Defining The incidence of Cardiac Involvement in Myositis Using Mapping Based Cardiovascular Magnetic Resonance Imaging

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Short title of study: DIFUS-T1

1. Declaration

I, Luke Henry Dancy confirm that the work presented in this thesis is my own. Where information has been derived from other sources it has been appropriately indicated and referenced.

Signature:

Name: Dr Luke Dancy

Date:

Research reference numbers

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2. Abstract and funding

2.1 Abstract

Myositis is the name of a group of rare conditions characterised by inflammation and fibrosis of skeletal muscle leading to pain and progressive weakness [1]. In a proportion of patients, extra skeletal muscle manifestations complicate the clinical course. In particular, cardiac muscle involvement is associated with worse outcomes [2, 3]. The pathophysiology of myocardial involvement is inflammation and fibrosis [4] which has proven difficult to identify by traditional investigations. The new techniques of T1 and T2 mapping in cardiovascular magnetic resonance (CMR) allow for assessment of myocardial fibrosis and oedema. Use of T1 and T2 mapping has been shown to offer improved sensitivity for mild and diffuse inflammation as well as subtle focal and diffuse fibrosis in inflammatory myocardial diseases such as viral myocarditis and sarcoidosis. This could help guide therapy and monitor treatment response [5, 6] in myositis.

We scanned healthy volunteers to establish normal T1 and T2 values for our particular scanner. Using both healthy volunteers and patients with myositis we devised and validated a novel method of analysing the ventricle following a multi-segment model. The model was based on the 12 basal and mid-level American Heart Association myocardial segments [7]. The apical segments were excluded due to well documented issues around reproducibility [8, 9].

Using the above method, we found that patients with myositis had significantly higher T1 and T2 than healthy volunteers, independent of blood troponin. We also found that in patients with myositis, a raised troponin was associated with higher myocardial T1 and T2 values than those patients whose troponin was not elevated.

We performed follow -up scans on patients with myositis and demonstrated a reduction in T1 and T2 values over time without any change in overall cardiac function. T2 values returned to baseline over the course of the study whereas T1 values remained significantly higher than that of healthy volunteers.

These studies show that measuring myocardial T1 and T2 values in myositis may have a role in both the identification of inflammation or fibrosis and in monitoring the response to

treatment. The sample size of the study did not allow for comparison of different treatments to be made.

We also undertook mapping of skeletal muscle tissue mapping using the same, gated, cardiac specific un-optimised sequences. The purpose was to look for trends in T1 and T2 values that might suggest a benefit in developing specific sequences to allow researchers or clinicians to reproducibly measure skeletal muscle disease activity non-invasively. We found that, despite large variability in the data set, T2 values decrease over time with treatment suggesting there may indeed be a benefit in working towards dedicated skeletal muscle sequences in the future.

2.2 *Funding*

This work was funded by a KMRT JRC fellowship from King's College London, and sponsored by King's College Hospital, UK.

3. Contents

3.1 *Tables*

***3.2** Figures*

4. Background, Aims and objectives

Myositis is a collective term for a group of conditions affecting skeletal muscle causing inflammation and progressive fibrosis. Patients usually present with progressive muscle pain and weakness. Muscle damage is usually immune mediated and if left untreated can lead to permanent weakness. This presents a particular problem as the slow onset and generalised symptoms of pain and weakness often lead to a delay in accurate diagnosis. In addition to muscle weakness, extra skeletal muscle manifestations (lung, skin, gastrointestinal and cardiac) are frequent and can cause additional morbidity and mortality [10].These are important as 20% of dermatomyositis and polymyositis deaths are reported to be secondary to cardiac causes [2].

Cardiac involvement in myositis is difficult to identify using current diagnostic tools (such as ECG, echocardiography and Holter monitoring) and the incidence is, therefore, poorly defined [11]. CMR, which is now the gold standard non invasive investigation for tissue characterisation of the myocardium[12, 13], has been used in myositis, but in small numbers. The presence of late gadolinium enhancement (LGE) is at best semi-quantitative and may not be able to identify subtle or diffuse change in the myocardium. More modern tissue mapping sequences (that use T1 and T2 relaxation times) are quantitative. A value for the variable is produced which can be compared to a normal range and hence opens the possibility for monitoring change over time and finding more subtle or diffuse disease. As compared to LGE assessment which is semi quantitative where LGE is either present or it is not and the only thing we can quantify is the amount of it present, not the actual underlying extracellular volume fraction. As T2 relaxation times increase in areas of increased water (oedema) and T1 in extracellular space expansion of all causes with the exception of fat, these sequences could be used to differentiate between acute and chronic change [14].

This thesis applies the now clinically accepted T1 and T2 mapping sequences to patients with myositis to attempt to identify those with acute cardiac involvement and those with evidence of chronic myocardial fibrosis. These patients were followed over time with serial CMR scans to observe changes in myocardial T1 and T2 with treatment.

The overall aims were as follows:

1. Establish normal ranges for T1 and T2 mapping by scanning healthy volunteers.
2. Validate a method for evaluating large areas of myocardium that is reproducible allowing for focal and diffuse change to be identified.
3. Correlate mapping values with biomarkers, functional capacity and patient assessment of symptoms using visual analogue scales (VAS) [15] to aid in the diagnosis of acute myocardial inflammation in patients with myositis.
4. Monitor treatment response using repeated tissue mapping CMR to look for any resolution in patients undergoing effective rheumatological management for their disease.

5. Introduction

5.1 Background

The individual conditions collectively referred to as myositis (polymyositis (PM), dermatomyositis (DM), immune mediated necrotising myopathy and sporadic inclusion body myositis (sIBM)) are characterised by gradual onset muscle pain and weakness. They can be complicated by damage to other organs with resulting increased morbidity and mortality. Myositis is known to be associated with both acute myocardial inflammation and more chronic fibrosis, but this has been poorly defined in vivo.

Myocardial inflammation and fibrosis are accurately and repeatably assessed using CMR and in particular newer T1 and T2 (tissue) mapping sequences [14, 16-18]. These sequences are moving from the research domain and into the clinical arena (in conditions like acute myocarditis and takotsubo cardiomyopathy) because of their ability to identify disease not seen with traditional LGE and non-mapping based T2 assessment, the previous non-invasive gold standard approach [19, 20].

The focus of this thesis is to assess patients with myositis using T1 and T2 mapping techniques to try to gain a more accurate picture of the incidence of myocardial involvement and how it changes with time. We will also design and validate a novel means of interpreting tissue maps to improve identification of both focal and diffuse disease.

The following chapter will explore the current position with regards to myositis and cardiac involvement. The use of tissue mapping sequences will be described and the potential benefits of its application in myositis discussed. A systematic review of the current literature relating to CMR in myositis will be presented. Finally, the rationale for the design of this thesis will be described.

5.2 *Myositis*

The term myositis refers to a collection of conditions characterised by progressive inflammation of muscle and fascia eventually leading to permanent muscle weakness mediated by fibrosis. The clinical course can be either progressive or relapsing remitting. The specific conditions making up this group of inflammatory myopathies include: polymyositis (PM), dermatomyositis (DM), immune mediated necrotising myopathy, cancer associated myositis, myositis in overlap with a further connective tissue disease (CTD) and sporadic inclusion body myositis (sIBM).

Myositis is estimated to affect 250,000 individuals worldwide with an estimated incidence of 4-10 cases/million population per year [21, 22]. Similar to other autoimmune rheumatic diseases, there is a female predominance. Myositis presents with a bimodal age at onset as it affects both children and adults. In adult disease, the median age of onset is 55, although the age range is wide. The aetiology of myositis remains unknown, but is thought to be due to a combination of environmental exposure to specific triggers in genetically predisposed individuals [23]. Other factors which support an autoimmune basis in myositis include the coexistence of other autoimmune and connective tissue disease, a strong association with circulating antibodies which themselves are associated with specific HLA genotypes, and the response to immunomodulatory agents.

Morbidity in myositis is caused predominantly by muscle damage but is contributed to by other organ involvement. Recognised complications include: Cancer, interstitial lung disease and cardiac involvement in adults, all of which can lead to premature death and significant disability. The side effects of steroid and the immunomodulating therapies to treat myositis can also contribute with infection being a major cause of mortality. Given the rarity of the condition, the often insidious onset of symptoms and difficulty recognising active muscle disease, the diagnosis is often delayed. This and the variable response to treatment can lead to irreversible muscle and other organ damage.

5.2.1 Diagnostics

A detailed clinical history and thorough clinical examination remain crucial in the diagnosis and management of myositis. Disease progression is monitored in the clinical setting using the manual muscle test (MMT8) which is a reproducible measure of muscle strength. It tests 8 different muscle groups, both distal and proximal, giving each a score out of 10. There are

seven bilaterally assessed groups that yield a maximum score of 140 and an additional 10 for neck flexion meaning a total score of 150.

Investigations that aid diagnosis include, detection of raised skeletal muscle enzymes such as Creatine kinase (CK), specific electromyographic (EMG) abnormalities and MRI imaging of affected muscles for detection of oedema. Muscle biopsy (usually from the quadriceps) can aid both diagnosis and sub-type analysis. For Example:

- CD4/CD8+ T and B lymphocytic inflammatory infiltrates and/or CD68+ macrophagic involvement in sIBM
- Complement (C5b-9) deposition in capillaries and perifascicular atrophy in DM
- The invasion of viable muscle cells by CD8 positive T-cells in PM
- Necrotic cells with only mild inflammation in immune mediated necrotising myopathies.

5.2.2 Classification

Myositis-specific serum autoantibodies can be measured and are also linked to the clinical and prognostic features of myositis. Myositis associated antibodies are also found in myositis but are not specific for the condition, also occurring in other connective tissue diseases.

Identification and characterisation of serum autoantibodies is vital in the diagnosis of autoimmune connective tissue disease (CTD)[24]. Autoantibody profile in combination with characteristic changes on muscle biopsy help to distinguish different sub-types of myositis and rule out inclusion body myositis (IBM) and non-inflammatory myopathies [25, 26]. In addition to aiding diagnosis, myositis-specific antibodies can help predict clinical manifestations or subsets. Cancer related myositis is most strongly associated with anti-TIF1-gamma and NXP2 (also associated with higher likelihood of calcinosis) antibodies. Interstitial lung disease associated with myositis is more common in patients with anti-MDA5 and antisynthetase antibodies. Immune mediated necrotising myopathy, which is often resistant to therapy is associated with anti-SRP and anti-HMG-CoA reductase antibodies. Despite these associations, on an individual basis, the combination of antibody profile and muscle biopsy is unable to accurately assess the severity of disease or the likelihood of extra-skeletal manifestations [27]. Pertinent to this study, there is not a specific

antibody that predicts myocardial involvement. Patients with anti-SRP antibodies have a proportionally higher incidence of myocardial involvement but cardiac involvement is not limited to this antibody specifically. Given the overlap in antibody profile of individual patients with myositis, much larger studies are needed to try to link specific antibodies to non-skeletal muscle manifestations.

5.2.3 Biomarkers

Elevated serum skeletal muscle enzyme concentrations, including Creatine kinase (CK), remain a cornerstone in the diagnosis and monitoring of myositis. Not infrequently, serum cardiac Troponin T (TnT) levels are raised in acute myositis, as TnT appears to be expressed in regenerating skeletal muscle cells [28]. The question, therefore, arises in the setting of a raised TnT in myositis monitoring, as to whether this represents skeletal or cardiac muscle inflammation, or both. Therefore, the presence of TnT can lead to an inaccurate assumption about the presence of cardiac involvement. However a further enzyme, Troponin I (TnI), is not expressed in regenerating skeletal muscle cells and thus appears to be more specific to the myocardium [28].

5.2.4 Imaging

MRI is a very sensitive, non-invasive, radiation free technique for localising oedema in the skeletal fascia or muscle [29]. It allows for sequential examination of large volumes of muscle, allowing focal areas of myositis to be identified. This also aids in planning for targeted muscle biopsy. The technique alone lack specificity for myositis because mitochondrial/metabolic myopathies, dystrophies, and even motor neuron disease can show secondary inflammatory changes on MRI. To date there is no prospective MRI data to:

a) Characterise MRI patterns of inflammation in specific myositis disease subtypes.
b) Use novel modalities of MRI including spectroscopy and whole-body imaging to characterise disease.
c) Validate the use of MRI muscle imaging in the quantification of disease activity.
d) Measure sensitivity to change over the course of disease.

5.2.5 Cardiac involvement in autoimmune disease

Extra-skeletal involvement in rheumatological conditions is well described. In many conditions a variety of different pathologies and severity of involvement is described from subclinical to severe [30]. With many of the rheumatological conditions the actual frequency of cardiac involvement is either unknown or poorly defined as is the case with myositis (**Table 1**). Recent advances in cardiac imaging have allowed more subtle disease to be detected and, in many cases, diagnosis is aided by the ability to tissue characterise cardiac muscle non-invasively. This allows the disease process to be detected directly rather than looking for the effects that the pathology has on heart function or rhythm, which will only generally occur in more severe and advanced disease. Being able to directly find changes in the myocardium allows for earlier disease detection and has potential monitoring applications [31, 32].

In autoimmune conditions such as sarcoidosis and systemic lupus erythematosus (SLE) where more is known about the cardiac manifestations than in myositis, the cardiac sequalae account for significant mortality and morbidity. The cardiac implications of sarcoidosis include significant LV impairment, ventricular arrhythmia and sometimes death [33, 34]. In SLE, 50% of patients are reported to have cardiac involvement [35]. Here, the presence of cardiac involvement carries a significant mortality risk. In one study of patients presenting with lupus myocarditis, 40% either died or were left with significant residual cardiac damage [36]. Patients with rheumatoid arthritis (RA) have improving cardiovascular mortality, although cardiovascular disease and cardiac disease remains the leading cause of premature death in RA sufferers [37]. In systemic sclerosis the presence of diastolic dysfunction is associated with significant mortality risk [38]. In non-malignancy associated myositis, cardiac involvement is the strongest prognostic factor [3]. While there is more data available on some of the less rare autoimmune conditions, much of this data is derived using less sensitive diagnostic techniques meaning it is possible the incidence of cardiac involvement is higher than currently published. Where myositis is concerned, some of the difficulties in defining an incidence has centred around sub-clinical and varied presentations. As mentioned above, when testing for the effect a pathology may have (fibrosis for example) the heterogeneity in clinical syndromes makes accurate diagnosis challenging.

Disease	% of patients with cardiac involvement	Cardiac Involvement
Rheumatoid Arthritis	Upto 50%	Coronary Artery Disease Pericarditis / Pericardial Effusion Myocarditis (rare) Valve disease
SLE	Around 50%	Valve disease Pericarditis Coronary artery disease Myocarditis
Systemic Sclerosis	?	Microvascular disease Myocardial fibrosis Pericardial disease Arrhythmia
Poly and Dermatomyositis	?	Myocarditis Myocardial Fibrosis Arrhythmia LV dysfunction
Sarcoidosis	40%	Myocardial Infiltration Myocarditis Myocardial Fibrosis Arrhythmia

Table 1: Incidence, where known, of cardiac involvement in Rheumatological conditions and the common pathologies seen

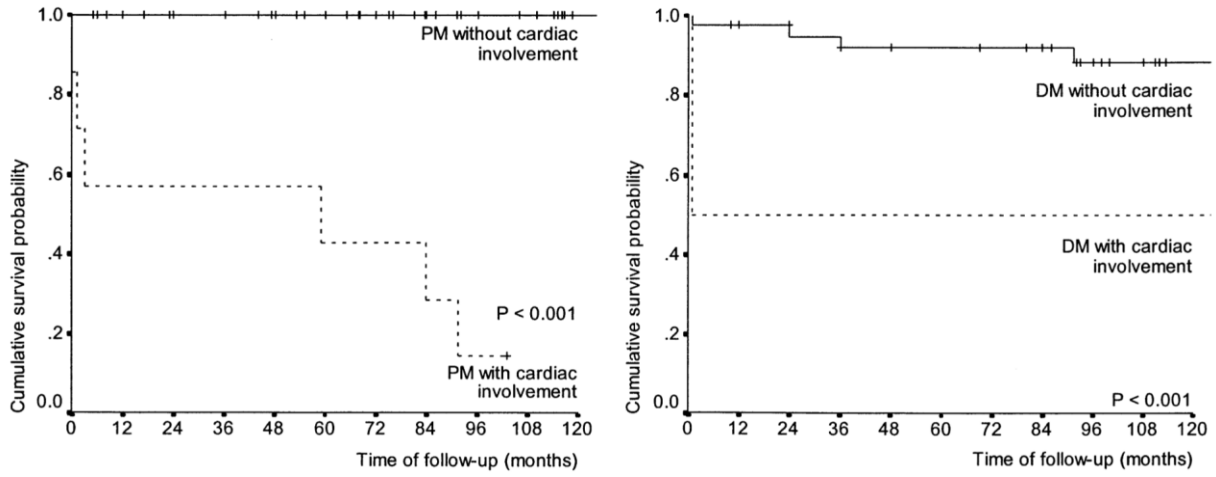

Figure 1: Survival curve of PM and DM patients with and without cardiac involvement by Danko et al (2004) [3]. Cardiac involvement was defined by the exclusion of other causes of rhythm disturbance, conduction disease, myocarditis, cardiomyopathy, and LV dysfunction. 8/15 had histological confirmation of cardiac involvement.

5.2.6 Different cardiac conditions associated with myositis

As mentioned above, the morbidity and mortality associated with cardiac disease in rheumatological conditions is significant but not yet well defined for all subtypes [3]. This is at least in part due to the heterogeneous nature of presentations within this group. Multiple different cardiac sequelae have been described within the myositis population, but the

overall incidence has yet to be adequately defined. Given the importance of identifying patients with cardiac involvement, the published prevalence of 9-72% is of little clinical use. This data is based on a systematic review of 26 studies (1530 cases) [11].
The most commonly reported abnormality is ectopy, both atrial and ventricular. Prolonged QT interval (compared to age matched controls) is also seen [39]. Diastolic dysfunction is seen more commonly than systolic dysfunction although both are described. The mechanism for this is linked to progressive replacement fibrosis (**Figure 2**) [4, 40]. Although less well studied, meta-analysis of observational studies suggests a significantly increased coronary artery disease risk in the myositis population [41]. It remains unclear what the mechanism of this increase is although one theory links inflammation to the atherosclerotic process [41].

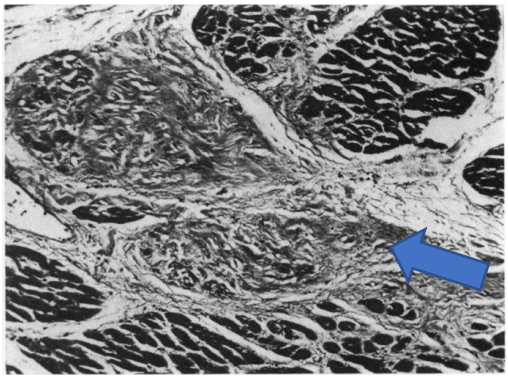

Figure 2: Replacement fibrosis of the right bundle branch (arrow) in patient with polymyositis taken at autopsy [4].

The other main cardiac disease state that manifests in the autoimmune rheumatology population is myocardial (myocarditis) or pericardial (pericarditis) inflammation. Acute myocarditis has frequently been described in myositis whereas pericarditis is not frequently seen [4, 5]. As mentioned above, it is becoming common practice to monitor troponin levels in patients with myositis and frequently these are found to be elevated. In fact, over an 18-month period in our hospital, 27% of 135 troponins (TnI) measured on patients with myositis were raised regardless of whether they had overt cardiac symptoms. Currently there is no established link between troponin elevation and underlying cardiac involvement, but the most likely cause, given the effect of the condition on skeletal muscle, would be that of an inflammatory process similar to that of other myocarditides.

The challenge of accurately identifying cardiac disease in myositis centres around the difficulty testing directly for the underlying pathology. This is particularly important as there appear to be a significant proportion of sub-clinical presentations.

Most traditional cardiac investigations look for abnormalities caused by a pathological process such as ectopics on an ECG. Echo can show accurately the structure and function of the heart but will not show fibrosis until it is sufficient to change contraction or relaxation. There are some more recent studies looking at tissue characterisation using echo, but these techniques remain in the academic domain currently [42, 43]. The use of speckle tracking and strain may allow earlier detection of disease by more accurate assessment of LV [44]. The identification of low-level fibrosis requires the ability to tissue characterise. CMR is the gold standard non invasive method for doing this. In doing so, it offers huge potential benefits in improving the sensitivity and specificity for diagnosing both acute and chronic cardiac involvement in myositis.

Test	LV function	Coronary Anatomy	Ischaemia	Radiation	Viability	Infarction	Making another diagnosis
Stress Echo	+	No	++	No	++	+ / -	+
CMR	++	+ (Proximal courses)	++	No	++	++	++
CT	+	++	Only in research	+	No	No	++
Invasive Angiography	+ / -	++	++ with use of pressure wire	+	No	No	No

Table 2: Table showing the applications of different common cardiological tests depending on the diagnostic question to show the relative strengths and weaknesses of each [45].

5.3 *Cardiac MR as a tool for assessing character of the myocardium*

5.3.1 Cardiovascular magnetic resonance

The human body is composed of around 95% water. When placed into a powerful magnetic field (such as a 1.5 Tesla MRI scanner) the hydrogen protons in water (and other tissues) behave as a series of mini magnets which will align and precess (at the Larmor frequency) [46]. Inside the MRI scanner, there is both transverse (Mx and My) magnetisation which is

perpendicular to the lie of the patient in the scanner and longitudinal (Mz) which is parallel to the patient (**figure 3**).

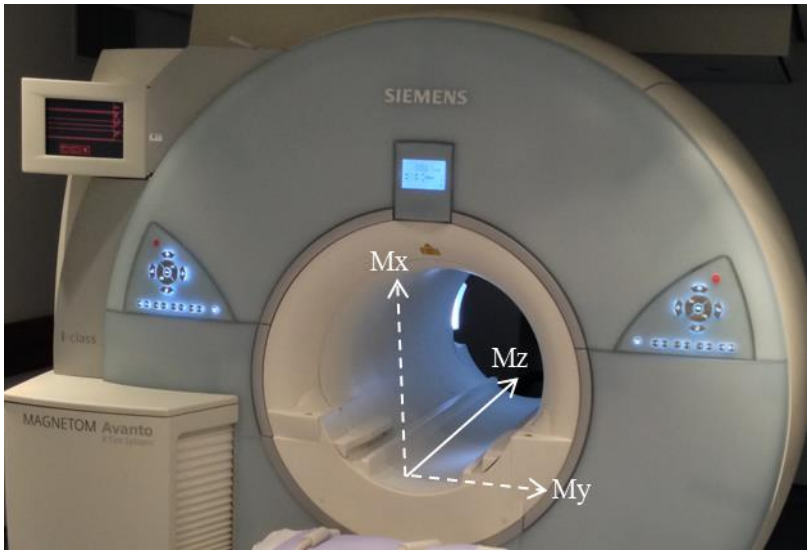

Figure 3: 3D magnetisation vectors in CMR. The figure shows the 1.5 Tesla Siemens Avanto. The longitudinal (Mz, arrow) and transverse (Mx and My, dotted arrows) magnetisation vectors are shown. *Image provided by and included with the permission of Dr Daniel Sado, Consultant Cardiologist, the author's research supervisor*.

When inside the scanner's magnetic field, protons in the human body lack phase coherence meaning the transverse component to their magnetisation sums to zero and all the net magnetisation is longitudinal. This does not produce a measurable signal. To produce a measurable signal, protons need to be excited by the application of an external radiofrequency pulse resulting in a change in their magnetic alignment towards the transverse plane. This creates phase coherence and a signal that can be measured by a receiver coil. Once the radiofrequency pulse is removed the protons return (relax) back to their original longitudinal magnetic alignment. This relaxation causes a change in the signal detection by the receiver coil according to known rate constants (for example T1 and T2).

The above process takes time to complete as it relies on repeated measures undertaken with different magnetic gradients to allow the location within the body to be identified for the purposes of image reconstruction. The issue with imaging the heart is that the motion means the information cannot be acquired in a single block and has to be acquired over a number heart beats. In order to ensure that as much as possible the heart is in the same position for each of the pulses, most sequences are performed with the patient holding their breath and are ECG gated.

The raw data from these acquisitions enter the K-space, a matrix which, when complete, undergoes Fourier transformation by computer software which produces a clinical MRI image. The difference in tissue density and intrinsic magnetic properties are displayed by varying 'shades of grey'.

T1 Relaxation

T1 relaxation describes the time taken to return to longitudinal plane following a radiofrequency pulse. The time taken is affected by the tissue composition in which the protons exist and can be very different between different tissues [47]. The relaxation pattern is an exponential recovery pattern and the absolute T1 value is quoted as the time required for the longitudinal magnetisation (Mz) to return to 63% of its pre-pulse value **Figure 4.**

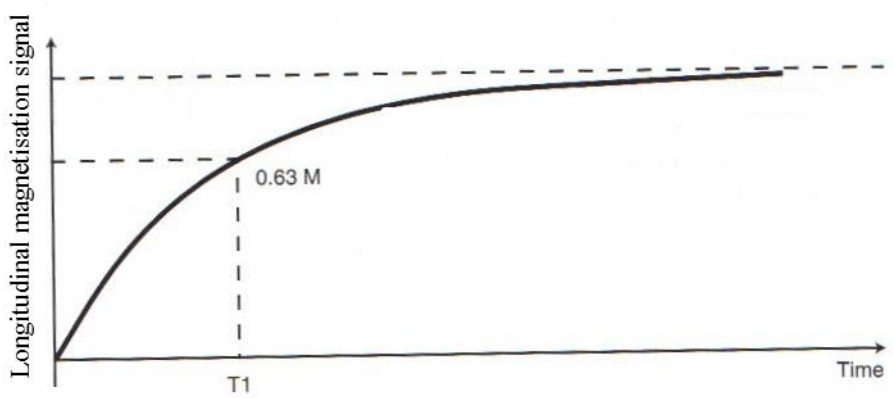

Figure 4: The T1 relaxation curve following a 90° radiofrequency pulse. The T1 time constant is defined as the time required for the longitudinal magnetisation to return to 63% of its maximal value. Taken from Lee V.S [46].

It should be noted that higher field strengths will increase the T1 of most tissues explaining the importance of magnet normal values. T1 relaxation times can be manipulated by the administration of paramagnetic contrast agents, such as gadolinium [48]. Gadolinium (contrast medium) molecules are too large to enter cells with intact membranes, but diffuse out of blood capillaries and into the interstitium [49]. The presence of gadolinium in a tissue allows hydrogen protons to relax 1 million times faster.

T2 Relaxation

T2 (or spin-spin) relaxation describes the time taken for protons to return to the zero coherence state in the Mx and My planes following the removal of a radiofrequency pulse [46]. Like T1 it relies on the local composition of tissues. The faster moving a proton is the less effect it has on its local environment meaning it takes longer to return to zero coherence

Fast moving protons have the smallest effect on the local environment and so the longest T2 (e.g., water). T2 relaxation decays in an exponential curve. Like T1, T2 values are quoted as the time taken for 63% decay to non-coherence (**Figure 5**).

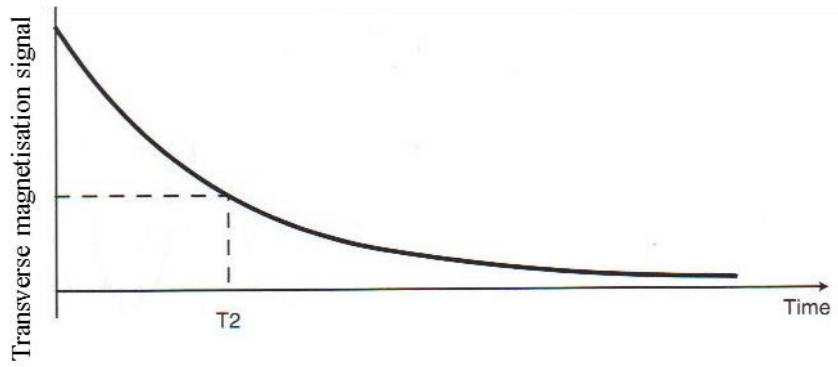

Figure 5: T2 decay. The T2 time constant is the time required for the transverse signal to decay by 63% of its maximal value following removal of radiofrequency pulse. Taken from Lee V.S. [46].

5.3.2 Myocardial fibrosis

In the developed world, cardiac disease is a major cause of morbidity and mortality. Much of this burden is due to changes in the myocardium leading to systolic and diastolic heart failure, arrhythmia, and death. The myocardium consists of three main components – the myocytes, the surrounding supporting structures (the interstitium) and capillaries. The interstitium, including the collagen architecture of the myocardium, is vital to normal myocardial function and its response to stress and injury [50]. Fibrosis represents the end product of a healing process necessary in the absence of myocyte division. It is fundamental to the process of cardiac remodelling and the potential development of heart failure [51, 52]. Fibrosis may be focal or diffuse [53]. Focal fibrosis or scar is most commonly the result of myocardial infarction but is caused by many other diseases such as myocarditis and

sarcoid [54-56]. It can lead to systolic heart failure, a worse prognosis, arrhythmia, and sudden death [57]. Diffuse fibrosis is also eventually associated with worsening systolic function, but also with decreased compliance leading to diastolic dysfunction [58] and increased incidence of atrial arrhythmia [59]. Left ventricular hypertrophy (LVH) leads to diffuse fibrosis and the level of fibrosis increases parallel to the heart weight [60]. Moreover, in cardiomyopathy, fibrosis cannot only be seen in areas of hypertrophy but also in apparently "healthy" myocardium [60]. Some current heart failure treatments in use today such as spironolactone produce at least some of their beneficial effects by acting to reduce progressive fibrosis seen within the myocardium [61-63]. While the LGE technique in CMR is now a well-established way to assess for focal fibrosis non-invasively, it is not as effective at identifying diffuse low-level disease. This is because it requires comparison with 'normal' areas of myocardium (**Figure 6**). In diffuse disease it is sometimes only possible to indirectly infer its presence using echocardiographic derived measures of diastolic dysfunction. Other techniques are useful, but prone to false negative results. For example, in-vivo biopsies are subject to sampling errors and biomarkers of collagen turn-over are non-specific and altered by other conditions such as growth or systemic diseases [64].

5.3.3. CMR imaging for focal fibrosis – 'The late gadolinium enhancement' technique

The LGE CMR technique is now a frequently used clinical tool and a well validated means to identify focal replacement fibrosis of the myocardium non-invasively [65-67]. Gadolinium is an extracellular marker that, following intravenous administration, is a small enough to diffuse out of capillaries but is too large to cross intact cell membranes. It therefore accumulates in the extracellular space. Gadolinium potently decreases T1, a fundamental magnetic relaxation constant inherent to all tissue. In areas where there is more extracellular space, such as areas with increased fibrosis, the kinetics of the gadolinium diffusion into that space will change. It will take longer to fill the region with expanded extracellular space due to reduced blood supply and, once accumulated, will take longer to 'wash out' than would be seen in adjacent healthy tissue. This results in a difference in the T1 relaxation times between the normal and abnormal areas (i.e., a greater inhibition of T1 relaxation time in the diseased area that in the healthy tissue). An MRI magnet can show

these differences by using imaging sequences which are T1 weighted and use the inversion recovery technique. By convention, the images obtained show normal myocardium as being black, with areas of focal extracellular space expansion as being white.

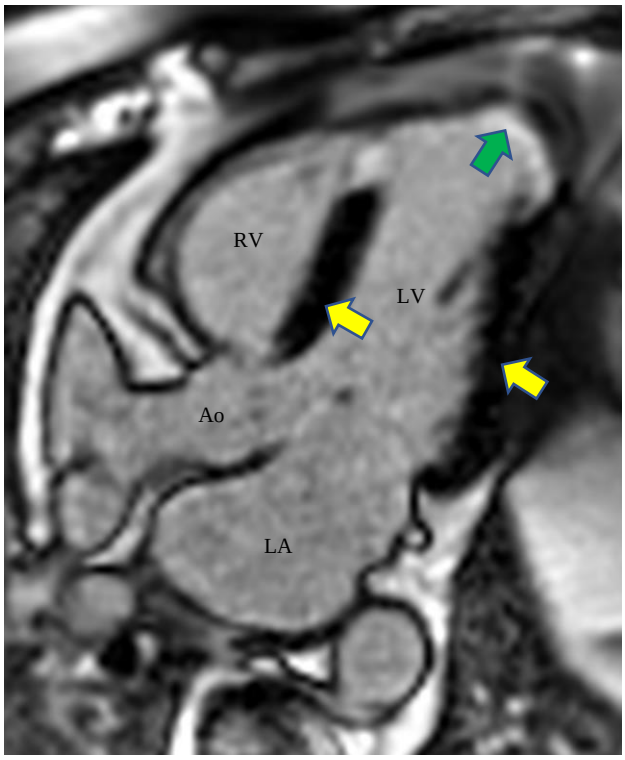

Figure 6: Apical three chamber view showing late gadolinium enhancement. In areas of focal extracellular space expansion, the myocardium appears white (green arrow) and in areas of healthy tissue the myocardium appears black (yellow arrows).

5.3.4. CMR imaging for focal myocardial oedema

Traditionally, focal myocardial oedema is assessed in two ways using CMR. In areas of oedema, the myocardial T2 will be increased. First, using the short tau inversion recovery sequence (STIR) produces greyscale images upon which the areas of oedema are brighter than surrounding normal tissue. This is a semi quantitative technique as it does not quantify the T2. Secondly, the LGE technique described above has some application here. In some areas of oedema there will be evidence of LGE, however, often in a less well circumscribed or monochrome way than in, for example, infarction (**Figure 6**) leaving it open to interpretation and to being missed.

5.3.5 CMR imaging for Diffuse Fibrosis and Oedema

There are limitations with STIR and LGE for the assessment of fibrosis and oedema. As mentioned above, these techniques are only semi-quantitative. The STIR technique does not calculate actual myocardial T2 values, but instead makes areas of oedema appear brighter on the image than the surrounding areas. This means that there is no healthy 'reference' range for comparison and so diffuse and milder disease will be missed. The sequence is also very prone to artefact. The LGE technique suffers many of the same problems – it is only semi-quantitative and therefore cannot be used to calculate the extracellular volume of the myocardium or assess diffuse disease.

Over the last 10 years, a number of 'parametric' mapping techniques have been designed allowing for direct quantification of myocardial T1 relaxation times (which is increased in any state where the extra cellular space is increased), T2 relaxation times (T2 as mentioned above is increased in the presence of increased water content reflecting oedema) and derived measurements of the extracellular volume (ECV) (which attempts to give an actual value to the amount of extracellular space). Importantly, these sequences produce a value for these relaxation times making them directly quantitative. This means, in turn, that normal ranges can be calculated allowing for assessment of diffuse disease. There is also the potential to monitor treatment response and disease progression with these sequences [16, 68-70].

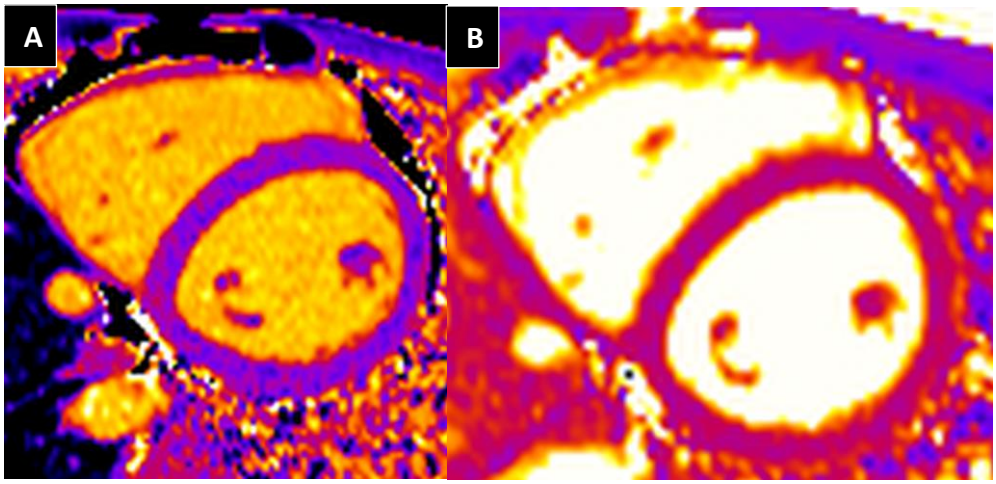

Figure 7: Short axis acquisition at the level of the papillary muscles showing colour maps representing normal T1 and T2 relaxation times. Panel [**A**] shows T1 colour map and panel [**B**] T2.

The importance of tissue mapping has yet to be fully realised but is an area of significant growth in research given its potential to allow for subtle and early changes to be reliably detected. **Figure 8** shows the increase year on year of the number of T1 mapping focused studies published and available through PUBMED [71].

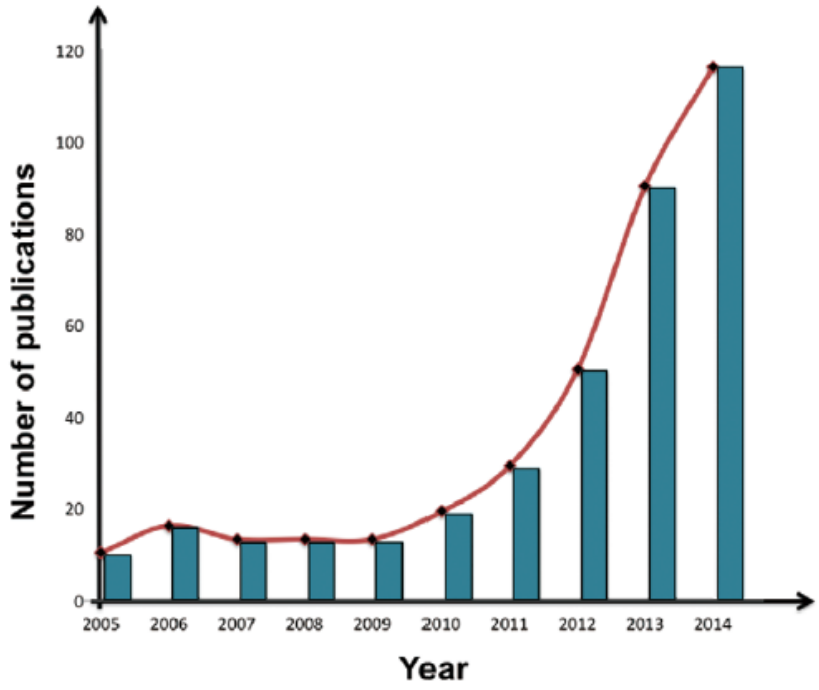

Figure 8: Exponential growth in PUBMED articles surrounding T1 mapping [71].

5.4 *Cardiac and skeletal muscle biomarkers*

5.4.1 Troponins

High sensitivity troponin titres have been sampled in most rheumatological conditions known to be associated with cardiac involvement as a potential tool for identifying or monitoring cardiac disease. Both troponin I and T seem to have potential in this field although Troponin I appears to have greater selectivity for myocardial tissue [72] since troponin T is also expressed by skeletal muscle [72-75]. In SLE, troponin T has been used to identify the presence of subclinical cardiac disease and to monitor treatment response [76]. One paper using troponin T in patients with sarcoidosis found troponin T elevation had a sensitivity and specificity of 87.5% and 75% respectively when compared with FDG- PET for identifying the presence of myocardial inflammation [77]. Likewise in rheumatoid arthritis, a

raised high sensitivity troponin I may help to detect sub-clinical cardiac involvement [78]. More recently an association between elevated troponin I and increased occult atherosclerotic plaque burden has been noted in both RA and SLE patients [79, 80]. In the myositis population, troponin I and T have been used to identify sub-clinical cardiac involvement [81]. Troponin T has also been cited as a possible skeletal muscle monitoring biomarker as it correlates well with skeletal muscle disease activity even in patients whose creatine kinase is not elevated [82]. Troponin I, being released only by cardiac myocytes, is the superior assay when trying to identify myocardial inflammation as both CK and troponin T may be raised by skeletal muscle disease. Systemic sclerosis appears to follow a similar pattern to that of the other conditions discussed [81].

In summary, it is likely that both troponin I and T have a role in the early identification of myocardial inflammation. Current understanding points to the use of troponin T in SLE and sarcoidosis whereas troponin I appears to have a more specific application in myositis, RA, and systemic sclerosis. However, the issue remains that in a large number of cases, no correlated cardiac pathology is identified using current clinical imaging techniques, leading to the question of the significance of a raised troponin.

B-type Natriuretic peptide (BNP/NT-pro-BNP)

While often elevated in the conditions being discussed, the association between disease presence and activity with the two common measures of B-type NP appear less convincing than with the troponin assays. This may be because the release of BNP is related to stretch, rather than inflammation and fibrosis which is the key pathophysiological feature of the process in rheumatological cardiac disease. There are currently no studies that correlate NT-pro-BNP with either disease activity or cardiac involvement in myositis.

Creatine Kinase (CK)

CK has long been the mainstay of biomarker monitoring in myositis. It is raised in inflamed skeletal muscle and is known to be elevated in cases where myocardial damage occurs. Given this, it has limited applications in delineating the presence of cardiac disease independently. Creatine kinase myocardial bound (CK-MB), which was historically used to try to specifically identify cardiac myocyte involvement, is also insufficiently specific [83, 84]. It is of note that recent work looking at the combination of CK measures with troponin titres

found a cohort of patients with myositis, normal CK, elevated troponin titres and clinical evidence of active skeletal muscle disease leading the researchers to conclude that CK in isolation may mislead clinicians treating patients with myositis [28, 81].

5.5 *Current position on the use of CMR and tissue mapping in myositis, a systematic review*

5.5.1 Introduction

Myositis is known to affect the heart in some cases. Currently the frequency with which this occurs is unclear. While a number of abnormalities have been described, myocardial inflammation and fibrosis appear to underpin them. Histological analysis of myocardial tissue in patients with myositis revealed inflammation in a similar pattern to that of the skeletal muscle [85, 86]. More chronic changes are mediated by fibrosis within the myocardium [86, 87]. Identification of these changes should allow for more accurate detection of cardiac involvement in myositis.

CMR is now the accepted gold standard non-invasive method with which to tissue characterise ventricular myocardium [56, 88, 89]. Understanding its current application in myositis is essential to understanding the true prevalence of cardiac involvement. Other cardiac investigations such as ECG, Holter monitoring, and echocardiography have been used to try to identify the presence of cardiac disease caused by underlying myocyte pathology. These techniques, however, have many limitations. In particular, they have limited sensitivity and specificity in detecting fibrosis and oedema which are the hallmarks of this disease process. Traditionally, myocardial fibrosis is diagnosed on CMR by LGE imaging. The pattern of LGE also gives a clue as to the aetiology of the fibrosis [90, 91]. In **Figure 6** the area of LGE involves the sub-endocardium (the area of interface between the blood pool and the myocardium) suggesting that the fibrosis is caused by an ischaemic insult. In the event that the LGE is seen either in the mid-wall or in the epicardium (the interface between the myocardium and the pericardial space) the aetiology is more likely to be non-ischaemic (potentially inflammatory, but with a wide differential diagnosis) (**figure 9**). Oedema assessment has been undertaken using T2 weighted MRI imaging [65]. T2 sequences are sensitive to the presence of water in tissue and have been shown to correlate well to areas of oedema in tissue. These sequences are effective, particularly where

pathology is focal. It has also been suggested that there may be applications in more subtle, diffuse, or sub-clinical presentations where a ratio between skeletal and cardiac muscle T2 are calculated. This allows a marginally more quantitative approach [92].

In a letter, Mavrogeni reported results of 16 patients with DM (9) and PM (7) scanned using standard CMR and found evidence of LGE in 9/16 patients (53%) [93]. Another study of 53 patients with myositis (excluding Inclusion Body Myositis (IBM)) found evidence of LGE in 62% of patients overall and 54.5% of patients with normal left ventricular function, adding further weight to the concept of a significant population with subclinical cardiac involvement [94]. This work has led to CMR with LGE becoming part of the recommended diagnostic pathway for patients with Myositis [95].

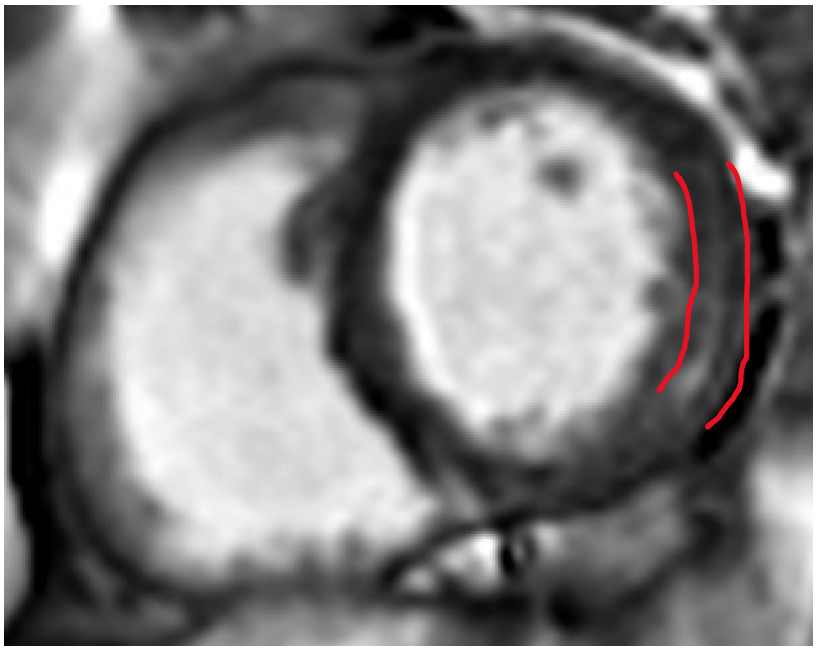

Figure 9: Image in short axis orientation of a patient with antisynthetase syndrome. An area of bright myocardium in the lateral and inferolateral walls (between red lines) that spares the sub-endocardium is suggestive of fibrosis caused by an inflammatory rather than an ischaemic process. In ischaemia the subendocardium is damaged first leading to scar appearing first at the interface between blood pool and the myocardium.

There are limitations to the current T2 weighted and LGE techniques in CMR. The main problem is that they are only semi-quantitative. T2 is a magnetic relaxation time constant that is an inherent property of all living tissue placed inside an MRI scanner. Current CMR T2 assessment techniques do not calculate actual myocardial T2 values in milliseconds but make areas of oedema appear brighter on the image than the surrounding areas [92, 96].

The problems with semi-quantitative approaches are that they may miss diffuse or subtle disease and make monitoring the effects of treatment difficult.

To address this problem, myocardial parametric tissue mapping sequences for T1 and T2 relaxation times are beginning to be applied to the myositis population. With the addition of a blood haematocrit, the myocardial extracellular volume can also be calculated. The data from these sequences is displayed as a colour map, with each pixel on the screen given a colour based on its underlying T1/T2/ECV value (depending upon which parameter is being measured) (**Figure 10**). A region of interest can then be drawn on the image to calculate the underlying T1/T2/ECV. In patients with conditions requiring monitoring over a long period of time the fact that a numerical value is produced makes disease monitoring more accurate.

A large amount of work has now been done in this field, using these techniques in healthy volunteers and patients with a number of different diseases [97]. There are now consensus guidelines for mapping techniques and the sequences have become commercially available [98]. Validation work has also been undertaken by comparing T1/T2 and extracellular volume values with myocardial biopsy to confirm correlation between sequences and direct tissue measurement [16, 99, 100]. Bull compared T1 values from a shortened modified Look-Locker mapping sequences with histological samples by calculating a collagen volume fraction. The collagen volume fraction showed a strong correlation with T1 values [16]. Bohnen demonstrated a correlation between T1 and T2 values and increased inflammation in the myocardium of subjects with acute myocarditis. In their prospective study T2 was shown to have the closest correlation with acute inflammation on endomyocardial biopsy [101].

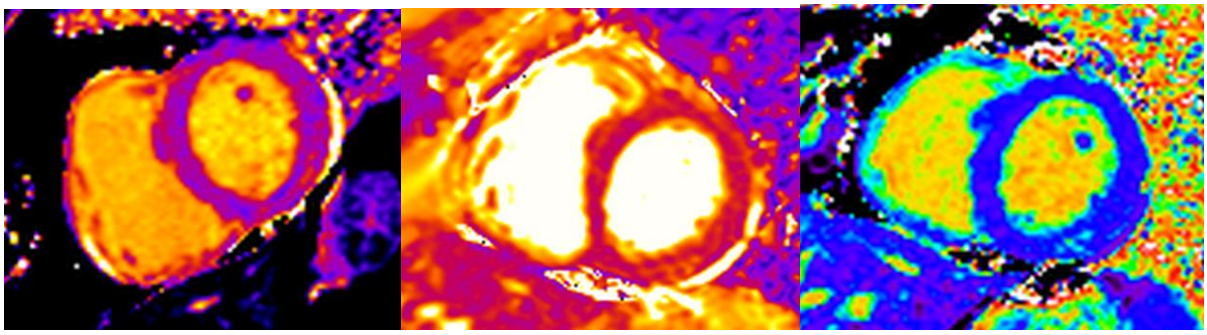

Figure 10: Short axis at the mid ventricular level showing the colour maps produced by parametric tissue mapping sequences. Left to right T1, T2 and ECV.

The benefits of these sequences are multiple. Subtle disease may not be picked up by reporting clinicians using traditional methods as the 'brightness' or 'whiteness' of the affected myocardium may not be 'different' enough from healthy tissue. In patients with balanced, diffuse disease the whole myocardium may appear slightly abnormal but the absence of 'healthy' myocardium for comparison may lead to the conclusion it is all 'normal'. As mentioned above the quantitative nature of these sequences is key to ensuring subtle disease is identified and that changes over time can be monitored. These sequences are beginning to be applied to patients with myositis and the results appear promising when attempting to identify the frequency of myocardial involvement.
What follows is a systematic review of the current literature to identify the current position with CMR in patients with myositis to attempt to systematically assess the current prevalence of cardiac involvement in myositis as defined by CMR.

5.5.2 Methods

A systematic review of literature was performed following the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) guidelines[102].

Search strategy & eligibility criteria

I conducted a literature search of Medline (2005 to 2019). The search was conducted using MeSH terms and keywords relating to myositis, CMR and T1 and T2, as follows (**Figure 11**):

☐	1	▶ myositis.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	41975	Advanced
☐	2	▶ IIM.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	2320	Advanced
☐	3	▶ idiopathic inflammatory myopathy.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	1457	Advanced
☐	4	▶ polymyositis.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	18595	Advanced
☐	5	▶ dermatomyositis.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	29068	Advanced
☐	6	▶ antisynthetase.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	1748	Advanced
☐	7	▶ T1.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	274813	Advanced
☐	8	▶ T2.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	231438	Advanced
☐	9	▶ Tissue mapping.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	182	Advanced
☐	10	▶ mapping.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	698897	Advanced
☐	11	▶ late enhancement.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	1989	Advanced
☐	12	▶ late gadolinium enhancement.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	10059	Advanced
☐	13	▶ LGE.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	8407	Advanced
☐	14	▶ Gad.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	24658	Advanced
☐	15	▶ 1 or 2 or 3 or 4 or 5 or 6	69960	Advanced
☐	16	▶ CMR.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	30740	Advanced
☐	17	▶ Cardiac MR.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	3105	Advanced
☐	18	▶ Cardiac MRI.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	13442	Advanced
☐	19	▶ cardiovascular magnetic resonance.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	49240	Advanced
☐	20	▶ Cardiac magnetic resonance.mp. [mp=ab, hw, ti, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	31082	Advanced
☐	21	▶ 16 or 17 or 18 or 19 or 20	77003	Advanced
☐	22	▶ 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14	1100289	Advanced
☐	23	▶ 15 and 21 and 22	65	Advanced
☐	24	▶ remove duplicates from 23	41	Advanced

Figure 11: OVID medline search terms

This was developed by Dr Daniel Bromage (DB) and myself in an iterative manner and was peer reviewed internally by members of the King's College London cardiovascular research fellows' group. Our search was limited to English language reports due to limited access and financial resources for translation. Duplicates were removed using Ovid and all remaining reports were screened for eligibility.

Study eligibility criteria were defined using a PICOS approach [103]. Review articles, letters and abstracts were excluded.

Reference	Subjects (n)	Diagnoses	Late gadolinium enhancement (LGE) assessed	LGE Present (n)	T2 weighted imaging performed	Elevated Signal intensity (n)	T1 Mapping performed	T1 elevated	T1 elevated (n)	T2 Mapping performed	T2 Elevated	T2 elevated (n)	ECV performed	ECV elevated	ECV elevated (n)	TnI measured	TnI elevated (n)	TnT measured	TnT elevated (n)	antibody association with presence or absence of cardiac disease
Bujo, S., E. Amiya, T. Kojima, S. Yamada, H. Maki, M. Ishizuka, M. Uehara, Y. Hosoya, M. Hatano, A. Kubota, T. Toda, and I. Komuro, *Variable Cardiac Responses to Immunosuppressive Therapy in Anti-Mitochondrial Antibody-Positive Myositis.* Can J Cardiol, 2019.	1	AMA positive Myositis	Yes	1	Yes	1	No		Not reported	No		Not reported	No		Not reported	Yes	1	No		No
Dieval, C., C. Deligny, A. Meyer, P. Cluzel, N. Champtiaux, G. Lefevre, D. Saadoun, J. Sibilia, J.L. Pellegrin, E. Hachulla, O. Benveniste, and B. Hervier, *Myocarditis in Patients with Antisynthetase Syndrome: Prevalence, Presentation, and Outcomes.* Medicine (Baltimore), 2015. **94**(26): p. e798.	12	aSS	Yes	7	Yes	3	No		Not reported	No		Not reported	No		Not reported	Yes	Not stated but likely 11/12	Yes	Not stated but likely 1/12	No
Huber, A.T., J. Lamy, M. Bravetti, K. Bouazizi, T. Bacoyannis, C. Roux, A. De Cesare, A. Rigolet, O. Benveniste, Y. Allenbach, M. Kerneis, P. Cluzel, A. Redheuil, and N. Kachenoura, *Comparison of MR T1 and T2 mapping parameters to characterize myocardial and skeletal muscle involvement in systemic idiopathic inflammatory myopathy (IIM).* Eur Radiol, 2019. **29**(10): p. 5139-5147.	20	NAM 7, aSS 5, PM/DM/overlap 8	Yes	7	No		Yes	Yes	Not reported	Yes	Yes	Not reported	Yes	Yes	Not reported	No		Yes	20	No
Jaworski, C., J.L. Looi, L.M. Iles, C.A. McLean, A.J. Taylor, and J.L. Hare, *Bright muscle, weak heart, bad start?* Heart Lung Circ, 2014. **23**(3): p. 293-4.	1	PM	Yes	1	Yes	1	No		Not reported	No		Not reported	No		Not reported	No		No		No
Khoo, T., M.B. Stokes, K. Teo, S. Proudman, S. Basnayake, P. Sanders, and V. Limaye, *Cardiac involvement in idiopathic inflammatory myopathies detected by cardiac magnetic resonance imaging.* Clin Rheumatol, 2019.	19	DM 4, PM 4, NAM 2, MNOS 4, DM/SSc 1, SSc with Myositis 2, IBM 2	Yes	9	Yes	Not reported	Yes (n= 15)	Yes	7	No		Not reported	No		Not reported	No		No		No
Mavrogeni, S., G. Markousis-Mavrogenis, L. Koutsogeorgopoulou, T. Dimitroulas, K. Bratis, G.D. Kitas, P. Sfikakis, M. Tektonidou, G. Karabela, E. Stavropoulos, G. Katsifis, K.A. Boki, A. Kitsiou, V. Filaditaki, E. Gialafos, S. Plastiras, V. Vartela, and G. Kolovou, *Cardiovascular magnetic resonance imaging pattern at the time of diagnosis of treatment naive patients with connective tissue diseases.* Int J Cardiol, 2017. **236**: p. 151-156.	5	DM	Yes	2	Yes	2	No		Not reported	No		Not reported	No		Not reported	No		Yes	0	No
Mavrogeni, S., P.P. Sfikakis, E. Gialafos, K. Bratis, G. Karabela, E. Stavropoulos, G. Spiliotis, E. Sfendouraki, S. Panopoulos, V. Bournia, G. Kolovou, and G.D. Kitas, *Cardiac tissue characterization and the diagnostic value of cardiovascular magnetic resonance in systemic connective tissue diseases.* Arthritis Care Res (Hoboken), 2014. **66**(1): p. 104-12.	19	IIM	Yes	7	Yes	0	No		Not reported	No		Not reported	No		Not reported	Yes	0	No		No
Mavrogeni, S., P.P. Sfikakis, G. Karabela, E. Stavropoulos, G. Spiliotis, E. Gialafos, S. Panopoulos, V. Bournia, D. Manolopoulou, G. Kolovou, and G. Kitas, *Cardiovascular magnetic resonance imaging in asymptomatic patients with connective tissue disease and recent onset left bundle branch block.* Int J Cardiol, 2014. **171**(1): p. 82-7.	4	IIM	Yes	1	Yes	0	No		Not reported	No		Not reported	No		Not reported	No		No		No
Rosenbohm, A., D. Buckert, N. Gerischer, T. Walcher, J. Kassubek, W. Rottbauer, A.C. Ludolph, and P. Bernhardt, *Early diagnosis of cardiac involvement in idiopathic inflammatory myopathy by cardiac magnetic resonance tomography.* J Neurol, 2015. **262**(4): p. 949-56	53	PM 34, DM 13, NSM 4, GM 2	Yes	33	No		No		Not reported	No		Not reported	No		Not reported	No		No		No
Sado, D.M., R. Kozor, L. Corr, and J.C. Moon, *Global Myocardial Edema in Antisynthetase Syndrome Detected by Cardiovascular Magnetic Resonance Mapping Techniques.* Circulation, 2016. **133**(3): p. e25-6.	1	aSS	Yes	0	Yes	0	Yes	Yes	Not reported	Yes	Yes	Not reported	Yes	Yes	Not reported	No		No		No

Yu, L., J. Sun, J. Sun, J. Li, Y. Dong, X. Zhou, A. Greiser, Y. Han, Q. Zhang, Q. Xie, and Y. Chen, Early detection of myocardial involvement by T1 mapping of cardiac MRI in idiopathic inflammatory myopathy. J Magn Reson Imaging, 2018. 48(2): p. 415-422.	25	PM/DM	Yes	5	No		Yes	Yes	Not reported	No		Not reported	Yes	Yes	Not reported	No		Yes	22	No

Table 3: Manuscripts included in systematic review of literature. Late gadolinium enhancement (LGE). Troponin I (TnI). Troponin T (TnT). Antimitochondrial antibody positive myositis (AMA). Granulomatous myositis (GM). Non-specific myositis (NSM). Idiopathic inflammatory myopathy (IIM). Polymyositis (PM). Dermatomyositis (DM). Inclusion body myositis (IBM). Anti-synthetase syndrome (aSS). Necrotising autoimmune myopathy (NAM). Systemic sclerosis (SSc). Non-specific overlap myositis (MNOS).

Data collection and synthesis

Results were screened for eligibility using the title and abstract (level 1 screening), followed by the full text (level 2 screening). Eligibility was assessed independently and blinded. Any disagreements were resolved by consensus between assessors in all cases.

Variables for extraction were selected *a priori* according to factors considered likely to impact on diagnostic accuracy, based on clinical experience, extrapolation from similar conditions. This also included patient demographics, details of clinical presentation, and specific CMR sequences. Data was extracted independently by the author using a sheet based on the Cochrane Consumers and Communications Review Group template [104]. As reports were expected to be confined to case reports and series, no study level quality assessment was made.

5.5.3 Results

65 papers were identified. Following removal of duplicates, 41 papers remained. These underwent further review (see PRISMA diagram (**Figure 12**)). The two Huber papers report the same patient cohort and so one of these papers was excluded. Eleven papers were included in the study. These yielded 160 individual cases covering PM, DM, antisynthetase syndrome (aSS), antimitochondrial antibody positive myositis (AMA), granulomatous myositis (GM), non-specific/overlap myositis, and necrotising autoimmune myopathy (**Table 3).**

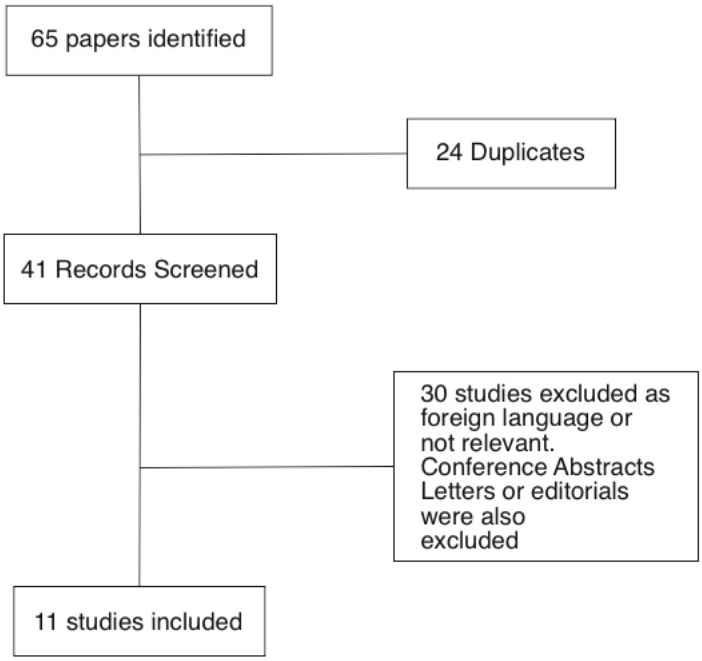

Figure 12: QUORUM diagram of the study selection process used to identify included articles in the systematic review

Late gadolinium enhancement

All studies in this review used LGE as the biomarker to assess for the presence of fibrosis. Of the 160 subjects reviewed, 73 had evidence of LGE (46%) [5, 94, 105-114]. The largest sample size in an individual study was 53 patients with a diagnosis of myositis (34 PM, 13 DM, 4 non-specific myositis and 2 GM) [94]. Of the 53, 62% had CMR evidence of myocardial fibrosis on the basis of LGE.

Three patients in the Khoo et al. paper had overlap systemic sclerosis [110] and 2 had inclusion body myositis (IBM), excluding them from analysis.

Native T1 and ECV

Yu et al. provide the largest sample size of patients who underwent native T1 and ECV assessment. They compare their findings in 25 participants (PM 13, DM 12) to age matched healthy volunteers [114]. Patients in this study were prospectively enrolled and any prior cardiac disease was an exclusion criterion. Eighty eight percent (n=22) of the subjects were found to have elevated Troponin T (TnT) at the point of enrolment. Twenty percent (n=5) of participants had evidence of late gadolinium enhancement. A statistically significant

increase in both native T1 and ECV between patients with myositis and healthy volunteers [108] was observed.

Khoo et al report an higher T1 relaxation time in 47% of 15 subjects compared to healthy volunteer derived normal values [110]. Huber et al also reported ECV in their cohort of 20 patients with myositis but failed to demonstrate a significant difference between myositis and healthy controls

T2 mapping

In the paper by Huber et al, 20 myositis subjects (7 necrotising autoimmune myopathy, 5 aSS and 8 with PM, DM or overlap myositis) with high troponin were analysed using T2 mapping [107, 108]. 7 of the 20 (35%) patients had LGE. This study found a significant difference in native T1 and T2 values between patients with myositis and healthy volunteers but no significant T2 difference between LGE positive and negative patients with myositis [108].

T2 Weighted imaging

Mavrogeni S et al. published a retrospective analysis of 19 patients with myositis separated into two groups (n=9 and n=10) [112]. The authors separated those presenting with typical or atypical cardiac symptoms and myositis. None of the patients in the study had an elevated TnI. These 19 patients represent the largest series of patients in which T2 weighted imaging was undertaken. None of the 19 patients with myositis were found to have elevated T2 signal intensity regardless of symptomatology.

Troponin elevation

Troponin levels are discussed in 6 of the papers with both troponin I (TnI) and troponin T (TnT) reported. Troponin elevation was a recruitment criterion in both the Huber et al. and the Dieval et al papers. Dieval et al. selected 12 subjects from 352 in the French National Registry. They required elevation of either TnI or TnT but did not stipulate which for each case. On review of the data table it appears, however, that the majority had TnI measured [106]. The Huber paper exclusively used TnT [108]. As previously stated, Yu et al. report elevated TnT in 88% (n=22) of their patients with myositis [114]. Bujo et al. describe a single case of AMA myositis with elevated TnI [105]. In both Mavrogeni papers, troponin was

measured. However none of the myositis subjects had elevated levels of troponin (TnI or TnT) [111, 112]. Troponin elevation is associated with LGE in 36% of 55 cases when both TnT and TnI are considered. Only one case clearly reported the association between TnI and LGE. Given the Dieval paper does not distinguish between troponins it was excluded from the troponin specific calculations.

5.5.4 Discussion

Identifying the incidence of cardiac involvement

Although the above describes several different studies approaching the same question with different methods, the presence of sub-clinical cardiac involvement in myositis is significant with 20% of DM/PM deaths reported to be secondary to a cardiac cause [2]. All studies using parametric tissue mapping found a significant increase in T1, ECV and T2 relaxation times as compared to either institutional normal values or to healthy volunteers, despite the subjects often having no symptoms and negative 'traditional' investigations.

The significance of extracellular space expansion has yet to be defined in myositis but in other diseases, measuring ECV, T1 and T2 has become an important tool in both diagnosis and disease monitoring [6, 115, 116]. Late gadolinium enhancement alone identified disease in 46% of subjects tested regardless of the presence of LV impairment, troponin elevation or symptoms. This is important as the presence of LGE in the general population, regardless of the cause, is an adverse prognostic marker (although how adverse differs according to the underlying disease process) [91, 117-122]. In acute viral myocarditis, tissue mapping has identified areas of myocardial involvement not seen with LGE imaging [123]. This is key in myositis as myocardial oedema/inflammation forms part of the pathophysiology of myocardial involvement. It is likely, therefore, that the actual incidence of cardiac involvement in myositis is significantly higher than the 46% figure based on LGE assessment alone.

The use of troponin as a discriminator does not significantly alter the proportion of patients identified as having cardiac involvement. Within the above cohort, filtering participants with raised troponin actually decreases the percentage with LGE identified and importantly only 29% of 42 patients with elevated TnT had LGE. There are a number of possible reasons for this. The first is the knowledge that TnT is released from healing skeletal muscle as well as cardiac muscle and is therefore less specific than TnI [28]. This is particularly so in patients

with myositis as healing skeletal muscle in this disease process is common. The second is the fact that the presence of LGE does not distinguish between acute and chronic myocardial injury whereas troponin levels are acute phase reactants. The third reason could be that the level of myocardial inflammation and eventually fibrosis is sufficient to elevate T2 then T1 quantitative values but insufficient to give visible changes using the semi-quantitative LGE technique.

Distinguishing between acute and chronic cardiac involvement

The presence of LGE does not distinguish between acute inflammation and scarring/fibrosis from a previous episode of acute inflammation. Late enhancement shows extracellular space expansion and so can be present due to the presence of scar (more collagen in the extracellular space) or acute oedema (more water in the extracellular space). T2 weighted imaging, which is much more specific for oedema, is seen in one of the case reports where troponin I is also positive, suggesting an acute process. In ¼ of patients in the Dieval et al series [106] T2 weighted images demonstrated signal hyperintensity. Given that all subjects had raised troponin and that it appears that most of these were the more specific TnI, a greater number of cases with a high signal intensity would be expected. This is potentially explained by the known limitations of the semi quantitative STIR sequence as mentioned above, but also by the fact that the sequence requires an area of 'normal' myocardium with which to compare. This is where fully quantitative T2 parametric mapping sequences have the potential to improve sensitivity. They produce a numerical value for T2 which allows for identification of more subtle and / or diffuse disease.

The Huber et al [108] paper demonstrated a significant difference in T1 and T2 values in their cohort of TnT positive patients compared with healthy volunteers. T1 is impacted by fibrosis, oedema, or infiltration. Whereas, T2 is much more specific for the assessment of oedema. The findings in the Huber et al paper therefore suggest the presence of an acute oedematous process within the myocardium.

The Yu et al [114] paper measured T1, ECV and troponin T and found a significant difference in T1 and ECV between myositis and controls. Interestingly they did not observe a difference in T1 between their TnT positive and negative patients with myositis. Thus, it would appear, even from the limited data available, that the combination of T1 and T2 mapping has the

potential to both identify the presence of myocardial disease but also distinguish acute and chronic disease.

5.5.5 Limitations

Given these are relatively new sequences being applied to this rare condition, the available evidence is limited. The sample sizes are small, and the imaging methods are varied making it difficult to compare like with like. In addition to the small sample sizes, the study population varied significantly, as did the indications for scanning, making a degree of bias in recruitment likely. This limited the depth of analysis that was possible. The varied use of troponin I and T makes correlation with levels and tissue characteristics difficult. None of the recruited patients had myocardial biopsy correlation assessment with T1 and T2 mapping values.

5.5.6 Future Research

Studies using TnI that directly compare T1 and T2 values in patients with myositis with positive and negative troponin are needed to identify the incidence of cardiac involvement. Validation of the accuracy of CMR mapping and TnI in identifying acute myocardial inflammation in myositis is a key step. At this point the clinical significance of low-level diffuse disease in this cohort is unclear. However, in other conditions the presence of sub-clinical disease is sometimes a precursor to progression of cardiac involvement. The importance of longitudinal studies is two-fold; first, to establish the pattern of progression of myocardial involvement with time and assess its significance, and second, to assess whether these sequences can have a meaningful role in monitoring treatment response.

5.5.7 Conclusion

Modern quantitative parametric tissue mapping CMR sequences may offer information above and beyond of that found by the LGE and STIR semi quantitative techniques. Acute myocardial inflammation in patients with myositis maybe seen even in asymptomatic patients, suggesting there could be a sub-set of patients with active heart muscle disease who are not being identified by traditional means. Even those with no biomarker evidence of active cardiac disease have evidence of chronic elevation in T1 suggesting underlying

myocardial disease. Further studies using the more specific troponin I and mapping sequences over time are needed to further evaluate the potential of this safe, non-invasive imaging method.

5.6 *Summary*

In this section, the manifestations, particularly in the heart, of myositis has been introduced. The current clinical work up for patients suspected of myocardial involvement was discussed, along with its limitations. The following chapters explore the potential to improve this with blood TnI and CMR based myocardial tissue mapping techniques.

6. Methods

6.1 Ethical approval

Ethical approval was granted (Essex REC – REF 17/EE/0294) to study healthy volunteers and patients with myositis. Original ethical approval permitted up to 4 CMR scans per recruit. Non-contrast scanning was approved for healthy volunteers, whereas permission was granted to administer intravenous (IV) gadolinium to patients with myositis during their first and last scans with non-contrast scans undertaken between these. Initially the protocol did not include the sampling of blood but did permit the use of blood results requested by the subjects' clinical team. I was granted a substantial amendment to allow blood samples to be drawn at the time of IV cannulation for the gadolinium enhanced scans as this ensured contemporaneous biochemistry results with the scan.

6.2 Study protocol

The overall study protocol is detailed in the following section. Within each of the specific results chapters the specific subjects or data sets used will be detailed.

6.2.1 Study population

Healthy Volunteers

Healthy volunteers were recruited from King's College Hospital and King's College London. A generic email was distributed to the cardiology department at King's College Hospital. Members of the radiology department were also recruited by word of mouth. All potential participants received written information (Healthy volunteer patient information sheet

appendix 4) about the nature of their participation and in the project as a whole and provided written consent.

Patients with myositis

King's College Hospital rheumatology department is a national centre for the treatment of myositis. Patients with a new diagnosis of myositis with either a raised troponin or cardiac symptoms (chest pain, palpitations, pre-syncope or peripheral oedema) as assessed by the clinical and research teams were approached for recruitment. Also, patients with existing established disease suffering a relapse, cardiac symptoms (as above) or troponin elevation were recruited. In order to compare, patients with quiescent disease were also recruited although the numbers of such patients was lower as the process of following patients over a period of months meant many of those already recruited attended for follow up scans with stable symptoms. Patients were highlighted for recruitment by the clinical teams or by inpatient referrals through the rheumatology service. All potential participants received written information (Participants' information sheet **Appendix 2**) about the nature of their participation and in the project as a whole and provided written consent only after having sufficient time to decide on their desire to participate.

Inclusion Criteria

- Healthy volunteers with no history of prior cardiac disease
- Patients with confirmed diagnosis of myositis by validated method [124]
- Adults aged 18 years old or over

Exclusion criteria

- Patients with a contraindication to MRI scanning
- Patients with a contraindication to IV gadolinium administration (e.g., eGFR <30)
- Pregnancy (testing for any female participants who were unsure)
- Healthy volunteers found to have an underlying cardiac condition
- Patients unable to give informed consent

6.2.2 Consent

All participants signed a consent form detailing their role in the study and the project outline (**Appendix 1**). Prior to giving consent, patients were provided with written information regarding the study objectives, design and contact details (**Appendix 2**). What was entailed was explained in detail and the participants' understanding and capacity to consent was assessed. It was stressed to patients that participation was voluntary and that if they chose not to be involved their clinical care would not be affected. It was also made clear that, except for skeletal muscle mapping (recorded from the same sequences as the cardiac mapping sequences) the CMR sequences are clinically indicated and they may undergo them as part of their routine clinical care either way.

6.2.3 Healthy Volunteer Protocol

All healthy volunteers had a detailed cardiac medical history taken to ensure that they had no previous history of heart disease. Participants underwent a non-contrast truncated CMR (detailed below). T1 and T2 mapping sequences were acquired in both long and short axis orientation with a large field of view for the four chamber acquisition for the purposes of measuring the T1 and T2 values in skeletal muscle. Full details of the CMR protocol are listed below.

FOLLOWING RECRUITMENT AND CONSENT	
Medical History	Single CMR with T1 and T2 mapping as part of a truncated protocol. No contrast was given

Figure 13: Healthy volunteer protocol schema

6.2.4 Myositis patient protocol

Following recruitment, subjects underwent the usual cardio-rheumatologic work up as advised by their clinical rheumatology team. This consisted of an ECG, 24-hour tape,

transthoracic echocardiogram (TTE) and blood tests. The results of these were made available for use in the study. The first CMR performed was a full LV volume and gadolinium study as would be undertaken for any patient referred in for a routine CMR. All the scans were performed by the author except for five. In addition to the full diagnostic protocol, T1 and T2 mapping was performed in long and short axis with additional large field of view imaging in the 4 chamber view to allow measurement of skeletal muscle T1 and T2. The full CMR protocol details are below.

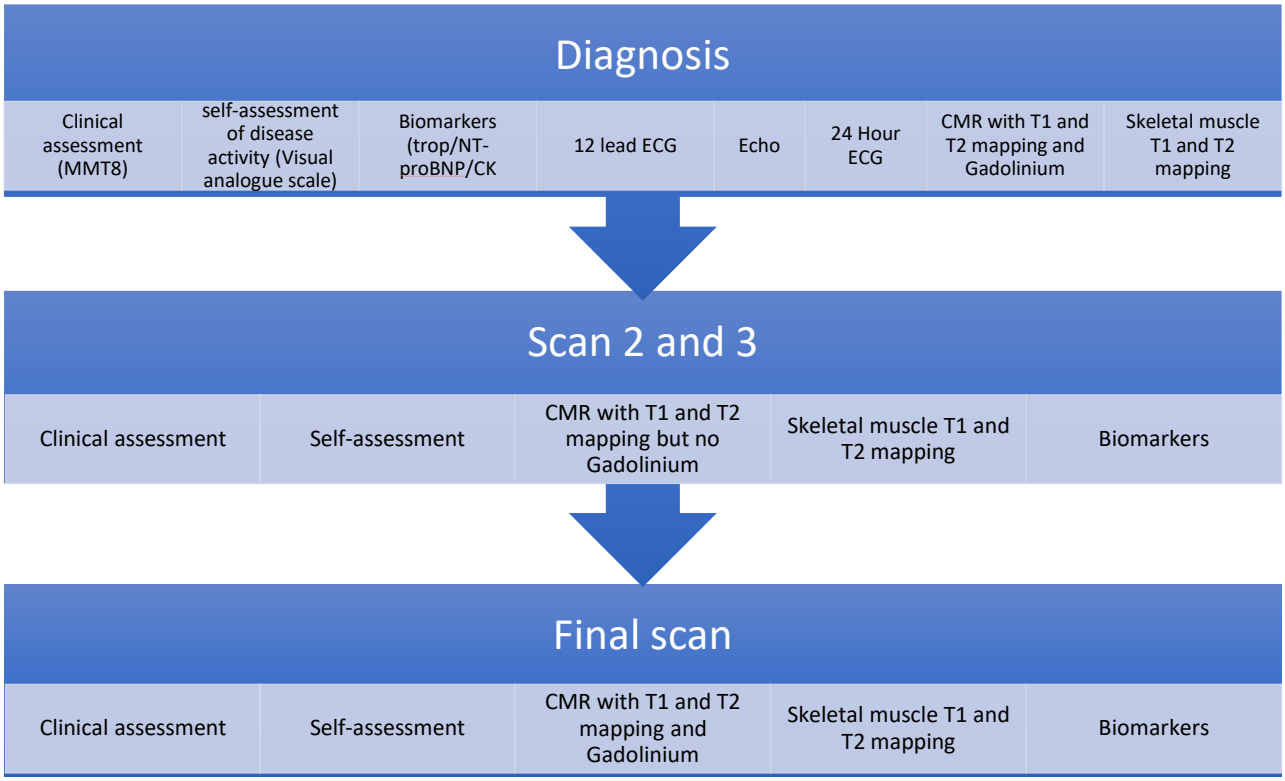

Figure 14: Flow chart showing journey of patients with myositis through the research protocol. Biomarker, patients on self-assessment of their condition using a visual analogue scale and clinical assessment was repeated at each attendance, as was the mapping component to the CMR protocol

Patients with myositis were invited to return for three further scans over the course of at least 12 months. The initial intention had been to confine the time course to 12 months, however, with limited scanning time available, this was extended to ensure the maximum number of patients could attend for repeated scans. The second and third scans followed the same protocol as that of healthy volunteers where no contrast was administered. Ethics had not been granted to sample blood at the same time, however, in the event there was a clinical indication for blood sampling as judged by the subjects' clinical team then these were arranged on the same day as the CMR. For subjects' final study, the same full

diagnostic protocol including early and late gadolinium images were undertaken for comparison. At this point, where possible, contemporaneous blood samples were taken.

6.2.5 Data collection and handling

All patients and healthy volunteers recruited were assigned a unique participant number to allow data anonymisation. This identifier was used to collate data from each study participant. Since the investigations undertaken were routine clinical investigations, the results were made available to the clinical team via the electronic patient records system (EPR) in all cases. Each CMR undertaken was formally reported by a Consultant Cardiologist with the results available to the clinical team. In the event of significant abnormality in scans from either group, participants were invited to attend for review in clinic with Dr Dan Sado, Consultant Cardiologist and lead for CMR at King's College Hospital. Images were stored in DICOM format (standard format) on the KCH PACs system in line with the KCH trust policy. This data, along with the clinical reports were stored on an encrypted trust computer and remain on trust property. The reports are available to the clinical team via EPR. Once the clinical scan was complete the information required by the study was anonymised and extracted for statistical analysis. This data has been stored on a biometrically (fingerprint) protected computer.

Research Subjects				Segment									Means and SD					
Subject	months	Scan number	Troponin (Negative=0,1	Inferoseptum	Inferior	Inferolateral	Anterolateral	Anterior	Septum	Inferior	Lateral	Anterior	16 segment	SD	12 segment	SD	Mid septal	SD
DNP001	0	1	0	1017	1030	1049	972	953	1050	1019	1011	1022	1012	27	1007	29	1011	8
	8	2	0	1027	979	1018	988	999	1007	959	986	938	1001	32	1011	27	1031	5
DNP002	0	1	2	1062	1027	1019	1017	1053	1046	1020	1025	1034	1033	34	1033	39	1035	38
	13	2	0	1030	971	1012	1042	981	982	1052	1028	1033	1012	25	1008	23	1010	29
DNP004	0	1	0	1053	1065	1017	1019	1021	999	1030	1078	1029	1027	23	1025	20	1034	28
	8	2	0	1034	1027	999	1012	960	1012	1039	1015	991	1012	20	1011	21	1020	20
DNP005	0	1	2	1085	1043	1052	1049	1153	1069	1061	1032	1033	1076	48	1085	51	1060	36
	8	2	3	1067	1050	1010	1047	999	1083	1052	1039	1025	1054	28	1056	29	1058	13
	12	3	0	1031	1059	1027	1007	983	1036	1023	1034	996	1029	21	1032	22	1019	18
DNP006	0	1	1	1161	1107	1114	1099	1060	1132	1121	1102	1128	1113	24	1110	27	1135	37
	7	2	3	1026	1002	1012	1040	1027	975	1044	1026	933	1009	30	1014	20	1021	7
	11	3	0	1008	1000	952	980	934	980	961	1021	1016	996	31	997	33	1020	16
DNP007	0	1	1	1028	1049	1007	1050	1040	1047	1033	1027	1016	1034	11	1035	11	1034	8
	4	2	1	1055	1018	1034	1044	1021	1050	1012	1035	986	1037	23	1043	20	1053	4
	8	3	1	1044	969	1000	1065	1041	1051	1090	1047	1014	1042	34	1040	35	1059	21
	12	4	0	981	989	974	989	964	982	949	970	980	981	14	984	13	988	9

Figure 15: Example of anonymised mapping data following extraction

Data from patient questionnaires or functional assessments is filed in a designated folder kept on hospital premises in a locked, key card access office. Data collected on paper does not contain patient identifiable data, rather the patients' unique identifier.

6.2.6 Blood sampling

All blood samples were collected by either the hospital's own phlebotomy department or by the author. Samples were sent for analysis to the King's College Hospital laboratory and no samples were stored. Results of all samples acquired were made available to the clinical team and reviewed by the author. In the event of significant or unexpected abnormality, I personally informed the individuals' clinical team. Initially, blood sampling was to be undertaken by the subjects' clinical team, however, it became apparent that contemporaneous samples were required. To this end the author applied for and was granted a substantial amendment to the protocol allowing for blood to be drawn at the point at which IV cannulation was undertaken when patients were having contrast enhanced scans. In the event no contrast was being given, blood samples were only collected in the event of a clear clinical indication.

6.2.7 CMR protocols

Patients attending for CMR completed a safety questionnaire to assess whether it was safe to enter the scanner. The safety of CMR in individuals without contraindication has long been established [125]. For scans where the full clinical protocol is required, including the administration of IV gadolinium, intravenous access was gained using an intravenous cannula. Participants underwent a CMR protocol of approximately forty minutes allowing all clinical sequences to be run. In that time, we performed T1 and T2 mapping of myocardium and skeletal muscle.

Full diagnostic, contrast enhanced study

Patients with myositis undergoing their first scan and their last scan were subjected to a full clinical protocol including tissue mapping sequences. In the event of ongoing clinical concerns from the patient's clinical team, additional contrast enhanced scans were offered. All scans were performed on the same 1.5 Tesla Siemens Magnetom Aera, (Siemens Healthcare GmbH Erlangen, Germany) at King's College hospital, London, UK.

Localiser imaging

Single shot pilot images with the following parameters:

Repeat time: (TR): 288ms

Echo time (TE): 1.13ms

Slice thickness: 8mm

Field of view: 400x400mm

Read matrix: 256

Flip Angle: 60°

HASTE imaging

Axial imaging through the thorax was undertaken in both black and white blood

TR: 700ms (black blood) and 500ms (white blood)

TE: 27ms and 1.22ms

Slice thickness: 8mm

Cine Images

Steady state free procession (SSFP) cine imaging was performed. First in the long axis following traditional planes used in echocardiography, namely 4, 3 and 2 chamber acquisition where the apex is presented and two of the LV walls are planned perpendicular to the imaging plane. In the 4 chamber the anterolateral and inferoseptal walls are seen. In the 3 chamber the inferolateral and anteroseptal walls and in the 2 chamber the anterior and inferior walls. In CMR there is a convention that a fourth long axis view is acquired through the left ventricular outflow tract. From the 4 and 2 chamber images a 'stack' of short axis slices are then planned through the left ventricle. These are planned perpendicular to the left ventricular walls and show all the LV walls from base to apex. The default imaging parameters below were adjusted according to the individual patient to ensure the highest quality imaging possible.

Cine imaging parameters:

TR: 36.82ms

TE: 1.1ms

Slice thickness: 8mm

Voxel size: 1.8x1.8mm

Flip Angle: 80°

Base resolution: 208

Phase resolution: 90%

Tissue mapping sequences

T1 and T2 maps were acquired pre contrast. As mentioned above, gadolinium potently reduces T1, so these sequences are performed before the administration of contrast.

T1 mapping was undertaken using a 5s(3s)3s Modified Look-Locker inversion recovery (MOLLI) protocol [126]. The acquisition parameters were:
Pixel bandwidth: 1085 Hz/pixel
Echo time: 2.7ms
Flip angle: 35°
Voxel size: 1.4x1.4mm
Slice thickness: 8 mm

T2 mapping was performed using a T2-prepared steady-state free precession (SSFP) sequence [127] with the following imaging parameters:
Pixel bandwidth: 1184Hz/Pixel
Echo time: 2.5ms
Flip angle: 70°
Voxel size: 1.9x1.9mm
Slice thickness: 8mm
T1 and T2 maps were performed in all three long-axis (4,2 and 3 chamber views) and 2 basal, 2 mid and 2 apical short axis slices. A large field of view (500mm) 4 chamber view was

acquired, both centred over the heart and centred over the left arm to try to capture skeletal muscle measurements at each scan. The rationale for the specific cardiac views is discussed in detail in **Chapter 7** of this thesis.

For each of the mapping sequences a manual shim was applied to the myocardium to reduce magnet bore heterogeneity caused by interference from metal or other magnetic fields in the local environment. For the skeletal muscle imaging the manual shim 'box' was moved to the edge of field over the area of interest.

Post contrast early and late (gadolinium) enhancement imaging

Default imaging parameters:

Flash Segmented Gradient Echo Inversion Recovery

Repeat time 737ms

Echo time 3.21ms

Flip angle 25°

Voxel size 1.3x1.3mm

Slice thickness 8mm

Field of View 320mm (adjusted as required)

Base resolution 256

Single shot FISP Non segmented Imaging (in the event of significant breath holding difficulty or arrhythmia)

Repeat time 863.2ms

Echo time 1.02ms

Flip angle 40°

Voxel size 2.2x2.2mm

Slice thickness 8mm

Intravenous gadolinium (Gadovist®) was administered as a bolus via a hand injection at a dose of 0.1ml/Kg followed by a normal saline flush. Following this both early and late enhancement imaging sequences were undertaken.

Early gadolinium (EGE) imaging was acquired immediately after administration of contrast. The purpose of the sequences is to identify area of reduced tissue perfusion such as in acute infarction with microvascular obstruction. Also, thrombus takes up no contrast and as such appears black against the backdrop of a bright blood pool (**Figure 16**). Early gadolinium imaging is performed in the three long axis views.

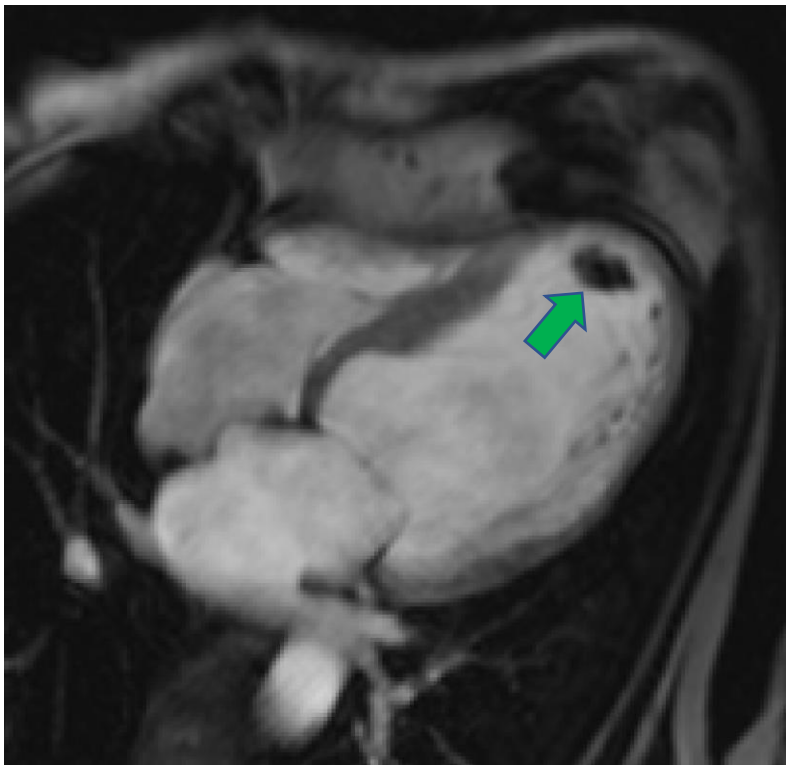

Figure 16: 'Early gadolinium' imaging showing bright, gadolinium rich blood pool contrasted against the black areas with no contrast uptake (arrow). In this case the area of no contrast uptake is a prominent thrombus in the LV apex.

Late gadolinium imaging was undertaken from around 5-15 minutes post injection (**Figure 17**). Long axis views were acquired first. This was followed by each of the short axis slices which were planned by directly copying the position of the short axis cine images. This allowed for direct comparison of the late enhancement images with their corresponding cine image to avoid mistaking blood pool for late enhancement. Following this, the long axis late gadolinium images were repeated at longer inversion times. Throughout the acquisition of late enhancement images, the inversion time was adjusted to ensure the optimum

contrast between myocardium (black) and gadolinium (White). In the event late enhancement was seen, correlation between long and short axis acquisitions was made to reduce/eliminate artefact. In the event further information was required, images were acquired again in phase-swap or systole.

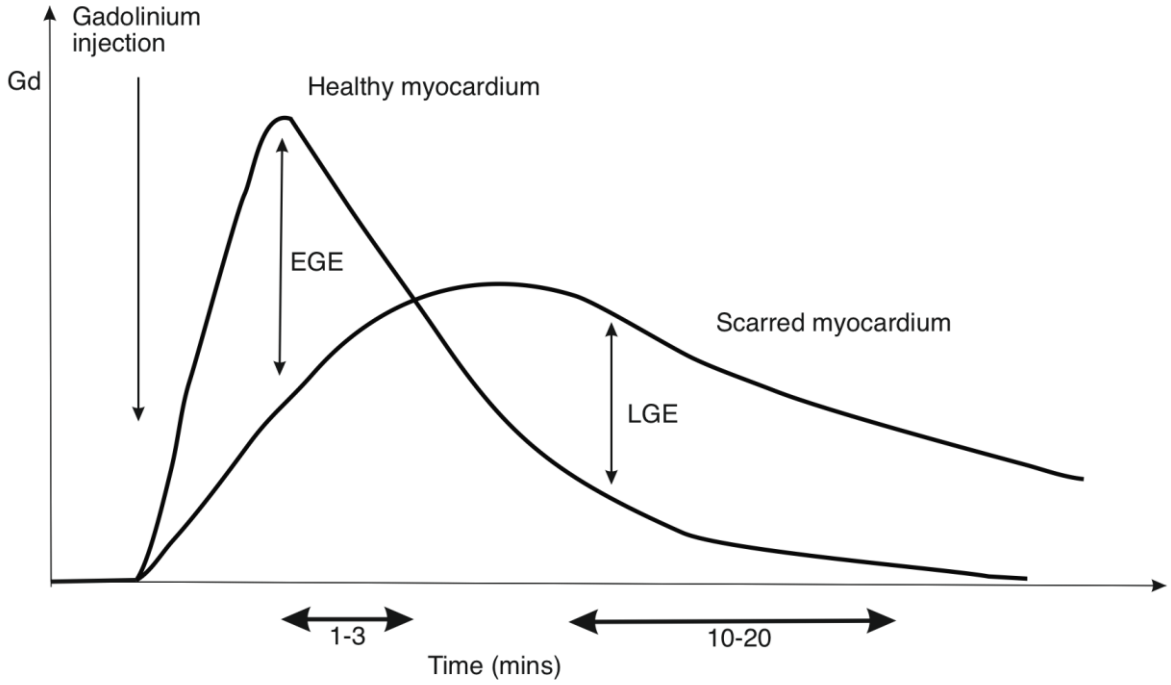

Figure 17: Schema of gadolinium (Gd) diffusion characteristics into and out of healthy and scarred myocardial tissue. Early gadolinium enhancement (EGE) shows the contrast in gadolinium concentrations between healthy and unhealthy tissue due to decreased perfusion. Late gadolinium enhancement (LGE) shows contrast between scarred, fibrosed or inflamed myocardium and healthy myocardium due to collection of late enhancement in the areas of increased extracellular space.

Truncated CMR study

Subjects recruited into the study underwent multiple CMR studies as detailed above. For the healthy volunteer subjects, no contrast enhanced imaging was undertaken. The localiser images were as detailed above with the exception of HASTE images which were not routinely performed as they are not related to myocardial assessment. The mapping sequences as detailed above were undertaken in full. The same protocol was observed for patients with myositis at their middle attendances. If there had previously been note of abnormality in the HASTE images then these were repeated to aid the subjects' clinical

team, however, in the event these had been normal initially they were not routinely repeated.

6.2.8 Offline image analysis

Images were stored as DICOM images and transferred to trust encrypted computers for analysis.

Healthy Volunteers

All DICOM images were interpreted using specifically designed reporting software (version 5.10.1, Circle Cardiovascular Imaging Inc., Calgary, Canada). All healthy volunteer studies were clinically reported by the author. Every scan resulted in a formal report made available to the individual subject at their request. Each of the clinical reports was finalised by Dr Dan Sado, consultant cardiologist, honorary senior lecturer at King's College London and the study chief investigator. The tissue mapping sequences were interpreted by the author using a manual contouring approach. This was performed in a single mid septal short axis slice (**Figure 18**) and in a multi-segment method involving 16 of 17 (the apical segment was omitted) American Heart Association segments conventionally used in echocardiography [7]. **Figure 19** shows an example of contours drawn across each of the myocardial segments in the basal, mid, and apical cavity. The data from the mid septal regions of interest was used to establish the reference normal values for our departmental scanner. As relaxation times can vary between individual scanners and with changes in scanner environments, four healthy volunteers were invited back for repeat scanning at a 3-month interval to ensure no changes in the T1 and T2 values had occurred.

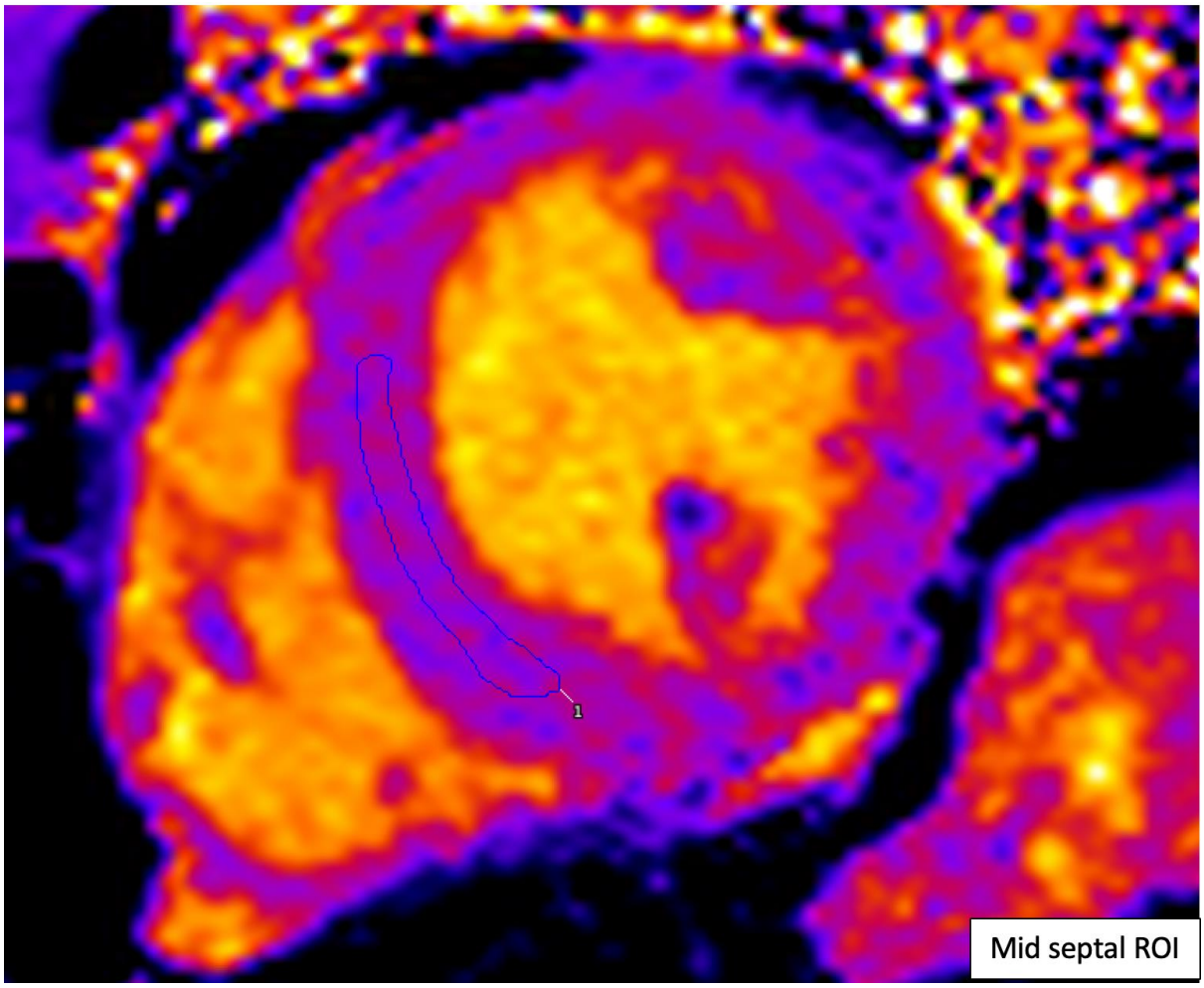

Figure 18: Mid-septal region of interest (ROI). Conventional T1 and T2 mapping measurements rely on the measurement of values in the basal or mid septum as they are the most reproducible

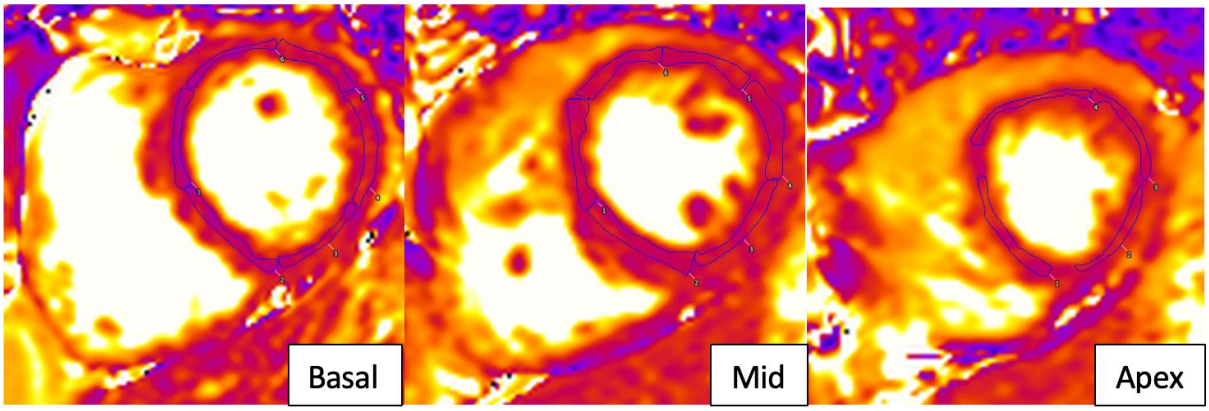

Figure 19: Basal, mid, and apical short axis T2 mapping images with manually drawn regions of interest positioned according to the American Heart Association (AHA) ventricular model.

Patients with myositis

Subjects with myositis had each of their scans, whether contrast or non-contrast enhanced, formally reported by a consultant cardiologist. All measurements presented in this thesis were undertaken by the author (with the exception of inter-operator variability assessment in **Chapter 7**). The reports were uploaded to the hospital electronic patient records, so they were available to the patients' own clinical team. Any significant abnormalities were highlighted to the rheumatology department by email.

Tissue mapping analysis was undertaken in the same way as detailed above in the healthy volunteer section.

6.2.9 Other Investigations

ECG: 12 lead Electrocardiogram was be performed on each patient.

Transthoracic Echocardiography: Patients underwent standard clinical transthoracic echocardiography

24-hour ECG recording: Patients were offered 24-hour Holter monitoring as part of their standard clinical work up for myositis

Functional assessment: Manual muscle testing (MMT8) is a validated assessment tool for muscle weakness which is performed at each clinical visit and involves a clinical assessment of eight muscle groups. The power of each group is assigned a score out of ten. Each score is added together to a total of 150 [128]. Patient global activity assessment tool is a visual analogue scale (VAS) of disease activity (**Appendix 7**) which patients completed at each CMR scan attendance [129].

7. Validation of a novel 12 segment approach to interpretation of parametric tissue mapping sequences based on the American Heart Association model

7.1 *Abstract*

Background: Parametric tissue mapping (T1 and T2) sequences in cardiovascular magnetic resonance (CMR) provide a quantitative means of identifying myocardial fibrosis and oedema without the need for contrast. Conventionally, values taken from a single septal region-of-interest (ROI) are evaluated. This can miss focal disease.

Hypothesis: A 12-segment model, based on the American Heart Association (AHA) 17-segment model, omitting the apical segments is reproducible and agrees with values from a mid-septal ROI.

Methods: 18 healthy volunteers and 11 patients with myositis underwent T1 and T2 mapping. 12 left ventricular segments were analysed by 2 readers and re-reported manually and using computer generated auto-segmentation. Coefficient of variation (CoV) and Bland-Altman plots were used to demonstrate correlation.

Results: Mean CoV of segment-by-segment comparison was 1.5% (range 0.1-6.7%) for T1 and 2.9% (0-9.9%) for T2 in healthy subjects and 3.1% (1.4-4.7%) for T1 and 5.68% (2.9-8.9) for T2 in myositis. CoV of 12-segment mean and mid-septal ROI was 0.82% (0.02-2.18%) for T1 and 1.86% (0.0-5.28%) for T2 in healthy volunteers and 0.19 $\pm$ 1.4% for T1 and -1.7 $\pm$ 4.5% for T2 in myositis.

Conclusion: The 12-segment approach is reproducible across each segment and provides a comparable overall mean to the mid-septal ROI used to establish normal. Therefore, abnormalities between values can be interpreted as suggesting the presence of disease.

7.2 *Introduction*

Currently, the majority of research papers in the T1 and T2 mapping field use a ROI in the mid-wall of the basal or mid septum. This has been shown to give highly reproducible values [133, 134]. In diseases where the process is diffuse (for example iron overload or amyloidosis) [115, 135], this approach works well. The rationale for selecting the septum at this level is to reduce the risk of inadvertent inclusion of blood pool or extracardiac structures within a ROI. The septum is also less prone to off-resonance artefact. Off-resonance artefact describes signal loss or geometric distortion caused by inhomogeneity in the scanners magnetic field. However, some cardiac diseases cause more focal inflammation. In this situation, a second ROI can be placed in the region of myocardium that appears most 'diseased' on the colour map to characterise the abnormality [136]. While effective, this method has a significant drawback: sampling a single segment gives insufficient information about the left ventricle as a whole. It also relies on the reader to identify the lesion.

Multi-segment analysis has been suggested as a way of quantitatively assessing the whole left ventricle [134, 137]. The potential benefits of this are two-fold. First, focal disease will be identified without the need for a qualitative assessment based on visual assessment of colour variations within a map, and second, an average value across all segments may identify subtle diffuse disease inadequately sampled by a single ROI [138]. Several studies have investigated a multi-segment approach in T1 and T2 mapping. Rogers et al compared mid-septal (MS) ROI with a mid-lateral wall ROI and a complete mid-septal slice and found a significant difference between all three values [133]. Similarly, Heiss et al. compared regions and ventricular levels as part of the AHA 17-segment model [7] and found regional differences between different parts of the myocardium [9]. These findings were mirrored in T2 by Bonner et al. who also used a 17-segment AHA approach to analysis, although they omitted segment 17 and found a significant difference between basal, mid, and apical T2 values [139]. Variability across the ventricle was also seen in a study by Wassmuth et al [8]. What is not clear from the published literature is whether mean T1 and T2 values from a multi-segment analysis is different from the value of the mid-septal ROI. This is key as if the two mean values are reproducibly comparable, then an observed difference is suggestive of focal or diffuse underlying disease.

The aim of this study was to demonstrate that a 12-segment myocardial model (**Figure 20**) is reproducible and is also comparable to septal ROI for acquiring a mean T1 or T2. If shown, this will allow its application in both diffuse and focal disease states.

7.3 *Methods*

Given the previously stated issues surrounding reproducibility of assessment of the left ventricular (LV) apex when tissue mapping, in this study we propose a 12-segment ventricular model. This is based on the 17-segment AHA model (**Figure 20**) [7], discounting the five apical segments, as these are difficult to accurately assess with mapping sequences.

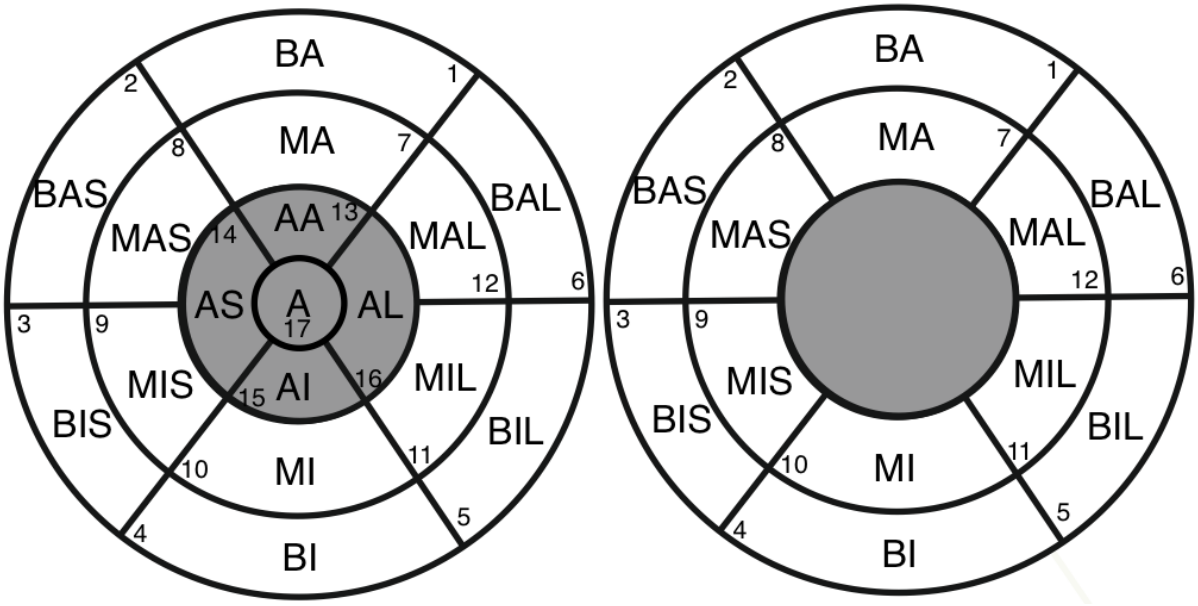

Figure 20: Schematic of AHA 17-segment model of LV myocardium (left). Segments in grey are omitted from the proposed 12-segment model (right). Basal anteroseptum (BAS), basal inferoseptum (BIS), basal inferior (BI), basal inferolateral (BIL), basal anterolateral (BAL), basal anterior (BA), mid anteroseptum (MAS), mid inferoseptum (MIS), mid inferior (MI), mid inferolateral (MIL), mid anterolateral (MAL), mid anterior (MA), apical septum (AS), apical inferior (AI), apical lateral (AL), apical anterior (AA), apex (A).

7.3.1 Population

Healthy volunteers and patients with myositis were included in this prospective study. All were recruited in accordance with the methods detailed above (**chapter 6**) and gave written informed consent in accordance with study protocol. The study obtained ethics committee approval (Essex REC – REF 17/EE/0294). Exclusion criteria for healthy volunteers included known underlying cardiac disease and contraindications to undergoing MRI scanning. In

patients with myositis the inclusion criteria mirror those discussed in the methods chapter above. Exclusion criteria were contraindications to undergoing CMR.

7.3.2 CMR protocol

All subjects were scanned on a 1.5T Magnetom Aera, (Siemens Healthcare GmbH Erlangen, Germany). Healthy subjects underwent a CMR protocol including localiser imaging and long and short axis cine sequences using the protocol described in the methods section of this thesis. T1 and T2 maps were performed in all three long-axis (4,2 and 3 chamber views) and 2 basal, 2 mid and 2 apical short axis slices. Apical slices were acquired as a reference to crosscut any long axis imaging to ensure a mid-chamber location within the ventricle. Patients with myositis underwent tissue mapping using the same method as for healthy controls but as part of a full CMR protocol as detailed in **chapter 6.**

7.3.3 Functional assessment

All subjects had formal assessment of LV structure and function by a Cardiologist experienced in CMR (Level 3 accredited). Left ventricular ejection fraction (EF) was calculated using proprietary software for short axis segmentation (Version 5.10.1, Circle Cardiovascular Imaging Inc., Calgary, Canada). End diastolic (EDV) and end systolic volumes (ESV) were calculated and indexed to body surface area. Any structural or functional abnormality at this point excluded any of the controls and resulted in follow up investigations and consultation being arranged. The patients with myositis underwent the same functional assessment but in conjunction with a formal clinical report of the full study with the results made available to the individuals usual clinical team. Abnormalities in function were not exclusion criteria in the patient group.

7.3.4 Analysis of parametric tissue maps

Analysis was undertaken using commercially available post-processing software (Version 5.10.1, Circle Cardiovascular Imaging Inc., Calgary, Canada). 3 readers (LD reader 1 (Level 3 CMR accreditation), DB reader 2 (Level 2) – control group, Dr Adam Nabeebacus (AN) (level 2) – disease group), blinded to the clinical details and to each other's contours, performed segmental analysis using manual ROI delineation (**Figure 21**) with care taken to ensure no contours included blood pool or extracardiac structures in accordance with

recommendations made by the T1 mapping development group [89, 98]. A single T1 or T2 value for each of segments 1-12 of the AHA 17-segment model [7] was recorded for each volunteer. Segments 13-17 were excluded (**Figure 20**). In the event a single short axis image was judged to be of insufficient quality, either the other acquisition in the same region was used or, if this was also of insufficient quality, the equivalent segment in the long axis was measured. Both long and short axis acquisitions were appropriately manually shimmed, and readers analysed the same segment for each subject. Analysis was then repeated by reader 1 following removal of all previously drawn contours and re-anonymising the images after 6 months. While convention at the time of the study was for manual contouring, early automated contouring was available so additionally the images were analysed using endo and epicardial delineation (with 10% endo- and epicardial offset). Automated segmental analysis of the basal and mid short axis slices using the anterior right ventricular (RV) insertion point as a reference was then performed for comparison with other methods. Normal T1 and T2 values for the scanner used in all studies was defined using the septal ROI mean ±2 standard deviations (SD) as per convention [89]. These became the standardised magnet normal values.

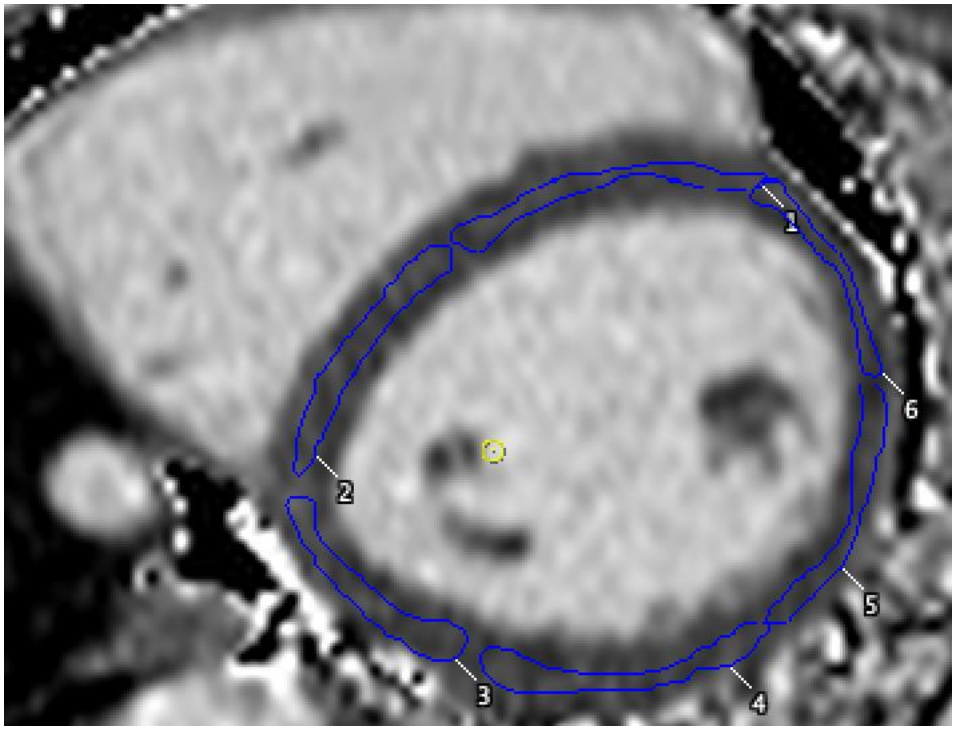

Figure 21: Example of manual ROI delineation in a basal short axis slice through the left ventricle

7.3.5 Statistical analysis

All statistical analyses were performed using GraphPad Prism 9 (GraphPad Software Inc, USA). Mean (±SD) T1 and T2 values of each subject were calculated for both the 12-segment model and mid-septal ROI. Bland-Altman analysis was performed comparing the T1 and T2 values for each segment and between the 12-segment and mid-septal ROI values. Coefficient of variation analysis (CoV) was performed between mid-septal ROI and the 12-segment model in both health and disease subjects. Bland-Altman [132] and CoV have been previously applied to different methods for analysing parametric tissue maps [140]. Further analysis in healthy volunteers compared repeated measures, interobserver variability and automated segmentation. The recruited patients baseline characteristics are detailed in **Table 4:**

	Healthy volunteers	Patients with myositis
Number (Gender M/F)	18 (9/9)	11 (4/7)
Mean age (years)	33 [25-38]	57 [31-78]
Mean height (cm)	173.5 [165-185]	173 [165-191]
Mean weight (kg)	75 [63.8-86.5]	92 [65-145]
Mean end-diastolic volume (EDV) (ml/m2)	81.1 ±11	72.5 ±19
Mean end-systolic volume (ESV) (ml/m2)	26.7 ±4	23.5±15
LVEF (%)	67.1 ±4.6	69±12
Native T1 (12 segment) (ms)	995 ±23.8	n/a
T2 (12 segment) (ms)	46 ±1.6	n/a

Table 4: Details of subjects. Overall mean native T1 and T2 value across all subjects. Left ventricular ejection fraction (LVEF)

7.4 *Results*

Of 19 healthy volunteers recruited, 18 (9 female) were included in the study. One female healthy volunteer was excluded due to abnormal LV volumes. 11 patients (7 female) with myositis were included. Mean T1 and T2 values were not compared across the disease patients. Full 12-segment analysis was undertaken 4 times in the 18 healthy subjects in

addition to mid-septal ROI assessment leading to a total of 882 T1 and T2 ROIs being interrogated. Mean (SD) T1 and T2 mid-septal ROI values were 1002 ±28 ms and 46 ±2 ms, respectively. These values ± 2 SD became the normal range for our scanner.

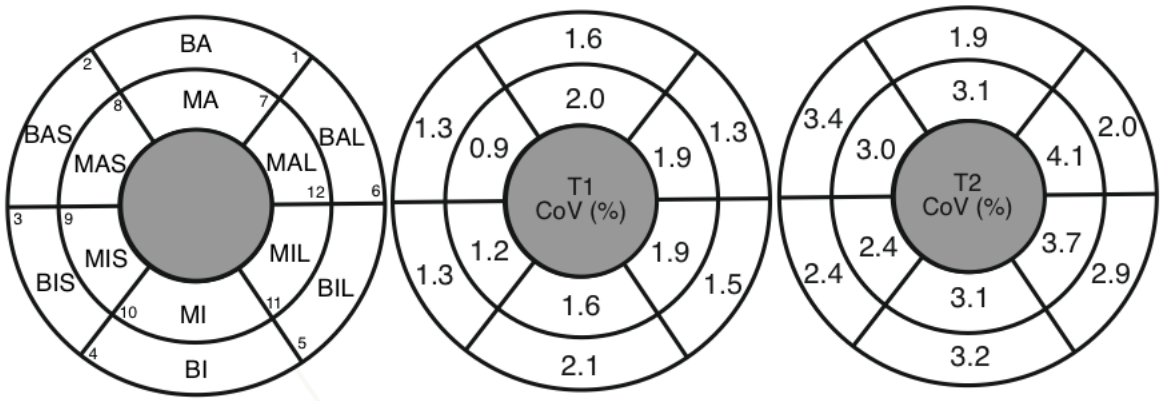

Figure 22: Mean Coefficient of Variability (CoV) (%) of both T1 and T2 across all methods of analysis by myocardial segments (segments 1-12 of the AHA 17-segment model). Basal anteroseptum (BAS), basal inferoseptum (BIS), basal inferior (BI), basal inferolateral (BIL), basal anterolateral (BAL), basal anterior (BA), mid anteroseptum (MAS), mid inferoseptum (MIS), mid inferior (MI), mid inferolateral (MIL), mid anterolateral (MAL), mid anterior (MA)

7.4.1 Multi-segment analysis

Coefficient of variation was performed for each segment in each subject across the 4 analysis methods for both T1 and T2 (**Tables 5 and 6**). Mean T1 CoV across the 4 methods segment-by-segment was 1.5% (0.1-6.7%). T2 CoV mean was 2.9% (0-9.9%). Mean CoV of each of the 12-segments is displayed in **Figure 22**. Bland-Altman plot of comparison between each method and reader 1 yielded mean bias of 0.3 ±2.6% for T1 and -0.5 ±5% for T2 (**Figure 23**). Bland-Altman plot for each segment between Reader 1 and 2 are displayed in **Figures 26 and 27**. CoV of the myositis patients 12 segment model was performed by two readers blinded to each other's ROI. The T1 and T2 CoV between segments was 3.08% (1.4-4.7%) and 5.68% (2.97-8.93%) for LD and 2.8% (1.7-4.8%) 5.31% (3.3-6.8%) for AN. Mean CoV of 12 segment analysis of LD and AN in patients with myositis were 0.78% (0-2.2%) for T1 and 3.0% (1.4-6.1%) for T2 respectively. These demonstrate acceptable agreement

between all methods of analysis where values should be equivalent and good agreement between different analysis of values in patients with myositis.

7.4.2 Multi-segment mean compared to mid-septal ROI

CoV between the 12-segment mean, by each method, and mid-septal ROI was 0.82% (0.02-2.18%) for T1 and 1.86% (0.0-5.28%) for T2 in healthy volunteers. A Bland-Altman plot of 12-segment mean and MS ROI with each reporting method resulted in a mean bias across the methods of -0.52 ±2.8 for T1 and -0.27 ±5.8 for T2. When patients with myositis were analysed using reader 1 (LD) the CoV for T1 was 0.8% (0.14-1.65%) and for T2 was 3.54% (0-8%). Bland-Altman plot showed a bias of -0.19 ±1.4% for T1 and -1.7 ±4.5% for T2 (**Figure 24**). The grater variation in T1 than T2 reflects the known overall reproducibility of the T2 mapping sequence compared to T1.

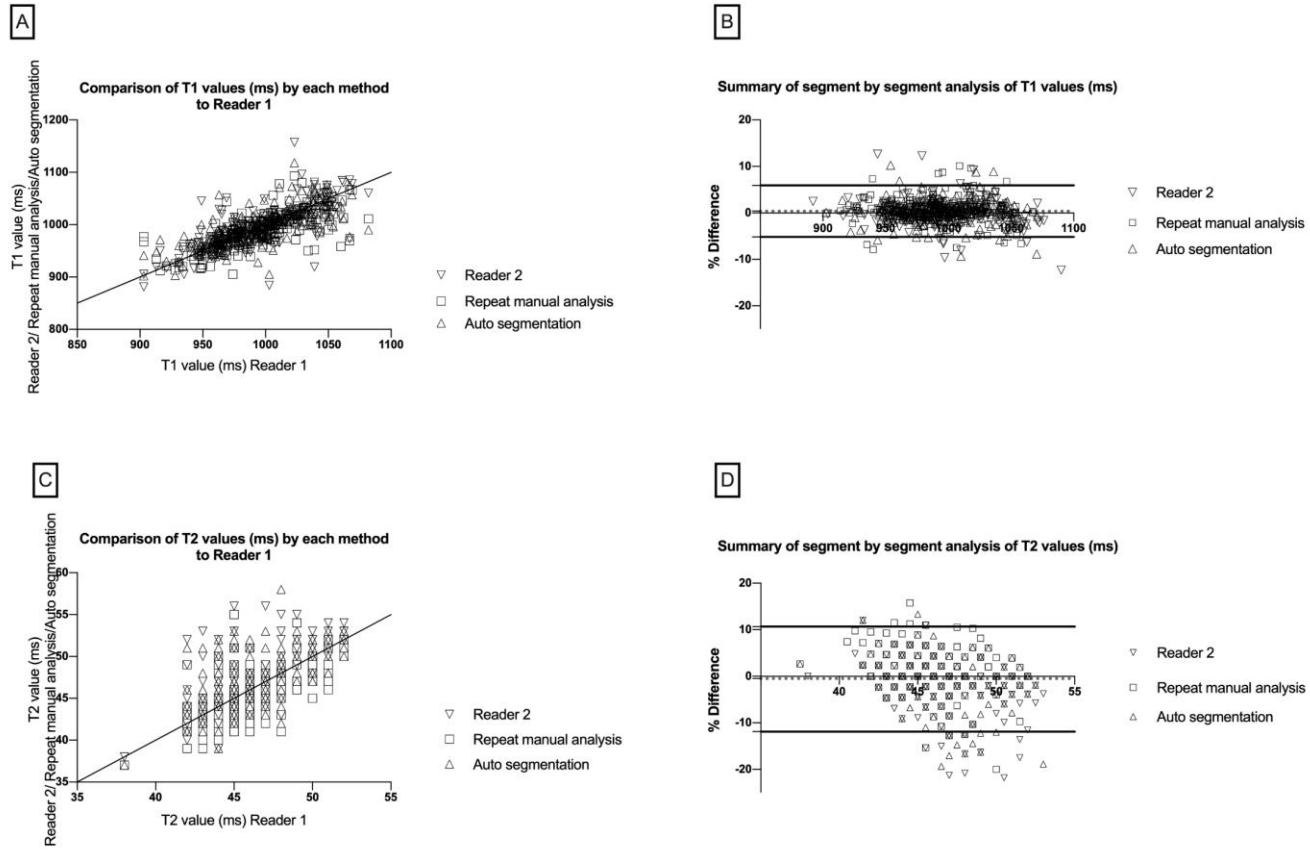

Figure 23: **A and C:** XY plot of segmental T1 and T2 values using the different analysis methods compared to Reader 1 (LD) around a line of fidelity. **B and D:** Bland-Altman plot of the percentage difference of each of the methods of analysis for each segment compared to reader 1 analysis. Bias displayed as dotted line. 95% limits of agreement solid lines

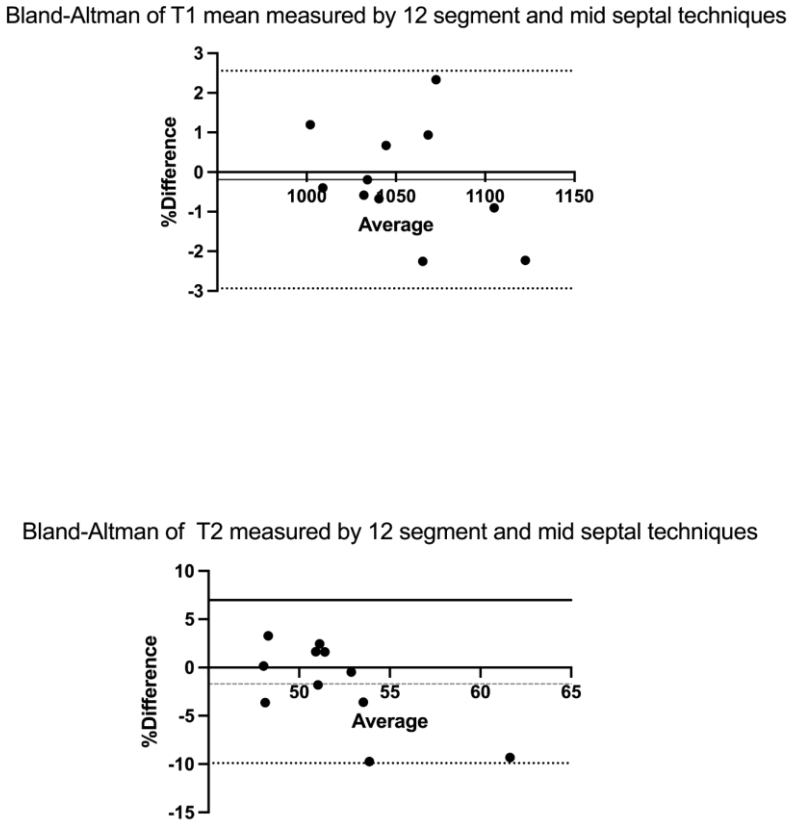

Figure 24: Bland -Altman plots of T1 [**Top**] and T2 [**Bottom**] of patients with myositis analysis. Both show good agreement between a 12 segment mean and the mean value of a mid-septal ROI which is the accepted standard assessment tool for measurement. Dotted line demonstrates 95% limits of agreement. Solid lines indicate bias.

7.5 *Discussion*

Parametric mapping is a reproducible and reliable means of assessing for focal and diffuse intra and extracellular myocardial disease processes [5, 97, 130]. This has been useful in a number of cardiac diseases [141, 142]. The data presented demonstrates that a 12-segment approach to analysing T1 and T2 is reproducible across different readers and different analysis techniques. Furthermore, the agreement between the T1 and T2 values of a mid-septal ROI and 12-segment mean is extremely important when considering the application of mapping techniques in both focal and low-level diffuse disease. That the agreement persists in disease states as well as healthy volunteers is also encouraging as patients will often struggle more than volunteers to breath hold for the sequence acquisition.

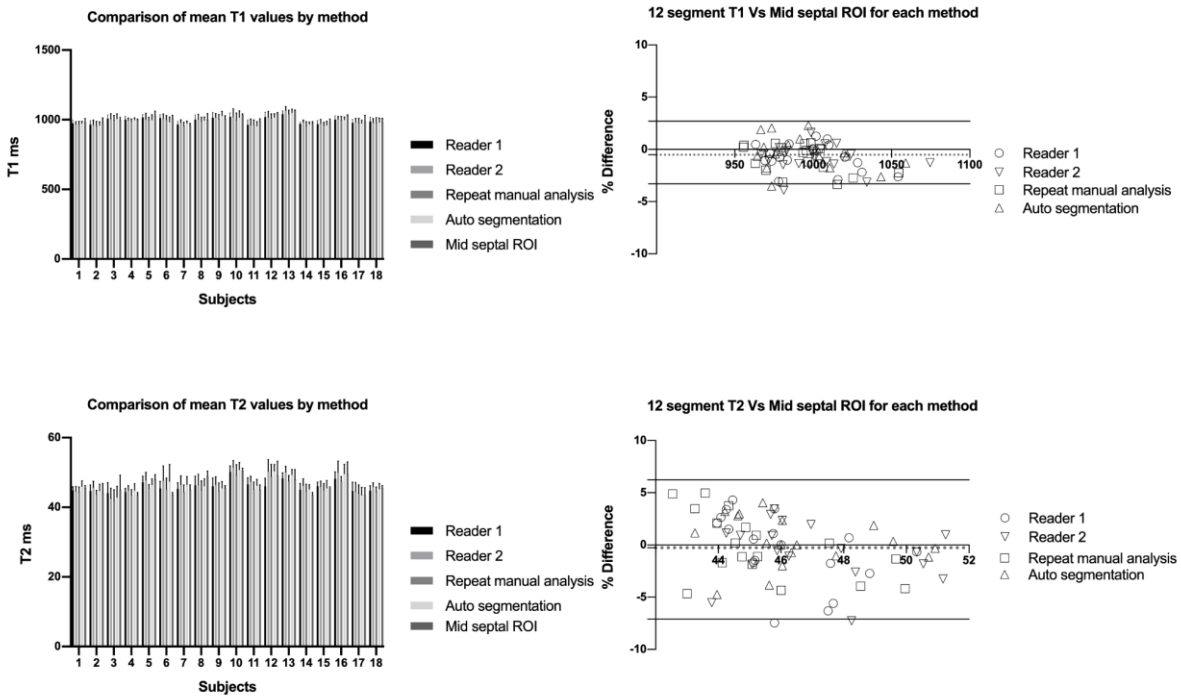

Figure 25: Mean ±SD of all four analysis methods and MS ROI for T1 and T2. Bland-Altman plot of percentage difference of each analysis method compared to MS ROI. %Bias of comparison methods -0.52 ±1.4 for T1 and -0.27 ±2.9 for T2 (dotted line). Solid lines represent 95% limits of agreement

7.5.1 The 12-segment approach.

We are the first to propose and validate a model that provides good ventricular coverage and gives comparable mean values to the standardised magnet normal as suggested by the Society of Cardiovascular Magnetic Resonance (SCMR) and European Association for Cardiovascular Imaging (EACVI) [89]. Previous studies such as Dabir et al measured all 17 AHA segments and found a significant difference between T1 means. T2 means were not found to differ significantly which may reflect the greater variability known to exist with the T2 sequence [143]. Our data confirms this, with a wider relative variation in T2 values compared to T1 but still with low bias.

Coefficient of variation of T1 values assessed by all methods by segment													
Subject (Anonymous identifier)	BAS	BIS	BI	BIL	BAL	BA	MAS	MIS	MI	MIL	MAL	MA	Subject mean
HV001	1.2	1.9	1.3	0.8	1.7	1.0	0.6	1.0	1.7	1.4	1.1	1.0	1.2
HV002	0.4	1.1	2.2	0.4	1.5	0.6	0.9	1.3	0.3	3.5	3.2	0.8	1.3
HV003	2.3	0.2	0.3	0.1	0.9	0.9	0.9	1.1	0.5	2.0	0.7	4.3	1.2
HV004	1.8	1.3	1.3	2.8	1.4	0.9	0.3	0.4	1.1	0.9	1.7	1.7	1.3
HV005	1.0	1.3	0.4	1.4	0.9	6.7	1.0	1.8	1.0	1.7	1.4	0.2	1.6
HV006	0.7	1.3	4.7	2.1	1.2	1.7	0.9	1.5	2.9	0.2	2.5	5.8	2.1
HV007	0.5	1.0	1.6	1.4	1.5	0.7	1.3	1.0	1.1	1.7	3.0	3.2	1.5
HV008	0.9	0.6	2.3	1.3	0.5	0.9	0.5	1.2	0.8	3.3	2.6	2.0	1.4
HV010	2.3	4.1	4.3	5.2	1.6	3.7	2.3	2.8	0.9	4.9	0.5	2.6	2.9
HV011	2.2	2.0	5.1	1.4	4.3	0.6	0.5	1.3	5.5	3.0	2.6	1.7	2.5
HV012	2.6	0.6	4.6	1.3	1.1	1.9	0.4	0.5	3.1	1.8	1.5	1.6	1.7
HV013	0.9	0.8	0.9	1.6	0.6	1.1	1.4	0.7	1.0	3.3	1.9	0.4	1.2
HV014	1.5	2.7	1.8	1.7	1.0	1.4	0.8	0.8	1.7	0.5	1.8	2.3	1.5
HV015	0.9	0.9	2.3	0.6	1.4	0.6	1.5	1.3	0.6	1.8	3.8	1.7	1.5
HV016	1.4	0.9	0.5	1.1	1.1	3.5	0.7	1.1	0.9	1.7	1.6	2.0	1.4
HV017	1.0	0.8	1.0	1.3	0.7	0.3	0.5	1.0	1.8	1.0	2.5	1.5	1.1
HV018	1.2	0.7	0.4	0.5	0.6	0.9	1.1	1.2	2.5	0.6	1.3	0.6	1.0
HV019	1.4	0.6	2.0	1.8	1.0	0.8	0.9	1.2	0.8	0.8	0.9	1.6	1.1
Segmental mean	1.3	1.3	2.1	1.5	1.3	1.6	0.9	1.2	1.6	1.9	1.9	2.0	1.5

Table 5: Coefficient of variability (%) of T1 for each segment by subject using each analysis method. Basal anteroseptum (BAS), basal inferoseptum (BIS), basal inferior (BI), basal inferolateral (BIL), basal anterolateral (BAL), basal anterior (BA), mid anteroseptum (MAS), mid inferoseptum (MIS), mid inferior (MI), mid inferolateral (MIL), mid anterolateral (MAL), mid anterior (MA)

7.5.2 Focal Disease

Currently, focal disease is assessed using a ROI in the affected area compared either to previously established means from healthy volunteers or with 'remote' myocardium adjudged to be normal [136, 144]. As highlighted above, this approach relies on the reader correctly identifying the area of disease. Equally problematic is the fact that significant differences between individual regions have been described, suggesting the observed differences could simply be due to off-resonance or susceptibility artefact. However, we demonstrate that the analysis of each segment is reproducible across different analysis techniques. This allows significance to be attributed to changes to these values over time as is expected with treatment of disease states [133, 145]. It is not new to suggest that even in focal disease the 'global' T1 values may be increased when compared to normal [144]. The

combination of this observation with a 12-segment approach has the potential to provide a fully quantitative method for analysing focal disease in both the acute state but also allow for accurate monitoring of disease.

Coefficient of variation of T2 values assessed by all methods by segment													
Subject (Anonymised identifier)	BAS	BIS	BI	BIL	BAL	BA	MAS	MIS	MI	MIL	MAL	MA	Subject mean
HV001	1.8	1.1	3.1	3.8	5.0	2.1	2.0	1.1	1.3	3.3	2.2	1.1	2.3
HV002	5.1	1.3	2.9	3.9	2.2	1.1	4.0	2.9	1.3	2.8	6.7	4.1	3.2
HV003	5.9	5.9	2.0	1.1	1.2	1.5	2.6	4.1	3.1	2.8	2.8	2.1	2.9
HV004	2.2	1.2	1.9	1.8	1.1	1.3	2.9	3.4	3.3	2.1	2.5	4.6	2.4
HV005	3.6	3.3	3.6	1.1	1.1	1.1	2.9	3.3	5.5	3.7	1.1	2.0	2.7
HV006	7.1	2.9	4.7	4.0	2.8	2.0	9.9	1.3	4.7	9.3	7.0	9.1	5.4
HV007	2.2	0.0	1.0	3.4	2.1	1.7	2.9	1.1	2.1	3.0	8.4	4.8	2.7
HV008	1.9	2.2	4.8	3.1	3.0	2.0	4.5	2.5	2.8	3.5	4.8	1.6	3.1
HV010	7.1	5.3	3.0	2.7	0.0	5.2	1.1	1.1	3.4	2.9	2.1	3.7	3.2
HV011	3.8	5.7	5.0	1.9	2.3	0.0	1.6	1.0	1.8	2.8	6.5	3.0	2.9
HV012	1.8	1.1	1.0	4.5	1.1	1.0	4.7	1.3	2.4	6.0	3.7	2.7	2.6
HV013	2.8	6.9	9.6	7.3	4.8	2.8	1.9	5.0	6.2	3.3	6.9	5.2	5.2
HV014	2.1	1.0	3.5	3.5	2.0	1.2	2.0	3.4	5.1	3.7	2.0	2.6	2.7
HV015	2.8	1.3	2.5	3.2	2.1	2.2	2.9	2.2	2.0	2.2	2.7	2.0	2.3
HV016	3.5	1.3	2.9	1.3	0.0	1.7	1.8	1.1	0.0	2.1	1.7	2.1	1.6
HV017	2.7	0.0	2.4	2.1	1.7	1.1	3.3	2.7	3.6	9.5	7.0	3.7	3.3
HV018	3.0	1.9	1.9	1.2	2.2	4.0	1.4	2.6	1.8	2.5	1.1	0.0	2.0
HV019	1.3	0.0	1.2	3.2	1.1	1.3	1.1	3.1	4.6	1.1	5.2	1.3	2.1
Segmental mean	3.4	2.4	3.2	2.9	2.0	1.9	3.0	2.4	3.1	3.7	4.1	3.1	2.9

Table 6: Coefficient of variability (%) of T2 for each segment by subject using each analysis method. Basal anteroseptum (BAS), basal inferoseptum (BIS), basal inferior (BI), basal inferolateral (BIL), basal anterolateral (BAL), basal anterior (BA), mid anteroseptum (MAS), mid inferoseptum (MIS), mid inferior (MI), mid inferolateral (MIL), mid anterolateral (MAL), mid anterior (MA)

7.5.3 Diffuse disease

When the assumption is that a particular disease process may result in diffuse myocardial involvement, convention for measurement is either a single mid-septal ROI in a mid-ventricular short axis segment with endo and epicardial delineation to provide a mean for the slice [138, 145, 146]. Alternatively, the whole ventricle is sampled with the potential issues raised above [147]. The measured relaxation time is then compared to a pre-determined mean. As discussed, in the absence of a comparable approach to assess the

ventricle in multiple segments, significant differences may either be identified incorrectly or missed altogether.

7.6 *Limitations*

The sample size is small. Image quality was deemed good in all segments, however, there is the risk of off-resonance artefact, and this was not formally assessed. The sequences available at our centre do not provide error maps meaning qualitative assessment was based on visual assessment of both greyscale and colour maps which has the potential to introduce greater variability due to inadvertent partial voluming. The use of a 12-segment model does not afford diagnostic information about the apical segments. As mentioned previously, accuracy of both acquisition and interpretation of the apical segments remains limited which is why they have been excluded from the 12-segment model.

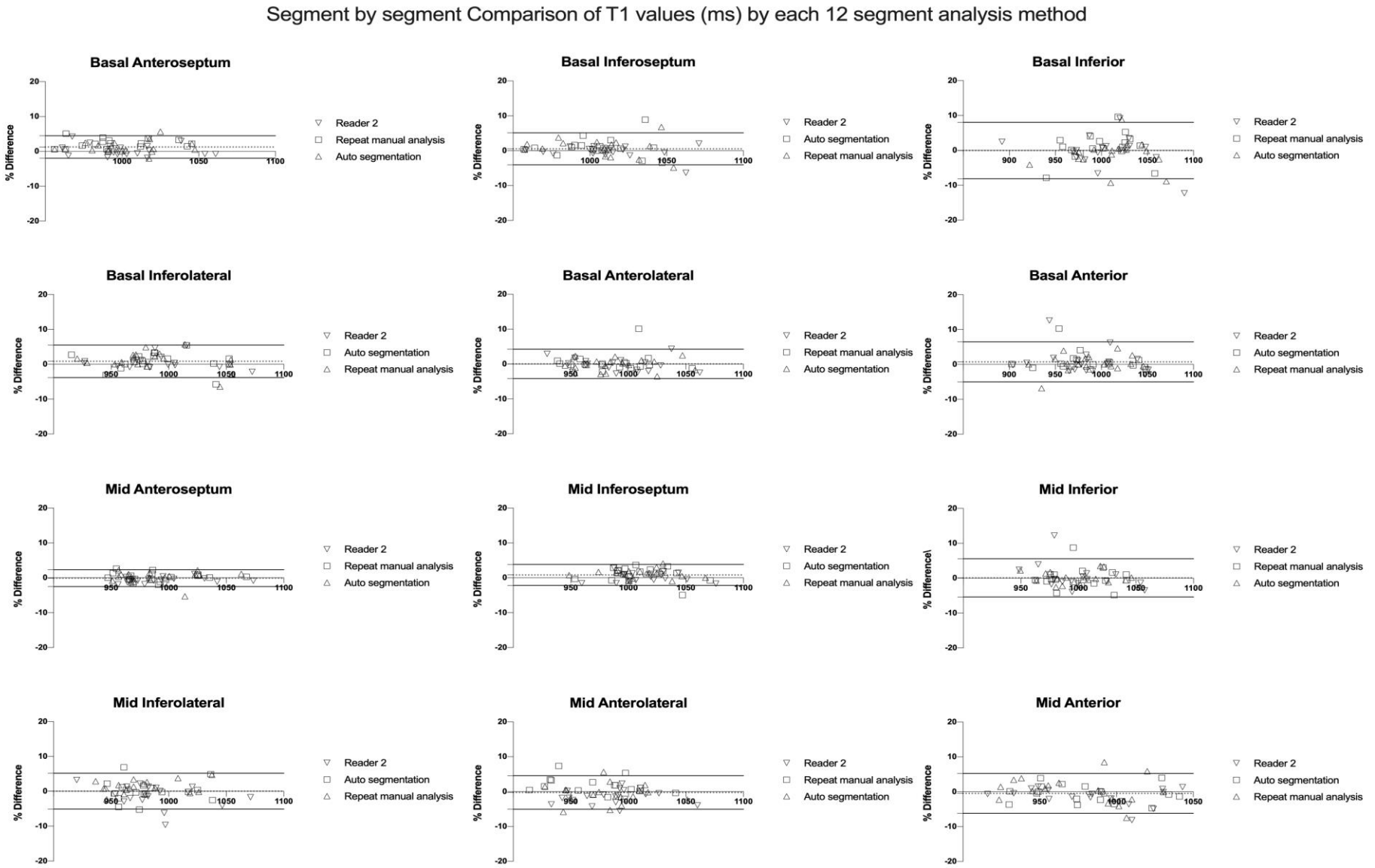

Figure 26: Segment by segment comparison of T1 values (ms) by 2 blinded readers, across repeated measures and auto-segmentation compared to reader 1 analysis

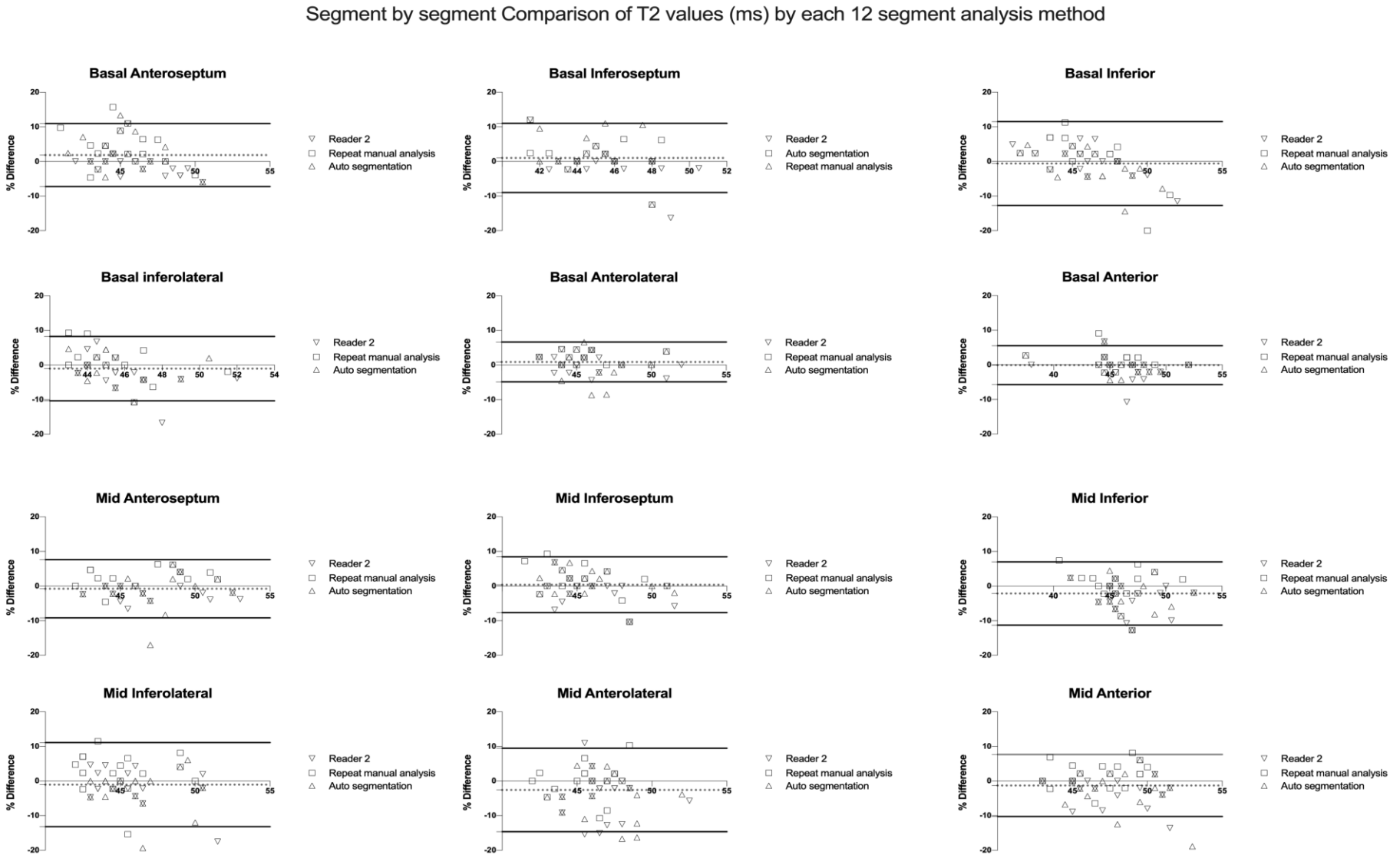

Figure 27: Segment by segment comparison of T2 values (ms) by 2 blinded readers, across repeated measures and auto-segmentation compared to reader 1 analysis

7.7 *Conclusions*

A novel 12-segment approach to analysing native myocardial T1 and T2 covering all basal and mid left-ventricular regions is presented. Firstly, we validated this method irrespective of whether a manual ROI technique or auto-segmentation is used in healthy subjects where the values are expected to be comparable. Secondly, the overall mean over 12-segments is equivalent to a traditional mid-septal ROI in both healthy and disease states. We also found agreement between different readers using the same technique to analyse myocardium in patients with myositis. Therefore, changes in average T1 and T2 relaxation times between disease and health states can be quantified, as well as monitored over time providing a potentially significant improvement in the clinical assessment of patients with myositis and, with further validation work, potentially in other disease states as well.

8. Application of parametric tissue mapping sequences in the identification of cardiac involvement in myositis

8.1Introduction

Identification of the presence of cardiac involvement in myositis remains challenging. The aim of this study was to apply both T1 and T2 mapping sequences to patients with myositis to try to identify the link between a raised troponin and the presence of myocardial inflammation or fibrosis. Other biomarkers and markers of disease activity were also assessed and compared to T1 and T2 values. Myocardial and skeletal muscle T1 and T2 values of patients with myositis were compared to the myocardial and skeletal muscle values in healthy volunteers.

8.2 Hypothesis

That parametric tissue mapping sequences will identify the presence of cardiac involvement in myositis and that an elevated troponin will correlate with acute cardiac involvement adding weight to the use of biomarkers to predict its presence.
Supplementary hypothesis: The pattern of late enhancement may provide insight into the diagnosis of IIM mediated myocarditis

8.3 Methods

Both healthy volunteers and patients with myositis were included in this study. The details of the healthy volunteers are found in **table 4** and those of the patients with myositis in **table 7**.

8.3.1 Healthy volunteers

Details of healthy volunteer recruitment are found in **chapter 6**. The healthy volunteers underwent a single non-contrast CMR with a truncated protocol (**chapter 6**). No blood tests were undertaken. The purpose of healthy volunteer scanning was to produce a control group but also to establish normal ranges for our departmental scanner for future clinical and research purposes.

8.3.2 Patients with myositis

Patients with myositis were recruited with different disease states ranging from new diagnosis to acute relapse through to quiescent disease states. The reason for this was to understand the potential relationship between acute skeletal muscle disease and myocardial involvement. The specific characteristics of the patients is shown in **table 7**. Each recruit underwent mapping sequences and completed a patient global assessment visual analogue scale (VAS) (**Appendix 7**) at each visit. Functional assessment of the patients (MMT8) was undertaken by the patients' clinical team (in each case by a senior rheumatologist) and documented as part of routine care at clinic visits. Each patient who underwent blood tests had the results published on the trust computer system for review by the clinical team. Any cardiac abnormalities identified resulted in referral for review in cardiology clinic. Patients were followed up for over a year or until they had completed 4 scans.

8.3.3 CMR

All CMR studies were performed on the same 1.5T Magnetom Aera, (Siemens Healthcare GmbH Erlangen, Germany). Assessment of LV function was undertaken at each visit using standard CMR assessment. Each scan involved full CMR T1 and T2 mapping protocol described in detail above (**chapter 6**). Three long axis acquisitions and 2 basal, 2 mid and 2 apical short axis segments were acquired for both T1 and T2. A large field of view image was acquired in both to try to ensure biceps and triceps was included in the field of view. The reason for 2 acquisitions in each short axis position was to increase the chances that diagnostic quality images were acquired. All mapping sequences were interpreted by the author using a manual contour approach (the accepted convention at the time of writing) over the 12 basal and mid-cavity segments. Any segment where image quality was deemed inadequate in both short axis slices the corresponding long axis acquisition was measured (following appropriate cross-cut correlation). As with the myocardial maps, skeletal muscle acquisitions were assessed for quality using both the colour maps and the grey scale images and if the quality was deemed insufficient the measurements were disregarded. Those that were included were measured with location of ROI limited to biceps or triceps in the 4-chamber view. Following analysis, all measurements were anonymised and tabulated for data interpretation.

Patients with myositis undergoing contrast enhanced studies had the LGE assessed by a level 3 accredited consultant cardiologist who was blinded to the troponin value to reduce the risk of bias.

8.3.4 Biomarkers and functional assessment

Patients with myositis were asked to complete a patient global assessment visual analogue scale at each visit. They were asked to grade their myositis severity as a whole rather than purely their cardiac symptoms. Manual muscle testing (MMT8) was recorded having been undertaken by experienced clinicians during routine clinical care. Initially blood samples for troponin, CK and NT-pro-BNP were used as collected by the clinical team but, following a substantial amendment to the original protocol, the author was able to sample blood from subjects when they attended for contrast enhanced scans. This was because they would need IV access in the form of a cannula for the administration of contrast. At attendances for non-contrast studies, sampling was only undertaken in the event of clear clinical indication (i.e., the patient was due a blood test for their usual clinical team or presented for a scan with overt clinical symptoms of cardiac involvement). These samples were analysed by the King's College Hospital biochemistry department and made available to the subject's clinical team. Troponin, NT-pro-BNP and CK levels were recorded and interpreted according to the KCH biochemistry department reference ranges. In the event a particular assay fell below the lower limit of its sensitivity the value was recorded as 1 (as opposed to <x).

8.3.5 Statistical analysis

All statistical tests were undertaken using GraphPad Prism 8 (GraphPad Software Inc, USA) All measurements analysed were assessed for normality or log normality where $p<0.05$ was considered in keeping with normality. In the event both lognormal and log normal scales were equally well fitting, the distribution that satisfied the most of four normality tests, Anderson-Darling, D'Agostino and Pearson, Shapiro-Wilk and Kolmogorov-Smirnov was ascertained. Data are displayed as mean ± SD unless stated otherwise. Non-normally distributed data are displayed as median ± range or interquartile range (IQR).

1. Parametric and non-parametric distribution data
    a. Unpaired t-test
    b. Mann-Whitney

2. Observed and expected variable assessment
    a. Chi-squared
3. Correlation
    a. Pearson's rank correlation coefficient
    b. Spearman's rank correlation coefficient

8.3.6 Data handling

All values were tabulated using Microsoft Excel and stored anonymously. DICOM files were stored within the hospital in a locked cupboard on DVD and on an external hard drive. DICOMS were anonymised when analysis was undertaken purely for research purposes. Example of the raw data is shown in **Figure 15.** All DICOM images were analysed using commercially available post-processing software (Version 5.10.1, Circle Cardiovascular Imaging Inc., Calgary, Canada.

8.4 *Results*

18 healthy volunteers (9/9) were included as one control arm of the study. Their specific characteristics are displayed in **table 4**. Healthy volunteer T1, T2, LV volumes and ejection fraction values were compared with 26 patients with myositis whose specific characteristics are displayed in **table 7**. The median ages (33 and 53) were statistically significantly different ($p<0.0001$). All healthy volunteers underwent a single non-contrast scan. Four of the healthy volunteers underwent repeat scanning. Twenty eight patients with myositis were invited for multiple studies. Two patients were excluded following their initial scan, one because the eventual diagnosis was not that of myositis and the other due to a large pre-existing myocardial infarction of the anterior wall rendering ventricular analysis impossible. Overall, 70 myositis studies and 18 healthy volunteer studies were undertaken. From each of these, volume assessment was undertaken and T1 and T2 mapping of 16 segments of myocardium were measured. In total 3168 segments of myocardium were analysed. Each was quality

assessed as described in the methods section above. All mapping segments were analysed by the author.

Variable (mean ± SD unless stated)	Healthy volunteers	Myositis	Difference(p)
Number (M/F)	18 (9/9)	26 (6/20)	-
Age (median, range)	33 (25-38)	53 (27-78)	**<0.0001**
Weight (Kg) [median, range]	75 (50-128)	81.5 [54-145]	0.19
Height (cm) [median, range]	173.5 [158-190]	170 [158-190]	0.5
EDV_i (mls/m²)	81.1 ± 11	73.2 ±15	0.07
ESV_i (mls/m²)	26.7 ± 5	23.3 ±12	0.27
EF (%)	67.1 ± 4.6	69.6 ±11	0.36
Mean heart rate ± SD (bpm)	70 ± 14	74 ± 12	0.38
Cardiac symptoms (chest pain/palpitations/ankle oedema/pre-syncope)	0	Chest pain: 4 Palpitations: 7 Ankle oedema: 3 Pre-syncope: 0	-
Sinus rhythm (%) [n]	18 [18]	25 [16]	-
Arrhythmia	-	AF: 1 NSVT: 2 Ectopy: 4	-
Past cardiac history	-	Hypertension: 4 AS: 1 IHD: 0 LVSD at recruitment: 2	-
Past medical history	-	DM type 2: 2 PVD: 1 Asthma: 3 Hypothyroidism:2	-
Cardiac medications	-	Betablocker: 3 ACE-i/ARB: 3 Diuretic: 3	-
Disease modifying antirheumatic drugs (DMARDS)	-	Prednisolone: 20 MMF: 17 Methylprednisolone: 4 Cyclophosphamide: 6 IVIg: 4 Tacrolimus: 4 Rituximab:2 Azathioprine: 1	-
Troponin (raised/normal) [n]	-	19/6 [25]	-
NT-pro-BNP (raised/normal) [n]	-	4/16 [20]	-
CK (raised/normal) [n]	-	19/6 [25]	-
T1	995 ± 24	1044 ±33	**<0.0001**
T2	46 ±2	50 ±3	**<0.0001**
MMT 8 out of 150 (Range)	-	144 (124-150)	
Visual analogue scale (VAS) cm (range)	-	5.2 (0.1-8.1)	-
Diagnoses (n)	-	DM (8) PM (6) Antisynthetase (3) Necrotising myopathy (4) Myositis overlap (5)	-
Antibodies (n) NOTE: Some subjects had more than one positive antibody	-	TIF-1(2), PL12(3), NXP2(1), PL7(2), Ro52(4), dsDNA (1), Jo-1(5), cardiolipin (3), HMG-CoA (1), SRP (3), TH/TO (1), Ku (1) ANA (1), Isoleucyl-tRNA synthetase (1), RhF (1), Mi-2(2). U1 RNP (1), Pm-Scl (1)	-

Table 7: Demographics of subjects at presentation for first scan. N=26 except where stated otherwise. Indexed end diastolic volume (EDV_i). Indexed end systolic volume (ESV_i). Ejection fraction (EF). Dermatomyositis (DM). Polymyositis (PM). Manual muscle test 8 (MMT 8). Creatine Kinase (CK). N-terminal pro b-type natriuretic peptide (NT-pro-BNP). Intravenous immunoglobulin g (IVIg). Mycophenolate mofetil (MMF). Angiotensin converting enzyme inhibitor (ACE-i). Angiotensin receptor blockers (ARB). Diabetes Mellitus (DM). Peripheral vascular disease (PVD). Left ventricular systolic dysfunction (LVSD)

8.4.1 Patients with myositis at initial attendance

Of the 26 patients with myositis included, 19 had raised troponin at first presentation (TnI/TnT =14/5). 4 had raised NT-pro-BNP and 19 had raised CK. Twenty five out of twenty six were in sinus rhythm (96%). One patient was in atrial fibrillation. Two subjects had evidence of non-sustained VT on their 24-hour ECG (8%). Frequent atrial and ventricular ectopics were identified in 4/26 recruited patients (15%). Median visual analogue scale (VAS) distance 5.2cm (Range 0.1-8.1). Median MMT8 was 144 (124-150). The specific myositis sub-type and antibody profiles are listed in **Table 7**.

8.4.2 Healthy volunteers compared to patients with myositis at initial scan

Mean (±SD) T1 and T2 values were derived using the 12-segment model described above. The mean values were T1: 995 ±24 ms and T2: 46 ±2 ms. This makes the upper limit of normal for T1 and T2 1043 and 50 respectively (Mean +2SD). The mean 12 segment T1 and T2 values for patients with myositis at their initial scan were 1048 ±37 and 50 ±3 respectively (Difference between healthy and myositis T1 and T2 both significant $p<0.0001$, **Figure 28**) regardless of disease state at presentation. As shown in **table 7** there was no significant difference between height, weight, EDV, ESV or EF between each group. There was, however, significant variability in the biomarker profile of the experimental group at presentation (positive/[n] trop:19/25, NT-pro-BNP 4/20, CK19/25). Given that a high troponin concentration is the most sensitive biomarker for cardiac myocyte damage, panel **[B]** of **figure 28** shows the difference between troponin negative patients with myositis and healthy volunteers at their initial scan (Mean 1015 ±10 and 47 ±3 for T1 and T2 respectively, $p=0.04$ for T1 and 0.03 for T2).

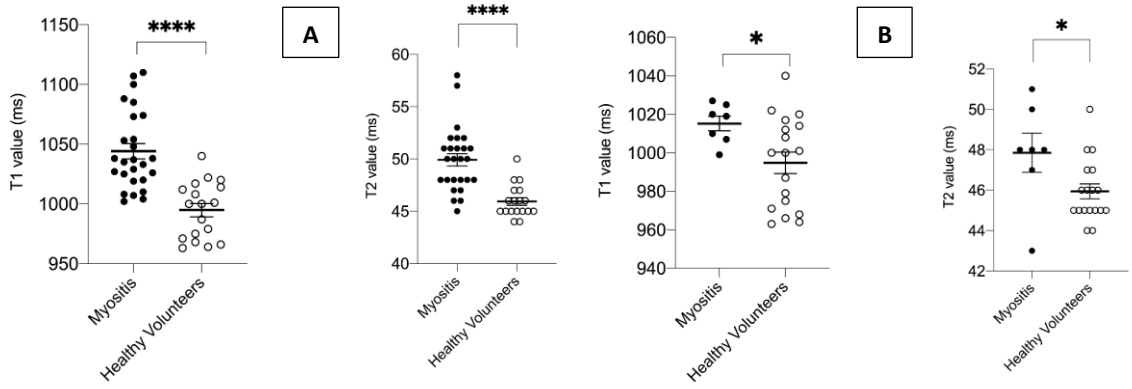

Figure 28: [A] Comparison of mean T1 and T2 values (ms) between healthy volunteers and patients with myositis ($p<0.0001$ for both T1 and T2) displayed as mean ± SEM. Myositis T1 1048 ±6.4, T2 50 ±1. **[B]** Comparison of mean T1 and T2 values (ms) between healthy volunteers and patients with myositis with a negative troponin at initial presentation. Troponin negative myositis T1 1015 ±3.9 T2 48 ±1. Both are compared to healthy volunteer T1 995 ±6 and T2 46 ±0.4 ($p<0.0001$ for both T1 and T2 in panel **[A]** and 0.04 for T1 and 0.03 for T2 in panel **[B]**. $p<0.0001$ (****)/$p<0.05$ (*).

Skeletal muscle T1 and T2 was also compared between patients with myositis (n=21 for T1 and 23 for T2) and healthy volunteers (n=11) at their initial recruitment scan. Median (range) T1 864 (131) and T2 34 (6) for healthy volunteers. Skeletal muscle T1 and T2 values of patients with myositis were not normally distributed. Median T1 and T2 at initial presentation were 931 (range 498) and 39 (45) respectively. The corresponding values in healthy volunteers were 864 (131) and 34 (6). **Figure 29** demonstrates a significant difference between the two groups ($p=0.002$ for T1 and 0.0006 for T2) irrespective of the severity of myositis at the time of scanning. The significance of the skeletal muscle differences needs to be interpreted in the context of a non-optimised sequence with a large spread of values.

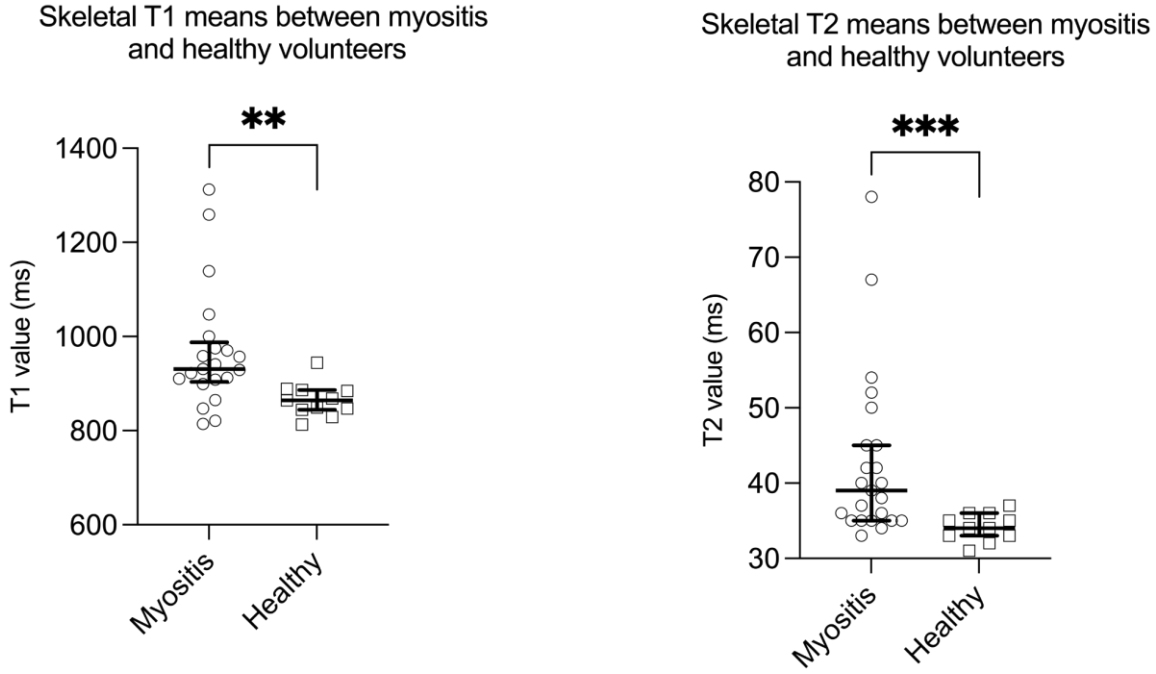

Figure 29: Median (range) of the skeletal muscle T1 (**left**) and T2 (**right**) values in both healthy and patients with myositis at the initial scan regardless of disease state. 931 (498) and 39 (45) for myositis T1 and T2 respectively and 864 (131) and 34 (6) in healthy volunteers. (p=0.002 for T1 and 0.006 for T2). Caution in interpretation is required given the large range and spread of the data from this non-optimised sequence.

8.4.3 Late gadolinium enhancement and myocarditis

Myositis recruits underwent contrast enhanced scans at their first and last study (with one exception in whom we could not gain IV access). 13 out of 25 (52%) studies had evidence of LGE (on blinded reporting). Of these, in one the cause was a previous myocardial infarction and in another the pattern of late enhancement was most consistent with an embolic phenomenon (it bears noting that the patient was found to be in atrial fibrillation at the time of scanning). The remaining studies had a pattern of LGE consistent with non-ischaemic (mid wall or epicardial) scarring such as that caused my myocarditis (see **Figure 9** which shows a short axis slice of a patient in the study with antisynthetase syndrome). Overall the percentage of patients with myositis in our study (52%) is similar to analysis of all the published literature showing a 46% of CMR studies in patients with myositis have LGE. The largest single cohort of patients with myositis subjected to LGE concluded 62% had evidence of myocardial fibrosis by this method [94].

The quantity and distribution of LGE within the LV cavity has been shown to follow distinct patterns in different conditions [90, 138]. Basal lateral and inferolateral epicardial or mid wall late enhancement is the most common pattern seen in myocarditis [149]. The

distribution of late enhancement in this study of patients with myositis demonstrates a pattern of predilection for the same regions (p<0.0001). The distribution of late enhancement is shown in **Figure 30**.

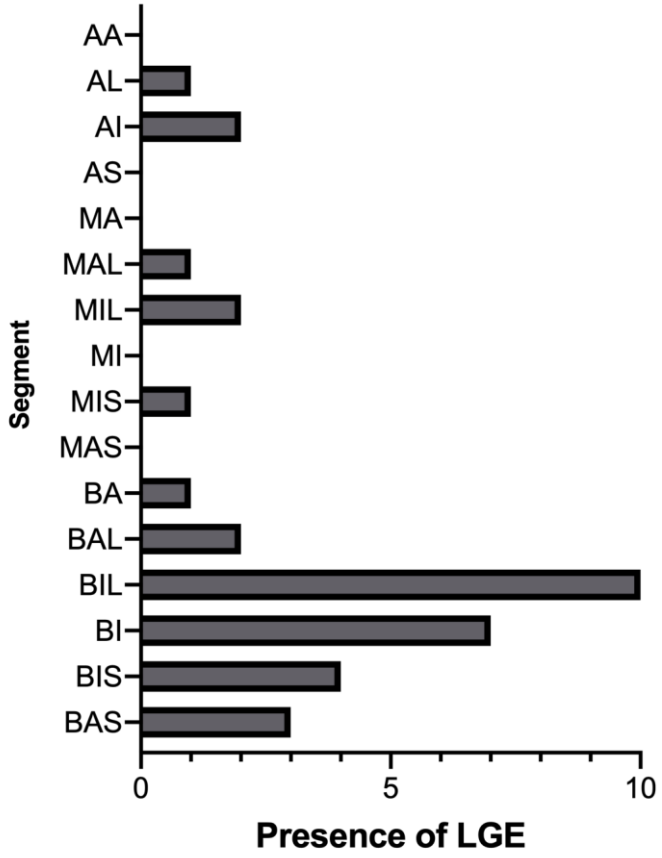

Figure 30: Graph of the frequency of occurrence of LGE in patients with myositis by AHA segment. Maximum frequency is found in the basal inferolateral wall (BIL) (10) (p<0.0001). Basal anteroseptum (BAS), basal inferoseptum (BIS), basal inferior (BI), basal inferolateral (BIL), basal anterolateral (BAL), basal anterior (BA), mid anteroseptum (MAS), mid inferoseptum (MIS), mid inferior (MI), mid inferolateral (MIL), mid anterolateral (MAL), mid anterior (MA)

8.4.4 Difference in T1 and T2 values of cardiac muscle with high or normal biomarker levels

The T1 and T2 values of patients with myositis with positive and negative values for each biomarker were compared at their initial scan. There was no significant difference in myocardial T1 or T2 values in patients with high or normal NT-pro-BNP (T1 p=0.2 and T2 p=0.4) or CK (T1 p=0.09 and T2 p=0.07). As some of the referring trusts used TnT and there was no contemporaneous TnI measure, only patients with a troponin I were included in the troponin specific analysis (n=20. 14 elevated). A significant difference between myocardial

T1 and T2 levels is seen when patients with elevated and normal troponin I (T1 p=0.02 and T2 p=0.03) **Figure 31.** Absolute values are shown in **table 8.**

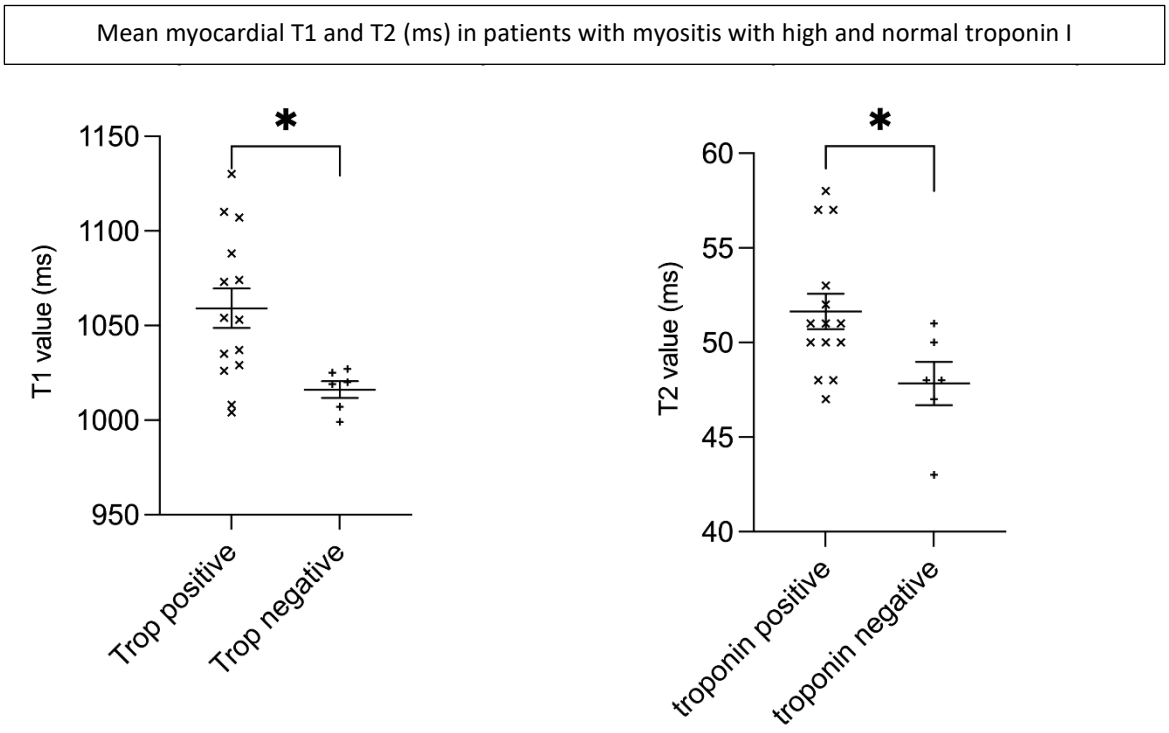

Figure 31: Difference in myocardial T1 (**left**)and T2 (**right**) values of patients with myositis with high (n=14) and normal (n=6) troponin I. T1/T2 values (ms) (mean ±SD) in troponin positive 1059 ±39/52 ±3 and 1016 ±11/48 ±3 in troponin negative (p=0.02 and 0.03 for T1 and T2 values respectively).

	Myocardial (mean ±SD)				Skeletal muscle (Median (range))			
	T1 initial		T2 initial		T1 initial		T2 initial	
Myositis	1048 ±37		50 ±3		931 (498)		39 (45)	
Healthy	995 ± 24		46 ±1.6		864 (131)		34 (6)	
p-value	**<0.0001**		**<0.0001**		**0.002**		**0.006**	
Trop +ve	1063 ±34		51 ±3		949 (491)		40 (43)	
Trop -ve	1015 ±10		47 ±3		894 (491)		39 (34)	
p-value	**0.002**		**0.02**		0.4		0.99	
NT-pro-BNP +ve	1070 ±31		52 ±3		1003 (404)		45(42)	
NT-pro-BNP -ve	1043 ±38		50 ±4		930 (445)		38 (34)	
p-value	0.2		0.4		0.2		0.1	
CK +ve	1055 ±37		51 ±3		957(498)		40 (43)	
CK -ve	1023 ±32		48 ±3		865 (75)		39 (12)	
p-value	0.09		0.07		0.1		0.7	

Table 8: Mean ±SD or Median (range) myocardial and skeletal muscle T1 and T2 of patients with myositis at initial presentation. P-values describe the comparison of the two values above (where specific biomarkers were high or normal) using either t-test or Mann-Whitney depending on normality.

8.4.5 Difference in T1 and T2 values of skeletal muscle with high or normal biomarker levels

There is no significant difference in skeletal muscle T1 or T2 values when biomarkers are raised vs normal (**Figure 32**).

Mean skeletal muscle T1 (left) and T2 (right) separated by high or normal initial biomarker levels

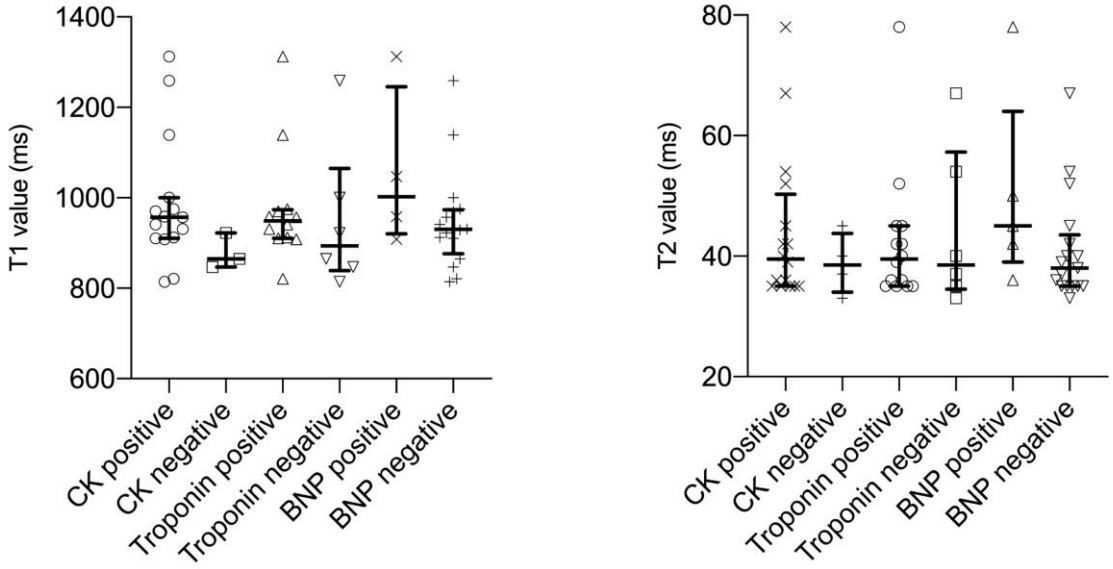

Figure 32: Skeletal muscle T1 and T2 of patients with myositis at first presentation. Error bars show median and IQR. No statistically significant differences between measures in patients with raised and negative biomarker. As with myocardium there is a trend towards lower mapping values in patients whose biomarkers are negative. As mentioned previously, note should be made of the significant spread in values making interpretation difficult.

8.4.6 Correlation between Biomarker values and T1 and T2 at initial presentation

Pearson's or Spearman's rank correlation coefficients were applied to the initial scan data to establish a correlation between T1 and T2 and biomarkers measured. Given the large variation in scales of different biomarkers and T1 and T2, lognormality testing was undertaken to allow for correlation to be assessed. Initially correlation was sought between log T1 and Log T2 of both myocardium and skeletal muscle. A strong correlation was observed between these 2 sets of variables (**Figure 33**) (myocardial T1 and T2 r^2=0.69 n=26 p<0.0001, skeletal muscle r_s=0.86 n=18 p<0.0001 (rs where spearman's)). This is to be expected as T1 values increase with increasing extracellular space including that caused by oedema. Myocardial T1 and T2 show a positive correlation with troponin I values (T1 r^2=0.57 n=20 p=<0.0001, T2 r^2=0.47 p=0.0009) (**Figure 34**).

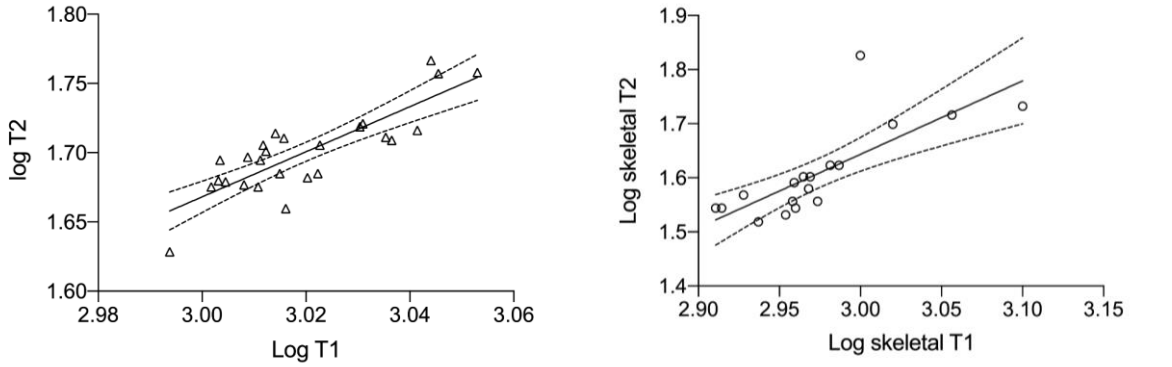

Figure 33: Scatter plot with mean and 95% confidence intervals of log T1 and Log T2 of myocardium (**left**) and skeletal muscle (**right**) at initial presentation. Myocardial T1 and T2 r^2=0.69 n=26 p<0.0001, skeletal muscle r_s=0.86 n=18 p<0.0001

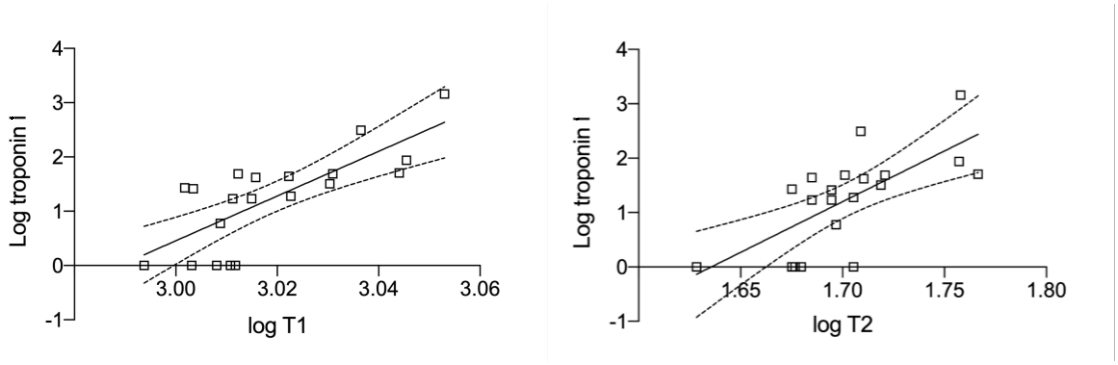

Figure 34: Scatter plot of log myocardial T1 [**left**]and T2 [**right**] values and log troponin I. (r^2=0.57 and 0.47 n=20 p<0.0001 and 0.0009 for T1 and T2 n=20 respectively)

Skeletal muscle T1 and T2 values did not show any correlation with troponin I, NT-pro-BNP or CK (N=21 for NT-pro-BNP and CK. Troponin I n=20). Both T1 and T2 correlated with the presence of LGE with T1 correlating more strongly than T2. T1/T2: r_s=0.6/0.45 p=0.002/0.02.

8.4.7 Correlation between myositis disease severity measures and T1 and T2 values at presentation

Patients' initial MMT8 value correlated to their visual analogue assessment (VAS) of their own disease (r_s=-0.63 n=18 p=0.005). There was no significant correlation between MMT8 and any of the biomarkers tested or myocardial or skeletal muscle T1 or T2. There is a weak correlation between MMT8 and ejection fraction (r_s=-0.5 n=19 p=0.05).

8.5 *Discussion*

8.5.1 The ability of T1 and T2 mapping to identify cardiac disease

Parametric tissue mapping sequences have established themselves in several conditions as reliable and reproducible means to identify and quantify myocardial and inflammation and fibrosis [116, 142, 150, 151]. In applying these sequences to patients with myositis we have not only demonstrated a significant extra cellular space expansion (secondary to inflammation, fibrosis, or both) when myositis is compared to healthy volunteers but also when patients with myositis are grouped by elevation of different cardiac biomarkers. As discussed above (**chapter 5**), there has only been limited previous work undertaken using CMR to support the diagnosis of cardiac involvement in myositis. Only 161 cases are published and of these only 61 report T1 mapping and 21 T2 mapping [5, 107, 114]. These data show a significant difference between T1 and T2 values in patients with myositis compared to healthy volunteers. However, none of these studies divides subjects by their biomarker values. Biomarkers are known to be of use in the diagnosis and management of myositis. CK is, and recently troponin has been suggested as, a useful marker of overall disease activity rather than purely of cardiac involvement. Lilleker et al found that troponin I and T were associated with more marked overall skeletal muscle disease activity in patients with myositis and that patients with cardiac involvement had significantly higher TnI values [28].

This is the first study to demonstrate a significant difference in levels of myocardial T2 (and hence likely inflammation) in patients with myositis (p=0.02) compared to healthy volunteers whether their troponin was elevated or not. This suggests that troponin measurements alone may miss a proportion of patients with ongoing myocardial inflammation. Initial scan sensitivity was 78% with a specificity of 38%. These data are,

however, undoubtedly influenced by the bias towards selecting myositis subjects with positive initial troponin. The T1 levels are also significantly different (p=0.002) as compared to healthy volunteers. T1 is elevated by anything (other than fat) that increases extracellular space and as such can measure of both acute and chronic changes. Without T2 values it is not possible to distinguish acute inflammation from chronic scarring. The greater difference between the T1 values and the T2 values may be explained by an acute on chronic process where previous episodes of myocardial inflammation have resulted in fibrosis leading to persistent elevation in T1 values, whereas the T2 values will remain elevated only while acute inflammation is present.

T1 and T2 values appear to be slightly more accurate at detecting low level myocardial inflammation than troponin levels alone. This may relate to the observation that troponin elevation tends to normalise very quickly when treatment of the underlying cause is initiated, however, the presence of inflammation as measured by T1 and T2 may remain for a significant period of time (this will be explored in greater detail in a later chapter). This phenomenon may lead to false negative results if troponin alone is sampled. T1 and T2 both correlate well with the presence of LGE in our patient cohort. T1 correlates more closely than T2 (r_s=0.6 p=0.002 vs r_s=0.45 p=0.02).

8.5.2 Troponin as a marker of myocardial disease activity

Since in most practice, clinicians only have ready access to one troponin titre, some of our patients had troponin T measured and others TnI. We know that TnT is released by healing skeletal muscle [148, 152] and so we only included patients with TnI in correlation assessments and biomarker specific measures. This study demonstrates a correlation between troponin levels and myocardial T1 and T2 (T1 r^2=0.57 n=20 p=<0.0001, T2 r^2=0.47 p=0.0009). Previously observed cases of high TnI levels in patients with myositis where other cardiac investigations were normal may have a degree of myocardial inflammation or fibrosis when investigated using tissue mapping techniques.

8.5.3 CK as a marker of cardiac involvement

Within our patient group there was a significant difference between myocardial T1 in patients with elevated CK compared to those with normal CK (p=0.0008). This was not reflected in T2 values suggesting that the presence of acute inflammation did not seem to

be responsible for elevated CK as T2 would then also be expected to be elevated. The presence of elevated T1 could suggest that where CK is elevated in patients it represents worse disease control overall meaning risk of prior or recurrent myocarditis is more likely. That being said, if there was a strong association between CK and myocardial fibrosis, given the quantitative nature of T1, a correlation would be expected, and this is not seen. Additionally, the lack of a correlation with either myocardial T1 and T2 suggests CK is not a useful biomarker for myocarditis in this setting. There is a weak correlation with CK and troponin at initial presentation (which is to be expected given CK is released by both myocardium and skeletal muscle (r^2=0.25 n=25 p=0.01).

8.5.4 Pattern of LGE

LGE is seen in 52% of initial scans. The pattern of LGE had been hypothesised as a means of determining myositis myocarditis as a cause over other myocarditides. However, as with most myocarditides, the overwhelming predilection was for the basal segments, and in particular the basal inferior and basal inferolateral walls.

8.5.5 Skeletal muscle tissue mapping

In this study, cardiac tissue mapping sequences were applied to the skeletal muscle. Since the heart moves with both systole and diastole the sequences need to be performed breath-held and as a result sacrifices in image resolution are made to ensure sufficient information can be acquired in the space of a single breath hold. This is not necessary in skeletal muscle where sequences can be run over several minutes with image resolution maximised. Also, cardiac scanning mandates that the heart is placed at the centre of the magnet bore, again, to maximise image quality. The peripheral skeletal muscles sampled in this study are close to the edge of field and subject therefore to artefact degrading the images. The purpose of sampling skeletal muscle was predominantly as a pilot for potential applications of dedicated skeletal muscle tissue mapping sequences. Despite these challenges we see a significant difference between T1 and T2 values when healthy volunteers and myositis subjects are compared (p=0.002 for T1 and 0.006 for T2). This is consistent with the only other study in this population which used skeletal muscle T1 and T2 values to distinguish between acute viral myocarditis and myositis myocarditis [107]. When myositis subjects are separated by biomarkers all significance is lost. This is likely as a result

of the wide variability on values as highlighted by the larger range of values. The data from this non-optimised sequence suggests hints at the potential for the development of a sequence allowing for quantitative analysis of the constitution of skeletal muscle by CMR, rather than pointing to the use of these specific cardiac sequences on the skeletal muscle routinely.

8.5.6 MMT8 and VAS as markers of disease severity

Both VAS and MMT8 are validated methods of assessment of disease activity. The study found there was a correlation between the two (r_s=-0.63 n=18 p=0.005), but no correlation between either and myocardial T1 or T2 values. They was no correlation with skeletal muscle T1 or T2 either.

8.6 *Limitations*

The sample size of this study was calculated to give sufficient power to the difference between healthy volunteers and patients with myositis. As such the sample size is small when data as variable as skeletal muscle values are concerned. When overall differences in values are considered, each scan undertaken offers greater power to the data, but these are not interpretable where correlation is being assessed limiting this to the initial presentation. Although prospective, this is an unblinded recruitment process meaning there is likely significant selection bias, particularly given 73% of subjects had elevated troponin at their first scan.

The most notable limitation of this study is the significant difference in age between the experimental group and the healthy controls. The difference in medians between the experimental group and the healthy controls is 20 years. Although T1 values have been shown to fall with advancing age [153] the effect on T2 remains contentious [139]. Future studies specifically prospectively recruiting age and gender matched controls would strengthen the case to use tissue mapping in this cohort. Note is made that current early clinical applications of parametric tissue mapping sequences advises a single normal range of both T1 and T2 be derived from mid septal ROI in normal myocardium and does not mandate different normal ranges dependent on age or gender [154].

Although the sequences themselves have been validated against biopsy, this is yet to be performed in this group of patients to confirm the findings are consistent with tissue diagnosis.

8.7 *Conclusions*

This study shows the ability of T1 and T2 mapping to identify myocardial changes in patients with myositis. It demonstrates that troponin I correlates well to cardiac disease activity. Previous work suggesting that TnT and TnI have a role in assessing severity of skeletal muscle disease independent of CK levels leads to the suggestion that troponin I levels should be routinely sampled rather than only in the event that cardiac disease is suspected [28].

The observation that even patients with normal troponin I had significantly higher T1 and T2 levels than healthy volunteers suggests an acute on chronic process that is not necessarily being represented by troponin elevation. In cases with isolated high T1 the suggestion would be of a previous cardiac insult causing chronic fibrosis, whereas a T2 elevation in conjunction with T1 is found when acute inflammation. The observation that low level inflammation may be present despite a normal troponin and the knowledge that the presence of LGE (seen in roughly 50% of patients with myositis across this study and the available data) worsens prognosis regardless of the cause [155] makes a strong case for the routine use, not only of CMR , but CMR with tissue mapping in patients with myositis.

The lack of correlation with MMT8 and VAS measures further highlights that clinical assessment and traditional assessments for the presence of cardiac involvement are insufficient to identify subtle low-level myocardial involvement.

9. Longitudinal application of CMR with tissue mapping as a tool for monitoring disease progression

9.1 Introduction

The presence of cardiac involvement in myositis has been discussed in detail above. The findings from the previous chapter (**Chapter 8**) highlight a role for mapping based CMR in identifying the presence of cardiac involvement in patients with myositis. While this has potential clinical applications, it is then additive to understand how cardiac involvement alters over time with treatment. Is it progressive or does the current rheumatological management effectively treat the cardiac involvement? As mentioned previously, the presence of LGE is an adverse prognostic sign whatever the cause [155] and as T1 elevation might be a precursor to the development of LGE, could timely identification help to reduce the risk of developing LGE following an acute inflammatory episode.
Currently, the significance of low-level myocardial inflammation in myositis as identified in the previous chapter is unknown. Aside from the development of scar (LGE) as a poor prognostic sign, the long-term impact of mild myocarditis has yet to be established. CMR with mapping is able to identify this low-level disease. In this chapter its validity as a tool for monitoring cardiac involvement in myositis will be assessed in order to better understand how a long term follow up study could be designed.

9.2 Hypothesis

That mapping based CMR will provide an accurate tool for monitoring disease progression or response to treatment in this cohort. Given not many repeated measure studies using T1 and T2 mapping exist it will also demonstrate the sequences' ability to sensitively delineate changes in myocardial extracellular space caused by inflammation or fibrosis.

9.3 *Methods*

All myositis subjects were invited to attend up to four times through the study. CMR with full mapping protocol was undertaken at each visit and at the first and last visit contrast enhanced (LGE) imaging was undertaken. In the event a subject had a clinical indication further contrast enhanced studies were undertaken (n=2). Following a substantial amendment, blood samples were collected at times where there was need for IV access or a clinical indication.

9.3.1 Population

Patients with myositis were recruited either from Dr Gordon's Rheumatology clinic at King's College Hospital or at the point where they were referred for CMR as part of an acute presentation with either a new diagnosis of myositis or a flare of their existing disease. Inclusion and exclusion criteria are displayed in detail above (**chapter 6**). The treatments the patients with myositis were on at recruitment are listed in **table 7**. Treatment throughout the study was altered according to the patient's own condition by their rheumatology team. This study was not powered to assess for response according to different treatment regimens. Patients were scanned on a clinical NHS CMR list by the author according to specific protocol. Appointments for scans were controlled by the same waiting list process as NHS patients and facilitated by the author.

9.3.2 CMR

All CMR studies were performed on the same 1.5T Magnetom Aera, (Siemens Healthcare GmbH, Erlangen, Germany). Assessment of LV function was undertaken at each visit using standard volume assessment. Each scan involved full CMR T1 and T2 mapping protocol described in detail above. 3 long axis acquisitions and 2 basal, 2 mid and 2 apical short axis segments were acquired for both T1 and T2. A large field of view image was acquired in both to try to ensure biceps and triceps was included in the field of view. The reason for 2 acquisitions in each short axis position was to increase the chances that diagnostic quality images were acquired. All mapping sequences were interpreted by the author using a manual contour approach over the 12 basal and mid-cavity segments. Any segment where image quality was deemed inadequate in both short axis slices the corresponding long axis

acquisition was measured. Skeletal muscle acquisitions were assessed for quality using both the colour maps and the grey scale images and if the quality was deemed insufficient the measurements were disregarded. Those that were included were measured with location of ROI limited to biceps or triceps in the 4-chamber view. Following analysis all measurements were anonymised and tabulated for data interpretation. Patients with myositis undergoing contrast enhanced studies had the LGE assessed by senior readers in CMR (consultant grade) to reduce the risk of bias.

9.3.3 Biomarkers and functional assessment

Patients with myositis were asked to complete a patient global assessment visual analogue scale at each visit. They were asked to grade their myositis severity as a whole rather than cardiac symptoms. Manual muscle testing (MMT8) scores were recorded having been undertaken by experienced clinicians. Initially blood samples for troponin, CK and NT-pro-BNP were collected by the clinical team but, following a substantial amendment to the original protocol the author was able to sample blood from subjects when they attended for contrast enhanced scans. At attendances for non-contrast studies, sampling was only undertaken in the event of clear clinical indication. These samples were analysed by the King's College Hospital biochemistry department and made available to the relevant clinical team.

9.3.4 Statistical analysis

All statistical tests were undertaken using GraphPad Prism 8 (GraphPad Software Inc, USA) All measurements analysed were assessed for normality or lognormality where $p<0.05$ was considered in keeping with normality. In the event both normal and log normal scales were equally well fitting, the distribution that satisfied the most of four normality tests, Anderson-Darling, D'Agostino and Pearson, Shapiro-Wilk and Kolmogorov-Smirnov was used. Data are displayed as mean ± SD unless stated otherwise. Data are displayed as mean ± SD unless stated otherwise. Non-normally distributed data are displayed as median ± range or interquartile range (IQR).

Other statistical tests undertaken were:

1. Parametric and non-parametric distribution data
    a. Unpaired and paired t-test

   b. Mann-Whitney
   c. Wilcoxon test
2. Change with time
   a. t test
   b. Mixed-effects analysis

9.3.5 Data handling

All values were tabulated using Microsoft excel and stored anonymously. DICOM files were stored within the hospital in a locked cupboard on DVD and on an external hard drive. DICOMS were anonymised when analysis was undertaken purely for research purposes. Example of the raw data is shown in **Figure 15.** All DICOM images were analysed using commercially available post-processing software (Version 5.10.1, Circle Cardiovascular Imaging Inc., Calgary, Canada).

9.4 *Results*

9.4.1 Population

Specific Characteristics of subjects are displayed in **table 7**. A total of 70 scans were undertaken on 26 myositis subjects. Each subject was scanned between 1 and 4 times (mode=3). The spread of myositis diagnoses was as follows; Diagnosis (n): DM (8), PM (6), Antisynthetase syndrome (AsS) (3), Necrotising myopathy (NM) (4) and Myositis overlap (5). The antibody profile is detailed in **table 7**. The most common antibodies observed were Jo-1 (n=5) and Ro-52 (n=4). There was no significant variation in T1 or T2 values between the different antibody profiles. There was no increase in the incidence of LGE or a myocarditis diagnosis with particular antibodies. Mean ejection fraction 69.6 ±11 as assessed by CMR (normal). This did not change significantly with time. Median patient VAS (cm) was 5.2 (8). This did not reduce significantly with time (**Figure 35**).

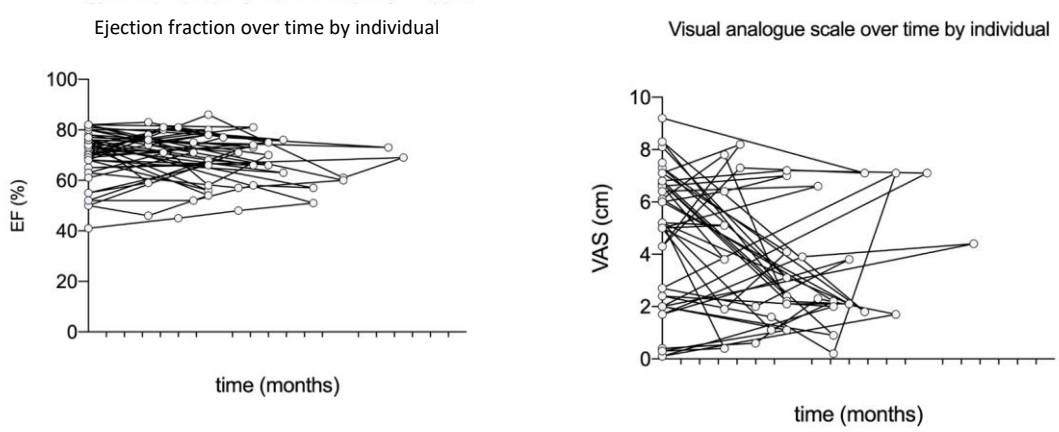

Figure 35: Ejection fraction (EF) (%) over time by individual [**left**]**.** There is no significant change in EF%. VAS (cm) by individual over time [**right**]**.** There is no significant change over time.

9.4.2 Late gadolinium enhancement

On initial scanning, 52% (13/25) of patients were found to have LGE. The final CMR protocol involved the administration of contrast. Two patients developed LGE over the course of the study. One of these was secondary to the development of pulmonary hypertension so outside the scope of this study. In 3 cases the LGE seen on initial scanning was not seen on the subsequent scan representing an 8% reduction in LGE with time.

9.4.3 Myocardial T1 and T2 changes over time

Myocardial T1 and T2 values fell over time in patients with myositis on treatment. In fact, for all patients recruited there was a significant fall over the course of the study (T1 $p<0.0001$ and T2 $p=0.0007$) (**Figure 36**). In subjects presenting with an initial T1 and T2 above the upper limit of normal for our scanner, a reduction in T1 and T2 represents a resolving process (either as a result of treatment or spontaneous recovery). In these subjects we saw a significant reduction in relaxation times over time using repeated measures assessment (T1 $p<0.0001$ and T2 $p=0.0002$) (**Figure 37**). If subjects presenting with elevated TnI were isolated (instead of presenting T1 and T2 values) a significant reduction with time was still seen (T1 $p<0.0001$ and T2 $p=0.0026$).

9.4.4 Skeletal muscle T1 and T2 changes over time

Skeletal muscle T1 and T2 values were measured at each visit (provided image quality was sufficient). We did not find a significant fall in skeletal muscle T1 with time in patients with myositis using repeated measures analysis (T1 p=0.23 n=24, missing values 35/96). There was a reduction in T2 values with time (p=0.04 n=26, missing values 44/104). The number of missing values highlights the caution with which these data should be interpreted and illustrates the challenges of acquiring images of high enough quality using these sequences.

9.4.5 Change in myocardial T1 and T2 over time compared patients with myositis and healthy volunteers

There was a significant reduction in T1 and T2 over time. If comparison of T1 and T2 over time was made with the healthy volunteer values, the difference in T1 remained significantly higher at the end of the follow up period (1022 ±27 vs 995 ±25 p=0.006). However there was no significant difference between T2 and healthy volunteers at the end of the follow up period (46 ±1.7 vs 46 ±1.5 p=0.6). This remained the case when we only considered patients where initial T1 and T2 values were above the upper limit of normal at presentation (T1 1032 ±27 vs 995 ±24 p=0.003, T2 47 ±1vs 46 ±2 p=0.06) (**Figures 36 and 37**). If those patients with elevated initial troponin were isolated, the significant difference remained present in T1 but not in T2 when compared to healthy volunteers (T1/T2 p=0.02/0.6).

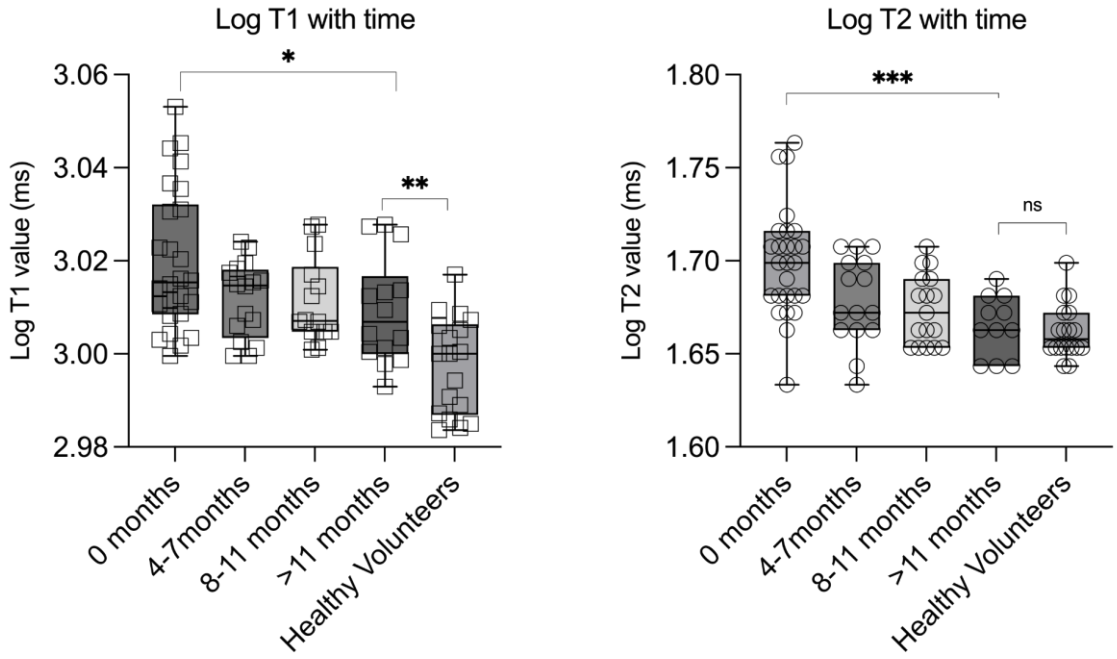

Figure 36: Box and whisker (min to max) plot of T1 and T2 over time of all recruits and compared with healthy volunteers. Significant difference between T1 values remains throughout despite treatment (1022 ±27 vs 995 ±25 p=0.006). Significant fall in both values over time with T2 values returning to the level of healthy volunteers (46 ±1.7 vs 46 ±1.5 p=0.6). Bars represent paired t test of values between each time point. P<0.05 *, p<0.001 **, p<0.001 ***p<0.0001 **** Repeated measures analysis showed a significant reduction with time in the experimental group (T1 p<0.0001 and T2 p=0.0007)

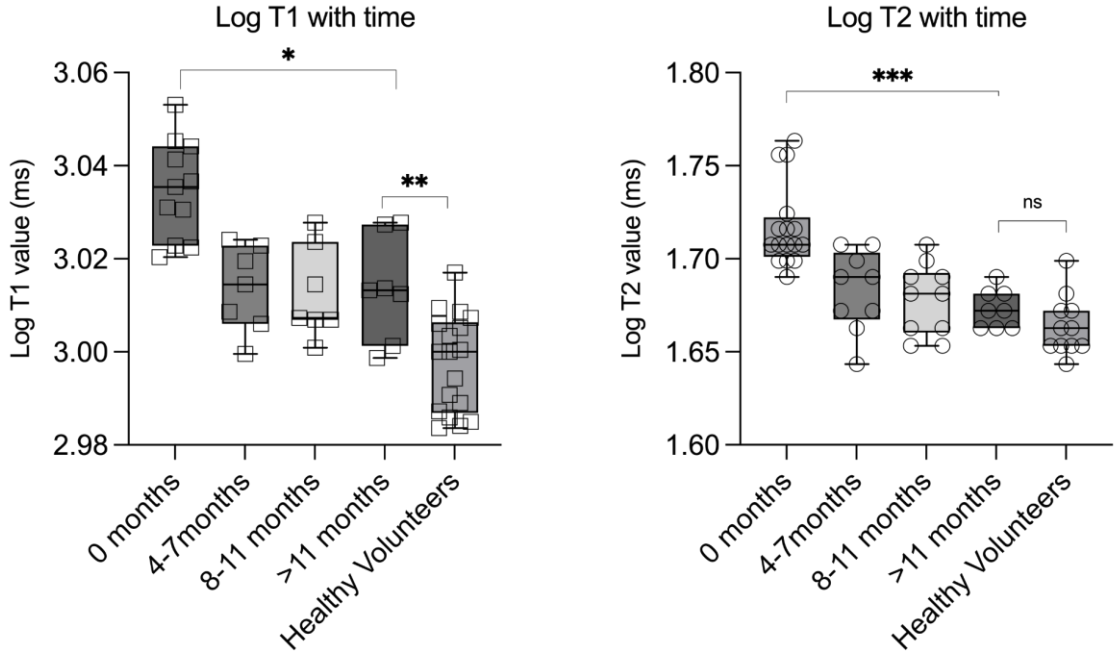

Figure 37: Box and whisker (min to max) plot of T1 and T2 values in patients with myositis presenting with elevated initial T1 and 2 over time compared with healthy volunteers. As with all subjects T1 does not return to the level of healthy volunteers but T2 values do (T1 1032 ±27 vs 995 ±24 p=0.003/T2 47 ±1vs 46 ±2 p=0.06). Bars represent paired t test of values between each time point. P<0.05 *, p<0.001 **, p<0.001 ***p<0.0001 **** Repeated measures analysis showed a significant reduction with time in the experimental group (T1 p<0.0001 and T2 p=0.0002)

9.4.6 Change in myositis skeletal T1 and T2 over time compared with healthy volunteers.

As shown above there was a significant drop in skeletal muscle T2 over time. If paired analysis of T1 and T2 values at initial presentation were compared with the final scan, a difference was seen (T1/T2 Median(range) 931(498) vs 894(242) p=0.001/39(45) vs 34(11) p=0.004). **Figure 38** shows the reduction over time of skeletal muscle T1 and T2 and compared with healthy volunteers. Unlike myocardial T1, the T1 value of skeletal muscle at the last scan was not statistically different to the value found in healthy volunteers (Median (range) 894(242) vs 864(131) p=0.09). The same pattern of T2 compared with healthy volunteers with time was seen in skeletal muscle as with myocardium (Median (range) 34(11 vs 34(6)). If subjects with elevated CK at presentation are considered, there remained a significant difference between T1 of myositis and healthy volunteers following treatment but not with T2 (T1/T2 Median(range) 923(242) vs 864(131) p=0.003/34(11) vs 34(6) p=0.7). One reason for repeated measures failing to show a significant reduction over time whereas individual analysis of paired data sets at initial scan and final scan is likely to be the heterogeneity of the data as produced by the non-optimised sequence.

9.5 *Discussion*

This is the first study to comment on the change in T1 and T2 over time in patients with myositis. Indeed the use of mapping sequences as a tool for disease monitoring (even in primary cardiac disease) is limited currently. This study has shown the potential benefit of sequential scanning for monitoring change in disease state over time.

9.5.1 Markers of 'overt' cardiac disease in myositis

One of the challenges to diagnosing underlying cardiac involvement in myositis is identifying the presence of sub-clinical disease. When overt measures such as overall LV function are examined over time a significant drop in function overall is not seen, and although there are studies (using echo in particular) that suggest subtle differences between function in comparison with healthy volunteers [30] this was not seen either at presentation or throughout the period of follow up. Changes in late gadolinium seen across the subjects,

namely that in 3 cases the LGE resolved, underlines the observation that cardiac involvement is a dynamic process meaning a single assessment for the presence of cardiac involvement using LGE only is limited.

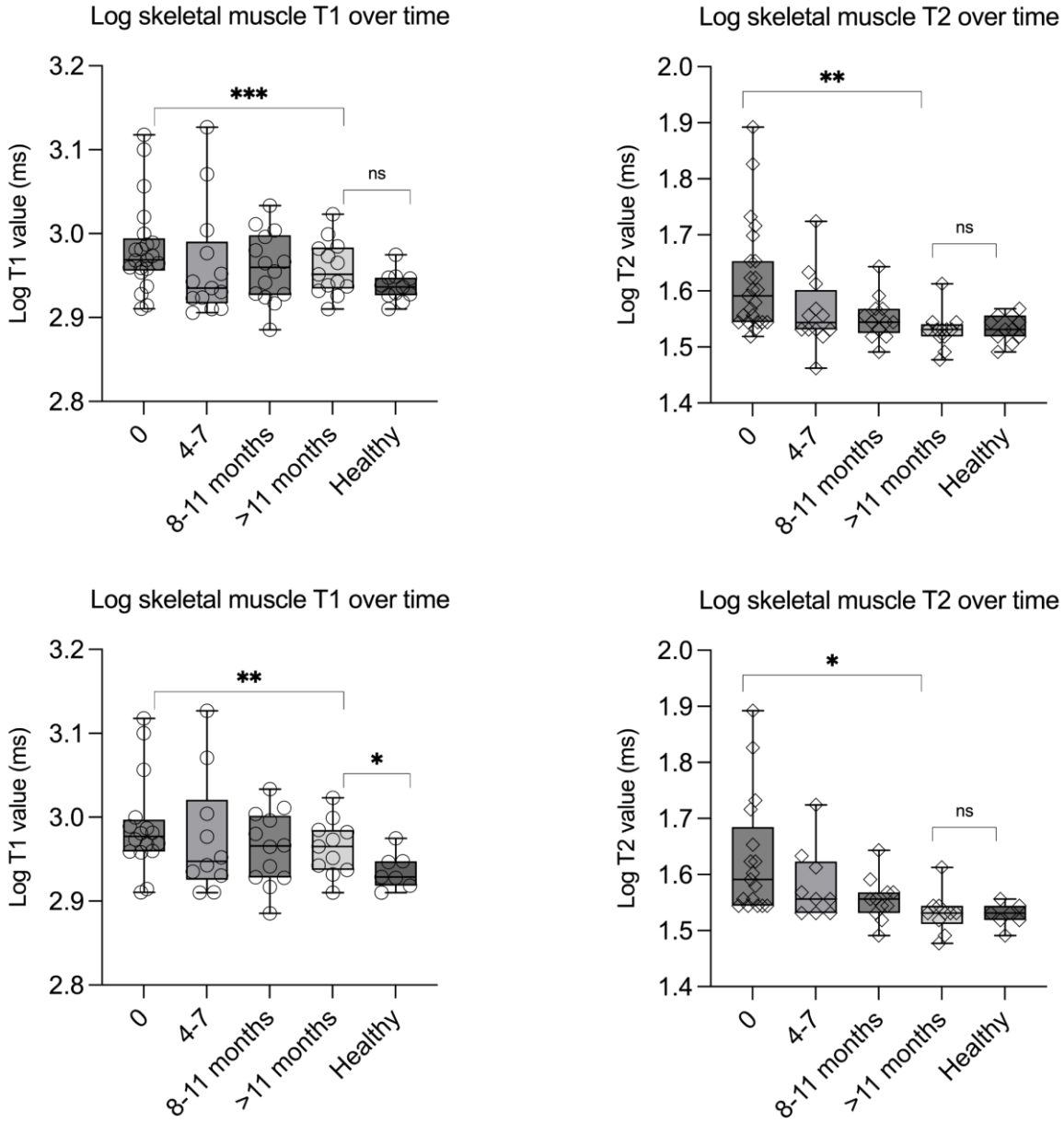

Figure 38: [**Top**] Change in skeletal muscle T1 and T2 over time of all myositis subjects compared with healthy volunteers (median ±IQR). There is a significant drop in values between the initial scan and the final scan in the myositis group (T1/T2 Median(range) 931(498) vs 894(242) p=0.001/39(45) vs 34(11) p=0.004). Both T1 and T2 values return to the level of healthy volunteers (p=0.09 and 0.6 for T1 and T2 respectively). [**Bottom**] Subjects with elevated CK at presentation are isolated. Significant reduction in relaxation times remain between first scan and last (T1/T2 Median(range) 949(498) vs 923(242) p=0.004/39(43) vs 34(11) p=0.02), however, in this group skeletal T1 remains elevated compared to healthy volunteers. T2 returns to normal (923(242) vs

864(131) p=0.03 for T1 and 34(11) vs 34(6) p=0.7 for T2. Bars represent Mann-Whitney comparison of each paired data set at each time point p<0.05 *, p<0.001 **, p<0.001 ***p<0.0001 ****

9.5.2 Changes in myocardial T1 and T2 over time

A significant reduction in relaxation times was seen over the course of the study. The relevance of this is particularly significant when considering those patients whose original presentation was suggestive of cardiac involvement. Cardiac involvement was defined as the presence of either elevated troponin I or elevated mapping values at initial presentation. In these patients T1 and T2 fell significantly with time. This likely reflects either a spontaneous resolution of myocarditis, or a response to the treatment.

Aside from merely observing a fall in relaxation times over the course of the study we compared the mapping values at the end of the study with healthy volunteer values, demonstrating that T2 returns to normal whereas T1 values remain significantly higher in patients with myositis than in healthy volunteers. This suggests that inflammation (based on high T2 values) resolves more completely with time, but that there may be a chronic fibrosis leading to persistently elevated T1 values. Myocardial scar of any cause is an adverse prognostic sign and while it has yet to be shown conclusively that T1 elevation is a similarly adverse sign, it is likely to be linked to adverse outcomes [156-159]. Likewise, T2 elevation in myocarditis of other causes is also associated with adverse outcomes [160]. Monitoring both T1 and T2 over time will allow for monitoring of ongoing inflammation but may also offer useful insight into the effect different treatments have on the chronic fibrosis seen in these patients. Our study was not powered to observe the effects of different treatments, particularly given the wide variety of different DMARDs utilised.

It seems likely that the persistent difference in T1 values between healthy volunteers and myositis patients represents the ‘sub-clinical’ cardiac involvement that has not been identifiable to date. Given the high likelihood that these findings represent a precursor to overt cardiac involvement, a contrast free non-invasive test able to identify it has significant potential in both the clinical and research fields.

9.5.3 Changes in skeletal muscle T1 and T2 over time

Skeletal muscle T1 and T2 values were measured (where image quality was sufficient) at each visit. As there are currently no normal values for skeletal muscle T1 and T2 using tissue

mapping sequences, for the purposes of this study, subjects were considered to have active skeletal myositis if they had a raised CK. When subjects with a CK elevation were analysed, a significant drop in skeletal muscle T2 over time was found.

While there is a significant difference between T1 and T2 values at presentation and at the final scan, if repeated measures are applied, there was no evidence of a pattern of reduction with time. In patients with CK levels suggestive of active muscle inflammation at recruitment, the T2 values by the final scan are no longer statistically different to the values seen in healthy volunteers. This may suggest that a skeletal muscle dedicated T2 sequence could have disease monitoring applications in patients with myositis. Given the difference between repeated measures and paired analysis it is unlikely that anything more than potential future applications of a dedicated sequence can be inferred. Where T1 values are considered, a similar pattern is seen as with T2. However, in patients with a high CK at presentation, the T1 did return to the level of healthy volunteers by the end of the follow up. While this mirrors what we see in the myocardium caution is required in interpretation. While it could represent a chronic fibrotic process, without dedicated sequences affording less variability and better image quality, this conclusion is beyond the reach of this study.

In summary, despite some significant changes over time, the skeletal muscle findings highlight the difficulties with applying these sequences outside of the centre of the magnet bore and that further CMR sequence development will be required.

9.6 *Limitations*

As myositis is a rare condition, sample size is low. However, the effect size is high enough for adequate power to be achieved. The protocol required repeated scanning and as a regional centre for myositis, many of the patients had a significant journey making drop-out rates higher than originally anticipated. The scanning of patients had to be arranged around clinical priority as no research dedicated scanner time was available making total flexibility for the research subjects needs difficult. We also did not perform a myocardial biopsy to validate the CMR findings.

As mentioned previously, the mapping sequences used were not designed for the skeletal muscle and this led to a significant scatter of data, which therefore of likely limited accuracy.

As with the previous chapter, the lack of age and gender matched controls may have a small impact on what can be inferred from the comparison with healthy volunteers.

9.7 *Conclusions*

This study shows that T1 and T2 mapping may both have a role in both identification and monitoring of myocardial involvement in myositis. The normalisation of T2 suggests resolution of acute inflammation with treatment, however, the persistent increase in myocardial T1 values suggests there are chronic 'sub-clinical' changes within the myocardium of patients with myositis.

10. Discussion

10.1 *Introduction*

Through this thesis the potential application of novel parametric tissue mapping sequences to the question of underlying cardiac involvement in myositis has been explored. The importance of cardiac muscle involvement is not to be understated but the variability of presentation has made accurate diagnosis difficult. This, combined with the large proportion of apparent sub-clinical presentations, has further muddied the water.

The work in this thesis has shown that a multi-segment model of analysis of tissue maps is reproducible and also provides comparable values to the current practice of analysing a single region in the septum. Adoption of this method will help prevent focal disease being missed in patients with myositis. Following on from this, T1 and T2 maps were shown to likely detect low-level inflammation and fibrosis within both the myocardium and skeletal muscle of patients with myositis. Myocardial involvement was shown to correlate with troponin values. Repeated measurements of T1 and T2 mapping we found to have the ability to monitor treatment response over time, showing that inflammation (assessed via myocardial T2), appears to resolve, but that subclinical myocardial fibrosis (assessed by myocardial T1) is likely to result. Myocardial T1 values were also higher in patients with myositis with normal troponin than healthy volunteers. This suggests that even if the troponin is negative, there is potentially a degree of chronic change within the myocardium. This chapter will bring together the findings from this thesis with the current understanding to offer insight into how this new information could be applied clinically and suggest areas of future work in the field.

10.2 *Validation of a novel 12-segment approach to interpretation of parametric tissue mapping sequences based on the American Heart Association model*

10.2.1 Summary of study validation and findings

The current convention for analysis of tissue mapping sequences is to draw a single ROI in the basal or mid-septum to measure a relaxation time (ms). In order to identify subtle disease, either patchy or diffuse, that may exist outside of the septum **chapter 7** describes a method using a 12 segment AHA model and found high reproducibility in health and disease when compared to a mid-septal ROI. This is the first time this approach has been applied to either healthy volunteers or disease states. The method proved robust whether interpreted by manually drawn segmental ROI or using the automated endo/epithelial derived contours provided by the reporting software (Version 5.10.1, Circle Cardiovascular Imaging Inc., Calgary, Canada).

10.2.2 Future clinical applications

This novel model for assessing T1 and T2 relaxation has potential clinical application. Currently, there is hesitation in the field to use multi-segment analysis as inclusion of segments that abut non-cardiac structures and those with high levels of through-plane motion (particularly the apex) have been seen as introducing too much variability. By removing the apical segments, a larger area of myocardium can be assessed without removing the quantitative nature of the sequences. In conditions where a focal myocardial process might occur (such as in myocarditis of any aetiology or in hypertrophic cardiomyopathy for example), this technique will allow for quantitative analysis and for monitoring of progression.

10.2.3 Future research applications

In future work within the myositis population this method will be beneficial to investigators wishing to research either acute myocarditis or treatment response, particularly given the predilection myositis appears to have for the basal inferolateral walls. This might also be of use in myocardial infarction for example, where infarct size, area at risk and myocardial

salvage index are measured. It has been suggested that the greater the ventricular coverage the more accurate the assessment in this disease process [146, 163].

10.3 *Application of parametric tissue mapping sequences in the identification of cardiac involvement in myositis*

10.3.1 Summary of study validation and findings

In **chapter 8** a comparison of mapping values of myositis subjects with healthy volunteers was performed and the results compared to other biomarkers. T1 and T2 values in patients with myositis were significantly higher than healthy volunteers. This is in keeping with the small body of work already in the literature. There is a degree of selection bias in both the current published literature and in the myositis subjects that make up this study (78% of patients had a positive initial troponin). Hence this study also compared patients with myositis with a negative troponin to healthy volunteers and then to TnI positive patients. This comparison showed a significant difference between troponin negative myositis and healthy volunteers at initial scan (T1/T2 p=0.04/0.03) and between high and normal TnI patients with myositis (T1/T2 p=0.02/0.03). Other biomarkers and clinical indicators of skeletal muscle disease severity (MMT8 and Patient VAS) did not correlate with mapping values. Myocardial T1 and T2 values were found to correlate with troponin levels. This is the first study to identify this correlation and suggests that the group of patients with myositis previously noted to have elevated troponin, but seemingly normal cardiac investigations will likely have subclinical myocarditis. Where previous studies have only compared mapping values with healthy volunteers, by comparing between troponin positive and negative patients this work provides evidence that troponin is a useful test to identify patients with active cardiac disease.

Skeletal muscle T1 and T2 relaxation times were also compared. A significant difference between health and disease was identified (T1/T2 0.002/0.006 respectively) but no

significant difference was found between myositis subjects when grouped by biomarker levels or a correlation to disease activity measures. The wide range of values may lead to a lack of significant correlation in the initial scans between either biomarkers or MMT8 and VAS. This is the first study to look at the comparison of patients with myositis by biomarkers and the signal found suggests potential value in further work in this area using a specific sequence tailored to skeletal muscle.

10.3.2 Future clinical applications

This study confirms the suggestion from the current literature that T1 and T2 mapping has value in myositis. This, however, is the first study to attempt to offer an explanation of the elevated TnI seen in patients with myositis that had previously not been associated with cardiac disease. Routine TnI measurements should be taken in the same way as other skeletal muscle measures. In the event troponin is elevated, CMR with tissue mapping should be considered as this will help to confirm the presence of myocardial inflammation or fibrosis. Also, as these measures are quantitative, they give a reference value to monitor disease progression. Given the difference between healthy volunteer values and patients even with negative troponin, a case can be made to offer a CMR with tissue mapping to all patients with myositis as part of their work up. Limited access to CMR in some countries and localities may make this less feasible.

10.3.3 Future research

This study, like most within the myositis population is limited by sample size due to the condition being rare. Although significance is found (given a relatively large effect size) between different mapping values where biomarkers are high or low, there are no significant differences around different antibodies and the presence of cardiac involvement. It is known, for example, that certain antibody profiles are linked to increased likelihood of lung involvement, whereas no such associations have been identified with cardiac involvement. The ability to use tissue mapping to identify cardiac involvement as shown here could be applied to a larger sample size in order to try to identify patterns of antibodies.

Initial findings from the skeletal muscle component to this study suggests potential benefit from further work. By applying these sequences (cardiac optimised) or developing dedicated sequences to image large muscles (quadriceps for example) specifically may present mapping sequences as a non-invasive quantitative measure of skeletal muscle involvement which would be a useful diagnostic tool. The current data suffers from significant artefact leading to a wide variation in values making significant difference difficult to establish.

10.4 *Longitudinal application of CMR with tissue mapping as a tool for monitoring disease progression*

10.4.1 Summary of study validation and findings

The work in **chapter 9** addresses how the myocardial T1 and T2 change with treatment over the course of year in patients with myositis. No previous studies to date have addressed this.

Seventy scans were performed through the course of the study. Patients were being treated as deemed appropriate by their own clinical teams. Improvement in MMT8 scores during the study confirmed the clinical improvement of the skeletal muscle disease. Interestingly there was no change in left ventricular ejection fraction with time, highlighting the limitation of using traditional echo-based measures of cardiac involvement. A significant reduction in myocardial T1 and T2 over time was seen in subjects presenting with initial evidence of cardiac involvement (either elevated troponin, mapping values, or both). The strongest correlation was seen when mapping values were elevated at presentation. This demonstrates a response within the myocardium over time. After a year of treatment there was no difference between healthy volunteer and patient myocardial T2, however there remained a significant difference between myocardial T1. This suggests resolution of acute inflammation over the course of the study, but resulting subclinical fibrosis.

Skeletal muscle T1 and T2 was also assessed at each time point. While a signal for reduction with time was seen, particularly in patients with high CK at presentation, interpretation of this requires caution for the reasons detailed through the thesis.

10.4.2 Future clinical applications

The above findings, combined with those in **chapter 8,** suggest a role for CMR in the monitoring of patients with myositis. For patients with suspected cardiac involvement, CMR with tissue mapping is indicated, because the suggestion is that the sensitivity is greater than with TnI alone. This longitudinal study suggests a further role for CMR in monitoring disease progression as T1 and T2 change with time. Given troponin levels drop faster than the T2 values, using TnI values alone to monitor disease activity may both falsely reassure clinicians and fail to identify the low-level scarring evidenced by the persistently elevated T1 values. Although currently there is no evidence to support or refute treatment escalation in patients with persistently elevated T2 on serial CMR, being able to identify its presence will allow for case-by-case discussion as to whether any therapeutic changes are warranted.

10.4.3 Future research

In order to understand the significance of the changes seen within the myocardium, further research work is needed. Having established CMR as a useful method of monitoring myocardial disease, a long term follow up study with both the development of LGE and cardiac events as an end point is required to establish what effect these 'sub-clinical' changes may have on outcomes. The next area of work would be to try to identify whether certain treatments reduce the 'gap' between myositis T1, and healthy volunteers seen even after a period of treatment. This could involve the use of different disease modifying agents or indeed the use of a drug to reduce myocardial fibrosis such as an angiotensin-converting-enzyme inhibitor (ACE-I) or spironolactone.

11. Novel outcomes from this thesis

1. A 12 segment American heart association model for analysing the ventricle is reproducible and its mean is comparable to the mean of a mid-septal region of interest.
2. Myositis myocarditis follows the same pattern of distribution through the ventricle as viral myocarditis.
3. T1 and T2 values in patients with myositis with elevated troponin are higher than those with negative troponin.
4. Troponin level correlates with T1 and T2 values.
5. T1 and T2 of patients with myositis change with time.
6. T2 values return to the level of healthy volunteers over time.
7. T1 values of patients with myositis do not return to the level of healthy volunteers.
8. Skeletal muscle T1 and T2 measurements may have future merit in disease identification and monitoring, although dedicated sequences are needed.

12. Conclusions

In this thesis, the CMR derived methods of myocardial T1 and T2 show great promise in the assessment of subclinical myocardial involvement in patients with myositis. Further work will look to build on this with the potential to make both measurements a useful clinical and research tool in this disease.

13. Supervision and Personal contribution

13.1 *Supervision*

First supervisor: Dr Dan Sado, Consultant Cardiologist

Second Supervisor: Professor Theresa McDonagh, Professor of Cardiology

Third Supervisor: Dr Patrick Gordon, Consultant Rheumatologist

13.2 *Personal contribution*

My contribution to this work is listed below

1. Devised and wrote research protocol
2. Obtained ethical approval
3. Recruited, scanned, reported, and performed research analysis on all healthy volunteers.
4. Recruited, scanned, and analysed all images in patients with myositis
5. Obtained major ethics amendment to allow for blood sampling
6. Obtained all blood samples taken as part of the amended research protocol
7. Gathered and collated all clinical information, including additional biomarker values, clinical assessment data, and antibody profiles for all subjects
8. Performed all statistical analysis presented in this thesis
9. Wrote this thesis

13.3 *Publications arising from this thesis*

A prospective longitudinal follow up study using T1 and T2 mapping sequences and twelve-segment myocardial assessment to identify and monitor myocardial inflammation in myositis. Dancy L., Bromage D., Nabeebaccus A., O'Gallagher K., Le K., Pearce L., Millin A., Kellman P., Gordon P., Sado D. *European Heart Journal Cardiovascular Imaging EuroCMR 2019. Italy. 20 (Supplement 2) (pp ii126-ii127), 2019*

13.4 *Awards*

- Myositis UK Fellow Bursary 2019
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16. Appendices

Appendix 1: Consent Form for patient participants

Appendix 2: Patient information sheet

Appendix 3: Letter to GP

Appendix 4: Healthy Volunteer Information sheet

Appendix 5: Poster for recruitment of healthy volunteers

Appendix 6: Email for recruitment of healthy volunteers

Appendix 7: Example Patients Global assessment tool

Appendix 1

Patient Consent Form

DIFUS-T1

Defining the incidence of Cardiac involvement in Myositis using mapping based Cardiovascular Magnetic Resonance Imaging

IRAS Number 222888

Chief Investigator: Dr D. Sado, Cardiology Consultant

Patient Identification Number for this trial:

		Please initial box
1	I confirm that I have read the information sheet dated..... (version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2	I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	☐
3	I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from the sponsor of the trial (King's College London/King's College Hospital NHS Trust) and responsible persons authorised by the sponsor, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
4	I understand that the information collected about me will be used to support other research in the future and may be shared anonymously with other researchers.	☐
5	I agree to my General Practitioner being informed of my participation in the study	☐
6	I agree to be contacted about ethically approved future research.	☐
7	I understand that samples of blood will be taken and sent to King's College Hospital biochemistry and haematology departments for further analysis.	☐
8	I agree to take part in the above study.	☐

_____ _____ _____

Name of Participant	Date	Signature

Name of Person taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

Appendix 2

Patient information sheet

DIFUS-T1

Defining the incidence of Cardiac involvement in Myositis using mapping based Cardiovascular Magnetic Resonance Imaging

PART 1

We'd like to invite you to take part in our research study.
Joining the study is entirely up to you before you decide we would like you to understand why the research is being done and what it would involve for you.
One of our team members will go through this information sheet with you, to help you decide whether or not you would like to take part and answer any questions you may have.
We suggest this should take about 15 minutes.
Please feel free to talk to others about the study if you wish.

The first part of the Participant Information Sheet tells you the purpose of the study and what will happen to you if you take part.

The second part will give you more detailed information about the conduct of the study.
Please ask if anything is unclear.

What is the purpose of the study?

- The purpose of this study is to discover if patients with Myositis get heart muscle inflammation. Myositis is a condition where the body attacks its own muscles causing swelling and discomfort. It can also affect other organs including the heart muscle which is what we are aiming to study.

- The study is asking what proportion of patients with Myositis have heart muscle inflammation and whether an MRI scan can pick up subtle or early involvement
- The overall aim of the study is to accurately define what proportion of patients get heart muscle involvement so we can predict which patients need the most aggressive treatment to prevent heart muscle damage from adding to their symptoms and also to devise a method that can become the standard for everyone treating patients with this condition to follow in future.

Why have I been invited?

- You have been invited to take part in the study because you have an autoimmune condition.

Do I have to take part?

It is up to you to decide. We will describe the study and go through this information sheet, which we will then give to you. You will be able to keep this information sheet and think about taking part. You are free to discuss the information with anyone you wish including your family and friends. If you agree, we will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive

What will happen to me if I take part?

If you agree to participate in the study, we will ask you to consent to a number of different investigations that will help us to understand better the nature of heart involvement and the way in which it affects you. All the tests we perform are in fact accepted investigations for people with your condition, however, we will use a more detailed approach to the heart than has been applied to your condition previously. Below is a list of what we would undertake.

Summary of study investigations

We will be using the investigations and assessment that are routinely performed when you attend clinic for review including your routine blood tests and heart investigations. We will also invite you to attend for an MRI scan of your heart. This is commonly undertaken for a number of conditions including rheumatological conditions. We will, however, invite you to attend for more than one scan over the course of a year. The timetable will be as follows:

Initially: Cardiac MRI scan as you would undertake as part of your usual clinical assessment. This involves an injection of intravenous contrast

Three months: Repeat MRI with only non-contrast images taken. This is a much shorter scan.

Six months: Third MRI scan, also without contrast.

Twelve months: Repeat full MRI with contrast the same as at your first attendance.

All the above tests would be arranged in conjunction where possible with your usual follow up appointments in clinic. If, however, that is not possible then we will work around you to ensure attendance is as convenient as possible.

What are the alternatives for treatment?

As mentioned above, the study uses already established tests to look for heart muscle involvement with your condition. If you decide not to take part in the study but on clinical assessment there is suspicion of heart involvement, then any and all of the above may be offered. The main difference remains the frequency with which these tests will be undertaken and the ability to correlate information from many different tests involving many patients. Therefore, there will be no loss to you by not undertaking this project in terms of necessary investigations

What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get from this study will help improve the future treatment of people with Autoimmune conditions and it will ensure that the most advanced tests to look for heart muscle swelling have been performed.

What are the possible disadvantages and risks of taking part?

Despite the number of tests, we plan to perform, only the contrast injection carries a recognised risk to you.

- There is a tiny risk of allergic reaction to the contrast, although this is usually minor and easily managed
- In people with very severe kidney damage there is a risk of severe side-effects from contrast injection. For this reason, anyone with severe kidney problems will not be able to take part in the study. The kidney function is a blood test which will have been checked in clinic as part of your routine tests so we will know before there is any risk to you.

Who is organising and funding this study?

The doctor in charge of this study is: Dr Dan Sado, Cardiology Consultant, and head of Cardiovascular MRI at King's College Hospital. The study is funded by King's College London.

How have patients and the public been involved in this study?

- Potential participants were involved in reviewing the Participant Information Sheet.
- In designing this study, we have taken into account patient opinions on the frequency of participant visits and the tests that we will carry out.

Who has reviewed this study?

- *All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by _____Research Ethics Committee. It has also been approved by the Health Research Authority.*

Expenses and Payments

There are no funds available for payments to those participating in this study.

This completes Part 1 of the Information Sheet.

If the Information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

PART 2

What if new information becomes available?

- What if new information that becomes available might specifically affect you and your health? If this happens, your study doctor might consider that you should withdraw from the study. He/she will explain the reasons for withdrawing from the study and arrange for your care to continue.
- If the study is stopped for any other reason, we will tell you and arrange for your continuing care.

What will happen if I don't want to carry on with the study?

- You are free to withdraw from the study at any time; and if you would like to do so; please speak to your study nurse or doctor.
- Your decision to withdraw from the study will not affect the care you receive.
- If you withdraw your consent;
    - We will ask you if you remain happy for the information from tests already undertaken to be used in future research
    - If not, you will be able to withdraw consent for any of your information to be used and it will therefore be removed from the research database but will remain available to your doctor and GP in case of clinical need
- If you do withdraw, we may wish to stay in touch through your GP to monitor your progress
- If you do decide to withdraw, we will document exactly what your wishes are so that the minimum of inconvenience is caused to you

What if there is a problem?

- If you have a concern about any aspect of this study, you should ask to speak to your study doctor who will do their best to answer your questions Dr Daniel Sado 02032999000. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints procedure by contacting your local Patient Advice Liaison Service (PALS) office. Details of your local office can be obtained by asking your study doctor, GP, telephoning your local hospital or looking on the NHS choices website. http://www.nhs.uk/pages/home.aspx

- Every care will be taken in the course of this study. However, in the unlikely event that you are injured by taking part, compensation may be available.

- In the event that something does go wrong, and you are harmed during the research, and this is due to someone's negligence then you may have grounds for a legal action for compensation against King's College Hospital, NHS Trust but you may have to pay your legal costs.

- Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated by members of staff or about any side effects (adverse events) you may have experienced due to your participation in the study the normal National Health Service complaints mechanisms are available to you. Please ask your study doctor if you would like more information on this.

Will my taking part be kept confidential?

Any information gathered from our investigations will be kept confidentially. All information will be collected in a clinical context meaning that we will remain bound by the same medical confidentiality afforded to all personal medical records. For the purposes of our research, your information will be assigned a unique identifier which will remain separate from any of your personal information allowing the data to be kept without association with individuals. The document containing the identifiers and your details will be kept in paper format to prevent electronic access from outside.
Information once anonymised may be shared with collaborators outside of King's College Hospital, but only those also bound by the same confidentiality protocols as we do, and in this particular case, only other clinicians within the NHS.

If you consent to take part in the research, any of the information collected about you may be inspected by the sponsor (including representatives of the sponsor). These inspections are solely for the purposes of the research and analysing the results. Your records may also

be looked at by the regulatory authorities or ethics committees to check that the study is being carried out correctly.

The organisations listed above will keep information about you confidential and secure. Your name will not be used in any reports about the study and all data is stored in accordance with the principle of the Data Protection Act 1998. However, your hospital doctor may tell your GP about your participation if you agree to enter the study.

Involvement of the General Practitioner/Family Doctor (GP)

- With your consent, your GP will be informed of your involvement in the trial.
- Any other medical practitioners who treat you, e.g., should you be admitted to hospital for any reason, will also be informed.

What will happen to any samples that I give?

- Blood samples collected during the study will be processed by the King's College Hospital laboratory and stored in conjunction with the trust policy on safe and confidential storage

Will any genetic tests be done?

- No

What will happen to the results of the research study?

- First and most importantly, as this is a clinical research trial, our results will be fed back to your Hospital Doctor and where relevant your GP. This will allow the findings to be applied to constructing the most personalised treatment plan possible for you.
- If the findings of investigations into your heart demonstrate that you would benefit from additional investigation or treatment by a heart specialist, then we will arrange follow up for you in Dr Sado's clinic. He is a consultant Cardiologistand the lead for this study. You should note that you may be reviewed by Dr Luke Dancy in Dr Sado's clinic. He is a senior Cardiology Registrar, Dr Sado's Research fellow and a key investigator in the trial.
- The information gathered from you and other participants will be analysed and published in medical literature. This will allow us to further the understanding of this field

and share our findings with the wider scientific community. It will also form the basis of a Research Thesis towards the award of a higher degree.

- Currently there are other studies into this condition ongoing involving multiple different centres throughout the country. We may wish, anonymously to share our results with them in order to ensure the maximum benefit from the tests is garnered

Thank you

Thank you for considering taking part and taking the time to read this information sheet. If you decide to take part in the study, we will give you a copy of the information sheet and a signed consent form to keep.

Further information and contact details

Patient Advice and Liaison Service. Patient Advice and Liaison Service, King's College Hospital NHS Foundation Trust, Denmark Hill, London SE5 9RS

Tel: 020 3299 3601, 9am to 4.30pm, Monday to Friday (not bank holidays)

INVOLVE, Alpha House, University of Southampton Science Park, Chilworth, Southampton, SO16 7NS

Telephone: 023 8059 5628. Email: involve@nihr.ac.uk

Local Contacts:

Dr Dan Sado, Cardiology Consultant,

Secretary: Marie MacCarthy: 0203 299 9000

Dr Luke Dancy Cardiology Clinical Research Fellow

Tel: 0203 299 9000

Appendix 3

06.12.2016

Dear Colleague

RE: Defining the incidence of Cardiac involvement in Myositis using mapping based Cardiovascular Magnetic Resonance Imaging

Patient Name and DOB

I am writing to inform you that your patient has agreed to participate in the above clinical trial at King's College Hospital. The trial involves the use of standardised clinical imaging techniques during an MRI of the heart.

The purpose of this study is to establish whether there is Cardiac involvement of their Myositis allowing us to investigate accurately what proportion of patients with Myositis have cardiac involvement

I have enclosed a copy of the Patient Information Sheet for your reference, however, if you have any queries or require further information please contact Dr Luke Dancy, Clinical Research fellow at King's College Hospital, (02032999000).

Yours Sincerely,

Dr D. Sado, Consultant and Senior Lecturer in Cardiology, BM, BSc, MRCP, PGCE, MD.

Clinical Research Fellow

King's College Hospital and King's College London

Encs: Patient Information Sheet, version 1.0

Appendix 4

Patient information sheet: Healthy Volunteers

DIFUS-T1

Defining the incidence of Cardiac involvement in Myositis using mapping based Cardiovascular Magnetic Resonance Imaging

PART 1

We'd like to invite you to take part in our research study.
Joining the study is entirely up to you before you decide we would like you to understand why the research is being done and what it would involve for you.
One of our team members will go through this information sheet with you, to help you decide whether or not you would like to take part and answer any questions you may have.
We suggest this should take about 15 minutes.
Please feel free to talk to others about the study if you wish.

The first part of the Participant Information Sheet tells you the purpose of the study and what will happen to you if you take part.

The second part will give you more detailed information about the conduct of the study.
Please ask if anything is unclear.

What is the purpose of the study?

- The purpose of this study is to discover if patients with Myositis get heart muscle inflammation. Myositis is a condition where the body attacks its own muscles causing swelling and discomfort. It can also affect other organs including the heart muscle which is what we are aiming to study.

- The study is asking what proportion of patients with Myositis have heart muscle inflammation and whether an MRI scan can pick up subtle or early involvement
- The overall aim of the study is to accurately define what proportion of patients get heart muscle involvement so we can predict which patients need the most aggressive treatment to prevent heart muscle damage from adding to their symptoms and also to devise a method that can become the standard for everyone treating patients with this condition to follow in future.

Why have I been invited?

- You have been invited to take part in the study because you are healthy with no evidence of a heart condition.

Do I have to take part?

It is up to you to decide. We will describe the study and go through this information sheet, which we will then give to you. You will be able to keep this information sheet and think about taking part. You are free to discuss the information with anyone you wish including your family and friends. If you agree, we will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason.

What will happen to me if I take part?

If you agree to participate in the study, we will ask you to consent to a number of different investigations that will help us to understand better the nature of heart involvement and the way in which it affects patients with myositis.

Summary of study investigations

1. MRI of the heart.
    a. A detailed way of looking at the heart muscle to determine if the muscle is inflamed.
    b. We will look at the muscles of the arm at the same time. This will not change the scan in any way but affords us additional valuable information
    c. Does not involve any radiation and poses no risk to you
2. ECG – heart rhythm tracing
    a. Performed when you attend for your first MRI scan

The above tests will be arranged around you to ensure attendance is as convenient as possible.

What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get from this study will help improve the future treatment of people with Autoimmune conditions and it will ensure that the most advanced tests to look for heart muscle swelling have been performed.

What are the possible disadvantages and risks of taking part?

There is no radiation or contrast injection required in this study.

Who is organising and funding this study?

The doctor in charge of this study is: Dr Dan Sado, Cardiology Consultant, and head of Cardiovascular MRI at King's College Hospital. The study is funded by King's College London.

How have patients and the public been involved in this study?

- Potential participants were involved in reviewing the Participant Information Sheet.
- In designing this study, we have taken into account patient opinions on the frequency of participant visits and the tests that we will carry out.

Who has reviewed this study?

- *All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by _____Research Ethics Committee. It has also been approved by the Health Research Authority.*

Expenses and Payments

There are no funds available for payments to those participating in this study.

This completes Part 1 of the Information Sheet.

If the Information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

PART 2

What if new information becomes available?

- What if new information that becomes available might specifically affect you and your health? If this happens, your study doctor might consider that you should withdraw from the study. He/she will explain the reasons for withdrawing from the study and arrange for your care to continue.
- If any unexpected abnormalities are identified during the studies, we will inform your GP and ensure that any and all investigations and treatments indicated are arranged for you.
- If the study is stopped for any other reason, we will tell you.

What will happen if I don't want to carry on with the study?

- You are free to withdraw from the study at any time; and if you would like to do so; please speak to your study nurse or doctor.
- Your decision to withdraw from the study will not affect the care you receive.
- If you withdraw your consent;
    - We will ask you if you remain happy for the information from tests already undertaken to be used in future research
    - If not, you will be able to withdraw consent for any of your information to be used and it will therefore be removed from the research database but will remain available to your doctor and GP in case of clinical need
- If you do withdraw, we may wish to stay in touch through your GP to monitor your progress
- If you do decide to withdraw, we will document exactly what your wishes are so that the minimum of inconvenience is caused to you

What if there is a problem?

- If you have a concern about any aspect of this study, you should ask to speak to your study doctor who will do their best to answer your questions Dr Dan Sado 02032999000. If you remain unhappy and wish to complain formally, you can do this

through the NHS Complaints procedure by contacting your local Patient Advice Liaison Service (PALS) office. Details of your local office can be obtained by asking your study doctor, GP, telephoning your local hospital or looking on the NHS choices website. http://www.nhs.uk/pages/home.aspx

- Every care will be taken in the course of this study. However, in the unlikely event that you are injured by taking part, compensation may be available.

- In the event that something does go wrong, and you are harmed during the research, and this is due to someone's negligence then you may have grounds for a legal action for compensation against King's College Hospital, NHS Trust but you may have to pay your legal costs.

- Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated by members of staff or about any side effects (adverse events) you may have experienced due to your participation in the study the normal National Health Service complaints mechanisms are available to you. Please ask your study doctor if you would like more information on this.

Will my taking part be kept confidential?

Any information gathered from our investigations will be kept confidentially. All information will be collected in a clinical context meaning that we will remain bound by the same medical confidentiality afforded to all personal medical records. For the purposes of our research, your information will be assigned a unique identifier which will remain separate from any of your personal information allowing the data to be kept without association with individuals. The document containing the identifiers and your details will be kept in paper format to prevent electronic access from outside.
Information once anonymised may be shared with collaborators outside of King's College Hospital, but only those also bound by the same confidentiality protocols as we do, and in this particular case, only other clinicians within the NHS.

If you consent to take part in the research, any of the information collected about you may be inspected by the sponsor (including representatives of the sponsor). These inspections are solely for the purposes of the research and analysing the results. Your records may also be looked at by the regulatory authorities or ethics committees to check that the study is being carried out correctly.

The organisations listed above will keep information about you confidential and secure. Your name will not be used in any reports about the study and all data is stored in accordance with the principle of the Data Protection Act 1998. However, your hospital doctor may tell your GP about your participation if you agree to enter the study.

Involvement of the General Practitioner/Family Doctor (GP)

- With your consent, your GP will be informed of your involvement in the trial.
- Any other medical practitioners who treat you, e.g., should you be admitted to hospital for any reason, will also be informed.

Will any genetic tests be done?

- No

What will happen to the results of the research study?

- As you are a healthy individual, your results are likely to be normal. If you wish, a formal report can be provided to you to be disseminated to your GP by you if you wish to.
- If the findings of investigations into your heart demonstrate that you would benefit from additional investigation or treatment by a heart specialist, then we will arrange follow up for you in Dr Sado's clinic. He is a consultant Cardiologistand the lead for this study. You should note that you may be reviewed by Dr Luke Dancy in Dr Sado's clinic. He is a senior Cardiology Registrar, Dr Sado's Research fellow and a key investigator in the trial.
- The information gathered from you and other participants will be analysed and published in medical literature. This will allow us to further the understanding of this field

and share our findings with the wider scientific community. It will also form the basis of a Research Thesis towards the award of a higher degree.

- Currently there are other studies into this condition ongoing involving multiple different centres throughout the country. We may wish, anonymously to share our results with them in order to ensure the maximum benefit from the tests is garnered

Thank you

Thank you for considering taking part and taking the time to read this information sheet. If you decide to take part in the study, we will give you a copy of the information sheet and a signed consent form to keep.

Further information and contact details

Patient Advice and Liaison Service. Patient Advice and Liaison Service, King's College Hospital NHS Foundation Trust, Denmark Hill, London SE5 9RS

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Telephone: 023 8059 5628. Email: involve@nihr.ac.uk

Local Contacts:

Dr Dan Sado, Cardiology Consultant, CI

Secretary: Marie MacCarthy: 0203 299 9000

Dr Luke Dancy Cardiology Clinical Research Fellow

Tel: 0203 299 9000

Appendix 5

Participate in Research

Can you spare 20 Minutes to participate in a Research trial being undertaken here at King's?

Our Cardiac MRI Department is undertaking research into Rheumatological conditions and needs Healthy Volunteers to undergo MRI scans of the Heart to help further their research

Please Consider Participating in a painless, risk-free scan

Your help will allow us to compare the hearts of normal healthy subjects with the hearts of people who are suffering from Rheumatological Conditions known to affect the heart

If you are interested, or for more information please contact:

Dr Luke Dancy, Clinical Research Fellow,

King's College Hospital NHS Foundation Trust

lukedancy@nhs.net

Appendix 6

Dear Colleague,

The King's Cardiovascular magnetic resonance team would like to invite you to participate in a research study that we are undertaking. We are looking for healthy volunteers to undergo an ECG and Cardiac MRI scan. This will allow us to compare healthy participants with patients that have autoimmune conditions. This study is looking to use modern MRI mapping techniques to hopefully identify earlier and more accurately if there is cardiac involvement in patients with Autoimmune conditions.

The scan is painless and takes around 30 minutes to perform. There is no need for injections or intravenous cannulation. We will be able to provide you with a report of the scan and updates on the progress of the research if you are interested.

If you are keen to take part, please reply to this email so we can arrange a time to arrange a scan.

Thank you for considering participation and we hope to see many of you in the MRI department soon.

Yours,

Dr Luke Dancy
Cardiology SpR and Clinical Research Fellow
King's College Hospital

Appendix 7

IMACS FORM 03: PATIENT/PARENT GLOBAL ACTIVITY ASSESSMENT

Subject's IMACS number _____

Assessor _____

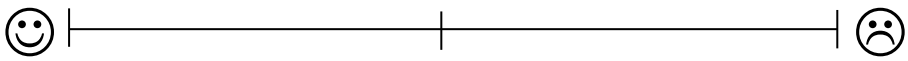
Assessor's relationship to subject: Patient__; Mother:__; Father__; Other (specify):_____

Date of assessment (mm/dd/yy) _____

Assessment number _____

Your myositis is the result of the combined effects of many disease processes. One of these is disease activity, which is active inflammation in your/your child's muscles, skin, joints, intestines, heart, lungs, or other parts of your body, which can improve when treated with medicines.

1. Considering all the ways that myositis affects you/your child, please rate the overall activity of your/your child's disease today by placing a mark on the line below.



No evidence of disease activity

Extremely active or severe disease activity