Cardiovascular abnormalities in immune-mediated inflammatory disease

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Intellectual 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 3 is based on work from a jointly authored publication by LA Bissell, Y Yusof and MH Buch. LA Bissell carried out the literature search, evaluated the results and wrote the manuscript, with supervision and input from MH Buch. Y Yusof evaluated the echocardiography literature and passed on the results to LA Bissell to incorporate into the manuscript.

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LA Bissell, Y Yusof, MH Buch. Primary myocardial disease in scleroderma – a comprehensive review of the literature to inform the UKSSSG (UK Systemic Sclerosis Study Group) cardiac working group. Rheumatology. In press.

LA Bissell, EMA Hensor, L Kozera et al. Improvement in insulin resistance is greater when infliximab is added to methotrexate during intensive treatment of early rheumatoid arthritis - results from the IDEA study. Rheumatology. 10.1093/rheumatology/kew306.

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My contributions to the work in this thesis

Chapter 2: I am solely responsible for this work.

Chapter 3: I was part of the team to design the research questions. I defined the search terms (confirmed by a University of Leeds librarian) and carried out the search. I summarised the raw data (barring the echocardiography data as described above) and wrote the chapter.

Chapter 4: The IDEA study had already been designed, recruited and completed, and the primary paper was submitted for publication, by the time I became involved in this study. My role was in preparation of the cardiovascular data (clinical and biological), performing the statistical analysis and writing the resulting publication. Dr Elizabeth Hensor, our departmental statistician, performed the multiple imputation during this process, and consequently repeated my analyses and provided me with the results to interpret.

Chapter 5: I was involved in the study design, along with my supervisors, and was solely responsible for recruiting the patents. I consented the participants and collected the clinical data (including pulse wave velocity by applanation tonometry) for the majority of the patients and controls (with help from the research nurses if I was unavailable). Although I learnt the basics of cardiac and carotid MRI interpretation, I depended on cardiology fellows as above for the measurement of the CMR outcomes. I performed the statistical analysis, which was checked by Dr Elizabeth Hensor for accuracy, before writing this chapter.

Chapter 6: With help from Prof Maya Buch and Dr Jacqueline Andrews, I wrote the CONVAS study protocol and assisted in its submission to the ethics committee. As per chapter 5, I was responsible for recruiting and consenting the patients with SSc, collecting the clinical data, and referring them for CMR. I performed the statistical analysis before writing this chapter.

Chapter 7: I was involved in writing the ELCASA study protocol and its submission to the ethics committee. I designed the study data collection forms (CRFs) and was responsible for recruiting and consenting the patients. I collected the baseline data, and year one data up until the time I took maternity leave. I prepared the collected data and performed the statistical analysis, before writing this chapter.

Chapter 8: I am solely responsible for this work.

List of publications and presentations arising from this thesis

Original articles

LA Bissell, Y Yusof, MH Buch. Primary myocardial disease in scleroderma – a comprehensive review of the literature to inform the UKSSSG (UK Systemic Sclerosis Study Group) cardiac working group. Rheumatology (Oxford) 2016 Dec 10. pii: kew364. [Epub ahead of print].

LA Bissell, M Anderson, M Burgess, et al. Consensus best practice pathway of the UK Systemic Sclerosis Study group: management of cardiac disease in systemic sclerosis. Rheumatology. In press.

LA Bissell, EMA Hensor, L Kozera et al. Improvement in insulin resistance is greater when infliximab is added to methotrexate during intensive treatment of early rheumatoid arthritis - results from the IDEA study. Rheumatology (Oxford) 2016 Dec;55(12):2181-2190.

Oral presentations (first author)

LA Bissell, EMA Hensor, SL Mackie et al. Improvement In Insulin Resistance Greater With The Use Of Infliximab Following Intensive Treatment Of Early Rheumatoid Arthritis. *Presented at American College of Rheumatology annual meeting, San Diego, USA in 2013.*

LA Bissell, R Dumitru, B. Erhayiem et al. First pilot study of an implantable loop recorder (ILR) in Systemic Sclerosis detects significant cardiac arrhythmias with CMR abnormalities. *Presented at European League Against Rheumatism annual meeting, London in 2016.*

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LA Bissell, SL Mackie, L Kozera et al. Improvement in some, but not all, surrogate measures of cardiovascular disease following intensive treatment of early rheumatoid arthritis. *Presented at European League Against Rheumatism annual meeting, Rome, Italy in 2012.*

LA Bissell, B Erhayiem, JP Greenwood et al. Characterisation of sub-clinical primary myocardial disease in systemic sclerosis - preliminary findings from a

cardiac magnetic resonance and electrophysiological study. *Presented at Scleroderma World Congress, Rome, Italy in 2014.*

LA Bissell, B Erhayiem, L Hensor et al. High prevalence of subclinical cardiovascular disease and abnormal left ventricular geometry detected by CMR in asymptomatic rheumatoid arthritis patents. *Presented at European League Against Rheumatism annual meeting, Paris, France in 2014.*

LA Bissell, B Erhayiem, L Hensor et al. Cardiovascular MR (CMR) evidence for reduced LV mass in Rheumatoid Arthritis (RA), suggesting pathology other than atherosclerosis for heart failure. *Presented at European League Against Rheumatism annual meeting, London in 2016*

LA Bissell, B Erhayiem, G Fent et al. Identification of at risk patients for cardiac MRI determined sub-clinical SSc-cardiomyopathy (CM). *Presented at European League Against Rheumatism annual meeting, London in 2016.*

LA Bissell, R Dumitru, G Abignano et al. First pilot study of an implantable loop recorder (ILR) in Systemic Sclerosis detects significant cardiac arrhythmias with CMR abnormalities. *To be presented at American College of Rheumatology annual meeting, Washington, USA in 2016.*

Abstract

Immune-mediated inflammatory disease (IMID) represents a group of diseases characterised by dysregulation of immune processes with a shared common inflammatory pathway, leading to end-organ damage, of which Rheumatoid Arthritis (RA) and Systemic Sclerosis (SSc) are two important examples. An accelerated risk of cardiovascular disease (CVD) with associated increased mortality is observed in those with IMID; the risk in RA being similar to those with diabetes mellitus. In addition, IMID can directly affect the myocardium independently of atherosclerosis, termed primary myocardial disease, causing further excess mortality; best described in SSc.

Determining the disease phenotype most at risk of either macrovascular or primary myocardial disease and having a greater understanding of the underlying pathophysiology is vital to develop effective screening strategies to prevent and manage its complications. Surrogate markers of CVD, including soluble cardiovascular (CV) biomarkers and cardiovascular magnetic resonance (CMR) imaging, can inform of subclinical CVD or risk of progression to clinical CVD; with currently limited data in IMID.

Using soluble CV biomarkers and CMR, this thesis demonstrates the presence of subclinical CVD in patients with RA and SSc free of clinical CVD. In RA, these abnormalities associate with traditional CV risk factors; emphasising the importance of their aggressive management. Using CMR, this thesis describes a reduction in left ventricular mass in RA; suggesting pathology other than atherosclerosis. This work investigates specific treatment strategies in the reduction of CV risk, reporting improvement in insulin resistance with TNF inhibition in a randomised controlled trial of early RA. In SSc, the utility of CMR in the assessment of primary myocardial disease is demonstrated, describing cardiac fibrosis in those free of known cardiac disease, associating with a poor prognostic phenotype. Finally, this thesis reports the novel use of an implantable loop recorder in SSc, detecting arrhythmias in patients free of known cardiac disease.

Lay summary

Rheumatoid arthritis (RA) and Systemic Sclerosis (SSc), types of immune-mediated inflammatory disease (IMID), are associated with significant cardiovascular-related illness and death; in RA the risk of cardiovascular disease (CVD) is as high as those with diabetes. CVD in IMID can either occur as a result of atherosclerotic heart disease (or 'hardening of the arteries') leading to angina and heart attacks, or can occur by the IMID process directly affecting the cardiac (heart) tissue; in SSc this can lead to inflammation and scarring in the heart, causing various problems including heart failure, abnormal cardiac rhythms (arrhythmias) and in some cases sudden death.

To effectively screen for such CVD and prevent its complications, more studies are required to be able to identify the patient most at risk and to better understand the underlying cause. At present, there are limited data; however with the use of surrogate measures of CVD, such as soluble biomarkers (bloods tests) and cardiovascular magnetic resonance (CMR) imaging, it is possible to improve understanding.

This thesis uses such surrogate measures and detects subclinical CVD in patients with RA and SSc, with no known history of CVD. It determines that the CV abnormalities are associated with the well-known 'traditional' CV risk factors, such as high blood pressure, reaffirming their need to be effectively managed in those with RA, and also describes a reduction in heart size in those with RA suggesting disease other than atherosclerosis occurring in the heart. In SSc, it demonstrates the utility of CMR in the detection of subclinical cardiac fibrosis, associating with poor prognostic SSc disease features, and describes the novel use of an implantable loop recorder (a device inserted under the skin of the chest wall to monitor the heart rhythm continuously), detecting serious arrhythmias in patients without known heart disease.

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List of abbreviations

ACA	anti-centromere antibody
ACPA	anti-cyclic citrullinated peptide antibody
ACR	American College of Rheumatology
AF	atrial fibrillation
AHA	American Heart Association
AIx	augmentation index
Apo	apolipoprotein
AV	atrioventricular
BP	blood pressure
Bpm	beats per minute
BMI	body mass index
BL	baseline
BSA	body surface area
CABG	coronary artery bypass graft
CCF	congestive cardiac failure
CDAI	clinical disease activity Index
CHB	complete heart block
CI	confidence interval
CIMT	carotid intimal-media thickness
CONVAS	CONnective tissue disease and VAScultis
CRP	C-reactive protein
CMR	cardiovascular magnetic resonance
CSS	cross-sectional study
CT	computerised tomography
CTD	connective tissue disease

CV	cardiovascular
CVD	cardiovascular disease
CXR	chest X-ray
DAS	disease activity score
dcSSc	diffuse cutaneous Systemic Sclerosis
DLCO	diffusing capacity of lungs for carbon monoxide
DLCO/VA	DLCO adjusted for volume
DM	diabetes mellitus
DMARDs	disease-modifying anti-rheumatic drug
DU	digital ulceration
ECG	electrocardiogram
ECV	extra-cellular volume
EDV	end-diastolic volume
ELCASA	ELectrophysiology and CArdiac imaging in SclerdermA
EP	electrophysiology
ESV	end-systolic volume
ESR	erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
FMD	flow-mediated dilatation
FVC	forced vital capacity
HAQ-DI	Health Assessment Questionnaire – Disability index
HCQ	hydroxychloroquine
HDL-C	high density lipoprotein-cholesterol
HOMA-IR	Homeostasis Model Assessment-estimated Insulin Resistance
HR	hazard Ratio
IACON	Inflammatory Arthritis Continnum

ICAM-1	intercellular adhesion molecule-1
ICD	implantable cardiac defibrillator
IDEA	Infliximab as Induction therapy for Early rheumatoid Arthritis
IFX	infliximab
IHD	ischaemic heart disease
IMID	immune-mediated inflammatory disease
IL	interleukin
ILD	interstitial lung disease
ILR	implantable loop recorder
IQR	interquartile range
IR	insulin resistance
JBS	Joint British Societies
lcSSc	limited cutaneous Systemic Sclerosis
LBBB	left bundle branch block
LDL-C	low density lipoprotein–cholesterol
LGE	late gadolinium enhancement
Lp(a)	lipoprotein A
LV	left ventricular
LVEDD	LV end-diastolic diameter
LVEF	LV ejection fraction
LVH	LV hypertrophy
LVP	late ventricular potentials
MCP-1	monocyte chemotactic protein-1
MEP	methylprednisolone
MetS	metabolic syndrome
MI	myocardial infarction

MMF	mycophenolate
MMP	metalloproteinase
MPO	myeloperoxidase
MPR	myocardial perfusion reserve
mRSS	modified Rodnan skin score
MTX	methotrexate
MVT	multivariate analysis
MWT	mean wall thickness
NFC	nail fold capillaroscopy
NO	nitric oxide
NOAR	Norfolk Arthritis Register
Ns	non-significant
NSAID	non-steroidal anti-inflammatory drug
NT-proBNP	N-terminal pro-brain natriuretic peptide
NYHA	New York Heart Association
OA	osteoarthritis
OR	odds Ratio
PAH	pulmonary arterial hypertension
PET	positron emission tomography
pi-HDL-C	Pro-inflammatory HDL-C
PMH	past medical history
PPM	permanent pacemaker
PVD	peripheral vascular disease
PWV	pulse wave velocity
PS	prospective study
PVD	peripheral vascular disease

RA	rheumatoid arthritis
RBBB	right bundle branch block
RF	rheumatoid factor
RP	Raynaud's phenomenon
RR	relative risk
RTX	rituximab
RV	right ventricular
RVEF	right ventricular ejection fraction
SAE	signal average ECG
sCD40L	soluble CD40 Ligand
Scl70	anti-topoisomerase antibody
SD	standard deviation
SHAQ-DI	Scleroderma Health Assessment Questionnaire – Disability index
SJC	swollen Joint Count
SSc	systemic sclerosis
SSc-CM	SSc-cardiomyopathy
SMR	standardised mortality ratio
SSZ	sulphasalazine
SV	stroke volume
SVE	supraventricular ectopic
SVT	supraventricular tachycardia
TC	total cholesterol
TFR	tendon friction rub
TG	triglycerides
TIA	transient ischaemic attack
TNFi	tumour necrosis factor-α inhibitor

UKSSSG	UK Systemic Sclerosis Study Group
US	ultrasound
UVA	univariate analysis
VAS	visual assessment score
VCAM-1	vascular cell adhesion molecule-1
VE	ventricular ectopic
VEGF	vascular endothelial growth factor
VF	ventricular fibrillation
VT	ventricular tachycardia
vWF	Von Willebrand factor
WHR	waist/hip ratio

Chapter 1 Introduction

1.1 Background

Cardiovascular disease (CVD) is the second largest cause of mortality in the UK. The British Heart Foundation reported 27% of all deaths in 2014 were secondary to CVD, with a cost to NHS England of £4.3 billion, not including production losses from those of working age [1]. Smoking, hypertension, dyslipidaemia, Diabetes Mellitus (DM) and a family history of premature CVD are well recognised risk factors for the development of CVD [2], and are often termed 'traditional CV risk factors'.

An accelerated risk of CVD is observed in those with immune-mediated inflammatory diseases (IMID). In RA, the risk of myocardial infarction (MI) is up to 1.6 times that of the general population [3], similar to the excess risk of those with DM [4], with an associated increased CVD-mortality [5]. Although RA disease activity and systemic inflammation are important contributors to this increased risk, traditional CV risk factors remain important in the development of CVD in RA [6].

Cardiac disease as a direct result of the IMID process, independent of atherosclerosis, termed primary myocardial disease, can be an additional insult, and is seen frequently in Systemic Sclerosis (SSc) [7, 8]. It has many presentations, for example, heart failure, pericardial effusions, arrhythmias and even sudden death [9, 10] and carries a poor prognosis [11].

Determining the disease phenotype most at risk of either macrovascular or primary myocardial disease is vital to allow more effective/intensive monitoring of cardiac health in IMID, in addition to learning more about the pathophysiology of the disease to more effectively prevent and manage its complications.

Given the absolute numbers of CV events in RA is modest, surrogate markers of CVD are often utilised to inform of the presence of subclinical disease or risk of progression to clinical disease. Commonly employed tools in the general population include soluble biomarkers reflecting endothelial dysfunction or dyslipidaemia [12], functional measures including that of arterial stiffness using applanation tonometry [13], and structural assessments such as echocardiography [14]. Cardiovascular magnetic resonance (CMR)

imaging is increasingly being utilised and is advantageous given that it provides a global anatomical and functional assessment of the heart with pathophysiological insight, including dimension, function, inflammation, fibrosis and arterial stiffness [15]. CMR has already demonstrated superiority over other imaging modalities in the detection and prognosis of ischaemic heart disease [16, 17].

Increasingly these surrogate measures of CVD are being applied to those with IMID, but data are limited, particularly with regard to who is most at risk, and how best to treat the at-risk patient. Until more is known regarding risk stratification, the European League Against Rheumatism (EULAR) recommend the multiplication of CV risk scores by 1.5 if two or more specific criteria are present, along with the suppression of disease activity for those with RA [18]; there is no specific guidance for patients with SSc. There remains an unmet need for further study in these areas.

1.2 Structure of the thesis

The hypotheses underlying this thesis are that subclinical CVD in IMID, namely RA and SSc, is prevalent, associated with a specific disease phenotype, and improved with optimal suppression of disease activity. A combination of surrogate measures for CVD and novel technology, can identify the at-risk patient, and can inform the underlying pathophysiology.

1.2.1 Thesis aims

The specific aims of this thesis are outlined below.

1.2.1.1 In Rheumatoid Arthritis

Prevalence of CVD in RA

- To describe the prevalence of CV biomarker abnormalities in early and established RA populations with no prior history of CVD, compared to healthy controls
- To describe the association of CV biomarker abnormalities with disease phenotype and disease activity in early and established RA populations with no prior history of CVD

Effect of RA disease control on CVD

- To determine whether effective suppression of disease activity in RA improves CV biomarkers

- To determine whether specific therapeutic agents have superior influence on CV biomarkers over others

Detailed disease phenotyping and CVD evaluation/risk in RA

- To demonstrate the global utility of CMR in the detection of subclinical CVD in RA
- To demonstrate the disease burden on CMR is greater in those with established RA than healthy controls
- To describe the phenotype of RA with the greatest burden of disease on CMR
- To demonstrate that MWT is greater in those with established RA than healthy controls, and determine the phenotype of RA associated with greatest MWT (and hence CV risk)

1.2.1.2 In Systemic Sclerosis

Prevalence of CVD in SSc

- To describe CV biomarker abnormalities in a SSc population with no prior history of CVD, and their association with disease phenotype and serology

Detailed disease phenotyping and CVD evaluation/risk in SSc

- To determine the utility of CMR in the evaluation of subclinical primary myocardial involvement in SSc; and detection of myocardial perfusion abnormalities and fibrosis.
- To determine if there is a relationship between myocardial perfusion abnormalities to areas of myocardial fibrosis detected on CMR.
- To evaluate the utility of an implantable loop recorder (REVEAL device) in the detection of arrhythmias in SSc
- To describe the prevalence of arrhythmias in SSc, and their relationship to disease phenotype

1.2.2 Thesis outline

The structure of the thesis is explained below.

Chapter Two: Literature review

A review of the literature of CVD in IMID was performed, with a focus on RA and SSc. It addresses the pathophysiology underlying atherosclerosis, the morbidity/ mortality associated with, and the various techniques used to assess, CVD in these populations.

Chapter Three: Primary myocardial disease in scleroderma – a comprehensive review of the literature.

A comprehensive literature review was performed to address the prevalence, nature and associated mortality of SSc-CM in SSc, and whether it is associated with a specific disease phenotype.

Chapter Four: Improvement in insulin resistance is greater when infliximab is added to methotrexate during intensive treatment of early RA - results from the IDEA study.

This chapter analysed stored soluble CV biomarkers from an already published randomised controlled trial treating patients with early RA with either methotrexate (MTX) and a tumour necrosis factor inhibitor, or MTX with intravenous methylprednisolone at induction. The difference between the treatment groups in change of soluble CV biomarkers was evaluated.

Chapter Five: Cardiovascular biomarkers and the use of cardiac and carotid MRI in established RA.

This chapter aimed to comprehensively evaluate patients with RA for subclinical CVD compared to healthy controls using a combination of clinical assessment, soluble CV biomarkers, measures of applanation tonometry and cardiac/carotid MR imaging.

Chapter Six: The use of CMR in SSc.

In this chapter, a comprehensive cardiac evaluation of patients with SSc free of known cardiac disease was performed, measuring lipid profile, and utilising applanation tonometry and late gadolinium enhancement-CMR with stress perfusion, with comparison to healthy controls.

Chapter Seven: Prospective study evaluating conduction abnormalities with correlation to cardiovascular magnetic resonance imaging in patients with scleroderma - the ELCASA study.

This chapter described the first use of the implantable loop recorder (ILR) as a research tool, in parallel to CMR, in the investigation of SSc-CM and associated arrhythmias.

Chapter Eight: Discussion

This last chapter brings together the body of work within this thesis and discusses its contributions to the published literature and future research directions.

Chapter 2 Cardiovascular disease in immune-mediated inflammatory disease

This review describes the pathogenesis of atherosclerosis, and how inflammation is inextricably linked. The impact of CVD on morbidity and mortality in patients with RA and SSc will be reported. The review will discuss the various tools at the disposal of researchers in determining the presence of CVD, and to what extent they have been applied in both the RA and SSc populations. The prevalence of the traditional CV risk factors and additional disease specific risk factors seen in RA will be discussed, along with the effect of disease suppression on CV mortality and markers of subclinical CVD.

2.1 Atherosclerosis

Cardiovascular (CV) events (myocardial infarction, angina, stroke, limb ischaemia) can occur due to a compromise in blood flow, which is most commonly the result of atherosclerosis. Atherosclerosis, or the atherosclerotic plaque, leads to a gradual narrowing of the vessel lumen, and can cause an abrupt occlusion of the vessel lumen due to rupture of the plaque. Historically, a build-up of cholesterol deposits within the vessel wall was thought to be the cause, but greater understanding suggests it is a much more complex process.

2.1.1 Pathogenesis of atherosclerosis

There are several stages in the pathophysiology of atherosclerosis, involving complex immune-inflammatory pathways, with key roles of both the innate and adaptive immune systems and their interaction with the vascular environment (see Figure 2-1) [19].

Stage 1: Endothelial activation

Smoking, hypertension, low-density lipoprotein cholesterol (LDL-C) modified by oxidation and diabetes mellitus, and other processes, can activate the endothelial (inner) lining of the vessel wall, usually occurring at bifurcation points of the vascular tree where flow turbulence is greatest. Activation of the endothelium leads to the increased expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), and the release of pro-inflammatory cytokines, increased permeability of the endothelium, and induction of clotting factors [20, 21].

Stage 2: Fatty streak formation

Macrophages and T-lymphocytes are attracted to the activated endothelium and migrate into the intima along with oxidised LDL-C. Monocyte chemotactic protein-1 (MCP-1) has been implicated as the chemoattractant for the monocyte migration [20]. There is now also data to support macrophage subtypes that vary in their pro-atherogenic capabilities [19]. The macrophages ingest oxidised LDL-C, accumulating cholesterol esters to become foam cells and also secrete pro-inflammatory cytokines that amplifies the inflammatory environment further. Many T-cell subsets are implicated; the pro-atherogenic effects of Th1 cells are clearer than the complex effects of Th2 cells, with recent evidence for regulatory T cell-driven anti-atherogenic effects and Th17-driven pro-atherogenic effects [19]. The interactions between foam cells, T-cells, and even B-cells subsets, cause the release of various cytokines including tumour necrosis factor (TNF-α) and IL-6 which encourage the migration of smooth muscle cells from the media into the intima with proliferation and thickening of the vessel wall leading to plaque formation [22]. Platelets aggregate on the sticky endothelial surface releasing cytokines and growth factors which can augment the migration of proliferation of the smooth muscle cells [21]. This process creates the 'fatty streak' within the intima. At this stage the vessel lumen patency is becoming compromised.

Stage 3: Fibrous cap formation

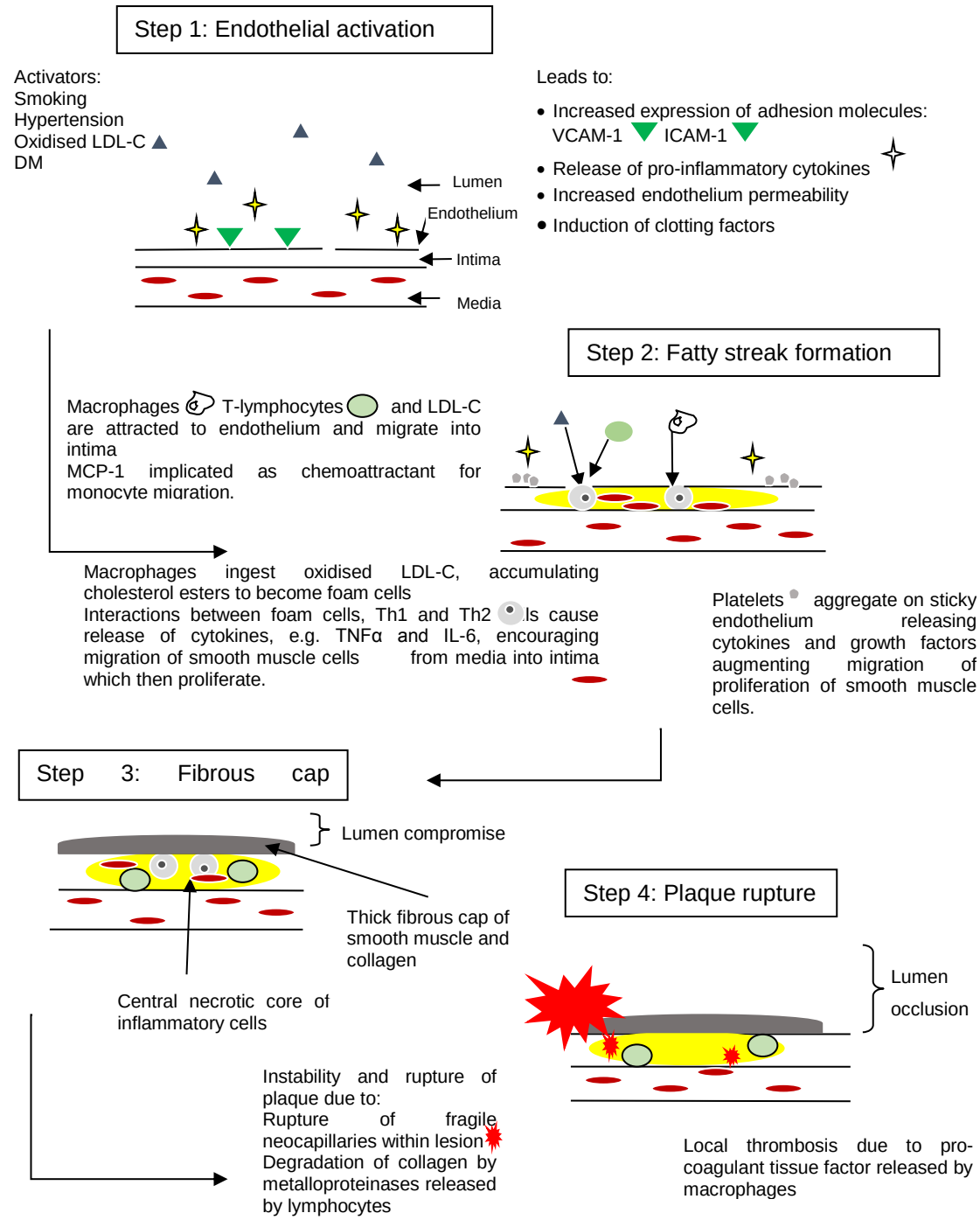
A thick fibrous cap of smooth muscle and collagen forms over the atherosclerotic lesion, which by now contains a central necrotic core of inflammatory cells [20]. Clinically, if a coronary vessel was involved, the patient may now present with chest pain.

Stage 4: Plaque rupture

Although many plaques can remain stable over years, fragile neocapillaries can rupture, and the release of metalloproteinases by lymphocytes can degrade the collagen, causing instability of the plaque leading to rupture, compromising the vessel lumen acutely [20, 21]. The release of pro-coagulant tissue factor by macrophages can also lead to local thrombosis [22]. If a coronary artery was involved, the patient could present with a myocardial infarction (MI) at this stage. Recent advances have also developed the notion of the 'vulnerable plaque' or 'thin-cap fibroatheroma' at higher risk of rupture [23]; featuring a thin fibrous cap, few smooth muscle cells, large lipid rich necrotic core, neovascularization and intra-plaque haemorrhage [24].

The atherosclerotic plaque is not a uniform lesion. Histological studies have shown heterogeneity in the composition of the plaque. As a result, in 1995, the American Heart Association (AHA) classified atherosclerotic plaques from type I (isolated macrophage foam cells) to type VIII (fibrous tissue changes), where type IV to VIII (demonstrating lipid cores, haemorrhage, thrombosis or calcification) can cause luminal compromise and clinical morbidity [25, 26]. Although these are based on histological findings, magnetic resonance imaging (MRI) has also been able to differentiate between the types of plaque; modifying the AHA's criteria slightly [27], demonstrating an increased risk of CV events with certain 'high-risk' plaque compositions [28].

Figure 2-1 Pathogenesis of atherosclerosis



ICAM-1: Intercellular adhesion molecule-1, LDL-C: Low-density lipoprotein cholesterol, MCP-1: Monocyte chemotactic protein-1, VCAM-1: Vascular cell adhesion molecule-1

2.1.2 Evidence for the role of inflammation in the atherosclerotic process

As described above, inflammatory mediators play a key role in the atherosclerotic process, and circulating levels of these mediators have been associated with the development of CVD. The most commonly studied marker is C-reactive protein (CRP), which has long been implicated in the pathogenesis of atherosclerosis. Histological studies have demonstrated the presence of CRP in atherosclerotic lesions, and its ability to induce adhesion molecule expression on endothelial cells and reduce endothelial nitric oxide synthase (a vasodilator). C-reactive protein has also been shown to augment monocytes migration into the atherosclerotic lesion, and mediate monocyte's uptake of LDL-C [29-31]. Many clinical studies have determined an association of CRP with CVD, and its ability to predict future CV events [32-39]. One notable study was the Physicians Health Study, which determined that healthy men with CRP in the highest versus lowest quartile had nearly triple the risk of MI [40]. More recent studies have suggested less inflated odd ratios; Danesh et al compared baseline CRP levels in 2459 patients with non-fatal MI or who had died from CVD to 3969 controls, and found the OR for ischaemic heart disease (IHD) was 1.45 (95% CI 1.25, 1.68) in those with a CRP in the top third compared to the lowest third, after adjustment for traditional CV risk factors; in line with their meta-analysis of 7068 patients with IHD [41].

The studies on the effects of statins have also implied that the benefit observed is not only attributable to lipid-lowering but also of lowering CRP levels. The Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) trial determined intensive statin therapy (target LDL-C<70mg/dl) was associated with better CV outcomes than standard statin management [42]; further analysis demonstrated, no matter what the LDL-C level, the improvement was better in those with a low CRP, and greatest in those who met both the LDL-C target and had a low CRP (<2mg/l) [43] suggesting statins have a dual effect. This observation has been replicated since, for example, in the Justification for Use of Statins in Prevention (JUPITER study) [44]. The JUPITER study also went on to show that the use of statins reduced vascular events even in those with an intermediate 10-year CV risk score (5-10%, and 10-20%) in those with an elevated CRP [45].

However, uncertainty remains whether the addition of CRP to standard CV risk models adds any further prognostic information due to its lack of independence from traditional CV risk factors [46]. There is also a school of thought that CRP is not causal in atherosclerosis; supported in genetic mouse model studies; but the result of associated obesity, or even

leak from atherosclerotic plaques into the vasculature [47]. In addition, a Mendelian randomisation study indicated inconsistent improvement of adding CRP to risk scores [48].

Other inflammatory mediators of interest include interleukin -6 (IL-6) and TNF-α. When long-term averaged IL-6 levels were examined in 2,138 patients who developed CVD compared to levels in 4,267 controls, Danesh et al determined an odds ratio (OR) for CVD of 2.14 (95% confidence interval (CI) 1.45, 3.15) per two standard deviation (SD) increase in IL-6. Their associated systematic literature review reported an OR of 3.34 (95% CI 2.45, 4.56) per two SD increase in IL-6 [49]. In support of this, the Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium demonstrated an IL-6 SNP interrupted IL-6 signalling consistent with IL-6 receptor blockade and was also associated with reduced IHD, suggesting a causal role in development of coronary heart disease [50]. Danesh et al also determined an increase in risk of non-fatal MI and CV mortality with increased levels of IL-8, matrix-metalloproteinase 9 (MMP-9), soluble CD40 ligand (sCD40L) and TNF-α [51].

The use of non-invasive imaging has helped support the notion of atherosclerosis as a chronic inflammatory disease. Positron emission tomography (PET) imaging using 18F-fluorodeoxyglucose (FDG), a radiolabelled glucose analogue, has illustrated inflammatory lesions within arterial wall plaques, correlating with CV risk factors, and inflammatory biomarkers such as CRP [24].

2.2 Immune-mediated inflammatory disease

Immune-mediated inflammatory disease (IMID) describes a group of diseases characterised by immune-dysregulation, with a common inflammatory pathway, leading to end-organ damage [52]. IMID includes a range of disease from inflammatory bowel disease (ulcerative colitis and Crohn's disease) to multiple sclerosis. This thesis focuses on two IMIDs; Rheumatoid Arthritis (RA) and Systemic Sclerosis (SSc) for the following reasons. RA is a relatively common IMID, affecting 0.5-1% of the population [53], causing significant morbidity and mortality including CVD, with additional significant individual and societal economic costs (work productivity losses and healthcare costs) [54]. Greater understanding in the development and management of CVD in RA would have great impact both in those with RA, and advances could potentially be applied and investigated in those with less prevalent IMID. Systemic Sclerosis (SSc), a rare disease, is of special pathophysiological interest due to co-existing vasculopathy and fibrosis in addition to

inflammation [55]; this process is recognised to directly affect the cardiac tissues; termed primary myocardial involvement or SSc- cardiomyopathy (SSc-CM). SSc-CM is inadequately understood and there is a great need to evaluate this further.

2.3 Cardiovascular disease in Rheumatoid Arthritis

2.3.1 Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is characterised by a symmetrical, sometimes erosive, inflammatory polyarthritis, associated with circulating rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA). Extra-articular manifestations can include rheumatoid nodules, interstitial lung disease, and peripheral ulcerative keratitis. Management is targeted at suppressing synovitis. Non-steroidal anti-inflammatories (NSAIDs) can be helpful, but immunosuppressive therapy is required with corticosteroids, synthetic disease modifying anti-rheumatic drugs (DMARDs) and/or biological DMARDs [56].

2.3.2 Cardiovascular mortality and morbidity

An accelerated risk of CVD exists in RA [57-60]. Del Rincon et al determined an increased risk of CVD in patients with RA not explained by traditional CV risk factors in 2001; incidence rate ratio after adjustment for CV risk factors was 3.17 (95% CI 1.33-6.36) [61]. This increased risk has been shown in many consequent studies; similar to the risk seen in that of DM [62]. Chung et al determined that patients with RA had a 38% greater chance of suffering a MI in a large retrospective study in Taiwan involving over 29,000 patients [58]. A meta-analysis reported an OR for patients with RA of 1.63 (95% confidence interval (CI) 1.34 to 2) for MI compared to the general population [3].

An increase in CV mortality is also observed. A large meta-analysis comprised of over 111,000 patients with RA reported a standardised mortality ratio (SMR) of 1.50 (95% confidence interval (CI) 1.39, 1.61), with SMR for IHD of 1.59 (95% CI 1.46, 1.73) and cerebrovascular disease (CVA) of 1.52 (95% CI 1.40, 1.67) (see figure 2-2) [5]. Another meta-analysis of over 120,000 patients provided a pooled estimate SMR of 1.77 (95% CI 1.65 to 1.89) for fatal MI, and 1.46 (95% CI 1.31 to 1.63) for fatal stroke [63].

Figure 2-2 Meta-analysis of studies on overall and cause-specific cardiovascular diseases in patients with rheumatoid arthritis.

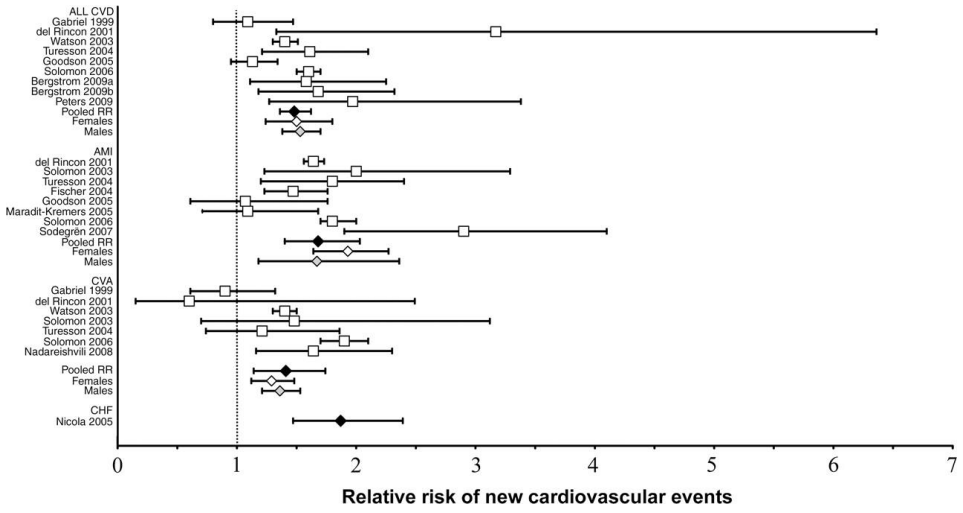


Image reproduced with permission from Avina-Zubieta JA, et al. Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies. Ann Rheum Dis 2012;71(9):1524-9.

The data for increased risk in early RA is conflicting. There is evidence for an increase in overt CVD in those with a recent diagnosis of RA [64]. The Rochester Epidemiology Project confirmed patients with recent onset RA had a 10-year CV risk profile similar to that of subjects five to ten years older without RA [65]. A Danish study followed early RA patients for an average of 4.6 years and determined the incidence rate ratio (IRR) of MI was 1.7 (95% CI 1.5 to 1.9), similar to the risk of diabetes mellitus (DM) (IRR 1.7 (1.6 to 1.8); p=0.64 for difference). In addition, those with less than three years of treatment had an increased risk of MI compared to healthy controls (OR of 1.7 (95% CI 1.3 to 2.2) [4].

Conversely, one meta-analysis concluded no increase in CV events and CV mortality in RA inception cohorts; although the authors suggest the numbers were too small in the analysis [66]. More recent prospective cohort studies have witnessed a decline in CV events in patients with RA with low disease activity [67], indicating perhaps earlier recognition of RA and the advent of early inflammatory arthritis clinics with more aggressive treatment, may be reducing the CV risk. Indeed, a large study in Finland of over 14,500 patients with recent onset RA (80% receiving RA medication) found patients had no increased risk for CV mortality compared to the general population in the early years of follow-up [68].

2.4 Cardiovascular (or surrogate cardiovascular) outcome measures in Rheumatoid Arthritis

As the absolute number of CV events in RA is modest, alternative methods of determining future CV risk have been developed and are often termed 'surrogate markers of CVD' or 'markers of subclinical CVD'. These investigative tools can either detect previous CV damage or highlight those at high risk of future CVD, and are often used as risk modifiers in the general population improving future CV risk prediction scores [69]. The commonly used CVD outcome measures in RA and the methods used to quantify them are described below.

2.4.1 Endothelial dysfunction

The endothelium plays a central role in regulating and producing mediators that affect vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation, and vessel wall inflammation [70]. With age, the endothelium's homeostatic capabilities suffer, however, atherosclerosis is thought to accelerate this decline, and efforts have been made to characterise the changes in greater detail [71].

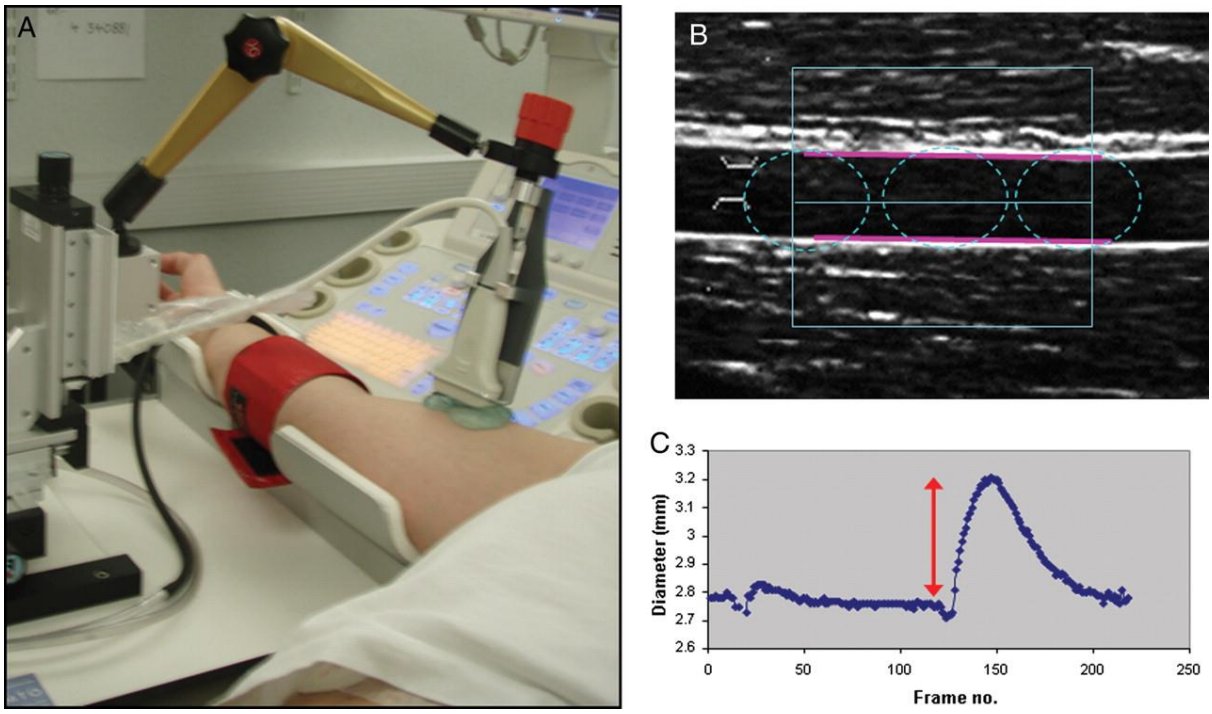
2.4.1.1 Soluble biomarkers

As described earlier, the pathogenesis of atherosclerosis is a complex inflammatory process [20]. Several mediators have pivotal roles to play and, consequently, they have been considered as potential biomarkers of subclinical CVD. Potential candidates include markers of endothelial activation such as monocyte chemotactic protein (MCP-1), and expression of adhesion molecules including soluble vascular cellular adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule (sICAM-1) and sE-selectin. These have been found to be increased in IHD and can predict CV events [12]. The limited evidence available suggests they are increased in patients with early and established RA [72-75]. Mediators implicated in the destabilisation of the atherosclerotic plaque, such as myeloperoxidase (MPO) and MMPs, and those released following plaque rupture, for example sCD40L, have been associated with CV events and mortality in the IHD population [12]. Again, limited data suggest an increase in these mediators in patients with RA, with some association with other markers of subclinical CVD [12].

2.4.1.2 Flow-mediated dilatation

Flow-mediated dilatation (FMD) is a non-invasive method of quantifying endothelial dysfunction in the peripheral vasculature, usually the brachial artery. In brief, the usual method is to place and inflate a cuff above the elbow for five minutes, and then release the cuff. The impact of the subsequent reactive hyperaemia and 'stress' to the brachial artery is measured using ultrasound (see figure 2-3). With an intact endothelium, the vessel dilates, mediated by nitric oxide (NO) [76]. Reduction in this dilatation is associated with aging and atherosclerotic vessels, thought secondary to a change in endothelial bioavailability of NO [76]. FMD is associated with traditional CV risk factors, and a meta-analysis has determined FMD can independently predict future CV events in the general population [77]. There is emerging evidence for a reduction in FMD in patients with RA [78, 79], including those with early disease [80].

Figure 2-3 Flow-mediated dilatation.



(A) Positioning of the sphygmomanometer cuff and use of the ultrasound probe holder. (B) Region of interest box for flow-mediated dilatation analysis. (C) Output generated to assess vasodilatory response. Reproduced with permission from Charakida M, et al. Assessment of atherosclerosis: the role of flow-mediated dilatation. Eur Heart J 2010;31(23):2854-61.

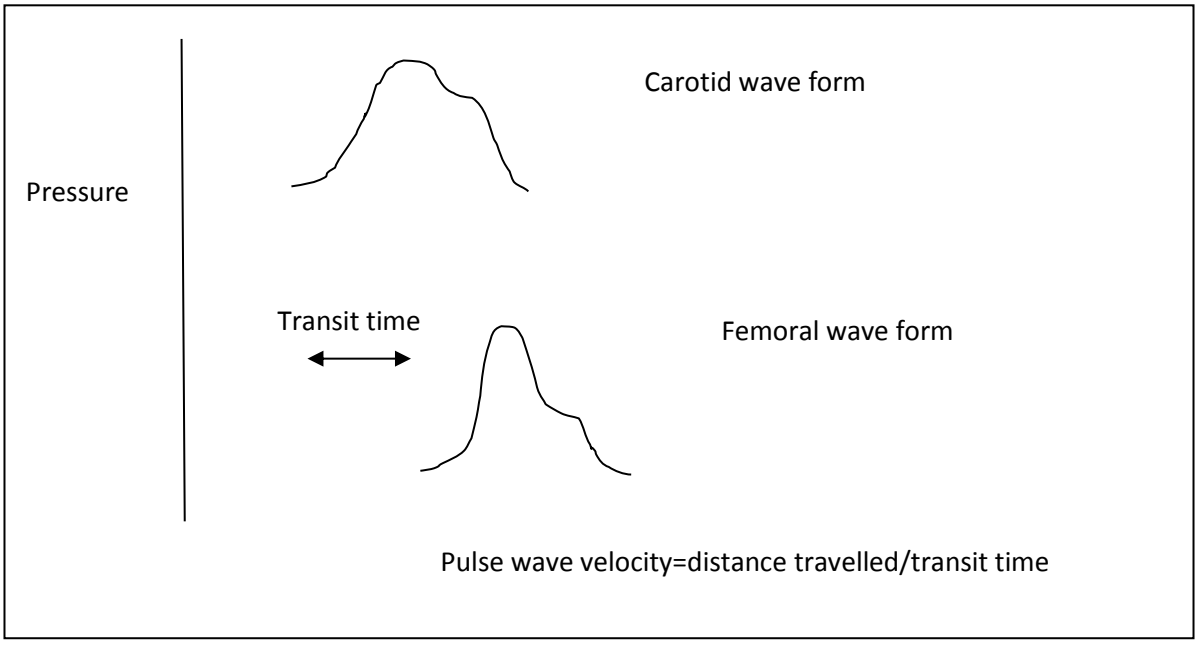
2.4.2 Arterial stiffness

As the atherosclerotic process advances, the usually compliant and elastic vasculature hardens and becomes stiffer [81]. As a result, the amplitude of the forward aortic pressure

wave reflected back by the vessel wall increases, and can augment the central systolic pressure, ultimately increasing LV afterload [82]. There are many ways to measure or estimate arterial stiffness, however, pulse wave velocity (PWV) and aortic distensibility are commonly utilised.

PWV describes the speed of blood flow, calculated by measuring the time taken for the systolic wave to reach a certain point in the vasculature, usually from LV ventricle to the femoral artery (transit time) and the distance travelled by the wave (see figure 2-4); higher values signify stiffer arteries. It is of greater value in older subjects but less sensitive to changes in heart rate and ventricular ejection than other methods, and importantly, can independently predict CVD [83]. The Rotterdam study produced a hazard ratio (HR) of 2.45 for IHD for those with a PWV in the third tertile after adjustment for age, gender, mean arterial pressure, and heart rate. The effect remained after adjustment for CV risk factors [13]. A meta-analysis determined a 1m/s increase in PWV led to an increased risk of 14%, 15%, and 15% for total CV events, CV mortality, and all-cause mortality respectively (adjusted for age, gender and risk factors) [84].

Figure 2-4 Measurement of pulse wave velocity



PWV is higher in patients with RA [85-94]. A recent meta-analysis of 25 studies (1,472 patients with RA, 1,583 controls) confirmed that patients with RA had a significantly higher aortic PWV (mean difference 1.32 m/s, 95% CI 0.77 to 1.88, $p<0.00001$) [95].

Stamatelopoulos et al also reported 84 patients with RA [mean (SD) disease duration 7.9 (6.8) years, 62% RF positive] with no history of Diabetes Mellitus (DM) or CVD compared to 84 age/sex matched controls and 48 patients with DM. PWV was higher in the RA patients (mean (SD) 10.02 (3.3) m/s vs. 8.74 (1.8) in controls, p=0.011) after adjustment for high-density lipoprotein- cholesterol (HDL-C), CRP and statin use. Importantly, there was no significant difference between patients with RA and DM [96]. A South Korean study demonstrated high PWV was associated with age over 65 years, systolic blood pressure greater than 140mmHg and steroid use [97]. There are limited data in early RA [86, 95, 98] and no longitudinal studies to date.

Aortic distensibility is a measure of how much an arterial vessel wall can expand; lower values signify stiffer arteries [82]. It can be measured using echocardiography, CT and more recently CMR; the latter with the advantage of mapping of the whole aorta with no radiation exposure [82]. Again aortic distensibility has shown utility in the prediction of CV events in the general population [99]. There are few studies assessing aortic distensibility in RA but it appears to be reduced when compared to controls [100, 101].

2.4.3 Vessel wall intimal thickness

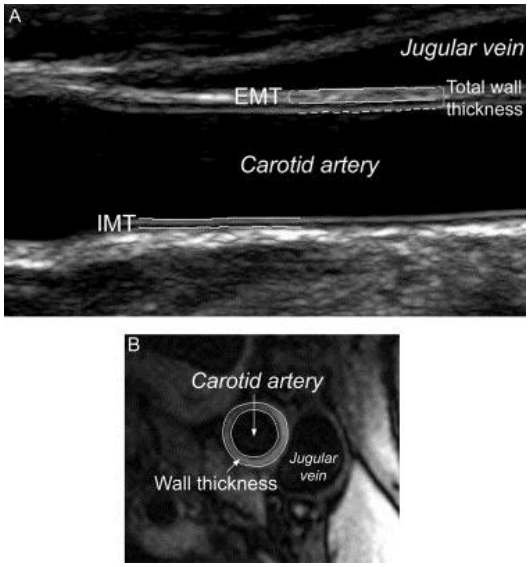
Common carotid intima-media thickness (CIMT) is a well-validated surrogate measure of CVD, and is used as an end-point in clinical trials for anti-hypertensive and lipid-lowering agents. CIMT has been shown to predict future CV events. A meta-analysis determined that in the general population the age and sex adjusted relative risk of MI was 1.15 (95% CI 1.12, 1.117) and stroke was 1.18 (95% CI 1.16, 1.21) per 0.1mm CIMT difference [102].

CIMT is higher in patients with RA. One meta-analysis determined a mean difference of 0.06mm (95% CI 0.05, 0.06mm) in those with rheumatic disease compared to controls [103]; another larger meta-analysis of 1384 patients with RA found the overall mean CIMT difference 0.09mm (95% CI 0.07, 0.11 mm) [104]. Furthermore, a small study of 47 RA patients followed over 5 years demonstrated higher CIMT was associated with CV events [105], and its use in combination with the calculation of CV risk scores in the assessment of the patient has shown some merit [106].

CIMT is usually measured by ultra-sound (US) and is operator dependent. Cross-comparisons of studies are difficult as varying scanning protocols are used. Mean wall thickness (MWT) as measured by cardiovascular magnetic resonance (CMR) imaging is an alternative method, correlating well with US-measured CIMT (see figure 2-5) [107-110].

CMR is advantageous as it allows the whole circumference of the artery to be measured at multiple levels. There is also evidence that MWT measured by CMR may correlate better with CV risk factor burden than US-measured CIMT, suggesting the adventitia plays an important role in CV risk [109]. MWT is yet to be measured in RA populations.

Figure 2-5 Carotid artery images using ultrasound.



(A) longitudinal section by B-mode ultrasound imaging, indicating the intimal-media thickness (IMT) (far-wall), extra-medial thickness (EMT) and total wall thickness; and (B) cross-section by magnetic resonance imaging, indicating the wall thickness Image reproduced with permission from Skilton MR, et al. Carotid intima-media and adventitial thickening: comparison of new and established ultrasound and magnetic resonance imaging techniques. Atherosclerosis 2011;215(2):405-10.

2.4.4 Coronary blood flow compromise

CT coronary angiography and invasive coronary angiography are methods of assessing epicardial coronary artery disease, visually estimating any luminal narrowing. They are accepted as standard investigative tools in the management of CVD in the general population [111], however, due to the ionising radiation involved and risks (albeit minimal) associated with the invasive procedure they are less commonly used research tools. Nevertheless, Karpouzas et al performed CT angiography on 150 patients with RA (free of CVD) and 150 matched controls and determined the burden of coronary plaque was greater in patients with RA (71% vs. 45% controls, $p<0.0001$), with greater multi-vessel disease after adjustment for CV risk factors [112].

2.4.5 Cardiac structural and functional impairment

2.4.5.1 N-terminal pro-brain natriuretic peptide

Released from heart muscle in response to ventricular stretch amongst other stimuli, N-terminal pro-brain natriuretic peptide (NT-proBNP) is an important predictor of LV function, CV events and mortality in those with and without CVD in the general population [113-115]. The neuro-hormone is raised in RA [116-118], and has been shown to predict future CV events in RA. In a study including patients with RA and patients with osteoarthritis, Ruff et al determined a HR for CV events of 3.53 (95% CI 1.89, 6.58) for NT-proBNP in the upper compared to the lower quartile (adjusted for age, gender, traditional CV risk factors and CRP) [119]. NT-proBNP is also an independent predictor of mortality in established RA. Provan et al monitored 182 patients with RA over a 10 year follow up period and reported NT-pro BNP, along with age, sex and disease activity, was an independent predictor of mortality [120].

2.4.5.2 Echocardiography

Echocardiography is a non-invasive, relatively low-cost investigation to perform, but relies on appropriately trained personnel to acquire optimal images and interpret appropriately. There have been many echocardiography studies in patients with RA that demonstrate systolic impairment and diastolic dysfunction in both early and established RA populations [121-123]; the latter in up to 32% of cases [124]. Many studies have also shown diastolic dysfunction [125] in the absence of impaired systolic function [126]; supported by clinical studies demonstrating an increased risk of heart failure after adjustment for IHD and CV risk factors suggesting an alternative pathophysiology other than that of atherosclerosis [127].

A meta-analysis of 1,614 patients with RA determined larger left atrial dimensions, higher LV mass index and a lower mitral E/A ratio (an indicator of diastolic dysfunction), differences remaining after inclusion only of those studies with matched data for co-morbidities. Interestingly, they found no difference in left ventricular ejection fraction (LVEF) [128]. Another meta-analysis confirmed an increase in LV mass index in patients with RA and suggested the measure could be considered for use as a surrogate end point in RA clinical trials [129].

2.4.5.3 Cardiovascular magnetic resonance imaging

Cardiovascular magnetic resonance (CMR) imaging is a valued tool in the assessment of CVD, and has demonstrated superiority over other imaging modalities in the detection of IHD in the general population [16]. A major advantage of CMR above other investigative tools is its ability to non-invasively assess both the structure and function of the heart and vasculature in one examination as well as tissue characterisation. Multiple techniques are employed, with the use of gadolinium-based contrast agents and/or adenosine (coronary vasodilator), to give information on dimensions, function, tissue viability, fibrosis, perfusion, coronary blood flow and arterial stiffness (Table 2-1) [15, 130]. Newer higher field strength scanners (3T) give higher signal-to-noise ratios potentially producing clearer images.

CMR can differentiate ischaemic from non-ischaemic cardiomyopathy based on;

- Location of late gadolinium enhancement (LGE); ischaemic in origin if in a vascular distribution (segmental, affecting subendocardial or transmural layers) or non-ischaemic in origin if non-segmental, or only affecting mesocardial and sub-epicardial layers.
- Pattern of LGE; focal, patchy or diffuse.
- Distribution of perfusion defects; segmental vs. non-segmental (non-ischaemic).
- T1 mapping and extracellular volume (ECV) fraction

Table 2-1Techniques used in CMR

Protocol	**Data acquired**	**Interpretation**
Low resolution survey, gradient and spin echo pulse sequences (cines; T1w and T2w imaging +/- fat suppression (pre- and post-contrast)	Left ventricular (LV) and right ventricular (RV) ejection fractions, % LV and RV end diastolic/systolic volumes, ml/min^2 LV mass, g/m^2 LV and RV stroke volumes, ml/min^2 Aortic distensibility, 10^{-3}mmHg^{-1} Carotid wall thickness (minimum, maximum, mean), mm Carotid wall and luminal volumes, uL	Anatomical and functional imaging: defining cavity dimensions, wall motion abnormalities. Arterial stiffness Carotid wall thickness, plaque quantification.
Phase contrast velocity mapping	Pulse wave velocity, m/s	Arterial stiffness
Tissue tagging	Peak twist, degrees Torsion, degrees Mid S' Mid A' Mid E'	Regional and global myocardial strain
T1 mapping, pre and post contrast	Native TI, m/s Extracellular volume (ECV) fraction, %	Myocardial inflammation and diffuse fibrosis
Stress-rest myocardial perfusion	Presence of gross perfusion defects Myocardial perfusion reserve	Macro and micro-vascular myocardial ischaemia
Late gadolinium enhancement (LGE)	Presence/distribution of LGE	Myocardial infarction and focal fibrosis

Research groups are increasingly employing CMR in the detection of subclinical CVD in RA. However, CMR protocols vary and minimal data describing the disease phenotype is offered. Few involve expertise of CMR cardiologists, with potential for over-reporting of artefactual abnormalities as pathology.

There are few published studies of CMR in RA [131-133]. However, in contrast to the echocardiography meta-analyses, the largest CMR study demonstrated an 18% reduction in LV mass in 75 patients with RA compared to 225 matched controls that underwent a 1.5T CMR. It is possible that the geometric assumptions relied upon during echocardiography to measure LV mass are less accurate in RA, whilst CMR provides detail on the whole of the myocardium making such assumptions redundant [134]. Patients with RA also had reduced LVEF, cardiac output and stroke volume compared to controls [135]. The reduction in LV mass was not however replicated in other (smaller) CMR studies [136, 137].

One of these modest sized CMR studies of 39 patients with RA, free of CVD, matched to 39 controls [median disease duration 7 (interquartile range (IQR) 4, 11) years, mean (standard deviation (SD)) DAS28 3.3 (1.3)] revealed focal fibrosis and focal myocardial oedema in 46% and 10% of patients with RA, respectively, compared to 0% in the control group [137]. Extracellular volume fraction (ECV) was also increased in the RA group (30.3% vs. 27.9% controls; $p<0.001$). They however, found no significant difference in LV volumes, mass and LVEF between the groups [137]. Mavrogeni et al performed a 1.5TCMR with LGE on 45 patients with RA with heart failure, and determined patterns of transmural and subendocardial fibrosis in up to 33% of patients [138].

2.5 Cardiovascular risk factors in Rheumatoid Arthritis

Many risk factors have been associated with CVD in RA and can be divided into traditional and non-traditional risk factors such as inflammation and disease specific RA features as discussed below.

2.5.1 Traditional cardiovascular risk factors

Traditional CV risk factors comprise hypertension, smoking, dyslipidaemia, DM and a premature family history of CVD. In RA, the evidence for an increased prevalence of these risk factors is inconsistent (see Tables 2-2 and 2-3) [60, 139]. However, the detection and management of traditional CV risk factors remains important; a meta-analysis confirmed hypertension, type 2 DM, smoking and hypercholesterolemia increased the risk of CVD in

RA [6]. Most of the studies assessing CV risk factors in RA involve established cohorts, with few looking at early RA [140], when perhaps the opportunity to recognise and act upon modifiable CV risk factors is greatest. A recent longitudinal study in early RA highlighted the interplay between traditional CV risk factors and disease activity [141].

Table 2-2Cross-sectional and case controls studies analysing the prevalence of traditional CV risk factors

Population	Study	Disease duration, years*	n	Results	Reference
Korea	Cross-sectional	Mean 10.2	198	Prevalence: Smokers=6.6%, hypertension =38.4%, DM = 11.6%, dyslipidaemia = 19.7%	[142]
ESPOIR	Case control	Early RA < 6 months duration ≥2 joints ≥6 weeks steroids & DMARD naive	442 cases 1821 controls	Diastolic BP, TC and LDL-C significantly lower, in cases vs. controls. Pulse pressure, TG and fasting glucose significantly higher in cases vs. controls LDL-C significantly lower and TG significantly higher in patients for upper tertile CRP vs. lower tertiles. No significant link with CRP for TC/HDL-C, blood glucose, diastolic or systolic BP, or pulse pressure	[140]
London, UK	Cross-sectional	Median 8	305	28 (9%) had a previous CV event Hypertension (57%), hyperlipidaemia (30%), DM (11%), ex/current smokers (52%) 1/3 without DM or hyperlipidaemia had ever had fasting glucose or lipid levels checked CV RFs undertreated: 52% on anti-hypertensives had ongoing hypertension Hypertension (OR 4.3, 95% CI 1.1, 16.8) retained significance in multivariate	[143]

				model for previous CV events	
Medellin, Colombia	Cross-sectional	Mean (SD) 12.53 (8.07)	538	16% hypertension, 9% dyslipidaemia Hypertension more likely if have extra-articular RA	[144]
QUEST-RA	Cross-sectional	Mean (SD) 11 (9)	4,363	Lifetime CV events prevalence 9.3%. Prevalence: 32% hypertension, 14% hyperlipidaemia, 8% DM, 43% ever-smoking Older age, male gender, hypertension, hyperlipidaemia, and ever-smoking independently associated with occurrence of CV events (multivariate Cox regression analysis)	[145]
Almada, Portugal	Case control	Median (IQR) 7.6 (0.5, 30)	98 RA 102 controls	44% hypertension vs. 32% controls 17% current smokers vs.19% controls 7% DM in RA and controls	[139]
PharMetrics Patient-Centric Database, USA	Case control	Not given	28,208 RA 112,832 controls	Prevalence of DM, hyperlipidaemia, and hypertension significantly higher in cases vs. controls ($p<0.01$)	[60]
West Midlands, UK	Case control	Mean 11.2 (9.7)	150 RA 100 OA	28 % on treatment for hypertension, 4% lipid-lowering therapy, 22% smoker, 5% DM (no different to OA) Mean Joint Societies Risk score; RA: 15.6 (11.0) vs. OA: 14.8 (9.3); p~0.63.	[146]

				More patients with RA and OA had CVD risk in the 15 – 29.9% or ≥30% risk categories vs. UK community based statistics	
National Data Bank for Rheumatic Diseases, USA	Case control	Not given	9093 RA 2479 OA	More smokers in RA: 13% vs. 6% Less hypertension in RA: 28% vs. 37%	[147]
MONICA study, UK	Case control	Median 12.5	76 RA 641 controls	Diastolic BP significantly higher and TC significantly lower in RA vs. controls. Prevalence of DM, systolic BP, smoking habits and exercise history similar	[148]

*unless otherwise stated

BP, blood pressure; CI, confidence intervals; CRP, C-reactive protein; CV, cardiovascular; DM, diabetes mellitus; HDL-C, high density lipoprotein-cholesterol; IQR, interquartile range; LDL-C, low density lipoprotein-cholesterol; OA, osteoarthritis; OR, odds ratio; RA, rheumatoid arthritis; SD, standard deviation; TC, total cholesterol; TG, triglyceride

Table 2-3 Prospective and retrospective studies describing prevalence of traditional CV risk factors among patients with RA

Population	Study	Disease duration	n	Methodology	Results	Reference
CORRONA	Prospective	Median at BL 84 months	10 156	18 829 patient-years follow-up	At BL: DM 7%, hypertension 30%, hyperlipidaemia in 9% and smokers 16%. 9% of subjects had prior CAD CV event rate = 3.98 (95% CI 3.08 to 4.88) To predict CV events use (c-statistic 0.71): 1. traditional CV RFs (DM, hypertension, hyperlipidaemia, smoking, known CVD, a FHx premature CVD, ≥75 years and male) 2. markers of RA severity (disease duration >5yrs, erosions, subcutaneous nodules, prior total joint replacement, modified HAQ score ≥2, Clinical Disease Activity Index score >22 and seropositivity) 3. age and gender 2+ traditional CV RFs & 3+ markers of RA severity: IR = 7.47 (95% CI 4.21 to 10.73) per 1000 person-years.	[149]
Swedish Rheumatoid Arthritis Registry	Prospective	<12/12 symptoms	442	5 years follow-up	At BL: 25% hypertension, 7% DM 30% smokers At 5 yrs: 37% hypertension (p< 0.001), 10% DM (p< 0.01), 22% smoking (p< 0.001) 48 patients suffered new CVE (MI/CABG, stroke/TIA/ DVT/PE, and	[141]

					ruptured aortic aneurysm) HR for hypertension predicting CV event = 3.597 (95% CI 2.028 to 6.380; *p*= 0.001) (cox multiple regression)	
Greece	Prospective	Median (IQR) at BL 2 years (1, 8.5)	325	Median follow-up period 10 yrs (range 6 to 17)	IR of CVD was 4 events per 3088 person-years in females and 14 events per 801 person-years in men (p = 0.001). Hypertension at BL increased the risk of CVD after adjustment for age, sex, and other factors; HR = 3.76 (95% CI 0.99 to15, p = 0.056).	[150]
MyEIRA (Malaysian Epidemiology in RA)	Retrospective	Mean 5.2 years	96		Prevalence at BL of: Hypertension =21.9%, DM= 8.3%, dyslipidaemia =11.5%, smokers =9.4% Prevalence at follow-up: Hypertension =27.1%, DM = 9.4%, dyslipidaemia = 32.3%	[151]
General Practice Research Database	Prospective	<2 years symptoms and DMARD naïve	1460	22 year follow-up	SIR for hypertension= 1.61; (95% CI 1.43 to 1.79), greater for females than males No difference in SIR for DM	[152]

BL, baseline; CABG, coronary artery bypass graft; CV, cardiovascular; DM, diabetes mellitus; DMARD, disease modifying anti-rheumatic drug; DVT, deep vein thrombosis; FHx, family history; HR, hazard ratio; IQR, interquartile range; IR, incidence ratio; MI, myocardial infarction; PE, pulmonary embolus; RFs, risk factors; SIR, standardized incidence ratio; TIA, transient ischaemic attack

2.5.1.1 Hypertension

Essential hypertension is prevalent amongst patients with RA, with some studies reporting hypertension in up to 57% [60, 139, 142, 143], although it is not clear if this prevalence is greater compared to the general population [147, 148]. The Swedish Rheumatoid Arthritis Registry determined that in 442 patients with RA, 25% at baseline had hypertension, 37% five years later, and importantly, hypertension predicted CV events (HR 3.67 (95% CI 2.0, 6.4, p0.001) [141]. Hypertension can be exacerbated by the use of corticosteroid and NSAIDs (discussed later).

2.5.1.2 Smoking

Smoking exposure in RA exemplifies a complex gene-environment interaction. In individuals with the HLA-DR4-positive ('shared epitope') genotype, smoking increases the citrullination of proteins and production of ACPA [153, 154]. Therefore not only does smoking increase CV risk through its 'traditional' effects on the endothelium and blood pro-coagulation, it is also an independent risk factor for developing RA [155], and predicting severe disease and poor treatment response [156]. The relationship of smoking with ACPA is especially important given that citrullinated proteins have been found within atherosclerotic plaques [157]. Smoking cessation is strongly advised in patients with RA [18].

2.5.1.3 Dyslipidaemia

Cholesterol homeostasis depends on hepatic cholesterol synthesis and absorption of cholesterol in the intestine from dietary sources [158]. It is the sum of very low-density lipoproteins (vLDL), low-density lipoproteins (LDL-C) and high-density lipoproteins (HDL-C), and is carried in chylomicrons in the circulatory system [159].

A high total cholesterol to HDL-C cholesterol (TC/HDL-C) ratio has long been recognised as a risk factor for CVD; clearly illustrated in the Framingham study [2]. Statins, which inhibit the HMG-CoA-reductase enzyme, and reduce the synthesis of cholesterol, are used routinely in the prevention and management of CVD in the general population [160, 161]. There is also evidence that the degree to which individuals absorb cholesterol in relation to how much they synthesise cholesterol is associated with higher risk of CVD; greater absorbers being at

greater risk, and a school of thought that non-statin responders should be given an inhibitor of cholesterol absorption [158].

Whereas the role of LDL-C is to transport cholesterol from the liver to peripheral sites, HDL-C facilitates the transport of cholesterol from tissues and the vasculature to the liver for excretion, and may protect from atherosclerosis further by decreasing LDL oxidation, increasing nitric oxide production and decreasing endothelial adhesion molecule expression [162].

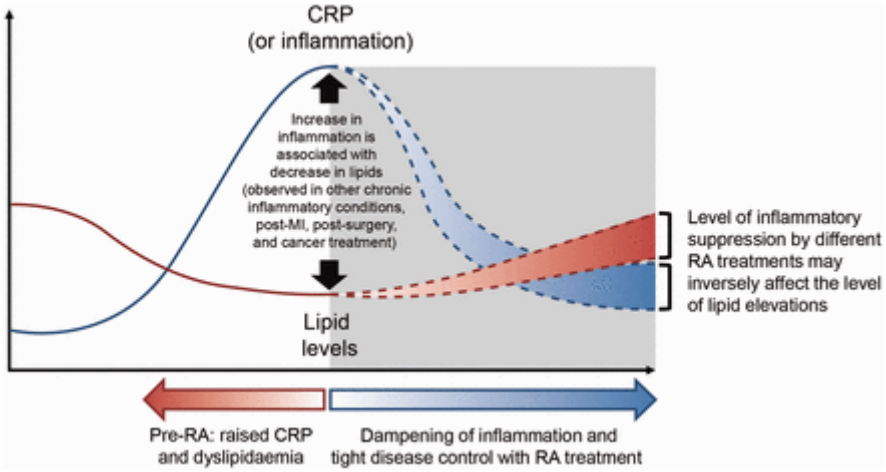
Dyslipidaemia is prevalent in RA; in up to 30% of cohorts [142-145]. However, the association of cholesterol and CV risk in RA is complex. Many studies report an increase in TC, LDL-C and reduction in HDL-C [163-168]; even seen within one year of its diagnosis [64]. Changes may also occur prior to the onset of RA. Van Halm et al described the lipid profile of 1078 blood donors; those who went on to develop RA had higher TC and lower HDL-C up to ten years before diagnosis compared to matched controls [169]. Some RA studies, however, report lower TC, LDL-C and HDL-C [140]. Evaluation of the atherogenic index (TC/HDL-C) as opposed to individual lipid values appears to be the clinically meaningful parameter; increased in patients with RA [170].

Hyperlipidaemia is associated with CV events in RA [6]. The QUEST-RA study determined hyperlipidaemia, along with age, male gender, hypertension and ever smoking independently associate with CV events [145]. However, the Apolipoprotein-related Mortality RISk (AMORIS) study complicated this when authors reported that although patients with RA had lower levels of TC and LDL-C compared to non-RA patients, they were more likely to suffer CVD; at least 1.6 times the rate (TC/HDL-C was not reported) [171]; an effect also seen in other studies [172].

This inverse relationship between lipid levels and CV risk in RA, or 'lipid paradox' may be explained by the changes in lipids in the presence of inflammation; CRP and disease activity tend to negatively correlate with TC, LDL-L and HDL-C (see Figure 2-6), whereas, a positive correlation (or sometimes none) is seen with TC/HDL-C [140, 168, 173-179], suggesting the latter may perform better as predictor of CV risk in RA. In addition, an interaction between lipids and inflammation has been identified in RA; both lower LDL-C and TC/HDL-C have

been associated with an even greater risk of CVD in the presence of high rather than low inflammatory markers [172].

Figure 2-6 Representation of the inverse relationship between changes in inflammatory and lipid parameters



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In addition to affecting lipid levels, some authors propose inflammation alters the function of the lipids. In particular, pro-inflammatory HDL-C (pi-HDL-C) or 'impaired HDL-C' has been identified, which may affect cholesterol metabolic pathways or other steps in the pathogenesis of atherosclerosis [180, 181], and could explain the paradox of lower levels of TC, LDL-C and TC/HDL-C associated with higher risk of CVD.

Apolipoproteins, particularly apo-B, have also attracted interest. Apo-B is present in all atherogenic particles including LDL-C and has been shown to be superior to LDL-C in the prediction of future CV events in the general population [182]. In addition, Lipoprotein (A) (lp(a)), an LDL-like particle to which Apoliprotein A (apo (A)) is covalently linked, has been associated with CVD [183]. Apo-A and B, along with Lp(a), are elevated in RA, but studies are limited in inception cohorts [173, 184, 185]. The apoB/apoA ratio has been shown to predict future CV events in RA [186].

2.5.1.4 Diabetes Mellitus

Cohort studies report the prevalence of DM in RA populations to range from 5 to 12% [31, 139, 141-143, 145, 146, 151, 187]; with conflicting data as to whether or not DM is more common in RA compared to the general population [60, 139, 148]. One notable study in over 28,000 patients with RA compared to over 112,000 controls found DM was more common in those with RA [60]. It hasbecome clear that the risk of CVD in RA is similar to that seen in DM [188, 189]. The CARRE (CARdiovascular research and RhEumatoid arthritis) study followed 294 patients with RA, 258 controls and 194 patients with DM for five years. The authors determined a relative risk of CVD, after adjustment for traditional CV risk factors, of 2 in DM and 2.7 in those with RA [188]. No study has clearly evaluated the impact on the occurrence of CVD in the patient with both RA and DM.

Insulin resistance, is considered a precursor to DM [190], and is commonly estimated by the homeostasis model assessment-estimated insulin resistance (HOMA-IR) index ((glucose x insulin)/405) [191]. HOMA-IR independently predicts CVD in the general population [192] and values have been found to be higher in patients with RA [193-195]. However, there have been no studies assessing the prognosis of increased HOMA-IR in the RA population to date. Established RA studies report an association with disease activity, although reports are mixed [194-197]. The evidence is inconsistent for an association of insulin resistance with CIMT [198, 199]. There are few studies demonstrating this in early RA [196, 200].

2.5.2 Inflammation – the non-traditional CV risk factor

The accelerated risk of CVD seen in RA has been shown to be independent of traditional CV risk factors [61]. Systemic inflammation, associated with active synovitis, is thought to play a key role. Synovitis, the hallmark of RA, is the result of a complex auto-immune process involving both innate and cell mediated immunity, with the activation of both T and B cells, macrophages, fibroblasts, endothelial cells and the release of cytokines such as IL-1, IL-6, IL-10, IL-12, IL-15, IL-17, IL-23 and TNF-α, TGF-β [201]. These cytokines are thought to initiate and augment the atherosclerotic process in the vasculature [202]. In support of this hypothesis, Aubry et al determined in a post-mortem study in those with CVD,

patients with RA had less severe coronary artery lesions but more evidence of vulnerable plaques, and more inflammatory change, compared to non-RA controls [203]; corroborated in other histological studies [204]. An increase in these inflammatory lesions in patients with RA when compared to controls has also been detected with PET imaging [205], correlating with CRP [206], and improving with TNF-α inhibitors (TNFi) [207].

As discussed previously, CRP, a measure of systemic inflammation, has been associated with increased CV events in RA, and can predict future CV events and CV related death [208, 209]. In the UK Norfolk Arthritis Register (NOAR) cohort of inflammatory polyarthritis, the HR for death from CVD with an elevated CRP level was 3.3 (95% CI 1.4, 7.6), adjusted for age, sex, smoking status, health assessment questionnaire (HAQ) score, RF positivity, and swollen joint counts (SJC) [209].

CRP correlates with NT-proBNP in patients with RA [117, 210, 211]. Few studies have assessed NT-proBNP in early RA [211]. Usefully, Provan et al, demonstrated repeated measures of CRP predicted NT-proBNP at 10 years in one early RA cohort [212].

CRP and ESR have also been associated with arterial stiffness [92], endothelial dysfunction [72, 73, 79] and with fibrotic change seen on CMR [131, 138]. In addition to influence by age, smoking, hypertension and DM, CRP has been associated with CIMT [187]. The Rotterdam study produced an odds ratio of 1.7 (95% CI: 1.0, 3.1) for progression of CIMT for CRP levels in the highest quartile, when adjusted for baseline CV risk factors [213]. Schau et al reported the echocardiographic findings of 157 unselected patients with RA; 23% were found to have evidence of heart failure compared to 6% of controls; of which CRP and ESR were both independent risk factors [122].

2.5.3 Disease specific cardiovascular risk factors

In order to correctly identify and manage those at high risk of CVD, efforts have been made to phenotype the patient with RA most at risk of CVD. Certain disease specific features have been associated with a higher CV risk. The CORRONA study found combining age, gender and traditional CV risk factors, with RA disease specific factors (disease duration greater than five years, presence of erosions, subcutaneous nodules, prior total joint replacement, modified HAQ score greater

than or equal to 2, Clinical Disease Activity Index (CDAI) score greater than 22 and seropositivity) yielded the strongest prediction for future CV events [149]. The following summarises the disease specific features associated with both overt and subclinical CVD.

2.5.3.1 Disease duration

Longer disease duration is recognised as a risk for CVD; consequently disease duration greater than 10 years is a criterion for multiplying CV risk scores as recommended by the EULAR cardiovascular guidelines [18]. A prospective cohort study followed over 114,000 women, of which 527 developed RA. The relative risk of MI compared to women without RA was 2.0 (95% CI 1.23 to 3.29) (figure adjusted for traditional CV risk factors and corticosteroid and NSAID use). The risk with disease duration greater than 10 years was even greater at 3.1 (95% CI 1.64 to 5.87). Interestingly no increased risk was seen for stroke events [214]. Along with age, systolic blood pressure and CRP, disease duration is an independent determinant of PWV in RA [85, 90, 96, 97, 215-217], and has been associated with reduction in FMD and increased CIMT [79, 218].

2.5.3.2 Serology

Positivity for Rheumatoid factor (RF) or ACPA has long been seen as a risk factor for accelerated CVD [18, 74]. However, in early RA the evidence is conflicting with some studies suggesting an increased risk [219, 220] and others not [141, 221]. A large study of 124 patients with RA reported no association of ACPA-2 and RF with either US-measured CIMT, carotid plaques or CT-detected coronary calcification scores [222]. Echocardiography studies showing abnormal LVEF and diastolic dysfunction [223] and CMR studies with reduced LV mass [135] and increased CIMT [223-225] suggest an association with ACPA.

In patients with RA, ACPA has been associated with inflammatory mediators such as IL-6, MCP-1, TNF-α, MMP-3, and *in vitro* studies have confirmed ACPA isolated from patients with RA alters the expression of pro-thrombotic and inflammatory markers from monocytes and leucocytes, including those implicated in the pathogenesis of atherosclerosis such as TNFα and MCP-1 [74]. Studies have also detected citrullinated proteins within atherosclerotic plaques, which bind and

form immune-complexes with RA-derived ACPA [157], providing a possible mechanism for their contribution to the atherosclerotic inflammatory process.

2.5.3.3 Disease activity

Disease activity in RA is commonly reported as a composite score of tender and swollen joints, a physician visual assessment score and an inflammatory marker, such as CRP or ESR. DAS28 (28 joint disease activity score), SDAI (Simplified Disease Activity Index) and CDAI are all universally accepted methods of assessing disease activity [226]. As disease activity fluctuates, it can be difficult to ascertain the effect on CV risk. Longitudinal studies that have attempted to estimate the burden of inflammation over a period of time have detected an association with overt CVD. Solomon et al followed 24,989 patients with RA for a median of 2.7 years, after which 534 met CV end-points (MI, stroke or death from CV causes). They determined a 10-point reduction in time average CDAI was associated with a 21% reduction in CV risk [227]. A Dutch study similarly followed an 855 strong cohort of patients with early RA and found time-averaged DAS28 was associated with CVD after adjustment for age, sex, BMI, baseline DAS28 and history of biologic use [228]. Other studies have used measures of subclinical CVD, such as FMD [79], CIMT [225, 229, 230], LV relative wall thickness [231] and CMR measures of LGE [138], myocardial T1 mapping and ECV [137], to determine a link with disease activity.

2.5.3.4 Subcutaneous nodules

The presence of subcutaneous nodules has been associated with a greater risk of CVD. Kaushik et al determined of 3908 patients with subcutaneous nodules at baseline out of 26,042 patients with RA, there was an increased risk of CV events, including MI, stroke and death from CV causes, after adjustment for age, sex, and traditional CV risk factors. However, this association was lost after additional adjustment for RA specific measures [232].

2.5.3.5 Erosions

Erosions are recognised as a marker of RA disease severity [233], however, there are few studies assessing the association of erosions on CV risk in RA. Erosions have been associated with higher CIMT measurements [225].

2.5.3.6 Health assessment questionnaire

The health assessment questionnaire–disability indexed (HAQ-DI) is used to evaluate disability in RA and seen as a marker of disease severity. Studies have reported higher values are associated with CV events [219, 234]. Higher HAQ scores have also been associated with CMR measured cardiac strain [132] and other measures of arterial stiffness [235].

2.5.4 Additional risk factors observed

2.5.4.1 Genetic loci similarities

The genome wide association studies have determined similarities between those associated with RA and atherosclerosis. Of particular interest are rs599839 A/G polymorphism (implicated in endothelial activation) and MIA3 rs17465637 A/C polymorphism, (involved in vascular adhesion molecule interactions and macrophage migration into intima). Various TNF-α polymorphisms have been identified as a link between the two disease processes. The HLA-DRB1*0404 allele, strongly associated with RA, has also been shown to affect endothelial function [236].

2.5.4.2 Sedentary lifestyle

Active synovitis and associated damage with consequent reduced mobility, pain and fatigue can lead to a more sedentary lifestyle in those with RA [237, 238], and this has been associated with a worse CV risk profile [239], although data in this area is sparse.

2.5.4.3 Medication

A recent large meta-analysis determined the use of corticosteroids were associated with an increased risk of CV events in RA (relative risk (RR) 1.47, 95% CI 1.34, 1.60, $p<0.001$) [240], however, the mechanism through which this occurs is unclear. It is proposed that corticosteroid use in the management of the patients with RA may exacerbate or induce hypertension, increase the risk of type 2 DM [241] and obesity, and affect lipid metabolism [242]. As a result, the European League Against Rheumatism (EULAR) advocates the use of 7.5mg or less per day of prednisolone to minimise CV risk [18]. The same meta-analysis detected an increase for all CV events and stroke with the use of non-steroidal anti-inflammatories (NSAIDs) [240], however, their analysis included the use of rofecoxib (now withdrawn from the market due to excessive adverse CV

outcomes). They found no increased risk for MI. Current practice is to minimise the use of NSAIDs, especially in those thought to be at higher risk of CVD [18].

2.6 Management of cardiovascular disease in Rheumatoid Arthritis

2.6.1 Current guidelines for reducing CV risk

In 2010, EULAR published guidance on how to manage CV risk in RA; recommending 1 to 3 yearly CV assessments dependent on individual risk, and multiplying the 10 year CV risk calculation by 1.5 if specific criteria were present; disease duration greater than 10 years, RF or ACPA, or presence of severe extra-articular features [18]. The suppression of disease activity was advised for the reduction of CV risk, with an acknowledgement that the use of methotrexate (MTX) and TNFi inhibitors had the best supporting evidence.

Despite these guidelines the management of CV risk in RA is suboptimal with studies determining hypertension inadequately treated and lipid levels unchecked [243-246]. The data assessing the effects of anti-hypertensives and statins on the risk of CVD in RA are limited, but the benefits seen in the general population are presumed to apply to those with RA [69]. A recent systematic review highlighted a few studies (mainly cohort) only, of low patient numbers, that showed a reduction in CV events in those with a history of statin use [247]. The Trial of Atorvastatin for the primary prevention of Cardiovascular Events in Rheumatoid Arthritis (TRACE-RA) was carefully designed to determine whether atorvastatin compared to placebo was superior in the primary prevention of CVD in RA [248]. 2986 patients with established RA were recruited, and although there appeared to be a lower risk of CVD in those on atorvastatin (not statistically significant), the study was stopped early due to a lower than anticipated overall event rate.

2.6.2 The effect of disease suppression

2.6.2.1 Cardiovascular morbidity and mortality

Disease modifying anti-rheumatic drugs (DMARDs), including MTX, have been shown to reduce CV deaths and events [145, 249]. A systematic review concluded in those with RA, psoriasis or polyarthritis, MTX lowered the risk of CVD and MI by

21% and 18% respectively [250]. A large meta-analysis reported a RR of all CV events with the use of MTX of 0.72; 95% CI 0.57, 0.91 [240].

TNFi can also reduce CV events in RA [251-254]. One meta-analysis suggested a pooled adjusted RR of 0.46 (95% CI 0.28, 0.77) for all CV events [253]; another meta-analysis quoted a RR of 0.70 (95% CI 0.54, 0.90) [240].

When comparing treatment strategies, Bozaite-Gluosniene et al found that an inception cohort of 1829 patients with RA using TNFi had a lower HR for incident coronary heart disease compared to those using MTX (0.33 versus 0.24) [252]. Also, a meta-analysis determined that while both TNFi and MTX use were associated with comparable reductions in risk of CV events, only TNFi use was associated with a reduced risk of stroke [240]. Although the mechanisms are unclear, the improvement is assumed as a consequence of a reduction in systemic inflammation rather than the specific action of therapeutic agents; Dixon et al found no difference in risk of MI in those using TNFi compared to DMARDs, but did see a reduction in TNFi responders compared to non-responders [255].

2.6.2.2 Traditional cardiovascular risk factors

2.6.2.2.1 Dyslipidaemia

Corticosteroids can manipulate the lipid profile, but studies are few especially in early RA [256]. A recent systematic review analysed the effect of low dose prednisolone (less than 7.5mg per day) in RA and found mixed reports; some reporting no change in lipids, some an increase in HDL-C, and others a decrease in LDL-C and TC [241].

DMARDs can improve dyslipidaemia [176, 257-259] although data in early RA is limited [260-262]. In the COBRA study, 154 patients [median duration of RA of 4 months] were randomised to combination therapy (sulphasalazine (SSZ), MTX and prednisolone) or SSZ alone. Combination therapy led to a more rapid and effective reduction in disease activity, and greater improvement in TC/HDL-C after 16 weeks of therapy [263]. In the TEAR trial of 459 early patients with RA, which compared MTX + etanercept (TNFi) to triple DMARD therapy to aggressively titrated MTX, TC, HDL-C and LDL-C significantly increased, and TC/HDL decreased in all three groups [264].

TNFi can also influence lipid profiles, although reports are inconsistent [265-267]. Recent meta-analyses, report an increase in TC and HDL-C (approximately 10% and 7% respectively), and no change in TC/HDL-C [268, 269], whilst another suggested no change in lipid levels [270]. Improvements in levels of pi-HDL-C have also been reported [271]. The change in lipid profile on TNFi may be dependent on the suppression of inflammation [265, 272-274]; an association seen in surgical and/or septic patients [275]. Few studies involve early RA.

Studies have not demonstrated any benefit of TNFi over DMARDS in improving the lipid profile. Two prospective cohorts found no difference between TNFi and DMARDs on dyslipidaemia [264, 266, 276]; suggesting again the improvement in lipids observed is related to the improvement in disease activity rather than a specific action of TNFi.

There is less data on other biological DMARDs. Tocilizumab, an IL-6 receptor inhibitor, can cause increases in lipids, including TC/HDL-C and triglycerides [277, 278], however, it is suggested these changes do not translate into an increased risk of CV events in RA [279]. Rituximab, an anti-CD20 antibody (found on B cells), has been shown to decrease the overall atherogenic index [280].

2.6.2.2.2 Diabetes Mellitus

There is more data evaluating the effect of disease suppression on surrogate measures of insulin resistance rather than the development of DM. However, one abstract publication investigating the CORRONA cohort, a large multi-centre observational study, found a reduced in incidence in DM in those treated with TNFi compared to other non-biological DMARDS (excluding MTX and hydroxychloroquine (HCQ) (adjusted HR 0.35, 95% CI 0.13, 0.91) [281].

The limited data studying the effect of DMARDs on IR suggests an improvement [282]. The effect of TNFi has been studied more, but with inconsistent results [283-287]. No superiority of TNFi over DMARDs has been observed in cross-sectional studies [288] and small unblinded RCTs [289, 290]; but there is yet to be a double-blind RCT study comparing the change in HOMA-IR with TNFi versus non-TNFi in early RA.

2.6.2.3 Outcomes measures of cardiovascular disease

2.6.2.3.1 Soluble markers of endothelial dysfunction

There is very little data on the effect of disease suppression of soluble markers of endothelial dysfunction, but what is available suggests that they improve [291, 292]. One small study (n=18) reported an improvement in sICAM-1, sVCAM-1, sE-selectin and VEGF when patients with RA were treated with etanercept [292]. Another (n=66) found improvement in sE-selectin and sVCAM-1 levels with aggressive anti-rheumatic therapy [293]. Other studies have not shown as favourable results [75].

2.6.2.3.2 Arterial stiffness

No studies have assessed the effect of DMARDs on arterial stiffness, but there is evidence for improvement of PWV [290, 294-298] and aortic distensibility [297] with TNFi. A systematic review found all but one study reported a decrease in PWV following treatment with TNFi, but did comment on study heterogeneity [299]. There are longitudinal data to support an improvement in PWV with treatment with Rituximab, a monoclonal antibody against B cells [298] and tocilizumab (anti-IL6 receptor antibody) [300].

2.6.2.3.3 Vessel wall intimal thickness

There is also a paucity of data regarding the effect of treatment of RA on CIMT. There are data to support an improvement when treated with MTX and prednisolone [301]. A few studies have shown an improvement with TNFi compared to DMARDs [302-304], whilst others demonstrate no change [295, 305]. The meta-analysis that found no change in lipid profiles with TNFi also found no significant decrease in CIMT [270].

2.6.2.3.4 Structural cardiac changes

The little data available for structural and functional changes seen on echocardiogram with disease suppression are conflicting. Some studies suggest improvement with TNFi, for example in LVEF and LV torsion [306, 307], others report no changes [308]. However, the numbers involved in these studies are small.

No group has specifically assessed the effect of steroids or DMARDs on NT-proBNP, however it may improve with TNFi [306, 308, 309]. There have been no studies in early RA.

2.6.2.3.5 Cardiovascular Magnetic Resonance Imaging

The emerging data on CMR in RA lacks analysis of treatment specific associations. One small study (n=20) found tocilizumab (an IL-6 inhibitor) was associated with an increase in LVEF and reduction in LV mass, as well as disease activity, over one year [136].

2.7 Cardiovascular disease in Systemic sclerosis

2.7.1 Systemic sclerosis

Systemic sclerosis (SSc) or scleroderma is a chronic systemic autoimmune disease characterised by inflammation, vasculopathy and fibrosis [55]. The course of the disease depends on the disease subtype; limited cutaneous systemic sclerosis (lcSSc) and diffuse cutaneous systemic sclerosis (dcSSc). Skin thickening is a cardinal feature and may present with an inflammatory phase, detected by the appearance of oedematous fingers, before a progressive fibrotic phase begins. Skin involvement that extends proximal to the elbows and knees and involves the trunk is characteristic of dcSSc [55], and the gold standard for measuring severity of skin involvement is the modified Rodnan skin score (mRSS), which grades 17 body areas using a 0 to 3 scale resulting in a maximum score of 51 [310].

Over 95% of the patients with SSc suffer from Raynaud's phenomenon (RP), characterised by vasospasm of the peripheral arterioles. Cold environments usually precipitate RP, leading to the classic triphasic response of pallor, cyanosis and subsequent hyperaemia. With the added complications of digital ulcers, patients are at risk of secondary critical ischaemia and/or infection, with risk of tissue/digit loss [311].

Other manifestations of the disease include interstitial lung disease (ILD), renal crisis, intestinal malabsorption and small bowel bacterial overgrowth, pulmonary arterial hypertension (PAH) and cardiac disease [55]. Specific auto-antibodies are

associated with the disease sub-types; anti-centromere antibody (ACA) with lcSSc and anti-topoisomerase (Scl70) antibody with dcSSc.

The management of SSc is tailored to the clinical manifestations. Invariably immunosuppression is required to halt progressive skin fibrosis or advancing interstitial lung disease. IV cyclophosphamide is used in severe cases, or in milder forms, mycophenolate mofetil (MMF) and MTX. Barring modest data in SSc related ILD, there is no robust evidence for any immunosuppressant agent in SSc [312]. Despite appropriate management, SSc is associated with a higher mortality, with one meta-analysis producing a SMR of 3.5 [313]. Cardiopulmonary complications are the leading cause of death [314].

2.7.2 Cardiovascular morbidity

Cardiac manifestations in SSc vary from palpitations, arrhythmias, diastolic dysfunction, heart failure, myocarditis, MI and even sudden death [315, 316]. Two distinct pathological processes are responsible for these manifestations; as a result of atherosclerosis and IHD, or as a direct consequence of the SSc disease process acting on the cardiac tissues; termed primary myocardial involvement or SSc-related cardiomyopathy. The cardinal feature of primary myocardial disease in SSc is myocardial fibrosis, which can affect the endocardium, myocardium and pericardium, explaining the varied clinical presentations [315, 316]. Myocarditis (with or without co-existing peripheral myositis) may also occur [317].

2.7.2.1 Macrovascular disease

The risk of atherosclerotic-related CVD does appear to be accelerated in SSc [318]. A large Australian cohort reported an OR of 3.2 (95% CI 2.3, 4.5) for IHD in SSc patients when adjusted for traditional CV risk factors [319]. A UK primary care database study of 865 patients compared atherosclerotic CVD rates of patients with SSc to healthy controls during the years 1986 to 2011; adjusted HRs for MI, stroke and peripheral vascular disease (PVD) were 1.80 (95% CI 1.07, 3.05), 2.61 (95% CI 1.54, 4.44) and 4.35 (95% CI 2.74, 6.93) respectively [320].

This increased risk is relatively understudied but endothelial dysfunction with microvascular fibro-proliferation, both hallmarks of the disease pathogenesis, may be accountable [321, 322], and unlike with RA, inflammation playing a much smaller role.

The prevalence of CV risk factors in SSc has also been understudied. Lipid studies report lower levels of HDL-C [323, 324]; lower in those with ACA, with no correlation with inflammatory markers [324]. Smoking has a detrimental effect on morbidity in SSc [325] and patients are actively encouraged to stop. Hypertension is aggressively managed due to its association with renal crisis [326].

2.7.2.2 Primary myocardial disease

The reported prevalence of primary myocardial disease varies greatly (from 5 to 52%) [8, 327-332]. Ferri et al described 30% of 1012 Italian patients with SSc had at least one of the following symptoms: pericarditis, congestive heart failure, severe arrhythmias and/or atrioventricular (AV) conduction abnormalities at diagnosis [331]. Definitions of cardiac involvement vary and difficulty lies in teasing out whether clinical manifestations are due to atherosclerosis or primary myocardial involvement. Studies have described the presence of abnormal electrocardiograms (ECGs) or of arrhythmias requiring intervention; with ventricular arrhythmias associated with sudden cardiac death [315, 316]. The most commonly reported abnormality across studies is diastolic dysfunction, followed by conduction disturbances and pericarditis/pericardial effusions.

Although present in both disease subtypes, myocardial involvement is more common in dcSSc [331-333] and those with positive serology such as Scl-70 [334]. No one antibody has a stronger association, unlike older age of onset of SSc [335] and the presence of tendon friction rubs [336]. Uncertainty remains regarding the association with mRSS [337, 338] and late vasculopathy pattern on nail-fold capillaroscopy (NFC, visualisation of nail-bed capillaries with light microscopy) [339].

2.7.3 Cardiovascular mortality

Once cardiac involvement has become clinically overt, prognosis is poor, with a 5-year survival of 13 to 59% [331, 340]. It is difficult to differentiate primary myocardial from atherosclerotic-related deaths. A EUSTAR group did so when reporting the mortality of 234 SSc patients [55% dcSSc]; whilst 14% died due to primary myocardial causes (6% due to arrhythmias), another 12% of deaths were secondary to atherosclerotic/thrombotic causes [341]. Meta-analyses have not made such a distinction, producing adjusted HRs of 2.8 (95% CI 2.1, 3.8) to 3.15

(95% CI of 2.33, 4.26) for death from cardiac involvement, broadly defined from major conduction disturbances to heart failure [11, 342], making interpretations difficult.

2.7.4 Surrogate measures of cardiovascular disease

Multiple investigative modalities can inform of cardiovascular health in patients with SSc [343].

2.7.4.1 Endothelial dysfunction

As endothelial dysfunction is a crucial step in the pathogenesis of SSc, much effort has been put in to find an effective biomarker of disease activity and prognosis. Notable proteins include von Willebrand factor (vWf), an endothelial cell protein, and thrombomodulin, endothelin (ET)-1 (a potent vasoconstrictor), VEGF, and adhesion molecules such as ICAM-1, soluble VCAM-1 and e-selectin have shown promise [344].

Increased levels of sVCAM-1, sE-selectin, VEGF and ET-1 have been seen in patients with SSc with systemic organ involvement compared to those without organ involvement [345], and evidence suggests some, for example, sICAM-1, sVCAM-1 and E-selectin, may correlate with disease activity [346, 347]. Data is limited regarding their association specifically with cardiac disease in SSc. Insulin-like growth factor binding protein 3 (IGFBP-3), ICAM-1, plasminogen activator inhibitor 1 active have been associated with the presence of plaque, and myeloid progenitor inhibitory factor 1 (MPIF-1), serum amyloid A and thrombomodulin with CIMT as measured by ultrasound [348].

2.7.4.2 Arterial stiffness

Fewer studies have assessed PWV in SSc, but evidence suggests a higher PWV [87, 349-355]; interestingly, more so in lcSSc, those with ACA and longer disease durations [355]. No studies have combined the use of soluble CV biomarkers with PWV in assessing CV risk.

2.7.4.3 Coronary blood flow compromise

Invasive angiography studies have confirmed a reduced coronary reserve in patients with SSc compared to controls [356, 357], and evidence of calcified

plaque using CT angiography [358]. However, as for RA, these modalities of investigation as not favoured due to the risks involved.

2.7.4.4 Electrophysiological testing

Many electrophysiology (EP) studies in SSc, including routinely performed 12-lead ECGs and 24 hour ambulatory monitoring, have reported cardiac abnormalities, including conduction defects, arrhythmias and autonomic dysfunction; in up to 51% of cases [359-361]. Intra-ventricular conduction disturbances in SSc have been associated with future AV block and mortality [359, 361].

2.7.4.5 Cardiac structural and functional impairment

2.7.4.5.1 Echocardiography

Echocardiography is routinely performed on patients with SSc as it remains the gold standard for screening for PAH. However multiple studies have reported the prevalence of subclinical manifestations of cardiac disease in SSc, including diastolic dysfunction and pericardial effusions [328, 362]. The investigation however, relies on highly trained sonographers and can be limited by body habitus.

2.7.4.5.2 Soluble markers

Creatine Kinase (CK) and troponin (C, I or T) are cardiac enzymes, which indicate damage to the cardiac muscle, and used routinely in the assessment of acute coronary syndromes. Troponin I has the added advantage of specifically reflecting cardiac muscle damage [363], whereas the remaining enzymes could reflect peripheral myopathies. Although, they do appear to be raised in patients with SSc [363, 364], and associated with CVD [365, 366], their role in routine screening for cardiac disease in SSc has not been validated.

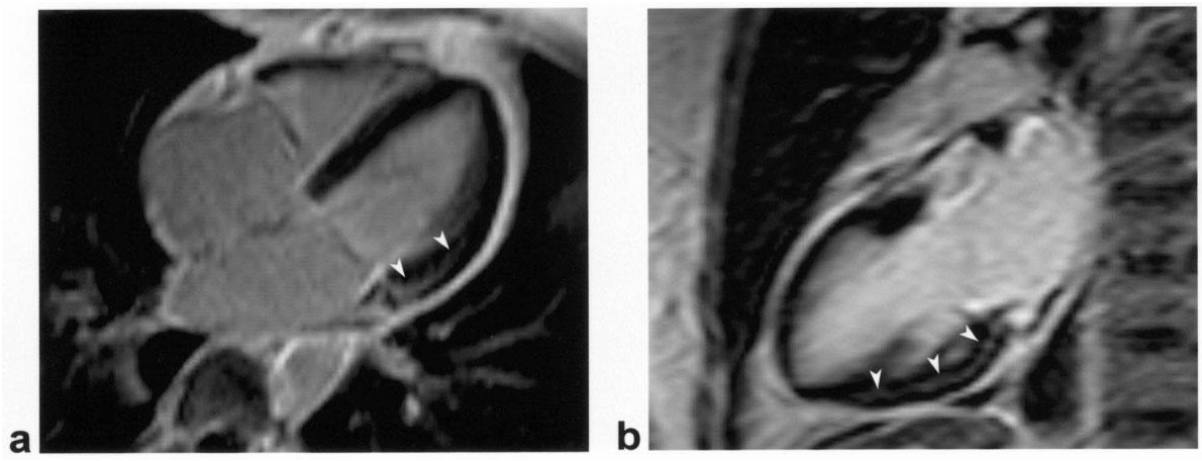
It is recognised that NT-proBNP is higher in SSc [367], especially in those with clinical cardiac involvement [362]. It is commonly associated with, and recommended in risk stratification for, PAH [368], however, an OR of 78 (95% CI 14, 424) has been reported for primary cardiac involvement (defined as haemodynamically significant arrhythmias, pericardial effusion or congestive cardiac failure) if NT-proBNP was greater than 50pmol/l [369]. It is associated with

markers of disease activity such as mRSS [370], higher IL-6 levels and echocardiographic abnormalities [371], however, study sizes are small.

2.7.4.6 Cardiovascular magnetic resonance imaging

The utility of CMR is better described in SSc [372-375]. The use of contrast and stress perfusion protocols gives insight into the pathogenesis of the disease. Studies report abnormalities in LGE, signifying fibrosis, in 15 to 66% of patients with SSc (see Figure 2-7); more severe in dcSSc [374, 376] and longer disease durations [377, 378]. Using CMR, fibrosis has been shown to spare the subendocardial layer, usually affected in atherosclerotic heart disease, consistent with an alternative (distinct) pathological process [379]. Perfusion defects are also commonly reported; up to 51-79% of subjects [374, 376]; with one study of 62 patients demonstrating a correlation with CRP [376]. There have been no longitudinal studies to determine the relationship (if any) between myocardial perfusion and fibrosis. Again, issues with lack of CMR expertise in scan performance and reporting remain.

Figure 2-7 Representative delayed enhanced magnetic resonance images in a patient with systemic sclerosis.



A: Four-chamber view showing linear midwall enhancement with spiculations (arrowheads) at the basal segment of the LV free wall. B: Long-axis view showing linear midwall enhancement (arrowheads) at the basal and midcavity segments of the LV inferior wall. Reproduced with permission from Tzelepis GE et al. Pattern and distribution of myocardial fibrosis in systemic sclerosis: A delayed enhanced magnetic resonance imaging study. Arthritis Rheum 2007;56(11):3827-36

2.7.5 Management of cardiovascular disease

Unlike in RA, there is little guidance in the management of atherosclerotic cardiovascular disease in SSc. Traditional CV risk factors are targeted in the same way as the general population, with more care given to the control of blood pressure, given the association with renal crisis [380]. As discussed, smoking is strongly discouraged due to its effect on RP.

Again, there is no formal guidance in the management of primary myocardial disease. The general school of thought is that any active disease should be treated with immunosuppression; however, it is not clear if certain chemotherapy agents have advantages over others [381]. Arrhythmias are treated in the usual manner with appropriate anti-arrhythmic medication, ablation therapy or implantable cardioverter defibrillators (ICDs) [382]. There remains an unmet need for well-designed studies in this area.

2.8 Summary

This review has described the now accepted model for the pathogenesis of atherosclerosis, in that it is an inflammatory process and not a bland lipid storage

disorder as originally thought. The dependence on inflammatory mediators for its initiation, to their role in endothelial activation and the now known composition of the final atherosclerotic plaque, provides evidence for the role of inflammation at every stage in its development. In keeping with this, the review has highlighted the literature supporting an association between systemic inflammation and an increase in CV events.

The review has summarised the impact of CVD on IMID, namely RA and SSc. Patients with RA appear to be at similar risk of CVD as patients with DM, with an accelerated risk also seen in SSc. Traditional CV risk factors play a role in the development of CVD in IMID, however, disease specific risk factors also exist; such as disease activity, disease duration and serology. Although, the underlying mechanism for the accelerated risk of CVD in IMID is still to be clearly defined, it is evident from the literature systemic inflammation contributes to this risk significantly.

This review also described the additional effect of the disease process on the heart in SSc, named primary myocardial disease or SSc-cardiomyopathy. This is independent of the macrovascular atherosclerotic disease process, and occurs as a result of disease pathology within the cardiac tissues. Although relatively understudied, primary myocardial disease accounts for a substantial proportion of deaths in SSc, and there is little known about the type of SSc patient most at risk or how best to monitor and treat such patient.

This review has described the various methods of determining CVD in both RA and SSc. Due to low absolute numbers and overt CV events taking many years to occur, surrogate markers are required to evaluate CV risk. Surrogate measures used in the general population are increasingly being applied to those with IMID, and reflect the various stages in the development of CVD; from early (potentially reversible) changes such as endothelial dysfunction, to arterial stiffness and thickening, and finally to structural and functional impairment of the heart. The use of CMR is an increasingly attractive option for examining CVD in IMID as it can offer information on many aspects of CV health in one examination; from arterial stiffness, cardiac function, chamber dimensions, perfusion defects and focal and diffuse fibrosis. The CMR examination is not dependent on adequate imaging ‘windows’ as in echocardiography studies, and lacks the ionising radiation

received in CT studies, or physical risks associated with invasive angiography. Most studies have reported the use of one or two surrogate markers of CVD, and therefore comparisons across all the surrogate measures are difficult due to cohort and disease heterogeneity. There are a paucity of data in IMID that combines multi-modality assessments of CVD, which would not only provide insight into the pathogenesis but may also help inform us of the disease phenotype most at risk of developing CVD.

2.8.1 Key messages

1. Atherosclerosis is a complex inflammatory process involving both the innate and adaptive immune system
2. The risk of CVD is increased in IMID
3. SSc-cardiomyopathy is prevalent and a major cause of morbidity and mortality
4. Surrogate measures of CVD can inform of CV risk in RA

Chapter 3 Primary myocardial disease in scleroderma – a comprehensive review of the literature

This chapter presents the results of a comprehensive literature review on myocardial disease in systemic sclerosis (SSc), which subsequently informed the UK Systemic Sclerosis Study Group (UKSSSG) best practice guidelines for the detection and management of myocardial disease in SSc in the UK.

3.1 Introduction

Cardiac disease in SSc carries a significant mortality risk but the pathogenesis is poorly understood. This is compounded by the difficulty in sometimes differentiating primary myocardial disease (herewith termed SSc-cardiomyopathy) from that of macrovascular cardiovascular disease.

The manifestations of SSc-cardiomyopathy are varied; includes myocarditis, conduction abnormalities, left ventricular (LV) dysfunction and pericardial effusions, and is a consequence of a pathological process distinct from pulmonary vasculopathy and its associated indirect cardiac effect. The SSc disease phenotype most at-risk of myocardial involvement and the tools best to detect and monitor for such disease are relatively under explored, leaving a lot of uncertainty in the management of the patient with SSc.

Expert bodies and specialist groups recognise these concerns. Recently, the UK Scleroderma Study Group initiative, (UKSSSG) producing the expert consensus best practice management for SSc, commissioned best practice guidelines for the detection and management of myocardial disease in SSc in the UK. The purpose of this comprehensive literature review was to inform these guidelines with a focus on the prevalence and nature of myocardial disease in SSc (or SSc-cardiomyopathy), its associated mortality and prognosis, and associations with clinical and serological phenotype. This would in addition serve to highlight knowledge gaps that could be used to determine a future research agenda.

3.2 Methods

This section outlines the remit of the literature search, the methods in which it was carried out, including the eligibility criteria.

3.2.1 Remit of literature search

The first meeting of the UKSSSG cardiac study group outlined the requirements for the literature search; namely to answer the following questions;

1. What is the prevalence of primary myocardial disease in SSc, and how does it present?
2. To what extent is primary myocardial disease associated with greater mortality in SSc?
3. Is primary myocardial disease associated with a specific disease subtype or serology?
4. Do poor prognosis SSc clinical features associate with increased evidence of primary myocardial disease?

3.2.2 Literature search

In September 2012, EMBASE, MEDLINE, and the Cochrane database were searched from 1946 with the help of an experienced librarian. The search terms 'scleroderma', 'systemic scleroderma' and 'CREST' were used, in combination with the terms 'myocardial', 'cardiac', 'cardiovascular', 'conduction defect' or 'arrhythmia'. MeSH terms and subject headings were used for all. The references of relevant and recent reviews, and the ACR, EULAR and Scleroderma World Congress abstracts from 2010 to 2012, were checked to ensure no other original relevant articles were missed. Therapeutic intervention studies were outside of the remit of the search.

3.2.3 Eligibility criteria and data extraction

One author (LA Bissell) screened the results and undertook detailed review. Any ambiguity was resolved following discussion with MH Buch.

3.2.3.1 Types of studies

Studies not published in English, not in adults and non-original articles were not included. Existing meta-analyses and SLRs were obtained. Where research questions were not covered by systematic reviews, original studies were found. Unless providing any additional information, publications already included in described meta-analyses/systematic reviews were excluded to avoid repetition. Studies were dismissed if not enough detail was

supplied for a thorough analysis. Case series were excluded. To improve the validity of the case study analysis, publications referring to therapeutic trials, sine scleroderma or scleroderma in specific cohorts such as women with breast implants or miners were excluded as they may have provided a biased perspective.

3.2.3.2 Study details

Studies with the following were excluded (1) Reports not covering scleroderma and cardiac disease (2) Articles purely referring to macrovascular disease (3) Genetic studies (4) Publications referring to the use of soluble biomarkers due to their relative lack of use in rheumatological practice; this included N Terminal-pro-Brain Natiuretic Peptide (NT-pro-BNP) addressed by a separate UKSSSG guideline working group in its role in the assessment of PAH (5) Less routinely used (nuclear medicine) imaging techniques (including scintigraphy, radionucleide ventriculography, SPECT and thallium studies), and angiography (specific to the assessment of macrovascular heart disease) (6) (Unless stated) Studies reporting less than 60 patients were excluded, however, due to the paucity of data, studies of 30 or more patients were considered for the CMR, conduction and autonomic analyses.

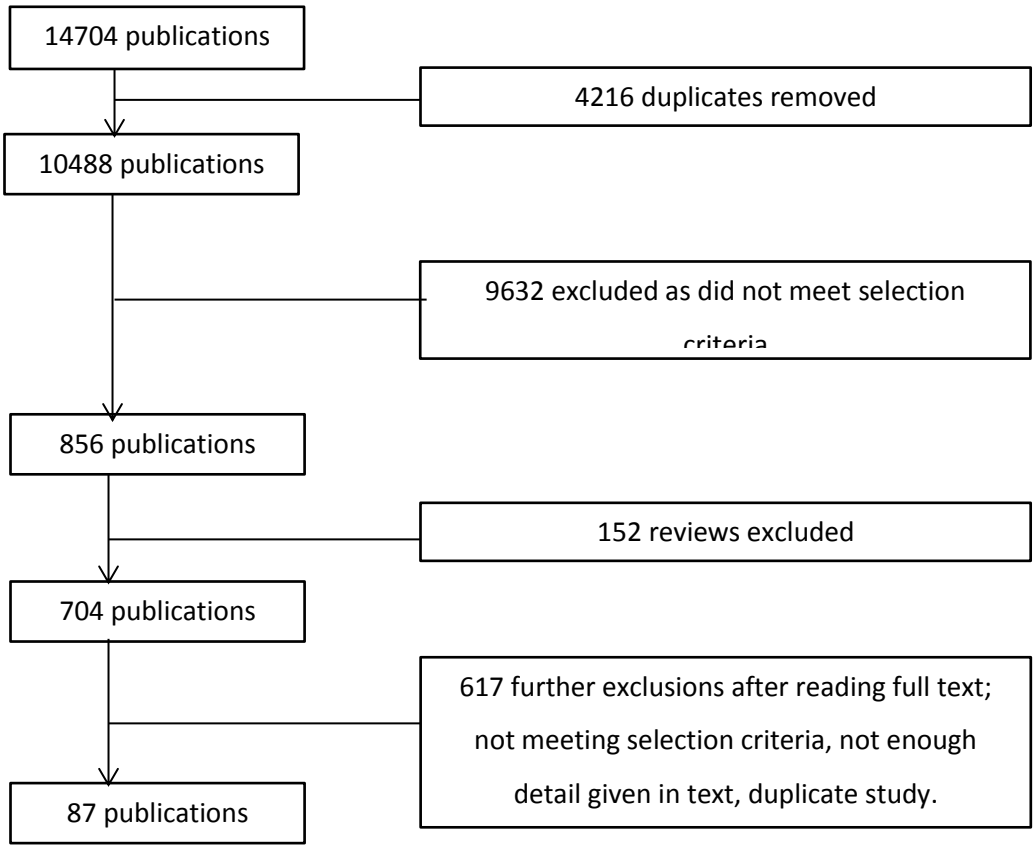
In an effort to minimise the inclusion of undifferentiated/other connective tissue diseases (CTDs), case studies reporting the prevalence of cardiac disease were only included if patients achieved the 1980 ACR criteria for SSc [383] (if published post 1980) or LeRoy criteria for SSc [384]; a criterion applied to echocardiography studies also.

Publications were dismissed if another from the same centre was already included, in order to avoid duplicate reporting, with the most recent and largest cohort kept.

3.3 Results

Figure 3-1 summarises the literature search. Many studies did not meet the criteria, either referring to children or genetics, with many publications pertaining to neural crest (identified as CREST was a search term). At the end of the process, 87 publications remained for analysis.

Figure 3-1 The literature search process



3.3.1 What is the prevalence of primary myocardial disease (PMD) in SSc, and how does it present?

3.3.1.1 Data from case studies

The literature search detected 18 cross-sectional and prospective case studies (see Table 3-1) describing 4854 patients with SSc. The prevalence of cardiac involvement differed greatly across the studies, from 7 to 39% [7, 8] although there was significant heterogeneity in the definitions of cardiac disease given.

Most notable publications included Ferri et al who described 30% of 1012 Italian patients with SSc [56% with limited cutaneous SSc (lcSSc), mean (SD) disease duration 5.1 (7.3) years] had at least one of the following symptoms: pericarditis, congestive heart failure, severe arrhythmias and/or atrioventricular (AV) conduction abnormalities at diagnosis, rising to 35% at follow-up (mean (SD) duration 7.1 (5.7) years) [331]. A Hungarian study reported 33% of a cohort of 114 patients with SSc [70% lcSSc, 30% diffuse cutaneous SSc (dcSSc), mean disease duration 10.7 years] had cardiac involvement defined as AV and intra-ventricular conduction disturbances, signs of myocardial ischaemia on electrocardiograph (ECG), congestive heart failure (CCF) (with normal fundus by ophthalmoscopic examination), and pericarditis (without uraemia) (all evaluated using clinical symptoms, ECG and occasionally echocardiography) [385]. A more modest prevalence was found in a 211 cohort of Japanese patients where only 7.1% of patients with SSc (55% lcSSc, mean disease duration 7.4 years) had cardiac involvement; defined as any of the following: symptomatic pericarditis, clinical evidence of left ventricular (LV) CCF, or arrhythmia requiring treatment[7]. Sampaio-Barros et al defined cardiac involvement as developing CCF, arrhythmia or a conduction defect requiring treatment (11.7% of 947 SSc patients affected) [386], whereas Riccieri et al also included pericarditis in the definition (18% of 92 SSc patients affected) [387].

Only one study referred to the modified Medgser severity scale (see Table 3-2) [388], which classifies severity of organ involvement (Table 3-2), and is predictive of mortality [389]. The study reported 12.6% of 103 patients (66% lcSSc, median disease duration 7 years) had mild cardiac involvement, 7.8% moderate, 1.9% severe and none had end-stage cardiac disease [339]. Steen and Medgser reported 15% of 913 patients with dcSSc in a prospective study developed severe heart disease which they defined as cardiomyopathy with decrease in LVEF and symptoms of CCF, symptomatic pericarditis (pericardial pain),

cardiac decompensation from effusion, or arrhythmia attributable to SSc requiring treatment [330]. They usefully also commented that those who developed severe cardiac involvement were more likely to do so in the first three years of disease onset, in line with their observations of severe skin and renal involvement; an occurrence not apparent with pulmonary or severe gastro-intestinal (GI) disease.

Where details were given, there was variation in the nature of cardiac disease reported. Palpitations were commonly recorded [10], along with pericarditis and pericardial effusions [9, 390]. Many studies reported abnormalities in conduction, from bundle branch block [10, 391] to arrhythmias requiring intervention [9]; others merely reported any abnormality in ECG [327, 392, 393]. Further manifestations included cardiomegaly [10, 391], diastolic dysfunction [328, 394] and abnormal LV ejection fraction (LVEF) [328, 394]. A Spanish prospective cohort determined that of 78 patients (71% lcSSc, mean disease duration 8.3 years), 18% developed cardiomyopathy and 9% pericarditis (both confirmed on echocardiogram), 12% atrial fibrillation (AF), 3% supraventricular tachycardia (SVT) requiring ablation, 2% ventricular tachycardia (VT), 1% left bundle branch block (LBBB), 8% right bundle branch block (RBBB) and 4%, 3%, and 3% developing first, second and third degree (atrio-ventricular) AV block respectively [9].

No case study differentiated SSc-cardiomyopathy from (coronary artery) macrovascular disease when reporting cardiac morbidity; cardiac manifestations may be a result of primary myocardial pathology but may also be a consequence of atherosclerotic ischaemic heart disease (IHD). Eloranta et al actually included present or previous cardiac infarction in their definition of cardiac involvement, along with heart failure, pleuritis or arrhythmia, when they reported 39% of their 70 SSc patient cohort having cardiac involvement [8]. In an ideal study, all patients with known IHD or key cardiovascular risk factors would be excluded (or at least separately reported) for a more accurate portrayal of the prevalence of primary myocardial disease in SSc.

Table 3-1 Case studies describing the prevalence and nature of cardiac involvement in Systemic Sclerosis

Population	**Study type**	**n**	**Age~***	**Female**	**LcSSc/ DcSSc**	**Disease duration ~**	**Antibody present**	**Cardiac involvement**		**Reference**
			years	%	%	years	%	Definition	%	
Sweden	CSS	100	At disease onset 41 lcSSc 48 dcSSc~~	67	63/37	3~~ (not defined)	64 ANA	Cardiomegaly on radiograph Abnormal ECG: non-specific ST or T wave changes, bundle branch block, hemiblock or myocardial infarction pattern	26 29	[391]
Iraq	CSS	75	Peak age incidence: 20-39	89	NR	NR	67 ANA	Abnormal ECG (not defined)	4	[327]
Spain	PS	78	59.8 (13.3) at disease diagnosis	79	71/30	8.3 (10.6) from Sx onset to diagnosis with 6.6 (4.7) follow-up	97 ANA 67 ACA 17 Scl70	Pericarditis (confirmed on echocardiogram) Cardiomyopathy (confirmed on echocardiogram) AV block; 1st, 2nd, 3rd degree LBBB, RBBB, Bilateral BBB Arrhythmias; AF, SVT requiring ablation, VT	9 18 3, 3, 4 1, 8, 1 12, 3, 2	[9]
Japan	CSS	211	51 (range 12-83)	91	55/45	7.4 not defined	87 ANA 29 ACA 32 Scl70	Any of following: symptomatic pericarditis, clinical evidence of LV congestive heart failure, or arrhythmia requiring Rx	7.1	[7]

UK	PS	280	45.7 (13.7)	77	53/47	17 (7.0-38.5)# from skin involvement onset to baseline	86 ANA 17.8 ACA 17.9 Scl70	Abnormal ECG (not defined) at baseline	10.2	[392]
Italy	CSS	103	54.3 (13.6)	88	66/34	7 (1-46)~~ not defined	53 ACA 39 Scl70	Medsger severity scale used to determine presence or not Mild, moderate, severe and end-stage	22.3 12.6, 7.8, 1.9, 0	[339]
Hungary	CSS	114	51.5 (13.3)	91	70/30	10.7 (8.7) not defined	NR	Defined as: AV and intraventricular conductory disturbances, signs of myocardial ischaemia on ECG, congestive heart failure (with normal fundus by ophthalmoscopic examination), and pericarditis (without uraemia)- all evaluated using clinical symptoms, ECG and occasionally echocardiography.	33.3	[385]
Sweden	CSS	70	60 (range 19-93)	81	77/23	9 (0–34)~~ not defined	34 ACA 13 Scl70	Present or previous cardiac infarction, heart failure, pleuritis or arrhythmia	39	[8]
Italy	PS	1012	50.5 (13.8) at enrolment	89	56/44	From disease onset at: Enrolment: 5.1	39 ACA 36 Scl70	At least one of the following symptoms: pericarditis, congestive heart failure, severe arrhythmias and/or AV conduction abnormalities:	30	[331]

						(7.3) Follow-up: 12.2 (9.0)		At baseline At follow-up	35	
China	CSS	100	Range 17-68	89	15/85^	UK	NR	Palpitations Cardiomegaly on XR (performed in 52 patients) Abnormal ECG (performed in 27 patients) (not defined but included LV hypertrophy, significant ST-T changes and/or arrhythmias as premature beats and RBBB	44 27 37	[10]
Canada	PS	237	47.1 + 14.1 at enrolment	83	57/43	3.8 (5.2) at enrolment	NR	According to the modified Medsger criteria	8.9	[395]
USA	CSS	210	Not given	UK	NR	UK	NR	Acute pericarditis Chronic pericardial effusion Other cardiac features (if present) not reported as not intent of publication	1.9 5.2	[390]
Hungary	PSS	131	55.9 (11.7)	90	69/31	8.1 (7.2) from 1st non-RP symptom	NR	Diastolic dysfunction LV ejection fraction ≤50%	51.9 3.1	[328]
Italy	CSS	62	52.8	92	44/56	9.1 not defined	40 ACA 32 Scl70	Pericarditis, congestive heart failure, or arrhythmias requiring Rx	18	[387]

UK	PS	84	Range 21-74	75	NR	NR	NR	Abnormal ECG (not defined)	49	[393]
Brazil	PS	947	42.6 (14.1) at disease onset	88	75/25	12.9 (8.5) at onset with 9.6 (7.7) follow-up	89 ANA 22 ACA 16 Scl70	CCF, arrhythmia, conduction defect requiring Rx	11.7	[386]
Brazil	CSS	87	48.5 (11.7)		78/22	NR	NR	Abnormal LVEF	4.8	[394]
USA	PS	953	30% symptom onset when > 50yrs old	80	0/100	NR	NR	Severe heart involvement reported only: cardiomyopathy with decrease in LVEF and symptoms of CCF, symptomatic pericarditis (pericardial pain) or cardiac decompensation from effusion, or arrhythmia attributable to SSc requiring Rx	15	[330]

~values given indicated mean (SD) unless otherwise stated
~~median(range)
#median (IQR)
^LcSSc/dcSSc distinction not made, but instead limited defined as scleroderma limited to the fingers
*at time of analysis unless otherwise stated
AF, atrial fibrillation; AV, atrioventricular; CCF; congestive cardiac failure; CSS, cross-sectional study; dcSSc, diffuse cutaneous SSc ECG, electrocardiogram; lcSSc, limited cutaneous SSc LBBB, left bundle branch block; LV, left ventricular; LVEF, LV ejection fraction; NR, not reported; PS, prospective study; RBBB, right bundle branch block; RP, Raynaud's phenomenon; Rx, treatment; SD, standard deviation; SSc, Systemic Sclerosis; SVT, supraventricular tachycardia; VT, ventricular tachycardia; XR, radiograph

Table 3-2 Modified Medsger Severity Scale for cardiac involvement

Grade	Characteristics
0 (No involvement)	Normal ECG, LVEF≥50%
1 (Mild)	Conduction defects on ECG, LVEF 45-49%
2 (Moderate)	Arrhythmias on ECG, LVEF 40-44%
3 (Severe)	Arrhythmias requiring treatment on ECG, LVEF 30-40%
4 (End-stage)	Congestive heart failure, LVEF<30%

ECG, electrocardiogram; LVEF, left ventricular ejection fraction

3.3.1.2 Data from studies using investigative tools

3.3.1.2.1 Electrophysiology

Electrophysiology (EP) studies in SSc commonly report cardiac abnormalities, including conduction defects, arrhythmias and autonomic dysfunction in up to 51% of cases [359-361, 396, 397]. Notable studies include that by Draeger et al who presented the results of ECGs analysed by cardiologists in 265 patients with early SSc (disease duration of five years or less) in the GENISOS (Genetics versus Environment in Scleroderma Outcome Study) cohort [359]. Fifty-one percent of patients had abnormalities; including 2.6% with sinus tachycardia, 7.2% sinus bradycardia, 5.3% first degree AV block and 7.6% fascicular block. A Swedish study determined 28% of 110 patients with SSc had abnormal ECGs compared to 17% of 105 age/gender matched controls ($p=0.05$), with 15% suffering conduction abnormalities (5% in control ECGs, $p=<0.01$) [397]. 49 patients underwent 24 hour ambulatory monitoring; abnormal in 28% compared to 17% controls, $p=0.05$) mainly due to extra-systoles. However, the study included those with existing cardiovascular disease (CVD); 12% with IHD, 2% with permanent pacemakers and 5% with LVEF less than 50%. Attempts have been made to reduce confounding from macrovascular disease; Kostis et al described SSc patients free of CVD; 43% of 183 patients with SSc had abnormal ECGs; 31% with non-specific ST-T changes and 20% with conduction defects [396]. Twenty-four hour ambulatory monitoring in these patients found premature atrial contractions in 61%, supraventricular tachycardia (SVT) in 21%, Wenckebach AV block in 1%, complete heart block in 1% and ventricular tachycardia (VT) in 7% amongst other abnormalities [396]. Intra-ventricular conduction disturbances in SSc are clinically relevant as they have been associated with the future development of AV block and other rhythm disturbances [361].

Late ventricular potentials (LVP) are variances of QRS complexes calculated using signal averaged ECGs and are thought to represent areas of depolarisation and myocardium at risk of re-entrant arrhythmias [398]. Reports of their use in SSc have been limited. Morelli et al found 20.5% of 77 patients with SSc free of clinical IHD had evidence of LVP, compared to 3% of controls ($p=0.02$), and those SSc patients with LVP were more likely to have abnormal myocardial scintigraphy ($p=0.002$) [399]. LVP using signal average ECG gave the highest diagnostic power in determining myocardial disease (33% sensitivity and 97% specificity) compared to resting ECG, 24 hour ambulatory monitoring and echocardiography [399].

3.3.1.2.2 Echocardiography

Echocardiography is a routine tool used in the assessment of SSc, usually as a screen for pulmonary artery hypertension (PAH), and has the advantages of being widely available and of relatively low cost to perform. Echocardiography studies evaluating those with SSc demonstrate a wide range of abnormalities [365, 400-402]. Again, these patients were not necessarily free of overt IHD or PAH. Most studies reported evidence of pericardial effusions [329, 362, 403-405], with one study reporting its presence in 18% of 63 patients with SSc [404]. However, when compared to controls, the evidence for an increased prevalence in SSc is inconsistent [402, 406-408].

Many studies confirm diastolic dysfunction in SSc [328, 329, 362, 365, 394, 400-407, 409-411], independently associated with disease duration, age, coronary artery disease, and systemic hypertension [401]. A Polish study reported diastolic dysfunction in 63% of 60 SSc patients [412], whilst others have been more modest [329, 411]. E/A ratio (early to late filling peak velocity ratio of tricuspid valve), an indicator of diastolic function, also tended to be lower in SSc compared to healthy controls [403, 404, 406, 408]. Aguglia et al referred to secondary causes of diastolic dysfunction in their study of 124 SSc patients [403]. They identified conditions that could affect diastolic function; pulmonary arterial systolic pressure (PASP) greater than 45mmHg, left ventricular hypertrophy (LVH), moderate or severe pericardial effusion, valvular heart disease, chronic renal insufficiency, arterial hypertension, and coronary heart disease, and on exclusion of these patients found no difference in LVEF, left ventricular (LV) mass or Doppler variables for diastolic dysfunction, including E/A ratio. Maione et al, on the other hand, found 9 out of 27 patients with no risk factors for diastolic dysfunction, such as pericardial effusions, PAH, arterial hypertension and LVH, still had an inverted E/A ratio (compared to 20 out of 48 patients with these risk factors) [402]. Logistic regression demonstrated independence of an inverted E/A ratio from these risk factors, suggesting primary myocardial pathology as the cause.

The evidence for impaired LV systolic function in SSc is not as strong with many echocardiography studies showing no difference between SSc patients and controls [405, 406, 408, 409].

3.3.1.2.3 Cardiovascular Magnetic Resonance Imaging

CMR imaging is an increasingly valued tool in the assessment of myocardial disease in SSc [372, 375-378, 413, 414]; in particular it can provide insight into the pathogenesis of cardiac disease. Standard Cine imaging techniques and black blood T1 weighted imaging

can demonstrate cardiac structure, function, chamber volumes and mass, and the use of contrast agents can inform of areas of myocardial focal fibrosis ('late gadolinium enhancement' (LGE)). The addition of adenosine stress perfusion imaging into the CMR protocol can identify areas of myocardial hypoperfusion ('ischaemia') [15, 130].

A CMR study by Bezante et al. confirmed the presence of diastolic dysfunction [413], with a reduced E/A ratio in 50 patients with SSc free of CVD [66% lcSSc, mean disease duration 12.2 years] when compared to 31 age/sex/body surface index(BSA) matched controls in a study by Bezante et al [372]. As with echocardiography studies, systolic function does not appear to be dramatically affected in SSc [375, 376]. Bezante et al also reported reduced LVEF and right ventricular (RV) EF [372], however, this was not replicated in a German cohort of 35 SSc patients (43% lcSSc, mean disease duration 8.4 years), also free of IHD, where LVEF was similar to 34 age/sex/cardiovascular risk factors controls (61.5% vs. 63.3%, p value not significant) [375].

CMR has the advantage of being able to detect myocardial oedema and fibrosis. A French study of 52 patients with SSc (64% lcSSc, mean disease duration 6.6 years) identified myocardial oedema in 12% and focal fibrosis in 21%; predominantly in a linear pattern in the midwall of the ventricles, with sparing of the subendocardium suggesting an alternative pathological cause to macrovascular IHD. LGE was worse with increasing SSc disease duration ($r=0.3, p<0.05$) [378]. A smaller Greek study demonstrated similar results but with 66% having evidence of LGE; again the changes were linear, seen in the mid-wall, sparing the subendocardium, within the basal and mid-cavity segments of the LV. In line with the previous study, LGE was worse in those with RP greater than 15 years ($p=0.017$) [377]. Interestingly there was a correlation with abnormal 24 hour ambulatory ECG monitoring ($p=0.035$).

Stress perfusion CMR can detect small perfusion defects in SSc. 79% of 62 Mexican SSc patients (53% lcSSc, mean disease duration 9.7 years) had evidence of subendocardial perfusion defects [376]. LGE was also identified in 45% (distribution: 18% patchy, 36% in bands, 11% subendocardial, 29% mixed, 7% transmural) [376].

3.3.2 To what extent is primary myocardial disease associated with greater mortality in SSc?

There are many studies describing mortality in SSc, but few teasing out the impact of cardiac disease. A large EUSTAR publication of 5860 SSc patients revealed 14% of deaths

were due to myocardial disease (subdivided into arrhythmias, left or right heart failure and biventricular heart failure, and pericarditis), and 12% secondary to cardiovascular disease (deemed non-SSc related) [341]. Although, this approach is helpful in determining the impact of primary myocardial disease, it may be difficult to determine SSc-related arrhythmias from IHD-related arrhythmias. A meta-analysis by Komosci et al described 18 studies comprising of 12829 patients (84% female, 38% dcSSc); 19.7% died of cardiac causes; 10% due to SSc-related cardiac disease [11]. A smaller meta-analysis, comprised of 2691 SSc patients, reported 29% of deaths were due to cardiac disease [313]. Six smaller studies have since reported cardiac-related disease causing 1.4-24.5% of deaths in SSc patients, with many lacking any definition of cardiac disease [386, 401, 415-418].

3.3.2.1 Cardiac manifestations associated with a greater mortality.

Cardiac disease in SSc is associated with a greater risk of mortality. Komosci et al determined an increased risk of death in those with cardiac disease; defined as pericarditis verified by echocardiogram, recurrent arrhythmia and/or conduction abnormality on ECG, or clinical signs of heart failure (hazard ratio (HR): 3.15, 95% confidence interval (CI): 2.33, 4.26) [11]. The GENISOS study also reported mortality was increased in those with fascicular block, independent of non-SSc related cardiac risk factors (HR 2.1; 95% CI 1.02, 4.28, p=0.04) [359]. Kostis et al determined that SVT and ventricular ectopic activity, including the presence of VT, was strongly associated with increased mortality in SSc; VT remaining significantly associated after multi-variate analysis, along with hepatojugular reflux, antiarrhythmic therapy, reduced forced vital capacity, serum creatinine level, increased intensity of pulmonic second sound, chest radiographic evidence of interstitial lung disease and increasing patient age [396]. Hinchcliff et al confirmed in 153 SSc patients, diastolic dysfunction (baseline tissue Doppler E' velocity) was independently associated with an increased risk of death (HR 3.2, 95% CI 1.1, 9.5, p=0.034 per each SD decrease in tissue Doppler E' velocity), along with age, disease duration, gender, interstitial lung disease (ILD) and PAH [401].

3.3.3 Is primary myocardial disease associated with a specific disease subtype or serology?

3.3.3.1 Disease subtype

Cardiac involvement is reported in both lcSSc and dcSSc subtypes although more frequently in those with dcSSc (see Table 3-3) [332, 333, 419-421]. In the study by Ferri et al, 23% of

lcSSc and 32% of dcSSc (p=0.05) had cardiac involvement at diagnosis [331]. There was a larger proportion of males in the dcSSc group as might be expected (2.8 female to male ratio vs. 12.3 female to male ratio in lcSSc, p=0.001) which may be a confounding factor, but the much shorter disease duration (mean (SD) 2.2 (4.0) years vs. 7 (9.4) in lcSSc) may re-affirm the aggressive presentation of dcSSc. The study also found patients with dcSSc were more likely to have oesophageal, lung and renal involvement than lcSSc patients. An increased risk of cardiac disease in dcSSc was also reported in the German Network for Systemic Scleroderma (DNSS) cohort; 23% of dcSSc compared to 12% of lcSSc had cardiac involvement (p<0.0001). When this figure was dissected there was similar prevalence of palpitations (20% dcSSc vs.23% lcSSc), but diastolic dysfunction (19% vs. 15% in lcSSc) and conduction block (19% vs. 11% in lcSSc) were more common in dcSSc [332]. Again a higher proportion of men in the dcSSc group may explain this difference. Considering the European EULAR Scleroderma Trials and Research (EUSTAR) study of 3450 patients, only the subjective complaint of palpitations was more common in dcSSc (27% vs. 23%, p=0.003), with a similar prevalence of conduction block and diastolic dysfunction; again more men and a shorter disease duration seen in dcSSc [422].

3.3.3.2 Serology

Studies evaluating the impact of serology on the prevalence of cardiac disease in SSc compare both those antibody positive to those antibody negative [423-429], and across antibody groups [430-433], again with varying definitions of cardiac involvement (see Table 3-4).

The German DNSS cohort determined a non-statistically significant trend for a greater prevalence of conduction disturbances and abnormal ECGs in those positive rather than negative for anti-Scl70 antibodies (49% vs. 38% respectively, p=ns) [424], although not confirmed in a Danish study of 230 patients who found no difference between antibody positive and negative groups [426]. Anti-Ku antibodies were associated with cardiac involvement in a Mexican study of 139 patients; 50% in those positive compared to 7% in those negative (p=0.04) [429]. There is also some evidence for an increased risk of cardiac involvement for those with anti-Histone [425] and anti-RNA polymerase (I, II and III) antibodies [427].

Comparing across the antibody groups, the Pittsburgh Scleroderma Databank determined of 963 patients those with anti-Scl70 and anti-U3 RNP antibodies had a higher prevalence of severe heart involvement (definition as used in Steen et al [330]) (16% and 18%

respectively) compared to anti-centromere (ACA) (4%), RNA polymerase III (7%), anti-U1 RNP (11%), anti-PmScl (6%) and anti-Th/To (7%) antibodies ($p<0.01$ ANOVA) [433]. Conversely, a large UK cohort of 1966 SSc patients reported no significant differences in prevalence of cardiac involvement (not defined) across anti-ACA, anti-Scl70, anti-RNA polymerase III, anti-U1RNP and anti-U3 RNP antibody groups [432]. A smaller study by Ceribelli et al determined of 216 SSc patients, those with anti-Th/To antibodies had a greater prevalence of pericarditis than those with anti-ACA antibodies (4.5% and 25% respectively, $p=0.058$) [430].

3.3.3.3 Data from studies employing investigative tools

Few EP studies have further dissected association of abnormalities by disease subtype or serology. The GENISOS study found no association of ECG abnormalities with disease subtype or serological profile [359], similar to findings by Nordin et al [397]. However, Kostis et al determined those with dcSSc were more likely to have episodes of SVT and VT [396], and LVP have been associated with dcSSc (30% vs. 9% lcSSc, $p=0.04$) [399]. There was no significant difference in diastolic dysfunction on echocardiogram between lcSSc and dcSSc in a large 570 patient study (74% lcSSc, mean disease duration 9 years) in France [400].

As expected, given increased likelihood of developing PAH, RVEF was reduced more in patients with lcSSc than dcSSc in the CMR study by Bezante et al ($p=0.03$) [372]. This was not replicated in the French study of 52 patients, where no difference in RVEF was detected between the subtypes; however, they did report a greater frequency of those with lcSSc had impaired LVEF compared to dcSSc (34% vs. 5%, $p=0.02$) [378]. The basis for this finding was not clear. The evidence is inconsistent for an increase in fibrosis detected by CMR in dcSSc [376, 377, 414].

Table 3-3 Case studies describing the prevalence and nature of cardiac disease per disease subtype in Systemic Sclerosis.

Study population	**Type of study**	**n**	**LcSSc/ DcSSc**	**Age ~ LcSSc/ DcSSc**	**Female LcSSc/ DcSSc**	**Disease duration ~ LcSSc/ DcSSc**	**Antibody present LcSSc/ DcSSc**	**Cardiac involvement**		**Reference**
			%	**years**	**%**	**years**	**%**	**Definition**	**% LcSSc/ DcSSc**	
Spain	CSS	413	NR	NR	NR	NR	NR	Clinical manifestations, alterations in echocardiography, stress myocardial perfusion SPECT, cold-induced myocardial perfusion SPECT, coronary arteries catheterization, CXR and ECG	45% in total: no difference between dcSSc, lcSSc or sine SSc	[434]
Italy	PS	1012	56/32	NR	88/76	At diagnosis: 7(9.4)/2.2(4)	ACA 53/11.3 Scl70 25.3/58.6	At least 1 of the following symptoms: pericarditis, congestive heart failure, severe arrhythmias and/or AV conduction abnormalities at diagnosis	23/32 (p=0.05)	[331]
USA	Case control	17 CREST 17 PSS	50/50	49.5 (10.9)/ 48.2 (10.9)	94/94 matched	12.0 (9.3)/ 11.2 (9.2)** matched	ANA 56/82%	Cardiomegaly on CXR Pericardial effusion Abnormal ECG	29/35 19/25 29/24 No significant	[435]

				matched					difference	
Germany	CSS	1158	58/42	58.5(12.7)/54.1(14.1)	87.8/76.1	Expressed as age at: RP onset 44.7(15.8) /44.5(15.2) Skin involvement 49.9 (14) /46.4(14.3)	ANA 92.9/93.9 ACA 61.5/11.2 Scl70 16.2/55.8	1 of the following: palpitations, conduction disturbance and diastolic dysfunction on echocardiogram. Sub-analysis: Palpitations Conduction block Diastolic dysfunction	12/23 (p<0.0001) 23.1/20.1 10.8/19.2 14.9/19	[332]
Serbia and Montenegro	CSS	105	48/52	54/52	UK	5.2/5.4 (not defined)	NR	Arrhythmia or myocardial ischaemia detected by ECG or myocardial fibrosis and pericarditis detected by echocardiogram	8/29 (p<0.001)	[419]
USA	PS	212	19/81	36 (5-71)/47 (3-79)***	80/74	NR	ACA 0% Scl70 100%	One or more of: LVEF <45%, left sided CCF, pericarditis, arrhythmia requiring Rx or complete heart block.	18/30	[436]
Iran	CSS	58	60/40	41.3 (14)/40.3 (13.4)	92/83	8.41/7.35	NR	One or more dichotomous variables, i.e. presence or absence of cardiomegaly, pericardial effusion, ventricular	20.6/17.6 (p=0.96)	[329]

								arrhythmias, conduction disturbances, axis deviations, + pathological Q waves Sub-analysis; Conductive disturbance, left axis deviation, arrhythmia, cardiomegaly (on CXR, echocardiography or ECG), and pericardial effusion.	14.7/8.7, 14.7/4.3, 2.9/4.3, 11.8/25, 14.7/16.7	
Spain	CSS	811	62/27	45.9 (15.6)/ 43.8 (15.4) at disease onset	89/83	NR	ACA 58/8.4% Scl70 9.4/52.7%	One or more of: pericarditis, ischaemic cardiomyopathy with no known cause, reversible thallium perfusion defects after cold stimulation, any disturbance on colour-Doppler echocardiography, ECG alterations with no other cause, LVEF< 50%, or RVEF < 40% on echocardiography or radionuclide ventriculography. Pericarditis Ischaemia Conduction alteration	31.1/32.5 3.4/7.8 (p=0.01) 10/8.2 (p=ns) 11.9/10.3(p=ns)	[420]
Greece	CSS	238	49/45	41.8	99/87	11.5 (0.9)/	ACA 34/0%	Conduction disturbances +/or nodal or	7/21 (p=0.0025)	[421]

				(1.3)/ 38.4 (1.4) at onset~~		9.1 (0.8) with 2.5(0.34)/ 3 (0.34) follow-up~~	Scl70 21/60%	ventricular arrhythmias, congestive heart failure not attributable to any other condition and/or moderate to severe pericardial effusion on echocardiogram.		
EUSTAR	PS	3450	57/37 (remaining6% overlap)	57.4(13.1)/ 52.3(13.7)	81/91	9.6(8.1)/ 7.4(6.9)*	ACA 47/6% Scl70 23/61%	Palpitations Conduction block Diastolic dysfunction Reduced LVEF	22.6/27.3(p=0.003) 10.4/12.7(p=0.12) 15.4/16.6 (p=0.42) 5.0/7.2 (p=0.59)	[333]
Mexico	CSS	139	57/43	47.4 (14.3)/42.7 (13.8)	99/87	13.2 (11)/7.9 (6) (first symptom attributed to SSc)	ANA 100/100 ACA 37/20 Scl70 15/45	Left sided congestive heart failure (FEVI<45%) or pericarditis on echocardiogram or CMR, arrhythmia requiring treatment or conduction defect	7/16 (p=NS)	[429]
USA	Retrospective cohort	398	47/31	51.8 (13.9)/46.7 (14.2)	100/100	NR	ANA 95/92 ACA 47/14 Scl70 15/18	Pericarditis	3.3/7.6 (p=0.05)	[437]

~values indicate mean (SD) unless otherwise stated
~~values indicate mean (standard error)
*Onset from first non-RP feature

**from first symptom
***age at first symptom attributable to SSc
AV, atrioventricular; CSS, cross-sectional study; CXR, chest radiograph; dcSSc, diffuse cutaneous SSc; ECG, electrocardiogram; lcSSc, limited cutaneous SSc LBBB, left bundle branch block; LV, left ventricular; LVEF, LV ejection fraction; NR, not reported; ns, non-significant; PAH, pulmonary artery hypertension; PS, prospective study; RBBB, right bundle branch block; RP, Raynaud's phenomenon; RVEF, right ventricular ejection fraction; Rx, treatment; SD, standard deviation; SSc, Systemic Sclerosis; SE, standard error; XR, radiograph

Table 3-4 Case studies describing the prevalence and nature of cardiac disease across serological subtypes in Systemic Sclerosis.

Study population	**n**	**1980 ACR/ LeRoy met**	**Anti-body, n (%)**				**Female %**	**Age, years~**	**Disease duration, years~**	**Cardiac involvement**		**Reference**
			ANA	**ACA**	**Scl70**	**Others**				**Definition**	**%**	
Denmark	230	yes	196 (85)	78 (34)	30 (13)	15 (6.5) Anti-U1 RNP 8 (3.5) Anti-U3 RNP 5 (2.2) Anti-Th RNP	82	59 (46, 86)~~	11 (5, 19)~~ from first SSc related symptom	Clinical or ECG abnormalities in the absences of other causes	ACA: 5 Scl70: 10 Anti-U1 RNP: 0 Anti-U3 RNP: 0 Anti-Th RNP: 0 ANA –ve: 11 P=ns vs. Ab and ANA -ve	[426]
UK	1966	yes	1654	618	683	Anti-RNA poly III: 77 Anti-U1 RNP: 102 Anti-U3 RNP:	82	54.2 (14.1)	NR	Not defined	ANA: 10.9 ACA: 9.1 Scl70: 12.4 Anti-RNA poly III: 6.4	[432]

						38					Anti-U1 RNP: 11.8 Anti-U3 RNP: 13.2 No difference between groups	
Italy	216	yes	NR	67 (31)	81 (38)	Anti-Th/To: 8 (4)	F:M ratio ACA: 66.1 Anti-Th/To 5.3	ACA: 66.6 (10.1) Anti-Th/To 54.5 (17.9)	ACA: 8.7 (5.9) Anti-Th/To 8.5 (6.5) (not defined)	Pericarditis	ACA: 4.5 Anti-Th/To: 25 p=0.028 between groups	[430]
Brazil	85	y	93	26 (31)	27 (32)	Anti- RNA poly III: 35 (41)	92	ACA: 54.6 (10.5) Scl70: 45.8 (12.5) Anti-RNA poly III:	ACA: 23 Scl70: 7 Anti- RNA poly III: 10 (not defined)	Palpitations Cardiac conduction blocks	ACA: 35, Scl70: 15, Anti- RNA poly III: 20, p=0.07 across groups ACA: 24, Scl70: 4, Anti- RNA poly III: 9, p=0.05 across groups	[431]

								47.2 (12.8)		Reduced LVEF Diastolic dysfunction	ACA: 8, Scl70: 4, Anti- RNA poly III: 12, p=ns across groups ACA: 38, Scl70: 28, Anti- RNA poly III: 33, p=ns across groups	
USA	963	yes		291 (30)	318 (33)	RNA poly III: 120 (12.5) Anti-U1 RNP: 71 (7.4) Anti-U3 RNP: 55 (5.7) PmScl: 36 (3.7) Anti-Th/To: 72 (7.5)	ACA: 92 Scl70: 73 RNA poly III: 81 Anti-U1 RNP: 79 Anti-U3 RNP: 71 PmScl: 81 Anti-	NR	ACA: 20 Scl70: 16.3 RNA poly III: 11.3 Anti-U1 RNP: 16.5 Anti-U3 RNP: 12.0 PmScl: 14.3 Anti-Th/To: 16.3 (not	Severe heart involvement reported only: cardiomyopathy with decrease in LVEF and symptoms of CCF, symptomatic pericarditis (pericardial pain) or cardiac decompensation from effusion, or arrhythmia	ACA: 4 Scl70: 16 RNA poly III: 7 Anti-U1 RNP: 11 Anti-U3 RNP: 18 PmScl: 6 Anti-Th/To: 7, $p<0.01$ by ANOVA for Anti-U3 RNP and Scl70	[433]

							Th/To: 81		defined)	attributable to SSc requiring Rx		
Mexico	139	84%	139 (100)	41 (30)	39 (28)	RNA poly III: 2 (1) Anti-U1 RNP: 15 (11) PmScl: 12 (9) Anti-Ku: 14 (10)	93.5	45 (14.2)	NR	Left sided congestive heart failure (FEVI<45%) or pericarditis on echocardiogram or CMRI, arrhythmia requiring treatment or conduction defect	Anti-Ku 50% vs. 7% if anti-Ku -ve, p=0.04 Other Abs not associated	[429]
Sweden	276	99.6%	232 (84)	51 (19)	26 (9)	Anti-U1 RNP: 59 (21) Anti-RNA poly (I, II, III): 60 (22) Anti-histone: 44 (16)	74	ACA: 48.8 Scl70: 48.8 Anti-U1 RNP: 45.1 Anti-RNA poly (I,	NR	Abnormal ECG Cardiomegaly	ACA: 66, Scl70: 48, Anti-U1 RNP: 48 Anti-RNA poly (I, II, III): 66, Anti-histone: 74* ACA: 10, Scl70: 28, Anti-U1 RNP: 17 Anti-RNA poly (I,	[425]

								II, III): 49.8 Anti-histone: 48.9			II, III): 22, Anti-histone: 28 *p<0.05 vs. Ab -ve	
Italy	105	92%	104 (99)	18 (17)	70 (67)	NR	NR	NR	ACA: 17.5 (7.4) Scl70: 11.2 (10.2) from first manifestation	Myocardial ischaemia or necrosis (by ECG or scintigraphy) Conduction defects Arrhythmias	ACA: 11, Scl70: 19 ACA: 17, Scl70: 23 ACA: 0, Scl70: 6 P=ns vs. Ab -ve	[428]
Japan	275	yes	NR	44 (16)	68 (26)	RNA poly (I, II, III): 14 (5) Anti-U1 RNP: 67 (27) Anti-U3 RNP: 10 (4) Anti-Ku: 7 (3) Anti-Th: 5 (2)	ACA: 100 Scl70: 90 RNA poly (I, II, III): 43 Anti-	ACA: 49 (11) Scl70: 90 (43 (14) RNA poly (I, II, III):	ACA: 5.5 (4.4) Scl70: 2.3 (2.7) RNA poly (I, II, III): 0.7 (0.5) Anti-U1 RNP: 3.5	Symptomatic pericarditis, clinical evidence of LV congestive heart failure not attributable to any other condition, or conduction defect or	ACA: 2, Scl70: 9, RNA poly (I, II, III): 50,* Anti-U1 RNP: 3, Anti-U3 RNP: 10, Anti-Ku: 0, Anti-Th: 0, *p<0.0001 vs. Ab -ve	[427]

							U1 RNP: 91 Anti-U3 RNP: 90 Anti-Ku: 100 Anti-Th: 80	51 (13) Anti-U1 RNP: 38 (11) Anti-U3 RNP: 36 (10) Anti-Ku: 30 (9) Anti-Th: 38 (13)	(2.2) Anti-U3 RNP: 2.2 (1.3) Anti-Ku: 1.5 (1.2) Anti-Th: 4.7 (4.8) from diagnosis	arrhythmias requiring treatment		
Germany	280	Not all - DNSS study		NR	67 (24)	NR	F:M ratio 243:37	56 (13.2)	7 (7.38) from diagnosis	2 of the following symptoms: diastolic dysfunction, conduction abnormalities, cardiomyopathy, reduced LVEF, valvular changes (latter two not due to	49% vs. 38% Scl70 -ve, p=ns	[424]

										other diseases), pericarditis. Abnormal ECG Conduction disturbance	 41% vs. 22% Scl70 -ve, p=0.007 37% vs. 21% Scl70 -ve, p=0.009	
USA	2579	81%	NR	NR	NR	Anti-U3 RNP: 108 (4)	Anti-U3 RNP: +ve: 71 -ve: 81	Anti-U3 RNP: +ve: 45.2 (15.6) -ve: 50.2 (14.3)	Anti-U3 RNP: +ve:5.3 (7.6) -ve:7.6 (9.4) from symptom onset	Any one of: left-sided heart failure (clinical/LVEF< 45%), pericarditis (pericardial pain + friction rub, effusion, or ECG evidence), arrhythmia requiring treatment, CHB, or CTD-related cardiac death.	23% vs. 20% Anti-U3 RNP -ve, p=ns	[423]

~values indicate mean (SD) unless otherwise stated

~~median (IQR)

Ab, antibody; ACA, anti-centromere antibody; CCF, congestive cardiac failure; CTD, connective tissue disease; dcSSc, ECG, electrocardiogram; LV, left ventricular; LVEF, LV ejection fraction; NR, not reported; ns, non-significant; Scl70, anti-topoisomerase antibody; SD, standard deviation; SSc, Systemic Sclerosis; +ve, positive; -ve, negative

3.3.4 Do poor prognosis SSc clinical features associate with increased evidence of primary myocardial disease?

3.3.4.1 Male gender

The literature is limited regarding the impact of gender on the prevalence of cardiac involvement in SSC, however, men may have a greater risk than females in line with the general population [438]. The EUSTAR cohort of 7073 SSc patients reported LVEF less than 55% was independently associated with male gender, along with age, myositis, digital ulcers, lung involvement and absence of previous treatment with calcium channel blockers [365]. Again, it is not possible to tease out confounding by macrovascular disease. LVP were not more likely to occur in men in the study by Morelli et al [399]. As alluded to earlier, some of the increased risk observed in dcSSc compared to lcSSc could be explained by an increased proportion of these patients being male [331, 332].

3.3.4.2 Ethnicity

There is limited data evaluating the impact of ethnicity on cardiac disease in SSc. In line with other clinical complications of SSc, cardiac involvement may be more prevalent in black SSc patients. A diffuse subtype and a younger age at diagnosis, along with pericarditis (OR =3.5, p=0.012 when adjusted for disease phenotype) were determined to be more common in black compared to white women in a retrospective cohort study of 514 SSc patients (47% lcSSc, 31% dcSSc, 77% meeting ACR criteria) in the US [437]. Other studies suggest no difference in prevalence across Caucasian, Asian and Hispanic groups [439, 440]; a cross-sectional US study of 165 SSc patients determined the prevalence of arrhythmias, congestive heart failure and pericardial effusions were comparative [440]. Similar results were seen in a cross-sectional study in Canada; although those of European decent were more likely to have joint and GI involvement and those of Chinese descent, anti-Scl70 and anti-Ro antibodies, there was no difference in the prevalence of cardiac involvement (defined as one of: pericarditis, cardiomyopathy, congestive heart failure or arrhythmia requiring treatment) between the two groups when matched for gender, disease sub-type and age at diagnosis [439]. Interestingly, EUSTAR reported a trend for eastern rather than western European centres to care for those palpitations (p=0.002), conduction blocks (p=0.03), diastolic dysfunction (p<0.001 adjusted for late onset of Raynaud's phenomenon (RP)) and LVEF (p=0.005) when analysing 3661 patients (62% lcSSc, 38% dcSSc) [422]. This

trend was also seen for anti-Scl70 antibody positivity, although no such association was found for disease sub-type, gender, age of first non-RP symptom, or modified Rodnan skin score (mRSS).

3.3.4.3 Age at disease onset

Increasing age at disease onset appears to be associated with a higher risk of developing cardiac manifestations. EUSTAR reported that in 1180 patients with SSc (50% lcSSc) with disease duration less than 3 years, older age was associated with cardiac conduction block, LV diastolic dysfunction ($p<0.0001$ for both) and low LVEF ($p=0.03$); all but the latter remaining significant with multi-variate linear regression [441]. A large US SSc study of 2084 patients (83% meeting 1980 ACR criteria) reported those who developed their first non-Raynaud's Phenomenon (RP) symptom at 65 years of age or older suffered cardiac disease (as defined as a score greater than one on the Medsger Severity Scale [388]) more often than those younger (odds ratio (OR) 2.69, 95% CI 1.92, 3.78 when adjusted for race, sex, disease subtype and duration, and smoking status) [335]. These findings were confirmed in a Spanish cohort (n=319) where cardiac involvement (defined by one or more of: clinical symptoms, reversible thallium perfusion defects after cold stimulation, any change on colour-Doppler echocardiography, ECG alteration, LVEF less than 50% or RVEF less than 40% on radionuclide ventriculography) was more prevalent in those aged 65 years and over at disease onset (70% vs. 50% if less than 65 years, $p=0.004$). Similar increases in cardiac involvement were seen in an Hungarian cohort (30% dcSSc) in those with disease onset older than 60 years of age than younger (55.6% vs. 31.4% respectively) [385]. All these studies again do not discriminate between IHD and SSc-cardiomyopathy.

3.3.4.4 Modified Rodnan skin score (mRSS)

The mRSS is a semi-quantitative method of scoring skin involvement in SSc with higher scores associated with worse prognosis in SSc [442], although there is little evidence for its association with cardiac disease. A large cross-sectional study suggests no association of mRSS with the occurrence of cardiac disease in SSc [338]. In this study, of 1200 patients within the German DNSS cohort (61% lcSSc, 82% met 1980 ACR criteria, mean duration of RP 14 years, mean duration since skin involvement 11 years, 33% anti-Scl70 positive) 14% had 'conduction alterations', 14% diastolic dysfunction and 22% complained of palpitations. When patients were categorised by mRSS (0 to 3, greater than 3, greater than 6 and greater than 14), there was no significant difference in the prevalence of these cardiac complaints between the groups, however differences were seen across the mRSS groups

for antibody, disease sub-type, digital ulcers and ILD, amongst other clinical features. Conversely, a smaller one year study of 55 patients found that mRSS correlated with diastolic dysfunction in dcSSc (no p-value given) and arrhythmias in lcSSc (p=0.04) [443].

3.3.4.5 Tendon friction rubs

The presence of tendon friction rubs (TFRs) is generally associated with active disease, and may also be associated with an increased risk of cardiac disease as was reported from the Pittsburgh cohort that described 1305 patents; 51% lcSSc, number meeting 1980 ACR criteria not given [336]. Those with TFRs (28% of patients) had a higher prevalence of cardiac involvement (not defined) (19% if TFR present vs. 8% with absence of TFR, p<0.0001); a sub-analysis found this distinction was only present in those with dcSSc (p=0.009) and any association was lost in those with lcSSc. As the association with cardiac involvement was an exploratory finding in this paper no attempt was made for adjustment for confounders, such as severity of skin involvement.

3.3.4.6 Nail-fold capillaroscopy

Nail-fold capillaroscopy (NFC) is a non-invasive method of evaluating the microcirculation *in vivo*, most commonly performed at the nail bed of the fingers. Specific patterns of vasculopathy have been described in SSc; early, active and late [444], and have been associated with the future development of severe organ involvement [445]. Evidence suggests it remains a poor prognostic indicator for the risk of cardiac disease [339, 387, 412]. A cross-sectional study of 103 patients with SSc (66% lcSSc, median disease duration 7 years), 39% anti-Scl70 antibody positive) suggested that a late pattern of vasculopathy was associated with an increased risk of moderate-to-severe cardiac involvement (as defined by the Medsger Severity Scale [388]) (OR 5.75, 95% CI 2.04, 16.21) [339]. This was consistent with findings from another cross-sectional 62 patient study (44% lcSSc, mean disease duration 109 months, 32% anti-Scl70 antibody positive) in which 18% had cardiac involvement (defined as pericarditis, congestive heart failure or arrhythmia requiring treatment). In this study, active and late NFC patterns were more frequently associated with cardiac involvement (21% and 29% respectively) than those with an early pattern (5%, p<0.05 for both active and late patterns) [387]. However, in a small study of 35 Turkish SSc patients (57% lcSSc, mean disease duration 9.7 years, 51% anti-Scl70 antibody positive), the prevalence of pericardial effusions did not differ between those with abnormal and normal nail-fold capillaries [446].

3.3.4.7 Quality of life measurements

A range of quality of life assessments suggest cardiac involvement is more prevalent in those with worse scores. Del Rosso et al reported Short Form 36 (SF-36) general health perception scores in 24 patients with SSc (63% lcSSc, mean duration from first non-RP symptom 8.3 years) were higher in those with cardiac involvement (50% of cohort; defined as abnormal ECG, 24 hour ambulatory monitoring or echocardiogram) ($p<0.039$) [447]. There was no difference in physical summary scores in those with and without cardiac involvement. Steen et al found that the Health Assessment Questionnaire – disability index (HAQ-DI) was higher in dcSSc patients with heart or kidney involvement (not defined) (n=74) compared with those without (n=573) (mean HAQ-DI score 1.36 and 0.68 respectively, $p<0.001$) [448]. A Japanese study (n=50, 76% lcSSc) reported the severity of cardiac involvement (based on ECG, ejection fraction and New York Heart Association (NYHA) findings) correlated with dressing, walking, hygiene, reach and activity and overall scores within the HAQ-DI (correlation co-efficient 0.50, $p<0.001$ for overall score); an association remaining significant after adjustment for skin and joint involvement and PAH ($p<0.001$) [449].

3.4 Discussion

The remit of this comprehensive review was to describe the prevalence and prognosis of primary myocardial disease in SSc and any association with clinical phenotype. SSc-cardiomyopathy is prevalent, however, the frequency of its occurrence varies greatly across studies; up to 40% in some reports. Heart failure, arrhythmias, diastolic dysfunction, pericarditis and pericardial effusions make up the majority of manifestations, with few reports of valvular pathology. The limited data available suggests its presence is associated with a significantly increased risk of mortality. This review highlights potential 'red flags' associated with cardiac disease in SSc; namely a dcSSc subtype, positive serology (in particular anti-Scl70, anti-U3 RNP, anti-Ku and anti-Th/To antibodies), black ethnicity, older age of onset, TFRs, abnormal NFC and worse quality of life scores. Also, this review demonstrates the advantages of employing non-invasive investigative techniques to detect cardiac involvement in SSc, in addition to allowing a better understanding of its pathophysiological basis.

3.4.1 Concerns

3.4.1.1 Identifying primary myocardial disease

However, conclusions are drawn cautiously. A major concern is the difficulty in differentiating primary myocardial disease morbidity and mortality from that of cardiac disease secondary to macrovascular heart disease. For example, arrhythmias could be the result of either pathology. Only if study populations are free of known macrovascular heart disease, with no or minimal risk factors for such, can a distinction be more confidently made. The cohort studies detected by the search generally referred to unselected cohorts, with the inclusion of all patients, with or without additional morbidity. Some EP, echocardiography and CMR studies did exclude those with known IHD [372, 375, 377, 396, 400, 407, 411, 450], and Tyndall et al also attempted to differentiate myocardial pathology from macrovascular heart disease in their mortality report; although no details were given as to how they made these distinctions; biventricular heart failure could occur as the result of either pathology [341]. Reporting cardiac manifestations in unselected cohorts in SSc is also confounded by the presence and complications of PAH. Not all studies reported the prevalence of such, and only one excluded these patients [362].

3.4.1.2 Heterogeneous definitions of cardiac disease

Accurate comparisons across the studies are difficult as there was great heterogeneity in the definition of cardiac involvement in SSc. Although the modified Medsger scale is available [388], few used this tool. Missing data in clinical databases many account for this; not all patients may have an ECG in their records. An example of how this inconsistency can influence the data was most notably demonstrated in a prospective study from Spain, which reported a large proportion (40%) of 204 patients had cardiac involvement (study excluded from the above review as only 67% met 1980 ACR criteria). However, the authors included PAH in its definition of cardiac disease. A sub-analysis revealed 30% had PAH whilst only 13% had pericardial effusion and 13% arrhythmias [451].

3.4.1.3 Disease phenotype inconsistencies

The SSc phenotype, including disease subtype and disease duration, varied greatly across all the studies. The ratio of lcSSc to dcSSc differed with some studies reporting only one subtype [330, 404]. Additionally, although the standard accepted definition of disease duration in SSc is time from first non-RP symptom, many studies either did not define disease duration, used time from onset of RP or time from first SSc symptom, or time from disease diagnosis. These factors consequently compromise cross study comparisons.

3.4.1.4 ACR classification criteria

The exclusion of studies if they did not meet ACR SSc or LeRoy classification criteria was done in an effort to exclude other connective tissue diseases (CTD), such as undifferentiated CTD. However, these are not diagnostic criteria, and additionally, this measure may miss those with early SSc when the disease has not fully differentiated but morbidity can still occur. The introduction of criteria to detect early [452] and very early [453] SSc reflects this unmet need to correctly identify and manage these patients. As a consequence of our strict eligibility criteria, a report from the German DNSS describing the clinical manifestations of 1483 patients of varying disease subsets (45% lcSSc, 33% dcSSc) was excluded. The 1980 ACR/Le Roy criteria were acknowledged but thought to be restrictive in such a heterogeneous disease. Their aim was to report their whole scleroderma cohort; including those with undifferentiated SSc, overlap syndrome and SSc sine scleroderma. Of the 1483 patients, 15% patients had heart involvement (12% of those with lcSSc, 23% of those with dcSSc) (defined as one of the following: palpitations, conduction disturbance and diastolic dysfunction on echocardiogram). This statistic was further dissected with 22.3% suffering palpitations (23% of lcSSc, 20% of dcSSc), 12.9% conduction blocks (11% of lcSSc, 19% of dcSSc) and 14.1% diastolic dysfunction (15% of lcSSc, 19% of dcSSc) [332].

3.4.1.5 CMR acquisition/interpretation

There is a need for a unified CMR protocol to allow cross study comparisons and appropriate translation of results/findings into clinical practice, with the avoidance of false positive changes. The detection of perfusion defects, for example, through CMR, has concerns over inconsistent CMR protocols and image quality [454]. There has recently been some work in this area with recommendations for CMR use and protocols produced by an international consensus group [455].

3.4.2 Significant relevant studies published since time of literature review

More recent publications have confirmed the increased prevalence of cardiac disease in dcSSc [456], and its association with increased mortality [457, 458]. Foochaoen et al reported 61% of 103 SSc patient with no history of cardiac disease or atherosclerotic risk factors had at least one cardiac related abnormality on ECG, echocardiography, radiographic, cardiac enzymes and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) testing, independently associated with dcSSc with increased mRSS and shorter

disease duration [459]. EUSTAR confirmed a greater risk of death in those with raised CK (HR 1.9, 95% CI 1.1, 3.3, p=0.02), diastolic dysfunction (HR 2.1, 95% CI 1.3, 3.3, p=0.002) and cardiac blocks (HR 2.1, 95% CI 1.3, 3.3, p=0.004) in 1188 SSc patients, along with mRSS, raised acute phase reactants and joint contractures; a raised CK significant in both disease subtypes, whilst only cardiac block significant in lcSSc [457]. A retrospective longitudinal CMR study of 124 patients also determined LVEF less than 45% and impaired right ventricular ejection fraction, but not LGE, was associated with greater mortality [460]. Reports now also suggest an association with severe GI disease [461] and digital ulceration [462].

In order to tease out the pathophysiological process underlying myocardial disease in SSc, Pieroni et al performed a comprehensive cardiac assessment, including LGE-CMR and endomyocardial biopsies, on seven of 181 SSc patients who had developed new symptoms of heart disease (defined as heart failure, chest pain and palpitations) [381]. Apart from raised cardiac enzymes, four patients had evidence of ventricular ectopics, three had cardiac wall motion abnormalities, six pericardial effusions, and LGE on CMR was seen in six (T2-hyperintensity in two). Interestingly, upregulation of endothelium adhesion molecules and activated T lymphocytes infiltration, with myocyte necrosis, was found in all patients. No evidence of atherosclerotic heart disease was found.

The use of CMR as a research tool is becoming more popular in SSc. More recent studies confirm previous findings [373, 463, 464]. Gargani et al report the results of 131 scans in SSc patients, finding normal systolic function on echocardiogram but non-ischaemic myocardial fibrosis in 28%; independently associated with E/E' on echocardiography, with no association with age, disease duration, disease subtype or mRSS [465]. Interest is also growing in the measurement of extracellular volume fraction (ECV) as an indicator myocardial fibrosis [466, 467]. Barison et al found that ECV was increased in SSc compared to controls, correlating with skeletal muscle ECV, however, found no correlation with disease phenotype. There was no difference in ECV in those with LGE compared to those without, and the authors suggest myocardial remodelling as well as a fibrotic process in SSc cardiac disease [468].

3.4.3 Significance of this review

The findings of this review were presented to the multi-disciplinary UKSSSG cardiac disease working group, aiding the production of best practice guidelines for the detection and management of SSc-related myocardial disease in the UK. However, in addition, it has

highlighted many areas in need of further research; for example, when, how and how often should the physician screen for cardiac involvement. Well-planned specific studies are required to minimise confounding by macrovascular disease, ideally designed in collaboration between rheumatologists and cardiologists. In the meantime, physicians should be alert to symptoms or signs of cardiac disease in SSc and employ a multi-disciplinary approach to their management.

3.4.4 Summary

To conclude, this comprehensive literature review has described the prevalence of SSc-cardiomyopathy, confirmed its association with greater mortality and its association with other poor prognostic indicators. The review has demonstrated that non-invasive investigative techniques are improving the understanding of its pathophysiological basis, and also differentiating SSc-CM from IHD should be a priority when designing future studies.

3.4.5 Key messages

1. SSc-cardiomyopathy is prevalent in SSc and is associated with poor SSc prognostic indicators.
2. Advancing non-invasive investigative techniques are helping to inform the pathophysiology of SSc-cardiomyopathy.
3. Differentiating SSc-cardiomyopathy from IHD requires well planned future studies.

Chapter 4 Improvement in insulin resistance is greater when infliximab is added to methotrexate during intensive treatment of early rheumatoid arthritis - results from the IDEA study

This chapter describes the results of a cardiovascular sub-study within a randomised controlled trial assessing two different treatment strategies in early Rheumatoid Arthritis (RA).

4.1 Introduction

An accelerated risk of cardiovascular disease (CVD) is well-recognised in established RA [3, 5, 63], however, there is emerging evidence for an increase in overt CVD in those even with a recent diagnosis of RA [64]. A report from the Rochester Epidemiology Project confirmed patients with recent onset RA had a 10-year CV risk profile similar to that of subjects five to ten years older without RA [65]. A nationwide Danish cohort study identified subjects who developed RA, and after a mean follow up time of 4.6 (SD 2.8) years determined that the overall incidence rate ratio (IRR) of myocardial infarction (MI) in RA was 1.7 (95% CI 1.5 to 1.9), similar to the risk in diabetes mellitus (DM) (IRR 1.7 (1.6 to 1.8); p=0.64 for difference). In addition, those with treatment duration of less than three years had an increased risk of MI compared to healthy controls (OR of 1.7 (95% CI 1.3 to 2.2) [4]. Having said this, a recent meta-analysis of two inception RA cohorts concluded no increase in CV events, and another of four inception cohorts showed no increase in CV mortality [5, 66].

The investigation of CVD in RA has seen an increase in the application of surrogate measures of CVD, in the form of soluble biomarkers, used in the general population [12]. However, there is difficulty in differentiating biomarkers that only reflect systemic inflammation from those that truly act as surrogate markers of underlying CVD.

Three biomarkers of CV risk commonly measured are total cholesterol/high-density lipoprotein cholesterol (TC/HDL-C) [469], the homeostasis model assessment-estimated insulin resistance (HOMA-IR) [191, 192] and N-terminal pro-brain natriuretic peptide (NT-pro BNP) [470]; the term “biomarker” used in line with the NIH working group definition [471]. In RA, the levels of these biomarkers are raised [64, 117, 194], and both TC/HDL-C and NT-proBNP have been associated with future CV events [119, 175]. In the general

population, HOMA-IR has been validated as a measure of insulin resistance (IR) and is proven to be independent predictor of CVD [192]. HOMA-IR has been shown to correlate with carotid intimal-media thickness (CIMT) in established RA [198], however, its clinical prognostic utility in RA remains to be determined. Each biomarker has been found to correlate with inflammation, although the correlation of inflammation with TC/HDL-C is weak [174, 197, 212].

The effect of RA disease suppression on biomarkers of CV risk has not been extensively explored, particularly in early RA, however, it is clear that CV morbidity and mortality are reduced with the use of disease-modifying anti-rheumatic drugs (DMARDs) [250] and tumour necrosis factor inhibitors (TNFi). A meta-analysis of TNFi use suggested a pooled adjusted relative risk (RR) of 0.46 (95% CI 0.28-0.77) for all CV events [253]. DMARDS have also been shown to reduce TC/HDL-C. Georgiadis et al described 58 patients with RA with disease duration of less than one year in whom there was an improvement in HDL-C and TC/HDL-C after commencement of MTX and steroids [261]. In the COBRA study, 154 patients with a median duration of RA of 4 months were randomised to combination therapy (SSZ, MTX and prednisolone) or SSZ alone. Combination therapy led to a more rapid and effective reduction in disease activity, but also a greater increase in TC and HDL-C, with a greater improvement in TC/HDL-C after 16 weeks of therapy [263]. The authors were unable to comment if this was due to the suppression of disease activity or direct actions of the medication. Meta-analyses report no improvement in TC/HDL-C with TNFi, however, reports suggest a complex process and TNFi use may alter the structure and function of lipids [269].

IR can be improved with DMARDs in established RA [282], but the effect of TNFi is unclear [284, 472]. Sixty-one patients with RA demonstrated an improvement in IR following 3 months of TNFi [472]. In a prospective study, Seriolo et al showed that although there were no significant changes in HOMA-IR 12 weeks after commencing TNFi, by week 24 there was an improvement (HOMA-IR: 1.445 at baseline vs. 1.733 at week 24, $p<0.01$) [287]. Kiortsis et al et al described how only those with the highest measures of IR improved following 6 months of IFX therapy [473].

The limited data on NT-proBNP suggest improvement with TNFi [306]. Peters et al described 171 patients with RA given adalimumab and showed NT-proBNP reduced significantly by week 16 [309]. Kotyla et al described a reduction in NT-proBNP in 23 patients with RA 12 months after IFX treatment [306]. Interestingly, Tomas et al showed

that NT-proBNP increased immediately after IFX infusion at baseline, 6 months and 12 months in 31 patients with autoimmune disease, 10 of which had RA. Over 12 months however, NT-proBNP decreased (not significantly). Parallel echocardiograms within the study did not reveal any deterioration in cardiac morphology and function [308]. There have been no studies in early RA to our knowledge.

Few studies have compared the change in biomarkers of CV risk with different treatment strategies. Cross-sectional and small open label studies have found no differences in lipid profile when comparing TNFi to DMARDs [266, 276, 290]. The TEAR trial is the only previous randomised controlled trial (RCT) to our knowledge to assess change in TC/HDL-C, randomising 459 early RA patients to MTX + etanercept (ETN) or triple DMARD therapy or aggressively titrated MTX, determining a similar decrease in TC/HDL-C at week 24 across the groups [264]. No superiority of TNFi over DMARDs has been found on changes in HOMA-IR in cross-sectional studies [288]; however, one unblinded RCT of 40 patients with early RA (disease duration less than 2 years) given MTX or infliximab (IFX) and MTX reported a lower fasting glucose at 12 months in the IFX arm [290]. However, the main goal in this trial was to achieve remission and the therapeutic regime was changed as necessary. A higher proportion of patients in the TNFi group achieved remission; therefore the difference could reflect the degree of systemic inflammation rather than the specific action of TNFi. More recently, a study reported a reduction in the incidence of diabetes mellitus (DM) in those treated with TNFi in RA compared to conventional synthetic DMARDs [281].

4.1.1 Study hypotheses and aims

The thesis hypotheses relevant to this chapter include that subclinical CVD in RA is prevalent, associated with a specific disease phenotype, and improved with optimal suppression of disease activity.

The aims of this chapter were;

- To describe the prevalence of CV biomarker abnormalities in RA with no prior history of CVD
- To describe the association of CV biomarker abnormalities with disease phenotype, serology and disease activity in early RA with no prior history of CVD
- To determine whether effective suppression of disease activity in RA improves CV biomarkers

- To determine whether specific therapeutic agents have superior influence on CV biomarkers over others

4.1.2 Study objectives

The primary objective in the study was to assess the change in biomarkers of CV risk in DMARD-naïve early RA patients, treated with either TNFi and MTX or MTX and methylprednisolone within a RCT. The primary endpoints of interest included the change in atherogenic index (TC/HDL-C), IR as estimated by HOMA-IR and NT-proBNP.

The secondary objective was to determine whether treatment strategy exerted any influence on these changes; i.e. to determine if TNFi yielded a benefit over and above the suppression of disease activity and systemic inflammation with a combination of MTX and corticosteroids, or if the suppression of inflammation irrespective of treatment was the key to a better cardiovascular profile.

4.2 Methods

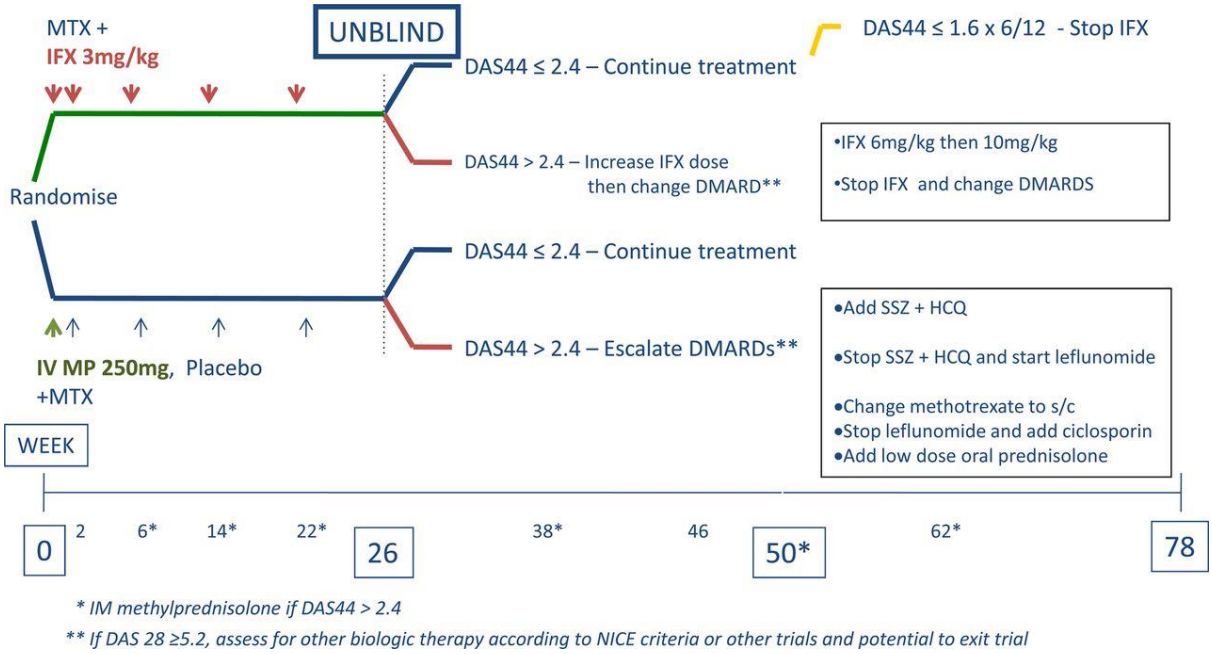
The Infliximab as Induction therapy for Early rheumatoid Arthritis (IDEA) study was a multicentre double-blind RCT performed in hospitals within the Yorkshire network [474], led by the research team in Leeds. The study was carried out with ethical approval from the Northern and Yorkshire Multi-Centre Research Ethics Committee (RR05/7092, Eudract-2005-005013-37 and ISRCTN48638981). This study was designed to compare, using a treat to target approach, the use of IFX with MTX to the use of MTX alone with intravenous (IV) methylprednisolone (MEP) at induction in early RA. The results of the RCT which have been published previously in 2013, reported the use of MTX and IFX was no more beneficial than MTX and MEP [474]. In addition to collecting clinical data relating to disease activity and response to treatment, a comprehensive CV assessment was performed at baseline, week 26 and week 78 which will be described in full below. To note, I was not involved in the design or delivery of this study; only part of the study research team with regards to the CV sub-study and thus my involvement was with the preparation and analysis of the already collected CV data.

4.2.1 Study design

Participants within the IDEA study, recruited from 2006 to 2008, were randomised to receive either IFX and MTX or MTX and a pulse of 250mg IV MP as induction therapy. One

hundred and twenty mg intra-muscular (IM) MP was given at weeks 6, 14, 22, 38, 50 and 62 if DAS>2.4 at that visit. Patients were unblinded at week 26 and subsequently treated pragmatically guided by disease activity according to a pre-determined therapeutic regime (see Figure 4-1). The study was powered to detect a difference between the two treatment groups in the primary endpoint of change in Sharpe van Der Heijde score at 50 weeks from baseline.

Figure 4-1 Study design.



**DMARD escalation protocol: sulphasalazine(SSZ) 500mg daily increasing to 1g twice daily over 4 weeks + hydroxychloroquine(HCQ) 200mg – 400mg daily to a maximum of 6.5mg/kg, then stop SSZ + HCQ and add leflunomide (LEF) 10mg daily for 1 month, then increase to 20mg daily, then one of the following: subcutaneous (s/c) methotrexate (MTX) + LEF or MTX + ciclosporin 2.5 mg/kg/day or MTX + LEF + prednisolone 5–7.5 mg daily. Reproduced with permission from primary IDEA publication; Nam et al 2013 ARD [474]

4.2.1.1 Endpoints

The primary endpoints for this CV sub-study was the difference in change in CV biomarkers between the two treatment groups; namely; TC/HDL-C, HOMA-IR and NT-proBNP at week 26 and 78.

4.2.1.2 Subject numbers

One hundred and twelve patients across Yorkshire were recruited into the IDEA study; however, the CV sub-study was only carried out on the 86 participants attending the Leeds Teaching Hospitals NHS Trust (LTHT) due to practical issues with biological sample

collection and processing. This sub-study was not powered to detected changes in CV biomarkers but was seen as an exploratory study within a field with limited data.

4.2.1.3 Eligibility criteria

Patients were required to be between 18-80 years of age, and capable of giving informed consent. They needed to fulfil the 1987 ACR Criteria for RA [475], with symptoms of >3 months and <12 months duration, have active disease as defined by DAS>2.4 and be DMARD/TNFi therapy naïve.

In this study, patients with hepatitis, tuberculosis, HIV, previous malignancy or significant haematological abnormalities were excluded, and also if they had received corticosteroids within 1 month prior to baseline. For the CV sub-study specifically, patients were excluded if they had a history of existing CVD (cardiac, cerebral or peripheral).

4.2.1.4 Informed consent

Appropriate patients were given a patient information sheet to read, and after time given to digest the material, the patients were contacted to determine their interest in participating, discuss the study in detail and answer any questions.

All participants had at least 24 hours to consider the study before consenting. A study co-investigator took consent. A copy of the consent was kept in the site file, with one copy filed in the notes, and another sent to the patent.

4.2.2 Study schedule

4.2.2.1 Screening visit

The screening visit occurred no more than 28 days prior to randomisation and baseline.

The following procedures took place:
Informed consent
Collection of demographic data and medical history obtained.
Pregnancy test performed for women of child bearing potential
A chest radiograph unless done in the preceding 3 months
Purified protein derivative (PPD) TB skin test
Urinalysis
Viral serology to exclude Hepatitis B and C unless done within the 3 months prior to baseline.
HIV serology done in those at high risk

4.2.2.2 Baseline and follow up visits

Randomisation occurred at baseline. Further study visits for the IDEA study occurred at weeks 6, 14, 22, 26, 38, 50, 62, and 78.

Specific to the CV sub-study, at baseline, weeks 26 and 78, the following additional data were recorded:

Presence of cardiovascular co-morbidity and traditional CV risk factors.

Blood pressure, height, weight, hip and waist circumference

Use of lipid-lowering therapy and antihypertensive therapy

Biological sample collection for those recruited in LTHT

Patients completed the Rose Angina Questionnaire [476] and Edinburgh Claudication questionnaire [477] at baseline and week 78.

4.2.3 Study procedures

Blood samples were collected into serum tubes, processed and stored at −80 °C until testing. Glucose, total cholesterol (TC), HDL-C, were determined in serum using the biochemical analyser Cobas INTEGRA 400, NT-proBNP using Cobas 6000 (immunochemistry module Cobas e601) and insulin using COBAS e 411 (all form Roche Diagnostics GmbH, Mannheim, GERMANY) with the appropriate assays supplied by Roche Diagnostics according to the manufacturer instructions. All measurements were performed at the Department of Biochemical Diagnostics, Clinical Hospital No. 1, Medical University of Lublin, Poland.

LDL-C was calculated using Friedwald's formula [478]. A complementary estimate of IR was computed by HOMA-IR (fasting insulin (μU/ml) × fasting glucose (mg/dl)/405) [191]. Higher HOMA-IR values indicate lower insulin sensitivity. A TC/HDL-C ratio (atherogenic index) greater than 6 was used as the cut-off for a high value in line with the Joint British Societies guidance [479]. For NT-proBNP, the manufacturer's guidance was followed, adjusting levels according to age and gender.

4.2.4 Statistical analysis

Analyses were performed in SPSS (version 21, IBM, NY, USA) and Stata (version 13.0, Statacorp, Texas, USA). Any missing data were managed using multiple imputations by chained equations. Results for both the observed and imputed data are provided.

Chi-squared tests, Mann-Whitney U and independent T tests as appropriate were used to detect differences between the subjects within the CV sub-study and those within the whole IDEA cohort. Associations between biomarkers and baseline clinical variables were assessed using Spearman's rank correlation. The clinical variables chosen for analysis were those with a previously reported association with biomarkers of CV risk, or were closely linked to such variables, for example, BMI and waist/hip circumference ratio (WHR).

Biomarkers HOMA and NT-proBNP were found to be highly skewed; values were ln-transformed prior to parametric analyses. The exponentiated differences between values on a log scale represent the ratio of one value to another. Therefore, changes between visits have been expressed as the ratio of the follow-up values to the baseline values, and differences between groups represent the ratio of values in the MTX+IFX group to those in the MTX+MP group.

A Mann-Whitney U test was used to determine differences in CV biomarkers at baseline dependent on RF, ACPA and current smoking status, and to compare the IA/IM steroid dose requirement per month between groups.

Multiple linear regression was used to show whether changes in biomarkers differed between the treatment groups, controlling for baseline values, and then repeated, controlling for baseline BMI, anti-citrullinated peptide antibody (ACPA) positivity, steroid dose and smoking status (current vs. ex-/non).

Multiple linear regression was used to assess whether changes in BMI during follow-up differed between groups, adjusting for baseline BMI, and to identify associations between changes in biomarker levels and changes in disease activity and BMI.

4.3 Results

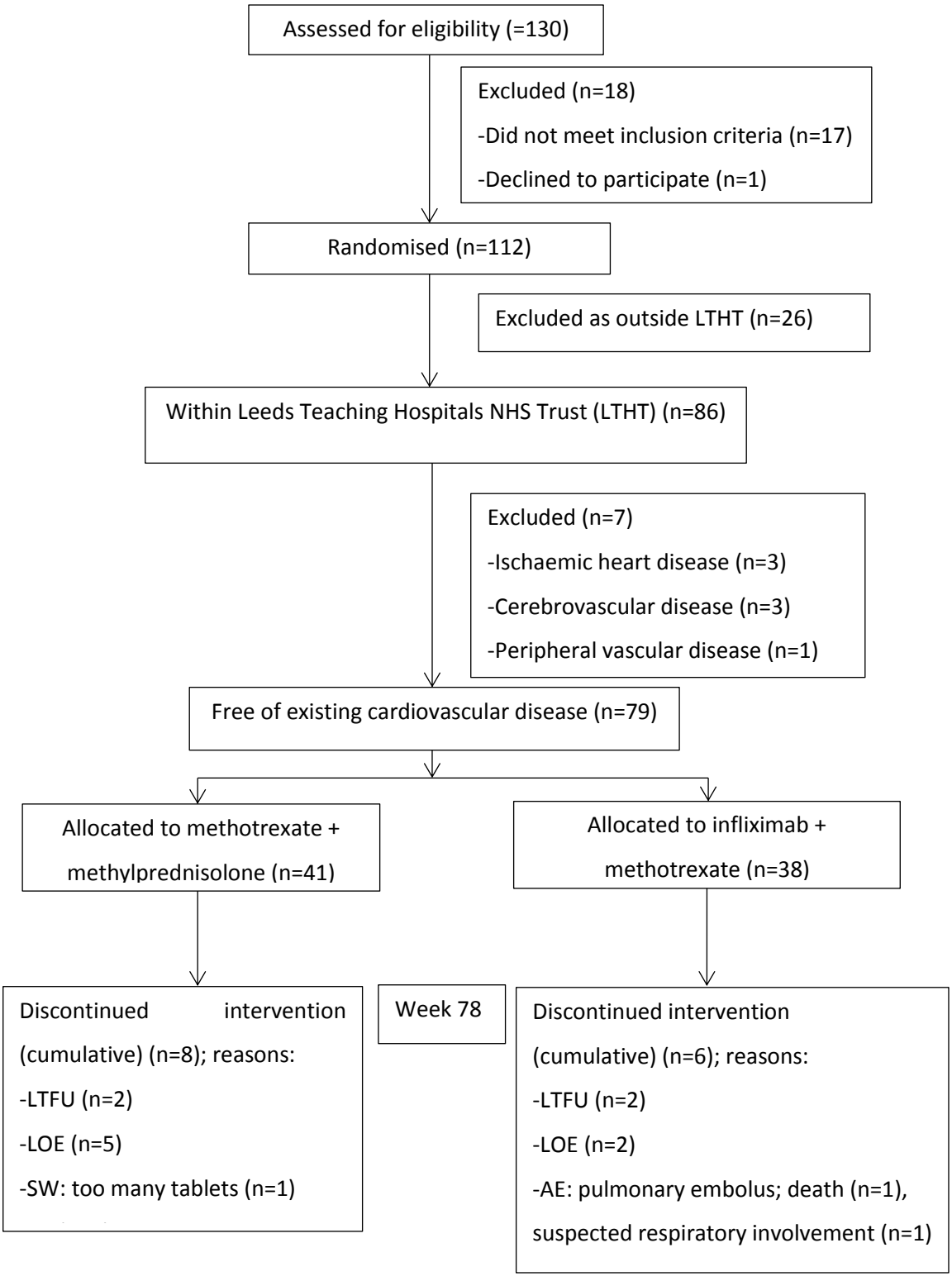
The following section describes the baseline characteristics of the study participants, and their change in soluble CV biomarkers over time, and difference between treatment groups.

4.3.1 Baseline characteristics

One hundred and twelve patients were recruited into the IDEA study, with biomarkers of CV risk data available for 86 patients. Of these 86, 7 patients (8%) had known existing CVD

at baseline; 4 (5%) had ischaemic heart disease, 3 (3.5%) cerebrovascular disease and 1 (1%) peripheral vascular disease, and were excluded from subsequent analyses (Figure 4-2).

Figure 4-2 Flowchart of participants within the cardiovascular sub-study of IDEA.



LTFU, lost to follow-up; LOE, loss of efficacy; AE, adverse events; SW, self-withdrawal.

Of the remaining 79 patients, the mean (standard deviation (SD)) age was 51.6 (12.7) years, range 19-75), 71% were female, 57% RF positive and 72% ACPA positive. The median (interquartile range (IQR)) 44 swollen joint count (SJC44) was 8 (5.5, 13) and median (IQR) CRP was 14 (0-0-28). Thirty-eight (48%) patients received IFX+MTX and 41 (52%) MTX+MP. When compared to the IDEA subjects with no biomarker data or those excluded from the analysis due to existing CVD (n=33 in total), those within the CV sub-study were younger (mean (SD) age in remaining 33 subjects: 57.3 (12.5) years, p=0.031) and had a lower SJC44 (median (IQR) SJC44 in remaining 33 subjects: 12 (6.5, 21), p=0.034). No differences in gender, serology or CRP were recorded. The baseline clinical characteristics and biomarkers of CV risk results are detailed in Table 4-1.

Table 4-1 Baseline clinical characteristics

Variable		**MTX+MP (n=41)**	**IFX+MTX (n=38)**
Demographics			
Age (years):	mean (SD), range	50.9 (12.6), 19 to 69	52.3 (13.0), 28 to 75
Female:	n (%)	30 (73)	26 (68)
RA characteristics			
Disease duration (months):	median (IQR)	1.01 (0.69, 1.71)	1.00 (0.72, 1.45)
Symptom duration (months):	median (IQR)	7.98 (5.03, 9.82)	7.01 (5.03, 10.38)
ESR (mm/hr, normal range <15):	median (IQR)	46 (20, 80)	34 (19, 51)
CRP (mg/L, normal range <5):	median (IQR)	14 (0, 35)	13 (0, 25)
DAS44:	mean (SD)	3.45 (0.91)	3.87 (1.03)
RF positive (≥40iu/ml):	n (%)	26 (63)	19 (50)
ACPA positive (≥10U/ml):	n (%)	31/39 (80)	24/37 (65)
HAQ-DI:	mean (SD)	1.37 (0.53) (n=40)	1.46 (0.51)
Co-morbidity			
Systolic BP (mmHg)	mean (SD)	122 (16)	125 (15)
Diastolic BP (mmHg)	mean (SD)	77 (11)	76 (10)
BMI	mean (SD), range	26.2 (4.2), 14.7 to 35.0	27.1 (6.0), 18.8 to 48.8
Waist/Hip circumference ratio	mean (SD)	0.89 (0.09) (n=36)	0.89 (0.15) (n=35)
Current smoker	n (%)	14 (34)	10 (26)
PMH Hypertension	n (%)	4 (10)	5 (13)
PMH Hyperlipidaemia	n (%)	6 (15)	1 (3)
PMH Diabetes Mellitus	n (%)	3 (7)	3 (8)

Family history of CVD*	n (%)	8/40 (20)	9 (24)
On anti-hypertensive therapy	n (%)	7 (17)	5 (13)
On lipid-lowering therapy	n (%)	5 (12)	0 (0)
Biomarkers of CV risk			
TC/HDL-C ratio	mean (SD)	5.71 (2.26)	5.12 (1.67)
TC/HDL-C ratio >6	n (%)	17 (42)	9 (26) (n=35)
HOMA-IR mean**	geometric	2.54	2.06
NT-pro-BNP (pg/ml) mean**	geometric	62.92	78.33 (70.15***)
High NT-pro-BNP (pg/ml)	n (%)	9/41 (22.0)	3/35 (8.6) [2/34 (5.9%)***]

ACPA, anti-citrullinated protein antibody; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; CVD, cardiovascular disease; DAS44, disease activity score (44 joints); ESR, erythrocyte sedimentation rate; HAQ-DI, Rasch-transformed health assessment questionnaire disability index score; HOMA-IR, homeostasis model assessment-estimated insulin resistance index; IFX+MTX, infliximab + methotrexate; MTX+MP, methotrexate and intravenous steroid; NT-proBNP, N-terminal pro-brain natriuretic peptide, PMH, past medical history; RF, rheumatoid factor; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio.
* defined as first degree relative less than 60 years old if relative is female, and 55 years old if relative is male
**It was not possible to calculate SD in original units for log-transformed variables
***minus extreme outlier

An extreme outlier was identified on analysis of NT-proBNP (3343pg/ml). This subject, who had a history of hypertension, was promptly referred to a cardiologist and managed accordingly. This subject also had a low HDL-C (34.13mg/dL), a TC/HDL-C ratio of 5.71 and relatively high HOMA-IR (2.94). Data for NT-proBNP with this patient excluded are also presented.

4.3.2 Relationship of biomarkers of CV risk with baseline variables

Table 4-2 describes the association of the baseline variables CRP, SJC44, HAQ-DI, BMI and WHR, with TC/HDL-C, HOMA-IR or NT-proBNP. HAQ-DI correlated positively with NT-

proBNP (rho=0.332, p=0.004), and BMI correlated positively with TC/HDL-C (rho=0.312, p=0.006) and HOMA-IR (rho=0.574, p<0.001). No other associations were seen.

There was no difference in TC/HDL-C, HOMA-IR or NT-proBNP according to RF or current smoking status at baseline. Those who were ACPA positive were more likely to have a lower NT-proBNP (median (IQR): 55.25pg/ml (34.02, 93.90) compared to 105.70pg/ml (49.84, 253.10) when ACPA negative (p=0.016); significance was sustained after removal of the ACPA negative NT-proBNP outlier (p=0.031). ACPA status was not associated with TC/HDL-C or HOMA-IR at baseline.

HOMA-IR was higher in those with a raised TC/HDL-C (median (IQR) HOMA-IR if TC/HDL>6: 2.28 (1.79, 3.41) vs. 1.82 (1.16, 3.66) if TC/HDL<6, p=0.017). NT-proBNP was not associated with either HOMA-IR or TC/HDL-C.

When reviewing the individual lipid measurements, HAQ-DI negatively correlated with TC (rho=-0.341, p=0.003), HDL-C (rho=-0.391, p=0.001) and LDL-C (rho=-0.350, p=0.002) (see Table 4-3). SJC44, CRP and WHR negatively correlated with HDL-C (rho=-0.291, p=0.0015, rho=-0.304, p=0.007 and rho=-0.269, p=0.027 respectively). There were no other significant correlations between these lipids with CRP, SJC44, BMI and waist/hip circumference.

Table 4-2 Spearman's rank correlations of biomarkers with variables at baseline

Variable	TC/HDL-C		HOMA-IR		NT-proBNP	
	Rho	**P value**	**Rho**	**P value**	**Rho**	**P value**
CRP	0.15	0.195	-0.035	0.765	0.182	0.116
SJC44	0.189	0.117	-0.159	0.189	0.12	0.918
HAQ-DI	0.107	0.362	0.093	0.426	**0.332**	**0.004**
BMI	**0.312**	**0.006**	**0.574**	**<0.001**	0.174	0.134
Waist/Hip circumference	0.162	0.188	0.076	0.538	-0.177	0.149

BMI, body mass index; CRP, C-reactive protein; HAQ-DI, Rasch-transformed health assessment questionnaire disability index score; HOMA-IR, homeostasis model assessment-estimated insulin resistance index; NT-proBNP, N-terminal pro-brain natriuretic peptide; SJC44, 44 swollen joint count; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio.

Table 4-3 Correlations of TC, HDL-C and LDL-C with baseline variables

Variable	TC		HDL-C		LDL-C	
	Rho	**P value**	**Rho**	**P value**	**Rho**	**P value**
CRP	-0.069	0.556	**-0.304**	**0.007**	-0.118	0.311
SJC44	-0.068	0.577	**-0.291**	**0.015**	-0.052	0.671
HAQ-DI	**-0.341**	**0.003**	**-0.391**	**0.001**	**-0.350**	**0.002**
BMI	0.185	0.110	-0.220	0.056	0.164	0.156
Waist/Hip circumference	-0.032	0.795	-0.269	0.027	-0.046	0.712

BMI, body mass index; CRP, C-reactive protein; HAQ-DI, Rasch-transformed health assessment questionnaire disability index score; HDL-C, High-density lipoprotein cholesterol ratio; LDL-C, low-density lipoprotein cholesterol ratio; SJC44, 44 swollen joint count; TC, total cholesterol.

4.3.3 Follow-up

Of the patients included in this sub-study, 14 out of 79 (6 IFX+MTX, 8 MTX+MP) did not complete the study treatment schedule for various reasons including failure to suppress

disease activity [474]. Three patients had missing data at baseline; data on TC/HDL-C, NT-proBNP and HOMA-IR were available for 76 patients at baseline and week 26, and 66 patients at week 78. Values of TC/HDL-C, ln-transformed HOMA-IR & NT-proBNP, and DAS44-CRP were imputed at weeks 26 and 78, whilst BMI and WHR were imputed at week 78 only. In addition to baseline values of each variable, gender, smoking status, ACPA positivity and mean steroid requirement were included in the imputation models. One patient developed ischaemic heart disease by week 78, but there were no new cases of DM or peripheral/cerebrovascular disease.

4.3.4 Differences between treatment groups

The following section describes the differences between the treatment groups in disease activity and soluble CV biomarkers and weeks 26 and 78 of the RCT.

4.3.4.1 Disease activity

Consistent with the findings in the full trial, in the patients included in this CV sub-study, changes in DAS44-CRP did not differ between groups at week 26 (mean (SD) change MTX+MP -1.52 (0.86), IFX+MTX -1.96 (1.10); adjusted mean difference -0.17 (-0.54, 0.19), p=0.345) or week 78 (mean (SD) change MTX+MP -1.94 (1.04), IFX+MTX -1.92 (1.13); adjusted mean difference 0.32 (-0.10, 0.74), p=0.137).

4.3.4.2 Soluble CV biomarkers

Substantive reductions in all three biomarkers were observed at week 26, irrespective of treatment, with no significant differences between the groups at week 26; TC/HDL-C decreased by 0.7-0.9 units, HOMA-IR values decreased by 28-29%, whilst NT-proBNP decreased by 16-17% (Table 4-5).

At week 78, TC/HDL-C continued to improve in both groups, whilst NT-proBNP values were similar to those recorded at 26 weeks. The IFX+MTX group showed further improvement in HOMA-IR at week 78 (by 55% relative to baseline), whilst there was some loss of the early week 26 improvements in HOMA-IR in the MTX+MP group. On average at week 78, IFX+MTX HOMA-IR values were 0.58 times as high as those treated with MTX+MP (p=0.003). The findings were similar in the observed data (see Table 4-6).

There were no differences in the proportion of patients with at-risk levels of TC/HDL-C or NT-proBNP. At week 26 the proportions of patients with at risk ratio of TC/HDL-C were 20.1% and 13.2% in the MTX+MP and IFX+MTX groups respectively (adjusted odds ratio

(OR) 0.89 (0.21, 3.78), p=0.876); at week 78 the proportions were 21.5% and 17.1% respectively (adjusted OR 1.14 (0.24, 5.28), p=0.870). At week 26 the proportions of patients with at risk levels of NT-proBNP were 8.0% and 13.2% in the MTX+MP and IFX+MTX groups respectively (adjusted OR 0.95 (0.15, 6.14), p=0.959); at week 78 the proportions in the observed data were 0% and 12.9%, (model for imputation failing given the 0%).

4.3.4.2.1 Individual lipid measurements

There were no significant differences in the change in TC, LDL-C and HDL-C changes after 26 and 78 weeks, adjusting for baseline values (see Table 4-7 for imputed data and Table 4-8 for observed values). Insulin levels were lower in the IFX+MTX group at week 78; difference in ln(insulin+1) between the groups-0.51 (-0.78, -0.23), p=0.001 (adjusted for baseline values).

Table 4-4 Imputed differences between treatment arms in TC/HDL-C, HOMA-IR and NT-proBNP changes after 26 and 78 weeks, adjusting for baseline values

Change	MTX+MP (n=41)	IFX+MTX (n=38)	Unadjusted difference (95% CI)	Adjusted* difference (95% CI), p-value
Week 26				
TC/HDL-C mean	-0.89	-0.67	0.23 (-0.47, 0.93)	0.13 (-0.43, 0.69), t=0.48, p=0.635
HOMA-IR mean ratio	FU/BL 0.72	FU/BL 0.71	IFX/MP 0.99 (0.64, 1.53)	IFX/MP 0.84 (0.62, 1.14), t=-1.14, p=0.259
NT-proBNP mean ratio	FU/BL 0.83	FU/BL 0.84	IFX/MP 1.06 (0.76, 1.49)	IFX/MP 1.18 (0.85, 1.62), t=1.01, p=0.314 **1.15 (0.84, 1.58), t=0.89, p=0.378
Week 78				
TC/HDL-C mean	-1.00	-0.89	0.11 (-0.64, 0.87)	-0.09 (-0.70, 0.51), t=-0.31, p=0.758
HOMA-IR mean ratio	FU/BL 0.84	FU/BL 0.55	IFX/MP 0.66 (0.40, 1.08)	IFX/MP 0.58 (0.41, 0.82), t=-3.17, p=0.003
NT-proBNP mean ratio	FU/BL 0.82	FU/BL 0.86	IFX/MP 1.02 (0.68, 1.54)	IFX/MP 1.19 (0.81, 1.75), t=0.91, p=0.367 **1.16 (0.79, 1.71), t=0.78, p=0.440

CI, confidence intervals; HOMA-IR, homeostasis model assessment-estimated insulin resistance index; IFX+MTX, infliximab + methotrexate; MTX+MP, methotrexate and intravenous steroid; NT-proBNP, N-terminal pro-brain natriuretic peptide; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio.

*Adjusted for baseline values

**Excluding patient with extremely high NT-proBNP value

Table 4-5 Observed differences between treatment arms in TC/HDL-C, HOMA-IR and NT-proBNP changes after 26 and 78 weeks, adjusting for baseline values

Change	MTX+MP (n=41)	IFX+MTX (n=38)	Unadjusted difference (95% CI)	Adjusted* difference (95% CI), p-value
Week 26				
TC/HDL-C mean	-0.85, n=38	-0.49, n=35	0.36 (-0.34, 1.06)	0.09 (-0.47, 0.64), p=0.761
HOMA-IR mean ratio	FU/BL 0.71, n=38	FU/BL 0.70, n=34	IFX/MP 0.99 (0.63, 1.55)	IFX/MP 0.86 (0.65, 1.15), p=0.298
NT-proBNP mean ratio	FU/BL 0.85, n=38	FU/BL 0.88, n=35	IFX/MP 1.03 (0.75, 1.42)	IFX/MP 1.12 (0.83, 1.50), p=0.462
Week 78				
TC/HDL-C mean	-1.02, n=34	-0.86, n=28	0.15 (-0.61, 0.92)	-0.14 (-0.68, 0.41), p=0.612
HOMA-IR mean ratio	FU/BL 0.88, n=35	FU/BL 0.64, n=29	IFX/MP 0.72 (0.43, 1.21)	IFX/MP 0.59 (0.42, 0.82), p=0.002
NT-proBNP mean ratio	FU/BL 0.79, n=35	FU/BL 0.98, n=29	IFX/MP 1.24 (0.85, 1.80)	IFX/MP 1.26 (0.89, 1.77), p=0.183

*Adjusted for baseline values

CI, confidence intervals; HOMA-IR, homeostasis model assessment-estimated insulin resistance index; IFX+MTX, infliximab + methotrexate; MTX+MP, methotrexate and intravenous steroid; NT-proBNP, N-terminal pro-brain natriuretic peptide; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio.

Table 4-6 Imputed differences between treatment arms in insulin, glucose, TC, LDL-C and HDL-C changes after 26 and 78 weeks, adjusting for baseline values.

Change	MTX+IV MP (n=41)	IFX+MTX (n=38)	Unadjusted difference (95% CI)	Adjusted* difference (95% CI), p-value
Week 26				
ln(Insulin+1) mean (SD)	-0.24	-0.24	-0.01 (-0.32, 0.31)	-0.15 (-0.38, 0.07), p=0.176
ln(Glucose) mean (SD)	-0.15	-0.08	0.08 (-0.04, 0.20)	0.04 (-0.03, 0.12), p=0.284
TC, mg/dL mean (SD)	6.94	7.11	0.18 (-18.74, 19.09)	3.29 (-14.88, 21.46), p=0.719
LDL-C , mg/dL mean (SD)	10.49	7.09	-3.40 (-17.85, 11.05)	-0.21 (-14.06, 13.64), p=0.976
HDL-C, mg/dL mean (SD)	7.97	8.35	0.38 (-5.46, 6.21)	-0.63 (-6.48, 5.21), p=0.829
Week 78				
ln(Insulin+1) mean (SD)	-0.08	-0.44	-0.35 (-0.73, 0.03)	-0.51 (-0.78, -0.23), p=0.001
ln(Glucose) mean (SD)	-0.04	-0.07	-0.03 (-0.16, 0.10)	-0.03 (-0.14, 0.07), p=0.535
TC, mg/dL mean (SD)	19.29	5.42	-13.87 (-32.50, 4.75)	-13.55 (-30.81, 3.71), p=0.121
LDL-C, mg/dL mean (SD)	18.73	5.74	-12.99 (-31.91, 5.94)	-12.38 (-29.73, 4.98), p=0.158
HDL-C, mg/dL mean (SD)	11.57	11.50	-0.07 (-6.72, 6.57)	-0.64 (-7.43, 6.14), p=0.850

*Adjusted for baseline values

CI, confidence intervals; HDL-C, high-density lipoprotein cholesterol ratio; IFX+MTX, infliximab + methotrexate; MTX+MP, methotrexate and intravenous steroid; LDL-C, low-density lipoprotein cholesterol ratio; TC, total cholesterol.

Table 4-7 Observed differences between treatment arms in insulin, glucose, TC, LDL-C and HDL-C changes after 26 and 78 weeks, adjusting for baseline values.

Change		MTX+MP	IFX+MTX	Unadjusted difference (95% CI)	Adjusted* difference (95% CI), p-value
Week 26					
ln(Insulin+1)	mean (SD)	-0.20 (0.77) (n=38)	-0.26 (0.64) (n=35)	-0.05 (-0.39, 0.28)	-0.16 (-0.39, 0.07), p=0.166
ln(Glucose)	mean (SD)	-0.17 (0.26) (n=38)	-0.07 (0.24) (n=34)	0.10 (-0.02, 0.21)	0.05 (-0.03, 0.12), p=0.200
TC, mg/dL	mean (SD)	7.28 (41.32) (n=38)	9.55 (39.52) (n=35)	2.27 (-16.63, 21.18)	2.81 (-14.91, 20.53), p=0.753
LDL-C, mg/dL	mean (SD)	11.26 (34.34) (n=38)	10.15 (26.20) (n=35)	-1.10 (-15.45, 13.25)	-0.88 (-14.60, 12.84), p=0.899
HDL-C, mg/dL	mean (SD)	8.00 (11.95) (n=38)	6.93 (12.11) (n=35)	-1.07 (-6.68, 4.55)	-0.70 (-6.37, 4.98), p=0.808
Week 78					
ln(Insulin+1)	mean (SD)	-0.14 (0.89) (n=35)	-0.39 (0.67) (n=29)	-0.26 (-0.66, 0.15)	-0.41 (-0.68, -0.14), p=0.003
ln(Glucose)	mean (SD)	-0.03 (0.20) (n=35)	0.00 (0.27) (n=29)	0.04 (-0.08, 0.16)	-0.03 (-0.12, 0.06), p=0.467
TC, mg/dL	mean (SD)	21.74 (38.81) (n=34)	6.24 (38.43) (n=28)	-15.50 (-35.22, 4.22)	-15.56 (-33.04, 1.91), p=0.080
LDL-C, mg/dL	mean (SD)	18.92 (37.74) (n=34)	6.84 (32.47) (n=28)	-12.08 (-30.18, 6.03)	-12.38 (-28.23, 3.47), p=0.123
HDL-C, mg/dL	mean (SD)	11.58 (11.30) (n=34)	11.27 (12.63) (n=28)	-0.31 (-6.39, 5.78)	-0.26 (-6.45, 5.92), p=0.932

*Adjusted for baseline values
CI, confidence intervals; HDL-C, high-density lipoprotein cholesterol ratio; IFX+MTX, infliximab + methotrexate; MTX+MP, methotrexate and intravenous steroid; LDL-C, low-density lipoprotein cholesterol ratio; TC, total cholesterol.

4.3.5 Additional steroid requirement

There was no significant difference between the groups in the median (IQR) intra-articular (IA)/IM steroid injection dose received per month (MTX+MP 20.1mg (6.7, 26.8), IFX+MTX 13.4mg (0.0, 20.4), p=0.189). Adjusting for the total IA/IM steroid received per month did not affect the overall results; for example, having adjusted for baseline BMI, smoking status, ACPA positivity and steroid dose, the between-group ratio of HOMA-IR values at week 78 was 0.61 (95% CI 0.43, 0.87, p=0.007).

During the first 26 weeks and between weeks 27-78; there were no statistically significant differences in the cumulative doses of IA and IM steroid injections received between the groups for either period of follow-up (Table 4-8). Seventeen patients (6 MTX+MP, 11 IFX+MTX) had received no additional IM steroid during the full 78 weeks of follow-up; although this subgroup was small, the results were comparable to those reported for the full cohort (mean ratio without adjusting for BMI, smoking or ACPA 0.39 (95% CI 0.18, 0.85); with adjustment for additional covariates 0.48 (95% CI 0.19, 1.23). A small number of patients received oral steroids; 3 in the IFX group (total doses 1320mg, 560mg, 150mg), 7 in the MP group (doses 916mg, 816mg, 1418mg, 952mg, 560mg, 280mg and another who received a short course of 30mg daily for a respiratory infection (exact number of days on steroid unknown)). Excluding these patients, along with those who received any IA/IM steroid, revealed there was still a difference between the groups for change in HOMA-IR at week 78 (IFX+MTX value 0.39 times as high as MTX+MP group value (95% CI 0.17, 0.88), p=0.027 (n= 16; 10 IFX+MTX, 6 MTX+MP).

Table 4-8 Cumulative doses of IA and IM steroid received during the first 26 weeks, and between 27 and 78 weeks, according to treatment group, in patients followed up for 78 weeks.

		MTX+MP (n=37)	IFX+MTX (n=34)	P value for difference
Intra-articular steroid				
Weeks 0-26	0mg	78.4% (29)	91.2% (31)	p=0.149
	1-120mg	16.2% (6)	8.8% (3)	
	121-240mg	5.4% (2)	- (0)	
Weeks 27-78	0mg	81.1% (30)	85.3% (29)	p=0.666
	1-120mg	16.2% (6)	8.8% (3)	
	121-240mg	2.7% (1)	5.9% (2)	
Intra-muscular steroid				
Weeks 0-26	0mg	21.6% (8)	32.4% (11)	p=0.305
	1-120mg	27.0% (10)	17.6% (6)	
	121-240mg	27.0% (10)	44.1% (15)	
	>240mg	24.3% (9)	5.9% (2)	
Weeks 27-78	0mg	45.9% (17)	55.9% (19)	p=0.359
	1-120mg	37.8% (14)	29.4% (10)	
	121-240mg	13.5% (5)	8.8% (3)	
	>240mg	2.7% (1)	5.9% (2)	

IFX+MTX, infliximab + methotrexate; MTX+MP, methotrexate and intravenous steroid.

4.3.6 Combined group analysis

Controlling for baseline values, and irrespective of treatment strategy, there was no evidence that changes in TC/HDL-C, HOMA-IR or NT-proBNP during 78 weeks of follow-up were associated with changes in DAS44-CRP over the same period (Table 4-9). However, patients achieving ACR70 responses had lower TC/HDL-C (differences between the means: 0.68 units, p=0.012) and their HOMA-IR values were 31% lower on average (p=0.042) than those who did not achieve a response. Change in BMI at week 78 was not associated with

changes in TC-HDL-C, HOMA-IR or NT-proBNP, but HOMA-IR increased by 35.2% for each additional 10% of WHR (p=0.033).

Table 4-9 Associations between changes in disease activity and BMI and changes in biomarkers over 78 weeks in the combined treatment groups (n=79), adjusting for baseline values

	Biomarker change over 78 weeks		
Covariate:	**TC/HDL-C**	**HOMA-IR**	**NT-proBNP**
DAS44-CRP, per unit	0.39 (-0.05, 0.84), p=0.081	11.2% (-12.5%, 41.5%), p=0.376	10.2% (-14.9%, 42.7%), p=0.452
ACR70 response	**-0.68 (-1.20, -0.15), p=0.012**	**-31.2% (-52.1%, -1.4%), p=0.042**	8.8% (-25.8%, 59.7%), p=0.660
BMI, per unit	0.11 (-0.10, 0.32), p=0.273	10.6% (-2.0%, 24.9%), p=0.096	-4.0% (-12.0%, 4.7%), p=0.342
WHR, per 10%	0.35 (-0.10, 0.80), p=0.121	**35.2% (2.5%, 78.1%), p=0.033**	-14.9% (-35.1%, 11.7%), p=0.238

ACR, American College of Rheumatology; BMI, body mass index; DAS44-CRP, 3-variable disease activity score based on CRP, RAI and SJC44; HOMA-IR, homeostasis model assessment-estimated insulin resistance index; IFX+MTX, infliximab + methotrexate; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio; WHR, waist/hip circumference

4.3.7 Use of CVD screening questionnaires

Eleven of 76 patients who completed the Rose Angina questionnaire at baseline, declared they had chest pain. However, 9 patients mapped the pain to joints, such as the sterno-clavicular joint. The sub-investigator marked eight questionnaires to indicate that the pain was not angina-related, including those from the two patients who did not map the pain to joints. Of 76 patients who completed the Edinburgh claudication questionnaire at baseline, 39 reported leg pain, however, 30 mapped the pain to joints - commonly knees and ankles, and the sub-investigator marked 31 to indicate that the pain was not related to intermittent claudication. A similar pattern for both questionnaires was seen at week 78.

4.4 Discussion

This RCT has demonstrated that treatment of early DMARD naïve RA, with either a combination of IFX and MTX or MTX and MP as induction therapy, combined with a T2T approach, can improve biomarkers of CV risk. In addition it has demonstrated a superiority of IFX with MTX over MTX and steroids in the margin of improvement in HOMA-IR.

The baseline analysis revealed no association of TC/HDL-C with markers of disease activity, similar to reports from established RA cohorts [174], supporting the opinion TC/HDL-C may be more reliable than other lipid measurements in calculating long-term CV risk. There is evidence to support the synthesis of NT-proBNP following inflammatory cytokine release [116]. No association of NT-proBNP was identified with inflammation and disease activity (excluding HAQ-DI); this is in contrast to several other studies (although these included patients with longer disease durations) [117, 211]. In one early RA study, NT-proBNP correlated with baseline CRP, predicting NT-proBNP with repeated measures at 10 years [212], however, CV co-morbidity data was not collected limiting its application to those without clinical CVD. The utility of NT-proBNP in early RA could be limited, as currently there is no solid evidence to support higher NT-proBNP levels in early RA.

An improvement was seen in TC/HDL-C and IR in both treatment groups after 26 weeks of treatment, sustained at week 78. The improvement in TC/HDL-C occurred as a result of an increase in both HDL-C and LDL-C (Tables 4-6 and 4-7). Similar magnitudes of change in TC/HDL-C have been reported previously [261]. The effect of anti-rheumatic treatment on HOMA-IR has been less well studied, with most reporting the effect of TNFi alone, involving small established cohorts [284, 287] or with short term follow-up [472]. The work in this chapter provides valuable longer-term data. NT-proBNP also improved at weeks 26 and 78, although the change was not statistically significant at the latter time point. The prospective studies reporting improvement in NT-proBNP with TNFi are in established RA cohorts with mean disease durations of at least 7 years, higher baseline NT-proBNP values, and no clear exclusion of all known previous CVD [306, 308, 309], and so again longer term follow-up data may be required for early RA.

Meeting ACR70 response criteria was associated with lower values of TC/HDL-C and HOMA-IR at week 78; suggesting aggressive treatment of RA can lead to a more favourable lipid profile and improved IR in the long-term. The findings with TC/HDL-C are supported by Park *et al* who showed that the improvement in HDL-C and LDL-C/HDL-C in 42 early RA

patients after treatment with steroids and DMARDs for one year was significantly more so in ACR20 responders than non-responders. They did not report the results for TC/HDL-C [260]. The COBRA study similarly revealed a greater improvement in disease activity and TC/HDL-C in the combination DMARD group compared to sulphasalazine group after 16 weeks of therapy [263]. Improvements in HOMA-IR have been associated with a reduction in DAS28 following TNFi in other studies [287].

There was no difference in improvement in TC/HDL-C between the treatment regimes, in line with previous cross-sectional studies [266, 276] and the TEAR trial, where a similar fall in non-fasting TC/HDL-C was seen in all treatment arms [264]. Unlike our study, however, it is not clear if disease activity was similar across the groups at this time-point, making comparisons between therapeutic regimes difficult. NT-proBNP did not differ between the groups, but again longer follow-up may be needed to identify any differences.

The major finding from the work within this chapter was that TNFi appeared to provide additional benefit in the improvement in IR above the use of MTX and MP when using a T2T approach. IR improved by nearly half as much in comparison. This is an important finding. Patients with RA do not appear to have a greater prevalence of DM [193] (also noted in our study with the prevalence of DM similar to that found in the general population), however, patients with RA are at higher risk of insulin resistance [152]. In addition, overlapping genetic loci between type 1 DM and RA have been identified [480, 481].

This is the first RCT, to my knowledge, to compare the change in HOMA-IR between treatment regimes in early RA. One cross-sectional study reported no difference in HOMA-IR in 37 RA patients treated with either i) TNFi, ii) MTX or iii) no treatment, but disease durations and activity varied across the groups [288]. In the open label study by Tam *et al*, the higher fasting plasma glucose in the non-TNFi arm could be explained by a higher proportion of patients in TNFi group achieving remission; therefore, the difference seen reflecting the degree of systemic inflammation [290]. An unusual advantage in this chapter's work is that disease activity at week 78 was similar in both treatment groups, and controlling for IM steroid use produced comparable results.

The role of adipose tissue may underlie the observations. Visceral (central) obesity is associated with a chronic, low-grade inflammatory state (including the production of TNF) and has been implicated in the development of insulin resistance [482]. In support of this, HOMA-IR positively correlated with BMI in this study. Central abdominal rather than gluteo-femoral adipose tissue has been shown to secrete higher levels of inflammatory

cytokines [483] strengthening the argument that distribution of fat (i.e. WHR) is important in determining future CV risk. There was no relationship between WHR and baseline HOMA-IR in this chapter's work, although an increase in WHR over time was associated with an increasing IR.

There have been some studies assessing TNFi in non-RA patients [484, 485]. One RCT of 40 obese subjects with metabolic syndrome (MetS) randomized subjects to ETN or placebo, performing glucose tolerance testing and subcutaneous fat biopsy for TNF mRNA expression at baseline. HOMA-IR was significantly associated with C-reactive protein and serum TNF-receptor 1, but not adipose TNF expression. At 6 months, ETN improved fasting glucose and CRP, although the change in latter was not significant [486]. In contrast, one RCT assessing IFX in nine obese young men with MetS found no change in IR, however, only 3 infusions of IFX were given [487]. Another study of 12 patients with psoriasis and risks factors for type two DM, determined no difference in insulin sensitivity or secretion between those given ETN or placebo, however, the study duration was only two weeks [488]. Interestingly, a RCT involving 56 MetS patients given ETN/placebo for 4 weeks reported no change in HOMA-IR, but also a decrease in the high-to-low molecular weight adiponectin ratio (associated with IR) suggesting more complex interactions of TNF in adipose tissue [489].

The use of the Rose angina and Edinburgh claudication questionnaires proved unreliable in this study; primarily because synovitis led to many false positive results. Although both validated screening methods for CVD in the general population, it appears from that their use is limited in RA, and more specific tools are required. Clinical history combined with objective measures, such as an electrocardiogram, ankle brachial pressure index or applanation tonometry to determine arterial stiffness may perform better in RA patients, and should be specifically addressed in future studies.

This study has several limitations. Firstly, it was not designed nor powered to investigate the change in biomarkers of CV risk. However, the magnitude of difference between the treatment groups for IR is an important finding and should encourage larger more focussed studies. Secondly, only a subset of patients had biomarkers collected due to the practicalities of sample processing and storage in the peripheral hospitals. However, no selection bias was encountered as each hospital cohort was independently randomised. Finally, long-term data are required to validate the improvement in lipid profiles and IR found, and to detect any reduction in CV outcomes and DM. This data would also help

determine the biomarker that best predicts CVD in the context of systemic inflammation, particularly assessing the use of TC/HDL-C.

4.4.1 Summary

In conclusion, this study confirmed the improvement of soluble biomarkers of CV risk with suppression of disease activity in early RA using a T2T approach. TNFi appeared to show additional benefit over MTX and IV MP in measures of IR. This raises the question of whether TNFi may confer additional protection in the prevention of CVD over and above the suppression of inflammation. Longer-term studies should be encouraged, employing overt CV outcomes, or validated surrogate measures of CVD (in the general population) such as arterial stiffness or carotid intima-media thickness, as end-points.

4.4.2 Key messages

1. A treat to target approach in early RA improves soluble biomarkers of CV risk
2. TNF inhibition may provide additional advantage over methotrexate and methylprednisolone in improving insulin resistance measures.

Chapter 5 Cardiovascular biomarkers and the use of cardiac and carotid magnetic resonance imaging in established Rheumatoid Arthritis

5.1 Introduction

The risk of cardiovascular disease (CVD) is accelerated in Rheumatoid Arthritis (RA) [34], with meta-analyses determining standardised mortality ratios (SMR) of 1.59 and 1.77 for ischaemic heart disease (IHD) [5, 63]. Traditional CV risk factors only partially account for this increased risk [61, 141], and systemic inflammation as a result of active synovitis in RA is thought to be a contributor [204, 205, 208]. Until more is known regarding risk stratification, the European League of Rheumatism (EULAR) recommend the multiplication of cardiovascular (CV) risk scores by 1.5 if two or more specific criteria are present [18].

Surrogate measures of CVD which can either detect previous CV damage (markers of subclinical CVD) or highlight those at high risk of future CVD are used in RA populations. Soluble markers such as lipid profiles and NT-proBNP can predict CVD in patients with RA [119, 175], and measures of insulin resistance [194] and arterial stiffness, such as pulse wave velocity (PWV) by applanation tonometry [95], are higher in patients with RA. Cardiovascular magnetic resonance (CMR) imaging, providing information on cardiac dimensions, function, tissue viability, fibrosis, perfusion, coronary blood flow and arterial stiffness (protocol dependent, see Table 5-1) [15, 130], has already demonstrated superiority over other imaging modalities in the detection of ischaemic heart disease (IHD) in the general population [16], and is increasingly being used as research tool in patients with RA [131-133, 137, 138], however, many without the involvement of expert CMR-cardiologists, risking over-reporting of artefact for pathology.

Most CMR studies in RA to date have involved small numbers of patients [131, 132]. The largest study described the 1.5T CMR results from 75 patients with RA compared to 225 matched controls. The authors' key finding was an 18% reduction in LV mass in those with RA. LVEF, cardiac output and stroke volume were also found to be reduced compared to controls [135]. A preliminary report of a large CMR study, by our research group has demonstrated a reduced LV mass, as well as reduced vascular function, in 66 patients with

treatment-naive early rheumatoid arthritis [490]. Both of these CMR studies are in contrast to the many echocardiographic studies in RA, where an increase in LV mass has been detected [128, 129], and smaller CMR studies have not replicated this reduction in LV mass [136, 137].

Meta-analyses confirm greater common carotid intima-media thickness (CIMT) in those with RA [103, 104] predicting future CV events [102, 105]. However, as discussed in chapter 2, CIMT, as measured by ultra-sound (US) has some limitations. CMR-measured mean wall thickness (MWT) has been shown to correlate well with US-measured CIMT [107-110], with the added advantage of allowing the whole circumference of the artery to be measured at multiple levels. There is also evidence that MWT may correlate better with CV risk factor burden, suggesting the adventitia plays an important role in CV risk [110]; supported by recent data demonstrating increased expression of inflammatory cytokines in the aortic adventitia of patients with RA undergoing coronary artery bypass grafts (CABG) compared to non-RA CABG patients [491]. CMR-carotid arterial wall measurements have been shown to predict future CV events in the general population [492], but have yet to be measured in RA populations. Therefore, any RA CMR-MWT studies must extrapolate from US-measured CIMT studies for guidance on study design and power calculations.

To improve on the EULAR recommendations for reducing CVD in RA, there is a need to accurately determine the phenotype of patient with RA most at risk of CVD. CMR, used as the gold standard assessment tool, can be used to identify CVD and subsequently inform of this high-risk phenotype. Soluble biomarkers could then potentially be applied to determine any correlation with this profile. In addition, given the unique evolving immune processes in early RA [493], these CV assessment should be ideally be performed in two distinct RA disease sub-groups; early RA and established RA. Although, the disease duration to define established RA is decreasing, for a long time, disease for greater than five years was accepted as such [494].

No CMR study in RA to date has comprehensively described the CV risk profile of the patients involved, and reported in parallel, soluble markers of CVD and measures of arterial stiffness (by applanation tonometry) to provide insight into the RA disease phenotype most at risk of CVD.

5.1.1 Study hypothesis and aims

My thesis hypothesis relating to is that subclinical CVD is more prevalent in RA, and CVD is associated with a specific RA disease phenotype as well as traditional CV risk factors.

The aims of this chapter were in an established population of RA with no prior history of CVD;

- To describe the CV risk profile and prevalence of subclinical CVD as measured by surrogate measures, compared to healthy controls
- To describe the association of abnormalities with disease phenotype, serology and disease activity.
- To demonstrate the global utility of CMR in the detection of subclinical CVD in patients with RA
- To demonstrate the novel utility of carotid wall MRI imaging in patients with RA

5.1.2 Study objectives

The primary objective was to assess the difference between patients with established RA (free of known CVD and diabetes mellitus) and healthy controls in lipid profiling, insulin resistance, and other soluble markers of CVD, PWV as measured by applanation tonometry and cardiac/vascular MRI.

5.2 Methods

The IACON (Inflammatory Arthritis disease CONtinuum longitudinal) study is a single-centre observational study within the Leeds Teaching Hospital NHS trust (LTHT) that assesses and follows the patient with inflammatory arthritis/RA across the disease continuum (REC 09/H1307/98, Leeds West ethics committee). A cardiovascular sub-study was designed as a 'bolt-on' to the main study to allow a comprehensive assessment of the patient's CV health.

The IACON study was employed to investigate the above hypotheses. Patients with RA and healthy controls were invited to participate in the CV sub-study, and undergo a cross-sectional comprehensive CV assessment; a clinical assessment with blood collection and pulse wave velocity (PWV) measurement, and a non-contrast CMR.

5.2.1 Study design

5.2.1.1 Endpoints

The primary endpoint was mean wall thickness (MWT) (mm) as measured by MRI.

Secondary endpoints included

Soluble markers

- Total cholesterol/High density lipoprotein cholesterol (HDL-C) ratio
- Homeostasis model of assessment for insulin resistance (HOMA-IR)
- N terminal pro-brain natriuretic peptide (NTproBNP) (pg/ml)

Applanation tonometry

- Pulse wave velocity (m/sec)

CMR measures (as outline in Table 2-1 in chapter 2):

- LV ejection fraction (LVEF) (%)
- LV end-diastolic volume (EDV) (ml)
- LV end-systolic volume (ESV) (ml)
- LV mass indexed to body surface area (g/m^2)
- LVmass/EDV *(indicator of cardiac remodelling)*
- Stroke volume indexed to body surface area (ml/m^2)
- Mid systolic strain rate (Mid S') *(precursor to reduced LVEF)*
- Peak twist *(measure of diastolic dysfunction)*
- Torsion *(measure of diastolic dysfunction)*
- Inferoseptal T1 (ms)
- Aortic distensibility ($10^{-3}mmHg^{-1}$)
- Pulse wave velocity (PWV) (m/s)
- Minimum carotid wall thickness (mm)
- Maximum carotid wall thickness (mm)
- Carotid artery volume (ml)

5.2.1.2 Subject numbers

The primary endpoint for the CV assessment was MWT. MWT is a relatively novel measurement using CMR, with no prior reports in the RA population; therefore, this study represented a pilot study with no formal power calculations.

In collaboration with the departmental statistician, using the PEAR technique [495], it was calculated 70 patients with RA and 25 healthy controls should be recruited. This was based on an assumption that R-squared for the multiple linear regression model would be 0.5; the sample size was set so that the R-squared in a future validation study would be expected to shrink by no more than 40% i.e. that R-squared would remain substantive (0.3) if the model

were validated in future. The estimated sample size would also allow sufficient numbers for logistic regression modelling within the RA group and for comparison of MWT with healthy controls. This calculation was based on extrapolating data from a meta-analysis suggesting average CIMT to be 0.71mm (0.65-0.77mm) in RA and 0.62mm (0.56-0.68mm) in controls, with a mean difference of 0.009 (0.007-0.11) [104].

5.2.1.3 Eligibility criteria

For patients with RA:

Inclusion criteria

- RA meeting 1987 American College of Rheumatology (ACR) criteria [475]
- RA disease duration greater or equal to 5 years
- Between ages of 18 and 80 years old
- Capable of understanding and signing an informed consent form

Exclusion criteria

- History of cardiovascular disease (CVD); cardiac, peripheral or cerebral
- History of diabetes (to minimise confounding)
- Contraindications for MRI scanning; Pregnancy, breastfeeding, non-MR conditional pacemakers, surgical clips within the head, certain inner ear implants, neuro-electrical stimulators or metal fragments within the eye or head.

For controls:

Inclusion criteria

- Between ages of 18 and 80 years old
- Capable of understanding and signing an informed consent form

Exclusion criteria

- History of RA or significant osteoarthritis that affected their mobility (and potentially increasing their CV risk)
- History of cardiovascular disease (CVD); cardiac, peripheral or cerebral
- History of diabetes (to minimise confounding)
- Contraindications for MRI scanning; Pregnancy, breastfeeding, non-MR conditional pacemakers, surgical clips within the head, certain inner ear implants, neuro-electrical stimulators or metal fragments within the eye or head.

5.2.1.4 Target population

Consecutive patients with RA attending the general or biologics specialist rheumatology clinics at LTHT were considered for the study.

Healthy controls were identified by asking patients with RA to 'bring a friend' to minimise demographic differences; although due to challenges with recruitment, university colleagues were also invited. In addition, a decision was made not to individually match the controls to subjects for age and gender, but to match as a group and adjust in later analyses. In addition, CMR data from two healthy controls already recruited by the CMR research team using separate ethics pertaining to 'Assessment of myocardial perfusion by Magnetic Resonance Imaging: 3T Optimization of acquisition and analysis methods in patients with heart disease' (REC 10/H1307/103, Leeds West Ethics committee) were used. No clinical assessment was available for these controls; only age, gender and blood pressure were recorded.

5.2.1.5 Informed consent

Appropriate patients were given a patient information sheet to read either prior to their appointment in clinic or sent to them in the post. Time was given for them to digest the material and then the investigator (LAB) contacted the patient either in clinic or by telephone to determine their interest in participating, discuss the study in detail and answer any questions.

All participants (patients and controls) had at least 24 hours to consider the study before consenting. A study co-investigator took consent. In most instances the study investigator was LAB, but on occasion a delegated research nurse took consent. A copy of the consent was kept in the site file, with one copy filed in the notes, and another sent to the patent.

5.2.1.6 Withdrawal

Patients were free to withdraw from the study at any time, and participate in as much of the study as they wished. For example, some patients consented and underwent the clinical assessment but then changed their mind about undergoing the CMR. This did not affect their usual clinical care. Any data/blood samples already collected was retained and remained for analysis.

5.2.2 Study schedule

5.2.2.1 Clinical visit

Patients and controls attended Chapel Allerton Hospital, Leeds, UK to see the study investigator. The following procedures took place:

5.2.2.1.1 Informed consent.

The study was discussed further with the patient or control and any questions answered. Inclusion and exclusion criteria were checked. The patient or control and the study investigator then signed the consent appropriately.

5.2.2.1.2 Clinical evaluation

The following aspects of the study participant were recorded:

Demographics

- The participant demographic history including their age, gender and ethnicity.

Cardiovascular risk evaluation.

- History of smoking habit, hypertension, dyslipidaemia and family history of premature cardiovascular disease.
- How many minutes of moderate exercise taken per week (defined as enough to make them feel out of breath or perspire) and how many days per week five or more fruit and vegetables per day were consumed.
- Weight, height, waist and hip circumference were measured, along with blood pressure.
- The heart was auscultated for any heart murmurs suggesting underlying heart disease.
- Rose Angina Questionnaire completed.

RA disease phenotype evaluation (patients with RA only).

- Year of RA diagnosis.
- Minutes of current early morning stiffness.
- Current and previous DMARD and biological DMARD medication use for RA disease
- History of surgical procedures undergone as a result of RA disease.
- Joint examination to calculate 28 swollen and tender joint count [496].
- Health Assessment Questionnaire Disease Index (HAQ-DI) completed [497].
- Most recent hand or foot X-ray assessed for the presence or absence of erosions.

5.2.2.1.3 Fasting blood collection

The study participant came fasting (at least eight hours) for the study visit; hence most attended in the morning for the study. Under aseptic conditions, the following bloods were taken from RA patients; antinuclear antibody (ANA), rheumatoid factor (RF), anti-citrullinated peptide antibody (ACPA), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR),total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG), glucose. Two 9ml clotted red tops, one 4ml sodium citrate, one 4ml EDTA and two 9ml lithium heparin Vacutainers were filled, and the resulting anonymised processed aliquots of blood stored (some at -30^{o}C and some at -80^{o}C) for later biomarker analysis. All samples but the RA serology samples and lithium heparin Vacutainers were collected from controls.

The stored serum samples were utilised to measure the following in the department of Biochemical Diagnostics, Medical University of Lublin, Poland; glucose using the biochemical analyser Cobas INTEGRA 400; NT-proBNP using Cobas 6000 (immunochemistry module Cobas e601) and insulin using COBAS e 411 (Roche Diagnostics GmbH, Mannheim, Germany) and appropriate Roche Diagnostics assays. From these results, HOMA-IR was calculated; fasting insulin (μU/ml) × fasting glucose (mg/dl)/405 [191]. Samples were also analysed in a Myriad Human CardiovascularMAP® multi-analyte profile assay (Myriad RBM, Austin, Texas, USA). The following soluble biomarkers were analysed in this study (chosen for their broad representation across the atherosclerotic process); Apolipoprotein (Apo) A1 and B, lipoprotein A (lp(a)), E-selectin, intercellular adhesion molecule-1 (ICAM-1), P-selectin, vascular cell adhesion molecule-1 (VCAM-1), vascular endothelial growth factor (VEGF), CD40-ligand (CD40-L), monocyte chemotactic protein-1 (MCP-1), matrix metalloproteinase-1 and -9 (MMP-1, MMP-9), thrombomodulin, von Willebrand factor (vWF), adiponectin, fetuin-A and leptin.

5.2.2.1.4 Pulse wave velocity by applanation tonometry.

VICORDER® (SMT Medical, Würzburg, Germany) software was employed to measure PWV. The methodology replicated that visually demonstrated by a SMT medical representative who visited the department in 2010. Patients, in a fasting state, rested on the examination couch for at least five minutes. A neck pressure cuff was placed around the neck with the pressure pad overlying the carotid pulse. A second pressure cuff was placed around the right thigh proximally. The path length was calculated by measuring and adding the distance from the neck pressure pad to the sternal notch together with the distance from this notch to the mid-point of the thigh pressure pad. The automated technology inflated

the cuffs to a level just above diastolic blood pressure and produced a graph of the pressure wave at the two sites. If the wave form produced was of high quality, the integrated software calculated the speed of blood flow using the path length and transit time of the wave.

Only once the study was underway, after reading the inbuilt software guidelines, it became apparent that this methodology was incorrect and the path length should only be the distance between the sternal notch to the femoral cuff, and the distance from the carotid cuff to the notch was not required. Changing the distance used for the future study patients at this point would introduce error into the study. Therefore, after discussion with SMT medical and the study team, as a compromise, the two discrete distances (neck cuff to notch, and notch to thigh cuff) for future patients were recorded. The average of the carotid to notch distances from these patients was calculated, and this distance was subtracted from the total distance recorded in the software for each individual participant; from these new values, an adjusted PWV was calculated. Although this technique is not ideal, it makes some correction for distance and still supports an analysis between patients and between patients and controls.

5.2.2.2 Cardiovascular Magnetic Resonance Imaging visit

The study participant attended, usually on a separate day, for a non-contrast CMR scan at the Leeds General Infirmary, Leeds, UK. The scan was performed on a dedicated 3 Tesla Philips Achieva TX scanner (Philips Healthcare, Best, The Netherlands), with participants in the supine position, lasting approximately one hour.

5.2.2.2.1 CMR protocol

Trained CMR radiographers and expert CMR cardiologists directed and supervised the scan, in which the following sequences were performed;

1. Low-resolution survey, reference scans and localizers. Following survey and reference scans, the heart's axes were defined.

2. Baseline T1 mapping. Using a single end-expiratory breath holding technique, this feature measures T1 relaxation within the myocardium. T1 mapping provides information on the extent of extracellular matrix, or the myocardial interstitial space, increased with inflammation and fibrosis [498]. Although the whole myocardium can be mapped, inferoseptum T1 mapping has been shown to be a good indicator for the whole myocardium, and this is used for 'native T1'.

3. Tissue tagging for strain analysis and diastology. Peak twist and torsion, measures of myocardial contractile dysfunction, including diastolic dysfunction [499], can be calculated from this pulse sequence.

4. Aortic distensibility. Cross-sectional, high temporal resolution cine images of the ascending and descending thoracic aorta are recorded at the level of the pulmonary artery bifurcation, and along with recording blood pressure and heart rate, measurements of aortic stiffness can be calculated.

5. Carotid arterial imaging. Using a dStream Flex small 10cm phased array coil (Philips Healthcare, Best, The Netherlands), a general survey located the bifurcation of the common carotid artery into the internal and external carotid artery. PD, T1 and T2 weighted black blood sequences (non-breath holding) were performed along the common carotid artery path.

5.2.2.2.2 CMR evaluation

With supervision from an expert CMR-cardiologist (JG), a cardiology fellow (BE) reviewed and reported the cardiac CMR scans using QMASS MR 7.5 technology (Medis, Leiden, The Netherlands). Any unexpected findings were discussed with senior CMR cardiologists (SP and JG), acted upon appropriately and the participant, attending physician and GP were informed as required. The following measures were provided; LVEF, LVEDV, LVESV, stroke volume, LV mass/BSA, T1 mapping value, peak twist, torsion, PWV and aortic distensibility.

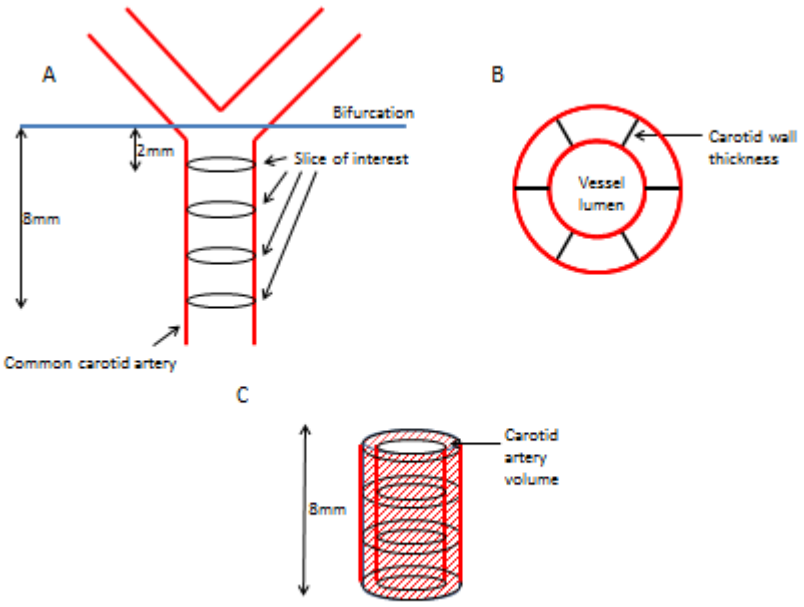
Another cardiology fellow (GF) evaluated the carotid artery pulse sequences in a blinded fashion, again using QMASS MR 7.5 software (Medis, Leiden, The Netherland). As MWT is a relatively novel measure, the detail of its evaluation is described below. The measurement methodology has close similarities to previous studies by other research groups [500-502].

Firstly, the carotid arteries were assessed on the general survey looking for the relative level of bifurcation of the common carotid arteries. Cross-sectional black blood T1 weighted static images of the right carotid artery were assessed in short function axis views. The bifurcation of common carotid artery was located, and the slices 2, 4, 6 and 8mm below this level were identified. For each of these slices, the carotid artery endothelium was manually marked out (using the same software and techniques used to mark out the left ventricular endocardium) and QMASS automatically produced an endovascular contour of best fit. The resulting tracing was adjusted manually if required. This contouring was repeated for the outer aspect of the adventitial layer (using the same

approach used to mark out the left ventricular epicardium) (see figure 5-1). A reference point was placed at the 12 o'clock position.

QMASS MR was then able to calculate the wall thickness within 6 segments around the wall for each slice. In this study, the minimum and maximum wall thickness, along with MWT of all 24 segments are reported. As part of the automated software output, the mass of the carotid wall was also produced; calculated by multiplying the wall volume by the density of the tissue (1.05mg/ul) (software meant for cardiac evaluation) [134]. Therefore, by working backwards, the carotid arterial wall volume is also reported in this study. The study investigators thought that this would provide a more accurate representation of the quantity of carotid arterial wall plaque, rather than discrete measurements of thickness. The total carotid wall volume was normalised for vessel size (carotid wall volume indexed) by calculating carotid wall volume/(carotid wall volume + luminal volume).

Figure 5-1 Diagrammatic representation of the carotid artery



A) Carotid artery at the level of bifurcation and the slices of interest B) a cross-sectional view of the carotid artery with wall thickness measurements C) carotid artery volume measured using data from the four slices of carotid artery.

Carotid arterial wall volume was only calculated for study participants with sufficient carotid data available. Although, the integrated software could compensate for missing data from slices 4mm and 6mm below the bifurcation, it required as a minimum dataset

from the slices 2mm and 8mm below the bifurcation. If the right carotid artery images were of poor quality or did not provide enough slices, the left carotid artery was used for measurement.

The reported CMR outcome measures were measured using established and validated protocols [130]. Intra and inter-observer variability testing (between GF and LAB) was performed for carotid MWT to demonstrate an acceptable co-efficient of variance.

5.2.3 Data collection

Clinical data for study participants were stored anonymously in an electronic database, accessed only by those involved in the study. Blood samples for storage were anonymised after processing. CMR scans were stored electronically and accessed only by those working within the CMR department.

5.2.4 Missing data

All efforts were made to avoid missing data. In the event of missing serology the most recent value found preceding the visit was carried forward into the data. This was not done for inflammatory markers such as CRP or ESR due to their capacity to vary depending on disease activity or for lipid/glucose profile, as a fasting state could not be verified and again their ability to differ with inflammatory load.

5.2.5 Statistical analysis

The statistical packages SPSS (IBM SPSS Statistics 22) and Stata/IC 13.1 were used.

5.2.5.1 Non-CMR data

The distribution of each variable was determined and a check for extreme outliers was performed. If any true outliers were found, the analysis was performed with them kept in and removed (both results shown). A descriptive analysis then followed to describe the cohort, abnormalities in lipid/glucose profile, soluble CV biomarkers and distribution of PWV, with comparison to healthy controls. The independent t test was used to determine unadjusted statistically significant differences in the soluble CV biomarkers and PWV between RA patients and controls. Non-normally distributed variables (TG, HOMA-IR, NTproBNP, Lp(a), MMP-1, vWF, leptin) were log transformed prior to analysis to allow the use of the independent t test; the resulting mean differences were exponentiated and results presented as a ratio of the value from the RA group to controls. Linear regression was used to determine differences between RA patients and controls when adjusted for 1)

age and gender, 2) age, gender, and CV risk factors. CV risk factors were defined as hypertension (either history of hypertension or anti-hypertensive agent), dyslipidaemia (either history of dyslipidaemia, on lipid-lowering medication or TC/HDL-C ratio greater than 6) and ever smoked. The log-transformed values were used for the non-normally distributed variables for this analysis; with the results representing the percentage change in CV soluble biomarker in patients with RA compared to controls.

Within the RA group, Pearson's/Spearman's correlation/univariate analysis (UVA) was used to determine associations between patient characteristics and CV soluble biomarkers and PWV. The log-transformed values were used for the non-normally distributed variables to allow Pearson's correlation testing when appropriate. 3 variable DAS28 was log-transformed prior to analysis due to lack of constance in residual variance (heteroskedasticity) in the UVA.

Any variables considered to be associated with PWV in the literature, or strongly correlated in an UVA (coefficient greater than 0.3), were put into a multivariate linear regression model to determine significant baseline variables (potentials in Table 5-1) associated with higher PWV.

The median differences (with corresponding 95% CIs) of ten year CV risk scores were calculated, followed by a Mann-Whitney U test to test for statistical significance between the RA patients and controls.

5.2.5.2 CMR data

Again, the independent t test was used to determine unadjusted statistically significant differences in CMR measures between RA patients and controls. Linear regression was used to determine differences between RA patients and controls when adjusted for 1) age and gender, 2) age, gender, and CV risk factors. CV risk factors were defined as for the non-CMR data.

Within the RA group, Pearson's correlation was used to determine associations between patient characteristics and CMR outcomes. Any variables considered to be associated with the CMR outcomes in the literature, or strongly correlated in an UVA (coefficient greater than 0.3), were put into a multivariate linear regression model to determine independently associated baseline variables (potentials in Table 5-1). For some variables heteroskedasticity was not improved by logarithmic transformation of the data, therefore standard errors were employed to compensate for this.

Correlation/univariate analyses were performed to detect associations between soluble CV biomarkers (only those significantly different in those with RA compared to controls) with CMR outcomes, and entered into the MVA described in the paragraph above if r was greater than 0.3.

Table 5-1 Potential variables incorporated into multivariate linear regression model

Demographic	Clinical	Laboratory	Functional
Age	Disease duration	ACPA	PWV
Gender	Smoking status (ever/never)		
	Systolic blood pressure		
	TC/HDL-C ratio		
	HAQ		
	3 variable DAS28		

Although this study is in essence an exploratory study, attempts to reduce false positive p values due to multiple comparisons was made. Holm's correction was employed when appropriate [503]. P-values were ranked from lowest to highest. The lowest one was compared to 0.05/(number of tests), repeated for the next lowest one calculating 0.05/(number of tests-1), and the next lowest one to 0.05/(number of tests-2) and so forth. The first time a p-value was higher than the value it was compared to determined the threshold for significance.

5.3 Results

95 consecutive patents with RA were recruited into the study; 76 of these underwent CMR, and 68 had carotid artery images acquired. Thirty-seven healthy controls were recruited; 26 undergoing the CMR but only 25 having carotid artery sequences performed. The reasons for not having the CMR are shown in Table 5-2.

Table 5-2 Reasons for study participants not having CMR scan

Reason	RA group (n=19)	Control group (n=11)
Participant claustrophobia	6	0
Unable to contact participant	6	1
Participant did not attend appointment	1	1
Participant changed mind	2	1
Non-MR conditional implant	1	0
CMR machine fault	1	0
Participant unable to physically fit into scanner	1	1
CMR scan not tolerated by participant	1	0
Participant deemed too young in comparison with the patients with RA	NA	7

5.3.1 Study participant characteristics

Table 5-3 outlines the demographic history and CV risk profile of the study participants who underwent a CMR scan, with table 5-4 describing the disease specific features of the patients with RA scanned.

The mean age (standard deviation (SD)) of the 76 patients with RA was 60 (9.2) years, with 74% being female. The majority were Caucasian. The patients with RA had a median (interquartile range (IQR)) disease duration of 16.5 (10.7, 25.7) years, 89.5% were seropositive for RF or ACPA and 78% had evidence of erosive disease on hand and foot radiographs. Twenty-one (27.6%) patients had a history of previous orthopaedic surgery, for example joint replacement or joint fusion; 11 (14.4%) of patients having 2 or more episodes of surgery. The disease activity score revealed patients overall were in remission; median (IQR) DAS28 was 2.59 (1.30, 3.33), early morning stiffness 10 (10, 37.5) minutes and CRP was 0 (0, 7.8) mg/L. Eighty-two percent were taking a conventional synthetic DMARD (csDMARD), with 21% taking two or more concurrently. Many had taken more in the past; median number of previously taken csDMARDs was two, ranging from none to seven. A large proportion was taking a biological DMARD; 29% using a TNF-α inhibitor and

34% rituximab. The median number of cycles for current rituximab users was four (ranging from one to nine cycles). Only 5% were currently taking regular oral prednisolone.

A significant proportion had CV risk factors; 33% had a history of hypertension, 25% of hypercholesterolaemia and 26% with a family history of premature CVD. Although 46% had never smoked, 13% were current smokers. 26.3% were taking 1 or more anti-hypertensive medications (10.5% on two or more) and 16% were on a statin to lower lipid levels. Mean systolic and diastolic blood pressure levels were within an acceptable range.

Control study participants were younger (mean (SD) age 51.8 (11.8) years). There were fewer females in the control group (54% female). There was less of a presence of CV risk factors in the control group; only 9% had a history of hypertension, 4% a history of hypercholesterolaemia and 18% with a family history of premature CVD. Again, a large proportion had never smoked, but there were fewer smokers (9%). Only 4% were taking anti-hypertensive agent and 4% a lipid-lowering agent. Average levels of systolic and diastolic blood pressure were lower in the control group. Waist/hip circumference and body mass index was similar in both the RA and control group.

Table 5-3 Study participant characteristics

Variable	**Expressed as**	**RA patients n=76**	**Controls n=26**
Demographics			
Age, years	mean, SD	60 (9.2) (range 31, 78)	52.2 (11.4) (range 35-80)
Female	n %	56 (73.7)	14 (53.8)
Ethnicity	n %	72 (94.7) Caucasian	23/24/ (95.8) Caucasian
CV risk profile			
PMH hypertension	n %	25 (32.9)	2/23 (8.7)
PMH Hypercholesterolaemia	n %	19 (25)	1/23 (4.3)
Smoking status: Never Ex Current	n %	35 (46.1) 31 (40.8) 10 (13.2)	12/23 (52.2) 9/23 (39.1) 2/23 (8.7)
Alcohol intake, units/week	Median (IQR)	2 (2, 8)	2 (2, 8)
FHx premature CVD*	n %	20 (26.3)	4/22 (18.2)
Five or more fruit/vegetables daily intake, days/week	Median (IQR)	5 (4, 7)	5 (4, 7)
Moderate exercise, mins/week	Median (IQR)	37.5 (0, 142.5)	60 (0, 255)
Number of current anti-hypertensives	n %	10 (13.2)on 1 drug 8 (10.5) on 2 drugs 2 (2.6) on 3 drugs	1/23 (4.3) on 2 drugs
Current use of statin	n %	12 (15.8)	1/23 (4.3)
BMI	mean, SD	26.1 (3.5)	25.0 (3.4)
Waist/Hip Ratio	mean, SD	0.84 (0.08) (n=74)	0.82 (0.09) (n=23)
Systolic BP, mmHg	mean, SD	135 (20)	127 (16) (n=25)
Diastolic BP, mmHg	mean, SD	80 (12)	72 (10) (n=25)

BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; FHx, family history of; PMH, past medical history of; RA, rheumatoid arthritis

*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 relative

Table 5-4 Disease specific characteristics of patients with Rheumatoid Arthritis

RA phenotype	**Data expressed as**	**RA patients n=76**
Disease duration, years	Median (IQR)	16.5 (10.7, 25.7) (range 4.2, 43.4)
Early morning stiffness, mins	Median (IQR)	10 (10, 37.5)
History of orthopaedic joint surgery	n %	21 (27.6)
Number of orthopaedic joint surgical episodes	n %	10 (13.2) - 1 episode 3 (3.9) - 2 episodes 6 (7.9) - 3 episodes 2 (2.6) - 4 episodes
Current use of oral prednisolone	n %	4 (5.3)
Current use of non-biological DMARD	n %	62 (81.6)
Number of csDMARDs currently taking	n %	48 (63.2) taking 1 8 (10.5) taking 2 8 (10.5) taking 3
Number of previously tried csDMARDs	Median (IQR)	2 (1, 3) (range 0, 7)
Current use of biological DMARD Current TNFI users Current Rituximab users	n %	51 (67.1) 22 (28.9) 26 (34.2)
Number of treatment cycles in current RTX users	Median (IQR)	4 (3, 5.25) (range 1, 9)
Number of previously tried biological DMARDs	Median (IQR)	0 (0, 1)
Patient general health VAS	Median (IQR)	31 (15, 52)
TJC28	Median (IQR)	2 (0, 6)
SJC28	Median (IQR)	0 (0, 1)
HAQ-DI	Median (IQR)	1.44 (0.53, 2.00)
3 variable DAS28CRP	Median (IQR)	2.59 (1.30, 3.33)
Erosions on hands/feet XR	n %	57/73 (78.1)

CRP (mg/L) (normal range <5)	Median (IQR)	0 (0, 7.8)
ESR (mm/hr) (normal range 1-15)	Median (IQR)	14 (6, 27)
RF positive (≥40iu/ml)	n %	53 (69.7)
ACPA positive (≥10U/ml)	n %	61/75 (81.3)

ACPA, anti-citrullinated peptide antibody; CRP, C-reactive protein; csDMARDs, conventional synthetic DMARDs; DAS28CRP, 28 joint disease activity score; DMARDs, disease modifying anti-rheumatic drugs; ESR, erythrocyte sedimentation rate; HAQ-DI, health assessment questionnaire-disability index; RF, rheumatoid factor; RTX, rituximab; SJC, swollen joint count; TJC, tender joint count; VAS, visual assessment score; XR, radiograph.

5.3.2 Soluble cardiovascular markers and pulse wave velocity

5.3.2.1 Routine fasting lipids and glucose measures

The fasting lipid and glucose profile results are shown in table 5-5. There was little difference in lipid and glucose levels between patients with RA and controls. In particular, the TC/HDL-C ratio in those with RA was very similar to the controls; 3.4 (1.0) vs. 3.3 (1.0) respectively, p=0.906.

Within the RA group, no lipid value was associated with CRP, 3-variable DAS28 or RA disease duration (data not shown); with similar findings if the patients on a statin (n=12) were excluded from the analysis. There was little difference in patients who were ACPA positive compared to those ACPA negative, although there was a non-statistically significant trend for higher TC/HDL-C in ACPA positive patients; mean (SD) TC/HDL-C ratio 3.4 (1.1) vs. 3.3 (0.8) in ACPA negative patients, mean difference 0.12 (95% CI -0.50, 0.73) p=0.706.

Table 5-5 Lipid, glucose and PWV profile of study participants

Variable	RA patients	Controls	Mean difference in RA from control group (95% CI)	Unadjusted p value for difference
Fasting glucose, mmol/L	4.9 (0.9) (n=72)	4.9 (0.8) (n=20)	0.06 (-0.39, 0.50)	0.805
Fasting total cholesterol, mmol/L	5.3 (1.1) (n=75)	5.1 (0.9) (n=21)	0.25 (-0.29, 0.78)	0.359
Fasting HDL-C, mmol/L	1.7 (0.4) (n=73)	1.6 (0.4) (n=21)	0.08 (-0.13, 0.29)	0.441
Fasting LDL-C, mmol/L	3.1 (0.9) (n=73)	3.0 (0.9) (n=21)	0.12 (-0.33, 0.57)	0.602
Fasting triglycerides, mmol/L*	1.16 (n=75) **	0.97 (n=21) **	1.20 (0.96, 1.49)***	0.108
Fasting TC/HDL-C ratio	3.4 (1.0) (n=73)	3.3 (1.0) (n=21)	0.03 (-0.46, 0.52)	0.906
PWV, m/sec	8.87 (2.0) (n=74)	8.30 (1.63) (n=22)	0.57 (-0.34, 1.48)	0.216

CI, confidence interval; CV, cardiovascular; PWV, pulse wave velocity; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio
Values expressed as mean (SD) unless stated otherwise
Mean differences calculated using independent t-test
*variables log transformed prior to analysis.
**values exponentiated to express as geometric mean (no SD available)
***values exponentiated to give ratio of difference of one group to another, with associated confidence intervals.

Table 5-6 Ten year cardiovascular disease risk scores (unadjusted and adjusted as per EULAR guidelines) of study participants

Ten year CVD risk score, %	RA patients	Controls	Median differences in RA group from controls (95% CI)	Unadjusted p value for difference**
Unadjusted				
Framingham	11.5 (5.4, 18.4)	5.0 (1.7, 13.3)	3.31 (-0.12, 7.20)	0.052
Joint British Societies 2	8.6 (4.1, 8.6)	4.2 (1.2, 10.5)	2.49 (-0.24, 5.61)	0.087
Adjusted as per EULAR guidelines*				
Framingham	17.2 (6.3, 26.7)	5.0 (1.7, 13.3)	6.48 (2.77, 12.40)	**0.004**
Joint British Societies 2	12.9 (12.8, 18.8)	4.2 (1.2, 10.5)	4.78 (1.83, 9.55)	**0.007**

CI, confidence interval.
Values expressed as median (interquartile range)
*Values multiplied by 1.5 if two or more of following present: disease duration greater than 10 years, RF or ACPA, or presence of severe extra-articular features [18]
**Mann Whitney U test

Using the lipid values, the ten-year CVD risk scores of the study participants were calculated. The Framingham risk scores in the RA group were double that of the control group, and nearly the same pattern was seen with Joint British Societies scores), however the difference did not show statistical significance. As might be expected, a greater difference was seen when the EULAR guidelines were followed and the risk scores of those patients with RA with two or more 1) disease duration greater than 10 years, 2) RF or ACPA, or 3) presence of severe extra-articular features, were multiplied by 1.5 (see Table 5-6); median (IQR) Joint British Societies 2 (JBS2) risk; 12.9 (12.8, 18.8)% vs. 4.2 (1.2, 10.5)% in controls, median difference 4.78 (95% CI 1.83, 9.55), p=0.007.

5.3.2.2 Soluble cardiovascular biomarkers

5.3.2.2.1 Differences between patients with RA and controls

Table 5-7 describes the differences in soluble CV biomarkers in RA compared to controls; including detailed lipid profiling, markers of endothelial dysfunction, plaque destabilisation/rupture, mediators within the clotting pathway, metabolic markers and of myocardial stretch.

Although there was a trend for higher values of lipids, particularly Lp(A), and less so for Apo B, in those with RA, the difference did not meet statistical significance, even after adjustment for age and gender, and adjusting for CV risk factors. ICAM-1 was increased in patients with RA; mean difference (95% CI) in RA vs. controls was 19.3 (8.3, 30.4)ng/ml, p=0.001), along with MCP-1 (mean difference (95% CI) 69.2 (10.0, 128.3)pg/ml, p=0.022). However, the statistical significances were lost after adjustment for age and gender, and CV risk factors. There appeared to be a trend for higher values of E-Selectin and VEGF in those with RA.

A non-statistically significant trend was also observed for higher levels of MMP-1 and MMP-9 in patients with RA. vWF was also increased in those with RA; values were 1.3 times as high (95% CI 1.1, 1.5, p=0.003) in patients with RA compared to controls, although it just lost statistical significance after adjustment for age and gender, and CV risk factors.

Considering metabolic biomarkers, only leptin was higher in those with RA compared to controls; values were 1.9 times as high (95% CI 1.2, 3.0, p=0.004) in RA, however, again statistical significance was just lost after adjustment for age and gender, and more so including CV risk factors. There was a trend seen for higher values of adiponectin in those

with RA (mean (SD) 7.8 (3.9)ug/ml in RA, 6.2 (2.2)ug/ml in controls, unadjusted p value for difference =0.057).

A similar trend for higher levels was seen for NT-proBNP (RA values were 137% of the values of controls (95% CI 81% to 231%, p=0.243).

Using Holm's method to attempt to correct for multiple comparisons, a new p value of 0.003 was calculated to determine statistical significance when comparing soluble CV biomarkers in patients with RA to controls. This new p value determined only ICAM-1 was significantly different between the groups, with vWF only just reaching significance.

5.3.2.2.2 Association with RA disease phenotype

An exploratory correlation analysis is detailed in Table 5-8.

There was evidence for the association of some soluble CV biomarkers with traditional CV risk factors in RA. In particular, strong associations (r>0.3) were seen with increasing age with vWF (r=0.339, p=0.003) and NT-proBNP (r=0.325, p=0.006), and increasing atherogenic index with higher ICAM-1 (r=0.409, p<0.001) and lower adiponectin (r=-0.320, p=0.006) levels. Lower adiponectin and leptin levels were found in males (p=0.019 and <0.001 respectively). Smoking was associated with lower leptin levels (p=0.008).

As expected increasing BMI was associated with increasing leptin (r=0.515, p<0.001), and increasing WHR with HOMA-IR (p=0.368, p=0.002) and lower adiponectin (r=-0.396, p=0.001).

PWV was associated with increasing levels of MCP-1 (r=0.336, p=0.004), vWF (r=0.281, p=0.016) but unexpectedly, lower MMP-1 levels (r=-0.391, p=0.001).

Increasing RA disease duration was associated with greater adiponectin levels (r=0.320, p=0.005); with a weaker association with increasing NT-proBNP (r=0.278, p=0.019), and of increasing HAQ-DI with VEGR (r=0.242, r=0.049). No other RA disease phenotype variable was significantly associated with the soluble CV biomarkers.

Table 5-7 Soluble cardiovascular biomarkers in patients with RA and controls

Biomarker	RA n=75	Controls n=24	Mean difference in RA from control group (95% CI), p value	Mean difference (95% CI), p value adjusted for age and gender	Mean difference (95%), p value adjusted for age/sex, CV risk factors^
Lipids					
Apo A1, mg/ml	2.47 (0.55)	2.33 (0.33)	0.15 (-0.09, 0.38), 0.220	0.06 (-0.19, 0.31), 0.640	0.04 (-0.23, 0.31), 0.776
Apo B, ug/ml	1737.8 (665.8)	1560.8 (365.3)	1477.0 (-106.1, 460.0), 0.103	93.6 (-222.2, 409.39), 0.558	111.7 (-228.9, 452.2), 0.516
Lp(a), ug/ml	157.1*	113.4*	1.4 (0.7, 2.7)**, 0.323	44.6 (-30.4, 200.4)***, 0.320	62.1 (-25.1, 251.1)***, 0.217
Endothelial function					
E-Selectin, ng/ml	8.96 (3.97)	8.20 (3.22)	0.76 (-1.01, 2.53), 0.394	1.11 (-0.86, 3.08), 0.265	0.99 (-1.07, 3.04), 0.341
ICAM-1, ng/ml	101.4 (37.4)	82.1(17.1)	**19.3 (8.3, 30.4), 0.001**	16.0 (-1.6, 33.6), 0.074	16.3 (-1.8, 34.3), 0.076
MCP-1 pg/mL	333.4 (128.8)	264.3 (121.6)	**69.2 (10.0, 128.3), 0.022**	54.7 (-10.26, 119.7), 0.098	52.2 (-16.5, 121.0), 0.135
P-Selectin, ng/ml	132.9 (42.2)	135.7 (42.1)	-2.8 (-22.5, 16.8), 0.774	3.1 (-18.4, 24.5), 0.778	0.2 (-22.3, 22.8), 0.983
VCAM-1, ng/ml	710.4 (162.7)	696.5 (145.0)	13.9 (-60.0, 87.7), 0.710	-32.6 (-111.9, 46.8), 0.417	-26.1 (-111.9, 59.6), 0.546
VEGF, pg/ml	283.6 (128.3)	236.8 (142.8)	46.9 (-14.5, 108.3), 0.133	59.8 (-8.6, 128.2), 0.086	57.6 (-16.1, 131.2), 0.124
Plaque destabilisation/post-rupture					
CD40L, ng/ml	2.38 (0.97)	2.45 (0.83)	-0.07 (-0.50, 0.37), 0.763	0.01 (-0.48, 0.50), 0.957	0.02 (-0.50, 0.53), 0.953
MPO, ng/ml	1085.0 (645.4)	1308.0 (770.3)	-223.0 (-538.2, 92.2), 0.163	4.5 (-333.4, 342.5), 0.979	8.0 (-354.8, 370.9), 0.965
MMP-1, ng/ml	11.88*	9.63*	1.23 (0.93, 1.64)**, 0.143	11.96 (-18.05, 52.81)***, 0.474	11.96 (-19.67, 55.89)***, 0.501

MMP-9, ng/ml	1079.3 (364.2)	995.8 (379.7)	83.6 (-87.7, 254.8), 0.335	183.4 (-3.4, 370.2), 0.054	200.5 (-0.6, 401.6), 0.051
Clotting pathway					
Thrombomodulin, ng/ml	4.35 (1.05)	4.33 (1.08)	0.02 (-0.47, 0.52), 0.932	-0.09 (-0.60, 0.42), 0.722	-0.20 (-0.73, 0.34), 0.470
vWF, ug/ml	109.2*	84.3*	**1.3 (1.1, 1.5)**, 0.003**	19.1 (-0.2, 42.3)***, 0.053	20.4 (-0.3, 45.4)***, 0.054
Metabolic					
Adiponectin, ug/ml	7.82 (3.91)	6.20 (2.23)	1.62 (-0.05, 3.29), 0.057	0.19 (-1.55, 1.92), 0.832	0.38 (-1.42, 2.18), 0.672
Fetuin-A, ug/ml	1021.3 (228.3)	1010.2 (178.8)	11.2 (-90.1, 112.5), 0.827	-8.9 (-122.4, 104.52), 0.876	-13.5 (-128.9, 102.0), 0.817
HOMA-IR	1.10* (n=71)	1.20* (n=22)	0.92 (0.62, 1.36)**, 0.661	7.25 (-30.37, 65.04)***, 0.749	-4.11 (-39.29, 51.59)***, 0.856
Leptin, ng/ml	11.14*	5.77*	**1.93 (1.23, 3.02)**, 0.004**	49.2 (-1.5, 125.9)***, 0.059	35.1 (-9.1, 100.8)***, 0.135
Myocardial stretch					
NT-proBNP, pg/ml	57.64* (n=71)	42.17*(n=22)	1.37 (0.81, 2.32)**, 0.243	-13.7 (50.8, 51.4)***, 0.606	-26.8 (-59.1, 31.0)***, 0.290

Apo, apolipoprotein; CD40-L, CD40 ligand; CI, confidence interval; HOMA-IR, homeostasis model of assessment of insulin resistance; ICAM, intercellular adhesion molecule, Lp(a); lipoprotein A; MCP; monocyte chemotactic protein; MMP; matrix metalloproteinase; MPO, myeloperoxidase; NT-proBNP, N-terminal pro-brain natriuretic peptide; RA, rheumatoid arthritis; VEGR, vascular endothelial growth factor, VCAM; vascular cell adhesion molecule, vWF, von Willebrand factor

*Geometric mean

**values exponentiated to give ratio of difference of one group to another, with associated confidence intervals.

***% change

^CV risk factors defined as: hypertension (history of hypertension or anti-hypertensive agent), dyslipidaemia (history of dyslipidaemia, on lipid-lowering medication or TC/HDL-C ratio greater than 6), ever smoked and family history of premature CVD

Table 5-8 Association of soluble cardiovascular biomarkers with disease phenotype in 75 patients with RA

Variable	Statistic	Lp(a)^	E-Selectin	ICAM-1	VEGF	MCP-1	MMP-1^	MMP-9	vWF^	Adiponectin	Leptin^	HOMA-IR (n=71)	NT-proBNP^ (n=71)
Age	r	-0.081	0.117	0.061	-0.162	0.225	-0.07	-0.079	**0.339**	**0.249**	-0.009	-0.100	**0.325**
	p-value	0.492	0.318	0.605	0.166	0.052	0.551	0.503	**0.003**	**0.031**	0.942	0.407	**0.006**
Male gender*	rho	-0.049	0.148	0.036	-0.104	0.052	-0.151	**0.234**	0.143	**-0.270**	**-0.606**	0.090	-0.205
	p-value	0.674	0.206	0.762	0.372	0.656	0.197	**0.043**	0.219	**0.019**	**<0.001**	0.455	0.086
Systolic BP	r	**-0.236**	0.224	-0.003	0.078	0.199	-0.139	0.17	0.135	-0.141	0.04	0.142	0.161
	p-value	**0.042**	0.054	0.977	0.504	0.087	0.235	0.145	0.249	0.227	0.736	0.239	0.181
Ever smoked*	rho	0.144	0.09	**0.264**	-0.066	-0.03	0.051	-0.028	-0.045	0.088	**-0.304**	-0.043	-0.059
	p-value	0.219	0.44	**0.022**	0.576	0.796	0.661	0.809	0.7	0.45	**0.008**	0.724	0.628
BMI	r	-0.202	0.105	-0.06	0.109	0.07	-0.143	-0.055	0.02	-0.144	**0.515**	**0.240**	0.196
	p-value	0.082	0.37	0.609	0.353	0.552	0.222	0.64	0.864	0.219	**<0.001**	**0.044**	0.101
Waist/hip circumference	r	-0.196 (n=73)	0.203 (n=73)	0.097 (n=73)	-0.052 (n=73)	0.076 (n=73)	-0.159 (n=73)	0.211 (n=73)	0.197 (n=73)	**-0.396** (n=73)	-0.137 (n=73)	**0.368** (n=69)	0.076 (n=69)
	p-value	0.097	0.086	0.415	0.66	0.521	0.179	0.073	0.095	**0.001**	0.247	**0.002**	0.533
TC/HDL-C	r	0.198 (n=72)	0.211 (n=72)	**0.409** (n=72)	0.209 (n=72)	-0.034 (n=72)	0.073 (n=72)	0.019 (n=72)	0.114 (n=72)	**-0.320** (n=72)	-0.017 (n=72)	0.027 (n=68)	0.002 (n=68)

	p-value	0.095	0.075	**<0.001**	0.078	0.776	0.542	0.877	0.342	**0.006**	0.884	0.827	0.99
RA disease	r	0.01	0.032	-0.161	-0.09	0.066	-0.184	0.034	0.021	**0.320**	-0.182	-0.102	**0.278**
duration^	p-value	0.932	0.783	0.168	0.441	0.574	0.113	0.77	0.857	**0.005**	0.118	0.395	**0.019**
3 variable	r	0.002	0.065	-0.042	0.148	0.015	-0.069	0.119	-0.107	-0.124	0.178	-0.069	0.027
DAS28^	p-value	0.989	0.58	0.721	0.206	0.897	0.554	0.308	0.359	0.291	0.127	0.57	0.824
ACPA*	rho	-0.182 (n=74)	0.183 (n=74)	0.212 (n=74)	0.065 (n=74)	0.096 (n=74)	0.04 (n=74)	-0.119 (n=74)	-0.134 (n=74)	0.144 (n=74)	0.16 (n=74)	-0.130 (n=70)	0.033 (n=70)
	p-value	0.121	0.119	0.07	0.584	0.415	0.732	0.314	0.255	0.222	0.173	0.283	0.788
HAQ-DI^	r	-0.033 (n=67)	0.08 (n=67)	0.202 (n=67)	**0.242** (n=67)	0.132 (n=67)	0.037 (n=67)	0.095 (n=67)	0.148 (n=67)	0.085 (n=67)	0.195 (n=67)	0.171 (n=64)	0.127 (n=64)
	p-value	0.791	0.52	0.1	**0.049**	0.287	0.764	0.447	0.231	0.494	0.113	0.177	0.318
Hx joint	rho	0.066	0.115	0.183	-0.026	-0.097	0.047	0.019	0.034	0.087	-0.088	0.079	0.082
surgery*	p-value	0.573	0.326	0.117	0.822	0.409	0.691	0.868	0.771	0.458	0.45	0.513	0.496
bioDMARD	rho	-0.022	0.099	0.118	0.038	-0.123	0.005	0.019	0.042	-0.054	0.11	0.054	0.081
current use*	p-value	0.854	0.399	0.312	0.747	0.291	0.964	0.872	0.722	0.648	0.348	0.656	0.5
PWV	r	-0.207 (n=73)	0.197 (n=73)	0.166 (n=73)	-0.115 (n=73)	**0.336 (n=73)**	**-0.391 (n=73)**	0.035 (n=73)	**0.281** (n=73)	-0.108 (n=73)	-0.096 (n=73)	0.024 (n=69)	0.202 (n=69)
	p-value	0.079	0.095	0.161	0.332	**0.004**	**0.001**	0.766	**0.016**	0.364	0.417	0.847	0.096

bioDMARD, biological disease modifying therapy; BP, blood pressure; HOMA-IR, homeostasis model of assessment of insulin resistance; Hx, history of; ICAM, intercellular adhesion molecule, Lp(a); lipoprotein A; MCP; monocyte chemotactic protein; MMP; matrix metalloproteinase; NT-proBNP, N-terminal pro-brain natriuretic peptide; RA, rheumatoid arthritis; VEGR, vascular endothelial growth factor, vWF, von Willebrand factor
*Spearman's correlation
^Logarithmic values used in analysis

5.3.2.3 Pulse wave velocity

Pulse wave velocity as measured by applanation tonometry was similar in both the RA and control group (mean (SD) 8.87 (2.0) m/sec vs. 8.30 (1.63) m/sec, mean difference 0.57 (95% CI -0.34, 1.48)m/s unadjusted p value=0.216). No difference was seen when adjusted for age, gender and CV risk factors (defined as history of hypertension or on anti-hypertensive medication, history of hypercholesterolaemia, on lipid lowering agent or TC/HDL-C greater than 6, ever smoked or family history of premature CV disease; mean difference (95% CI) -0.072 (-0.921, 0.777)m/s, p=0.866).

A univariate analysis (UVA) in those with RA found PWV correlated with age (coefficient 0.502, p<0.001) (see figure 5-2), male gender (coefficient 0.231, p=0.048), systolic blood pressure (coefficient 0.544, p<0.001) and hip/waist circumference (coefficient 0.371, p<0.001), but no association was detected with RA disease specific features such as disease duration, CRP, 3-variable DAS28, ACPA positivity, previous joint surgery or use of biological DMARDs (see Table 5-9). Variables considered to be associated with PWV in the literature, or were strongly associated in the UVA (coefficient greater than 0.3), were put into a multivariate linear regression model; only increasing age (B (95% CI): 0.070 (0.020, 0.120), p=0.007) and systolic blood pressure (B (95% CI): 0.042 (0.019, 0.065), p=0.001) were independently associated with PWV (R^2=0.472).

Table 5-9 Univariate and multivariate analysis of variables associated with pulse wave velocity by applanation tonometry in patients with RA.

Variable	**Univariate analysis (number of observations=74 unless otherwise stated)**			**Multivariate analysis R^2=0.472 (n=66)**	
	Correlation coefficient (r)	**B (95% CI)**	**p value**	**B (95% CI)**	**p value**
Age*	0.502	0.105 (0.063, 0.148)	<0.001	**0.070 (0.020, 0.120)**	**0.007**
Male gender*	0.236	1.031 (0.035, 2.027)	0.043	0.619 (-0.452, 1.690)	0.252
Systolic blood pressure*	0.544	0.057 (0.037, 0.078)	<0.001	**0.042 (0.019, 0.065)**	**0.001**
Ever smoked	0.062	0.243 (-0.669, 1.154)	0.597	-	-
BMI	-0.034	-0.019 (-0.147, 0.110)	0.772	-	-
Waist/hip circumference	**0.371**	9.173 (3.697, 14.650) (n=72)	0.001	3.123 (-2.901, 9.147)	0.304
TC/HDL-C*	0.047	0.098 (-0.396, 0.591) (n=71)	0.694	-0.032 (-0.445, 0.380)	0.877
HOMA-IR	0.024	0.073 (-0.140, 0. 286) (n=69)^	0.497	-	-
NT-proBNP	0.202	0.002 (-0.003, 0.008) (n=69)	0.397	-	-
RA disease duration*	0.097	0.006 (-0.037, 0.050)	0.773	-0.030 (-0.070, 0.011)	0.145
Ln(3 variable DAS28)*	-0.067	-0.003 (-0.012, 0.007)**	0.570	0.004 (-0.002, 0.018)**	0.339
ACPA*	0.121	0.593 (-0.555, 1.740) (n=73)	0.3067	0.786 (-0.364, 0.708)	0.523

HAQ-DI*	0.065	0.161 (-0.436, 0.759) (n=70)	0.592	0.172 (-0.364, 0.708	0.523
History of orthopaedic joint surgery	-0.128	-0.551 (-1.552, 0.450)	0.276	-	-
Current use of biological DMARD	-0.069	-0.284 (-1.245, 0.676)	0.557	-	-

ACPA, anti-citrullinated peptide antibody; BMI, body mass index; CRP, C-reactive protein; DAS28CRP, 28 joint disease activity score; DMARD, disease modifying anti-rheumatic drug; HAQ-DI, health assessment questionnaire-disability index; HOMA-IR, homeostasis model of assessment of insulin resistance; Ln, natural logarithm; NT-proBNP, N-terminal pro-brain natriuretic peptide; RA, rheumatoid arthritis; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio

Pearson's correlation coefficients greater than 0.3 (highlighted in bold) also entered into the linear regression model.

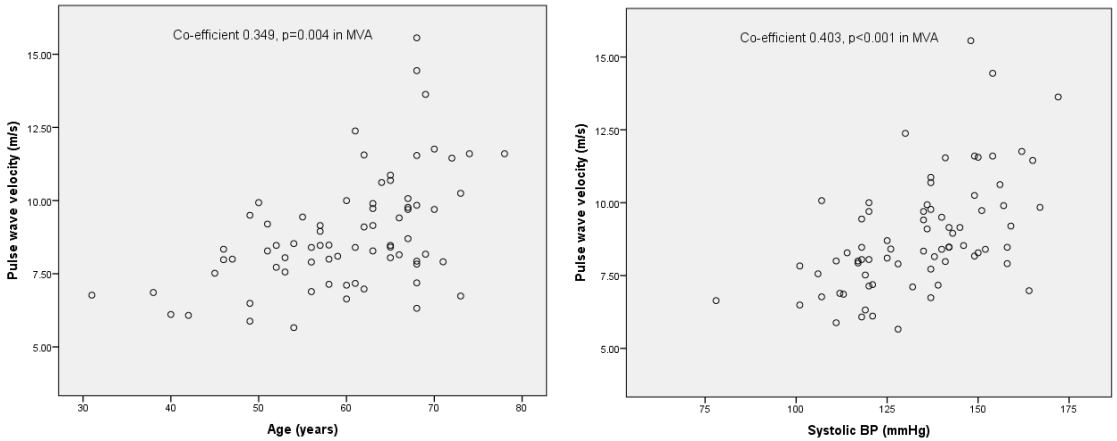
*variable entered into linear regression model as associated with LV mass in the literature

** Values presented are the exponentiated results of the analysis (B*Ln(1.01))

^Excluding high outlier: Correlation coefficient -0.053 B -0.106 (95% CI -0.505, 0.293) p=0.597 (robust standard errors employed due heteroskedasticity)

Figure 5-2 Scatter plot to show association of pulse wave velocity in patients with rheumatoid arthritis with age and systolic blood pressure.

A) Age B) Systolic blood pressure



MVA, multivariate analysis.

5.3.3 Cardiovascular magnetic resonance imaging

5.3.3.1 Differences between patients and controls

The CMR outcome measures are shown in table 5-10. The differences between patients with RA and controls were checked, and then adjusted for age and sex, and then again including CV risk factors; defined as hypertension (history of hypertension or anti-hypertensive agent), dyslipidaemia (history of dyslipidaemia, on lipid-lowering medication or TC/HDL-C ratio greater than 6) and ever smoked.

LV volumes and dimensions: LVmass/BSA was reduced in the RA group (mean (SD) 36.35 (10.52) vs. 44.06 (14.49)g/m^2 in controls, p=0.005), a difference remaining after adjustment for age, sex and traditional CV risk factors (mean difference (95% CI) -4.558 (-8.917, -0.199) p=0.041). LVEDV, LVESV and stroke volume (all indexed to BSA) were reduced in patients with RA but only stroke volume/BSA remained significant after adjustment for age and sex, and no significant difference remained after adjusting for CV risk factors.

LV function: Patients with RA had a lower LVEF compared to controls (mean (SD) LVEF in RA 59.1 (4.6) vs. 59.7 (4.8)% in controls); clinically and statistically significant after adjustment for age, sex, and CV risk factors (mean difference -2.858 (-5.167, -0.550)%, p=0.016). Mid systolic strain rate (mid S') was greater in RA patients compared to controls (mean (SD) -1.205 (0.209) vs. -1.398 (0.237) in controls, p<0.001, remaining significant after adjustment for age, sex and CV risk factors. Looking to signs of diastolic dysfunction, peak twist was found to be lower in patients with RA (mean difference (95% CI) -2.451 (-4.823, -0.080) p= 0.043 after adjustment for age, sex, and CV risk factors), however torsion values, which take into account heart length and diameter, were similar in both groups. Although early diastolic strain rates were similar between the groups, active/late diastolic strain rates were reduced; mean difference (95% CI) -0.45 (-0.67, -0.23) p<0.001 after adjustment for age, sex, and CV risk factors.

LV extracellular matrix: Native T1 values, an indicator of extracellular volume, were lower in patients with RA compared to controls (1156.82 (53.07) vs. 1186.00 (49.30)ms in controls, p=0.017); a difference remaining significant after adjustment for age, sex and CV risk factors (mean difference (95% CI) -34.776 (-64.058, -5.495)ms p=0.021).

Aortic stiffness: CMR measures of aortic stiffness were inconsistent; whilst aortic distensibility appeared lower in patients with RA (i.e. stiffer artery) (2.60 (1.82) vs. 3.83 (1.56)$10^{-3}$$mmHg^{-1}$, p=0.003), PWV was similar in both groups. The difference in aortic distensibility did not remain after adjustment for age, sex and CV risk factors (p=0.538).

Correction for multiple comparisons

Using Holm's method to attempt to correct for multiple comparisons, a new p value of 0.016 was calculated to determine statistical significance when comparing secondary CMR outcomes in patients with RA to controls, when adjusted for age, gender and CV risk factors. This new p value determined only mid S' and mid E' was significantly different between the groups, with LVEF only just reaching significance.

Table 5-10 Cardiovascular magnetic resonance imaging measures in study participants

Variable	RA patients n=76	Controls n=26	Unadjusted p value for difference	Mean difference (95% CI), p value adjusted for age and gender	Mean difference (95%), p value adjusted for age/sex, CV risk factors*
LVEF, %	59.1 (4.6) (n=74)	59.7 (4.8)	0.560	-2.100 (-4.268, 0.068), 0.057	**-2.858 (-5.167, -0.550) 0.016**
LV EDV/BSA, ml/m^2	79.09 (14.59) (n=74)	91.08 (24.32)	**0.024**	-5.15 (-12.908, 1.878) 0.142	-4.683 (-12.275, 2.909) 0.224
LV ESV/BSA, ml/m^2	32.45 (7.60) (n=74)	36.88 (12.00)	**0.032**	-0.545 (-4.311, 3.222) 0.775	1.187 (-2.491, 4.865) 0.523
LVmass/BSA, g/m^2	36.35 (10.52) (n=74)	44.06 (14.49)	**0.005**	**-5.421 (-9.615, -1.227), 0.012**	**-4.558 (-8.917, -0.199) 0.041**
LV mass/EDV, g/ml	0.46 (0.10) (n=74)	0.48 (0.10)	0.266	-0.035 (-0.075, 0.005), 0.086	-0.033 (-0.075, 0.010) 0.129
Stroke volume/BSA, ml/m^2	46.29 (7.26) (n=74)	53.47 (11.20)	**0.005**	**-4.273 (-7.998, -0.548), 0.025**	-0.3551 (-7.516, 0.413) 0.078
Mid S'	-1.205 (0.209) (n=69)	-1.398 (0.237) (n=23)	**<0.001**	**0.200 (0.089, 0.311) 0.001**	**0.227 (0.104, 0.349) <0.001**
Mid E'	0.70 (0.21) (n=69)	0.69 (0.29) (n=23)	0.794	0.02 (-0.003, 0.01), 0.791	0.04 (-0.09, 0.17), 0.511
Mid A'	1.65 (0.36) (n=68)	2.11 (0.50) (n=23)	**<0.001**	**-0.44 (-0.64, -0.45), <0.001**	**-0.45 (-0.67, -0.23), <0.001**
Peak twist, degrees	10.77 (4.31) (n=69)	12.38 (3.74) (n=23)	0.113	-2.026 (-4.162, 0.110) 0.063	**-2.451 (-4.823, -0.080) 0.043**
Torsion, degrees	10.94 (3.86) (n=68)	10.80 (4.41) (n=23)	0.886	-0.284 (-2.331, 1.764) 0.784	-0.889 (-3.139, 1.360) 0.434
Native (inferoseptal) T1, ms	1156.82 (53.07) (n=68)	1186.00 (49.30)	**0.017**	**-30.088 (-56.300, -3.875) 0.025**	**-34.776 (-64.058, -5.495) 0.021**
PWV (m/sec)	7.8 (2.9) (n=73)	7.2 (2.3)	0.339	-0.289 (-1.483, 0.905) 0.632	-0.443 (-1.639, 0.753) 0.464

Distensibility, 10^{-3}mmHg^{-1}	2.60 (1.82) (n=75)	3.83 (1.56) (n=25)	**0.003**	-0.340 (-0.996, 0.316) 0.307	-0.222 (-0.936, 0.492) 0.538

A'=Late/active diastolic strain rate; BSA, body surface area; CV, cardiovascular; E'=Early diastolic strain rate; EDV, end-diastolic volume; ESV, end-systolic volume; LV, left ventricular; LVEF, left ventricular ejection fraction; PWV, pulse wave velocity; S'=Peak systolic strain rate

Values expressed as mean (SD) unless stated otherwise

*CV risk factors defined as: hypertension (history of hypertension or anti-hypertensive agent), dyslipidaemia (history of dyslipidaemia, on lipid-lowering medication or TC/HDL-C ratio greater than 6), ever smoked and family history of premature CVD.

5.3.3.2 Association with disease phenotype

Three CMR measured variables were chosen further analysis; LV mass/BSA, LVEF and native T1. These measures were significantly different between patients with RA and controls, even after adjustment for CV risk factors. In addition, together they provide information on cardiac structure, function and myocardial tissue composition. Others measures such as mid S', mid A' and peak twist (measures of diastolic dysfunction) were not assessed further as their interpretation can be complex when done so in isolation.

An UVA analysis (see Table 5-11) in those with RA found that male gender (co-efficient 0.665, p<0.001), systolic blood pressure (coefficient 0.344, p= 0.003), waist/hip circumference ratio (coefficient 0.327, p=0.005) and PWV (coefficient 0.261, p=0.027) were associated with LVmass/BSA. A multivariate linear regression analysis, again using variables considered to be associated with LV mass in the literature (age, gender, systolic blood pressure, lipid ratio, disease duration, ACPA and PWV), or strongly associated in the UVA (coefficient greater than 0.3), revealed male gender (B (95% CI) 13.993 (9.166, 18.779), p<0.001), systolic blood pressure (B (95% CI) 0.138 (0.023, 0.253), p=0.020) and a history of ever smoking (B (95% CI) 3.595 (0.029, 7.160), p=0.048) were independently associated with increasing LV mass/BSA. No RA specific variables were significant.

No variables were associated with LVEF on UVA (see Table 5-12), however, male gender was independently associated with LVEF within a multivariate analysis (B (95% CI) -3.255 (-5.756, -0.754), p=0.012, R^2=0.132) which included age, systolic blood pressure, history of ever smoking, TC/HDL-C ratio and PWV. A history of ever smoking (coefficient 0.257, p=0.035), lower TC/HDL-C ratio (coefficient-0.257, p=0.039) and Ln(NT-proBNP) (coefficient 0.355 p=0.004) were associated with native T1 on UVA (see Table 5-13). However, a multivariate linear regression analysis, using variables considered to be associated with T1 mapping values in the literature (age, gender, systolic blood pressure and PWV), or strongly associated in the UVA (coefficient greater than 0.3) determined only ever smoking remained associated (B (95% CI) 27.105 (1.263, 52.948) p=0.04, R^2=0.143).

Table 5-11 Univariate and multivariate analysis of variables associated with CMR measured LV mass/BSA.

Variable	**LVmass/BSA**				
	Univariate analysis (number of observations =74, unless otherwise stated)			**Multivariate analysis R^2=0.620, n=66**	
	Correlation coefficient	**B (95% CI)**	**p value**	**B (95% CI)**	**p value**
Age*	0.139	0.157 (-0.106, 0.420)	0.238	-0.037 (-0.276, 0.203)	0.759
Male gender*	**0.665**	15.657 (11.50, 19.783)	**<0.001**	**13.993 (9.166, 18.779)**	**<0.001**
Systolic blood pressure*	**0.344**	0.184 (0.066, 0.302)	**0.003**	**0.138 (0.023, 0.253)**	**0.020**
Ever smoked*	0.186	3.905 (-0.948, 8.758)	0.113	**3.595 (0.029, 7.160)**	**0.048**
Body mass index	0.005	0.017 (-0.738, 0.773)	0.964	-	-
Waist/hip circumference	**0.327**	43.661 (13.571, 73.750) (n=72)	**0.005**	1.276 (-26.066, 28.618)	0.926
TC/HDL-C*	0.059	0.608 (-1.874, 3.091) (n=71)	0.626	0.840 (-1.004, 2.684)	0.365
HOMA-IR	0.137	0.642 (-0.489, 1.773) (n=69)^	0.261	-	-
NT-proBNP	0.062	0.009 (-0.27, 0.045) (n=69)	0.615	-	-
RA disease duration*	0.172	0.175 (-0.060, 0.411)	0.142	0.052 (-0.130, 0.234)	0.571
3 variable DAS28	0.084	0.739 (-1.319, 2.797)	0.476	-	-
ACPA*	-0.156	-4.2121 (10.294, 2.053) (n=73)	0.187	1.793 (-2.870, 6.456)	0.444
HAQ-DI	-0.140	-1.883 (-5.097, 1.331) (n=70)	0.247	-	-

History of joint surgery	0.112	2.587 (-2.825, 7.998)	0.344	-	-
Current use of biological DMARD	-0.008	-0.169 (-5.413, 5.075)	0.949	-	-
PWV*	0.261	1.428 (0.170, 2.685) (n=72)	**0.027**	0.442 (-0.825, 1.708)	0.488

ACPA, anti-citrullinated peptide antibody; CRP, C-reactive protein; DAS28CRP, 28 joint disease activity score; DMARD, disease modifying anti-rheumatic drug; HAQ-DI, health assessment questionnaire-disability index; HOMA-IR, homeostasis model of assessment of insulin resistance; NT-proBNP, N-terminal pro-brain natriuretic peptide; PWV, pulse wave velocity; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio

*variable entered into linear regression model as associated with LV mass in the literature.

Coefficients greater than 0.3 (highlighted in bold) also entered into the linear regression model.

^Excluding high outlier: Correlation coefficient B -0.025 (95% CI -2.732, 2.195) p=0.828

Table 5-12 Univariate and multivariate analysis of variables associated with CMR measured LVEF.

Variable	**LVEF**				
	Univariate analysis (n =74, unless otherwise stated)			**Multivariate analysis R^2=0.132, n=69**	
	Correlation coefficient	**B (95% CI)**	**p value**	**B (95% CI)**	**p value**
Age*	0.152	0.075 (-0.039, 0.190)	0.195	0.073 (-0.063, 0.208)	0.288
Male gender*	-0.281	-2.897 (-5.219, -0.574)	0.015	**-3.255 (-5.756, -0.754)**	**0.012**
Systolic blood pressure*	0.107	0.025 (-0.030, 0.080)	0.365	0.023(-0.049, 0.094)	0.528
Ever smoked*	0.034	0.314 (-1.847, 2.474)	0.773	-0.002 (-2.191, 2.187)	0.999
Body mass index	0.092	0.129 (-0.200, 0.458)	0.437	-	-
Waist/hip circumference	-0.022	-1.279 (15.390, 12.833) (n=72)	0.857	-	-
TC/HDL-C*	0.072	0.319 (-0.746, 1.384) (n=71)	0.553	0.376 (-0.742, 1.494)	0.504
HOMA-IR	-0.008	-0.017 (-0.522, 0.489 (n=69)^	0.410	-	-
NT-proBNP	0.064	0.004 (-0.012, 0.020) (n=69)	0.850	-	-
RA disease duration	-0.024	-0.011 (-0.115, 0.094)	0.851	-	-
3 variable DAS28	-0.082	-0.317 (-1.218, 0.584)	0.374	-	-
ACPA	0.020	0.235 (-2.523, 2.994) (n=73)	0.866	-	-
HAQ-DI	-0.050	-0.293 (-1.722, 1.136) (n=70)	0.448	-	-

History of joint surgery	-0.001	-0.014 (-2.398, 2.369)	0.990	-	-
Current use of biological DMARD	0.128	1.250 (-1.027, 3.527)	0.277	-	-
PWV*	0.148	0.279 (-0.287, 0.846) (n=72)	0.329	0.061 (-0.673, 0.796)	0.868

ACPA, anti-citrullinated peptide antibody; CRP, C-reactive protein; DAS28CRP, 28 joint disease activity score; DMARD, disease modifying anti-rheumatic drug; HAQ-DI, health assessment questionnaire-disability index; HOMA-IR, homeostasis model of assessment of insulin resistance; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; PWV, pulse wave velocity; S'=Peak systolic strain rate; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio

*variable entered into linear regression model as associated with LVEF in the literature.

Correlation coefficients greater than 0.3 (none) would have been also entered into the linear regression model.

^Excluding high outlier: Correlation coefficient 0.108 B 0.521 (95% CI -0.585, 1.627) p=0.350

Table 5-13 Univariate and multivariate analysis of variables associated with CMR measured inferoseptal T1.

Variable	**Inferoseptal T1**				
	Univariate analysis (n =68, unless otherwise stated)			**Multivariate analysis R^2=0.143, n=66**	
	Correlation coefficient	**B (95% CI)**	**p value**	**B (95% CI)**	**p value**
Age*	0.152	0.881 (-0.526, 2.289)	0.216	1.519 (-0.113, 3.151)	0.067
Male gender*	-0.072	-8.427 (-37.209, 20.356)	0.561	-8.977 (-38.272, 320.318)	0.542
Systolic blood pressure*	-0.053	-0.142 (-0.803, 0.519)	0.670	0.183 (-0.659, 1.025)	0.665
Ever smoked*	0.257	27.328 (2.023, 52.633)	**0.035**	**27.105 (1.263, 52.948)**	**0.040**
Body mass index	0.000	-0.003 (-3.625, 3.618)	0.999	-	-
Waist/hip circumference	-0.141	-93.403 (-256.269, 69.464) (n=67)	0.256	-	-
TC/HDL-C	-0.257	-13.786 (-26.825, -0.747) (n=65)	**0.039**	-	-
HOMA-IR	-0.085	-1.881 (-7.512, 3.750) (n=63)^	0.507	-	-
NT-proBNP	0.232	0.139 (-0.010, 0.287) (n=63)	0.067		
RA disease duration	0.040	0.199 (-1.032, 1.430)	0.748	-	-
3 variable DAS28	-0.079	-3.314 (-13.568, 6.941)	0.521	-	-
ACPA	0.113	15.178 (-17.791, 48.147) (n=67)	0.361	-	-

HAQ-DI	-0.006	-0.375 (-17.357, 16.607) (n=65)	0.965	-	-
History of joint surgery	-0.076	-8.881 (-37.655, 19.893) (n=68)	0.540	-	-
Current use of biological DMARD	0.089	10.309 (-17.996, 38.613) (n=68)	0.470	-	-
PWV*	-0.070	-2.878 (-9.677, 3.922) (n=66)	0.401	-7.384 (-15.922, 1.155)	0.089

ACPA, anti-citrullinated peptide antibody; CRP, C-reactive protein; DAS28CRP, 28 joint disease activity score; DMARD, disease modifying anti-rheumatic drug; HAQ-DI, health assessment questionnaire-disability index; HOMA-IR, homeostasis model of assessment of insulin resistance; NT-proBNP, N-terminal pro-brain natriuretic peptide; PWV, pulse wave velocity; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio

*variable entered into linear regression model as associated with native T1 in the literature.

Correlation coefficients greater than 0.3 (none) would have been also entered into the linear regression model.

^Excluding high outlier: Correlation coefficient -0.112 B -5.755 (95% CI -16.002, 4.492) p=0.266

5.3.3.3 Association with CV soluble biomarkers

Increasing leptin was associated with reducing LV mass (B -0.338 (95% CI -0.518, -0.158) p<0.001) (see table 5-14). However, no independent association was seen when added into the same MVA of LVmass/BSA described above which included age, gender, systolic blood pressure, ever smoking, WHR, TC/HDL-C, RA disease duration, ACPA positivity and PWV); B -0.004 (95% CI -0.187, 0.178) p=0.962. A weaker association of leptin was seen with increasing LVEF (B 0.107 (95% CI 0.025, 0.190) p=0.012).

Table 5-14 Association of soluble CV biomarkers with CMR outcomes in RA

CV soluble biomarker	**LVmass/BSA** (n=73)			**LVEF** (n=73)			**Native T1** (n=67)		
	Correlation coefficient (r)	**B (95% CI)**	**p value**	**Correlation coefficient (r)**	**B (95% CI)**	**p value**	**Correlation coefficient (r)**	**B (95% CI)**	**p value**
ICAM-1	-0.106	-0.029 (-0.095, 0.036)	0.373	0.201	0.024 (-0.004, 0.053)	0.088	-0.054	-0.072 (-0.404, 0.259)	0.665
MCP-1	-0.034	-0.003 (-0.022, 0.016)	0.773	0.025	0.001 (-0.007, 0.009)	0.832	-0.138	-0.055 (-0.153, 0.043)	0.266
vWF	0.017	0.004 (-0.051, 0.059)	0.884	0.023	0.002 (-0.022, 0.026)	0.844	0.096	0.105 (-0.166, 0.377)	0.441
Leptin	**-0.406**	-0.338 (-0.518, -0.158)	**<0.001**	**0.294**	0.107 (0.025, 0.190)	**0.012**	0.117	0.457 (-0.505, 1.419)	0.347

BSA, body surface area; CI, confidence interval; ICAM, intercellular adhesion molecule, LVEF, left ventricular ejection fraction; MCP; monocyte chemotactic protein; RA, rheumatoid arthritis; vWF, von Willebrand factor

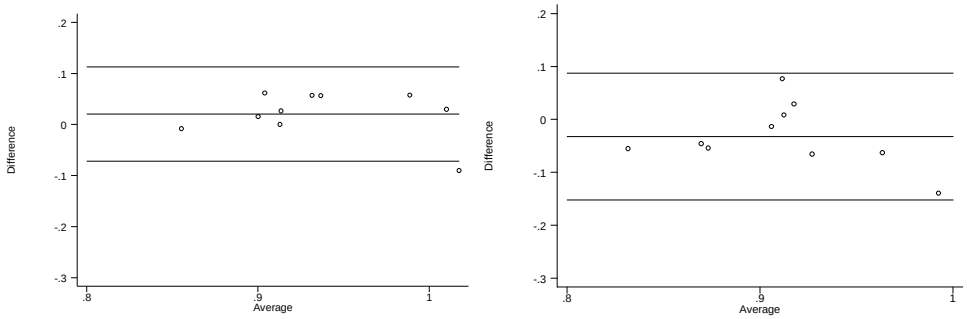
5.3.4 Carotid artery magnetic resonance imaging

Of the 76 patients with RA who underwent CMR, only 64 scans provided information on the carotid arterial wall; 7 missing due to protocol errors, 2 missing due to image processing errors, and 3 as the images were of poor quality. 60 patients provided data on carotid arterial wall volume measurements; 4 missing as the carotid artery bifurcated too low to allow measurement up to 8mm below the bifurcation.

Inter-observer and intra-observer variability were within acceptable ranges. The mean difference for inter-observer assessments was 0.032 (95% CI -0.075, 0.010)mm, and all values lay within the limits of agreement (2SD of the difference from the mean) (see figure 5-3). The coefficient of variance was 4.93%. The mean difference for intra-observer variability was 0.021 (95% CI -0.012, 0.054)mm. and all but one value lay within the limits of agreement (see figure 5-3). The coefficient of variance was 3.66%.

Figure 5-3 Bland Altman Plot to show inter-observer variation and intra-observer variation in mean wall thickness

A) intra-observer variation B) inter-observer variation



5.3.4.1 Differences between patients with RA and controls

There were no significant differences between patients with RA and controls for carotid arterial wall measurements, including maximum wall thickness, MWT, wall volume, luminal volume, wall volume index, and wall volume indexed/BSA, although there was a trend for an increases in values for those with RA, particular in respect to wall volume and luminal volume (mean difference (95% CI) -5.479 (-

28.979, 18.021)ul (p=0.644) and -8.792 (-71.799, 54.215)ul (p=0.782) respectively after adjustment for age, gender and CV risk factors (see table 5-15).

Table 5-15 Carotid wall measurements in in study participants

Carotid artery variable	RA patients n=64	Controls n=24	Unadjusted differences in RA group from controls		Mean difference adjusted for age and sex		Mean difference adjusted for age, sex and CV risk factors**	
			Mean (95% CI)	p value	B (95% CI)	p value	B (95%)	p value
Mean wall thickness, mm	1.05129 (0.12510)	1.02889 (0.12892)	0.02239 (-0.03762, 0.08241)	0.460	0.002 (-0.060, 0.064)	0.940	-0.009 (-0.079, 0.060)	0.790
Minimum wall thickness, mm	0.74670 (0.09610)	0.71797 (0.10747)	0.02873 (-0.01851, 0.07596)	0.230	0.013 (-0.036, 0.063)	0.591	0.000 (-0.056, 0.056)	0.990
Maximum wall thickness, mm	1.49828 (0.33022)	1.52963 (0.31131)	-0.03135 (-0.18612, 0.12343)	0.688	-0.050 (-0.217, 0.118)	0.556	-0.045 (-0.237, 0.148)***	0.646
Wall volume*, ul	234.52810 (45.18726) (n=60)	225.61349 (46.75117)	8.91460 (-13.00970, 30.83891)	0.421	-5.053 (-26.236, 16.131)	0.636	-5.479 (-28.979, 18.021)	0.644
Luminal volume*, ul	383.40817 (116.27667) (n=60)	368.75292 (105.62544)	14.65525 (-39.8249, 69.13536)	0.594	-0.430 (-55.585, 54.726)	0.988	-8.792 (-71.799, 54.215)	0.782
Wall volume indexed*	0.38549 (0.04590) (n=60)	0.38455 (0.04049)	0.00095 (-0.02041, 0.02231)	0.930	0.001 (-0.023, 0.024)	0.963	0.001 (-0.026, 0.027)	0.952
Wall volume	0.2157 (0.0357)	0.22057	-0.00481 (-0.02150,	0.567	-0.11 (-0.029,	0.201	-0.008 (-0.028,	0.430

indexed/BSA	(n=60)	(0.03205)	0.01187)		0.006)		0.012)	

BSA, body surface area; RA, rheumatoid arthritis

*of 6mm length of carotid artery. Carotid wall volume indexed calculated by carotid wall volume/(carotid wall volume + luminal volume)

**CV risk factors defined as: hypertension (history of hypertension or anti-hypertensive agent), dyslipidaemia (history of dyslipidaemia, on lipid-lowering medication or TC/HDL-C ratio greater than 6), ever smoked and family history of premature CVD

*** Heteroskedasticity of residuals therefore robust standard errors employed to compensate.

Values represent mean (SD) unless otherwise state

5.3.4.2 Association with disease phenotype

In patients with RA, of the variables measured, carotid wall volume and luminal volume correlated well with ten year CV risk scores (adjusted as per EULAR guidelines); correlation coefficient (p value) for JBS2 risk 0.370 (0.005) and 0.428 (0.001) respectively; for Framingham risk 0.376 (0.004) and 0.432 (0.001) respectively (see Table 5-16).

Tables 5-17-5-20 report the UVA and MVA of RA disease phenotype with carotid artery outcome measures. An UVA revealed no variables (traditional CV risk factors or RA disease specific factors) were associated with carotid mean wall thickness. A history of smoking, lower BMI, and lower HOMA-IR correlated with higher maximum wall thickness (r=0.247 p=0.049, r=-0.245 p=0.026, r=-0.132 p=0.014), however, HOMA-IR lost significance when the high outlier was excluded. As no coefficient was greater than 0.3 none were placed in the MVA (other than age and gender as identified as important associated factors in the literature; no independent association revealed). An UVA revealed increasing age (r=0.346, p=0.007), a history of smoking (r=0.329, p=0.010) and PWV (r=0.262, p=0.047) were associated with carotid wall volume, although only age remained independently associated in a MVA (B 1.126 (95% CI 0.32, 1.93) p=0.007 R^2=0.245. Increasing HOMA-IR and a history of previous joint surgery was associated with lower carotid wall volume index (r=-0.282 p=0.035 and r=-0.262 p=0.043 respectively), although, again, HOMA-IR lost significance when the high outlier was excluded. No coefficient was greater than 0.3 and so none were placed in the MVA (other than age and gender; no independent association revealed).

Table 5-16 The association of carotid wall variables in patients with RA with ten year cardiovascular risk scores (adjusted as per EULAR guidelines)

Variable	**Adjusted Joint British Societies 2 cardiovascular risk score**			**Adjusted Framingham cardiovascular risk score**		
	Spearman correlation coefficient	**95% CI**	**P value**	**Spearman correlation coefficient**	**95% CI**	**P value**
Mean wall thickness	0.085	-0.170, 0.330	0.514	0.098	-0.157, 0.342	0.451
Minimum wall thickness	0.129	-0.127, 0.369	0.322	0.117	-0.139, 0.359	0.368
Maximum wall thickness	0.044	-0.210, 0.293	0.734	0.064	-0.191, 0.311	0.622
Wall volume*	**0.370**	**0.121, 0.575**	**0.005**	**0.376**	**0.127, 0.579**	**0.004**
Luminal volume*	**0.428**	**0.188, 0.619**	**0.001**	**0.432**	**0.193, 0.623**	**0.001**
Wall volume indexed*	-0.228	-0.461, 0.035	0.088	-0.224	-0.458, 0.039	0.094
Wall volume indexed/BSA	-0.256	-0.484, 0.005	0.055	-0.243	-0.474, 0.018	0.068

Number of observations=61 unless otherwise stated. CI (confidence intervals) are of correlation coefficient
*number of observations=57

Table 5-17 Univariate and multivariate analysis of variables associated with mean carotid wall thickness in patients with RA.

Variable	**Mean wall thickness (mm)**				
	Univariate analysis (n =64, unless otherwise stated)			**Multivariate analysis R^2=0.031, n=64**	
	Correlation coefficient	**B (95% CI)**	**p value**	**B (95% CI)**	**p value**
Age*	0.159	0.002 (-0.001, 0.005)	0.209	0.002 (-0.001, 0.005)	0.235
Male gender*	0.088	0.024 (-0.045, 0.093)	0.491	0.020 (-0.049, 0.089)	0.568
Systolic blood pressure	-0.033	0.000 (-0.002, 0.001)	0.795	-	
Ever smoked	0.208	0.052 (-0.010, 0.114)	0.099	-	
Body mass index	-0.124	-0.005 (-0.014, 0.005)	0.328	-	
Waist/hip circumference	0.110	0.169 (-0.224, 0.561) (n=62)	0.393	-	
TC/HDL-C	-0.105	-0.012 (-0.042, 0.018) (n=61)	0.422	-	
HOMA-IR	-0.135	-0.007 (-0.021, 0.007) (n=60)^	0.303	-	
NT-proBNP	0.007	1.622E-005 (-0.001, 0.001) (n=60)	0.961	-	
RA disease duration	0.080	0.001 (-0.002, 0.004)	0.528	-	
3 variable DAS28	-0.016	-0.002 (-0.021, 0.017)**	0.861	-	

ACPA	0.021	0.007 (-0.072, 0.085) (n=63)	0.868	-	
HAQ-DI	-0.023	-0.004 (-0.046, 0.039) (n=60)	0.859	-	
History of joint surgery	-0.182	-0.049 (-0.117, 0.018)	0.150	-	
Current use of biological DMARD	0.033	0.009 (-0.058, 0.076)	0.798	-	
PWV	0.138	0.009 (-0.007, 0.025) (n=62)	0.286	-	

ACPA, anti-citrullinated peptide antibody; CI, confidence intervals; CRP, C-reactive protein; DAS28CRP, 28 joint disease activity score; DMARD, disease modifying anti-rheumatic drug; HAQ-DI, health assessment questionnaire-disability index; HOMA-IR, homeostasis model of assessment of insulin resistance; MWT, mean wall thickness; NT-proBNP, N-terminal pro-brain natriuretic peptide; PWV, pulse wave velocity; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio

**variable entered into linear regression model as associated with MWT in the literature

** heteroskedasticity of residuals therefore robust standard errors employed to compensate

^Excluding high outlier: Correlation coefficient -0.169 B (95% CI) -0.024 (-0.062, 0.013) p=0.200

Table 5-18 Univariate and multivariate analysis of variables associated with maximum carotid wall thickness in patients with RA.

Variable	**Maximum wall thickness (mm)**				
	Univariate analysis (n =64, unless otherwise stated)			**Multivariate analysis** R^2=0.001, n=64**	
	Correlation coefficient	**B (95% CI)**	**p value**	**B (95% CI)**	**p value**
Age*	0.035	0.001	0.783	0.001 (-0.009, 0.012)	0.820
Male gender*	0.008	0.006 (-0.176, 0.188)	0.947	0.004 (-0.193, 0.200)	0.971
Systolic blood pressure	-0.121	-0.002 (-0.006, 0.002)**	0.346	-	-
Ever smoked	0.247	0.163 (0.001, 0.326)	**0.049**	-	-
Body mass index	-0.245	-0.024 (-0.046, -0.003)**	**0.026**	-	-
Waist/hip circumference	0.030	0.122 (-0.918, 1.162) (n=62)	0.815	-	-
TC/HDL-C	-0.132	-0.040 (-0.0116, 0.035)** (n=61)	0.289	-	-
HOMA-IR	-0.132	-0.019 (-0.033, -0.004)** (n=60)^	**0.014**	-	-
NT-proBNP	-0.074	-0.0005 (-0.002, 0.001) (n=60)	0.574	-	-
RA disease duration	0.064	0.002 (-0.006, 0.010)	0.616	-	-
3 variable DAS28	-0.013	-0.004 (-0.063, 0.055)**	0.9103	-	-

ACPA	0.008	0.006 (-0.201, 0.214) (n=63)	0.951	-	-
HAQ-DI	0.018	0.008 (-0.104, 0.119) (n=60)	0.893	-	-
History of joint surgery	-0.186	-0.134 (-0.312, 0.045)	0.141	-	-
Current use of biological DMARD	-0.077	-0.053 (-0.230, 0.123)	0.547	-	-
PWV	0.008	0.001 (-0.038, 0.041)** (n=62)	0.948	-	-

*variable entered into linear regression model as associated with maximum wall thickness in the literature

** heteroskedasticity of residuals therefore robust standard errors employed to compensate

^Excluding high outlier: Correlation coefficient -0.120 B (95% CI) -0.046 (-0.118, 0.027) p=0.217**

Table 5-19 Univariate and multivariate analysis of variables associated with carotid wall volume in patients with RA.

Variable	**Carotid wall volume (ul)**				
	Univariate analysis (n =60, unless otherwise stated)			**Multivariate analysis** R^2=0.250, n=60**	
	Correlation coefficient	**B (95% CI)**	**p value**	**B (95% CI)**	**p value**
Age*	**0.346**	**1.630 (0.470, 2.790)**	**0.007**	**1.126 (0.32, 1.93)**	**0.007**
Male gender*	0.254	24.494 (0.008, 48.980)	0.050	15.990 (-10.62, 42.60)	0.234
Systolic blood pressure	0.128	0.280 (-0.290, 0.850)	0.329	-	-
Ever smoked	**0.329**	**29.710 (7.262, 52.158)**	**0.010**	19.430 (-1.48, 40.34)	0.068
Body mass index	-0.087	-1.164 (-4.664, 2.337)	0.508	-	-
Waist/hip circumference	0.184	100.401 (-43.199, 244.001) (n=58)	0.167	-	-
TC/HDL-C	-0.067	-2.920 (-14.739, 8.899) (n=57)	0.622	-	-
HOMA-IR	0.087	1.658 (-0.610, 3.927)** (n=56)^	0.149	-	-
NT-proBNP	-0.092	-0.083 (-0.328, 0.162) (n=56)	0.499	-	-
RA disease duration	**0.307**	**1.356 (0.250, 2.462)**	**0.017**	0.776 (-0.24, 1.79)	0.131

3 variable DAS28	0.003	0.100 (-6.859, 7.058)**	0.977	-	-
ACPA	0077	8.576 (-21.004, 38.157) (n=59)	0.564	-	-
HAQ-DI	0.065	3.780 (-11.975, 19.535) (n=57)	0.633	-	-
History of joint surgery	-0.018	-1.757 (-27.453, 23.940)	0.892	-	-
Current use of biological DMARD	-0.016	-1.5821 (-25.836, 23.794)	0.905	-	-
PWV	0.262	5.684 (0.081, 141.288) (n=58)	**0.047**	-	-

**variable entered into linear regression model as associated with carotid wall volume in the literature

** heteroskedasticity of residuals therefore robust standard errors employed to compensate

^Excluding high outlier: Correlation coefficient -0.073 B (95% CI) -3.817 (-15.449, 7.815) p=0.513**

Table 5-20 Univariate and multivariate analysis of variables associated with carotid wall volume index in patients with RA.

Variable	**Carotid wall volume index**				
	Univariate analysis (n=60, unless otherwise stated)			**Multivariate analysis R^2=0.026, n=60**	
	Correlation coefficient	**B (95% CI)**	**p value**	**B (95% CI)**	**p value**
Age*	-0.142	-0.001 (-0.002, 0.001)	0.278	-0.001 (-0.002, 0.001)	0.316
Male gender*	-0.091	-0.009 (-0.034, 0.017)	0.490	-0.007 (-0.033, 0.018)	0.572
Systolic blood pressure	-0.168	0.0004 (-0.001, 0.0002)	0.199	-	
Ever smoked	0.007	0.001 (-0.023, 0.025)	0.957	-	
Body mass index	-0.083	-0.001 (-0.005, 0.002)	0.530	-	
Waist/hip circumference	0.024	0.013 (-0.137, 0.164) (n=58)	0.860	-	
TC/HDL-C	-0.087	-0.004 (-0.015, 0.008)	0.520	-	
HOMA-IR	-0.282	-0.005 (-0.011, -0.0004) (n=56)^	**0.035**	-	
NT-proBNP	0.080	0.00007 (-0.0002, 0.0003) (n=56)	0.559	-	
RA disease duration	-0.253	-0.001 (-0.002, 0.000004)	0.051	**-**	
3 variable DAS28	-0.005	0.0002 (-0.008, 0.008)**	0.963	-	

ACPA	-0.060	-0.007 (-0.037, 0.023) (n=59)	0.651	-	
HAQ-DI	-0.154	-0.009 (-0.024, 0.007) (n=57)	0.252	-	
History of joint surgery	-0.262	-0.026 (-0.051, -0.001)	**0.043**	-	
Current use of biological DMARD	0.055	0.005 (-0.020, 0.031)	0.679	-	
PWV	-0.034	-0.001 (-0.007, 0.005) (n=58)	0.797	-	

ACPA, anti-citrullinated peptide antibody; CRP, C-reactive protein; DAS28CRP, 28 joint disease activity score; DMARD, disease modifying anti-rheumatic drug; HAQ-DI, health assessment questionnaire-disability index; HOMA-IR, homeostasis model of assessment of insulin resistance; MWT, mean wall thickness; NT-proBNP, N-terminal pro-brain natriuretic peptide; PWV, pulse wave velocity; S'=Peak systolic strain rate; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio

*variable entered into linear regression model as associated with carotid wall volume index in the literature

** heteroskedasticity of residuals therefore robust standard errors employed to compensate

^Excluding high outlier: Correlation coefficient -0.154 B (95% CI) -0.008 (-0.022, 0.006) p=0.262

5.4 Discussion

This study provided a comprehensive CV evaluation of patients with long-standing established RA with comparison to healthy controls. The study combined a clinical assessment with measurement of lipid profile, insulin resistance and other soluble biomarkers of CVD, along with the determination of arterial stiffness using pulse wave velocity by applanation tonometry and a thorough assessment of the heart and vasculature with CMR imaging. The key findings from this study were a reduction in LV mass in patients with RA compared to healthy controls, and the association of surrogate measures of CVD with traditional CV risk factors rather than RA disease specific features.

After adjusting for age and gender, LV mass/BSA, along with stroke volume, native T1, and active/late diastolic strain rate were decreased and mid peak systolic strain rate increased in patients with RA compared to controls. These differences remained even after adjustment for traditional CV risk factors (except for stroke volume) and in addition, LVEF and peak twist were reduced. Within those with RA, only traditional CV risk factors were associated with the CMR outcome measures; with male gender, systolic blood pressure, history of smoking, TC-HDL-C ratio and PWV remaining significant factors in a multivariable analysis. No RA specific features were associated with CMR outcome measures, including ACPA seropositivity.

The only similar sized CMR study in patients with RA also found a reduced LV mass [135]. Giles et al employed a 1.5T CMR scanner and found an 18% reduction in LV mass (reducing to 14.7% when using LVmass/BSA) in 75 patients with RA free of known CVD compared to 225 age, gender and ethnicity matched controls, after adjustment for systolic and diastolic blood pressure, heart rate, HDL-C, triglycerides, habitual exercise and coronary calcium score. Age, smoking habits and statin use were similar in Giles' cohort and this study; however there were fewer females (52%), the inclusion of patients with diabetes (4%), and a higher proportion with a history of hypertension (55%) in their study. The patients with RA may have been subjected to a smaller burden of inflammation as evidence by the shorter disease duration (median 7 (IQR 4-17) years) and smaller number taking a biological DMARD (49%), however, overall, these patients were not in

remission; disease activity was higher (mean (SD) DAS28 CRP 3.51 (1.10). Our study demonstrates similar results with a mean reduction of LVmass/BSA of 15.5% for patients with RA (Intercept 29.456, B -4.558 (95% CI -8.917, -0.199) p= 0.041). Similar to our study, Giles et al also determined a reduction in LVEF, cardiac output and stroke volume in those with RA.

In support of our findings, this research group has reported preliminary findings of lower LV mass in treatment naive early RA patients (n=66) compared to controls; becoming statistically significant in those seropositive [490]. Giles et al also determined increasing ACPA titre was associated with a lower LV mass [135].

In contrast to our findings, other smaller CMR studies have not supported a reduction in LVmass [136, 137]. It is noteworthy that the 39 patients with RA in the study by Nutsi et al tended to be younger (mean age 50 years), fewer with a history of hypertension, have a shorter disease duration, although they did have higher DAS28 scores [137]. Kobayashi et al found a higher LVmass/BSA in those with RA, along with a lower LVEF and higher LVEDV, although all study participants were females and numbers were small (n=20) [136].

Meta-analyses of echocardiography studies in RA have demonstrated an increase in LV mass in patients with RA, however, CMR imaging is much more sensitive to detect small differences in LV mass [504]. Echocardiography relies on the assumption that the LV is a truncated ellipsoid revolving around its major axis and as such the equations used for LV mass calculation are cubic functions, which potentially magnify any small measurement error. CMR does not require such geometric assumptions, as there is full coverage of the LV, such that it is now widely accepted as the reference standard for LV and RV mass and volumes measurement [134]. This may explain the discrepancy between the two modes of investigation.

The reason for reduced LV mass in RA is unclear, especially given that LV mass usually increases with CV risk [134]. Indeed, the Framingham and JBS2 ten-year CV risk scores in this study both correlated strongly with increasing LV mass and LV mass/EDV (correlation coefficients; 0.464 and 0.581 respectively for Framingham scores, and 0.481 and 0.592 respectively for JBS2 scores, $p<0.001$ for all). Previous authors propose possible physical deconditioning, or previous myocarditis [135] of which the latter is prevalent in RA [138]. Microvascular

dysfunction in RA [505], leading to myocardial ischaemia may also be key; Ciftci et al demonstrated coronary flow reserve, representing microvascular dysfunction, was reduced in patients with RA [506], and microvascular dysfunction has been associated with systemic inflammation [505, 507]. An alternative proposal is that LV mass is reduced as a consequence of cardiac remodelling; the latter defined as 'as genome expression, molecular, cellular and interstitial changes that are manifested clinically as changes in size, shape and function of the heart after cardiac injury' [508]. In our study, native T1 (a composite score derived from the myocyte and interstitial space [498]) was reduced in those with RA, perhaps a reflection of myocyte loss, rather than reduction in extracellular volume (ECV). The addition of late gadolinium enhancement to the CMR protocol and measurement of T1 post contrast would help tease this out further. Abnormal geometry in RA has been reported previously using echocardiography [509].

To add another layer of complexity, the Multi-Ethnic Study of Atherosclerosis (MESA) CMR study (n=1464) reported that higher levels of leptin were associated with lower LV mass, independently of other variables including traditional CV risk factors, IR, physical activity, and CRP [510]. The authors hypothesise that leptin inhibits the myocardial deposition of triglycerides, reducing ectopic fat accumulation. A similar association was seen in those studied in the Framingham Heart Study [511]. Given that leptin is higher in those with RA (as also shown in this chapter), this adds another possible reason for the change in LV mass.

This study exemplifies, for the first time, the utility of MRI-carotid assessments in RA, with measures correlating with ten-year CV risk scores. No significant difference in carotid wall measurements between those with RA and controls were found. However, there was a trend for larger values in those with RA. The patient numbers were smaller than anticipated in this analysis due to MR protocol error or poor image quality; a larger number may have demonstrated statistical significance. Studying three-dimensional volumes rather than two-dimensional wall thickness may provide a more accurate representation of the carotid wall, as it utilises more data in its calculation. Again, the traditional CV risk factors remained important; only age was associated with increasing carotid wall volume in a MVA, although a history of smoking, PWV, and RA disease duration showed promise in a UVA.

Other carotid MR studies have confirmed an association with traditional CV risk factors in the general population. The largest study (n=1670) reporting from the 'Atherosclerosis Risk in Communities' study, revealed carotid wall volume, thickness, and normalised wall index were positively associated with lipids, including total cholesterol, LDL-C, and ApoB [512]. Li et al determined men and increasing age were associated with MWT and maximum wall thickness in 196 study participants without CVD [513]. A smaller study assessing those with obstructive sleep apnoea (n=42) found an association of maximum wall thickness with waist/hip circumference, mean arterial blood pressure, Framingham risk scores, HDL-C, HOMA-IR, insulin and CRP [514]. There have been no previous carotid MR studies in RA.

Increases in carotid volumes may reflect carotid arterial remodelling; in particular, outward remodelling where a compensatory increase in lumen diameter occurs, together with an increase in wall thickness, in an effort to reduce the development of luminal stenosis. Outwards remodelling has been associated with 'softer plaque', i.e. less calcification, and greater plaque instability [515]. Interestingly, Van Sijl et al determined, using ultrasonography, patients with RA (n=96) compared to controls (n=274), despite having similar CIMT values, had a larger vessel lumen diameter, increased adventitia and greater wall stress and tension, after adjustment for CV risk factors [516], suggestive of an outward remodelling process.

Moving away from the CMR findings, despite published literature, no differences were determined in lipid profile, insulin resistance, NT-proBNP or PWV between patients with RA and controls. All but insulin resistance have been associated with inflammation in RA [92, 117, 168, 176], and therefore, perhaps, the absence of active disease in this cohort (illustrated by a mean DAS28 less than 2.6) may have contributed to the lack of difference. Having said this, there is evidence to suggest the atherogenic index (TC/HDL-C ratio) may not be as affected by inflammation as its component parts [174, 175].

This exploratory study has suggested the potential benefit through testing of soluble CV biomarkers in the assessment of CVD in RA. Increased levels of ICAM-1, MCP-1 (both reflecting endothelial dysfunction), vWF and leptin were detected in those with RA, although significance lost after adjustments for CV risk factors.

Keeping in mind the consequences of multiple testing, there was evidence to support soluble CV biomarkers tracking with traditional CV risk factors. There was little association with RA specific features, barring an association of adiponectin and NT-proBNP with increasing RA disease duration, and HAQ-DI with increasing VEGF.

The increase in leptin, and trend for higher adiponectin, is of interest. Known as adipocytokines and secreted from adipose tissue, they have been associated with insulin resistance, metabolic syndrome (MetS) and CVD [517]; lower adiponectin levels associated with CVD and increasing leptin predicting future events [12]. They are increased in RA; paradoxically so for adiponectin [518-520], however, levels, do not improve with TNFi therapy [517, 518] suggesting independence from TNF-mediated systemic inflammation. With its association with reduced LV mass as discussed earlier, perhaps, leptin has a role to play in 'RA-cachexia' (loss of body muscle mass with an increase in body fat mass) [521], distinct from the 'classic' cachexia (reduction in body muscle and fat mass) also seen in RA associated with active disease [522]. Although evidence suggest RA cachexia is a cytokine (predominantly TNF-alpha) driven process, studies to date fail to demonstrate its improvement following suppression of RA disease activity [521, 523], perhaps suggesting a resistance to reversal of the catabolic state and alternative pathological processes maintaining this state.

PWV in patients with RA was associated with traditional CV risk factors, such as age, male gender, systolic blood pressure and waist/hip circumference, however, no association was found with RA specific features such as disease activity or seropositivity. This has been mirrored in previous studies [95, 97].

5.4.1 Limitations

This study being cross-sectional was not able to quantify the burden of inflammation the patients with RA were exposed to over their disease duration. The RA patient cohort clearly had established severe disease as indicated by the lengthy disease duration and high proportion of seropositivity and erosive disease. However, the patients were also clinically in remission, as demonstrated with a DAS28 of less than 2.6. Few required oral prednisolone to control disease and systemic inflammatory markers were low. Although, disease activity was quiescent at the time of the study participation, it is likely the patients had

suffered a burden of inflammation over the years; many patients had required more than one DMARD in an effort to control disease, and a large majority of patients were dependent on biological DMARDs (only prescribed if disease activity was shown to be high on more than one occasion). In addition, a significant number of patients had a history of orthopaedic surgery as a consequence of uncontrolled synovitis. In the absence of previous regular measures of disease activity and inflammation, disease duration in this cohort is looked to as the proxy measure for the burden of disease.

There are also a few limitations with the use of PWV. Although it is routinely used and validated outcome measure in trials for anti-hypertensive and lipid lowering medication, errors can occur. Calculating the distance from the sternal notch to the femoral pressure cuff is affected by central obesity, and the overweight patient may have a larger distance recorded as result.

Measuring carotid wall thickness using MRI also depends on good quality images, with movement artefact or oblique vessels compromising the images. Given the differences in carotid wall thickness are so small, our inter-observer and intra-observer variability limits of agreement are relatively wide, spanning 0.085mm and 0.066mm respectively. In any future larger study, the difference between patients with RA and controls detected would need to be greater than 0.085mm to be meaningful.

5.4.2 Future research agenda

Larger and longitudinal studies of CMR in RA are required to validate these early findings. In particular to confirm the lower LV mass, and determine at which point the changes occur, and if LV mass is amenable to change with the treatment of RA. There would be advantages to assessing those with established yet more active disease to tease out the effect of inflammation on these measures. Further work is required to understand the underlying pathophysiology of the reduced LV mass, including its relationship with leptin and other adipocytokines. Larger cross-sectional studies and prospective longitudinal studies are also required to validate the use of MR in measuring carotid wall disease in RA. In addition, the use of contrast which can characterise carotid plaque further, differentiating unstable from stable plaques, lipid rich necrotic cores from intra-plaque

haemorrhage, could help inform of the pathophysiology of CVD in RA and in identifying those at risk of cerebrovascular disease [524].

5.4.3 Summary

In summary, this study has primarily demonstrated the importance of traditional CV risk factors, and not RA disease specific features, in the determination of markers of subclinical CVD in RA. In addition, PWV by applanation tonometry may have a role to play in improving the CV risk management in patients with RA in a clinical setting. This study has demonstrated the global utility of CMR as a research tool in the investigation of CVD in RA providing information on structure, function, tissue composition and the vasculature, and insight into the pathogenesis of cardiac disease in RA; in particular demonstrating reduced LV mass after adjustment for traditional CV risk factors, with an association with leptin. It has also demonstrated the utility of MRI carotid scanning in patients with RA.

5.5 Key messages

1. Traditional CV risk factors, and not RA disease specific features, appear to be key determinants of markers of subclinical CVD in RA.
2. CMR is a valued tool in the investigation of CVD in patients with RA, with potential for MRI-carotid imaging.
3. LV mass is reduced in those with established RA after adjustment for traditional CV risk factors.

Chapter 6 The use of cardiovascular magnetic resonance imaging in Systemic Sclerosis

6.1 Introduction

Systemic sclerosis (SSC) is a multi-organ autoimmune disease that comprises three pathological features; vasculopathy, inflammation and fibrosis. The cardinal sign is skin tightening, with over 90% suffering from Raynaud's phenomenon (RP) that can be associated with digital ulceration and critical ischaemia and gastro-intestinal motility disturbances. Major internal organ involvement typically comprises interstitial lung disease (ILD), and until recently, renal crisis (incidence reduced since the recommendation of ACE-inhibitors). Primary myocardial disease (SSc-cardiomyopathy (SSc-CM)) is also prevalent [7, 8] and can lead to various complications, such as heart failure, pericardial effusions, arrhythmias and even sudden death [9, 10]. Once clinically evident, the prognosis is poor [11]. For example, Medsger et al reported a 13% survival at 7 years in those with cardiac involvement [340]. The pathogenesis appears to be distinct to atherosclerosis, with autopsy studies demonstrating pathology in areas of the myocardium unrelated to compromise in blood flow from the coronary arteries [525].

SSc-CM is poorly understood; screening for myocardial involvement and even the management of clinically overt myocardial involvement is relatively evidence free, and no guidelines exist to help guide the physician. Determining the phenotype of patients with SSc most at risk is vital to allow more effective/intensive monitoring of their cardiac health, in addition to learning about the pathophysiology of the disease to more effectively prevent and manage its complications.

Surrogate markers of CVD and primary myocardial disease provide a method of studying cardiac involvement. Cardiovascular magnetic resonance (CMR) imaging with the use of contrast agents, as previously described in Chapters 2 to 4, can provide comprehensive information on cardiac structure, function, and arterial stiffness, and is a well validated tool in the investigation of ischaemic heart disease in the general population [16]. With the addition of contrast agents and pharmacological stress, information on myocardial perfusion can be derived and the presence of myocardial fibrosis can be evaluated; of particular value in the investigation of those with SSc. To date, few studies exist reporting

CMR abnormalities in patients with SSc; however, the description of disease phenotype had been limited, different levels of specialist CMR skills, with limited collaboration between rheumatology and specialist CMR-cardiology teams.

The majority of SSc-CMR studies do not show a reduction in LV ejection fraction (LVEF), but do show a high prevalence of diastolic dysfunction [372, 376]. Focal myocardial fibrosis (as measured by late gadolinium enhancement (LGE)), is subjectively identified with the use of contrast and is frequently reported; in up to 66% of cases, sparing the subendocardium suggesting an alternative pathology to atherosclerosis [376-378, 395]. There is evidence for increased fibrosis in those with dcSSc [374, 376] and with longer disease durations [377, 378]. Extracellular volume fraction (ECV), a reflection of the extracellular matrix, may detect early subclinical diffuse myocardial fibrosis, with evidence for correlation with disease activity and severity of heart failure [526]; and is increasingly being used to quantitatively measure myocardial fibrosis [435, 436]. Stress perfusion protocols have determined perfusion defects in up to 79% of patients with SSc [376]. Myocarditis has been identified frequently using T2 weighted CMR imaging; up to 12% of patients with SSc, mainly in the LV myocardium and transmural regions [378].

Few CMR studies in SSc to date have comprehensively described the SSc disease phenotype with relationship to myocardial involvement to provide insight into the patient most at risk of primary myocardial disease.

6.1.1 Hypothesis and aims

My hypothesis relating to this study was that CVD, and more specifically, primary myocardial disease, were more prevalent in SSc, and associated with a specific SSc disease phenotype as well as traditional CV risk factors.

The aim of this study was in patients with SSc free of CVD using surrogate measures of CVD;

- To describe the CV risk profile and prevalence of subclinical CVD and primary myocardial disease in patients with SSc, compared to healthy controls
- To describe the association of CV abnormalities with disease phenotype.
- To demonstrate the global ability of CMR in the evaluation of subclinical primary myocardial involvement, specifically looking to measures of fibrosis and myocardial perfusion abnormalities

- To explore the relationship between fibrosis and myocardial perfusion abnormalities and their relation to disease phenotype, to identify the patient most at risk.

6.1.2 Study objectives

The primary objective was to assess the difference in CMR outcome measures between patients with SSc (free of known CVD and diabetes mellitus) and healthy controls, and to examine the relationship of these abnormalities with SSc disease phenotype.

6.2 Methods

The Leeds Teaching Hospitals NHS Trust Connective Tissue Disease and Vasculitis Cohort Cross-sectional and Longitudinal Clinical and Basic Science Evaluation (CONVAS) study is an observational longitudinal study following patients with any/all auto-immune disease, including SSc, from early to established disease, capturing disease phenotype, treatment regimes and outcomes together with a biosample bank. A cardiovascular sub-study is included to the main study to allow a comprehensive assessment of CV health and permit additional research investigation. CONVAS has full ethical approval from Leeds (East) Research Ethics Committee (REC 10/H1306/88).

The ELectrophysiology and CArdiac imaging in SclerodermA (ELCASA) Study is a three year longitudinal study evaluating patients with SSc. Study participants undergo a comprehensive CV assessment, the insertion of an implantable loop recorder which continuously monitors the heart rhythm and CMR. ELCASA also has full ethical approval from Leeds (East) Research Ethics Committee (REC 12/YH/0298).

The CONVAS and ELCASA studies (baseline data) were interrogated to investigate the above hypotheses.

6.2.1 Study design

6.2.1.1 Endpoints

The primary endpoint was the presence of CMR-measured extra cellular volume (ECV) (marker of inflammation/ fibrosis)

Secondary endpoints included

Soluble markers

- Total cholesterol/High density lipoprotein cholesterol (HDL-C) ratio

Applanation tonometry

- Pulse wave velocity (m/sec)

CMR measures:

- LV ejection fraction (LVEF) (%)
- LV end-diastolic volume (EDV) (ml)
- LV end-systolic volume (ESV) (ml)
- LV mass indexed to body surface area (LVmass/BSA) (g/m2)
- LVmass/EDV (indicator of cardiac remodelling)
- Stroke volume indexed to body surface area (ml/m2)
- Peak twist (measure of diastolic dysfunction)
- Torsion (measure of diastolic dysfunction)
- Native T1
- Late gadolinium enhancement (LGE) (marker of fibrosis)
- Presence of myocardial perfusion abnormalities
- Aortic distensibility (10-3mmHg-1)

6.2.1.2 Subject numbers

This was an exploratory study and so no formal power calculations were carried out. However, it was considered at least 30 patients with SSc compared to 30 controls would provide a good bank of data to explore the hypotheses proposed.

6.2.1.3 Eligibility criteria

For patients with SSc:

Inclusion criteria

- Meet 1980 American College of Rheumatology (ACR) criteria [383] or 2013 ACR/European League of Rheumatism (EULAR) criteria [527]
- Between ages of 18 and 80 years old
- Capable of understanding and signing an informed consent form

Exclusion criteria

- History of cardiovascular disease (CVD); cardiac, peripheral or cerebral
- History of diabetes (to minimise confounding)
- Contraindications for CMR scanning; Pregnancy, breastfeeding, non-MRI conditional pacemakers, surgical clips within the head, certain inner ear implants, neuro-

electrical stimulators or metal fragments within the eye or head, an eGFR less than 45 ml/min/1.73m^2 (exclusion for gadolinium use) and asthma requiring oral steroids or previous hospital admission.

For controls:

Inclusion criteria

- Between ages of 18 and 80 years old
- Capable of understanding and signing an informed consent form

Exclusion criteria

- History of SSc
- History of cardiovascular disease (CVD); cardiac, peripheral or cerebral
- History of diabetes (to minimise confounding)
- Contraindications for MRI scanning; Pregnancy, breastfeeding, non-MRI conditional pacemakers, surgical clips within the head, certain inner ear implants, neuro-electrical stimulators or metal fragments within the eye or head, an eGFR less than 45 ml/min/1.73m^2 (exclusion for gadolinium use) and asthma requiring oral steroids or previous hospital admission.

6.2.1.4 Target population

Consecutive patients attending the SSc specialist clinic or general connective tissue disease (CTD) clinic at LTHT were considered for the study.

In this study, two groups of controls were recruited. The healthy controls recruited into the volunteer arm of the IACON (Inflammatory Arthritis disease CONtinuum longitudinal) study for the established RA study (described in detail in Chapter 5) underwent the same comprehensive CV assessment (clinical assessment with blood collection and PWV) as the patients with SSc, however their CMR was done without the use of contrast or stress perfusion; in line with the cardiac/carotid protocol for established RA patients. Rather than asking these controls (termed 'biomarker-controls') to return for a second CMR (with stress perfusion and contrast) within a short space of time, data from a second set of healthy controls were utilised. These controls (termed 'CMR-controls) were identified by the CMR team at the Leeds General Infirmary, using separate ethics pertaining to 'CE-MARC 2: Optimization of Image Acquisition and Analysis Methods' (REC 12/YH/0551). Age, gender, smoking status and blood pressure were recorded for these participants.

6.2.1.5 Informed consent

Appropriate patients with SSc were given a patient information sheet to read either prior to their appointment in clinic or sent to them in the post. Time was given for them to digest the material and then the investigator (LAB) contacted the patient either in clinic or by telephone to determine their interest in participating, discuss the study in detail and answer any questions.

All patients had at least 24 hours to consider the study before consenting. Consent was taken by a study co-investigator. In most instances the study investigator was LAB, but on occasion a delegated research nurse took consent. A copy of the consent was kept in the site file, with one copy filed in the notes, and another sent to the patient.

6.2.1.6 Withdrawal

Patients were free to withdraw from the study at any time, and participate in as much of the study as they wished. This did not affect their usual clinical care. Any data/blood samples already collected was retained and remained for analysis.

6.2.2 Study schedule

6.2.2.1 Clinical visit

Patients with SSc and, as already described in chapter 3, biomarker-controls, attended Chapel Allerton Hospital, Leeds, UK to see the study investigator. The following procedures took place:

6.2.2.2 Informed consent

The study was discussed further with the patient or control and any questions answered. Inclusion and exclusion criteria were checked. The patient or control and the study investigator then signed the consent appropriately.

6.2.2.3 Clinical evaluation

The following aspects of the study participant were recorded:

Demographics

- The participant demographic history including their age, gender and ethnicity.

Cardiovascular risk evaluation.

- History of smoking habit, hypertension, dyslipidaemia and family history of premature cardiovascular disease.

- How many minutes of moderate exercise taken per week (defined as enough to make them feel out of breath or perspire) and how many days per week five or more fruit and vegetables per day were consumed.
- Weight, height, waist and hip circumference were measured, along with blood pressure.
- Rose Angina Questionnaire completed.

SSc disease phenotype evaluation (patients with SSc only)

- Duration of RP
- Time since first non-RP symptom
- History of disease complications such as interstitial lung disease (ILD), digital ulceration (DU).
- Medication history
- Physical findings, including modified Rodnan skin score (mRSS), presence of DU, calcinosis, and tendon friction rubs.
- Nail-fold capillaroscopy (NFC)
- Scleroderma Health Assessment Questionnaire – disability index (HAQ-DI) and visual assessment scores (VAS)
- Details of most recent echocardiogram and pulmonary function tests (or test done within 8 weeks of baseline if an up to date test was clinically required)

Notes on S-HAQ analysis

Each of the 8 domains were given a score from 0 to 3. To do this, each question within the domain was given a score of 0 to 3; where without any difficulty scored 0 and unable to do scored 3. The largest score was taken to represent the domain. The use of aids/help from another person within a domain, increased that domain score of 0 or 1 to 2. The scores from the 8 domains were added together and divided by 8 to give the SHAQ-DI [383].

6.2.2.4 Fasting blood collection

The study participant (patient with SSc and biomarker-control) came fasting (at least eight hours) for the study visit; hence most attended in the morning for the study. Under aseptic conditions, the following bloods were taken from SSc patients; antinuclear antibody (ANA), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG), glucose. No blood samples were taken from the controls undergoing CMR.

6.2.2.5 Pulse wave velocity by applanation tonometry

VICORDER® (SMT Medical, Würzburg, Germany) software was employed to measure PWV. The investigation was carried out in the same way as described in Chapter 5.

6.2.3 Cardiovascular Magnetic Resonance Imaging visit

The study participant (patient with SSc and CMR-control) attended usually on a separate day, for a CMR scan at the Leeds General Infirmary, Leeds, UK. The scan was performed on a dedicated 3 Tesla Philips Achieva TX scanner (Philips Healthcare, Best, The Netherlands), with participants in the supine position, lasting approximately one hour. Patients were asked to avoid caffeine for 24 hours prior to the CMR.

6.2.3.1 CMR protocol

The CMR protocol closely followed that described in Chapter 5, and was performed by the same clinical research CMR-cardiology team. There were some differences; carotid arteries were not scanned, and the CMR involved the use of gadolinium-based contrast agent to identify areas of myocardial perfusion defects, detect LGE (focal fibrosis) and to allow measurement of ECV (diffuse fibrosis). The protocol in full detail has been published by the CMR-cardiology team [101] and is summarised below:

- Low-resolution survey, reference scans and localizers.
- Baseline T1 mapping. As described in Chapter 5.
- Adenosine stress first-pass myocardial perfusion imaging. Intravenous adenosine (given at 140 mcg/kg/min for three minute) under continuous ECG monitoring and assessment for adequate haemodynamic response, and gadolinium-DTPA (diethylene triamine pentaacetic acid (0.1 mmol/kg main bolus, plus 10% dilute pre-bolus dose).
- Resting wall motion and LV function.
- Tissue tagging for strain analysis and diastology. As described in Chapter 5.
- Aortic distensibility. As described in Chapter 5.
- Resting first-pass myocardial perfusion study. As described in step 3, without the use of adenosine. Cardiac perfusion is visually compared to the stress perfusion images (step 3) to detect defects.
- Late gadolinium enhancement (LGE). Performed between 10 and 15 minutes after step 7; bright areas signifying cardiac fibrosis.
- Post-contrast T1 mapping performed as per step 2, 15 minutes after step 7; allows calculation of ECV.

6.2.3.2 CMR evaluation

CMR-cardiology fellows (BE and GF) reviewed and reported the cardiac CMR scans using QMASS MR 7.5 software (Medis, Leiden, The Netherlands). GF was only responsible for the tagging measurements (producing strain rates, peak twist and torsion values), whilst BE measured the remaining outcomes. Any unexpected findings were discussed with senior CMR cardiologists (SP and JG), acted upon appropriately and the participant informed. The following measures were calculated; LV and RV; EF, EDV, ESV and stroke volume; LV mass/BSA, ECV, torsion, peak twist, mid-S', presence of LGE and myocardial perfusion defects and aortic distensibility.

6.2.4 Data collection

Clinical data for study participants were stored anonymously in an electronic database, accessed only by those involved in the study. Blood samples for storage were anonymised after processing. CMR scans were stored electronically and accessed only by those working within the CMR department.

6.2.5 Missing data

All efforts were made to avoid missing data. In the event of missing serology the most recent value found preceding the visit was carried forward into the data. This was not done for inflammatory markers such as CRP or ESR due to their capacity to vary depending on disease activity and only done for lipid/glucose profile if a fasting state could be verified.

6.2.6 Statistical analysis

Using the statistical package SPSS (IBM SPSS Statistics 22) and STATA/IC 11.1 (StataCorp, Texas, USA) the distribution of each variable was determined and a check for extreme outliers was performed.

A descriptive analysis then followed to describe the cohorts (SSc, biomarker-controls and CMR-controls), abnormalities in lipid/glucose profile, and distribution of PWV.

The independent t test was used to determine unadjusted statistically significant differences in the lipid levels and PWV between SSc patients with SSc and biomarker-controls. The non-normally distributed variable TG was log transformed prior to analysis to allow the use of the independent t test; the resulting mean difference was exponentiated and result presented as a ratio of the value from the SSc group to biomarker-controls.

Linear regression was used to determine mean differences between patients with SSc and CMR-controls when adjusted for 1) age and gender, 2) age, gender, systolic blood pressure and history of ever smoking. Logistic regression was employed to determining the odds ratios for the presence of LGE between patients and CMR-controls, adjusting as above.

Within the SSc group, Pearson's correlation and a univariate analysis (UVA) was used to determine associations between patient characteristics, lipid measures and PWV with continuous CMR outcome measures. MRSS was log-transformed prior to analysis due to lack of constance in residual variance (heteroskedasticity) in the UVA. Variables considered to be associated with CMR outcome measures in the literature, i.e. age and gender, or strongly associated in the UVA (correlation coefficient greater than 0.3), were put into a multivariate linear regression model to determine independently associated variables. To determine which (if any) patient phenotype was associated with the presence of LGE, the independent t-test (providing mean differences) was performed for continuous variables, and exact odd ratios (with 2-sided Fisher's exact P value) were calculated for categorical variables.

This study was an exploratory study, and so no power calculation was performed. Holm's correction [503] was employed to correct for multiple comparisons (described in chapter 5).

6.3 Results

42 patients with SSc were consented into this cardiac study; 20 into CONVAS and 22 into ELCASA. 30 controls were recruited for CMR.

6.3.1 Study participant characteristics

Table 6-1 outlines the demographic history and CV risk profile of the study participants, with table 6-2 describing the disease phenotype of SSc patients. The mean age of the 42 patients with SSc was 55.2 (standard deviation (SD) 12.3) years, with 69% being female. 83% were Caucasian, 12% Asian and 5% Afro-Caribbean descent.

Patients with SSc had a median (interquartile range (IQR)) disease duration of 9.0 (1.8, 18.9) years (defined as time from first non-RP phenomenon), 45% had dcSSc, 36% had previous digital ulceration (DU) and 43% had known interstitial lung disease (ILD). No patient had a history of pulmonary hypertension. Forty-eight percent were using a DMARD, and 31% had previously been treated with cyclophosphamide. Thirty-eight percent had

received iloprost in the past. Sixty percent were taking an ACE-inhibitor and 69% a calcium channel blocker (CCB). The median (IQR) MRSS was 2 (2, 6), 26% had evidence of calcinosis and 43% of digital pits. Thirty-one percent and 29% were ACA and Scl70 positive respectively. The most recent pulmonary function tests performed revealed the mean (SD) forced vital capacity (FVC) was 95 (21)% and transfer factor (DLCO) 61 (14)%. The mean (SD) right ventricular-right atrial (RV-RA) gradient from the most recent echocardiogram was 23(5)mmHg (data only available for 24 patients; lack of tricuspid regurgitation limited reporting).

A proportion of patients with SSc had traditional CV risk factors; 17% had a history of hypertension, 17% with a family history of premature CVD, 12% were currently smoking and 52% were ex-smokers. Although no patient had a history of hypercholesterolaemia, 5% were being treated with a statin. Mean systolic and diastolic blood pressure levels were within normal range.

Biomarker-control participants were slightly younger (mean (SD) age 49.2 (13.5 years), and there were fewer females (57%). A greater proportion had never smoked (59 vs. 36% patients with SSc). Although, there were fewer controls with a history of hypertension, mean systolic blood pressure was a little higher (128 vs. 118mmHg in SSc patients). This may reflect the use of CCB and ACE-I in those with SSc used routinely in the prevention of renal crisis and management of RP.

CMR-control study participants were again slightly younger (mean (SD) age 50.2 (15.1) years), and there were fewer females (63%). There was a reduction in smoking exposure in controls; only 13% were ex-smokers and 17% current smokers. Blood pressure values were similar to those with SSc.

Table 6-1 Study participant characteristics

Variable	**Expressed as**	**SSc patients n=42**	**Biomarker-controls n=35**	**CMR-controls n=30**
Demographics				
Age, years	mean, SD	55.2 (12.3) (range 26, 78)	49.2 (13.5)	50.2 (15.1) (range 20, 78)
Female	n %	29 (69)	20 (57)	19 (63)
Ethnicity Caucasian Asian Afro-Caribbean	n %	35 (83) 5 (12) 2 (5)	34 (97) 1 (3) 0 (0)	-
CV risk profile				
Smoking status: Never Ex Current	n %	15 (36) 22 (52) 5 (12)	20 (59) 11 (32) 3 (9) (n=34)	21 (70) 4 (13) 15 (17)
PMH hypertension	n %	7 (17)	3 (9) n=34	-
PMH Hypercholesterolaemia	n %	0 (0)	2 (6) n=34	-
FHx premature CVD*	n %	7 (17) (n=41)	4 (12) n=33	-
Waist/Hip Ratio	mean, SD	0.83 (0.09) (n=39)	0.82 (0.08)	-
BMI	mean, SD	24.9 (5.1)	24.6 (3.7)	27.2 (5.7)
Systolic BP, mmHg	mean, SD	118 (17)	128 (18)	120 (13)
Diastolic BP, mmHg	mean, SD	72 (10)	76 (11)	65 (13)
Five or more fruit/vegetables daily intake, days/week	Median (IQR)	5 (3, 7) (n=40)	5 (4, 7)	-
Moderate exercise, mins/week	Median (IQR)	0 (0, 0) (n=39)	60 (0, 225)	-
Alcohol intake, units/week	Median	3 (0, 8) (n=38)	1 (0, 8.5)	-

	(IQR)			
Current use of statin	n %	2 (5)	-	-

BMI, body mass index; BP, blood pressure; CMR, cardiovascular magnetic resonance; CVD, cardiovascular disease; FHx, family history of; IQR, interquartile range; PMH, past medical history of; SD, standard deviation; SSc, Systemic Sclerosis.

*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 relative

Table 6-2 Disease specific characteristics and lipid profile of patients with SSc

SSc phenotype	**Data as expressed as**	**SSc patients n=42**
Disease history		
Disease subtype LcSSc DcSSc	n (%)	23 (54.8) 19 (45.2)
Presence of RP	n (%)	42 (100.0)
Time since onset of RP, years	Median (IQR)	11.5 (3.0, 29.0)
Time since onset of first non-RP symptom, years	Median (IQR)	9.0 (1.8, 18.9)
History of: Digital ulceration GORD Non-GORD GI involvement ILD Palpitations	n (%)	15 (35.7) 35 (83.3) 7 (16.7) 18 (42.9) 17 (40.5)
Medication history		
Current use of DMARD	n %	20 (47.6)
Name of current DMARD	n %	Mycophenolate 11 (26.2) Methotrexate 3 (7.1) Hydroxychloroquine 3 (7.1) Azathioprine 1 (2.4) Cyclophosphamide 2 (4.8) Sulphasalazine 1 (2.4)
Previous use of cyclophosphamide Of these, number of previous cyclophosphamide infusions	n (%) Median (IQR)	13 (31.0) 6 (6, 12)
Current use of prednisolone	n (%)	1 (2.4) on 4mg 3 (7.1) on 5mg 1 (2.4) on 8mg 3 (7.1) on 10mg 1 (2.4) on 15mg
Previous use of iloprost	n (%)	16 (38.1)
Current treatment with: Sildenafil	n (%)	6 (14.3)

Bosentan		2 (4.8)
ACE inhibitor		25 (59.5)
Calcium channel blocker		29 (69.0)
Examination findings		
Total modified Rodnan skin score	Median (IQR)	2 (2, 6)
Presence of: Digital pits	n (%)	18 (42.9)
Digital ulceration		3 (7.1)
Tendon friction rubs		3 (7.1)
Calcinosis		11 (26.2)
TJC28	Median (IQR)	0 (0.0, 1.0)
SJC28	Median (IQR)	0 (0.0, 0.0)
NFC vasculopathy pattern (n=40)	n (%)	4 (10.0) non-specific
		17 (42.5) early
		8 (20.0) active
		9 (22.5) late
SHAQ-DI	Mean (SD)	1.27 (0.80) (n=39)
VAS Pain	Median (IQR)	57 (22, 66)
GI		22 (2, 60)
Breathing		34 (5, 65)
RP		70 (25, 77)
DU		4 (0, 53)
Overall		52 (22, 70)
Routine investigations		
Antibody positive: ANA	n %	35 (83.3)
ACA		13 (31.0)
Scl70		12 (28.6)
CRP (mg/L) (normal range <5)	Median (IQR)	0 (0.0, 2.5) (n=41)
ESR (mm/hr) (normal range 1-15)	Median (IQR)	13.5 (5.8, 30.8) (n=30)
CK (iu/L) (normal range 25-200)	Median (IQR)	58 (0.0, 99.3) (n=38)
Forced vital capacity, %	Mean (SD)	95.0 (21.3)
Total lung capacity, %	Mean (SD)	88.4 (17.3)
DLCO, %	Mean (SD)	60.9 (14.1)
DLCO/VA, %	Mean (SD)	79.1 (14.4)

RV-RA gradient, mmHg	Mean (SD)	23 (5) (n=24*)

ACE, angiotensin converting enzyme; ACA, anti-centromere antibody; ANA, anti-nuclear antibody; CK, creatine kinase; CRP, C-reactive protein; DcSSc, diffuse cutaneous SSc; DLCO, diffusing capacity of the lungs for carbon monoxide; DLCO/VA, DLCO adjusted for volume; DMARD, disease modifying anti-rheumatic drug; ESR, erythrocyte sedimentation rate; IQR, inter-quartile range; LcSSc, limited cutaneous SSc; NFC, nail-fold capillaroscopy; RF, rheumatoid factor; RP, Raynaud's phenomenon; RTX, rituximab; RV-RA; right ventricular-right atrial; Scl70, anti-topoisomerase antibody; SD; standard deviation; SHAQ-DI, Scleroderma health assessment questionnaire-disability index; SJC, swollen joint count; SSc, systemic sclerosis; TJC, tender joint count; VAS, visual assessment score

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

6.3.2 Lipids and pulse wave velocity

Table 6-3 describes the fasting lipid levels and PWV in patients with SSc and biomarker-controls. There were no clinical or statistically significant differences between fasting lipid levels and glucose between the two groups, with exception to triglycerides. Patients with SSc had increased triglycerides; values in those with SSc were 1.32 (95% CI 1.05, 1.66) times higher than the biomarker-controls (p=0.019).

PWV was marginally higher in patients with SSc compared to controls, however the difference did not reach statistical significance, and did not change when adjusted for age and gender; mean difference (95% CI) -0.02 (-0.62, 0.57)m/s, p=0.939, or when adjusted for age, gender, and CV risk factors (defined as history of ever smoking, history of hypertension, dyslipidaemia (history high cholesterol, on statin or TC/HDL>6) family history of premature CVD); mean difference (95% CI) 0.08 (-0.54, 0.70)m/s, p=0.797.

Using the lipid values, the ten year CVD risk scores of the study participants were calculated (see Table 6-4). The Framingham and Joint British Societies 2 risk scores in the SSc group were similar in both patients with SSc and biomarker-controls.

Table 6-3 Fasting lipid levels and PWV in SSc patients and controls

Fasting CV investigations	**Data as expressed as**	**SSc patients n=40**	**Biomarker-controls n=32**	**Mean difference SSc vs. controls (95% CI)**	**Unadjusted p value for difference***
Fasting glucose, mmol/L	Mean (SD)	4.8 (0.6) (n=38)	4.8 (0.7) (n=27)	-0.01 (-0.34, 0.31)	0.932
Fasting total cholesterol, mmol/L	Mean (SD)	4.9 (1.0)	5.1 (0.9) (n=29)	-0.23 (-0.70, 0.24)	0.333
Fasting HDL-C, mmol/L	Mean (SD)	1.5 (0.42)	1.6 (0.4) (n=29)	-0.12 (-0.32, 0.07)	0.206
Fasting LDL-C, mmol/L	Mean (SD)	2.8 (0.8)	3.1 (0.8) (n=29)	-0.25 (-0.65, 0.15)	0.211
Fasting triglycerides, mmol/L	Geometric mean	1.3 (n=40)*	1.0 * (n=29)	1.32 (1.05, 1.66)**	**0.019**
Fasting TC/HDL-C ratio	Mean (SD)	3.6 (1.0)	3.4 (0.9) (n=29)	0.21 (-0.27, 0.69)	0.394
PWV, m/sec	Mean (SD)	8.5 (1.6)	8.1 (1.6) (n=32)	0.47 (-0.29, 1.22)	0.224

CV, cardiovascular; CI, confidence interval; HDL-C, high density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low density lipoprotein cholesterol; SD, standard deviation; SSc, systemic sclerosis; TC, total cholesterol; PWV, pulse wave velocity.

*variable log transformed prior to analysis, and value exponentiated to express as geometric mean (no SD available)

**values exponentiated to give ratio of difference of one group to another, with associated confidence intervals.

Table 6-4 Ten year cardiovascular disease risk scores of study participants

Ten year CVD risk score, %	SSc patients n=40	Biomarker-controls n=28	Median differences in SSc from controls (95% CI)	Unadjusted p value for difference*
Framingham	4.9 (3.3, 10.8)	4.4 (0.9, 13.3)	0.5 (-3.1, 3.3)	0.609
Joint British Societies 2	4.4 (3.0, 9.3)	3.4 (0.7, 10.5)	0.7 (-2.2, 3.0)	0.470

CI, confidence interval; SSc, systemic sclerosis
Values expressed as median (interquartile range)
*Mann Whitney U test

6.3.3 Cardiovascular magnetic resonance imaging

Thirty-five patients with SSc underwent CMR; with contrast enhanced images acquired in 33. The reasons for patients not having the CMR are shown in Table 6-5. There were no significant differences in demographics, SSc profile or traditional CV risk factors between those who had CMR compared to those who did not.

Table 6-5 Reasons for SSc study participants not having CMR scan

Reason	CMR not done	CMR done but no contrast enhancement
Poor intra-venous access	3	2
Participant claustrophobia	2	-
Participant twice did not attend appointment	1	-
CMR abandoned due to unrelated medical reason	1	-

CMR, cardiovascular magnetic resonance scan

6.3.3.1 Differences between patients with SSc and CMR-controls

The CMR outcome measures are shown in table 6-6. The differences between patients with SSc and CMR-controls were checked, and then adjusted for age and sex, and then again for CV risk factors (defined as systolic blood pressure and ever smoking).

Volumes and dimensions: LVmass/BSA was reduced in patients with SSc (mean (SD) 44.45 (11.10) vs. 49.17 (7.73)g/m^2 in controls, but the difference did not reach statistical significance; p=0.055). After adjustment for age, sex, systolic blood pressure and ever smoking, the mean difference (95% CI) was -4.27 (-9.18, 0.64) g/m^2 p=0.087; again not statistically significant). LVmass/EDV was lower in those with SSc (mean (SD) 0.56 (0.10) vs. 0.62 (0.1)g/ml in controls, p=0.02). However, the difference was not statistically significant after adjustment for age, sex, CV risk factors (mean difference (95% CI) was -0.053 (-0.108, 0.001)g/ml p=0.054. There was no difference seen between the two groups in LV or RV EDV, LVESV or stroke volume (all indexed to BSA).

Cardiac function: Patients with SSc had a similar mean LVEF compared to controls and they had an increased RVEF (mean (SD) 57.2 (12.0) vs. 54.7 (6.2)% in controls, p=0.152); statistically significant after adjustment for age, sex, and CV risk factors (mean difference 5.6 (0.6, 10.5)%, p=0.028). Patients with SSc also had a higher mid-systolic strain rate (Mid S'); mean (SD) 1.29 (0.21) vs. 1.15 (0.13) in controls, p=0.002; mean difference after adjustment for age, sex and CV risk factors 0.14 (0.04, 0.24), p=0.006. Considering signs of diastolic dysfunction both peak twist and torsion were reduced in SSc patients; significance remaining after adjustment. Mean (SD) torsion in SSc patients was 12.98 (4.06) vs. 14.95 (4.46) in controls, p=0.079; mean difference (95% CI) -2.45 (-4.56, -0.35) degrees after adjustment for age, sex and CV risk factors (p=0.038). Early diastolic strain rate was increased in those with SSc; mean difference (95% CI) 0.19 (0.004, 0.038) p=0.045 after adjustment for age, sex, and CV risk factors. Active/late diastolic strain rate was similar in both groups.

Markers of cardiac fibrosis: Whereas native T1 values were similar in both groups, ECV was increased in patients with SSc; mean (SD) 30.6(3.8) vs. 25.1 (2.8)% in controls, p<0.001. The difference remained after adjustment for age, sex and CV risk factors (mean difference (95% CI) 05.0 (3.2, 6.8)% p<0.001). LGE was seen in nine patients with SSc and one control (p=0.014); after adjustment for age, sex and CV risk factors the difference became statistically insignificant (odds ratio (95% CI) 8.7 (0.7, 102.2), p=0.085. The majority of

lesions were found in the lateral or basal wall of the heart, and were focal in nature (see table 6-7). Although the most lesions were found in the mid-wall, LGE was also detected across the whole wall including the subendocardium. In those with LGE compared to those without, there was a non-statistically significant trend for higher ECV; mean (SD) ECV 31.9 (0.05)% vs. 30.1 (0.04)% without LGE, p=0.257.

Myocardial perfusion: In one patient a perfusion defect was seen, while all others were reported as normal. This patient with SSc had a stress induced perfusion defect in the right coronary artery distribution suggestive of epicardial coronary artery disease, and was referred to a cardiologist for further investigation of underlying IHD. This patient also had subendocardial LGE in the basal inferior segment of the LV. No non-segmental defects were recorded.

Aortic stiffness: Aortic distensibility was lower in those with SSc (i.e. greater arterial stiffness) although not statistically significantly so; mean (SD) 3.78 (2.25) vs. 4.70 (1.95)$10^{-3}$$mmHg^{-1}$ in controls, p=0.087, although this difference was minimised by the adjustment for age, sex and CV risk factors (mean difference -0.61 (95% CI -1.48, 0.26)$10^{-3}$$mmHg^{-1}$ p=0.163).

6.3.3.1.1 Correction for multiple comparisons

Using Holm's method to attempt to correct for multiple comparisons, a new p value of 0.006 was calculated to determine statistical significance when comparing secondary CMR outcomes in patients with SSc to controls, when adjusted for age, gender and CV risk factors. This new p value determined that ECV remained significant, with mid systolic strain rate just reaching significance, whilst the rest did not.

Table 6-6 Cardiovascular magnetic resonance imaging findings in study participants

Variable	SSc patients n=35	CMR-Controls n=30	Unadjusted p value for difference	Mean difference (95% CI), p value adjusted for age and sex	Mean difference (95% CI), p value adjusted for age, sex, ever smoked and systolic BP
Left ventricular measures					
LVEF, %	61.46 (4.59)	61.80 (4.58)	0.769	-1.072 (-3.218, 1.074) 0.322	-0.513 (-2.778, 1.752) 0.652
Mid S'	1.29 (0.21) n=32	1.15 (0.13) n=29	**0.002**	**0.15 (0.06, 0.24) 0.002**	**0.14 (0.04, 0.24) 0.006**
LV EDV/BSA, ml/m^2	79.51 (15.07)	79.78 (10.77)	0.935	1.637 (-4.598, 7.872) 0.601	-0.633 (-7.300, 6.033) 0.850
LV ESV/BSA, ml/m^2	30.87 (7.98)	30.63 (6.22)	0.897	1.567 (-1.639, 4.773) 0.332	0.222 (-3.155, 3.600) 0.896
LV stroke volume/BSA, ml/m^2	48.65 (8.43)	49.15 (6.61)	0.793	0.071 (-3.761, 3.902) 0.971	-0.854 (-4.985, 3.276) 0.681
LVmass/BSA, g/m^2	44.45 (11.10)	49.17 (7.73)	0.055	-3.554 (-8.058, 0.950) 0.120	-4.270 (-9.176, 0.636) 0.087
LV mass/EDV, g/ml	0.56 (0.10)	0.62 (0.10)	**0.020**	**-0.059 (-0.108, -0.010) 0.020**	-0.053 (-0.108, 0.001) 0.054
Right ventricular measures					
RVEF, %	57.18 (11.98)	53.67 (6.17)	0.152	3.304 (-1.419, 8.027) 0.167	**5.553 (0.614, 10.492) 0.028**
RV EDV/BSA, ml/m^2	85.38 (18.01)	92.35 (16.51)	0.111	-4.234 (-12.278, 3.809) 0.297	-6.530 (-15.205, 2.145) 0.137
RV ESV/BSA, ml/m^2	38.78 (12.54)	43.10 (10.73)	0.145	-2.324 (-7.587, 2.939) 0.381	-4.079 (-9.732, 1.574) 0.154
RV stroke volume/BSA, ml/m^2	47.75 (8.19)	49.25 (8.64)	0.475	-0.418 (-4.480, 3.644) 0.838	-0.084 (-4.505, 4.336) 0.970
Diastolic function					

Mid E'	0.71 (0.44) n=32	0.50 (0.15) n=29	**0.013**	**0.19 (0.02, 0.37), 0.033**	**0.19 (0.004, 0.38), 0.045**
Mid A'	1.54 (0.59) n=31	1.50 (0.26) n=29	0.762	0.08 (-0.15, 0.31), 0.489	0.09 (-0.16, 0.34), 0.459
Peak twist, degrees	13.11 (4.22) n=32	15.87 (4.40) n=29	**0.015**	**-3.21 (-5.37, -1.04) 0.004**	**-3.19 (-5.54, -0.83) 0.009**
Torsion, degrees	12.98(4.06) n=34	14.95(4.46) n=29	**0.079**	**-2.45 (-4.56, -0.35) 0.023**	**-2.41(-4.68, -0.14) 0.038**
Extracellular measurements					
Native T1 (inferoseptal)	1192 (64)	1202 (35)	0.417	-13 (-40, 13), 0.320	-22 (-50, 7), 0.133
ECV, %	30.6 (3.8) n=33	25.1 (2.8)	**<0.001**	**5.2 (3.5, 6.9) <0.001**	**5.0 (3.2, 6.8) <0.001**
Presence of late gadolinium enhancement	9 (25.0)* (n=33)	1 (3)*	**0.014**	**13.652 (1.472, 126.61) 0.021****	8.705 (0.742, 102.174) 0.085**
Measures of aortic stiffness					
Distensibility, $10^{-3}mmHg^{-1}$	3.78 (2.25) n=34	4.70 (1.95)	0.087	-0.494 (-1.330, 0.342) 0.242	-0.613 (-1.481, 0.255) 0.163

BP, blood pressure; BSA, body surface area; CI, confidence interval; CMR, cardiovascular magnetic resonance; Mid A', active/late diastolic strain rate; Mid E', early diastolic strain rate; ECV, extracellular-volume fraction; EDV, end-diastolic volume; ESV, end-systolic volume; LV, left ventricular; LVEF, LV ejection fraction; Mid S', peak systolic strain rate; RV, right ventricular; RVEF, RV ejection fraction; SSc, systemic sclerosis;
Values expressed as mean (SD) unless stated otherwise
*n (%)
**logistic regression analysis, therefore mean difference represents odds ratio

Table 6-7 Descriptive analysis of LGE seen in study participants

Study participant	SSc patient	Age	Gender	Disease subtype	Disease duration*, years	Antibody present	LGE features		
							Location	Position in wall	Pattern
1	Yes	38	Female	Diffuse	7.4	Sm, SmRNP, RNP-68, chromatin	Anteroseptal	Mid-wall	Focal
2	Yes	77	Female	Limited	18.7	ANA speckled	Lateral	Trans-mural (epicardium to subendocardium)	Focal
3	Yes	52	Female	Diffuse	1.2	Scl70	Inferolateral	Mid-wall	Focal
4	Yes	46	Male	Diffuse	1.1	Scl70	Inferolateral	Trans-mural (epicardium to subendocardium)	Focal
5	Yes	44	Male	Limited	15.8	Sm, SmRNP, RNP68, chromatin	Basal lateral	Epicardium	Focal
6	Yes	69	Male	Diffuse	1.1	Scl70, Ro-52	Basal inferior	Subendocardium	Diffuse, patchy
7	Yes	60	Male	Diffuse	2.3	Scl70	RVIP	Mid-wall	Focal
8	Yes	50	Female	Diffuse	32.2	Scl70	RVIP	Mid-wall	Focal
9	Yes	57	Male	Limited	9.0	Ro-52	Basal anterolateral and inferolateral	Epicardium	Diffuse, patchy

10	No	70	Female	-	-	-	Basal lateral	Mid-wall	Patchy, focal

LGE, late gadolinium enhancement; RP, Raynaud's phenomenon; RVIP, right ventricular insertion point
*defined as time from first non-RP symptom

6.3.3.2 Association with disease phenotype

CMR outcome measures chosen for detailed analysis included; LV mass/BSA, torsion, ECV and LGE. The latter three showed significant differences between controls and the patients with SSc, whilst the former outcome measure is of interest given a trend for reduction in LV mass was observed as well as the previous findings of reduced LV mass in RA (see chapter 5). Again, in line with the RA analysis, the four measures provide information on cardiac structure, function and tissue (fibrosis).

LVmass/BSA was significantly associated with male gender (correlation coefficient (r) 0.575, p<0.001) and lower BMI (r -0.399 p=0.018). Non-statistically significant associations worthy of mention include ever smoking (r 0.301, p=0.079), a diffuse disease subtype (r 0.308 p=0.072), and decreasing RV-RA gradient on echocardiogram (r-0.320 p=0.169) (see table 6-8). In a multivariable analysis, which also included age and gender, only male gender was independently associated with LVmass/BSA (B (95% CI) 10.88 (2.59, 19.17), p=0.012, R^2=0.446).

Decreasing torsion was associated with a known history of ILD (r -0.447, p=0.012) and Scl70 positivity (r -0.454, p=0.010), with a strong association (although not statistically significant) with male gender (r -0.304, p=0.096), previous cyclophosphamide use (r -0.305, p=0.096) and increasing RV-RA gradient on echocardiogram (r -0.354, p=0.150) (see table 6-9).

Increasing ECV in patients with SSc was associated with previous digital ulceration (r 0.397, p=0.022), decreasing waist/hip circumference (r-0.510, p=0.004) & decreasing TC/HDL-C (r-0.376 p=0.037) (see Table 6-10), however none of these variables remained independently associated in a multi-variable analysis with age and gender.

Table 6-11 describes the differences in patients with SSc in those with and without LGE. Given the small numbers involved in this exploratory study, no attempt was made to adjust for confounders. LGE was significantly associated with ILD (78% vs. 33%, p0.047), absence of ACA (0% vs. 42%, p0.032), reduced systolic blood pressure (mean difference (95% CI) -14 (27, -1)mmHg, p0.034) & lower BMI (mean difference (95%CI) -4.13 (-8.24, -0.02), p0.049). Although not statistically significant, other observations included an association of LGE with male gender (56% vs. 17% without LGE), dcSSc (56% vs. 38% without LGE), Scl70 positivity (56% vs. 29% without LGE) & shorter disease duration (median (IQR) 7.4 (1.2, 15.8) vs. 10.9 (2.5, 19.7) years without LGE).

Table 6-8 Univariate and multivariate analysis of variables associated with CMR measured LV mass/BSA.

Variable	**LVmass/BSA**						
	Univariate analysis (number of observations=35, unless otherwise stated)			**Multivariate analysis R^2=0.488 n=20**		**Multivariate analysis without RV-RA gradient (due to small numbers) R^2=0.446, n=35**	
	Correlation coefficient	**B (95% CI)**	**p value**	**B (95% CI)**	**p value**	**B (95% CI)**	**p value**
Age*	-0.033	-0.03 (-0.34, 0.28)	0.849	0.27 (-0.21, 0.76)	0.246	0.11 (-0.17, 0.39)	0.411
Male gender*	**0.575**	**14.39 (7.14, 21.64)**	**<0.001**	5.39 (-7.38, 18.15)	0.379	**10.88 (2.59, 19.17)**	**0.012**
Systolic blood pressure	-0.135	-0.09 (-0.32, 0.14)	0.438	-	-	-	-
Ever smoked	**0.301**	6.94 (-0.84, 14.72)	0.079	4.18 (-7.78, 16.14)	0.464	2.58 (-4.39, 9.56)	0.455
Body mass index	**-0.399**	**-0.83 (-1.51, -0.15)**	**0.018**	-0.68 (-2.41, 1.05)	0.410	-0.61 (-1.22, 0.001)	0.050
Waist/hip circumference	-0.065	-9.98 (-67.24, 47.27) (n=32)	0.724	-	-	-	-
TC/HDL-C	-0.296	-2.98 (-6.51, 0.54) (n=33)	0.094	-	-	-	-
SSc disease duration	-0.233	-0.26 (-0.65, 0.13)	0.178	-	-	-	-
DcSSc subtype	**0.308**	6.80 (-0.65, 14.25)	0.072	5.90 (-6.44, 18.24)	0.320	3.58 (-3.97, 11.14)	0.340
Hx of palpitations	-0.023	-0.51 (-8.42, 7.39)	0.896	-	-	-	-
Hx of DU	-0.128	-2.89 (-10.84, 5.06)	0.464	-	-	-	-
Hx of ILD	0.026	0.568 (-7.26, 8.39)	0.883	-	-	-	-

Scl70 positive	0.166	3.83 (-4.22, 11.88)	0.340	-	-	-	-
ACA positive	-0.169	-3.98 (-12.20, 4.25)	0.332	-	-	-	-
NFC pattern Normal	0.023	(n=33)	-	-	-	-	-
Non-specific		-7.30 (-28.77, 14.18)	0.492				
Early		2.21 (-15.66, 20.07)	0.802				
Active		3.50 (-15.36, 22.37)	0.706				
Late		-1.81 (-20.40, 16.79)	0.844				
Previous cyclophosphamide use	-0.017	-0.41 (-8.98, 8.16)	0.923	-	-	-	-
Ln(mRSS)	0.163	2.38 (-3.00, 7.75) (n=32)	0.374	-	-	-	-
S-HAQ-DI	-0.116	-1.66 (-6.95, 3.64) (n=32)	0.528	-	-	-	-
FVC	0.009	0.01 (-0.21, 0.22)	0.960	-	-	-	-
Echo RV-RA gradient	**-0.320**	-0.60 (-1.49, 0.28) (n=20)	0.169	-0.82 (-1.83, 0.19)	0.102	-	-
PWV	-0.080	-0.57 (-3.19, 2.05) (n=33)	0.660	-	-	-	-

ACA, anti-centromere antibody; BSA, body surface area; CI, confidence interval; DU, digital ulceration; FVC, forced vital capacity; ILD, interstitial lung disease; LVmass/BSA, left ventricular mass indexed to body surface area; mRSS, modified Rodnan skin score; NFC, nail-fold capillaroscopy; PWV, pulse wave velocity; RV-RA; right ventricular-right atrial; SHAQ-DI, Scleroderma health assessment questionnaire-disability index; Scl70, anti-topoisomerase antibody; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio

*variable entered into linear regression model as associated with LV mass in the literature.

Coefficients greater than 0.3 (highlighted in bold) also entered into the linear regression model.

Table 6-9 Univariate and multivariate analysis of variables associated with CMR measured torsion.

Variable	**Torsion**						
	Univariate analysis (number of observations =31, unless otherwise stated)			**Multivariate analysis R^2=0.656, n=18**		**Multivariate analysis without RV-RA gradient (due to small numbers) R^2=0.273, n=31**	
	Correlation coefficient	**B (95% CI)**	**p value**	**B (95% CI)**	**p value**	**B (95% CI)**	**p value**
Age*	0.215	0.07 (-0.05, 0.18)	0.247	-0.09 (-0.23, 0.05)	0.175	0.01 (-0.11, 0.13)	0.823
Male gender*	**-0.304**	-2.67 (-5.9, 0.51)	0.096	-2.41 (-7.00, 2.18)	0.272	-0.24 (-4.18, 3.70)	0.900
Systolic blood pressure	0.250	0.06 (-0.03, 0.15)	0.175	-	-	-	-
Ever smoked	0.192	1.58 (-1.48, 4.63)	0.300	-	-	-	-
Body mass index	0.284	0.22 (-0.06, 0.51)	0.121	-	-	-	-
Waist/hip circumference	0.077	3.98 (-16.74, 24.70) (n=28)	0.696	-	-	-	-
TC/HDL-C	-0.128	-0.53 (-2.16, 1.10) (n=28)	0.510	-	-	-	-
SSc disease duration	-0.032	-0.013 (-0.16, 0.14)	0.864	-	-	-	-
DcSSc subtype	-0.292	-2.33 (-5.24, 0.57)	0.111	-	-	-	-
Hx of palpitations	0.024	0.19 (-2.88, 3.27)	0.898	-	-	-	-
Hx of DU	0.088	0.72 (-2.38, 3.82)	0.639	-	-	-	-
Hx of ILD	**-0.447**	**-3.57 (-6.29, -0.86)**	**0.012**	-1.69 (-5.58, 2.20)	0.359	-1.85 (-5.46, 1.75)	0.299

Scl70 positive	**-0.454**	**-3.72 (-6.50, -0.95)**	**0.010**	-2.54 (-7.54, 2.46)	0.287	-2.24 (-6.05, 1.57)	0.237
ACA positive	0.142	1.29 (-2.14, 4.72)	0.448	-	-	-	-
NFC pattern Normal	-0.112	(n=29)	-	-	-	-	-
Non-specific		0.40 (-7.74, 8.54)	0.920				
Early		-0.07 (-6.92, 6.79)	0.984				
Active		-1.09 (-8.37, 6.19)	0.759				
Late		-0.92 (-8.07, 6.23)	0.793				
Previous cyclophosphamide use	**-0.305**	-2.60 (-5.69, 0.49)	0.096	-1.51 (-5.70, 25.68)	0.446	-0.78 (-4.58, 3.02)	0.676
Ln(mRSS)	-0.258	-1.33 (-3.33, 0.68) (n=28)	0.185	-	-	-	-
S-HAQ-DI	-0.195	-0.97 (-2.91, 0.96) (n=29)	0.311	-	-	-	-
FVC	0.164	0.04 (-0.04, 0.11)	0.378	-	-	-	-
Echo RV-RA gradient	**0.354**	0.22 (-0.09, 0.54) (n=18)	0.150	0.23 (-0.09, 0.54)	0.145	-	-
PWV	0.238	0.61 (-0.38, 1.60) (n=29)	0.215	-	-	-	-

ACA, anti-centromere antibody; CI, confidence interval; DU, digital ulceration; FVC, forced vital capacity; ILD, interstitial lung disease; mRSS, modified Rodnan skin score; NFC, nail-fold capillaroscopy; PWV, pulse wave velocity; RV-RA; right ventricular-right atrial; Scl70, anti-topoisomerase antibody ; SHAQ-DI, Scleroderma health assessment questionnaire-disability index TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio

* variable entered into linear regression model as associated with torsion in the literature

Coefficients greater than 0.3 (highlighted in bold) also entered into the linear regression model.

Table 6-10 Univariate and multivariate analysis of variables associated with CMR measured ECV.

Variable	**ECV**				
	Univariate analysis (number of observations=33)			**Multivariate analysis R^2=0.281, n=28**	
	Correlation coefficient	**B (95% CI)**	**p value**	**B (95% CI)**	**p value**
Age*	0.041	0.0001 (-0.010, 0.0012)	0.819	0.0002 (-0.0015, 0.0011)	0.754
Male gender*	-0.044	-0.004 (-0.035, 0.027)	0.807	0.004 (-0.033, 0.040)	0.839
Systolic blood pressure	-0.149	0.0003 (-0.0011, 0.0005)	0.409	-	-
Ever smoked	-0.006	0.000 (-0.029, 0.028)	0.973	-	-
Body mass index	-0.265	-0.002 (-0.004, 0.001)	0.135	-	-
Waist/hip circumference	**-0.510**	**-0.232 (-0.384, -0.081)** n=30	**0.004**	-0.193 (-0.414, 0.027)	0.083
TC/HDL-C	**-0.376**	**-0.015 (-0.029, -0.001)** n=31	**0.037**	-0.005 (-0.022, 0.013)	0.580
SSc disease duration	-0.046	0.0002 (-0.0016, 0.0012)	0.797	-	-
DcSSc subtype	0.044	0.003 (-0.025, 0.031)	0.807	-	-
Hx of palpitations	-0.032	-0.002 (-0.031, 0.026)	0.861	-	-
Hx of DU	**0.397**	**0.031 (0.005, 0.056)**	**0.022**	0.005 (-0.029, 0.039)	0.742
Hx of ILD	-0.046	-0.003 (-0.031, 0.024)	0.801	**-**	-
ACA positive	-0.251	-0.021 (-0.050, 0.008)	0.158	**-**	-

Scl70 positive	-0.001	-0.00006 (-0.029, 0.029)	0.997	-	-
NFC pattern Normal	0.225	(n=31)	-	-	-
Non-specific		-0.02 (-0.09, 0.04)	0.516		
Early		-0.00 (-0.06, 0.05)	0.854		
Active		-0.02 (-0.08, 0.04)	0.548		
Late		0.02 (-0.04, 0.07)	0.549		
Previous cyclophosphamide use	-0.179	-0.015 (-0.044, 0.015)	0.319	-	-
Ln(mRSS)	-0.032	-0.002 (-0.021, 0.018)	0.867	-	-
S-HAQ-DI	0.232	0.010 (-0.006, 0.026) n=30	0.217	-	-
FVC	-0.142	0.0003 (-0.0010, 0.0004)	0.429	-	-
Echo RV-RA gradient	0.128	0.001 (-0.003, 0.004) n=18	0.613	-	-
PWV	-0.00008	0.0001 (-0.009, 0.009) n=31	0.99986	-	-

ACA, anti-centromere antibody; CI, confidence interval; DU, digital ulceration; ECV, extracellular volume fraction; FVC, forced vital capacity; ILD, interstitial lung disease; mRSS, modified Rodnan skin score; NFC, nail-fold capillaroscopy; PWV, pulse wave velocity; RV-RA; right ventricular-right atrial; Scl70, anti-topoisomerase antibody; SHAQ-DI, Scleroderma health assessment questionnaire-disability index; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio

* variable entered into linear regression model as associated with ECV in the literature

Coefficients greater than 0.3 (highlighted in bold) also entered into the linear regression model.

Table 6-11 Association of disease phenotype with LGE on CMR.

Variable	**LGE present N=9**	**LGE Absent N=24**	**Group difference**	
	n (%)	**n (%)**	**Mean difference or exact OR*** (95% CI)**	**P value**
Age, years, mean(SD)	54.8 (12.4)	55.2 (13.2)	-0.4 (-10.7, 10.0)	0.939
Male gender*	5 (56)	4 (17)	6.25 (0.85, 46.88)	0.073
Systolic blood pressure, mmHg, mean(SD)	107 (18)	121 (15)	**-14.1 (-27.1, -1.2)**	**0.034**
Ever smoked*	7 (78)	14 (58)	2.5 (0.4, 28.9)	0.429
Body mass index, mean(SD)	21.9 (4.3)	26.0 (5.4)	**-4.13 (-8.24, -0.02)**	**0.049**
Waist/hip circumference, mean(SD)	0.81 (0.05) n=7	0.83 (0.08) n=23	-0.02 (-0.09, 0.05)	0.638
TC/HDL-C, mean(SD)	3.5 (1.1)	3.6 (1.0) n=22	-0.11 (-0.91, 0.68))	0.773
SSc disease duration, years, median (IQR)	7.4 (1.2, 15.8)	10.9 (2.6, 19.7)	2.0 (-4.7, 10.6)**	0.293^
DcSSc subtype*	5 (56)	9 (38)	2.1 (0.3, 13.3)	0.442
Hx of palpitations*	4 (44)	8 (33)	1.6 (0.2, 9.9)	0.691
Hx of DU*	4 (44)	9 (38)	1.3 (0.2, 8.1)	1.000
Hx of ILD*	7 (78)	8 (33)	**7.00 (0.96, 79.09)**	**0.047**
ACA positive*	0 (0)	10 (42)	-	**0.032**
Scl70 positive*	5 (56)	7 (29)	3.0 (0.5, 19.9)	0.230

NFC pattern*: Normal	1(13)	1 (4)	-	0.403
Non-specific	0 (0)	3 (13)		
Early	5 (63)	8 (35)		
Active	0 (0)	5 (22)		
Late	2 (25)	6 (26)		
Previous cyclophosphamide use*	3 (33)	7 (29)	1.2 (0.2, 7.9)	1.000
mRSS, geometric mean	3.2	2.9	1.1 (0.6, 2.1)**	0.814
S-HAQ-DI, mean(SD)	1.5 (0.8) n=8	1.2 (0.8) n=22	0.26 (-0.44, 0.95)	0.456
FVC, mean(SD)	93 (18)	97 (19)	-4.1 (-19.4, 11.2)	0.589
Echo RV-RA gradient, mean(SD)	22 (10) n=5	22 (4) n=13	0.25 (-11.73, 12.22)	0.959
PWV, mean(SD)	8.6 (2.0) n=8	8.6 (1.5) n=23	0.04 (-1.32, 1.41)	0.948

ACA, anti-centromere antibody; CI, confidence interval; DU, digital ulceration; FVC, forced vital capacity; ILD, interstitial lung disease; LGE, late gadolinium enhancement; mRSS, modified Rodnan skin score; NFC, nail-fold capillaroscopy; OR, odds ratio; PWV, pulse wave velocity; RV-RA; right ventricular-right atrial; Scl70, anti-topoisomerase antibody; SHAQ-DI, Scleroderma health assessment questionnaire-disability index; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol ratio

*Categorical data

**median difference (95%CI) ^Mann-Whitney U test

***continuous variables reported as mean difference (95% CI) with independent t test p value, categorical variables reported as exact OR (95% CI) with 2-sided Fisher's exact P value.

6.4 Discussion

This cross-sectional exploratory study performed a comprehensive CV assessment of patients with SSc, utilising contrast enhanced CMR, with comparison to healthy controls. The study determined patients with SSc have evidence of subclinical cardiac involvement, compared to controls, with these outcome measures associating with poor prognostic markers of SSc.

A proportion (25%) of patients with SSc, despite having no known cardiac disease had evidence of fibrosis with the presence of LGE. This is similar to the findings of Gargani et al who reported 23% of 171 patients with SSc having LGE on CMR [414]. Hachulla et al also reported 21% of 52 patients with SSc had LGE [378], however, other studies have reported higher prevalence of LGE. Rodriguez-Reyna et al determined LGE in 45% of their larger SSc cohort (n=62) predominantly in the mesocardium, sparing the subendocardium [464]. There were some differences in the cohort; 97% were female, and the mean age was nearly 15 years younger than those in this study. In addition, 79% had subendocardial perfusion defects suggesting confounding by IHD. The authors also reported that those with LGE were more likely to have a reduced LVEF (56% vs. 63% without DE, p=0.0009. In this chapter, a similar but non-significant trend was seen (LVEF in those with LGE 59% vs. 62% without LGE, p=0.092). Tzelepis reported 66% of their 36 SSc patient cohort had LGE; also sparing the subendocardium, with those with RP for longer than 15 years having more involved cardiac segments. However, there were a greater percentage of patients with dcSSc disease (64%) in their study which may account for the difference [377]. Finally Nutsi et al reported 53% of 19 patients with SSc had LGE, in a non-ischaemic pattern; their cohort was similar to this study however 95% were female [466]. A principal concern regarding LGE reporting, similar to perfusion defect reporting, is its subjectivity; cross-study comparisons are therefore difficult, in addition to the real risk of reporting artefact as pathology.

This study determined patients with known ILD and absence of ACA were most likely to have LGE, with a suggestion men with recent onset dcSSc and Scl70 positivity would be at greater risk. Indeed, this is in agreement with Rodriguez-Reyna et al who determined a greater proportion of patients with dcSSc had LGE than lcSSc (59% dcSSc, 33% lcSSc p=0.04) [464]. However, Gargani et al did not find any such association of LGE with these variables or age, duration of disease, mRSS or activity score in their 131 strong CMR study [414], and

neither did Sano et al with disease duration, subtype or antibody status in a study of 40 patients [528]. No cohort details were given in the abstract to allow comparison.

Whereas LGE detects focal fibrosis, ECV detects diffuse fibrosis, and given its quantitative nature, ECV may be a better marker of (early) fibrosis. Even after adjustment for key CV risk factors, in this study patients with SSc had increased ECV, suggesting diffuse fibrosis. This is in agreement with other reports [467, 468]. Nutsi el at determined an unadjusted ECV of 35% in patients with SSc compared to 28% in controls $p<0.001$ [466]. They also reported no difference in ECV in those patients with and without LGE, and suggest the increase in ECV was not driven by LGE presence (focal fibrosis). The work presented in this chapter found no statistically significant difference in ECV between those with LGE and without LGE, although there was a trend for higher ECV in those with LGE.

With regards to the phenotype of SSc most at risk, patients with a history of DU were more likely to have higher ECV (although this did not remain significant in the MVA; perhaps due to small numbers). Nutsi et al did report an increased ECV in those with dcSSc (37% vs. 33% lcSSc, $p=0.002$), and detected an association of ECV with MRSS ($p=0.03$) and SSc Valentini-disease activity index (VDAI) ($p=0.04$) [466]. However, other groups have tested for and not found any association of ECV with clinical data such as dcSSc and mRSS [467, 468]. Interestingly, there was an association of lower BMI and systolic blood pressure with the presence of LGE, and of lower TC/HDL-C and waist/hip ratio with increased ECV. This may reflect general cachexia and RP-medication use in severe disease. In contrast, increasing BMI has been associated with increasing ECV in the general adolescent population [529].

There was a trend for lower LV mass in patients with SSc, perhaps needing larger numbers to reach statistical significance. Although a trend for lower LV mass was seen in a study by Thuny et al [467], most other CMR studies, albeit including small numbers of patients, have found no difference [466, 468, 530].

One perfusion defect was seen in this cohort of patients with SSc, which is surprising given the prevalence of its reporting in other CMR studies [464]. Rodriguez-Reyna et al reported perfusion defects in 79% (mainly in the subendocardial region); a cohort similar to the one presented in this chapter in terms of SSc-comorbidity [464]. The reliable visual interpretation of myocardial perfusion CMR requires good image quality and experienced observers [454]. There is a significant risk of interpreting artefacts as pathology; for example, artefacts created by cardiac motion within a single cardiac cycle, or ‘Gibb’s ringing’ creating a dark rim between myocardium and blood pool in ventricle (high contrast

area) requiring filtering of images and expert interpretation [531]. For this reason we performed our studies in a world leading CMR centre with 20 years of expertise in myocardial perfusion CMR. Some of the data presented by less experienced groups may not meet the same quality standards.

To summarise, this study demonstrates the SSc phenotype which appears to be most at risk of SSc-CM is the male patient with recent onset diffuse disease, absence of ACA, with Scl70 positivity, and a history of DU and ILD; all regarded as poor prognostic markers generally in SSc.

6.4.1 Limitations

This was an exploratory study and much larger numbers are required to accurately tease out the association of disease subtype and serology and cardiac involvement in SSc. Despite small numbers this study is still relevant as it provides an accurate and comprehensive description of the SSc disease phenotype and its association with CMR measures.

A major limitation in interpreting the data is differentiating the underlying pathology of primary myocardial disease from that of atherosclerotic macrovascular disease. CV risk factors were minimised; diabetes was an exclusion criterion, and ELCASA patients were not allowed more than one traditional CV risk factor. Any pathology seen would be therefore less likely to be due to macrovascular disease. Having said this, however, the one perfusion defect seen in this study was subendocardial and located in an area thought secondary to atherosclerotic disease within the right coronary artery. Differentiating between the two pathologies will always be challenging when reporting primary myocardial disease in SSc.

Another limitation is that myocardial perfusion was assessed by the visual detection of perfusion defects only. Myocardial perfusion reserve would have offered a quantitative assessment of myocardial blood flow, however, this technique is still being evaluated in the general population [532].

6.4.2 Future research agenda

These data suggest an at risk group for further evaluation and validation, that would justify more intensive monitoring for SSc-CM & its complications. Longitudinal studies are required to determine the significance of sub-clinical diastolic dysfunction and association of fibrosis (ECV and LGE) to the development (if any) of arrhythmias and heart failure.

Further evaluation of CMR measured ECV compared to that of skeletal muscle would also be valuable. Barison et al determined an association between cardiac ECV and skeletal ECV in their CMR study [468]. If parallels between the pathogenesis of SSc-CM and peripheral myopathy/myositis do exist, this would serve as an advantage in the understanding of the pathophysiology of SSc-CM given the feasibility of obtaining tissue samples for histology.

6.4.3 Summary

This study has shown that subclinical myocardial involvement is more prevalent in patients with SSc compared to controls, and is associated with a poor prognostic SSc phenotype. This study highlights the need for much larger scale longitudinal studies to assess the impact and prognosis of subclinical myocardial disease in SSc.

6.4.4 Key messages

1. SSc-cardiomyopathy is prevalent and associated with a poor prognostic SSc phenotype.
2. CMR has utility in the assessment of SSc-cardiomyopathy

Chapter 7 Prospective study evaluating conduction abnormalities with correlation to cardiovascular magnetic resonance imaging in patients with Scleroderma. Electrophysiology and Cardiac Imaging in Scleroderma- the ELCASA study

7.1 Introduction

SSc-cardiomyopathy (SSc-CM) is characterised by myocardial fibrosis, with or without myocardial inflammation [375, 376, 381]. SSc-CM can present in various ways, from pericardial disease to LV dysfunction, but of perhaps most notable significance is the development of arrhythmias and associated sudden death [331, 385]. Historical electrocardiogram (ECG) and 24 hour ECG monitoring studies have demonstrated abnormalities in up to 53% of SSc cohorts [359]. These abnormalities include an increase in the usually benign supraventricular (SVEs) and ventricular ectopics (VEs) compared to control populations, and also the presence of significant arrhythmias such as supraventricular tachycardia (SVT), and more serious arrhythmias such as ventricular tachycardia (VT) and complete heart block (CHB) [359, 396, 397]. In addition, signal averaged ECG (SAE) studies in SSc have reported increased late ventricular potentials (which denote myocardium at risk of arrhythmia) in SSc and have been associated with cardiac fibrosis [399].

SSc-CM is associated with an increase in mortality. Komocsi et al reported a hazard ratio of 3.15 (95% confidence interval (CI) 2.33, 4.26) for patients with SSc with pericarditis verified by echocardiogram, recurrent arrhythmia and/or conduction abnormality on ECG, or clinical signs of heart failure [11]. A large European Scleroderma Trials and Research (EUSTAR) study of 5860 patients with SSc described 14% of deaths secondary to myocardial disease, whilst 12% were secondary to atherosclerotic heart disease (or cardiovascular disease (CVD), deemed non-SSc related) [341]. Sudden death from arrhythmias is a recognised phenomenon, with 6% of all deaths in the EUSTAR cohort due to arrhythmias [341]. Electrophysiological (EP) studies have reported an increase in mortality in patients with SSc with EP abnormalities [359, 396].

The implantable loop recorder (ILR) is a tool used by cardiologists for patients symptomatic of infrequent palpitations/symptoms suggestive of arrhythmias not captured using routine 24 hour or seven day ECG monitoring, and is recommended for the investigation of unexplained syncope in the general population [533]. This wireless device, inserted under the skin on the chest wall, records EP data remotely, and can remain in-situ for up to three years, at which point the battery life ends.

Cardiovascular magnetic resonance (CMR) imaging, as discussed in previous chapters, is an increasingly valued tool in the assessment of global cardiac function and also changes occurring at tissue level. Techniques using gadolinium based contrast agents can detect late gadolinium enhancement (LGE) providing information on focal cardiac fibrosis, and T1 and extra-cellular volume (ECV) measures can give detail on milder levels of inflammation and presence of more diffuse fibrosis [372, 377, 378, 466, 534].

Detecting EP abnormalities in patients with SSc earlier may prevent their associated complications, such as cardiac decompensation or even sudden death by the timely intervention with appropriate drug therapy or implantable cardiac defibrillator (ICD). However, there remains a clinical unmet need for clear guidance/algorithm as how best to screen for EP disturbances and how frequently to perform such screening tests. It is also unclear as to underlying pathophysiology of SSc-CM and associated arrhythmias. There is autopsy evidence to suggest sparing of the conduction system in myocardial fibrosis [535].

7.1.1 Hypotheses and aims

The hypotheses relating to this study was that cardiac arrhythmias are prevalent in patients with SSc, free of clinical SSc-CM, and that these arrhythmias are associated with sub-clinical CMR abnormalities.

This study aimed;

- To evaluate the feasibility of an ILR (REVEAL® device) in the detection of arrhythmias in SSc
- To describe the prevalence of arrhythmias in SSc
- To describe the relationship of arrhythmias to the SSc disease phenotype and to findings on CMR

As a result, this study may eventually inform effective and streamlined strategies in the management of patients with SSc, whilst also providing insight into the pathophysiology of arrhythmias in SSc-CM.

7.1.2 Study objectives

The primary objective was to assess for cardiac conduction abnormalities with the use of an ILR in patients with SSc free of CVD and minimal CV risk factors, and examine the relationship of any conduction abnormalities with disease phenotype and CMR.

7.2 Methods

The following section outlines how the study was carried out.

7.2.1 Study design

The ELCASA (ELectrophysiology and CArdiac imaging in SclerodermA) study is a single centre 144 week long prospective pilot study currently running within Leeds Teaching Hospitals NHS trust (LTHT) (REC: 12/YH/0298, NRES Committee Yorkshire & The Humber - Leeds East ethics committee). Patients with SSc, with minimal CV risk factors, underwent a comprehensive CV assessment at baseline, including a clinical assessment with blood collection and pulse wave velocity (PWV) measurement, and a stress-perfusion CMR, and electrophysiology testing, followed by the insertion of an implantable loop recorder (REVEAL® device). Baseline tests are repeated yearly up to 3 years (144 weeks), at which point the REVEAL® device is removed and the study ends.

Study participants continue to receive usual standard of care for their SSc as those outside of the study.

Currently, all patients have been recruited, and have passed the year 1 time point. An interim analysis was carried out at year 1 (48 weeks) to investigate the feasibility of the REVEAL® device and any unexpected findings, and perform a preliminary analysis to investigate the above aims. These one-year findings are included here.

7.2.2 End-points

The primary end-point was the presence of conduction abnormalities detected using the REVEAL® device.

The secondary end-points were:

Presence and extent of myocardial fibrosis on CMR, as measured by LGE and ECV mapping.

CMR measures:

- LV ejection fraction (LVEF) (%)
- LV end-diastolic volume (EDV) (ml)
- LV end-systolic volume (ESV) (ml)
- LV mass indexed to body surface area (g/m^2)
- LVmass/EDV
- Stroke volume indexed to body surface area (ml/m^2)
- Mid systolic strain rate (Mid S')
- Peak twist
- Torsion
- Inferoseptal T1 (ms)
- Aortic distensibility ($10^{-3}mmHg^{-1}$)

Applanation tonometry

- Pulse wave velocity (PWV) (m/sec)

7.2.3 Subject numbers

Twenty patients were recruited in this study; a figure decided upon given that this was a feasibility study using a device never before used as a research tool in SSc.

7.2.4 Eligibility Criteria

Inclusion criteria

- Between ages of 18 and 80 years old (inclusive)
- Capable of understanding and signing an informed consent form
- Meet 1980 American College of Rheumatology (ACR) criteria [383], 2013 ACR/European League of Rheumatism (EULAR) criteria [527] or the LeRoy classification [384] with in particular the following features (but not exclusively):
    - Diffuse cutaneous SSc: including patients with evidence of early internal organ involvement (e.g. lung)
    - Limited cutaneous SSc: include any with Scl-70 positive antibody
    - Earlier disease onset (within 5 years of first non-Raynaud's phenomenon symptom onset)

Exclusion criteria

- Prior diagnosis of CVD (cardiac, peripheral or cerebral)
- History of diabetes mellitus (to minimise confounding)

- More than one of 4 other traditional cardiovascular risk factors (current smoker, hypertension, hypercholesterolaemia/hypertriglyceridaemia, family history of CVD)
- For the CMR imaging component: pregnancy, breastfeeding, non-MR conditional 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, and an eGFR less than 45 ml/min/1.73m^2.

7.2.5 Target population

Patients with SSc, attending the LTHT connective tissue disease clinic, in particular the specialist-SSc clinic were approached to enter the study. Patients approached were those that demonstrated either diffuse cutaneous SSc (dcSSc) with evidence of internal organ involvement (e.g. lung) or limited cutaneous SSc (lcSSc), and particularly patients with early disease.

7.2.6 Withdrawal

Again in line with the previously described studies, patients were free to withdraw from the study at any time. This did not affect their usual clinical care. Any data/blood samples already collected was retained and remained for analysis.

7.2.7 Study Schedule

After screening, patients underwent clinical assessments at baseline and return at weeks, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 144 to assess clinical activity of disease (see Table 7-1). Whenever possible, the same investigator performed the rheumatological assessments (for example, modified Rodnan skin score (MRSS)) during all visits to reduce potential investigator bias; LAB up to September 2014, and GA or RBD thereafter.

Table 7-1 Study schedule

Week	-1	0	12	24	36	48	60	72	84	96	108	120	132	144
Visit	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Inclusion/exclusion criteria	X													
Informed consent	X													
Urinalysis	X													
Clinical data collection	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SHAQ-DI and VAS		X		X		X		X		X		X		X
Ten-year CV risk scores calculation and Rose Angina Questionnaire		X				X				X				X
Nail-fold capillaroscopy		X				X				X				X
Biological sample collection[1]	X	X[2]		X[2]		X[2]		X[2]		X[2]		X[2]		X[2]
Chest X-Ray	X[3]													
Doppler echocardiogram		X				X				X				X
ECG	X	X				X				X				X
Signal averaged ECG		X				X				X				X
24 hour ECG monitor		X				X				X				X
CMR imaging		X				X				X				X

Autonomic testing		X				X				X				X
ILR Insertion		X												
ILR interrogation			X	X	X	X	X	X	X	X	X	X	X	X
ILR removal														X
PWV		X				X				X				X

[1]Haematology, blood chemistry, CRP and ESR, Fasting plasma glucose, lipid and triglycerides, and serological tests (ANA, ACA, Scl-70) (latter to be done at screening and annual visits only).
[2]Research blood biomarker collection also included
[3]Only if not done in preceding 24 weeks
CV, cardiovascular; CMR, cardiovascular magnetic resonance imaging; ECG, electrocardiogram; ILR, implantable loop recorder; PWV, pulse wave velocity; SHAQ-DI, Scleroderma Health Assessment Questionnaire – disability index; VAS, visual assessment scores

7.2.8 Study visits

7.2.8.1 Screening

Potential study participants were screened no more than 4 weeks prior to assessment at baseline. Informed consent was taken and a patient number was assigned. A clinical assessment then collected data; physical examination (including height, weight and vital signs), urinalysis, concomitant medication and past medical history. A fasting blood sample was collected and set for haematology, blood chemistry, CRP and ESR, serologic tests (ANA, ACA, and Scl-70), fasting plasma glucose, lipid and triglycerides testing. A 12-lead ECG was performed and chest X-Ray (if not done in previous 24 weeks). Inclusion/exclusion criteria available at the time were evaluated.

7.2.8.2 Baseline (visit 2, week 0)

At baseline, the following were checked/recorded.

- Results of laboratory tests conducted at screening visit were in agreement with the inclusion/exclusion criteria
- Patient's eligibility confirmed, based on all inclusion/exclusion criteria

The following was also carried out; a standard clinical and physical examination (including measurement of weight, waist and hip circumference, vital signs, and usual SSc assessments (as in chapter 6), and nail-fold capillaroscopy. Fasting blood samples were taken and patients completed the Scleroderma Health Assessment Questionnaire – disability index (SHAQ-DI), visual assessment scores (VAS) and Rose Angina questionnaire. Ten-year cardiovascular risk scores were later calculated (Framingham, Joint British Societies and QRISK 2).

The following cardiac procedures were performed within 4 weeks of the baseline visit:

- 12-lead ECG
- Signal averaged ECG
- 24-hour ECG monitor
- Doppler echocardiography
- PWV by Applanation tonometry
- CMR
- Autonomic testing
- Insertion of Reveal® ILR (always on completion of the above)

7.2.8.3 Follow-up

At visit 3-14 (weeks, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 144) the following information was/will be taken; a standard clinical and physical examination (including measurement of weight, waist and hip circumference, vital signs, and usual SSc assessments (as described in chapter 6), and every 6 months; fasting blood sample collection was taken, and patients completed the SHAQ-DI and VAS.

At visit 3a-14a (weeks, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, and 144) patients attended or will attend the electrophysiology department for interrogation of the ILR, or if they preferred this was/is done over the telephone.

At the annual visits (visit 6, 10 and 14 or weeks 48, 96 and 144) the following procedures occurred/will occur:

Ten-year cardiovascular risk scores calculation

Rose Angina Questionnaire

Nail-fold capillaroscopy

Cardiac procedures

- 12-lead ECG
- Signal Averaged ECG
- 24-hour Holter monitor
- Doppler echocardiography
- PWV by Applanation tonometry
- CMR
- Autonomic testing
- Interrogation of Reveal® ILR

At visit 14 (week 144) the Reveal® ILR was/will be removed after interrogation.

7.2.9 Study procedures

7.2.9.1 Informed consent

Informed consent was taken in the same way as the previously described studies within this thesis; a patient information sheet was given, time was allowed for the patient to consider the study and then the patients were contacted to find out if they were interested in participating and/or had any questions. A copy of the consent was kept in the site file, with one copy filed in the notes, and another sent to the patent.

7.2.9.2 Biological sample collection

Patients were asked to fast for the preceding 8 hours before venepuncture. The samples were taken as already described in the study schedule.

With regards to the screening visit, if the fasting plasma glucose (FPG) was ≥7.1mmol/L the test was repeated. If the FBG was again ≥7.1mmol/L, diabetes mellitus was confirmed and the patient excluded from entering the study. The patient's GP was informed. If 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 informed. If the local laboratory did not process the lipids as the same test was done too recently to warrant their repeat, then the previous results were used.

In addition to already specified routine bloods, biological blood samples (total amounting maximum 56mls) were taken at baseline and follow-up visits at week 24, 48, 96 and 144, using EDTA, lithium heparin, red clotted, serum, citrate, PAXGENE and RNA tempus tubes. The resulting anonymised processed aliquots of blood were stored (some at -30^{o}C and some at -80^{o}C) for later cardiovascular biomarker analysis.

7.2.9.3 Comprehensive standard clinical data collection

In a similar fashion to the data collection in the cross-sectional CMR study described in chapter 6 of this thesis, the following aspects of the study participant were recorded:

Demographics

- The participant demographic history including their age, gender and ethnicity.

Cardiovascular risk evaluation.

- History of smoking habit, hypertension, dyslipidaemia and family history of premature cardiovascular disease.
- How many minutes of moderate exercise taken per week (defined as enough to make them feel out of breath or perspire) and how many days per week five or more fruit and vegetables per day were consumed.
- Weight, height, waist and hip circumference were measured, along with blood pressure.
- Rose Angina Questionnaire completed.

SSc disease phenotype evaluation

- Duration of RP
- Disease subset
- Time since first non-RP symptom (disease duration)

- History of disease complications/internal organ involvement such as interstitial lung disease (ILD), digital ulceration (DU).
- Medication history
- Physical findings, including modified Rodnan skin score (mRSS), presence of DU, calcinosis, and tendon friction rubs.
- Nail-fold capillaroscopy (NFC) using light microscopy +/- videocapillaroscopy
- HAQ-DI and VAS
- Details of most recent echocardiogram and pulmonary function tests (or test requested if an up to date test was clinically required).

7.2.9.4 Non-invasive cardiac testing

Tables 7-2 to 7-4 describe the data recorded from the 12-Electrocardiogram (ECG), echocardiogram and 24 hour ECG monitor. A SAE was performed to detect the presence of late ventricular polarisation. Criteria for the presence of late ventricular polarisation defined as meeting two of the following criteria [398]: total QRS duration (filtered) ≥114ms, duration of HFLA signals that are less than 40uV ≥39ms, root mean squared (RMS) voltage in terminal 40ms <20uV.

Table 7-2 Data recorded from 12-ECG.

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 LV hypertrophy: No/Yes
Strain: 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

Table 7-3 Data recorded from echocardiogram

Left atrium: Normal/Dilated (mm)	Right atrium: Normal/Dilated (mm)
LV end-diastolic diameter (mm)	LV end-systolic diameter (mm)
LV intraventricular septum (ED) (mm)	LV posterior wall (mm)
LV ejection fraction % (biplane)	Tricuspid valve annular motion, mm
Valvular stenosis/regurgitation? (>grade 1)	
Aortic stenosis: grade, gradient	Aortic regurgitation: grade
Mitral stenosis: grade, gradient	Mitral regurgitation: grade
Tricuspid regurgitation: grade	
IVC: Not dilated/Dilated	RV: Normal/Dilated: grade
RV function: Normal/Depressed: mild,	RV-RA gradient: mmHg

IVC, inferior vena cava, LV, left ventricle; RV, right ventricle;

Table 7-4 Data recorded from 24- hour ECG monitor

Heart rate: Average/Maximum/Minimal (beats/min)	Rhythm
Total number of heart beats	Number of supraventricular ectopics
Number of premature ventricular ectopics	Supraventricular tachycardia (≥3 beats): Yes/No
Presence of:	*Supraventricular tachycardia*
Couplet	*Triplet*
Drop beat	*Ventricular tachycardia*
Brady	*Pauses >3 seconds*

7.2.9.5 Cardiovascular magnetic resonance (CMR) protocol

The CMR scan was carried out on a 3 Tesla Magnetic Resonance scanner (Philips Achieva TX scanner (Philips Healthcare, Best, The Netherlands) at baseline before insertion of the MR-conditional ILR, at year 1, 2 and at year 3 after removal of the ILR. However, the first few CMR scans at year one revealed excessive artefact relating to the ILR, prohibiting accurate evaluation of the heart, and so an amendment was made to the study to perform a CMR scan at baseline and after the removal of the ILR at year 3 only. A single venous cannula was inserted for the administration of intravenous MR contrast agent (Gd-DTPA) and pharmacological stress with adenosine. The CMR lasted approximately 60 minutes. Patients were asked to avoid caffeine for 24 hours prior to the CMR.

The CMR protocol was the same as that described in chapter 6, and performed by the same clinical research CMR-cardiology team. As described previously in chapter 5, the protocol includes:

- Low-resolution survey, reference scans and localizers.
- Baseline T1 mapping.
- Adenosine stress first-pass myocardial perfusion imaging.
- Resting wall motion and LV function.
- Tissue tagging for strain analysis and diastology.
- Aortic distensibility.
- Resting first-pass myocardial perfusion study.
- Late gadolinium enhancement (LGE).
- Post-contrast T1 mapping

The CMR scans were evaluated in the same way as described in chapter 6, involving the expertise of CMR-cardiologists (supervision by SP) using QMASS MR 7.5 software (Medis, Leiden, The Netherlands).

7.2.9.6 Implantable loop recorder

The MR-conditional implantable loop recorder (ILR; Medtronic Reveal® XT) was implanted subcutaneously in a left pectoral position (local anaesthesia). The implantation was typically completed within 15 minutes. The ILR device is interrogated every 3 months and also if the patient has symptoms suggestive of arrhythmia. Interrogation is carried out remotely using Care Link® requiring the patient to hold a receiver over the device whilst data is transferred to a secure databank, or within hospital. The ILR is to be removed after 3 years. Data is stored securely in the pacemaker clinic/electrophysiological department with the usual Leeds Teaching Hospitals NHS Trust security measures.

The events that are recorded by the device are either patient triggered (patient had a remote control to trigger a recording) or auto-triggered.

7.2.9.7 Autonomic testing

This was determined using ECG monitoring and performed in the electrophysiology lab before implantation of the ILR. The following parameters were measured, of which normal values are age-related.

7.2.9.7.1 Respiratory RR interval variation

The shortest RR interval during inspiration and the longest RR interval during expiration were measured over two minutes. The E/I ratio was calculated: mean RRmax (mean)/mean RRmin (mean).

7.2.9.7.2 Valsalva

The Valsalva test measured the shortest RR interval during Valsalva for 15 seconds and longest RR interval within 30s after Valsalva. The longest/shortest RR ratio was then calculated.

7.2.9.7.3 Maximum/minimum 30:15 ratio

The maximum/minimum 30:15 ratio was calculated by measuring the longest RR interval between beat 20 and 40, divided by the shortest RR interval between beat 5 and 25 after standing up.

7.2.9.7.4 Orthostatic dysregulation

The difference in systolic blood pressure (sysBP) was measured from sysBP in the supine position to the lowest sysBP within three minutes of being in the upright position. An abnormal result is a drop of more than 28 mmHg.

7.2.9.7.5 Global assessment

An autonomic dysfunction was present if two or more of the four tests were abnormal.

7.2.9.8 Pulse wave velocity by Applanation Tonometry

PWV was measured using VICORDER® (SMT Medical, Würzburg, Germany), using the same methodology described in the previous chapters of this thesis.

7.2.10 Statistical analysis

As this was a pilot study, no formal power calculation for determining sample size was carried out. Twenty was determined by the study team to provide sufficient data in this novel feasibility study.

If specific dates of diagnosis or onset of RP were uncertain, the 15th day and/or 6th month of the year were chosen as the default to minimise bias. No imputation was carried out for missing values.

The statistical packages SPSS (IBM SPSS Statistics 22) and Stata/IC 13.1 (StataCorp, Texas, USA) were used to perform statistical tests. In line with the statistical analysis described in Chapter 6, the distribution of each variable and outcome measure was determined and checked for extreme outliers, followed by a descriptive analysis to describe the cohort and outcome measures.

Independent t-tests and linear regression, adjusting for age and sex, was used to determined differences in CMR outcomes in those with and without ILR abnormalities and significant arrhythmias. For the LGE analyses, relative risk calculations were performed to measure the difference between those with and without ILR abnormalities. Logistic regression was employed to represent odds ratios when adjusting for age and gender.

7.3 Results

Twenty-seven patients were screened for ELCASA. Five patients did not meet the inclusion criteria; four had two or more CV risk factors and one had a low eGFR (a CMR contrast

exclusion criterion). Two patients declined ILR implantation after their baseline visit as they were worried about the procedure and wearing the ILR, therefore two more patients were recruited (after submitting an amendment to and gaining permission from the ethics committee) to ensure the target number of 20 ILR insertions was achieved.

One patient died six weeks after baseline from causes unrelated to the study. No data from her ILR was extracted as she died abroad. Only data from the 19 patients who underwent the ILR insertion and had data extracted are reported below.

Fifteen out of the 19 patients with ILR data underwent the CMR scan, and contrast enhanced data were available in 14 patients; IV access was unobtainable in two patients, one patient was claustrophobic and couldn't tolerate the scan, and another CMR was abandoned due to urgent ILR data findings (discussed in detail below).

7.3.1 Baseline characteristics

The baseline characteristics (demographic, disease phenotype and CV risk factors) are reported in Tables 7-5 and 7-6.

Sixty-three percent of the patients were female and had a mean (standard deviation (SD)) age of 53 (12) years, 16 (84%) were Caucasian, 2 (11%) Asian and 1 (5%) Afro-Caribbean descent. Median (interquartile range (IQR)) disease duration was 7.5 (1.8, 19.5) years (defined as time from first non-RP phenomenon). 7 (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.

Eleven (58%) were on a DMARD, and 7 (37%) had previously been treated with cyclophosphamide. Five (26%) had received iloprost in the past. Eleven (58%) were taking an ACE-inhibitor and 15 (79%) a calcium channel blocker (CCB). The median (IQR) MRSS was 2 (2, 6), 5 (26%) had evidence of calcinosis and 9 (47%) of digital pits. Six (32%) and 4 (21%) were ACA and Scl70 positive respectively. The most recent pulmonary function tests performed revealed the mean (SD) forced vital capacity (FVC) was 92 (25)% and transfer factor (DLCO) 63 (16)%.

As expected given the inclusion criteria for this study, few patients with SSc had traditional CV risk factors; none had a history of hypertension or dyslipidaemia, and only 2 (11%) had a family history of premature CVD. No patient currently smoked, however, 11 (58%) were ex-smokers. No patient was being treated with a statin. Mean systolic and diastolic blood pressure levels were well within the normal range.

Table 7-5 Study participant characteristics

Variable	Expressed as	ELCASA patients, n=19
Demographics		
Age, years	mean, SD	52.8 (11.8) (range 26, 73)
Female	n %	12 (63)
Ethnicity: Caucasian	n %	16 (84)
Asian		2 (11)
Afro-Caribbean		1 (5)
CV risk profile		
Smoking status: Never	n %	8 (42)
Ex		11 (58)
Current		0 (0)
PMH hypertension	n %	0 (0)
PMH Hypercholesterolaemia	n %	0 (0)
FHx premature CVD*	n %	2 (11)
Waist/Hip Ratio	mean, SD	0.82 (0.09)
BMI	mean, SD	25.1 (5.5)
Systolic BP, mmHg	mean, SD	115 (13)
Diastolic BP, mmHg	mean, SD	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)

BMI, body mass index; BP, blood pressure; CMR, cardiovascular magnetic resonance; CVD, cardiovascular disease; FHx, family history of; IQR, interquartile range; PMH, past medical history of; SD, standard deviation; SSc, Systemic Sclerosis.
*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

Table 7-6 Disease specific characteristics of patients with Systemic Sclerosis

SSc phenotype	**Data as expressed as**	**SSc patients n=19**
Disease history		
Disease subtype LcSSc	n (%)	12 (63.2)
DcSSc		7 (36.8)
Presence of RP	n (%)	19 (100.0)
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: Digital ulceration	n (%)	6 (31.6)
GORD		17 (89.5)
Non-GORD GI involvement		3 (15.8)
Interstitial lung disease		8 (42.1)
Palpitations		9 (47.4)
Medication history		
Current use of DMARD	n %	11 (57.9)
Name of current DMARD	n %	
Mycophenolate		6 (31.6)
Methotrexate		2 (10.5)
Hydroxychloroquine		1 (5.3)
Cyclophosphamide		2 (10.5)
Sulphasalazine		1 (5.3)
Previous use of cyclophosphamide	n (%)	7 (36.8)
Of these, number of previous cyclophosphamide infusions	Median (IQR)	6 (6, 12)
Current use of prednisolone	n (%)	2 (10.5) on 5mg
		1 (5.3) on 8mg
		2 (10.5) on 10mg
		1 (5.3) on 15mg
Previous use of iloprost	n (%)	5 (26.3)
Current treatment with: Sildenafil	n (%)	2 (10.5)
Bosentan		1 (5.3)

ACE inhibitor		11 (57.9)
Calcium channel blocker		15 (78.9)
Examination findings		
Total modified Rodnan skin score	Median (IQR)	2 (2, 6)
Presence of: Digital pits	n (%)	9 (47.4)
Digital ulceration		2 (10.5)
Tendon friction rubs		1 (5.3)
Calcinosis		5 (26.3)
TJC28	Median (IQR)	1 (0.0, 4.0)
SJC28	Median (IQR)	0 (0.0, 0.0)
NFC vasculopathy pattern Non-specific	n (%)	1 (5.3)
Early		13 (68.4)
Active		3 (15.8)
Late		2 (10.5)
SHAQ-DI	Mean (SD)	1.39 (0.87)
Visual Assessment scores Pain	Median (IQR)	59 (38, 68)
Gastrointestinal		21 (1, 62)
Breathing		34 (4, 68)
RP		68 (22, 78)
Digital ulceration		11 (0, 55)
Overall		59 (28, 72)
Routine investigations		
Antibody positive: ANA	n %	15 (78.9)
ACA		6 (31.6)
Scl70		4 (21.1)
CRP (mg/L) (normal range <5)	Median (IQR)	0 (0.0, 1.3)
ESR (mm/hr) (normal range 1-15)	Median (IQR)	9.0 (4.3, 32.3) (n=16)
Forced vital capacity, %	Mean (SD)	91.5 (24.6)
Total lung capacity, %	Mean (SD)	91.5 (20.0)
DLCO, %	Mean (SD)	63.3 (15.8)
DLCO/VA, %	Mean (SD)	78.3 (16.8)

ACA, anti-centromere antibody; CRP, C-reactive protein; dcSSc, diffuse cutaneous Systemic Sclerosis; DLCO, diffusing capacity of the lungs for carbon monoxide; DLCO/VA, DLCO adjusted for volume; DMARDs, disease modifying anti-rheumatic drugs; ESR, erythrocyte

sedimentation rate; GORD, gastro-oesophageal reflux disease; HAQ-DI, health assessment questionnaire-disability index; lcSSc, limited cutaneous Systemic Sclerosis; NFC, nail-fold capillaroscopy; RF, rheumatoid factor; RTX, rituximab; RP, Raynaud's Phenomenon; Scl70, anti-topoisomerase antibody; SJC, swollen joint count; TJC, tender joint count
*many values missing due to poor tricuspid regurgitation/image quality

7.3.2 Baseline cardiovascular outcome measures

Tables 7-7 to 7-10 describe the various soluble, structural and functional CV outcome measures in the ELCASA participants. Patients in ELCASA had a mean (SD) fasting TC/HDL-C of 3.7 (1.3) and glucose of 4.8 (0.5)mmol/L, as shown in Table 7-7. Mean (SD) PWV was 8.1 (1.1)m/s.

Baseline echocardiograms reported mean (SD) LVEF where measured (n=14) of 56.1 (8.8)% and RV-RA gradient 22.5 (5.6)mmHg (see Table 7-8). Baseline ECGs revealed sinus rhythm (SR) in all (see Table 7-9) with two showing a left axis deviation (LAD), one patient with incomplete right bundle branch block, one with complete left bundle branch block (LBBB) and one with left anterior fascicular block. Twenty-four hour ECG monitoring revealed a mean (SD) heart rate of 80.3 (10.6) beats per minute (see Table 7-9), with one episode of supraventricular tachycardia (SVT) detected in two patients (7 and 17 beats duration). The median (IQR) number of supraventricular ectopics (SVEs) and ventricular ectopics (VEs) was 7.0 (0.5, 43.5) and 10.5 (2.3, 23.0) respectively. Six (43%) of 14 patients had globally abnormal autonomic tests and 4 (21%) of 13 patients had abnormal SAE tests. CMR-measured mean (SD) LVEF was 60.1 (4.5)%, LVmass/BSA 44.8 (10.6)g/m^2 and ECV 30.1 (3.2)%. Five (26%) patients had evidence of LGE on CMR.

Table 7-7 Baseline cardiovascular bedside outcome measures in ELCASA participants

Variable	ELCASA patients, n=19
Fasting glucose, mmol/L	4.8 (0.5)
Fasting total cholesterol, mmol/L	4.9 (1.0)
Fasting HDL-C, mmol/L	1.5 (0.4)
Fasting LDL-C, mmol/L	2.8 (0.8)
Fasting TC/HDL-C ratio	3.7 (1.3)
Fasting triglycerides, mmol/L	1.6 (0.8)
PWV, m/sec	8.1 (1.1) (n=17)

HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; PWV, pulse wave velocity
Values expressed as mean (standard deviation)

Table 7-8 Baseline echocardiogram outcome measures in ELCASA participants

Echocardiogram measure	ELCASA patients, n=19		
Left atrium Dilated* If dilated mean (SD) size	 5 (26.3) 47.8 (8.4)	Right atrium Dilated*	 1/18 (5.5) (no data for size)
LVEDD, mm	42.6 (9.8) n=16	LVESD, mm	30.6 (5.2) n=13
LVIS, mm	13.9 (13.1) n=15	LVPW, mm	9.9 (1.5)
LV ejection fraction, %** Comment in remaining examinations	56.1 (8.8) n=14 2 (10.5) were >55% 1 (5.3) was >50% 5 (26.3) were good/preserved or normal	Tricuspid valve annular motion, mm	21.9 (3.4) n=8
Valvular stenosis/regurgitation (>grade 1)*	1 (5.3) Aortic regurgitation (grade 2)	IVC Dilated*	6 (50) n=12
RV function depressed*	2/17 (11.7) (1 mild, 1 moderate)	Right ventricle dilated*	0/16 (0) - redo
RV-RA gradient, mmHg	22.25 (5.6) n=12		

LVESD, LV end-systolic diameter; LVEDD, LV end-diastolic diameter; LVIS, LV intraventricular septum; LVPW, LV posterior wall;
Values expressed as mean (standard deviation) unless otherwise stated

*Values expressed as n (%), ** where value measured

Table 7-9 Baseline electrophysiological outcome measures in ELCASA participants

Investigation	**n (%) unless otherwise specified**		**n (%) unless otherwise specified**
12-lead ECG (n=19)			
Heart rate, bpm*	70 (12)	Rhythm	Sinus 19 (100)
Axis: Normal Left	17 (89.5) 2 (10.5)	AV-block present	0 (0)
PR interval, ms*	161 (22)	QRS duration, ms*	91 (19)
QTc, ms	427 (25)	P-pulmonale present	0 (0)
P-mitrale present	0 (0)	Signs of LV hypertrophy present	0 (0)
Strain present	0 (0)		
Right bundle branch block present	Incomplete 1 (5.3)	Left bundle branch block present	Complete 1 (5.3)
Left anterior fascicular block present	1 (5.3)	Left posterior fascicular block present	0 (0)
Signal Averaged ECG (n=13)		Total QRS duration (filtered) ≥114ms	4 (21.1)
HFLA signal duration less than 40uV for ≥39ms	5 (26.3)	RMS voltage in terminal 40ms <20uV	4 (21.1)
Presence of late ventricular polarisation***	4 (21.1)		
24-hour ECG monitor (n=16)			

Minimum heart rate, bpm*	61.4 (8.4)	Maximum heart rate, bpm*	131.9 (14.4)
Average heart rate, bpm*	80.3 (10.6)	Total number of heart beats	115097 (17944)
Presence of sinus rhythm	16 (100)		
Supraventricular ectopics**	7.0 (0.5, 43.5)	Ventricular ectopics**	10.5 (2.3, 23.0)
Presence of SVT ≥3 beats	2 (10.5)		
Presence of: SVT Couplet Triplet VT Drop beats Bradycardic episodes Pauses >3 seconds	2 (10.5) 3 (15.9) 1 (5.3) 0 (0) 3 (15.9) 0 (0) 1 (5.3)		
Autonomic testing (n=14)			
Respiratory RR interval variation* E/I ratio [mean RRmax (mean)/mean RRmin (mean)]	1.19 (0.15)	Abnormal	6 (42.9)
Valsalva Longest/shortest RR ratio** [shortest RR interval during Valsalva for 15 seconds and longest RR interval within 30s after Valsalva]	1.21 (1.07, 1.60)	Abnormal	7 (50.0)
Maximum/minimum 30:15 ratio* [longest RR interval between beat 20 and 40, divided by shortest RR interval between beat 5 and 25 after standing up]	1.26 (0.15)	Abnormal	3 (21.4)

Orthostatic dysregulation greater than 28 mmHg drop [difference in supine to lowest within 3 minutes in upright position]	0 (0)		
Abnormal autonomic test [2 or more of the 4 above tests abnormal]	6 (42.9)		

Bpm, beats per minute; ECG, electrocardiogram; LV, left ventricular; ms, milliseconds; RMS; root mean squared; SVT, supraventricular tachycardia; VT, ventricular tachycardia
*Values expressed as mean (standard deviation)
** Values expressed as median (interquartile range)
***defined as 2 or more of individual SAE measures abnormal

Table 7-10 Baseline cardiovascular magnetic resonance imaging in ELCASA participants

Variable	**ELCASA patients n=15**
Left ventricular measures	
LVEF, %	60.11 (4.48)
Mid S'	1.20 (0.16) n=13
LV EDV/BSA, ml/m^2	83.71 (17.91)
LV ESV/BSA, ml/m^2	33.869 (9.23)
LV stroke volume/BSA, ml/m^2	50.03 (9.59)
LVmass/BSA, g/m^2	44.79 (10.57)
LV mass/EDV, g/ml	0.54 (0.07)
Right ventricular measures	
RVEF, %	57.69 (17.14)
RV EDV/BSA, ml/m^2	91.74 (20.20)
RV ESV/BSA, ml/m^2	43.60 (14.17)
RV stroke volume/BSA, ml/m^2	50.82 (8.11)
Diastolic function	
Mid E'	0.66 (0.37) n=13
Mid A'	1.36 (0.29) n=13
Peak twist, degrees	13.48 (5.4) n=13
Torsion, degrees	12.98 (5.08) n=13
Extracellular measurements	
Native T1 (inferoseptal)	1197 (74)
ECV, %	30.1 (3.2) n=14
Presence of late gadolinium enhancement	5 (26.3)* n=14
Measures of aortic stiffness	
Distensibility, $10^{-3}mmHg^{-1}$	4.26 (2.72)

Mid A', active/late diastolic strain rate; BP, blood pressure; BSA, body surface area; CI, confidence interval; Mid E', early diastolic strain rate; ECV, extracellular-volume fraction; EDV, end-diastolic volume; ESV, end-systolic volume; LV, left ventricular; LVEF, left ventricular ejection fraction; Mid S', peak systolic strain rate; RV, right ventricular; SSc, systemic sclerosis;

Values expressed as mean (SD) unless stated otherwise *n (%)

7.3.3 Implantable loop recorder

Patients activated the ILR download mechanism infrequently, although one patient did so 61 times in the first 12 months (see Table 7-11). The most common reason for activation was palpitations, revealing mainly sinus rhythm with supraventricular (SVEs)/ventricular ectopics (VEs) on ECG interrogation. One patient did have three episodes of non-sustained ventricular tachycardia (VT) (longest lasting 15 beats, fastest reaching 220 beats per minute.

The ILR itself auto-activated more frequently (median (IQR) 3 (0, 22)), and commonly reported apparent asystole and AF amongst others such as VT or ventricular fibrillation (VF). However on ECG interrogation, the apparent asystole was due to loss of signal or occurring during the time of implant of the ILR. Other findings were usually artefact or sinus rhythm with occasional sinus tachycardia. Of the 19 patients, the ILR autoactivation detected one episode of atrial flutter in one patient, another atrial flutter going into AF, multiple non-sustained SVT in one patient, and one case of complete heart block (CHB) (who went on to receive a permanent pacemaker (PPM)).

Table 7-11 ILR outcomes over first 12 month period

Variable	Total number, median (IQR), range	Reasons for activation	ILR interrogation results	Medical outcome
Patient auto-activations	0 (0, 4), 0-61	Palpitations Chest pain Fast heart rate Pre-syncope, Generally unwell Short of breath	SR +/- SVEs/VEs 3 episodes of VT 1 couplet 1 salvo (7 beats)	1 patient commenced on B-Blocker for non-sustained VT
ILR auto-activations	3 (0, 22), 0-2608	Asystole AF Pause Bradycardia VT VF	Loss of signal Occurred during implant Artefact SR/sinus tachycardia SVEs and VEs Bigeminy and couplets 1 atrial flutter 1 atrial flutter going into AF SVT (multiple in 1 patient) 1 CHB	CHB patient admitted same day for PPM insertion SVT and AF/flutter patients no treatment as patients asymptomatic

AF, atrial fibrillation; CHB, complete heart block; ILR, implantable loop recorder; PPM, permanent pacemaker; SR, sinus rhythm; SVEs, supraventricular ectopics; SVT, supraventricular tachycardia; VF, ventricular fibrillation; VT, ventricular tachycardia

Altogether, 11 (58%) ELCASA participants had evidence of altered rhythm, ranging from SVEs/VEs to VT and CHB (see Table 7-12). Seven (37%) patients had SVEs, 2 (11%) with VEs, 5 (26%) with significant arrhythmias of which 1 atrial flutter, 1 atrial flutter leading into atrial fibrillation (AF), 1 SVT, 1 VT & 1 complete heart block (CHB).

Table 7-13 describes the characteristics of patients with SSc and significant arrhythmia. Of the five ELCASA participants with significant arrhythmias; three were female, four were Caucasian, mean (SD) age 49.8 (5.5) years, median (IQR) time from first non-RP symptom 9.0 (1.9, 12.8) years. Three had dcSSc, two had known ILD, and two had a history of DU. Three were taking MMF (remaining two on no DMARD), two previously received cyclophosphamide and two iloprost. None were receiving sildenafil or bosentan. Two participants were ACA positive, one Scl70 positive and two ANA negative, and four had a SSc-pattern of vasculopathy on NFC (3 early, 1 active). Three had a history of palpitations, which had never been previously investigated in the form of a 24-hour ECG monitor (although recall bias may play a role here). On baseline questioning, one patient had regular palpitations 1 to 3 times per month, another had regular palpitations 1 to 5 times per day and the last patient irregular palpitations 1 to 5 times per day. Three out of the five had globally abnormal SAE (test not done in the other two), one patient had left axis deviation with complete LBBB and another patient had left anterior fascicular block on ECG. Two out of the three patients who underwent autonomic testing had globally abnormal results, and a 24-hour ECG monitor detected a median (IQR) number of SVEs and VEs of 15 (1.8, 65.0) and 6 (1.3, 23.0) respectively. LVEF on baseline echocardiogram was described as ‘good’ for one, and 40%, >55%, 60% 70% for the remaining, with a mean (SD) RV-RA gradient of 21.5 (3.9)mmHg.

The participant with complete heart block was a 41-year old Caucasian 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-RP symptom of 3.9 years, with ILD, a history of DU and irregular palpitations. He was managed on 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/VEs & three couplets only. However, he had complete LBBB with left axis deviation on baseline ECG, a LVEF of 40% on baseline echocardiogram (RV-RA gradient 21mmHg), and had globally abnormal SAE and autonomic tests. The patient had noticed increasing fatigue in the weeks preceding the CHB detection but no other new symptoms had developed.

The participant with non-sustained VT was a 50-year old Caucasian male who was an ex-smoker (2 pack years history), with no history of hypertension or family history of CVD. He had Scl70 positive dcSSc, with a time since first non-RP symptom of 2.3 years, with ILD, no history of DU but regular palpitations occurring one to three times per month (never previously investigated). Again he was managed on MMF with previous cyclophosphamide use (12 pulses in total). The baseline ECG was normal except for T wave inversion on lead III, global autonomic testing was normal, and echocardiogram reported a LVEF of 60% (no TR was detected to measure RV-RA gradient). His baseline 24-hour ECG monitor nine months earlier had revealed sinus rhythm with a few VEs & one drop beat only. However he had globally abnormal SAE. This patient was symptomatic with palpitations at the time of his arrhythmia; the only patient of the five with significant arrhythmias to be symptomatic.

Table 7-12 Type of ILR abnormalities over first 12 month period

ILR abnormality	**Present in study participants, n (%)**
Any abnormality	11 (57.9)
Patients with significant arrhythmia potentially requiring medical intervention	5 (26.3)
Patients with serious arrhythmia requiring pharmacotherapy/device	2 (10.5)
Supraventricular ectopics	7 (36.8)
Ventricular ectopics	2 (10.5)
Bigeminy	3 (15.8)
Couplets	1 (5.3)
Triplets	0 (0.0)
Salvos	1 (5.3)
Atrial fibrillation	1 (5.3)
Atrial flutter	2 (10.5)
Supraventricular tachycardia	1 (5.3)
Ventricular tachycardia	1 (5.3)
Complete heart block	1 (5.3)

Table 7-13 Characteristics of SSc patients with significant arrhythmias

	Atrial flutter to AF	**Atrial flutter**	**Non-sustained SVT**	**Non-sustained VT**	**Complete heart block**
Age, Sex	27, female	55, female	54, female	50, male	41, male
CV risk factors	Family history	Ex-smoker	Family history	Ex-smoker	None
DcSSc	x	x	✓	✓	✓
Time from 1st non-RP, years	2.4	21.8	1.4	2.3	3.9
ILD	x	x	x	✓	✓
Previous DU	x	✓	x	x	✓
Palpitations	x	x	✓	✓	✓
DMARD	x	x	MMF	MMF, previous CYC	MMF, previous CYC
ANA	ACA	ACA	Negative	Scl70	Negative
NFC pattern	Active	Early	Early	Non-specific	Early
ECG	Left anterior fascicular block	Normal	Normal	Normal	Complete LBBB with LAD
24 ECG	*Not done*	6 SVE, 12 VEs	2 SVEs, 11 VEs, 1 couplet, 2 triplets	14 VEs, 1 dropped beat	6 SVE, 29 VEs, 3 couplets
Abnormal autonomic test	*Not done*	*Not done*	✓	x	✓

SAE abnormal	*Not done*	*Not done*	✓	✓	✓
Echo: LVEF	Good	>55%	70%	60%	40%
RV-RA gradient	18mmHg	20mmHg	27mmHg	Not measured	21mmHg

ACA, anti-centromere antibody; ANA, anti-nuclear antibody; 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; RA, right atrial; RP, Raynaud's Phenomenon; RV, right ventricular; SAE, signal average ECG; Scl70, anti-topoisomerase antibody; SVEs, supraventricular ectopics; SVT, supraventricular tachycardia; VE, ventricular ectopics; VT, ventricular tachycardia

7.3.4 Association of ILR abnormalities with CMR

Differences in CMR outcome measures between study participants with any abnormality detected on ILR compared to those without are described in Table 7-14. Only three of the five participants found to have serious arrhythmias underwent a CMR; IV access was not possible for the patient with SVT, and the CMR was abandoned within minutes of starting for patient with CHB after CMR staff received a call that ILR interrogation had detected CHB).

There was a trend for reduced LV and RV EDV/BSA, ESV/BSA and stroke volume/BSA in those with ILR abnormalities ($p>0.05$ for all), and also reduced LV mass/BSA (mean difference (95% CI) after adjustment for age and sex was -1.84 (-14.67, 11.00)g/m^2, p=0.759). There was also a trend for increased ECV in those with abnormalities; (mean difference (95% CI) after adjustment for age and sex was 3.1 (-1.4, 7.6)%, p=0.158). ECV tended to be higher in those with SVEs (unadjusted mean difference (95% CI) 0.9 (-3.2, 4.9)% p=0.648) and VEs *(*unadjusted mean difference (95% CI) 1.5 (-6.1, 9.0)% p=0.679). Two (22%) of those with ILR abnormalities had evidence of LGE compared to three (50%) of those without. Aortic distensibility appeared to be greater (i.e. less stiff arteries) in those with abnormalities on ILR (mean difference (95% CI) after adjustment for age and sex 2.93 (-0.38, 6.24)$10^{-3}mmHg^{-1}$, p=0.077).

When comparing those with significant arrhythmias (n=5) to those without (see Table 7-15), there was little difference in the key CMR outcomes with exception to ECV and aortic distensibility, which appeared greater in those with significant arrhythmias. The mean difference (95% CI) after adjustment for age and sex for ECV was 2.8 (-2.0, 7.6)% p=0.220 and aortic distensibility (1.96 (-1.84, 5.75)$10^{-3}mmHg^{-1}$ p0.277. No participant with a significant arrhythmia had evidence of LGE on CMR.

Table 7-14 CMR measures in SSc patients with/without ILR abnormalities.

CMR Variable	ILR normal N (%)=6 (40)	ILR abnormal N (%)=9 (60)	Unadjusted analysis		Adjusted for age and sex analysis	
			Mean difference (95% CI)	P value	Mean difference (95% CI)	P value
Left ventricular measures						
LVEF, %	58.60 (3.84)	61.11 (4.81)	2.51 (-2.58, 0.59)	0.306	-0.21 (-5.85, 5.43)	0.937
Mid S'	1.21 (0.18) (n=5)	1.20 (0.15) (n=8)	-0.01 (-0.22, 0.19)	0.879	-0.02 (-0.61, 0.26)	0.858
LV EDV/BSA, ml/m^2	86.16 (24.36)	82.08 (13.54)	-4.08 (-29.84, 21.68)	0.720	-4.85 (-27.04, 17.34)	0.640
LV ESV/BSA, ml/m^2	35.95 (11.75)	32.17 (7.52)	-3.78 (-16.33, 8.77)	0.505	-2.30 (-13.63, 9.03)	0.663
LV stroke volume/BSA, ml/m^2	52.69 (9.98)	49.58 (6.97)	-3.12 (-12.52, 6.29)	0.487	-2.55 (-144.56, 9.46)	0.650
LVmass/BSA, g/m^2	46.26 (15.09)	43.81 (7.09)	-2.45 (-18.30, 13.41)	0.723	-1.84 (-14.67, 11.00)	0.759
LV mass/EDV, g/ml	0.54 (0.08)	0.54 (0.08)	0.003 (-0.086, 0.091)	0.948	0.02 (-0.10, 0.13)	0.754
Right ventricular measures						
RVEF, %	60.19 (27.59)	56.02 (5.51)	-4.17 (-33.07, 24.72)	0.729	0.40 (-17.49, 18.29)	0.962
RV EDV/BSA, ml/m^2	96.17 (29.81)	88.80 (11.60)	-7.37 (-38.58, 23.85)	0.585	-6.59 (-30.09, 16.91)	0.550
RV ESV/BSA, ml/m^2	50.16 (9.98)	39.22 (8.00)	-10.94 (-31.20, 9.32)	0.236	-8.84 (-22.04, 4.37)	0.169
RV stroke volume/BSA, ml/m^2	52.69 (9.98)	49.58 (6.97)	-3.12 (-12.52, 6.29)	0.487	-0.45 (-11.99, 11.08)	0.933
Diastolic function						

Mid E’	0.41 (0.34) (n=5)	0.82 (0.39) (n=8)	**0.41 (0.01, 0.81)**	**0.047**	0.41 (-0.15, 0.98)	0.129
Mid A’	1.43 (0.34) (n=5)	1.31 (0.28) (n=8)	-0.11 (-0.49, 0.26)	0.515	-0.11 (-0.63, 0.41)	0.633
Peak twist, degrees	12.18 (4.46) (n=5)	14.30 (6.01) (n=8)	2.12 (-4.78, 9.01)	0.514	0.55 (-8.83, 9.93)	0.897
Torsion, degrees	12.07 (4.55) (n=5)	13.55 (5.62) (n=8)	1.49 (-5.10, 8.08)	0.630	-0.86 (-9.48, 7.73)	0.823
Extracellular measurements						
Native T1 (inferoseptal)	1198.2 (54.2)	1196.4 (87.5)	-1.7 (-88.7, 85.3)	0.967	-45.19 (-1476.68, 56.31)	0.348
ECV, %	28.42 (4.07)	31.44 (1.75) (n=8)	3.02 (-0.45, 6.49)	0.082	3.10 (-1.42, 7.63)	0.158
Presence of LGE	3 (50.0)*	2 (22.2)* (n=8)	0.50 (0.12, 2.12)**	0.341	0.46 (0.02, 12.67)***	0.644
Measures of aortic stiffness						
Distensibility, $10^{-3}mmHg^{-1}$	3.66 (1.66)	4.48 (3.46) (n=8)	0.82 (-2.54, 4.18)	0.603	2.93 (-0.38, 6.24)	0.077

Mid A’, active/late diastolic strain rate; BSA, body surface area; CI, confidence interval; Mid E’, early diastolic strain rate; ECV, extracellular-volume fraction; EDV, end-diastolic volume; ESV, end-systolic volume; LGE, late gadolinium enhancement; LV, left ventricular; LVEF, left ventricular ejection fraction; RV, right ventricular; SSc, systemic sclerosis; Mid S’, peak systolic strain rate;

Values=mean(SD)

*n (%), **relative risk (95% CI) with fishers exact p value , ***logistic regression analysis therefore mean difference represent odds ratio

Table 7-15 Difference in CMR measures in ELCASA participants with significant arrhythmias compared to without significant arrhythmias

CMR Variable	No arrhythmia N 12 (%)= (80)	Arrhythmia N (%)=3 (20)	Unadjusted analysis		Adjusted for age and sex analysis	
			Mean difference (95% CI)	P value	Mean difference (95% CI)	P value
LVEF, %	60.11 (4.97)	60.12 (2.16)	0.01 (-6.48, 6.50)	0.997	0.30 (-5.52, 6.12)	0.912
LVmass/BSA, g/m^2	44.90 (11.66)	44.33 (5.80)	-0.57 (-15.86, 14.71)	0.937	-0.12 (-13.43, 13.18)	0.984
LV mass/EDV, g/ml	0.54 (0.08)	0.52 (0.05)	-0.03 (-0.13, 0.08)	0.592	-0.03 (-0.15, 0.09)	0.612
RVEF, %	57.30 (19.18)	59.23 (5.35)	1.94 (-22.84, 26.71)	0.869	0.18 (-18.28, 18.65)	0.983
Torsion, degrees	12.57 (5.78) (n=10)	14.36 (0.98)	1.79 (-2.45, 6.03)	0.615	1.64 (-6.31, 9.58)	0.652
ECV, %	29.55 (3.31) (n=11)	32.32 (2.0)	2.77 (-1.67, 7.20)	0.199	2.82 (-1.99, 7.63)	0.220
Presence of LGE	5 (41.7)*	0 (0.0)*	-	0.258**	-	-
Distensibility, $10^{-3}mmHg^{-1}$	3.69 (2.25) (n=11)	5.73 (4.46)	2.04 (-8.17, 12.24)	0.277	1.96 (-1.84, 5.75)	0.277

BSA, body surface area; CI, confidence interval; ECV, extracellular-volume fraction; EDV, end-diastolic volume; LGE, late gadolinium enhancement; LV, left ventricular; LVEF, left ventricular ejection fraction; RV, right ventricular

Values expressed as mean (SD) unless stated otherwise

* N(%), **Fisher's exact test

7.3.5 Medical outcomes for participants

Two of the 19 ELCASA participants with ILR data retrieved had a change in their medical treatment as a direct result of the study. The patient with CHB was admitted the same day the arrhythmia was detected, and a permanent pacemaker was inserted. He was subsequently withdrawn from the study as he had met a clinical endpoint (and the ILR had been removed). As a result of active ILD and presumed myocardial involvement, he went on to receive 6 pulses of cyclophosphamide (22.5mg/kg), followed by Rituximab, but unfortunately died of his disease 10 months after his baseline visit.

The patient with non-sustained VT was commenced on a beta-blocker. After careful consideration from the cardiologists, the three patients with atrial flutter, atrial flutter going into AF, and non-sustained SVT, received no change in their treatment given that they were asymptomatic at that time and had a low risk of stroke (relevant for AF patient).

7.4 Discussion

This pilot study describes the first use of the ILR as a research tool in the investigation of SSc-CM and associated arrhythmias. The study is still in progress and the final analysis is awaited, however this interim analysis at year one has demonstrated the potential feasibility of ILR in the incidental detection of conduction abnormalities, including significant cardiac arrhythmias, with a suggestion that ILR abnormalities and/or arrhythmias are associated with abnormalities in CMR outcomes.

Five of the 19 patients were found to have significant arrhythmias with four of them picked up incidentally as the patients were asymptomatic. The arrhythmias were detected over the course of the year, not within the first day, week or month, highlighting the limited value of routine 24 hour ECG monitoring or 30 day event recorders to detect clinically relevant abnormalities in an at risk asymptomatic patient.

Excluding those with significant arrhythmias, six patients demonstrated other ILR abnormalities, mainly SVEs and VEs. SVEs and VEs are usually considered benign, and are routinely detected in populations free of underlying heart disease [536, 537]; Kostis et al demonstrated 39% had at least one VE on 24 hour ECG monitoring [536]. Nevertheless, VEs have been associated with increasing LV mass in the hypertensive population [538], and in the context of ischaemic heart disease (IHD) frequent VEs have prognostic value for increased mortality, associating with more serious arrhythmias, such as ventricular

fibrillation (VF) [539]. Very frequent VEs, i.e. one or more VE on a standard ECG tracing or 30 or more per hour on a longer recording have been associated with a higher risk of death in the general population; a recent meta-analysis reported a hazard ratio for total cardiac death of 2.07 (95% CI 1.71, 2.50) in the general population [540]. The prognostic implications remain uncertain in SSc; a cohort at heightened risk for cardiac disease. One prospective cohort study by De Luca et al performed 24 hour ECG monitoring on 100 patients with SSc, with a mean follow-up of 23 (SD16) months, and found that a higher number of VEs were associated with both sudden cardiac death and requirement for ICDs; increased troponin-T and right bundle branch block on ECG were independent predictors of more than 1190 VEs in a 24 hour period [541].

This study demonstrated the more serious arrhythmias of VT and CHB occurred in the patients with poor prognostic markers for SSc (male, dcSSc, with ILD), mirroring that already suggested in the literature [332, 365]. The remaining three arrhythmias were supraventricular in origin, all atrial, occurring in females; two with lcSSc, none with ILD. None of these had known usual causes such as IHD, hypertension or mitral valve disease. Whilst two of these patients were over 50, perhaps suggesting the possibility of age-related changes, one was in their mid-twenties; implying in this patient particularly, SSc-CM could be the underlying cause. Further study is required to confirm whether there is an association of lcSSc with atrial arrhythmias, and then into determining the pathophysiological basis for this, keeping in mind CMR studies report fibrosis more commonly affecting the ventricles and not atria [377], possibly as focal fibrosis in atria is very difficult to detect on CMR.

The study also suggested an association of ILR abnormalities and arrhythmias with abnormalities on CMR, in particular with ECV as a marker of diffuse fibrosis. ECV is a relatively novel measure, increasingly being applied to those with SSc; greater ECV has been reported in SSc compared to healthy controls [466, 467]. Nutsi et al determined an unadjusted ECV of 35% in patients with SSc compared to 28% in controls, $p<0.001$ [466]. Both autopsy and LGE CMR studies suggest myocardial fibrosis to be the hallmark of SSc-CM, either occurring independently, or as a consequence of microvascular-perfusion abnormalities [315, 375, 376]. ECV may provide a more sensitive and quantitative measure of diffuse and focal fibrosis, which could be used to determine those at higher risk of arrhythmias in SSc; although further work is required to determine if any differences exists between those at risk of atrial or ventricular arrhythmias. The absence of LGE in

those with significant arrhythmias is consistent with early data suggesting the conduction system is spared in focal fibrosis [535].

Although the final analysis at year three is required, this preliminary data would support more aggressive cardiac monitoring in those with poor prognostic markers for SSc. This study has numerous implications for the future. With further study/work, the 'at risk' phenotype can be further refined. It may be possible to screen patients with SSc for SSc-CM, using a composite of tools (EP testing/echocardiography/CMR) to determine those most at risk for the development of arrhythmias requiring more intensive cardiac monitoring with or without the insertion of an ILR, and provide reassurance to those found to be at lower risk. Detecting arrhythmias earlier may mean interventions, such as ICDs or permanent pacemakers, may reduce risk of sudden death in SSc. In addition, detection of SSc-CM at an earlier stage could also help halt/slow down disease progression with the introduction of intensive pharmacotherapy/immunosuppression. Finally, the study provides insight into the pathogenesis of SSc-cardiomyopathy, associating myocardial fibrosis with arrhythmias. Longitudinal studies will have to refine this association further, providing data for the prognostic implications of CMR abnormalities in SSc.

7.4.1 Limitations of study

The major limitation is the size of the study. A 20 patient study is small, however, this was a pilot study. Its first aim was to demonstrate the feasibility of the ILR as a research tool in an asymptomatic SSc population. The next step is to undertake a much larger study to validate the initial findings of prevalent arrhythmias and association with CMR abnormalities, and determine the patient in whom an ILR would be indicated in clinical practice.

The complexity of the study and practicalities of cross-department (rheumatology/EP/CMR/echocardiography departments) procedures led to some cardiac testing not being performed and hence the presence of missing data. Perhaps providing the study participant with a logbook to present to the various departments explicitly detailing what is expected at each visit would help minimise any study errors. This study can also help rationalise testing in future studies.

7.4.2 Summary

In summary, this pilot study has demonstrated the feasibility of using an ILR in the detection of arrhythmias in SSc. It has demonstrated the presence of significant and

serious arrhythmias in a population free of clinical SSc-CM and therefore the importance of active screening for SSc-CM. The association of ILR abnormalities with CMR has suggested that a model/algorithm for screening for SSc-CM could be designed in the future to determine those at greatest risk of arrhythmias, and consequently, who would benefit from more intensive monitoring and the insertion of an ILR as part of their routine SSc management.

7.4.3 Key messages

1. Use of the ILR in SSc appears feasible in the incidental detection of cardiac conduction abnormalities, including serious cardiac arrhythmias
2. Cardiac conduction abnormalities are associated with CMR abnormalities
3. This study supports the need for identification of patients at risk that would benefit from ILR

Chapter 8 Discussion

8.1 Overview

Cardiovascular disease (CVD) is the second largest cause of mortality in the UK, and an accelerated risk of CVD is observed in those with immune-mediated inflammatory diseases (IMID). In RA, the risk is similar to the excess risk of those with Diabetes Mellitus (DM) [4], with an associated increased CVD-mortality [5]. Although, RA disease activity and systemic inflammation are important contributors to this increased risk [149], traditional cardiovascular (CV) risk factors are also key [6].

Research to determine the specific phenotype of the patient with RA most at risk of CVD is on-going, but it is hampered by the low absolute numbers reaching primary clinical outcomes, e.g. myocardial infarction. Surrogate markers of CVD are being looked to as alternative indicators of CVD, as are also studied in the general population. Soluble biomarkers have shown potential in the general population with evidence to support their use in RA. Cardiovascular magnetic resonance (CMR) is another such tool, giving global information on cardiac structure, function, tissue characterisation (inflammation, fibrosis) and ischaemia. CMR studies are slowly emerging in IMID, and have highlighted the need for collaborative work between cardiologists or radiologists and rheumatologists in order to achieve appropriate study design and data interpretation in the context of IMID. Few research groups have combined the use of soluble biomarkers and CMR, with detailed RA phenotyping, to determine a model most associated with CVD.

In addition to atherosclerosis, IMID can directly affect the heart, as observed in Systemic Sclerosis (SSc). Up to 14% of deaths are secondary to SSc-CM, with another 12% secondary to CVD, with 6% of all patients with SSc dying from arrhythmias [341]. Despite these significant statistics, there is relatively limited knowledge on the phenotype of patients with SSc most at risk of SSc-CM, along with how best to screen for such disease, how frequent and then how best to manage any disease seen. There is also a lack of understanding of the underlying pathological disease process leading to the development of SSc-CM and its complications. Although it is postulated SSc-CM is the clinical consequence of microvascular-perfusion defects preceding myocardial fibrosis [376, 542], there are no longitudinal studies to confirm this.

The body of this thesis aimed to describe the prevalence of abnormalities in surrogate measures of CVD, using soluble measures of CV risk and CMR, in early and established RA, with no prior history of CVD, compared to healthy controls, and describe the association of abnormalities with disease phenotype. It aimed to determine whether effective suppression of disease activity in RA improved metabolic biomarkers of CV risk, and whether specific therapeutic agents had superior influence over others on any improvement seen. The thesis also aimed to determine the presence of subclinical SSc-CM using CMR, and determine any association with disease phenotype. Along with the novel use of an implantable loop recorder (ILR), the thesis aimed to demonstrate the ability of novel technology to inform pathophysiology and risk stratify patients for arrhythmias and SSc-CM in SSc.

8.1.1 Thesis synopsis

Chapter Three: Primary myocardial disease in scleroderma – a comprehensive review of the literature.

This comprehensive literature review demonstrated a high prevalence of SSc-CM, presenting in various forms, such as heart failure, arrhythmias, diastolic dysfunction, pericarditis and pericardial effusions, and associated with a significantly increased risk of mortality. The review highlighted the difficulty differentiating primary myocardial disease from atherosclerotic heart disease and the relative absence of investigation that can conclusively distinguish the two. The review also determined potential 'red flags' associated with SSc-CM, which mirrored the more generally accepted poor prognostic markers for SSc; such as a dcSSc subtype, and positive SSc-specific serology. In addition, the review demonstrated the advantages of employing non-invasive investigative techniques to detect SSc-CM, such as electrophysiological (EP) testing and CMR, in addition to allowing a better understanding of its pathophysiological basis.

Chapter Four: Improvement in insulin resistance is greater when infliximab is added to methotrexate during intensive treatment of early RA - results from the IDEA study.

This randomised controlled trial demonstrated an improvement in atherogenic index (total cholesterol/high-density lipoprotein cholesterol, TC/HDL-C), insulin resistance (IR) as estimated by the homeostasis model assessment-estimated IR (HOMA-IR) and N-terminal pro-brain natriuretic peptide (NT-proBNP) in the treatment of patients with DMARD-naïve early RA, following a treat-to-target (T2T) strategy. An analysis between the two arms of

treatment; methotrexate (MTX) with methylprednisolone (MEP) at induction, or MTX with infliximab (IFX, a tumour necrosis factor inhibitor (TNFi), demonstrated that although the improvement in TC/HDL-C and NT-proBNP did not differ between the two treatment groups, a greater improvement in IR was seen in those patients in the TNFi arm of the study.

Chapter Five: Cardiovascular biomarkers and the use of cardiac and carotid MRI in established RA.

This cross-sectional exploratory study performed a comprehensive CV evaluation of patients with established RA with comparison to healthy controls; combining a clinical assessment with measurement of lipid profile, determination of arterial stiffness using pulse wave velocity by applanation tonometry and a thorough assessment of the heart and vasculature with CMR imaging. The study primarily demonstrated the importance of traditional CV risk factors, and not RA disease specific features, in the determination of markers of subclinical CVD in RA. It also demonstrated the value of CMR in the investigation of CVD in patients with RA, providing insight into the pathogenesis of cardiac disease in RA; in particular demonstrating reduced LV mass, and demonstrated the novel utility of CMR carotid scanning in patients with RA.

Chapter Six: The use of CMR in SSc.

This cross-sectional exploratory study performed a comprehensive CV assessment of patients with SSc, with comparison to healthy controls, focussing on contrast enhanced CMR. The study determined patients with SSc have greater evidence of subclinical cardiac involvement, particularly in measures of myocardial fibrosis, with these outcome measures associating with poor prognostic markers of SSc.

*Chapter Seven: Prospective study evaluating conduction abnormalities with correlation to c*ardiovascular *magnetic resonance imaging in patients with scleroderma - the ELCASA study.*

This study described the first use of the implantable loop recorder (ILR) (REVEAL® device) as a research tool in the investigation of SSc-CM and associated arrhythmias. The interim analysis at year one described in this thesis has demonstrated the potential feasibility of the ILR in the incidental detection of conduction abnormalities, including significant cardiac arrhythmias, in a population of patients with SSc free of clinical SSc-CM, with a suggestion

that ILR abnormalities and/or arrhythmias are associated with abnormalities in CMR outcomes.

8.2 Discussion

The following section discusses the work in this thesis in the context of recent advances in the field of CVD in IMID. In particular, it will focus on its contribution to the understanding of the pathogenesis of CVD in IMID and how novel technology can help inform this, along with the various approaches to its prevention.

8.2.1 Rheumatoid Arthritis

As outlined below, novel technology can improve the understanding of CVD in RA; informing pathophysiology but also in identifying those at great risk. This section also discusses the various approaches in reducing the risk of CVD in patients with RA; through RA disease suppression and the management of traditional CV risk factors, and describes the recent advances in knowledge in this area.

8.2.1.1 Novel technology in the evaluation of CVD in RA

Choosing the best technology and outcome measure to determine CV risk in this population remains to be determined. The outcome measure should ideally be a true surrogate endpoint [471], predicting future CVD, however, only few longitudinal trials required for this are available in RA and often the measures are extracted from that seen in the general population. Having said this, a recent study by Ikdahl et al of 138 patients with RA of less than four years duration reported increasing CIMT and presence of carotid plaque (both US-measured), and increasing arterial stiffness (aortic augmentation index and PWV), were associated with CV events over a mean follow-up period of 5.4 years [543].

This thesis used soluble CV biomarkers and CMR to describe subclinical CV abnormalities in patients with RA. The main advantage of CMR is that it provides a non-invasive comprehensive assessment of the heart; including dimensions, function, tissue characterisation and arterial stiffness. However, one concern is the need for a unified CMR protocol across IMID to allow cross study comparisons and appropriate translation of results/findings into clinical practice. There has recently been some work in this area with recommendations for CMR use and protocol produced by an international consensus group [455]. Although not reported in the published literature previously, this thesis employed CMR in the evaluation of the carotid arteries, and even in the general population the

protocol and best outcome measure (for example, maximal wall thickness or carotid wall volume) for carotid MRI imaging remains to be advised.

Given the well documented atherosclerotic process, biomarkers, or surrogate endpoints, as also described in chapter five of this thesis, can include soluble biomarkers representing endothelial dysfunction or plaque instability [12, 75], or functional outcome measures such as flow mediated dilatation [78]. Newer potential surrogate measures in RA include fetuin-A (glycoprotein which induces insulin resistance) [544] or even PET assessment for arterial wall inflammation [545].

8.2.1.1.1 Insights into pathogenesis of CVD in RA

The use of CMR, particularly within the work of this thesis, has suggested more than one potential pathological process affecting the heart in RA. CV abnormalities were detected despite the patients with RA evaluated in this thesis having no history of CVD. In addition, although the CV abnormalities did not strongly associate with a specific RA disease phenotype, they did favour an association with traditional CV risk factors, suggesting an accelerated process of atherosclerosis as a key driving factor.

The finding of a reduced LV mass in the established RA cohort (chapter five) goes against the expectation for a greater LV mass given the heightened CV risk in RA and as seen in previous echocardiography studies [504]. The findings are however consistent with the findings by the only other comparably sized CMR study at the time by Giles et al [135] and a more recent report (n=60) which found a non-statistically significant reduction in LV mass in those with RA [546]. As discussed in chapter five, it is possible that this reduction in LV mass represents alternative pathology to atherosclerotic heart disease; in particular, the result of microvascular changes driven by endothelial dysfunction [505], a distinct cardiometabolic remodelling effect of RA [521], subclinical myocarditis or deconditioning due to reduced mobility.

Learning more about this second cardiac insult is essential. For example, a non-atherosclerotic process may explain the increased risk of heart failure in those with RA unexplained by traditional CV risk factors [547]. Distinguishing between the pathologies may also help direct therapy in the management of CVD in RA. CMR is an ideal tool to help do so. In 2015, Ntusi et al suggested microvascular dysfunction in RA reporting non-segmental perfusion defects (i.e. not the result of atherosclerotic heart disease) in 47% of 55 patients with RA with no defects seen in 55 matched controls. Furthermore, the

myocardial perfusion reserve correlated with disease activity [548]. Further work combining CMR with soluble biomarkers such as leptin may also help tease out the effect (if any) of RA-cachexia on the heart.

8.2.1.2 The effect of disease suppression in RA

It is a generally accepted view that reduction of RA disease activity with conventional synthetic DMARDs (csDMARDs) or biological DMARDs can reduce CV events and deaths [240, 549], although there is limited data in early RA. Interestingly, a recent publication by Kerola et al reported that no increase in CV mortality was seen when evaluating over 14,000 patients with early RA receiving consistent treatment with one to seven years follow-up [68], suggesting the modern approach of prompt recognition and management of early RA is improving CV outcomes in RA.

In addition, a better response to RA treatment is associated with a reduction in CV events [550] and surrogate markers such as CIMT [551]. The reduction of systemic inflammation dampening the accelerated atherosclerotic process observed in IMID is thought to be the underlying reason for the reduced CV risk, and as a result, trials are now in progress to assess the effect of reducing inflammation in non-RA populations; for example, using MTX in those with type 2 DM [552].

Using alternatives to clinical outcomes, the IDEA study (chapter four) demonstrated that suppression of early RA disease activity was associated with a reduction in soluble biomarkers of CV risk, including TC/HDL-C, NT-proBNP and HOMA-IR, with those meeting the ACR70 response criteria associated with lower values of TC/HDL-C. Although, the effect of disease suppression on change in TC/HDL-C in early RA has been studied previously [260], NT-proBNP and HOMA-IR have been less well evaluated [553]. A recent systematic literature review assessing the change in HOMA-IR with the use of TNFi in RA (established or early) identified eight studies involving 260 subjects with a follow-up duration of a maximum of 12 months; although the study offered minimal data on the RA phenotype, the study concluded HOMA-IR decreased with TNFi [554]. Therefore, the IDEA study provides valuable longitudinal data in early RA for both changes in HOMA-IR and NT-proBNP.

8.2.1.2.1 Treatment strategy

There are suggestions that certain therapies may have an additional benefit in reducing CV risk compared to other RA therapies. For example, Sharma et al reported a hazard ratio of

0.28 (95% CI 0.12, 0.63) for incident CVD for hydroxychloroquine (HCQ) users compared to non-users in a cohort of 1266 patients with RA [555]. The authors postulated this was due to the beneficial effects of HCQ on lipid profiles, risk of DM and its anti-platelet effects. However, this was a retrospective study, and although the authors adjusted the results for CV risk factors, seropositivity, MTX and TNFi use, a potential difference in disease activity over the years between the groups remains a huge confounder.

There are relatively more reports of TNFi reducing CV events compared to other DMARDs [252]; a recent meta-analysis determined that both TNFi and MTX use were associated with comparable reductions in risk of CV events; however, TNFi use was also associated with a reduced risk of stroke [240]. The IDEA study (chapter four) found no difference in change in TC/HDL-C (in line with previous studies [264]) or NT-proBNP between the treatment groups, but did appear to show additional benefit of MTX and TNFi over MTX and MEP in measures of IR, despite similar disease activity in both treatment arms. This is the first RCT to compare the change in HOMA-IR between treatment regimes in early RA, with previous open label/cross-sectional studies compromised by varying disease activity between the treatment arms [286, 288]. Specifically designed longitudinal studies to address this are required, and to also confirm translation into a reduction in risk of DM/CV events. Interestingly, Lillegraven et al recently reported from a longitudinal study a reduced incidence of DM in those treated with TNFi compared to other biological DMARDs or non-biological DMARDs [281].

In this thesis, it was suggested the effect of TNFi on the low grade inflammatory state of adipose tissue could improve IR, given the key role adipose tissue plays in the development of IR [482, 483]. Another possible mechanism is derived from recent data suggesting TNFi plays a role in platelet deactivation; Manfredi et al demonstrated that the effects of TNFα-induced platelet activation, which lead to thrombin activation and clot formation, were restricted by the use of TNFi [556].

In the era of personalised medicine, there is a need to adequately determine any individual advantage or disadvantage for certain RA treatment strategies. For example, although tocilizumab is recognised to cause increases in lipids, including TC/HDL-C and triglycerides [277, 278], which may deter its use in those with severe dyslipidaemia, it is suggested these changes do not translate into an increased risk of CV events in RA [279]. Therefore, in addition to addressing CV risk factors and suppressing disease activity, and despite improving CV outcomes in RA, there remains a need for continued evaluation into the

management of CVD in RA. Key issues include the optimal timing of use of RA therapies, for example, determining any advantage for biological DMARDs as first line therapy that would improve outcomes for those with RA at greater risk of CVD, and also the evaluation of any unique RA treatment-specific effects.

8.2.1.3 Assessment and management of CV risk factors in RA

The IACON study (chapter five) indicated that it was the traditional CV risk factors that appeared to be most relevant in the determination of arterial stiffness and CMR outcome measures of subclinical CVD in a population of established RA. This would suggest that although reduction of disease activity is important in the reduction of CV risk in RA, reflected in the reduction of CV events with disease modifying therapy, aggressive management of modifiable traditional CV risk factors is paramount. In addition, testing multiple soluble CV biomarkers did not lead to significant advances in determining CV risk, an important observation given the lack of access to these tests in routine clinical practice.

The recent European Guidelines on CVD prevention in clinical practice provide clear recommendations on the management of CV risk factors applicable to those with RA as well as the general population [69], and as discussed earlier, there is data to support lower CV event rates with CV risk factor management in RA [69, 247, 248]. However, the management of CV risk in RA is suboptimal with studies determining hypertension inadequately treated and lipid levels unchecked [243-246, 557]. This should therefore become a priority for the reduction of CVD in RA; concentrating on aggressively managing CV risk factors, regularly calculating CV risk scores. Attention has started to turn to the delivery of effective CV risk reduction programs. Akenroye et al demonstrated that simple electronic reminders for the physician were not adequate in improving screening for dyslipidaemia and improving blood pressure [558].

Behavioural patterns have been recognised to impede the ability of the individual to adopt a healthier lifestyle and recommendations advise these are addressed in the prevention of CVD [69]. Svensson et al recently published a RCT study design for a multifactorial intervention to prevent CVD in early RA. Patients with LDL>2.5mmol/L will be randomised to receive standard care through their general practitioner or be entered into an intensive programme, with the introduction of statin and anti-hypertensive therapy if appropriate. In the intensive programme, a study nurse will counsel patients every six months in areas concerning behavioural change, for example, advise on a healthy diet, smoking cessation, appropriate alcohol intake and exercise habits (advising vigorous exercise on most days of

the week). The primary outcome measure will be a composite measure of death from CVD, non-fatal MI/stroke and cardiac revascularisation at 5 years [559].

However, there is emerging evidence for the presence of psychological barriers to any treatment adherence and positive behavioural change, such as belief as to the cause of the illness or lack of support [560, 561]; factors not addressed with simple advice/prescription. Various techniques have been developed to overcome these barriers; motivational interviewing being one example. Motivational interviewing involves a constructive conversation with patients about a particular behavioural change and has been shown to be effective in increasing physical activity, fruit and vegetable consumption, medication adherence, improving dyslipidaemia and decreasing systolic blood pressure in the general population [562]. To date the application of motivational interviewing to those with RA is limited [562] and needs to be explored.

8.2.2 Systemic Sclerosis

The following section discusses the important issues in distinguishing between atherosclerotic heart disease in SSc and SSc-CM, the use of novel technology in the evaluation of SSc-CM, and approaches to the detection of SSc-CM in clinical practice.

8.2.2.1 SSc-CM versus atherosclerotic heart disease in SSc

Although the focus of this thesis in SSc was on SSc-CM, there is debate as to the possible increased risk of atherosclerotic CVD in this population. The EULAR Scleroderma Trials and Research (EUSTAR) database reported 12% of all deaths in SSc were secondary to cardiovascular disease [341]. A UK primary care database study found patients with SSc were at greater risk of developing MI, stroke and peripheral vascular (PVD) [320], whilst a large Australian cohort reported a greater risk for ischaemic heart disease (IHD) in SSc patients when adjusted for traditional CV risk factors [319]. A matched incident cohort study of 1239 patients with SSc by Aviña-Zubieta et al this year (2016) determined adjusted HRs of 3.49 (95% confidence interval (CI] 2.52, 4.83) and 2.35 (95% CI 1.59, 3.48) for MI and stroke, respectively; with the risk highest within the first year following diagnosis (HR 8.95 and 5.25 respectively) [563]. In addition, two meta-analyses have demonstrated greater CIMT in patients with SSc [103, 318]. This increased risk is relatively understudied but endothelial dysfunction with microvascular fibro-proliferation, both hallmarks of the disease pathogenesis, may be accountable [321, 322].

Teasing out SSc-CM from that of atherosclerotic disease will always be a challenge, as seen in the comprehensive literature review (chapter three), but is important as such distinction may determine very different treatment strategies. It also is required in order to more accurately understand the natural history of SSc-CM. Ideally, studies assessing SSc-CM should assess those with no or very minimal traditional CV risk factors, but to date most studies still fail to acknowledge and prioritise this need. A recent EP study by Muresan et al reported that 61% of 100 patients with SSc had arrhythmias or conduction disturbance on ECG or 24 hour ECG monitoring [564]. However, there was a strong presence of CV risk factors (38% had dyslipidaemia, 27% arterial hypertension, 4% DM), 8% had known IHD, 1% with previous stroke and 41% had pulmonary arterial hypertension (PAH), therefore the EP abnormalities may have been the result of CVD or SSc related PAH rather than true SSc-CM. Although the EP abnormalities do still need to be addressed, their management may differ depending on the underlying cause. Distinguishing between the two pathologies should become a priority and be addressed early in study design.

8.2.2.2 Novel technology in the detection of SSc-CM

Novel technology is advancing the understanding of SSc-CM, with the possibility of even newer techniques used in the general population (such as optical coherence tomography in the morphological assessment of coronary arteries) being applied in SSc [565]. CMR and the ILR are two very promising research tools as discussed below.

8.2.2.2.1 CMR

The comprehensive literature review (chapter three) demonstrated the difficulty in assessing SSc-CM; in particular, cross-study comparisons of SSc-CM were difficult due to the heterogeneous definitions of SSc-CM used. Surrogate measures of SSc-CM may then offer an advantage over clinical reporting, and CMR is increasingly being utilised to do so given the global assessment of the heart and vasculature that it provides. The CONVAS study (chapter six) demonstrated the presence of subclinical SSc-CM in patients with SSc apparently free of clinical SSc-CM or atherosclerotic heart disease. One quarter of patients had evidence of focal fibrosis by the presence of late gadolinium enhancement (LGE), and a 5% increase (after adjustment for age, gender and traditional CV risk factors) in extra cellular volume (ECV), an objective measure of diffuse fibrotic change [566].

There are inherent concerns in using a subjective measure such as LGE to determine cardiac fibrosis, which may explain the varied reporting of LGE in SSc [464]. ECV may

therefore be preferred to LGE. Also, ECV has shown prognostic value in predicting all-cause mortality in the general population [567], and increasing ECV has been associated with type 2 DM and its associated risk of heart failure [568]. Recent CMR studies in SSc have reported differences in ECV between patients with SSc and controls between 2 and 3% [467, 468], however these studies had a smaller representation of dcSSc; 7 to 21% dcSSc compared to 45% dcSSc in CONVAS. Nutsi et al reported a difference of 7% (along with LGE in 53%); although similarly 47% had dcSSc, the patients evaluated had longer disease duration and greater modified Rodnan skin scores (mRSS), which may reflect a greater disease burden [466].

Using subjective methods to detect perfusion defects may also be unreliable. In the hands of expert CMR-cardiologists, the CONVAS study reported only one segmental perfusion defect (secondary to atherosclerotic heart disease), whilst others report a much higher prevalence. The recent study by Rodriguez-Reyna et al reported perfusion defects in 79% of their cohort [464], although these were described as subendocardial so may be the result of IHD. As discussed previously, the detection of perfusion defects through CMR requires expertise and consistent acquisition and analysis methods, which are not available everywhere [454]. Myocardial perfusion reserve (MPR) offers a more objective measure of myocardial perfusion; a recent CMR study of 46 patients with dcSSc reported impaired MPR compared to controls, correlating with digital ulceration; MPR deteriorating again two years later along with increased LGE, despite patients being asymptomatic [569]. The gold standard however is absolute quantification of myocardial blood flow, potentially available with CMR, although limited to a few centres.

8.2.2.2.2 ILR

As described in chapter three, sudden death from SSc-CM associated arrhythmias is a recognised phenomenon, but relatively less well studied aspect of SSc. The ELCASA study (chapter seven) used an ILR as a research tool for the first time in SSc, and demonstrated a high prevalence of EP abnormalities, including arrhythmias, in a population free of clinical SSc-CM and with minimal risk factors for atherosclerotic heart disease. The abnormalities detected included atrial and ventricular ectopics, which are usually seen as benign, however, a recent prospective cohort study using 24 hour ECG monitoring in SSc has suggested a higher number of VEs were associated with both sudden cardiac death and requirement for implantable cardiac defibrillators (ICDs) [541]. Aside from ectopics, arrhythmias were detected in five of the 19 patients; with one patient requiring

pharmacotherapy and another, an emergency permanent pacemaker. Four of the arrhythmias were picked up incidentally as the patients were asymptomatic at the time. The use of an ILR as a research tool in SSc has not been reported before. The advantage of its use includes continuous monitoring of EP activity in an 'at risk' asymptomatic patient, rather than snapshot assessments in patients with evolving/changing disease (although these would be entirely appropriate as an initial evaluation), and also allows a more accurate assessment of the prognostic implications for ectopics and arrhythmias in this population.

8.2.2.3 Detection of SSc-CM in clinical practice

8.2.2.3.1 Identifying the clinical disease phenotype at risk of SSc-CM

Identifying the patient with SSc most at risk for SSc-CM and associated arrhythmias is essential in the early detection of such disease. The limited data available from the comprehensive literature review (chapter three) did identify the following patient characteristics as potential 'red flags; for SSc-CM; dcSSc subtype, positive serology (in particular anti-Scl70, anti-U3 RNP, anti-Ku and anti-Th/To antibodies), black ethnicity, older age of onset, TFRs, abnormal NFC and worse quality of life scores. These characteristics are perceived as reflecting more severe disease generally. The results of this review went on to inform the consensus best practice pathway for the management of cardiac disease in SSc produced by the UK Systemic Sclerosis Study Group (UKSSSG) (manuscript submitted).

In agreement with the review in chapter three, the cross sectional CMR and EP studies, CONVAS (chapter six) and ELCASA (chapter seven), within this thesis determined similar findings, along with more recent clinical cohort studies [570]. The CONVAS study determined an association between interstitial lung disease (ILD), digital ulceration and absence of anti-centromere antibodies (ACA) with the presence of cardiac fibrosis on CMR (presence of LGE and greater ECV). The study also suggested an association of cardiac fibrosis with recent onset dcSSc and Scl70 antibody positivity, although these findings were not statistically significant. The ELCASA study reported the patients who developed serious arrhythmias were men with diffuse disease and interstitial lung disease (ILD). These variables are generally considered markers of poor prognosis in SSc [331].

Recent CMR studies in SSc have varied in their findings. Rodriguez-Reyna et al found those with dcSSc were more likely to have LGE than lcSSc (59% dcSSc vs. 33% lcSSc) [464], although other CMR studies have found no association of LGE with disease subtype,

duration, antibody status or skin score [528], including a 131 strong cohort of which 23% had LGE in an abstract publication by Gargani et al [414]. Although no detail on the SSc disease phenotype was given in this abstract, perhaps the inherent concerns in the subjective detection of LGE played a role in the absence of any associations found. Increased ECV was associated with dcSSc, as well as mRSS and the SSc Valentini-disease activity index (VDAI) in the study by Nutsi et al [466]. The EP study by Muresan et al reported that patients with EP abnormalities were more likely to be older, have pulmonary hypertension, valvular heart disease and higher NT-proBNP levels [564]. However, as discussed the distinction between SSc-CM and that of atherosclerotic heart disease or SSc related PAH is not clear in this study.

Unexpectedly, the CONVAS study determined an association of lower BMI and systolic blood pressure with the presence of LGE, and of lower TC/HDL-C and waist/hip ratio with increased ECV. These findings may reflect the general cachexia and Raynaud's phenomenon medication use in severe disease. This area requires further study; as in contrast, increasing BMI has been associated with increasing ECV in the general adolescent population [529].

8.2.2.3.2 Screening strategies for SSc-CM

This thesis has demonstrated a high prevalence of SSc-CM in a population free of clinical cardiac disease, and minimal traditional CV risk factors, with a significant number developing arrhythmias. The thesis, therefore, highlights the importance of active screening for SSc-CM. The UKSSSG consensus best practice pathway for cardiac disease in SSc (manuscript submitted) will help guide the physician, however, the evidence base is poor and requires further study. The common practice of routine ECG, with or without a 24 hour ECG monitor, may not be sufficient in the patients with SSc deemed to be at relatively higher risk of SSc-CM related arrhythmias; in the ELCASA study (chapter seven) the arrhythmias were detected over the course of the year, not within the first day or month, and so physicians and patients may be falsely reassured with short-term EP monitoring. There is the potential for more intensive monitoring including the use of ILR insertion in those at higher risk.

Determining which patient warrants such a device could become clear with the development of a screening algorithm (as discussed in the section above), with the use of cardiac biomarkers such as NT-proBNP, echocardiography, routine EP testing and CMR. NT-proBNP has previously shown an association with SSc-CM [369], including abnormalities on

echocardiography [371], and is already recommended in risk stratification for PAH [368]. However, study sizes are small and longitudinal data are required. The ELCASA study found that EP abnormalities and arrhythmias were associated with CMR abnormalities, in particular, diffuse myocardial fibrosis (ECV). Supporting this finding, a recent study by Mavrogeni et al determined 37 of 38 patients with SSc with no previous cardiac history, but with Q waves evident on ECG, had patchy focal myocardial fibrosis (LGE) on CMR [571]. Longitudinal data is required to determine true cause and effect, however if confirmed, it would support CMR as a key tool in the assessment for SSc-CM.

8.3 Limitations of thesis

There are several limitations with this thesis. Primarily, all the studies were exploratory in nature and not powered to detect a difference in the primary outcome. However, the studies provide a comprehensive evaluation of the patients offering detailed clinical phenotyping with the use of soluble biomarkers, CMR and in EP testing in various combinations. Therefore, the results are valuable to the wider research field and may help inform future appropriately powered studies.

In the assessment of SSc-CM it was difficult to completely exclude the possibility of pathology due to atherosclerotic heart disease. However, effort was made to minimise any confounding with the exclusion of patients with DM and the inclusion of those into ELCASA with no more than one traditional CV risk factor. Also, results were adjusted for traditional CV risk factors. Any pathology identified would be therefore less likely to be due to macrovascular disease. Having said this, however, the one perfusion defect seen in the CONVAS study (chapter six) was a focal defect suggestive of ischaemia due to atherosclerotic disease within the right coronary artery. Differentiating between the two pathologies will always be relevant when reporting SSc-CM. The tendency for atherosclerotic heart disease to cause focal defects within coronary artery perfusion territories whilst more diffuse changes are observed in non-atherosclerotic heart disease may help distinguish between the two pathologies.

Working across departments and sites proved to be an area of difficulty. Particularly with ELCASA, a complex study involving many investigations at baseline, there were concerns with missing data, which arose primarily due to suboptimal communication between rheumatology, non-invasive cardiology testing and the study participant. There was very little missing data for CMR and ILR downloads, presumably as the departments only

needed to perform the one test. However, the non-invasive cardiology department was responsible for the echocardiography and multiple EP testing (autonomic, signal averaged ECG and 24 hour ECG monitoring). Although appointments were made for all these tests by the study co-investigators, and given to the patient, some investigations were not performed; on questioning it was usually due to the department or patient not realising what was required. This highlights the need to rationalise testing in future studies. Perhaps providing the study participant with a logbook to present to the various departments explicitly detailing what is expected at each visit would help minimise any study errors. Despite the missing data, the results in chapter seven do provide sufficient information to help design the larger study required.

Finally, the main CMR studies (chapter five and six) are cross-sectional studies, and the IDEA (chapter four) and ELCASA studies (chapter seven) report short-term data only. To sufficiently determine and validate the CMR outcome measures in IMID, longitudinal studies are required to assess relationship to clinical CV outcomes such as MI or arrhythmias. Also, longer-term data is required to adequately assess the effect on clinical CV outcomes with early disease suppression of RA.

8.4 Future research agenda

This thesis has identified many areas in need of further study, and is discussed below.

8.4.1 Disease mechanisms

The thesis has provided insight into the pathophysiology behind CVD and cardiomyopathy within IMID. As well as the usual traditional CV risk factors tracking with CMR outcome measures, the suggestion of altered remodelling/microvascular abnormalities of the myocardium in RA, with reduced LV mass, requires further study, particularly the relationship with adipocytokines. Longitudinal studies are required to determine when the reduction starts to occur, i.e. is the reduction evident before the onset of clinical symptoms of RA ('pre-RA' status) which may suggest an alternative process other than a consequence of systemic/direct inflammation or deconditioning, and also to determine the prognostic implications of those with reduced LV mass, i.e. are these patients at greater risk of heart failure in the future.

In SSc, larger and longitudinal studies are required to determine an association (if present) of fibrosis (ECV and LGE) with the development (if any) of arrhythmias and heart failure, in

addition to the evaluation for possible changes in cardiac geometry as seen in RA. In addition, given the possible association of cardiac ECV with skeletal muscle ECV found by Barison et al [468], further assessment of this relationship would be valuable in the understanding of pathogenesis of SSc-CM given the greater feasibility to obtain histological samples of peripheral muscle in comparison to cardiac tissue.

8.4.2 CMR in IMID

This thesis reported the results of multiple CMR outcome measures; however, the optimal CMR outcome measure to determine CV risk in IMID remains to be determined. This measure would ideally detect subclinical CVD, be sensitive to longitudinal change and be predictive of morbidity/mortality. Measures of LV geometry, used in the general population to determine CV risk, for example, greater LVmass/EDV, may be compromised in patients with IMID if an intrinsic process of remodelling is occurring independently to accelerated atherosclerosis. Longitudinal studies will help determine which outcome measure (or combination of measures) is most predictive of CVD in IMID. These studies and any consequent recommendations should involve both rheumatologists and CMR-cardiologists to ensure correct interpretation and significance of the data.

This thesis also evaluated the novel use of carotid-MRI in RA. This work requires validation, with perhaps direct comparison to ultrasound- measured CIMT. In addition to the validating the ability of carotid-MRI to reflect and determine CV risk in RA, an expert consensus on the MR protocol and method of assessment/measurement is required. Studies using contrast may also help to characterise any differences in carotid plaque in RA compared to the general population; differentiating unstable from stable plaques, those with lipid rich necrotic cores from those with intra-plaque haemorrhage which may help in the understanding of the accelerated risk of CVD observed.

8.4.3 Prediction and prevention of CVD in IMID

This work suggests a subset of patients with SSc at higher risk of SSc-CM could be identified. Larger studies are required to more definitively determine the SSc phenotype most at risk of SSc-CM and associated arrhythmias; to be able to justify more intensive monitoring for SSc-CM & its complications. The ELCASA working group are currently collaborating with other research centres to set up a larger national trial using CMR and EP testing, including the use of ILR, to address this need. The goal is to establish an iterative screening algorithm using a composite of tools, such as soluble CV biomarkers (such as

Troponin I, NT-proBNP), echocardiography, CMR and basic EP testing to determine the patients requiring more intensive cardiac monitoring with the use of an ILR, and provide reassurance to those found to be at lower risk. Detecting arrhythmias earlier may guide cardiac interventions, both pharmacological and device such as implantable cardiac defibrillators (ICDs) or permanent pacemakers, to minimise complications including sudden death. Whilst escalation in immunosuppression is commonly instituted, to limit further disease progression, mainly in the context of myocarditis, there is minimal evidence base. Studies are therefore needed to evaluate the effect of intensive immunosuppression and/or pharmacotherapy in SSc-CM and its ability to halt or slow down disease progression.

As discussed earlier in this chapter, research is also required to evaluate the effect of immunosuppression on the development of CVD in IMID. Although the suppression of disease activity in IMID is associated with a reduced risk of CVD, this thesis suggests that TNF inhibitors may have an advantage over MTX in measures of insulin resistance. Therefore, further work is required to validate this, and also determine more broadly if specific disease modifying therapies have advantages over others in the prevention of CVD. In RA, CMR studies are required to determine if reduced LV mass is amenable to change with the treatment of active disease. Studies are also needed to evaluate the effect of intensive immunosuppression and/or pharmacotherapy in SSc-CM and its ability to halt or slow down disease progression.

8.4.4 Personal future research plans

The current standard approach for CV risk reduction in RA involves discussion with the patient of their heightened risk of CVD and need to reduce traditional risk factors. However, due to the complexities of RA management and limits on time in clinic, this education may not occur, and there is generally no formal follow-up to ensure patients have made the advised lifestyle changes. Intensive CV risk reduction programmes have been shown to be effective in the general population [572] and DM [573], however, there is little data on their efficacy in IMID, particularly RA [559]. Psychological and individual reported barriers, as discussed, also play a key role in any positive behavioural change [561] and have not been adequately evaluated in RA. In addition, our Leeds Biomedical Research Unit (LMBRU) CV research group have recently reported observational data from the Leeds Inflammatory Arthritis Continuum (IACON) study suggesting patients with early

RA do not increase their level of physical exercise, even if disease activity and levels of disability have improved [574].

Therefore, following on the completion of my PhD, I wish to investigate the patient-reported barriers to improving modifiable CV risk behaviours such as physical exercise, with an aim to develop an effective and feasible CV risk reduction programme in those with RA, with possible wider application across the other IMIDs. To do this, I will apply for a NIHR-clinical lecturer award (CL), to enable me to prepare for a NIHR-clinician scientist award (CSA) within the next two years. My main objectives would be to perform a systematic literature review (SLR) on the patient-reported reasons and barriers behind positive behavioural change to reduce CV risk factors within RA, and to perform a qualitative study to again determine IMID/RA specific barriers to positive behavioural change with regards the reduction of CV risk factors.

This proposed research programme will be undertaken in collaboration with the Academic Unit of Psychiatry and Behavioural Sciences, Leeds Institute of Health Sciences, University of Leeds; expert in the field of behavioural change, particularly in CVD risk reduction [575-578]. The results of the SLR and qualitative study will tailor a RA-specific CV risk reduction feasibility study in Leeds, which will then lead on to a larger randomised controlled trial (RCT) to demonstrate if this RA-specific CV risk programme is superior to the current standard approach. These studies would form the basis of my NIHR-CSA application.

8.5 Conclusions

The work within this thesis has demonstrated the presence of subclinical CVD in IMID using a combined approach of clinical assessment, soluble CV biomarkers and CMR. It has demonstrated abnormalities in RA associate with traditional CV risk factors; further emphasising the need for these to be aggressively managed in RA, alongside improving RA disease activity. CMR has been shown to be a valuable research tool in the investigation of CVD in RA; demonstrating a reduction in LV mass in those with RA free of known CVD; reported once only previously by a similarly sized CMR study, suggesting pathology other than atherosclerotic heart disease in RA. In addition, it has demonstrated for the first time, in a RCT, improvement in IR with the use of TNFi within a treat-to target management strategy in early RA. This thesis has also reported the global use of CMR in the assessment of SSc-CM, reporting increased levels of fibrosis in those free of known cardiac disease,

associating with a poor prognostic phenotype. For the first time, it has reported the use of an ILR in SSc, detecting arrhythmias in patients with SSc free of known cardiac disease.

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