopathy (123). This would suggest that, as described in the myocardium, interstitial fibrosis of the peripheral muscle can be a common subclinical finding, present in both SSc subtypes (165, 167).

Moreover, the study showed that patients presenting with a non-inflammatory myopathy had significantly greater ECV compared to the non-myopathy group, demonstrating the predictive value of T1 mapping in detecting low level muscle

pathology. A muscle ECV of 22% was found to best identify myopathy in this cohort. Whilst the study is exploratory and the data should be interpreted conservatively, demonstration of peripheral muscle ECV provides pathophysiological insights and suggests possible prognostic value, and a guide for future studies.

CK also associated with ECV, albeit only one patient had a CK above the normal value and 3 more patients had intermittently minimally raised CK, suggesting the potential clinical importance of low level CK in detecting muscle abnormalities. The process could be explained by the expansion of the interstitial space as a result of interstitial fibrosis with/without oedema and subsequent muscle injury with the release of CK. Other studies have documented the association of low level CK with impaired muscle strength, abnormalities on EMG as well as biopsy findings of fibrosis (123, 283).

The association of myositis and pSSc-HI has been commonly described (63, 114, 125), however the association of the non-inflammatory myopathy and pSSc-HI is less clear. Whilst Barison et al reported a strong relationship between the ECV in the skeletal and cardiac muscle by using CMR (127), the present study showed an association of CMR-ECV and peripheral muscle native T1 (stigmata of a more inflammatory process). The study indicates thus the coexistence of interstitial remodelling of both the cardiac and peripheral muscle in SSc patients, acknowledging the fact that the heart involvement might precede the muscle involvement. Purely speculative, but a low-level inflammatory process (represented by native T1) is indicated, which may lead to processes driving fibrosis in the peripheral muscle, but which may already be established in the heart. Larger studies are needed to clarify this further. Nonetheless, no association of hs-TnI with the muscle remodelling parameters or with CK was found, confirming that hs-TnI does not reflect muscle injury and is a specific biomarker for identifying cardiac involvement in SSc (185, 186).

In conclusion, this study demonstrated the feasibility of applying T1 mapping in the peripheral muscle of SSc patients, confirming the presence of interstitial fibrosis in the peripheral muscle of SSc patients, which might represent the substrate of the non-inflammatory myopathy in SSc. The study also showed the presence of interstitial remodelling in both the cardiac and skeletal muscle, speculating that the process in the

heart might precede that in the peripheral muscle. Further research is needed to validate the findings of this pilot study as well as identifying the prognostic value of interstitial fibrosis in the muscle.

7.4.1 Limitations

The main limitation of the study is the sample size. However, the study was a novel, pilot, hypothesis-generating study, aiming to demonstrate the feasibility of T1 mapping in the peripheral muscle of SSc patients. A larger cohort is required to accurately assess the association with disease phenotype and CMR findings. The study remains relevant, being the first to use T1 mapping in the peripheral muscle of SSc patients.

Another limitation of the study is that the HV were not matched by age and gender with the SSc patients. A matched cohort would have provided more reliable results. However, the recruitment of the HV was difficult mainly because the MRI implied the administration of contrast, thus younger HV were more interested to be part of the study. However, muscle native T1 values were similar between the SSc patients and HV and ECV was significantly higher in SSc compared to HV, thus unlikely for the results to change substantially if a matched HV cohort would have been used. Nonetheless, current evidence from CMR studies suggest that T1 mapping measures are not influenced by gender and T1 native values increase only in individuals over 60 years, but not between age category 20–39 years and 40–59 years (297). These might also apply in the peripheral muscle.

A T2 weighted or fat suppression MRI sequence (STIR) would have provided information with regards to the existence of muscle oedema. The scan did not include the oedema sequences mainly because of the long scanning time that was required for the development of the new T1 mapping sequence. However, the diagnosis of myositis was excluded based on clinical and biological markers. The study was not designed to include muscle biopsy, albeit this would have provided the opportunity to confirm the MRI findings of interstitial fibrosis.

7.4.2 Key messages

1. T1 mapping can be implemented in the peripheral muscle in the general population and in SSc
2. SSc patients have evidence of diffuse fibrosis in the peripheral muscle which could explain the noninflammatory myopathy
3. Interstitial remodelling is present in both the cardiac and peripheral muscle

Chapter 8. Discussion

This thesis focuses on primary systemic sclerosis heart involvement (pSSc-HI) and highlights the key clinical and research unmet needs. Whilst associated with significant mortality, understanding the nature of pSSc-HI, effective risk stratification and early diagnosis to ensure timely intervention is lacking. This programme of work was thus designed to provide a comprehensive characterisation of pSSc-HI by using sensitive tools such as the implantable loop recorder (ILR) to detect electrophysiological (EP) abnormalities and cardiovascular magnetic resonance (CMR) to phenotype subclinical pSSc-HI and inform a first step in risk stratification of pSSc-HI. Clinical and disease phenotyping together with serum cardiac biomarker testing were applied to identify predictors of CMR detected pSSc-HI and clinically overt pSSc-HI. CMR was also utilised to understand the dynamics of subclinical pSSc-HI and its clinical significance, and finally to explore for the presence of a relationship between CMR parameter of fibrosis and peripheral skeletal muscle fibrosis.

8.1 Implantable loop recorder

The implantable loop recorder (ILR) is used in cardiological practice for the evaluation of recurrent syncope of unknown origin, when a cardiac cause is highly suspected (298), recording data for up to three years; thus providing long term monitoring for any rhythm disturbances compared to the commonly employed methods such as ECG and 24-hour Holter. Its value in other cardiac pathologies such as recurrent atrial fibrillation, structural heart disease and haemodialysis patients with complicated rhythm disturbances has been recently demonstrated (299-302). No studies to date evaluated the value of ILR in SSc.

Chapter 4 has demonstrated the feasibility of the ILR in patients with SSc and showed significant EP abnormalities in eight patients of whom 6 required medical treatment and 3 developed serious arrhythmia/AV block including 2 with non-sustained ventricular tachycardia (NSVT) and one with complete heart block (CHB). The median (IQR) time from ILR implantation to detection of significant EP abnormality was 12 (4.5, 24) months.

There are multiple studies employing ECG/24h Holter to investigate rhythm disturbance however, the limitations of using short term monitoring apply here, with the majority of studies recording supraventricular/ventricular ectopics (SVE/VE), which unless of high burden, are generally considered benign (128, 129, 139, 303). Ferri et al detected resting ECG abnormalities in 22 of 53 patients, comprising conduction defects in 10, rhythm disturbances in 16, of which SVE in 2 and VE in 9, and ST-T changes in 3. When 24 hour ECG was used, conduction defects were identified in 16 patients, rhythm disturbances in 52, ST-T changes in 18 and ventricular arrhythmia including multiform ventricular ectopic beats (VEB) and non-sustained VT in 48 patients (303). The high prevalence of conduction abnormalities in the manuscript is likely the result of the high frequency of reporting Holter abnormalities, including benign findings. In addition, the study enrolled consecutive SSc patients attending the SSc clinic, including patients with CVD and PAH. Thus, the results of this study need to be interpreted with caution. The ELCASA study aimed to identify EP changes associated with primary pSSc-HI; hence minimised the role of cardiovascular disease (CVD) excluding ischemic heart disease (IHD), diabetes, PH and allowing patients with only one CV risk factors.

The ELCASA study also looked at the association of CMR and significant EP abnormality showing an association of CMR ECV with ILR findings. Myocardial fibrosis is a known risk factor for the development of arrhythmias, however, only one patient out of 5 with CMR LGE developed non-sustained ventricular arrhythmias (NSVT). The location, modest extent of myocardial fibrosis as well as the pilot sample size might explain the lack of association between arrhythmias and fibrosis. Reinforcing the literature, the more serious EP findings, NSVT and CHB associated with poor prognostic factors of SSc, including dcSSc, Scl70, male gender, ILD and early disease (96, 304, 305).

Only a few studies have assessed the association between CMR and EP changes, however none employed more advanced investigative tools including ILR, CMR T1 mapping and quantitative myocardial perfusion. One study showed no association between LGE focal fibrosis and 24h Holter findings including total number of VEB, ventricular arrhythmia severity (including couplets, triplets and runs) and conduction abnormalities (131). Tzelepis et al showed that 19/41 SSc patients included in the study had 24h-Holter abnormalities including 12 with VEB, 2 with supraventricular

tachycardias, 3 with supraventricular ectopic beats (SVEB), 2 with atrial fibrillation, 2 with NSVT, two with right bundle branch block, and 1 with left bundle branch block. These patients had a greater number of enhancing myocardial segments and a greater total volume percentage index of fibrosis compared to those with no conduction abnormalities [2.1 ± 1.9 vs 0.8 ± 1.2, p=0.019]. However, the study did not exclude patients with pulmonary hypertension (PH) and patients with abnormal Holter results were more likely to have PH [11 (58%), vs 2 (12%)] thus the study does not exclude secondary causes which can account for both the CMR and 24h-Holter findings (15).

The study also showed an association of cardiac biomarkers hs-TnI and NT-proBNP with ILR EP abnormalities, pinpointing myocardial injury as a substrate in the development of conduction abnormalities in SSc.

Thus, the ELCASA study was a first to demonstrate the feasibility of the ILR use in SSc as well as its value in detecting pSSc-HI manifesting as arrhythmia or AV block, detected in a poor prognosis SSc group. Cardiac biomarkers hs-TnI, NT-proBNP, CMR ECV, as well as poor prognosis factors of SSc could be of use for risk stratification for primary SSc EP abnormalities.

In a recent paper, of which I am the co-author, we undertook a secondary analysis of ELCASA study and demonstrated that SSc patients with ILR significant arrythmias/AV blocks were more likely to have multiple abnormal short-term EP tests, including signal average ECG (3/4 (75%) vs. 1/9 (11%) with no arrhythmia), autonomic testing (3/5 (60%) vs. 3/9 (33%)) and 24-hour Holter ECG findings (5/6 (83%) vs. 3/10 (30%)). These findings suggest that a composite of short-term EP tests could inform the use of ILR for the detection of pSSc-HI (306).

8.2 CMR

The use of the CMR in pSSc-HI is increasingly emphasised, as highlighted in the review chapter, however there are important discrepancies reported in the literature particularly with regards to the prevalence of CMR abnormalities and association of CMR findings with disease phenotype.

One of the main reasons for these inconsistencies is that many studies do not exclude patients with CVD, thus including IHD and/or pulmonary arterial hypertension (PAH). These might account for the high prevalence of subendocardial LGE reported in the literature. Subendocardial LGE was reported in 11 of the 17 patients with LGE by Meduri (160), in 2 out of 3 SSc patients with LGE by Kobayashi (181) and in 3 out of 11 with LGE by Hachulla (158). Subendocardial LGE is a typical finding of ischaemic pathology and should be thoroughly assessed in the clinical context to exclude common causes of IHD.

Right ventricle insertion point fibrosis LGE is a non-specific finding and is reported in healthy individuals with no cardiac pathology (260). However, CMR studies in SSc commonly describe insertion point fibrosis, included as a specific CMR-SSc pattern (162, 179, 182).

Visual perfusion defects are also commonly described in SSc CMR studies, including perfusion defects that are confined to LGE-fibrosis area (14). These abnormalities are highly specific for macrovascular disease and reflect IHD. Detection of perfusion defects requires CMR-cardiology expertise and consistent acquisition. A true perfusion defect should last for more than 5 dynamic frames and should not be present at rest (if myocardial infarction has been ruled out). A very frequent perfusion artefact is the “dark rim artefact” or Gibbs ringing which is produced as a result of magnetic susceptibility, cardiac motion and spatial resolution and appears in the subendocardial area, mimicking a perfusion defect (307). Rodriguez-Reyna et al in their study reported 49 of a total of 62 patients with subendocardial perfusion defects (14) and Moroncini reported 14/24 with perfusion defects (177) whilst Gyllenhamar reported no perfusion defects but lower myocardial perfusion reserve (MPR) in SSc patients compared to HC (179). SSc patients in CONVAS study had no visual perfusion defects but lower stress MBF and MPR compared to HC.

In addition, there are no standardised CMR protocols for the detection of pSSc-HI and not all studies use validated methods for CMR analysis. One study used LGE quantitative analysis albeit there was no focal fibrosis on visual assessment of LGE images, perhaps with the aim of quantifying diffuse fibrosis. At follow up, patients with

LGE quantitative fibrosis developed established LGE fibrosis on visual assessment (18). The LGE quantification method is inappropriately used as there was no visual LGE fibrosis, thus the results of the manuscript need to be cautiously interpreted.

Thus, there is a strong need for the use of established standardised CMR protocols and validated CMR analysis methods, with the use of two CMR expert readers for LGE and perfusion images to ensure inter-observer reliability. Specific protocols for pSSc-HI as well as joined Rheumatology and CMR-cardiology article reviewers need to be further established (13).

CONVAS study did not allow patients with IHD, PAH, diabetes, other inflammatory musculoskeletal diseases, and minimised the presence of traditional CV risk factors, thus reducing the risk of including cardiac fibrosis associated with IHD or other conditions. The study had 2 CMR readers for LGE and myocardial perfusion analysis: myself and the second was AK, consultant Cardiologist with more than 5 year experience in CMR reporting. Professor Sven Plein also reviewed the LGE and perfusion CMR images including cases challenging to adjudicate. Myocardial focal fibrosis was detected in only 18 of the 83 SSc patients, after excluding RV insertion point fibrosis, which was present in 16 SSc patients. As highlighted above, this represents a normal variant and has no prognostic significance. The CONVAS study was thus a first study to date to employ a particularly comprehensive assessment to detect all pathophysiological aspects of CMR pSSc-HI; and included LGE and LGE scar mass, using a validated quantification method (5SD) (232), as well as T1 mapping with ECV quantification and quantitative perfusion. The study demonstrated that subclinical focal and diffuse fibrosis as well as reduced myocardial perfusion represent the pathophysiological substrate in pSSc-HI.

8.3 Surrogate markers of pSSc-HI

8.3.1 Disease phenotype

Identifying credible clinical surrogate markers for CMR pSSc-HI to inform better clinical management is challenging. Whilst myocarditis and more serious arrhythmias are associated with poorer prognostic markers of SSc, the literature shows inconsistent

findings in regards to the association of CMR abnormalities with disease phenotype. Whilst some of the CMR studies report no association between LGE and ECV with clinical findings (127, 158, 172) several studies document the association of higher ECV with mRSS (171) and dcSSc (159) and of LGE fibrosis with longer history of Raynaud's (15) and Scl70 (183). Only one previous study explored the association of quantitative myocardial perfusion and disease phenotype in SSc, including age, gender, duration of Raynaud's, ANA SSc specific antibodies or NFC pattern, with no association found (178).

History of digital ulcers (DU) has been previously associated with worse cardiac outcomes. In a study examining the EUSTAR database, a history of DU was predictive of elevated systolic pulmonary arterial pressure on echocardiography but also of LV failure and cv events (308). No studies thus far have reported an association of DU with CMR abnormalities.

Our study was the largest study to confirm an association of mRSS with focal fibrosis, including quantified scar mass and diffuse fibrosis as well as to indicate an association of ECV and MPR with DU. These findings suggest a concurrent fibrotic process of both the skin and the heart as well as microvascular impairment as a pathological substrate of pSSc-HI.

8.3.2 Cardiac serum biomarkers

This thesis has also demonstrated the potential utility of hs-TnI and NT-proBNP in detecting both focal and diffuse CMR fibrosis as well as ILR-detected arrhythmias and AV block. One previous study has demonstrated the association of NT-proBNP and ventricular arrhythmia as assessed by 24h Holter-ECG. According to this study, NT-proBNP showed a positive association with VEB (r=0.445, p=0.006), total number of isolated PVC (r=0,493, *P*=0.002), total number of VEB (r=0.379, *p*=0.021) and the number of VEB morphologies (r=0.501, *P*=0.002). NT-proBNP levels above 287 pg/ml predicted a high frequency of VEB (> 100 PVC/24 h), complex ventricular arrhythmias (including ventricular bigeminy, couplets or triplets) and the presence of polymorphic VEB (195).

De Luca showed that out of 100 SSc patients with new onset cardiovascular symptoms including dyspnoea, palpitations, chest pain, and/or signs of heart failure and/or increase in cardiac biomarkers, 68% had ECG abnormalities including complete or incomplete right bundle branch block (n=19), VEB (n=10) and SVEB (n=12); and 56 patients had 24h ECG findings: 42 had VEB of whom 24 had frequent VEB, 14 had episodes of SVT and 11 presented short runs of NSVT. Both SVEB and VEB correlated with hs-TnT, CK-MB and NT-proBNP levels (309). Moreover, a total number of VEB of >1190/24h showed 100% sensitivity and 83% specificity in predicting cardiac complications (either sudden cardiac death or indication for implantable cardiac defibrillator) and these patients had higher hs-TnT. The study did not allow patients with a history of coronary artery disease (CAD) however, other confounders such as diabetes, PAH and other CVD were not excluded, thus these might account for the high prevalence of the ECG abnormalities.

The association between CMR finding and cardiac biomarkers has been rarely investigated (163, 195). One retrospective study that looked at CMR abnormalities reported LGE in 27/49 SSc patients. BNP levels were significantly higher in those with LGE compared to those without LGE [23.1 (19.9-28.3) vs 13.2 (10.5-20.3), p<0.001] and showed significant correlation with left ventricle mass index (rho = 0.75, P < 0.001). A BNP level of ≥16.7 pg/mL had a sensitivity of 81% and a specificity of 60% in detecting LGE (183).

The results of the CONVAS study suggest that both NT-proBNP and hs-TnI are sensitive tools in detecting diffuse and focal fibrosis respectively. NT-proBNP associated with ECV which might be explained by interstitial remodelling and expansion of the interstitial space with the subsequent release of the biomarker whilst hs-TnI associated with LGE, thus reflecting myocardial injury. A hs-TnI≥ 5.5 ng/l had a sensitivity of 65% and a specificity of 70% to predict the presence of focal LGE in our study.

8.4 Predicting pSSc-HI outcomes with CMR and cardiac biomarkers

This was the first longitudinal study to investigate the prognostic role of CMR, including LGE, T1 mapping and myocardial perfusion indices and cardiac biomarkers for the

future development of pSSc-HI CV outcomes, defined as arrhythmias/AV block, systolic or diastolic dysfunction on echocardiography and/or myocarditis. Cardiac biomarkers, in particular NT-proBNP appeared to predict pSSc-HI CV outcomes and CMR-ECV above the normal reference range (>29%) almost reached statistical significance; thus these 2 markers could be of further use for screening patients at risk of clinically overt pSSc-HI.

Only one previous study included longitudinal data and investigated the prognostic value of CMR and disease phenotype for predicting CV outcomes in 62 SSc patients. CV outcomes were defined as heart failure, coronary artery disease, arrhythmias, peripheral vasculopathy, elevated pulmonary systolic artery pressure and death. Myocardial fibrosis and older age were associated with heart failure at follow up [(p = 0.01, OR: 11.49, 95% CI(1.6, 83) (p = 0.02, OR: 1.11, 95% CI (1.01,1.22) respectively], mRSS associated with coronary artery disease (p = 0.02, OR: 1.2, 95% CI (1.02, 1.38)], whilst mortality [(p = 0.004, OR: 11.9, 95% CI (2.1, 65.7)] associated with CRP and recurrent digital tip ischemic ulcers (p = 0.001, OR 26.8, 95% CI 3.9,181.3)]. The study also looked at the predictive value of insertion point fibrosis (p = 0.001, OR: 12.5 95% CI (2.7, 56.6)), which associated with a history of digital ulcers (310). The main limitation of this work is the definition of CV outcomes, the study including both primary and secondary disease as an outcome but also death, which comprised causes other than solely CV related death. In addition, the study included and looked in particular at insertion point CMR fibrosis, which is known to be a normal variant.

A more recent large cross-sectional study of 201 SSc patients with no known cardiac related disease looked at the relationship between LGE CMR and 24h Holter ECG and showed association between myocardial fibrosis and ventricular arrhythmias as defined by Lown classification grade ≥ 2 and (chi-square test = 9.5; $p < 0.01$) and higher LGE scar mass in those with ventricular arrhythmias compared to those with no arrhythmia (2.6 ± 2.1 g vs. 1.2 ± 1.5 g; $p < 0.01$). (162) In our study, LGE focal fibrosis did not predict the development of CV outcomes including arrhythmia, which may be explained by the smaller sample size and event rate of our cohort but also low scar mass values observed in our study population (210, 257).

Larger longitudinal studies are required to confirm the finding of our study and inform risk stratification for pSSc-HI.

8.5 CMR natural course

Only one previous study included longitudinal CMR data in 11 of a total 44 SSc patients that otherwise had a single CMR, and showed further deterioration of myocardial perfusion and fibrosis that was detected only by quantitative analysis at the first visit and progressed into established fibrosis at follow up (18). However, as previously mentioned, this study inappropriately used a quantification method for LGE with visually no evidence of fibrosis. Our study showed that only 1/4 patients with LGE at V1 had an increase in LGE at follow-up and only one patient progressed to clinically overt CMR finding in the form of myocarditis. New LGE was however detected in 2 patients. An association of poor prognostic factors of SSc with a reduction in systolic function and new or increased fibrosis was also noted, albeit subclinical in all, except for the patient that developed myocarditis.

The CONVAS study was a first to also investigate the dynamic changes of biomarkers and CMR findings in SSc. An association of hs-TnI and NT-proBNP with a reduction in LVEF and increase in the end-diastolic and end-systolic volume was noted and both biomarkers were sensitive to change and associated with systolic function deterioration, indicating the potential utility of dynamic changes of both biomarkers for identifying patients at risk of CMR pSSc-HI progression.

No studies to date investigated the effect of treatment on CMR findings. Neither DMARD, vasodilator treatment or ACEI treatment seemed to influence myocardial fibrosis, remodelling or myocardial perfusion in our cohort. Larger longitudinal studies are thus needed to investigate whether immunosuppressive or vasodilator treatment can alter the course of pSSc-HI. More recent experimental studies on rats have documented the benefit of Nintenanib on myocardial fibrosis, LV dilatation, hypertrophy and collagen type III (311). Future research could clarify whether anti-fibrotic treatment has any beneficial role on myocardial fibrosis.

8.6 Association between myopathy and cardiac involvement in SSc

The prevalence of myopathy varies widely between studies, the main reason being the lack of a consensus for defining and classifying myopathy (123, 124, 126, 284). The literature describes an inflammatory pattern which is associated with poor prognosis factors of SSc and myocarditis and a non-inflammatory pattern, characterised by low level CK and muscle weakness, but it's association with cardiac involvement in SSc is not clear (123, 283). Chapter 7 describes higher peripheral muscle ECV in SSc compared to HC, being the first study to implement novel T1 mapping sequence in the peripheral muscle, indicating the presence of diffuse fibrosis in the skeletal muscle of SSc patients. An association of myocardial ECV and muscle native T1 was also found, suggesting the coexistence of interstitial remodelling in both the heart and the muscle. Larger cohort studies are required to validate the results of this pilot project. Only one previous study has explored this area, looking at T1 mapping and ECV quantification in the myocardium and thoracic muscle, showing good correlation between each other as well as higher values in SSc compared to controls (127). However, the main limitation of this study is that skeletal muscle ECV was measured at the edge of the CMR field, where artefacts are likely to occur. Also, ECV was measured in the thoracic muscle as opposed to peripheral muscles, which are the most commonly muscles affected in SSc and other IMID (292, 293).

8.7 Future research agenda

The thesis has provided valuable data with regards to the feasibility as well as the use of ILR in SSc. The ELCASA study showed ILR significant findings in almost half of the study population and serious arrhythmia/AV block in 3 patients, of whom all had early dcSSc and known ILD, 2 had Scl70 and 2 were males. Thus, a next step would be to validate these findings using a larger, multicentre design, enriched for a high-risk group, thus including patients with poor prognosis factors of SSc such as early dcSSc, ILD, male gender, Scl70 positive. These patients would mostly benefit from an ILR, enabling timely intervention with antiarrhythmic treatment, and or/ICD or permanent pacemaker and thus preventing arrhythmia related mortality in this group.

The CMR CONVAS study has provided a comprehensive assessment of CMR abnormalities detected in SSc patients free of CVD and identified potential surrogate clinical and cardiac serum markers for CMR defined SSc. The surrogate markers that were identified, Hs-TnI, NT-proBNP as well as mRSS and DU could thus be validated in a larger cohort study which can then be applied in clinical practice for early identification of patients at risk of CMR defined pSSc-HI. A larger CMR study would also provide more insights on pSSc-HI pathophysiology. Larger longitudinal studies would be also valuable for looking at the predictive value of CMR fibrosis in the form of LGE and ECV and cardiac biomarkers for the development of formal risk model and prediction of overt pSSc-HI. This would also allow early identification of those with higher risk of CV outcomes and close monitoring and early intervention.

Little is known whether CMR changes over time. The results of our CONVAS CMR longitudinal study showed that CMR does not significantly change over time, albeit SSc patients with poor prognostic factors and DMARD treatment have a greater deterioration of the CMR parameters. Firstly, a larger cohort, with longer follow-up is needed to confirm these findings and to establish whether treatment including DMARD or vasodilator treatment could influence the trajectory and outcome. Depending on these results, future randomized clinical trial to test the above-mentioned treatments on myocardial fibrosis and myocardial perfusion would be of great benefit to improve long term outcome of SSc patients. Another important issue that is currently debated is the use of anti-fibrotic treatment in SSc (312). Such treatments, including tyrosine-kinase inhibitor molecule Nintedanib could perhaps be also of use for myocardial fibrosis. In fact, pre-clinical studies have shown it's efficacy in myocardial fibrosis (311). Moreover, myocarditis per se is associated with a poor prognosis, with many patients not responding to conventional steroid or even DMARD treatment in SSc. Nonetheless, data on the use of DMARD treatment, including biological DMARDs in SSc myocarditis is scarce, with only case reports described in the literature. Randomized, multicentric studies, to test the efficacy of DMARD as well as bDMARD such as B-cell depleting therapy Rituximab or anti IL-6 agent Tocilizumab for treating SSc-myocarditis would be of great benefit for these patients and will reduce pSSc-HI associated mortality.

Lastly, a larger cohort to validate the findings of the T1 mapping peripheral muscle and cardiac MRI association study is needed. This would provide a greater understanding of SSc-myopathy and the significance of low level CK as well as a better understanding of the relationship between cardiac and muscle pathology in SSc. Since we have implemented T1 mapping in the peripheral muscle, other departmental studies have started to use this method in various other rheumatological conditions. T1 mapping is thus a promising tool for investigating muscle involvement in SSc and other muscular conditions.

A potential algorithm for use in future clinical trials and practice that results from the current work is illustrated below (Figure 8.1).

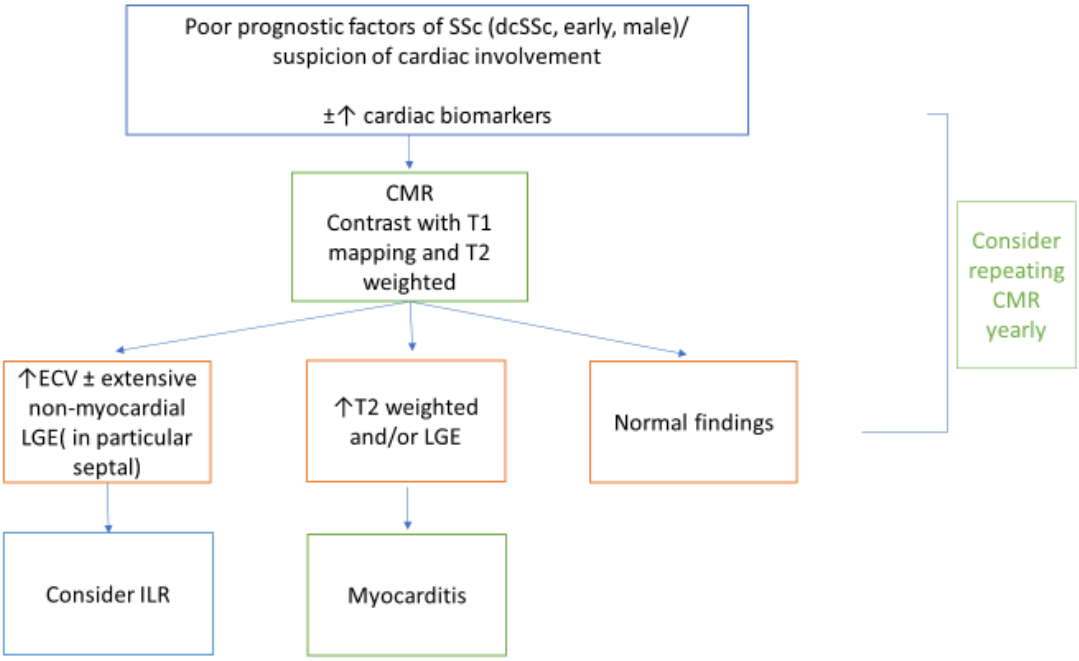


Figure 8-1. Algorithm for identifying pSSc HI by using CMR, cardiac biomarkers and ILR. CMR should be considered in SSc patients with poor prognosis or in SSc patients where there is a suspicion of cardiac involvement (abnormalities on echocardiography/ECG, in the absence of other CVD). The presence of increased cardiac biomarkers should also prompt performing a CMR. The CMR should include sequences for fibrosis (LGE), difusse fibrosis (T1 mapping) and T2 weighted (inflammation). In SSc patients with an increase in ECV and/or extensive LGE, ILR should be considered. Increase in T2 weighted (2:1; myocardial: peripheral muscle) denotes myocarditis. Close monitoring,

including yearly CMR is advisable for SSc patients with normal CMR, but in the presence of clincal red flags and increased cardiac biomarkers.

CMR, cardiovascular magnetic resonance; dcSSc, diffuse cutaneous systemic sclerosis; ECV extracellular volume; ILR, implantable loop recorder; LGE, late gadolinium enhancement.

8.8 Conclusion

The thesis has given a comprehensive overview on pSSc-HI using sensitive investigative tools- ILR and CMR in a well-defined cohort of unaffected SSc patients (with no CVD, IHD, PAH, allowing no more than 1/2 cv risk factors) and provided a first step of risk stratification for pSSc-HI. The thesis has demonstrated that ILR serious arrhythmias as well as CMR findings and progression of CMR findings are associated with poor prognosis factors of SSc including worse mrSS, ILD, Scl-70 positivity, as well as early disease and male gender for those with ILR serious arrhythmias.

ILR-arrhythmias associated with cardiac biomarkers and CMR-ECV. These findings were also confirmed in the longitudinal dataset (Chapter 5), looking at CMR and the development of future pSSc-HI CV outcomes, including arrhythmias, systolic/diastolic impairment and myocarditis. NT-proBNP was the only variable that predicted the development of CV outcomes, albeit higher CMR-ECV also showed a trend towards an association. These markers could be of use for risk stratification of clinically overt pSSc-HI.

In addition, new surrogate markers for CMR defined pSSc-HI have been suggested in the form of cardiac serum biomarkers (hs-TnI and NT-proBNP) and clinical markers mRSS and DU that could be of further use in both clinical and research setting. No significant change in CMR parameters was noted over time and only one patient developed clinically overt CMR abnormality, thus sequence CMR in a low risk group should be reconsidered.

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