INFLAMMATORY CARDIOMYOPATHY – DIAGNOSIS AND RISK STRATIFICATION IN MYOCARDITIS

Thesis submitted for the degree of *Doctor of Philosophy*

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Statement of Originality

I declare that this thesis represents my own work other than where appropriately referenced or duly acknowledged.

Copyright Declaration

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Personal Contributions to Work

I was appointed as a Clinical Research Fellow in the Cardiovascular Research Centre at Royal Brompton Hospital in October 2015 under the supervision of Professor Sanjay Prasad, Dr James Ware and Professor Stuart Cook. Under their guidance, I designed and conducted the work presented in this thesis over a period of three and a half years with mentorship provided by Professor John Cleland and Professor Dudley Pennell.

Whilst writing my initial grant application, I became aware of the distinct lack of contemporary epidemiological data on acute myocarditis within the UK. I actively pursued opportunities to access national hospital admission data from NHS Digital, formerly known as the Health and Social Care Information Centre (HSCIC). I created and submitted an information request for all hospital episode statistics (HES) relating to myocarditis from all hospitals across England over the last 20 years. This application included detailed scientific justification, hypotheses, proposed analytical methods, expected benefits and outcomes. I responded to multiple rounds of reviewers' comments over 18 months to justify our data request, particularly in light of the General Data Protection Regulations introduced in 2018. I worked closely with information technologists to minimise the size of our data request. Following receipt of this dataset in 2019, I personally filtered and constructed a database linking episodes into admissions and subsequently performed all the epidemiological analyses presented in this thesis. I drafted a manuscript from this chapter and I am listed as first author on this.

Together with Professor Prasad, I formulated the hypotheses and designed the prospective and retrospective myocarditis study. I wrote an ethics application that was successfully approved for this study. I wrote two grant applications, one to the British Heart Foundation and one to the Medical Research Council. I was awarded the former whilst the latter was still under consideration, which was therefore withdrawn. With the assistance of Professor Prasad, I set up collaborations with colleagues from a network of surrounding hospitals that acted as participant identification centres. With only a handful of exceptions, I recruited and conducted all study visits personally, which amounted to approximately 500 visits over 3 years (120 prospective myocarditis patients at 3-time points, 120 retrospective myocarditis patients and 40 infarct patients). I received invaluable support from our research nurse team to help

complete patient questionnaires, 12-lead ECGs, blood sample processing, co-ordinate follow-up study visits and collect patient outcome data. I personally supervised, analysed and reported all the CMR scans. I assisted with DNA extraction from venous blood samples and subsequent preparation for DNA sequencing. Whilst I received specialist bioinformatic support to extract, filter and annotate genetic variant call files, I subsequently formulated, planned and performed all statistical analyses described and presented in Chapters 4 and 5 relating to CMR parameters and burden testing of genetic variants with guidance from Professor Prasad and Dr Ware. I drafted three manuscripts from these chapters and I am listed as first author on each.

Together with Professor Prasad, I formulated the hypothesis and designed the FINALISE study presented in Chapter 6. I filtered, collected and assimilated CMR data 750 patients with and without non-ischaemic patterns of late gadolinium enhancement. I supervised a group of Imperial College medical students that sought patient consent and outcome follow-up data by postal and telephone interviews. I subsequently convened an independent expert panel, who adjudicated all clinical outcome events. I performed analyses of baseline characteristics and basic survival modelling, which was confirmed by a medical statistician. I drafted a manuscript from this chapter and was listed as first author on this.

Acknowledgements

I am grateful to my supervisors for allowing me the opportunity to undertake this PhD. My primary supervisor, Professor Sanjay Prasad, has shaped and championed my academic development over the last four years. He has provided me with invaluable support, encouragement and mentorship throughout this journey and I have learnt a huge amount from him, which will no doubt be instrumental to my future career as an academic clinician.

I wish to thank my co-supervisors, Dr James Ware and Professor Stuart Cook, for their encouragement and guidance. I thank my mentors, Professors John Cleland and Professor Dudley Pennell, for their invaluable support and direction. This work would not have been possible without the full support and infrastructure of the Cardiovascular Research Centre. I am thankful to all the members of staff, especially our dedicated research nurses, Rebecca Wassall and Sara Salmi, and CMR technologists, Rick Wage and George Matthew.

During my PhD, I have learnt a huge amount from my colleagues, Brian Halliday and Paz Tayal, as well as my predecessors, Vassilis Vassiliou and Tevfik Ismail. I thank them for their practical guidance when first starting, moral support and insightful scientific discussions.

I am grateful to the patients and volunteers who took part in this research. Clinical research would not be possible without the enthusiasm and altruism of these individuals. I equally wish to thank our funders, the Alexander Janson Foundation and the British Heart Foundation.

Finally, I wish to thank my family for their love and unwavering support throughout my studies. I am grateful to my big sister for always being there for me, and my niece and nephew for bringing so much joy and happiness over the last four years. I owe a special thanks to my wife for her unconditional support, understanding and encouragement. Submitting this PhD will mean we can finally spend our evenings together again and look forward to becoming parents ourselves later this year.

Above all, I wish to thank my father and late mother for all their hard work and sacrifice to provide me with the opportunities and experiences that have made me the person I am today – I dedicate this milestone to them.

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Abbreviations

AF – Atrial fibrillation

ARVC – Arrhythmogenic right ventricular cardiomyopathy

BSA – Body surface area

BNP – Brain natriuretic peptide

CI – Confidence intervals

CMR – Cardiovascular magnetic resonance

DCM – Dilated cardiomyopathy

DENSE – Density encoding with stimulated echoes

DSP – Desmoplakin gene

ECG - Electrocardiogram

ECV – Extracellular volume

ESC – European Society of Cardiology

ExAC – Exome Aggregation Consortium

FWHM – Full-width at half maximum

GWAS – Genome wide association study

HF – Heart failure

HR – Hazard ratio

HVOL – Healthy volunteer

ICD – Implantable cardioverter defibrillator

IHD – Ischaemic heart disease

IQR – Interquartile range

LA – Left atrial

LAVi – Left atrial volume indexed to body surface area

LBBB – Left bundle branch block

LGE – Late gadolinium enhancement

LV – Left ventricular

LVEDVi - Left ventricular end diastolic volume indexed to body surface area

LVEDD – Left ventricular end diastolic dimension

LVEF – Left ventricular ejection fraction

MAF – Minor allele frequency

MOLLI – Modified Look-Locker inversion recovery sequence

NGS – Next-generation DNA sequencing

NHS – National Health Service

NSVT – Non-sustained ventricular tachycardia

NYHA – New York Heart Association

ONS – Office of National Statistics

OPCS-4 - Office of population censuses & survey classification of interventions & procedures

RVEF – Right ventricular ejection fraction

SAX – Short axis view

SCD – Sudden cardiac death

SD – Standard deviation

SSFP – Steady state free precession imaging

STIR – T2 weighted Short-Tau Inversion Recovery sequence

SV – Stroke volume

TTNtv – Truncating variant in titin gene

VCF – Variant call file

VF – Ventricular fibrillation

VT – Ventricular tachycardia

VUS – Variants of uncertain significance

WES – Whole exome sequencing

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Abstract

Background:

Acute myocarditis remains a challenging clinical diagnosis with limited epidemiological data and poorly defined markers of adverse risk. We sought to build a better understanding of myocarditis epidemiology and to integrate clinical, genetic and advanced imaging data to generate new insights into myocarditis pathobiology.

Methods and Results:

(1) Evaluation of population-level hospital admission data from NHS England from 1998-2017 revealed a rising incidence of myocarditis, at least two-fold greater than that reported from pathological registries, most commonly amongst men with median age 36 years and women aged 46 years, with distinct peaks over Winter and the greatest burden in London.

(2) Cardiovascular magnetic resonance (CMR) phenotype study of a 114 prospectively recruited patients with acute myocarditis demonstrated the natural history of changes in left ventricular parameters over 12 months and revealed a correlation between change in myocardial mechanics, specifically circumferential strain, and extent of myocardial oedema by T2 mapping (R=-0.70, p=0.01).

(3) Genetic sequencing of 231 unrelated patients recruited with acute myocarditis revealed the presence of truncating variants in key cardiomyopathy genes in 4.8% of the cohort, particularly linked to arrhythmogenic ventricular cardiomyopathy (AVC) with significant enrichment compared with 1054 healthy volunteers indicating a potential overlap between myocarditis and AVC (odds ratio 8.2; 95% CI 2.4-28.3; p=0.001).

(4) Retrospective long-term clinical outcome study of 401 patients with mid-wall/subepicardial late gadolinium enhancement (LGE) but otherwise normal LV volumes and function suggestive of healed myocarditis demonstrated a low risk of actual or aborted sudden cardiac death over a median follow-up of 4.3 years (incidence rate per 100 patient-years of 0.05%).

(5) Psychological study of post-traumatic stress disorder amongst 231 patients with acute myocarditis compared with 44 patients with acute myocardial infarction highlighted the profound and long-lasting psychological morbidity associated uniquely with myocarditis.

Conclusion:

Myocarditis is a heterogeneous disease that remains vastly underestimated in prevalence. Integration of advanced CMR techniques with genomic data may provide incremental value in early diagnosis, non-invasive surveillance and identification of high-risk individuals who may benefit from a more personalised approach with close monitoring and targeted therapy.

Selected Awards and Publications

Awards

London Cardiovascular Society (JRSM) Young Investigator Award Runner-up (2018).

NHLI Best Clinical Poster Presentation Prize (2017).

Original articles

Halliday BP, Wassall R, **Lota** AS, et al. Withdrawal of pharmacological treatment for heart failure in patients with recovered DCM (TRED-HF). Lancet. 2019;393(10166):61-73.

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1st Author Oral Abstract Presentations

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Lota A, Mohan P, Arzanauskaite M, et al. Cardiac Inflammation in the Septum and Right Ventricular Free Wall: Lymphoma vs Sarcoid.

Society of Cardiovascular Magnetic Resonance Annual Scientific Session 2017; Washington DC

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1. INTRODUCTION: MYOCARDITIS

1.1. Overview

Myocarditis is defined as an inflammatory disorder of the myocardium, which typically occurs in response to environmental or endogenous triggers, most commonly acute viral infection.[1] Diagnosis is challenging due to a spectrum of clinical and histopathological manifestations with limited understanding of epidemiology and long-term outcomes.[2] The WHO Global Burden of Disease Study reported an age-standardized incidence rate of 22 cases per 100,000 of the world's population.[3] However, myocarditis has been implicated as the cause for 3% to 12% of all sudden cardiac deaths on post-mortem examination, representing the fourth most common cause of SCD.[4, 5]

Whilst spontaneous recovery occurs in approximately two thirds of patients not experiencing SCD, progressive left ventricular dilatation and systolic dysfunction, referred to as inflammatory cardiomyopathy, occurs in the remainder.[6] The burden of myocarditis as the underlying trigger for heart failure varies by age and region but has been linked to as many as 9% of cases of idiopathic DCM.[7] Specific forms of myocarditis, such as giant cell or eosinophilic myocarditis, occur less frequently but can be rapidly progressive. In general, there is unmet need to improve our understanding of who gets myocarditis, why and what happens in the long-term to improve diagnosis, risk stratification and treatment (figure 1-1).

Figure 1-1. Outline of current unanswered questions in myocarditis.

1.2. Historical perspective

The Greek word '*kardia'* appeared commonly throughout medical terminology and was often attached to other Greek suffixes, such as term '*itis'* meaning disease or inflammation. As a result, for many centuries the term *carditis* was widely associated with the premature death of most affected individuals. The more recent addition of the prefix '*myo'* to '*carditis'* was based on the expanding use of light microscopy, invented during the Renaissance in the 1700s, combined with the increasing practice of *autopsy* ('to see for oneself'). Subsequently, the term myocarditis was first reported by Jean-Nicolas Corvisart in 1812 working as the primary physician of Napoleon to specify disease of the heart muscle.[8] However, the concept of isolated heart muscle inflammation was more specifically described by German physician Joseph Friedrich Sobernheimin in 1837.[9] Several years later, Carl Ludwig Alfred Fiedler also described the fatal syndrome of giant cell or granulomatous myocarditis from the identification of giant multinuclear cells.

Many new and important concepts developed from the 1900s (figure 1-2) with the increasing utilisation of 12-lead ECG and X-ray, and Herrick's recognition of the clinical features of acute coronary thrombosis with associated myocardial infarction in 1919.[10] This heralded the separation of coronary artery disease from myocarditis as two distinct entities. During the Second World War, clinical investigation techniques rapidly advanced, including bacteriology, virology, electron microscopy and immunohistochemistry. As a result, the suspicion of a viral aetiology of myocarditis became a reality with the isolation of Coxsackie virus in 1945.[11]

Given the limited end-stage histological features (mainly fibrosis) common to many myocardial diseases, there was much interest in the development of a safe technique for myocardial biopsy. Existing practices of open surgical biopsy or direct transthoracic needle biopsy were rare and frequently associated with complications. Following the development of cardiac catheterisation, Sakakibara and Konno successfully reported the first use of a

transcatheter approach for endomyocardial biopsy in 450 patients in Japan in 1962.[12] This provided an opportunity to assess the premorbid presence of myocardial inflammation and also led to the recognition of cardiac involvement in a large number of systemic diseases.[13] The technique was further refined over time, driven primarily by the need to monitor graft rejection in cardiac transplant patients.[14] Within myocarditis, the Dallas criteria were described in 1986 to provide histopathological categorisation to establish a diagnosis of myocarditis.[15]

Use of non-invasive imaging in the form of echocardiography played a small role in advancing our understanding of myocarditis. First introduced by Edler in 1953, M-mode echo generated little interest until the first commercially available 2-D echo in the 1970s.[16] Mild to moderate forms of left ventricular (LV) dysfunction could be detected prior to the development of overt cardiomegaly on chest radiography. However, myocarditis was often overlooked as a contributory diagnosis to heart failure. The advent of cardiovascular magnetic resonance (CMR) imaging and the ability to visualise myocardial oedema and late gadolinium enhancement (LGE) patterns vastly improved the detection of myocardial involvement and thereby clinicians' awareness of this disease. In 2009, the Lake Louise Criteria recommended a standard protocol that would best identify myocarditis in 80% of cases where two out of three criteria were present; oedema, hyperaemia and necrosis.[17] This generated renewed interest in inflammation of the heart muscle and the use of CMR has continued to rapidly expand in all aspects of cardiology over the last two decades.[18]

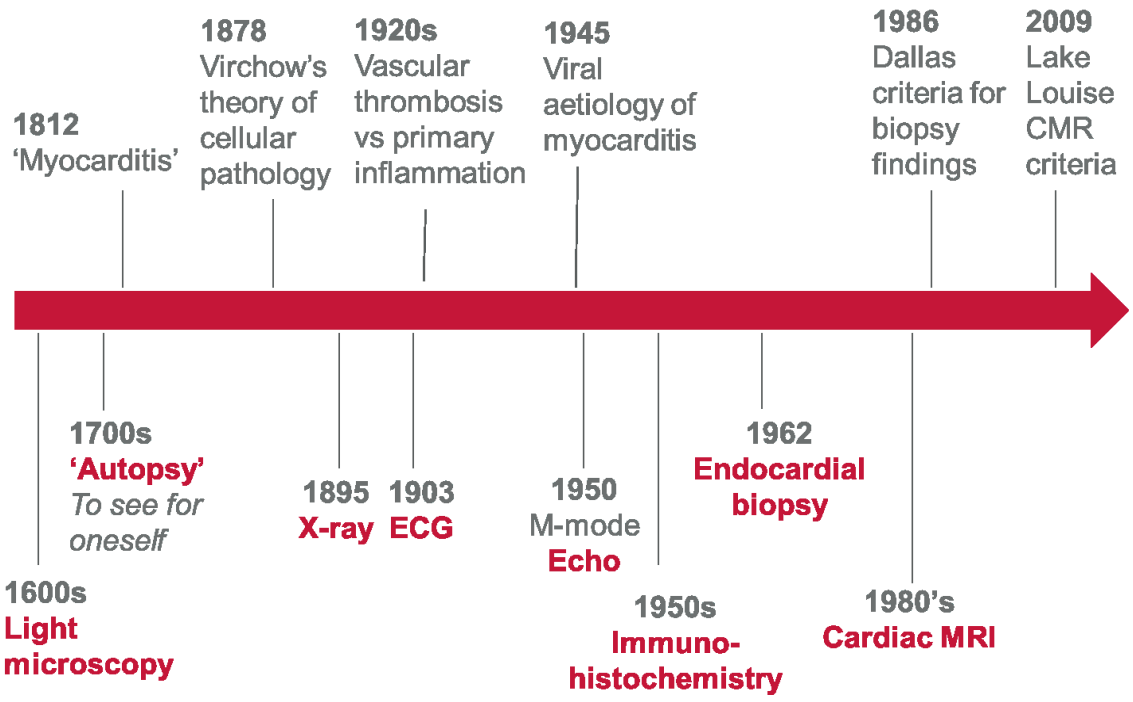


Figure 1-2 Timeline of major advances in concepts (top) and technology (bottom) in myocarditis.

More recent studies have enhanced our pathophysiological understanding of the complex interactions between viral injury and host immune responses.[19] In parallel, novel molecular techniques have facilitated the detection of viral genomes in patients with suspected myocarditis.[20] As a result, myocarditis continues to represent an active area for scientific research and discovery, with many unmet needs in diagnosis, risk stratification and treatment.

1.3. Classification

Myocarditis can be classified in a number of different ways by aetiology, clinical presentation, histological findings or clinicopathological descriptions of prognostic or therapeutic relevance.[21] Within these categories, patients may be diagnosed with possible, probable or definite myocarditis (table 1-1).

Common Classification Schemes	Criteria
Aetiology	Infectious Toxic Immune-mediated Systemic disease
Clinical presentation	Chest pain Syncope / Arrhythmia Acute heart failure Myopericarditis
Histological findings	Lymphocytic Eosinophilic Giant Cell
Clinicopathological	Subacute Acute Fulminant Chronic active Chronic persistent

Table 1-1 Common classification schemes for myocarditis

1.4. Aetiology

Myocarditis can be caused by a wide variety of infectious agents, drugs, toxins and systemic diseases although the exact cause in any given patient often remains unknown (figure 1-3). Acute viral infection represents by far the leading aetiology. Early studies implicated Coxsackie B and adenovirus as causative agents by serologic demonstration of rising antibody titres with acute symptomatic presentation.[22, 23] Following the development of molecular

techniques that allowed direct detection of viral genomes within myocardial tissue, the spectrum of most frequent viruses shifted from classic enterovirus and adenovirus to mainly parvovirus B19 (PVB19) and human herpes virus 6 (HHV6).[20, 24] These viral agents were also identified amongst patients with idiopathic dilated cardiomyopathy (DCM), which suggested an important link with viral myocarditis as environment trigger for DCM.[25, 26] Other viruses sometimes identified include H1N1 strains of influenza, cytomegalovirus and Epstein-Barr virus.[2] In a CMR study of 19 patients with biopsy confirmed acute myocarditis, nested polymerase chain reaction on myocardial tissue from regions of contrast enhancement by CMR identified Parvovirus B19 in 13 (68%) and HHV6 in 6 cases (32%).[27] In a follow-up study of 128 patients with myocarditis, PVB19 was found in 49 patients (38%), HHV6 in 16 patients (13%) and combined PVB19/HHHV6 infections in 15 patients (12%).[28] The remaining patients were diagnosed with healing myocarditis without presence of viral genomes (n=15) or did not have myocarditis by histopathology (n=26). The reason for the shift away from Coxsackie and adenovirus remains unclear and it is possible that geographical differences of virus infections between North America and Europe may also play a role.[29] Of note, the frequency of detection of viral genomes varies widely between studies and is likely driven by the differences in the number and location of biopsy samples obtained in each patient.[30] Furthermore, there are often uncertainties regarding the causality of a detected virus.

Bacterial diseases are less commonly associated with myocarditis, particularly in immunocompetent hosts. Lyme disease due to Borrelia burgdorferi from tick bites is perhaps the most recognised bacterial infection, and is associated with typical signs and symptoms, such as the bulls-eye rash.[31] Chagas disease, caused by the parasitic protozoan *Trypanosoma cruzi*, represents the predominant worldwide cause of myocarditis. Up to 20% of patients are reported to develop heart failure in the long-term.[32] It is ranked as the most serious parasitic

disease of the Americas with many public health initiatives aimed at eliminating domestic insect vectors.

Drug-induced myocarditis can occur in two settings; (i) by a direct toxic effect on cardiomyocytes or (ii) through immune-mediated hypersensitivity mechanisms.[33] Oncological agents, such as anthracyclines, fluorouracil, trastuzumab and also radiation therapy, have a direct toxic effect.[34] Cocaine is also toxic through its well-known vasospasm effects. In a post-mortem study of 40 patients with detectable levels of cocaine, myocarditis with mononuclear infiltrates due to aggressive myocardial necrosis from microvascular injury was found in 8 patients (20%) compared to only 1 patient with total thrombotic occlusion of an epicardial vessel.[35] Amphetamines represent the second most common widely abused illicit drug after cannabis and are linked with myocarditis through mitochondrial injury and catecholamine excess.[36]

Drug-induced hypersensitivity reactions may be encountered with many common prescription medications, such as penicillins, methyldopa, hydrochlorothiazide, furosemide, azithromycin, aminophylline and phenytoin.[37] Most cases develop early and are characterised by low-grade fever, sinus tachycardia, a drug rash and mild eosinophilia. However, in some cases, myocarditis can develop up to 2 years after the initiation of drug therapy, for example with clozapine use.[38] Hence, drug-induced myocarditis often goes undetected. Vaccination against smallpox infection is also associated with myocarditis in 6 per 10,000 vaccines.[39] More recently, novel checkpoint inhibitors used to direct the immune system to target cancerous cells have also been associated with immune-mediated adverse events, particularly fulminant myocarditis within 3 months of starting therapy.[40, 41] Examples of such agents include ipilimumab, an anti–cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) antibody, and nivolumab, an anti–programmed death-1 (PD-1) antibody, which both have favourable effects on tumour regression but this requires a careful balance with cardiovascular risk.

It is increasingly recognised that auto-immune myocarditis can also occur in the context of multi-systemic autoimmune disease, such as rheumatoid arthritis, systemic sclerosis and systemic lupus erythema, and is generally associated with poorer prognosis.[42] In patients with extra-articular features of rheumatoid arthritis, the incidence of myocarditis was estimated to be as high as 39%, although this is likely to have diminished with the advent of disease-modifying and biological therapy.[43] A low-threshold for investigation with any cardiac complaint is required in these systemic conditions to detect subtle myocardial involvement prior to downstream heart failure and arrhythmic complications.

1. Infectious myocarditis	
Bacterial	*Staphylococcus*, *Streptococcus*, *Pneumococcus*, *Meningococcus*, *Gonococcus*, *Salmonella*, *Corynebacterium diphtheriae*, *Haemophilus influenzae*, *Mycobacterium* (tuberculosis), *Mycoplasma pneumoniae*, *Brucella*
Spirochaetal	*Borrelia* (Lyme disease), *Leptospira* (Weil disease)
Fungal	*Aspergillus*, *Actinomyces*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Mucormycoses*, *Nocardia*, *Sporothrix*
Protozoal	*Trypanosoma cruzi*, *Toxoplasma gondii*, *Entamoeba*, *Leishmania*
Parasitic	*Trichinella spiralis*, *Echinococcus granulosus*, *Taenia solium*
Rickettsial	*Coxiella burnetii* (Q fever), *R. rickettsii* (Rocky Mountain spotted fever), *R. tsutsugamuschi*
Viral	RNA viruses: Coxsackieviruses A and B, echoviruses, polioviruses, influenza A and B viruses, respiratory syncytial virus, mumps virus, measles virus, rubella virus, hepatitis C virus, dengue virus, yellow fever virus, Chikungunya virus, Junin virus, Lassa fever virus, rabies virus, human immunodeficiency virus-1 DNA viruses: adenoviruses, parvovirus B19, cytomegalovirus, human herpes virus-6, Epstein-Barr virus, varicella-zoster virus, herpes simplex virus, variola virus, vaccinia virus
2. Immune-mediated myocarditis	
Allergens	Tetanus toxoid, vaccines, serum sickness Drugs: penicillin, cefaclor, colchicine, furosemide, isoniazid, lidocaine, tetracycline, sulfonamides, phenytoin, phenylbutazone, methyldopa, thiazide diuretics, amitriptyline
Alloantigens	Heart transplant rejection
Autoantigens	Infection-negative lymphocytic, infection-negative giant cell Associated with autoimmune or immune-oriented disorders: systemic lupus erythematosus, rheumatoid arthritis, Churg-Strauss syndrome, Kawasaki's disease, inflammatory bowel disease, scleroderma, polymyositis, myasthenia gravis, insulin-dependent diabetes mellitus, thyrotoxicosis, sarcoidosis, Wegener's granulomatosis, rheumatic heart disease (rheumatic fever)
3. Toxic myocarditis	
Drugs	Amphetamines, anthracyclines, cocaine, cyclophosphamide, ethanol, fluorouracil, lithium, catecholamines, hemetine, interleukin-2, trastuzumab, clozapine
Heavy metals	Copper, iron, lead (rare, more commonly cause intramyocyte accumulation)
Miscellaneous	Scorpion sting, snake, and spider bites, bee and wasp stings, carbon monoxide, inhalants, phosphorus, arsenic, sodium azide
Hormones	Phaeochromocytoma, vitamins: beri–beri
Physical agents	Radiation, electric shock

Figure 1-3. Detailed list of causes of myocarditis/inflammatory cardiomyopathy (reproduced with permission from the European Society of Cardiology – see appendix).[1]

1.5. Pathophysiology of Myocarditis

Pathophysiology for the various aetiologies of myocarditis is not completely understood, although for viral myocarditis, murine models of Coxsackie-B3 (CVB3) myocarditis have provided much of our current mechanistic understanding with three phases of disease activity.[7]

Coxsackie viruses belong to the *Enterovirus* genus within the *Picornaviridae* family. Of the six serotypes of CVB, only CVB1, 3 and 5, are notably cardiotropic.[44] CVB3 poses the greatest threat to host cells through a combination of virulence factors, including viral proteases, host protein shut-off and cleavage of dystrophin, leading to host cell death, apoptosis or necrosis. In most cases, the innate and adaptive immune responses effectively eliminate the viral agent and facilitate myocardial recovery. However, long-term viral persistence or the development of autoimmunity lead to chronic myocardial inflammation with continuous remodelling and matrix turnover leading to pump failure.

ACUTE PHASE (DAYS 0-3)

In the acute phase, mice injected intraperitoneally with CVB3 exhibited cardiomyocyte death within 3 days of inoculation.[45] After gaining entry into the myocyte through coupling of the virus to host-cell receptors, viral RNA is translated and transcribed. The RNA genome and structural proteins assemble to form a complete virion. Release of the viral progeny completes the life cycle and results in cardiomyocyte death and release of troponin.[44] Activation of the complement system leads to a cascade of reactions that contribute to inflammation, with rapid amplification of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-a and interleukin (IL)-6.

Inflammation is traditionally defined by the presence of *calor, dolor, rubor,* and *tumor,* meaning heat, pain, redness, and swelling. These changes reflect the actions of cytokines and other inflammatory mediators to promote local blood flow with increased capillary

permeability to facilitate the arrival of further immune cells, such as macrophages, natural killer cells and monocytes. Transmembrane or intracellular Toll-like receptors (TLR) expressed by dendritic cells within the myocardium also activate pro-inflammatory signalling cascades in response to both pathogen-associated molecular patterns but also damaged self-proteins.[46] These cells also transport antigens to lymphoid tissues to commence the induction of the adaptive immune response.

Myocardial oedema has been characterised histologically in acute myocarditis.[47] Whilst fundamental to the innate immune response, there is increased regional wall thickness with reduction in systolic contraction and diastolic relaxation, and more importantly, conduction disturbance and microvascular compression leading to arrhythmia and chest pain.[48, 49]

Depending on the infective agent, the mechanism of myocardial injury and resulting patterns of inflammatory mediators differ. For example, in Chagas myocarditis, tissue damage also occurs through the parasitic release of bioactive compounds promoting oxidative stress.[50] In contrast, parvovirus B19 predominantly infects endothelial cells rather than myocytes, leading to acute microvascular ischaemia and secondary myocyte necrosis.[51] A similar mechanism is also seen with cocaine use with profound vasospasm of the microvasculature.[35]

SUBACUTE PHASE (DAYS 4-14)

The second phase evolves over days 4 to 14 and involves further activation of the innate immune response. Recruitment of natural killer (NK) cells, macrophages and other antigen-presenting cells, including eosinophils and polymorphonuclear cells, drives ongoing cytokine and chemokine production (figure 1-4). An important action of NK cells activated by IL-2 is to limit further viral replication, as demonstrated by increased viral titres in NK-cell-deficient mice.[52] Nitric oxide (NO) generated in response to various interleukin also promotes control of enterovirus replication.[53]

Whilst the innate immune response is reliant on germline-encoded receptors to recognise micro-organisms, viruses bearing no surface molecules are rarely recognised by macrophages. Therefore, to overcome the constraints faced by the innate response, dendritic cells use a process known as micropinocytosis to take up antigens rather than receptor binding. This antigen presentation then activates the host's adaptive immune response and leads to antibody production from B lymphocytes targeted against viral proteins from day 8.[46] In parallel, cytotoxic T lymphocytes lyse virus-infected cardiomyocytes with viral proteins presented by the major histocompatibility complex class I, although this effect can lead to further myocardial injury.[54] Overall, viral titres that were maximal on day 4, rapidly decrease and disappear by days 10-14.[55]

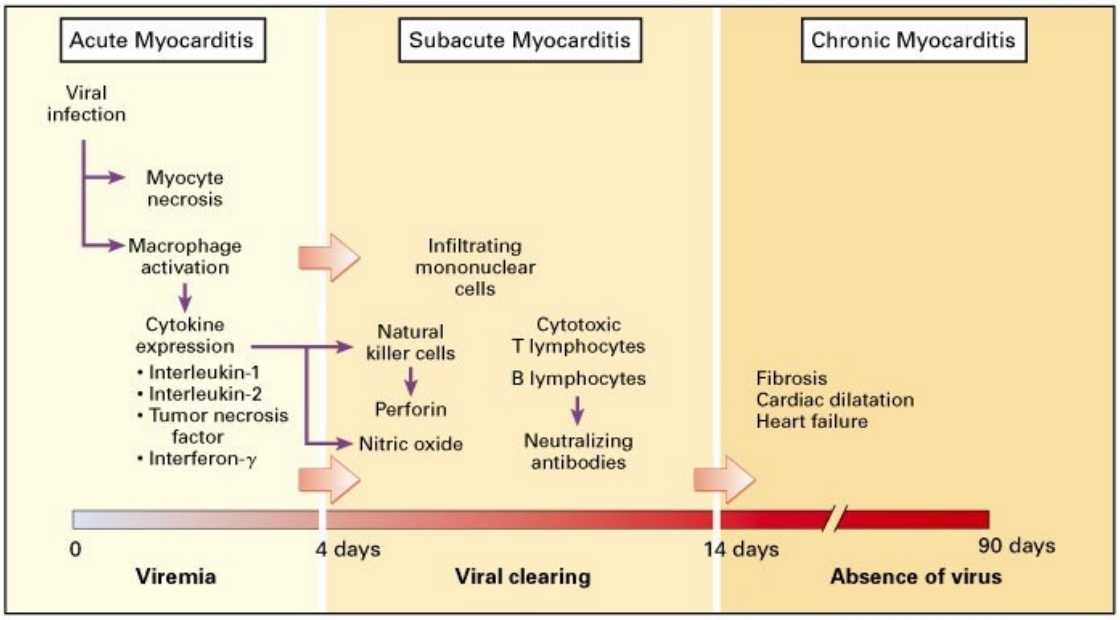


Figure 1-4. Time Course of Experimental Viral Myocarditis in Mice. Reproduced with permission from Feldman et al.[56] Copyright Massachusetts Medical Society.

CHRONIC PHASE (DAYS 15 TO 90)

The immunological responses outlined above lead to recovery in most cases, often leaving mild residual fibrosis in regions of affected myocardium. This state is often referred to as healed myocarditis. However, in a small subset of cases, there is chronic myocardial inflammation, which causes continuous remodelling and matrix turnover leading to contractile dysfunction and chamber dilatation resembling dilated cardiomyopathy. There are two main hypotheses for this; (i) low-level persistence of the virus as a proinflammatory stimulus, or (ii) the development of autoimmunity. Both hypotheses reflect opposite ends of the exquisite balance between effective viral clearance and overaggressive auto-immunologic activation.

In mice strains with ineffective host defence mechanisms, persistent viral replication resulted in chronic myocarditis with cardiac dilatation and failure.[57] Viral replication foci co-localised with regions of myocardial inflammation. Persisting viral RNA has also been detected in the myocardium of healthy mice by amplification using polymerase chain reaction 90 days after inoculation, in the absence of detectable virus titres.[58] Ongoing replication is likely to be restrained by viral mechanisms that remain unknown but at a level sufficient to produce new antigenic material to incite ongoing myocardial injury.[7] Alternatively, it has been shown that the virus may persist in extracardiac reservoirs, such as the spleen, lymph nodes, liver and pancreas, leading to recurrence of infection under conditions when host defense mechanisms are supressed.[59]

Autoimmune pathogenic mechanisms have been implicated in the development of chronic active myocarditis leading to dilated cardiomyopathy (figure 1-5). Following CVB3 myocarditis, many cardiac-specific antibodies have been identified in murine models, likely due to 40% overlap in the amino acid sequence of CVB3 capsid protein and cardiac myosin protein.[60] It was shown that isolation of such autoantibodies from CVB3 infected mice led to myocardial damage when injected into susceptible strains of mice.[61] Whilst the initial immune

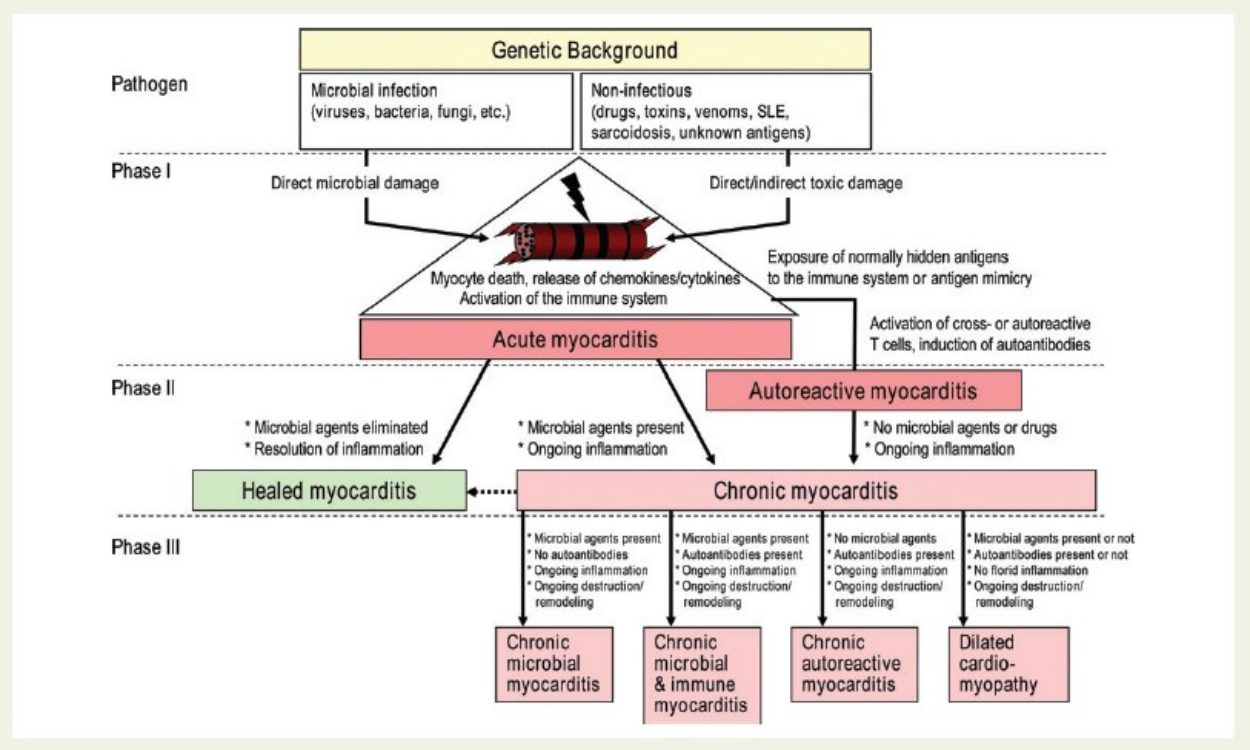
response may be beneficial in eliminating CVB3, cross-reacting autoantibodies through molecular mimicry may therefore play an important role in perpetuating myocardial injury. Studies in humans with idiopathic dilated cardiomyopathy have similarly identified antibodies against alpha myosin cardiac protein in 25% of cases, suggesting the importance of autoimmunity in driving LV dysfunction, potentially triggered by a previous episode of clinically silent viral myocarditis.[62] T cells have also been shown to dictate the course of disease progression. Early animal studies showed that T-cell deprivation surprisingly had a protective role in preventing fatal myocarditis.[63] There are two main subtypes of T lymphocytes; CD4 helper cells and CD8 cytotoxic cells. The CD4 type are potent producers of cytokines and can be further subdivided into Th1 or Th2. This fundamental difference determines the specific cytokines that are produced, which in turn either drives the immune response towards a Th1 or Th2 dominant pattern. The Th1 response occurs acutely and is characterised by macrophage activation and production of IFN-gamma and IL12, which both drive inflammation and differentiation of autoreactive T cells. In contrast, Th2 polarity is associated with B cell activation and humoral immunity, occurring between days 13-20, and eventual recovery following the acute episode.[64] The administration of exogenous IL12 seen in the Th1 response was indeed found to enhance myocarditis severity in murine models.[65] Thus, Th1/Th2 imbalance is considered to play an important role in the pathogenesis of myocarditis.

1.6. Gender differences in myocarditis

Men are twice as more likely to be affected by myocarditis than women.[66] Similarly, animal studies consistently demonstrate that male mice experienced greater inflammation and necrosis than female mice.[67] A mechanism underlying this difference relates to the imbalance in CD4 Th cells, with the Th1 subsets being dominant in male mice leading to increased IFN-γ and IL-2, compared with Th2 cells in female mice leading to IL-4 and IL-5 production.[64] Treatment

of male mice with oestradiol or female mice with testosterone before CVB3 infection lead to reversal of these patterns of Th cell differentiation. Further work is required to understand how these underlying differences in the immune system may be harnessed therapeutically.

Figure 1-5. Overview of pathogenetic mechanisms involved in myocarditis and progression to DCM (reproduced with permission from the European Society of Cardiology – see appendix). [1]



1.7. Clinical Presentation

Acute myocarditis can present with a range of symptoms from mild chest discomfort and breathlessness, to cardiogenic shock and sudden cardiac arrest. Many patients are likely to experience mild or transient symptoms, for example, chest discomfort or breathlessness in the context of a flu-like illness and may not seek medical attention. Of those seen within primary care, opportunities for myocarditis detection are often missed as chest discomfort is labelled as musculoskeletal or pleuritic. Hospital admissions are predominantly driven by chest pain mimicking an acute myocardial infarct, prompting direct presentation to hospital. Patients are often assessed according to local acute coronary care pathways, including primary coronary angiography. Sub-acute presentation with new-onset heart failure represents a diagnostic challenge, as conventional disease markers of myocarditis may have normalised at the time of clinical presentation. Patients presenting with fulminant heart failure generally require transfer to the nearest cardiac transplantation centre for advanced heart failure management, including consideration of mechanical circulatory support. These patients seen in hospital are likely to represent the top of an iceberg of patients affected by myocarditis, with an even smaller number at the tip presenting with fatal ventricular arrhythmia, detected for the first time on post-mortem examination (figure 1-6).

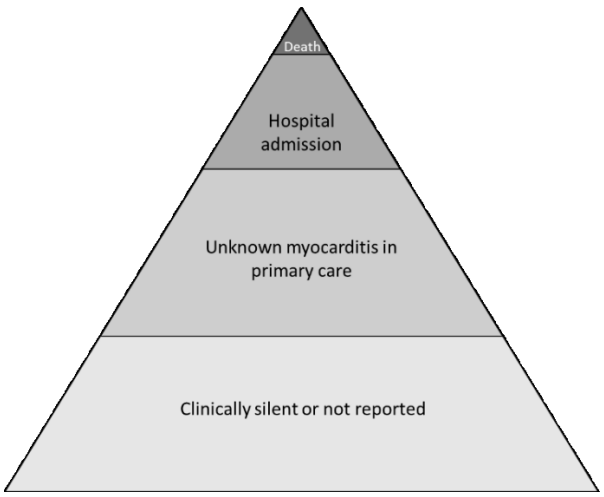


Figure 1-6. Iceberg phenomemon in detection of myocarditis cases

1.8. Current diagnostic evaluation

FIRST LINE TESTS

The European Society of Cardiology (ESC) Working Group on Myocardial and Pericardial Diseases published a position statement on the diagnosis and management of patients with suspected acute myocarditis in 2013.[1] First line recommendations for diagnosis include 12-lead electrocardiography (ECG), echocardiography, and assessment of cardiac troponin. ECG findings consist of; (i) ST segment elevation, similar to that seen with acute myocardial infarction, but typically concave and without reciprocal change,[68] (ii) T-wave inversion seen after ST segment normalisation, unlike in the setting of myocardial infarction where T-wave inversion often occurs with ST-segment elevation, (iii) PR-depression suggestive of associated pericarditis and (iv) ventricular arrhythmia arising from regions of myocardial injury, including atrio-ventricular conduction block commonly seen with Lyme disease or giant cell myocarditis.

Echocardiography defines cardiac morphology and ventricular function and is readily available, but lacks sensitivity and specificity.[69] Whilst the presence of a pericardial effusion may suggest associated pericarditis, regional wall motion abnormalities do not differentiate myocarditis from myocardial infarction and increased wall thickness can resemble hypertrophic cardiomyopathy, particularly in the apex. In cases of fulminant myocarditis, echocardiography plays an important role in the assessment of ventricular size and function and is helpful to exclude other cardiac diseases, such as valve disease. Repeat echocardiography also allows for monitoring of serial change in chamber size and function, relevant to ongoing clinical management.

Cardiac troponin elevation can be profound in myocarditis, beyond the level expected for a given clinical presentation.[70] However, troponin elevation also does not differentiate myocarditis from myocardial infarction and conversely, a normal troponin does not exclude myocarditis.[71] Erythrocyte sedimentation rate and C reactive protein levels are also

recommended as first line investigations, but do not confirm a diagnosis of myocarditis and are often raised with pericarditis.[72]

Viral serology is often assessed in cases of suspected myocarditis, although this investigation is not supported by the ESC 2013 recommendations. Serological testing is only recommended in cases of suspected Lyme disease. The reasons for this primarily relate to causation. Circulating IgG antibodies to cardiotropic viruses are prevalent in the general population but this does not equate to viral disease of the myocardium and may only indicate previous exposure to the virus.[73] Viral presence should ideally be evaluated on myocardial tissue samples by the detection of viral DNA or RNA material. However, in real-world practice, polyclonal stimulation of IgM antibodies to a known cardiotropic viral infection at the time of acute presentation does strengthen the likelihood of viral myocarditis over and above other potential aetiologies and is commonly used in real-world practice.

ROLE OF ENDOMYOCARDIAL BIOPSY

Endomyocardial biopsy (EMB) is perceived as the gold standard diagnostic test in current ESC recommendations for suspected myocarditis.[1] The working group recommended that 'all patients with clinically suspected myocarditis should be considered for selective coronary angiography and EMB.' By detailed histochemical analysis of myocardial tissue, it is possible to confirm a diagnosis of myocarditis and identify underlying aetiology with important therapeutic implications. For example, steroids may be given for Giant cell and hypereosinophilic myocarditis, but would be avoided in cases of lymphocytic myocarditis with high viral titres. Therefore, differentiating these specific forms of myocarditis can affect management decisions. However, the limitations of EMB are numerous. These include not only peri-procedural risks, such as cardiac tamponade (0.7%) and death (0.4%), but also sampling errors from insufficient biopsy sites[74] and high inter-observer variability in histopathological interpretation.[75] The high false negative rate arises from the focal nature of inflammatory

infiltrates and involvement of regions inaccessible to the bioptome, for example, within the left heart when accessing the venous system. Therefore, EMB must be performed in a biventricular manner to have greatest yield but remains 'positive' in only 70% of patients meeting current ESC diagnostic criteria for myocarditis.[76]

These limitations have prevented the broad use of EMB as a gold-standard and resulted in wide variation in clinical practice.[77] Within the UK, EMB is rarely performed outside of a cardiac transplant setting and is predominantly reserved for new-onset fulminant heart failure of unknown aetiology within tertiary level centres. In 2017, a total of 717 EMB procedures were reported to have been performed that year through a national survey in the UK, of which only 7 (0.9%) were indicated for the diagnosis of suspected myocarditis.[78] The 2016 ESC guidelines for heart failure diagnosis and treatment suggested that EMB may be considered in cases of rapidly progressive heart failure when myocardial samples may provide a specific diagnosis for which specific therapy is available and effective (Class IIb, Level C).[79] This would include myocarditis and complements the earlier ACC/AHA practice guidelines that outlined specific patient settings in which EMB may be considered, including heart failure with hemodynamic compromise of <2 weeks duration (class I recommendation) or <3 months duration if associated with heart block or new ventricular arrhythmias (class I recommendation).[80] Routine EMB surveillance has a well-established role in the surveillance of cardiac transplant rejection but use in myocarditis and heart failure in general remains variable.

ROLE OF NON-INVASIVE IMAGING

Cardiovascular magnetic resonance (CMR) plays an increasingly important role in the non-invasive diagnosis of myocarditis. The distribution, nature and severity of myocarditis can be assessed from tissue characteristics including; (i) interstitial oedema, (ii) hyperaemia and inflammatory infiltration and (iii) myocyte necrosis and replacement fibrosis affecting the mid-wall and subepicardial layer.[28] These key features were combined to form the CMR Lake

Louise Criteria, first described in 2009, with a diagnostic accuracy for myocarditis of 78% (sensitivity 67%, specificity 91%) when at least 2 out of 3 are present.[17] Numerous studies have sought to evaluate the diagnostic and prognostic utility of these criteria acute myocarditis. Most recently, in a study of 670 patients with suspected myocarditis, it was shown that the presence of LGE was associated with a more than doubling risk of MACE (hazard ratio 2.22; 95% CI 1.47-3.35; p<0.001), which remained significant after adjustment for LVEF and other clinical variables.[81] Previous work from our group has also demonstrated acute myocarditis on CMR by LLC in 50% of patients with troponin-positive chest pain and unobstructed coronary arteries by these criteria.[82] However, according to the 2013 ESC recommendations, which remain the most up-to-date version, tissue characterisation of the myocardium by CMR can only support the diagnosis of myocardium, and 'is reasonable' to be performed prior to EMB in stable patients. CMR does not constitute a first line diagnostic test.

Great strides have been made in diagnostic evaluation of myocarditis by CMR with the emergence of new quantitative mapping techniques, such as T1 and T2 mapping. These techniques have further increased diagnostic accuracy to 82% and 81%, respectively, in biopsy-proven myocarditis patients.[83] Moreover, these techniques reveal myocardial injury not seen on conventional imaging sequences[84] and potentially distinguish between acute and convalescent disease.[85] As indicated in the 2013 ESC consensus statement on CMR mapping, there was an important need for further research into T1 and T2 mapping techniques prior to large-scale application to understand their clinical significance.[86] Numerous studies performed from 2013 to 2017 were recently reviewed in two large meta-analyses. One meta-analysis pooled data for 867 myocarditis patients and 441 control subjects from 17 studies (table 1-3). Native T1 was shown to have a significantly higher sensitivity than standard LLC (85% versus 74%, P=0.025), with overall diagnostic performance of native T1, T2, and ECV mapping comparable to LLC.[87] In parallel, another meta-analysis pooled data for 22 studies and similarly

demonstrated that native T1 proved to be superior in terms of diagnostic accuracy.[88] In summary, both meta-analyses concluded that the accuracy of standard LLC could be improved with the addition of novel parametric mapping. As a result, the LLC were ultimately revised to incorporate parametric mapping in December 2018 (figure 1-7), although implementation into routine clinical myocarditis scan protocols remains variable due to the challenges of widespread variation in sequence parameters, partly driven by the vendor-specific platforms, resulting in lack of normal reference ranges for health and threshold values for disease in T1 and T2 values.[89]

First Author	Year Published	Subjects (n)	Study Design	Validation	Parameters	Scanner	Vendor	Field Strength (T)	Interval From Admission to CMR (d)	Interval From Onset to CMR (d)
Baeßler[12]	2017	84	Retrospective	Clinical	LLC, T2	Achieva	Philips	1.5	n/a	4.8±4.4*
Galea[24]	2017	54	Retrospective	EMB	LLC	Magnetom Avanto	Siemens	1.5	n/a	9.5±5.1*
Imbriaco[25]	2017	61	Not reported	Clinical	LLC	Gyroscan Intera	Philips	1.5	6.8±4*	n/a
Luetkens[26]	2017	83	Prospective	Clinical	LLC	Ingenia	Philips	1.5	2.7±1.9*	n/a
Nadjiri[27]	2017	171	Retrospective	Clinical†	LLC, ECV, T1	Magnetom Avanto	Siemens	1.5	n/a	n/a
von Knobelsdorff-Brenkenhoff[28]	2017	36	Prospective	Clinical	ECV, T1, T2	Magnetom Avanto	Siemens	1.5	n/a	<7
Lurz[11]	2016	61	Prospective	EMB	LLC, ECV, T1, T2	Intera CV	Philips	1.5	< 1.5	n/a
Luetkens[13]	2016	84	Prospective	Clinical	LLC, ECV, T1, T2	Ingenia	Philips	1.5	2.6±1.9*	n/a
Schwab[29]	2016	78	Retrospective	Clinical	LLC	Intera CV	Philips	1.5	1–17	n/a
Hinojar[30]	2015	101	Prospective	Clinical	T1	Achieva	Philips	1.5 and 3.0	n/a	2–8
Bohnen[9]	2015	31	Not reported	EMB	T2	Achieva	Philips	1.5	3 [1–6]‡	n/a
Radunski[10]	2014	125	Not reported	Clinical	LLC, ECV, T1, T2	Achieva	Philips	1.5	n/a	14 [7–49]‡
Luetkens[31]	2014	66	Prospective	Clinical	LLC, ECV, T1	Ingenia	Philips	3.0	2.6±2.2*	n/a
Ferreira[32]	2014	110	Prospective	Clinical	T1	Avanto	Siemens	1.5	3 [1–6]‡	n/a
Lurz[33]	2012	70	Prospective	EMB	LLC	Intera CV	Philips	1.5	n/a	3 [1–7]‡
Chu[34]	2013	45	Not reported	Clinical	LLC	Magnetom Avanto	Siemens	1.5	n/a	7±10*
Abdel-Aty[35]	2004	48	Not Reported	EMB	LLC	Signa CV	GE	1.5	n/a	5.6±4.2*

CMR indicates cardiac magnetic resonance; ECV, extracellular volume; EMB, endomyocardial biopsy; and LLC, Lake Louise Criteria.
*Expressed as mean with SD.
†Final diagnosis based on troponin >10× upper limit of normal.
‡Expressed as median with interquartile range.

Table 1-2. Characteristics of studies included into a meta-analysis evaluating the diagnostic accuracy of parametric CMR mapping techniques compared with standard LLC. Reproduced with permission.[87]

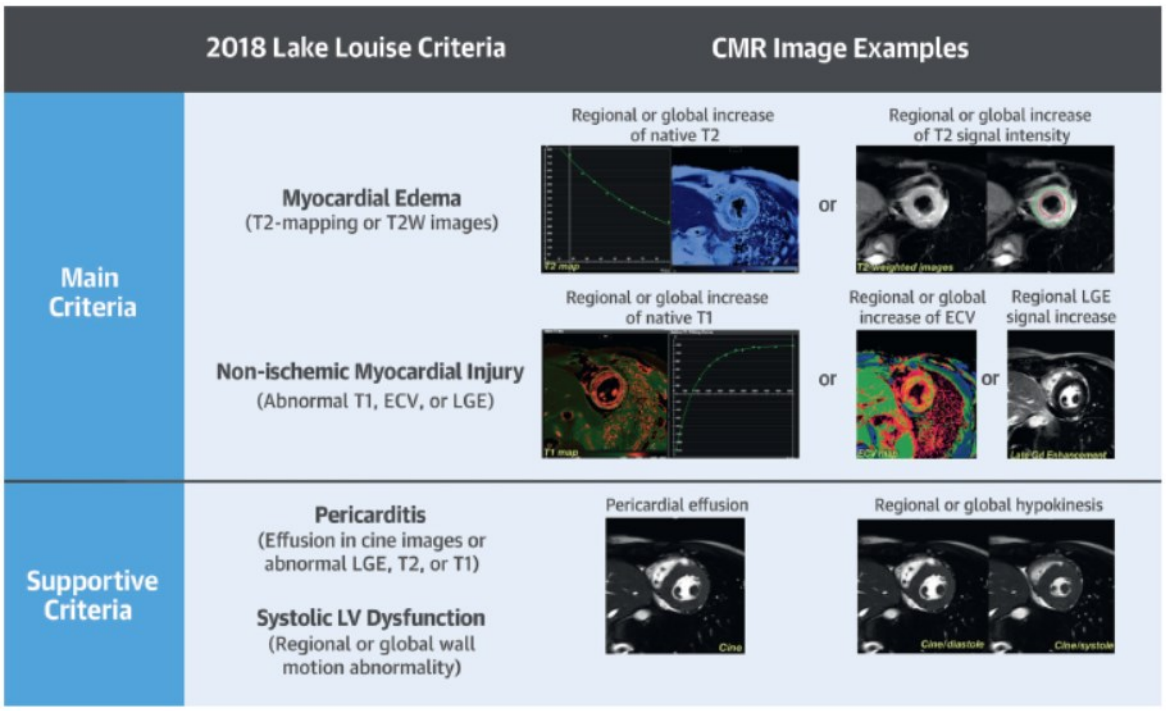


Figure 1-7. Overview of updated Lake Louise Criteria (December 2018). Reproduced with permission.[89]

NUCLEAR IMAGING

Positron emission tomography (PET) using radiolabeled glucose analogue [^{18}F]-2-deoxy-2-fluoro-d-glucose (FDG) plays an important role in the detection of cardiac inflammation, primarily due to cardiac sarcoidosis, according to the 2013 ESC recommendations.[1] For myocarditis, EMB is perceived as the gold-standard test and nuclear imaging is therefore discouraged. Sarcoidosis is a multisystem, granulomatous disease that most commonly affects young adults and cardiac involvement is the second most common cause of death. Whilst late gadolinium enhancement readily detects non-viable myocardial tissue,[90] the detection of active myocardial inflammation by CMR faces similar challenges as with myocarditis. As a result, most cases of cardiac sarcoidosis continue to be detected for the first time on post-mortem examination.[91] However, unlike myocarditis, a strong evidence-base supports the early use of steroids and other immunosuppressive agents to reverse active inflammation and prevent further deterioration in cardiac function and scar formation.[92, 93] Therefore FDG-PET has

emerged as an important tool to accurately detect active cardiac, but also non-cardiac, disease to guide the need for immunosuppression.[94] Although it is important to note that systematic comparisons show that CMR correlates better with clinical disease manifestations [95] and has greater specificity.[96] Further investigation is ongoing to define the role of parametric mapping in detection of cardiac sarcoidosis.

ADDITIONAL BIOMARKERS

Existing biomarkers (troponin and BNP) play a useful role but do not specifically assess pathways of inflammation and fibrosis, which are the hallmarks of this myocarditis. Circulating biomarkers of myocardial inflammation and fibrosis are providing new insights into clinical diagnosis, surveillance, and follow-up. Inflammatory cytokines such as interleukin-10[97] and interleukin-6[98] have been linked to adverse outcomes in limited human studies and animal models of myocarditis. Furthermore, blockade of IL-6 preserves cardiac function by reduction in viral load.[99] Characterisation of inflammatory and fibrogenic pathways, particularly exploration of the differences between patients showing spontaneous recovery versus progression to DCM, represents an opportunity to improve our understanding. There are also potential cost savings through unnecessary follow-up of the 'recovered' patient, and conversely, more targeted surveillance of the 'at-risk' patient.

GENETIC TESTING

The role of genomic data continues to rapidly expand in all areas of medicine. At present, there is insufficient clinical evidence to guide the use of genetic evaluation of myocarditis. In future, genomic data may be important to help understand if there is a 'vulnerable' patient cohort at risk of developing myocarditis and also understanding if myocarditis is an epigenetic trigger for the development of subsequent LV dysfunction and inflammatory cardiomyopathy.[100] These areas are discussed and investigated in more detail in chapter 5.

1.9. Clinical outcomes

Approximately two thirds of patients with acute myocarditis recover spontaneously within 2-4 weeks with no long-term impairment in LV systolic function. This scenario is often referred to as 'healed myocarditis.' Amongst this group, outcomes are generally favourable, particularly those with a normal CMR, although there is uncertainty regarding the future risk of recurrence of myocarditis and arrhythmia arising from regions of myocardial scar.[1]

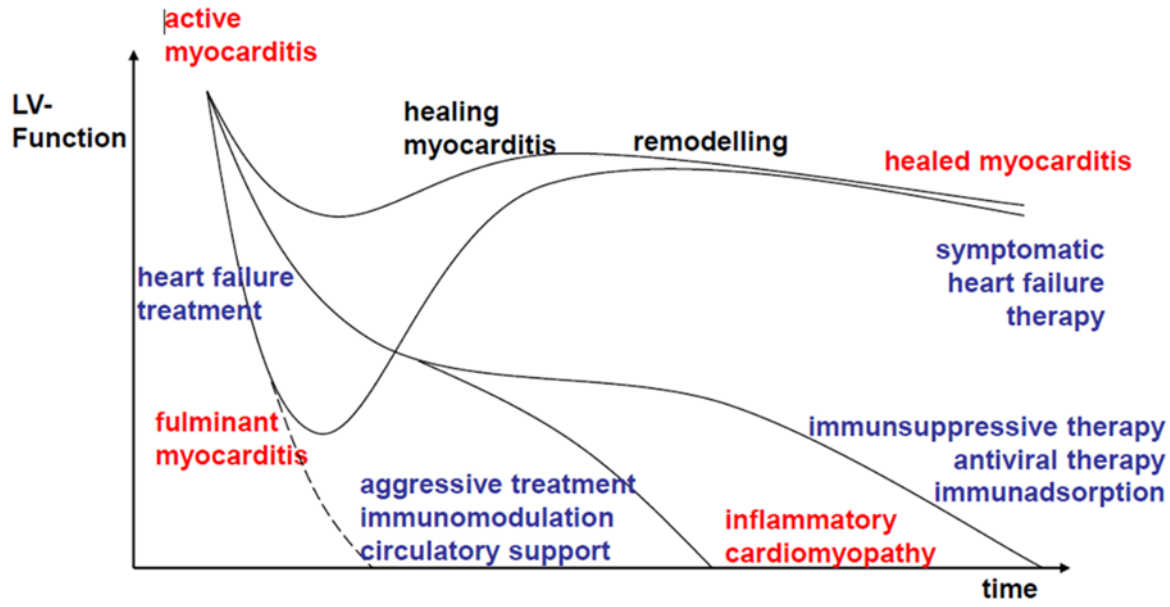


Figure.1-8 Illustration of clinical trajectories and possible outcomes (labelled in red) and related therapies (labelled in blue) following an episode of acute myocarditis.

Unfortunately, in a small but important subset of patients, myocarditis can lead to fulminant heart failure requiring critical care and advanced heart failure management. Amongst this group, outcomes have been generally variable with a one-year mortality of 15%, although in some patients LV recovery can be dramatic with aggressive heart failure treatment and circulatory support, resulting in paradoxically better outcomes than those patients presenting with sub-acute onset of LV dysfunction.[101] As discussed earlier, chronic active forms of myocarditis can result in gradual progression to inflammatory cardiomyopathy due to auto-

immune mechanisms. In a landmark study of 222 patients (median age 52 years) with biopsy-confirmed acute viral myocarditis and median LVEF 45%, cardiovascular mortality occurred in 15% of the cohort that were available for clinical follow-up over a median of 4.7 years.[6] After adjustment for LVEF, LV end-diastolic volumes and NHYA class, the presence of LGE remained associated with this outcome (hazard ratio 8.4; p= 0.004). SCD or aborted SCD was also reported in 9.9% of this cohort, with all affected individuals having median LVEF of 35%. The risk of SCD in patients with normal LV structure and function is yet to be defined, but myocarditis remains a common finding on post-mortem examination following sudden cardiac death in the young.[5] In summary, long-term outcomes in patients with LV dysfunction is no different from that of idiopathic DCM, and the key unmet need is to identify the markers of high-risk within these patients compared to the much larger number of patients that recover spontaneously, and also to elucidate predictors of fatal ventricular arrhythmia.

1.10. Clinical management

There is no targeted therapy for myocarditis. Finding a 'magic bullet' poses a number of challenges due to the various different aetiologies and underlying pathophysiological pathways. Management is therefore supportive with acute hospital admission recommended by current ESC guidelines for clinical monitoring and diagnostic evaluation.[1]

In patients with normal LVEF, simple analgesia and avoidance of exercise is recommended. This is based on historical studies in animals and the recognition that myocarditis is often lethal in young athletes experiencing sudden cardiac death.[102] The presence of atrial or ventricular arrhythmia may require appropriate pharmacological therapy, usually in the form of beta-blockade. Any potential cause for drug-induced myocarditis should also be eliminated. Non-steroidal anti-inflammatory drugs (NSAIDs) were initially avoided in myocarditis due to

animal studies showing worsening in inflammation, myocyte necrosis and mortality with NSAIDs compared to placebo.[103] In contrast, NSAIDs have traditionally formed the cornerstone of treatment for acute pericarditis and patients with myopericarditis have been retrospectively investigated to show that overall NSAIDs have been safe and effective.[104] However, controlled trials are still needed for NSAIDs in myocarditis as noted in current ESC guidelines, which also remains the case for colchicine, traditionally used as an anti-inflammatory agent to treat gout.[1]

In patients with LV dysfunction, management follows current guideline recommendations for heart failure with gradual titration of neurohormonal-blocking medications and beta-blockers. Diuretics may be used to optimize intravascular volume. In fulminant cases presenting with cardiogenic shock, admission to intensive care units for inotropic support, ventricular assist devices (VAD) or extracorporeal membrane oxygenation (ECMO) is often required as a bridge to recovery or transplant. In a pivotal study of myocarditis patients receiving VA-ECMO, 73% of the cohort recovered to hospital discharge without transplantation demonstrating the utility of this approach as a first-line strategy for mechanical circulatory support prior to VAD implantation.[105, 106] In the long-term, the optimal duration of HF mediation once LV function has recovered remains unclear. A recent study from our group investigating therapy withdrawal in patients with recovered DCM provided many useful insights.[107]

Various other treatment strategies have been evaluated in small, single-centre studies with limited evidence of clinical benefit. Controlled trials of antiviral therapy in virus-positive cases have been ineffective despite the success of ribavarin and interferon alpha in improving survival in animal models when administered at the time of inoculation.[108] Attempts have also been made at disrupting viral entry into myocytes through the use of modified coxsackie and adenovirus receptor (CAR) fragments to aid immune activation in animal models.[109] In a phase II study of 22 patients with chronic viral disease, it was found that treatment with subcutaneous

interferon-B resulted in improved viral elimination paralleled by improvement in LVEF over a period of 24 weeks.[110] Administration of exogenous interferons to promote natural defences may therefore represent a promising treatment strategy and further trials are underway in patients with inflammatory cardiomyopathy.[111]

Immunosuppressive treatment regimes in virus-negative cases have yielded mixed results and remain an active area of investigation. A single-centre study of 85 patients with LVEF <45% in the setting of virus-negative myocarditis showed that those receiving prednisolone (1mg/kg per day) showed significant improvement in LVEF at 6 months (assessed by 2-D echo) compared to azathioprine or placebo.[112] Interestingly, none of the untreated patients showed improvement in LVEF at follow-up. In a related study of 202 patients with biopsy confirmed chronic active myocardial inflammation and persistent LV dysfunction, randomisation to steroids and azathioprine in addition to standard HF therapy resulted in significant and sustained improvement in LVEF over 2 years of follow-up.[113] Given that perpetuating immunological mechanisms are likely independent from the initial clinical presentation of myocarditis, there is much interest in the role of immunomodulation both for acute myocarditis but also the large number of patients potentially labelled with 'idiopathic' rather than 'inflammatory' DCM.[114] High-dose intravenous immunoglobin and immunoadsorption have also been trialled in myocarditis but with no evidence of actual clinical benefit and therefore are not recommended in current ESC guidelines pending the results of future randomised trials. In the setting of Giant cell myocarditis, the use of high-dose intravenous steroid therapy was established many years ago and continues to be the mainstay of treatment.[115]

1.11. Unmet Needs

As highlighted above, myocarditis is a heterogeneous disease predominantly affecting young individuals with a significant burden of morbidity and mortality. A disease with such complexity requires contemporary, personalised and precision approaches to guide risk prediction and management. Whilst progress has been made in the invasive histopathological evaluation of myocarditis, there is limited understanding of additional non-invasive, mechanistic markers that identify subsets of high-risk individuals in whom further monitoring and medical therapy are key. Amongst the large numbers of patients with healed myocarditis, defined by normal LV structure and function but with small areas of scar, there is no evidence regarding need for ICD implantation. As a result, current strategies for risk prediction and subsequent follow-up remain uncertain.

With the advent of multiparametric T1 and T2 mapping techniques, there is unmet need to define the clinical role of such *in vivo* tissue characterization. Genomic data is similarly yet to be harnessed within myocarditis to establish its role in understanding risk of both initial susceptibility and determinants of downstream recovery versus progression to DCM. In recent years, genetic sequencing costs have exponentially fallen, partly driven by national programmes such as the UK Biobank. In the current era of big data, coupled with a clear focus on technology in the latest NHS Long Term Plan, there is a pressing need to leverage electronic healthcare data to update current epidemiological estimates otherwise based on historical post-mortem data or international datasets not necessarily be transferrable to patients seen within the National Health Service. Such information would likely have implications on myocarditis awareness, detection, resource utilization and inform myocarditis best clinical practice guidelines within the UK, which currently do not exist and hence the wide regional variation in diagnosis and management.

1.12. Aims, Objectives & Thesis Overview

Given the paucity of clinical evidence in a number of different areas relating to myocarditis, we designed a series of studies to provide novel insights in epidemiology, advanced imaging, genomics and long-term outcomes with specific hypotheses summarised below and re-presented over the following chapters. Our overall aim was to build a better understanding of myocarditis epidemiology and to harness mechanistic insights from the latest advances in cardiovascular magnetic resonance (CMR) and next-generation DNA sequencing to ultimately improve myocarditis awareness and clinical outcomes.

Chapter 3: Epidemiology of myocarditis

- Acute myocarditis is an increasingly prevalent disease.
- Men are affected at a younger age compared to women.
- Disease incidence shows seasonal variation throughout the year.
- Myocarditis is predominantly a disease of towns and cities, rather than rural areas.
- Diagnostic evaluation is diverse and varies by region.
- Morbidity and mortality are underestimated.

Chapter 4: Clinical outcomes and observations in myocarditis

- Deep phenotyping in acute myocarditis by harnessing recent advances in CMR multiparametric mapping and strain assessment may provide in-depth mechanistic insights into disease progression and assessment of treatment response.
- Following index presentation with myocarditis, patients at risk of progression to dilated cardiomyopathy may be identified by subclinical markers of disease characterised by:
    - Persistently elevated T1 & T2 values, as measured by multiparametric mapping.
    - Impaired strain, as measured by DENSE.

Chapter 5: Genetic determinants of myocarditis

- Amongst patients with myocarditis progressing to a DCM phenotype, there is a greater prevalence of titin truncating variants (TTN-tv) compared to those without adverse remodeling, suggesting that viral myocarditis acts as an environmental modifier that unmasks the DCM phenotype.

Chapter 6: Long-term outcomes in healed myocarditis

- Myocardial fibrosis attributed to healed myocarditis with normal left ventricular size and function portends increased risk of fatal ventricular arrhythmia in the long-term.

Chapter 7: Psychological impact of myocarditis

- Patients experience high levels of ongoing psychological distress many years after index hospital admission with acute myocarditis.

Chapter 8: What this thesis adds and future work

- Overview of key scientific findings and contributions in advancing the field.

2. BACKGROUND TO COMMON METHODOLOGY

Extracts from this chapter are based on my own work which has been published:

Lota AS, Gatehouse PD & Mohiaddin RH. T2 mapping and T2* imaging in heart failure. Heart Fail Rev. 2017 22(4):431-440.

2.1. Introduction

In this chapter, the general methods used for cardiovascular magnetic resonance (CMR) imaging, genetic sequencing and ethical considerations relating to these techniques are described. More detailed methodology sections focusing on the specific approaches used for data analysis and limitations are included in subsequent chapters. Epidemiological analyses are also discussed separately in the next chapter.

2.2. Basic physics of CMR

Cardiovascular magnetic resonance (CMR) generates images of the heart by radiofrequency excitation of hydrogen nuclei, or protons, within the magnetic field of the scanner. Following the application of radiofrequency energy, excited protons emit radiofrequency signals as they recover back towards resting equilibrium within the magnetic field (figure 2-1).

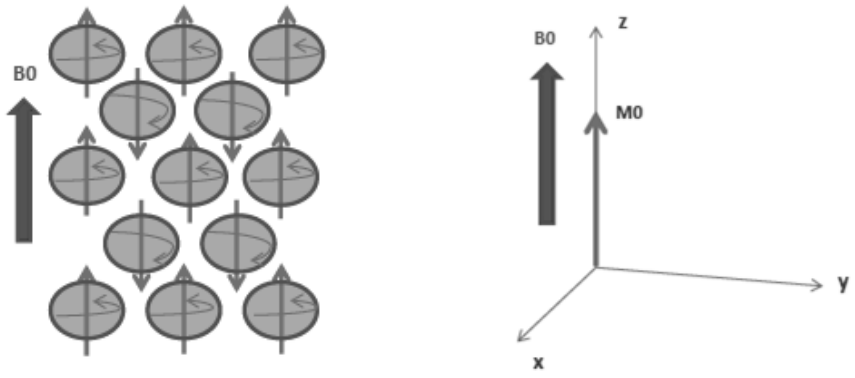


Figure 2-1. Hydrogen nuclei arrangement in the magnetic field along the B0 axis. At equilibrium, there is a small net magnetisation in the longitudinal z direction (modified with permission from Halliday et al).

The relaxation consists of two types: *recovery* of the longitudinal component of magnetisation (the T1 relaxation time) towards equilibrium, known as spin-lattice coupling, and the *decay* of the transverse magnetisation (the T2 relaxation time), known as spin-spin coupling. The

inherent T1 and T2 values vary depending on the composition of different biological tissues, primarily increased by greater water content, and these fundamental differences form the basis of the intrinsic contrast used to generate images. These signals are recorded and converted into an image using the principle of Fourier transformation.

2.3. CMR System Components

In order to achieve these processes, the CMR scanner consists of three major electromagnetic components; (i) a superconducting magnet to align the protons, (ii) radiofrequency coils to receive emitted radiofrequency signals, and (iii) gradient coils to create imaging planes.

SUPERCONDUCTING MAGNET

The superconducting magnet consists of a coil of niobium-titanium wire wound around a cylindrical core. Liquid helium within the core ensures continuous current flows with no electrical resistance at (-)270^{o}C in a 'superconducting' state. This generates a strong and constant magnetic field along the axis of the magnet bore known as the z-axis, which is denoted by the symbol B_0. The nominal strength of the CMR system is defined by the strength of this field and is measured in the units of Tesla (T), with 1 Tesla equal to 20,000 times the earth's magnetic field. The patient is positioned within the central bore of the magnet along the B_0 axis. Most clinical CMR systems operate at 1.5T, although higher field strengths, such as 3T, offer greater signal-to-noise ratio and are increasingly common. More recently, 7T systems have also been introduced with a much smaller bore size due to the size of the magnet required and these are generally used for animal studies in the research environment. Whilst signal-to-noise ratio improves with increasing field strength, the main limitations include greater B0 field inhomogeneity, higher radiofrequency power use, more frequent artifacts and ECG distortion.

RADIOFREQUENCY COILS

Radiofrequency coils emit radiofrequency pulses necessary to excite protons in the tissue being examined (transmission coils), and subsequently receive radiofrequency signals emitted during the relaxation phase as protons re-align to the resting magnetic field on the B_0 axis (receiver coils). Modern scanners have fixed coils around the central bore within the scanner that only transmit (body coils). Separate receiver coils are located within the scanner table and also placed on the patient's chest to maximize signal detection (figure 2-2).

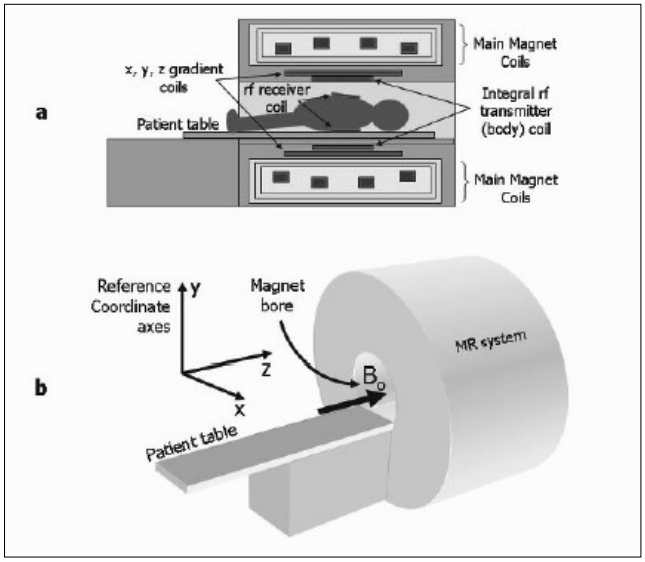


Figure 2-2. MR system components. (A) Diagram showing relative locations of the main magnet coils, gradient coils (x, y and z), radiofrequency transmitter body coil and receiver coils. (B) Diagram showing cylindrical magnet bore and reference coordinate axes. Reproduced with permission.[116]

GRADIENT COILS

Unlike computed tomography (CT) scanners, which acquire a 3-dimensional volume of data that can be reconstructed in any plane, CMR systems generally perform image acquisition on a single imaging plane that is defined and optimized by the operator prior to image acquisition. This is due to the complexity of the acquisition and subsequent post-processing required to generate an image. A reference co-ordinate system of 3 orthogonal axes (x, y and z) is used to define the magnetic field direction around the scanner table, which represents the sagittal,

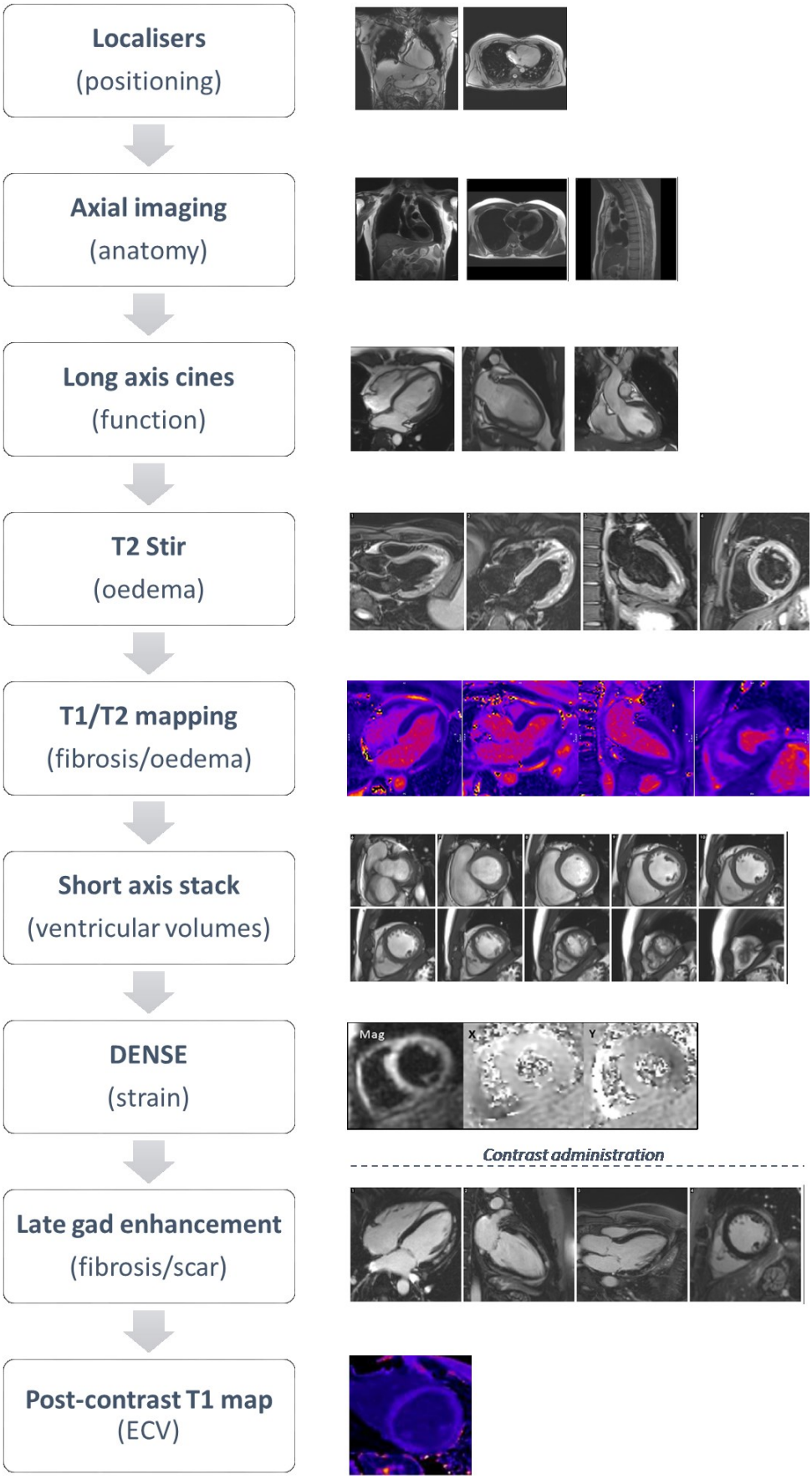
coronal and transverse planes respectively. Three orthogonal gradient coils are mounted inside the main magnet and can be activated to linearly modify the strength of B0 along the x, y and z directions depending on the gradient coil that is activated. The strength of the gradient magnetic field reflects the 'steepness' of its slope and is measured in millitesla per metre (mT/m). In this way, the gradient fields allow slice selection and spatial localisation of emitted radiofrequency signals during image acquisition.

2.4. CMR Protocol

A CMR scan consists of multiple imaging sequences, each used to generate short video loops ('cines') or static images of different tissue characteristics, which may be interrogated by adjusting the pulse sequence for T1- and T2-weighting. For example, T2-weighted imaging has an established role in depicting myocardial oedema due to the effect of increased interstitial free water on lengthening T2 relaxation times with particular relevance to inflammatory conditions, such as a myocarditis, and acute ischaemic injury. Similarly, the presence of increased iron reduces T2 and T1 by local magnetic field distortion. Myocardial image contrast can also be extrinsically modified through the intravenous administration of gadolinium contrast agent, which usually remains extracellular, where T1-weighted imaging shows areas of injured myocardium with expanded extracellular space due to shortened T1 recovery times.

Specific CMR sequences are included depending on the pre-determined protocol for the given scan indication. This requires a balance between acquiring sufficient information to address the clinical or research question whilst minimizing scan times and potential patient discomfort. A complete clinical CMR study would usually last up to one hour. We added a number of research sequences to a standard clinical CMR protocol for suspected acute myocarditis, which would include assessment of volumes, myocardial oedema and scar formation by late gadolinium enhancement (figure 2-3). These sequences are discussed in further detail below.

Figure 2-3. CMR protocol for our myocarditis research study



2.5. T2-STIR

T2-weighted imaging shows increased myocardial signal from myocardial oedema based on the prolongation of T2 relaxation caused by accumulation of interstitial water. This was first demonstrated in 1983 in a canine model of acute myocardial infarction.[117] T2 relaxation refers to the natural interactions causing irreversible dephasing of transverse magnetisation at atomic or molecular scale. Spin-echo sequences are used with a re-focusing (180°) radiofrequency pulse to re-phase reversible loss of transverse magnetisation due to local magnetic field inhomogeneity at larger scales, which can be considered stationary over the relevant duration involved during measurement. Signal from fat and the blood pool is suppressed to improve image quality. Sequences typically use a short-Tau inversion recovery (T2-STIR) nulled to suppress the shorter T1 of fat with a double inversion-recovery method aiming to suppress blood signal, overall known as 'triple-inversion recovery,' in preparation for a fast spin-echo sequence with T2-image contrast weighting identified loosely hereafter as T2-STIR (figure 2-4). In this way, pronounced contrast is created between bright oedema (longer T2) and hypointense normal myocardium (shorter normal T2).

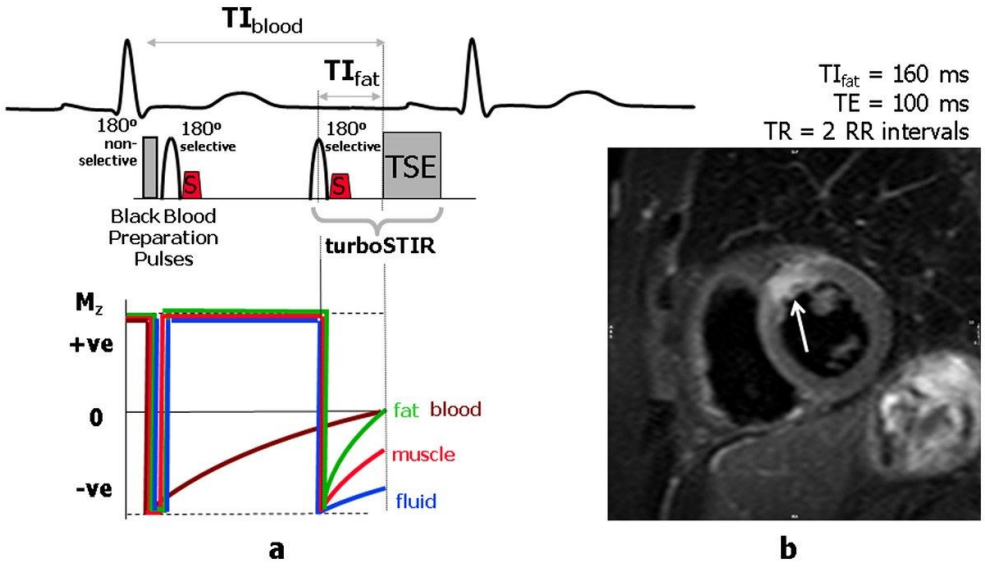


Figure 2-4. Triple inversion recovery turbo spin echo pulse sequence (T2-STIR) commonly used for oedema imaging. (A) The pulse sequence timing is shown together with Z-magnetisation curves. Black blood preparation is achieved through two 180° pulses, followed by a third slice-selective 180° pulse to provide

the STIR contrast. (B). Representative image acquired in the short axis plane using the black-blood turbo STIR technique. Myocardial oedema can be seen as an area of increased signal (arrow). Reproduced with permission.[118]

Preclinical and human studies have demonstrated a range of clinical applications for T2-STIR, for example, in acute myocardial infarction and acute myocarditis (figure 2-5).[119] However, limitations are well known to include low signal-to-noise ratio, loss of signal due to cardiac motion (not only the spin-echo method but also the complex triple-IR preparation sequence), imperfect blood suppression in areas of slow blood flow and subjective visual interpretation. While focal T2 increases may be easily visualised as image resolution of T2-STIR is finer than that of T2 mapping, larger regions are more challenging as they are easily confounded by myocardial signal darkening linked to motion and incorporate many other uncontrolled factors in MRI signal brightness – as yet, no standardised calibration of MRI magnitude values is routinely possible.

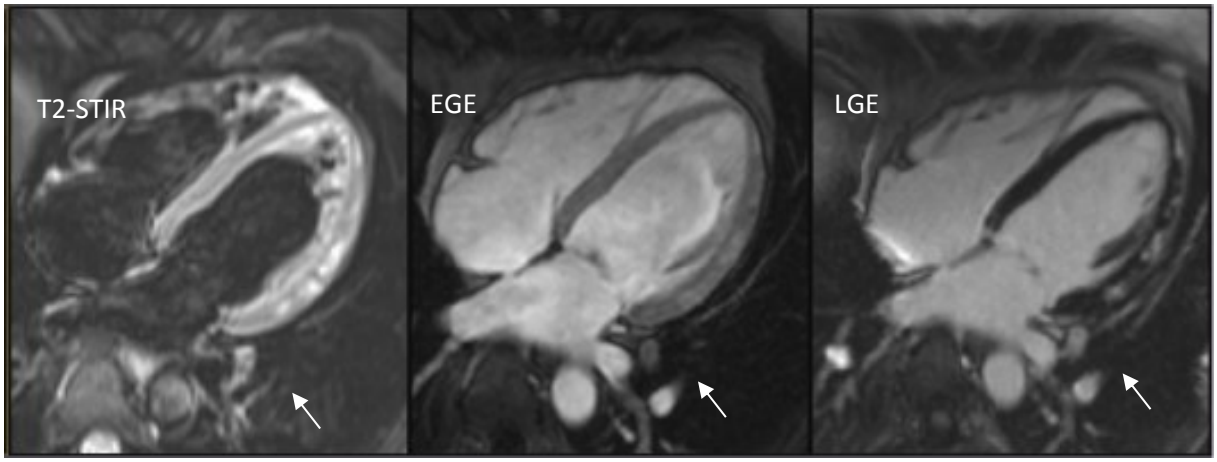


Figure 2-5. Standard Lake Louise Criteria for acute myocarditis showing focal regions of myocardial oedema on T2-STIR, reactive hyperaemia on early gadolinium enhancement, and myocyte necrosis/fibrosis on late enhancement in the inferolateral wall (arrowed).

2.6. Late gadolinium enhancement

Gadolinium-based contrast media allow direct visualisation of focal myocardial necrosis and fibrosis. In healthy myocardium, the volume of distribution of gadolinium contrast is low. Image acquisition using an inversion recovery gradient echo sequence after 10-20 minutes would show no areas of enhancement due to wash out of gadolinium. However, in acute myocardial infarction (or myocarditis), disruption of cell membranes allows troponin release and entry of gadolinium into the intracellular space (figure 2-6). Similarly, in the chronic setting, regions of myocyte necrosis are replaced with myocardial fibrosis and expansion of the extracellular space. Both processes delay the kinetics of gadolinium and imaging during the 10-20 minute window after the standard dose of gadolinium (Gadobutrol) 0.1 mmol/kg allows visualisation of regions of enhancement.[120] As with T2-STIR imaging, signal intensity ratios in these conventional CMR imaging sequences are displayed on an arbitrary grey scale, and therefore are not suited to quantitative measurement or comparison between patients and serial examinations. Subjective visual analysis susceptible to interobserver variation represents the main limitation of conventional CMR imaging.

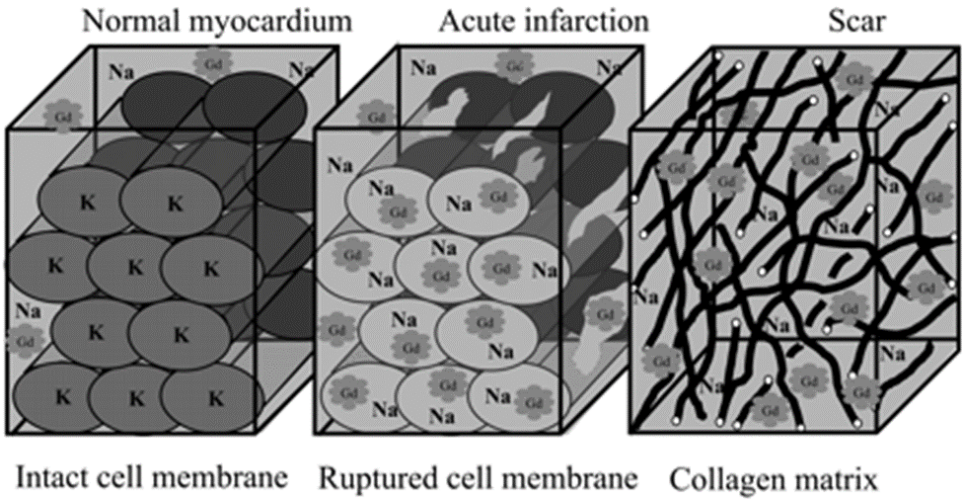


Figure 2-6. Mechanism of late gadolinium enhancement. Gadolinium accumulates in regions of myocardial injury due to disruption of cell membranes and extracellular expansion resulting in higher focal concentrations, shortening T1 recovery and creating increased signal intensity on T1 weighted imaging. Reproduced with permission.[121]

2.7. T2 mapping

T2 mapping, or T2 transverse relaxation time mapping, is a technique used to construct a 'parametric image' or 'map' in which the intensity of each voxel is the output of a calculation performed independently at each corresponding spatial pixel from a series of input images. The map value reflects the calculated T2 relaxation time at each pixel.

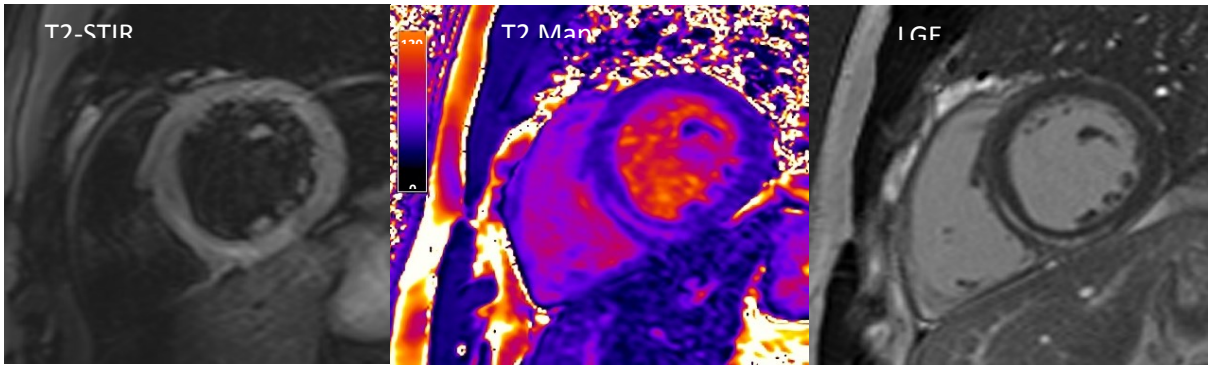


Figure 2-7. T2-STIR and T2 mapping at the basal short-axis level in a patient with acute myocarditis affecting the inferoseptal wall. Some caution would be required in cardiac walls adjoining the lung, particularly the inferolateral wall, due to B0-distortion effects in some types of sequence, particularly at 3-Tesla field strengths. The late gadolinium enhancement image is provided for reference.

T2 maps can be analyzed visually on a grey (or colour) scale but can also be analyzed quantitatively by defining regions of interest relevant to the particular pathology being studied. Various different sequences have been used for T2 mapping. In principle, at least 3 separate single-shot images are acquired at increasing T2 preparation times to construct a transverse relaxation curve from these separate echo times (figure 2-8).[122] A long repetition time of 2-4 RR intervals is used to achieve maximal T1 longitudinal recovery, which otherwise is capable of distorting the calculated T2 presented in the map without any warning.[123] Motion-correction algorithms are often used given that at least 3 T2-weighted images are acquired over multiple heart beats during a single breath-hold. Parametric mapping can be performed in any cardiac slice and position, but most commonly data is acquired on a short axis view at the basal and

mid-ventricular level. Long axes views may also be acquired because short axis slices at the apical level are prone to partial volume effects.[124] Other limitations include the need for increasing the number of RR intervals between each acquisition at faster heart rates to allow complete T1 relaxation.[125]

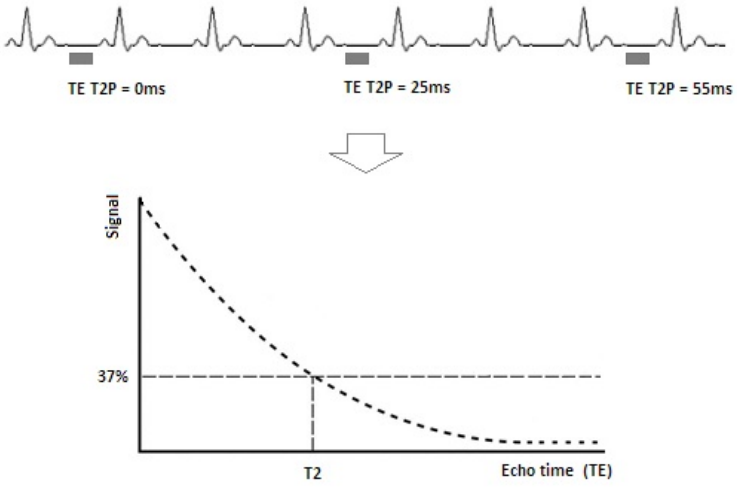


Figure 2-8. Principles of T2 mapping with different T2 preparatory durations with a long repetition time between the used cardiac cycles, crucial to allow as complete T1 recovery as possible, followed by reconstruction of the transverse relaxation curve in each pixel assuming satisfactory registration. T2 is defined as the time in milliseconds for the transverse magnetisation to decay to 37% of the original value.

T2 mapping gives access to global T2 changes as well as to nominally measured values for T2 rather than an uncalibrated T2-STIR report. However, the measurement is subject to sequence parameters without a standard, requiring care against changes. For example, some but not all protocols include a T2 preparation image at 0 milliseconds to avoid potential errors invoked by the T2-preparation. As mentioned above, the spatial imaging resolution of T2-STIR is finer than T2 mapping and this could be important for detailed focal disease visualisation on T2 maps. T2* imaging for iron overload employs a similar approach and has evolved from a specialist research technique to a clinically-validated tool in widespread general use. T2 mapping in the assessment of patients with cardiac inflammation and heart failure is likely to follow a similar trajectory.

2.8. T1 mapping

Whereas T2 mapping provides a quantitative approach in the assessment of myocardial oedema, T1 mapping emerged in parallel as a quantitative approach to assess replacement fibrosis but also earlier forms of diffuse interstitial fibrosis not typically seen on standard LGE imaging. The T1 parametric map can be created using a number of different inversion sequences to create a longitudinal relaxation curve from which biological T1 relaxation times can be quantified within each voxel of the map.[126] The Modified Lock-Locker Imaging sequence (MOLLI) was the first clinically applicable method for single-breath hold T1 mapping and was shown to have good correlation with histologically identified collagen volumes fraction (figure 2-9).[127, 128] Pulse sequence refinements led to reduced breath-hold durations from these early sequences, with imaging performed in our study over 11 R-R intervals to assess native T1 times (5(3)3 MOLLI).[129] In addition to pre-contrast T1 mapping, the MOLLI sequence can be repeated following the administration of gadolinium (4(1)3(1)2 MOLLI) to derive the myocardial extracellular volume (ECV), by estimating the amount of contrast in the extracellular compartment in the pre- and post-contrast maps relative to the blood pool at steady state according to this equation.[128]

$$ECV = (1 - haematocrit) * \frac{\left(\frac{1}{T1\ myo\ post} - \frac{1}{T1\ myo\ pre}\right)}{\left(\frac{1}{T1\ blood\ post} - \frac{1}{T1\ blood\ pre}\right)}$$

The multiplication by (1-hematocrit) represents the blood volume of distribution and converts the partition coefficient into myocardial ECV. ECV fraction has been shown to correlate well with interstitial fibrosis across a range of diseases including DCM, HCM and aortic stenosis and represents a powerful additional prognostic marker within these conditions.[128, 130, 131]

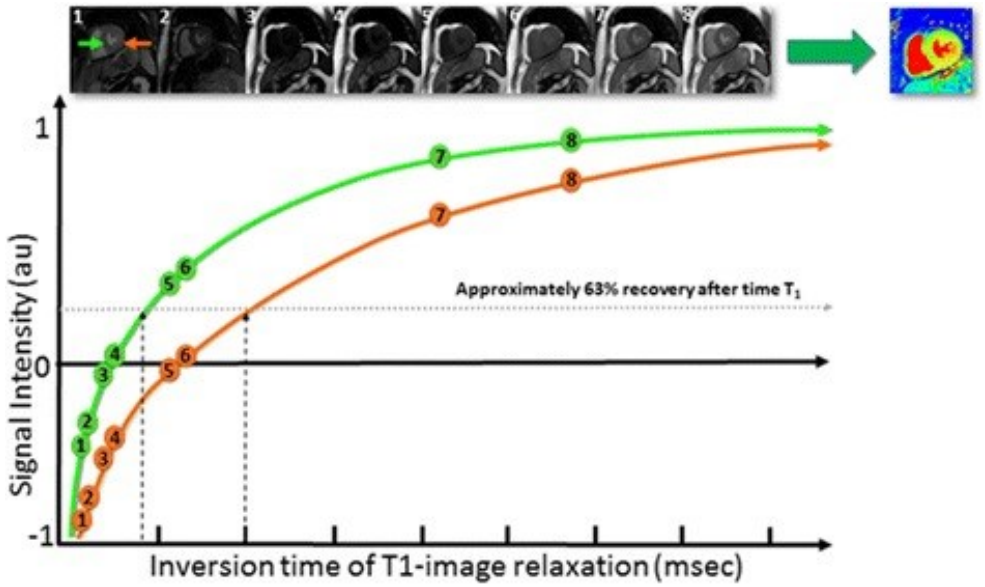


Figure 2.9. Modified Look-Locker Inversion Recovery (MOLLI) scheme for T1 mapping. Two inversions are used to acquire 8 images over 11 heart beats, referred to as 5(3)3. The green relaxation curve refers to normal healthy myocardium. The orange curve refers to an area of myocardial infarction with elevated native T1 times. Reproduced without alteration under the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). [126]

2.9. CMR safety considerations

CMR is generally considered a safe investigation due to the lack of ionizing radiation. All patients are screened for the presence of any implanted medical devices of foreign bodies through the completion of a paper questionnaire (see appendix), and appropriate safety precautions are followed to ensure ferromagnetic objects (e.g. wheelchairs and oxygen cylinders) are not brought into the scanner room. Most research patients with implantable cardiac devices are generally excluded from undergoing a CMR scan due to concerns about potential disruption of electrically or magnetically activated devices or risk of dislodgement or heating of ferromagnetic objects with little direct benefit to the patient from undergoing the scan.[132] Within the clinical setting, MRI conditional devices may be scanned if the benefit in terms of diagnostic assessment outweighs the risk, and there are rapidly increasing numbers of such patients safely undergoing CMR scans with implantable devices.[133]

Use of gadolinium contrast is generally well tolerated by most patients. Nephrogenic systemic fibrosis (NSF) is the main risk, although the actual incidence is exceedingly low with the current generation of gadolinium chelate based agents (e.g. gadobutrol, Gadovist).[134, 135] This is a progressive condition characterized by a widespread multi-system fibrotic reaction in the subcutaneous tissue, joints, skeletal muscle, and other solid organs such as the lungs, heart and liver. All patients undergo screening of renal function, and gadolinium contrast is withheld for research patients with eGFR less than 30 ml/min/1.73m^2.

2.10. Genetic Sequencing

Medical genetics has rapidly evolved since the DNA double helix was first reported by Watson and Crick in the 1950s. DNA sequencing is the basic process of determining the order of nucleotides (A, T, C and G) which determine the composition and sequence of specific amino acids that are assembled into larger proteins (figure 2-10). The principal sequencing technique was the Sanger method developed in the 1970s.[136] This was based on the incorporation of fluorescent nucleotides through cycles of denaturation and polymerisation to mark the ends of target DNA, which were read through electrophoresis. After decades of refinement, the entire human genome was published in 2001 through an international collaborative effort using Sanger sequencing.[137] The scale of The Human Genome Project cannot be underestimated and all sequencing data was made publicly available for research centres worldwide. During this 10-year project, sequencing costs dropped from $10 per nucleotide base to 9 cents due to the development of rapid processing and automation. This approach continues to represent the gold standard for sequencing accuracy but provides, what is now considered, low-throughput and thereby lacks cost-effectiveness.

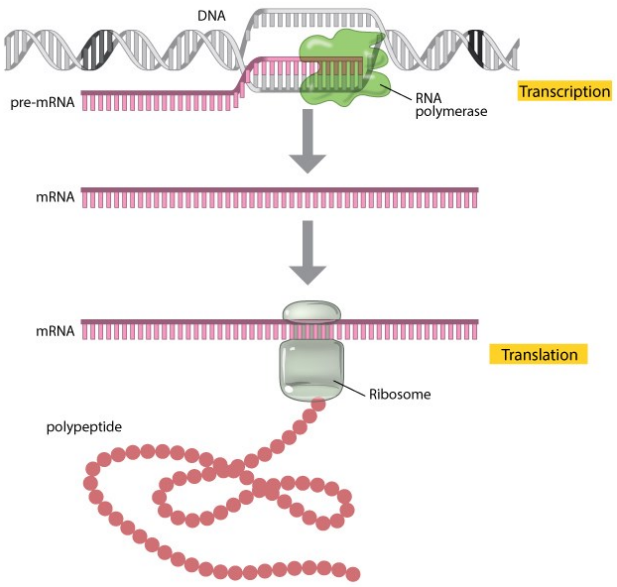


Figure 2-10. Overview of DNA transcription into messenger RNA, and mRNA translation into amino acids that are assembled into larger proteins. (Reproduced with permission from Nature Education.)[138]

Modern-day automated platforms rely on the concept of sequencing multiple copies of fragments of DNA in a massively parallel high-throughput approach, known as next-generation sequencing (NGS). This paradigm shift enabled around 50 billion bases to be sequenced per day from multiple individuals, as opposed to 2 million bases per day using even the latest Sanger sequencing machines, thereby significantly improving cost-efficiency. The term NGS originally referred to the generation of sequencing devices that followed the 'first generation' automated Sanger method, but since then, multiple generations have passed, and hence NGS refers to all high-throughput, massively parallel sequencing platforms.

In order to improve efficiency further, NGS sequencing can be targeted to the genes of interest rather than the whole genome or exome. This is important because unlike the Sanger sequencing method, NGS otherwise has no innate specificity. Target enrichment is achieved using off-the-shelf products that contain a library of pre-specified DNA fragments, which attach to the corresponding sections of predefined genes with evidence linking them to the disease of interest. These molecules are then clonally amplified, fixed to a solid surface and sequenced in parallel through complementary DNA synthesis. It is also possible to increase sequencing capacity by combining different patient samples in the same run by tagging each sample with a unique DNA "barcode" attached to a magnetic bead, which can be added or removed when required.

Bioinformatic pipelines are then used to analyse the large amount of data generated, which involves quality control of the raw sequence data before alignment of the 'reads' against a reference genome. Variants are subsequently called, annotated, filtered and interpreted for potential clinical significance.

2.11. Genetic variants and protein consequences

A genetic variant can occur anywhere in the genome and fall into three broad categories:

- Single base-pair substitution, also known as a single nucleotide polymorphism (SNP). This can be a transition (interchange of Adenine/Guanine or Cytosine/Thymine) or a transversion (interchange of a purine and pyrimidine nucleic acid), and the consequence of this change can vary significantly (discussed below).
- Insertion or deletion (Indel) of a DNA sequence 2 or more base-pairs in length.
- Structural variant occurring over a larger DNA sequence, including copy number variation and chromosomal rearrangement events (these variants are not discussed).

Point genetic variants occurring in the coding region of a gene can further categorised depending on the effect on amino acid sequence:

- Synonymous, where the change in nucleotide sequence does not change the amino acid sequence due to redundancies in the genetic code,
- Nonsense, where the change in nucleotide sequence can result in a stop codon, which will result in a truncated protein,
- Missense, where the change in nucleotide sequence results in a change in the amino acid, which may or may not alter the protein – this can be predicted using various software modelling tools, such as the Variant Effect Predictor (VEP) by Ensembl.

Of note, indels with a length divisible by 3 (given that 3 nucleotides code each amino acid), result in 'in-frame' insertions or deletions of whole amino acids, the effects of which can be predicted using the software above. Indels of lengths not divisible by 3 may result in missense, nonsense or whole frameshift variants, which often result in malformed protein that is subject to nonsense-mediated decay as a mechanism to reduce errors in gene expression.

Point variants occurring in the non-coding region of a gene (e.g. in a promotor or intron) may still exert a potentially pathogenic effect by altering gene regulation, but these variants are less well understood at present.

2.12. Challenges in the interpretation of genetic variants

NGS identifies large numbers of genetic variants per individual. However, the interpretation of potentially disease-causing variants is made challenging by the high frequency of benign variation seen within a population. A genetic variant that is not seen commonly within a population is typically described as being rare. Evolutionary theory predicts that these disease alleles should be rare due to their potentially deleterious protein consequences leading to disease states. Purifying selection tends to exceed mutation rates to keep the allele frequencies of these variants low. A minor allele frequency (MAF) of <1% is the usual definition of what constitutes rarity, although the exact frequency cut-offs vary in the literature.[139]

Rare variants tend to have large effects, due either to haplo-insufficient or gain of function alleles, where the risk of disease is elevated 2-fold or more over background. They account for typical Mendelian disease, such as hypertrophic or dilated cardiomyopathy, and show segregation within families allowing for cascade family screening. On the contrary, commonly encountered variants are thought to have small effect sizes and account for complex traits such as eye colour, and more recently, hypertension.[140] Many debates continue over the main source of genetic variation for disease. However, it is generally agreed that complex diseases are likely to occur from a number of common variants providing a background liability, with environmental and rare variants providing the extra impetus to exceed the defined threshold for disease.

To assign pathogenicity to a novel variant, two main levels of evidence are required. Firstly, there must be a discernible effect on protein structure or function. Secondly, this effect must be shown to be deleterious and cause disease, which often requires additional tissue or animal model studies. The American College of Medical Genetics and Genomics (ACMG) publishes standards and guidelines for the interpretation of sequence variants.[141] Understanding of the clinical significance of any variant falls along a spectrum, ranging from pathogenic or likely

pathogenic, to uncertain significance, likely benign or benign (table 2-1). This classification is based on various types of evidence (e.g. population, computational or segregation data).

	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very Strong**
Population Data	MAF is too high for disorder *BA1/BS1* **OR** observation in controls inconsistent with disease penetrance *BS2*			Absent in population databases *PM2*	Prevalence in affecteds statistically increased over controls *PS4*	
Computational And Predictive Data		Multiple lines of computational evidence suggest no impact on gene /gene product *BP4* Missense in gene where only truncating cause disease *BP1* Silent variant with non predicted splice impact *BP7*	Multiple lines of computational evidence support a deleterious effect on the gene /gene product *PP3*	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before *PM5* Protein length changing variant *PM4*	Same amino acid change as an established pathogenic variant *PS1*	Predicted null variant in a gene where LOF is a known mechanism of disease *PVS1*
Functional Data	Well-established functional studies show no deleterious effect *BS3*		Missense in gene with low rate of benign missense variants and path. missenses common *PP2*	Mutational hot spot or well-studied functional domain without benign variation *PM1*	Well-established functional studies show a deleterious effect *PS3*	
Segregation Data	Non-segregation with disease *BS4*		Co-segregation with disease in multiple affected family members *PP1*	Increased segregation data →		
De novo Data				*De novo* (without paternity & maternity confirmed) *PM6*	*De novo* (paternity & maternity confirmed) *PS2*	
Allelic Data		Observed in *trans* with a dominant variant *BP2* Observed in *cis* with a pathogenic variant *BP2*		For recessive disorders, detected in *trans* with a pathogenic variant *PM3*		
Other Database		Reputable source w/out shared data = benign *BP6*	Reputable source = pathogenic *PP5*			
Other Data		Found in case with an alternate cause *BP5*	Patient's phenotype or FH highly specific for gene *PP4*			

Table 2-1. ACMG framework for classifying pathogenic and benign variants by the type and strength of evidence

2.13. Population reference dataset

Understanding the significance of an individual's genetic variation would be challenging without comparison to a reference or control dataset due to the high degree of variability amongst healthy individuals. Highly pathogenic variants with a large effect should be rare and seen with a lower frequency in the general population. The Exome Aggregation Consortium (ExAC) represents an invaluable resource for the efficient filtering of candidate disease-

causing variants and their subsequent interpretation through the inclusion of sequencing data on 60,706 unrelated individuals from multiple international population genetic studies.[142]

2.14. Gene selection

Of the 174 genes linked to cardiovascular disease in the Illumina targeted panel,[143] our analysis focused on 11 DCM genes and 5 ARVC genes with robust evidence of disease association (table 2-2). This evidence was recently compiled and curated into a sem-automated, web-based decision support tool developed by other members within our group and known as CardioClassifier.[144] Whilst the ACMG rules provide a useful framework to standardise variant interpretation, they were intentionally broad to allow adoption across the full spectrum of genetic disorders. CardioClassifier provides a powerful new tool to automatically annotate variants across 17 computational criteria, each of which was individually parametrised for specific gene-disease pairs using expert disease-specific knowledge for these key genes (tabulised below). This was made possible using a number of highly curated datasets of disease cases and healthy controls.[145] As a result, variant pathogenicity is presented interactively to users to enhance interpretation of variant pathogenicity linked to cardiomyopathy.

Disease	**Genes**
DCM	LMNA, TNNT2, SCN5A, TTN, TCAP, MYH7, VCL, TPM1, TNNC1, RBM20, BAG3
ARVC	DSP, PKP2, DSG2, DSC2, JUP

Table 2-2. Details of gene-disease pairs analysed by CardioClassifier

3. EPIDEMIOLOGY OF MYOCARDITIS

Extracts from this chapter are based on my own work, which I presented at the AHA Scientific Sessions 2019 under a session entitled, 'Contemporary trends and epidemiology of heart failure:'

Lota AS, Halliday B, Tayal U, Salmi S, Shakur R, Hammersley D, Jones R, Daubeney P, Ware James S, Cleland John G, Cook Stuart A, Pennell Dudley J and Prasad Sanjay K. Abstract 11463: Epidemiological Trends and Outcomes of Acute Myocarditis in the National Health Service of England. Circulation. 2019;140:A11463-A11463.

A formal license is not required from Wolters Kluwer to reproduce these unmodified figures, also available here: https://www.ahajournals.org/doi/abs/10.1161/circ.140.suppl_1.11463.[146]

3.1. Aims and Hypotheses

The primary aim of this chapter is to evaluate the real-world burden of myocarditis on a population level to provide novel insights into disease heterogeneity, clinical outcomes, seasonal and geographical variation.

The hypotheses are as outlined:

- Acute myocarditis is an increasingly prevalent disease.
- Men are affected at a younger age compared to women.
- Disease incidence shows seasonal variation throughout the year.
- Myocarditis is predominantly a disease of towns and cities, rather than rural areas.
- Diagnostic evaluation is diverse and varies by region.
- Morbidity and mortality are underestimated.

3.2. Background

Epidemiology is the study of the distribution and factors that determine the presence and absence of disease. Accurate epidemiological data on acute myocarditis are lacking due to heterogeneity in clinical presentation, aetiology, diagnostic standards and geographical variation. Many historical estimates are based on the finding of myocarditis on post-mortem examination. Published studies investigating imaging and biomarker measures linked to patient outcomes (discussed in the introduction) are subject to bias amongst recruited individuals and provide a narrow window into possible outcomes over the short-term. As a result, these small groups of recruited study participants represent the 'tip of an iceberg.' Few studies have investigated the true clinical burden of myocarditis on a population level, and there is no epidemiological data on myocarditis within the UK.

POST-MORTEM STUDIES

Myocarditis has been implicated as the cause for 3% to 12% of sudden cardiac deaths on post-mortem examination. In an unselected national registry of 377,841 post-mortem studies in Japan, 434 patients (0.11% or 1:1000) were found to have myocarditis.[147] Amongst those that experience a sudden cardiac death, the prevalence of myocarditis was 3%.[4] In a series of 10,199 post-mortem studies from Sydney, when focusing on the 193 (1.9%) sudden deaths in people aged <35 years, myocarditis was found in 12% of cases and represented the fourth most common pathological finding as the potential cause of sudden death.[5] The most common cause was a structurally normal heart likely with a primary arrhythmogenic disorder (31%), followed by coronary artery disease (24%) and hypertrophic cardiomyopathy (15%). In the UK, investigators critically appraised Office of National Statistics data for causes of death in patients aged 1-34 years from 2002-2005.[148] There were 419 SCD events on average each year, which equated to 1.8 per 100,000 of the population under 35 years. This translated to 8 deaths each week in England and Wales due to SCD in individuals aged 1-34 years. The most prevalent causes were coronary artery disease (34%), cardiomyopathies (27%), sudden arrhythmic death syndrome (14%), myocarditis (11%), valvular heart disease (5%) and hypertensive cardiomyopathy (2%). Based on our extrapolation from this data, we estimate that myocarditis accounts for one sudden cardiac death each week in an individual aged <35 years of age in England and Wales.

NATIONAL STUDIES

In contrast to estimates of fatal myocarditis, the epidemiology of symptomatic myocarditis remains largely unknown. Following the documentation of SCD amongst young Finnish military conscripts in 1976, a national registry was established to investigate potential cardiac causes.[149] Acute myocarditis was diagnosed on the basis of chest pain with typical ECG changes and concurrent troponin elevation in 98 individuals out of 672,672 Finnish military

conscripts (14.6 cases per 100,000) from 1977-1996, who were all men with mean age 20 years (range 17-29 years). This heralded an incidence of 0.17 per 1000 patient years.[150] Over the follow-up period, there was one SCD due to myocarditis out of a total of 10 sudden deaths (10%; incidence 0.02 per 1000 patient years), consistent with other post-mortem studies. Finland offers publicly-funded healthcare for most of its residents and hospital admission data is stored centrally in a similar manner to the NHS. As a result, there have been a number of nationwide studies in other cardiovascular diseases, for example, acute MI and pericarditis.[151, 152] From this group, there was one study investigating childhood myocarditis, which reported 213 admissions for myocarditis out of 882,253 paediatric admissions (24.1 cases per 100,000) from 2004-2014.[153]

WHO GLOBAL BURDEN OF DISEASE STUDY

In 2013, the WHO conducted a systematic review of all available data in acute myocarditis as recognised cause for premature death in the young across 188 countries from 1990 to 2013. A writing committee manually reviewed all publications and hospital dismissal databases, where available, relating to myocarditis.[154] It was acknowledged that the definition of myocarditis used in published studies varied widely with many specifying the need for endocardial biopsy, surgical heart specimens or autopsy. However, the writing committee included all available studies reflective of real-world practice and recognising the imperfect distinction between acute myocarditis and acute dilated cardiomyopathy.

The overall global incidence of myocarditis was estimated at 22 cases per 100,000 of the world's population in 2013. During this window, there was a 53% increase from 961,000 cases in 1990 to 1,481,000 cases in 2013. Myocarditis was included as one of 65 causes for acute disease and injury with more than 1 million cases per year in 2013. Whilst this estimate provided a useful benchmark, it did not include; (i) myocarditis cases managed in the

community, (ii) cases presenting with fatal arrhythmia or (iii) cases misdiagnosed as idiopathic dilated cardiomyopathy.

The WHO study also highlighted clear geographical variation. For example, hepatitis C appeared to be an important cause of myocarditis in Japan.[155] In Australasia, there were clear associations of myocarditis with CVB and enterovirus epidemics.[156, 157] In certain regions of North America and Western Europe, Lyme disease was prevalent.[158] The rate of myocarditis as cause of heart failure also showed some variation by region, likely related to such underlying aetiological difference. A study of 18 African men in Nairobi with idiopathic DCM showed that 9 patients (50%) had evidence of healing myocarditis on EMB.[159] Similar studies from Mexico and Chile reported prevalence of histological myocarditis in idiopathic DCM of 13% and 65% respectively.[160, 161] Whereas in North America, the incidence of histological myocarditis in cases of idiopathic DCM was estimated at 9.6%.[162] No studies to date have looked at geographical variation within a country.

There is pressing unmet need to evaluate the true clinical burden of suspected acute myocarditis resulting in hospital admission within the NHS to inform healthcare service provision, but also direct future clinical standards and national guidelines, particularly with the advent of high-sensitivity troponin, and research directions.

3.3. Methodology

DATA SOURCE

NHS Digital is the national provider of information, data and IT systems for commissioners, analysts and clinicians in health and social care. As highlighted in the recent NHS Longterm Plan, there is a strong need to improve the use of digital technology throughout the NHS.

Hospital Episode Statistics (HES) represent one of the longstanding services provided by NHS digital, formerly known as the Health and Social Care Information Centre (HSCIC). This data has been accrued since 1997 from all NHS Clinical Commissioning Groups (CCGs) in England for all admitted patients as part of the Commissioning Data Set (CDS). In summary, administrative and clinical information collected locally by healthcare providers is submitted to the Secondary Uses Service (SUS), which makes it available to commissioners for Payment by Results (PbR), but also sends the information to HES (figure 3-1). The HES data quality team validate and clean data extracts with an extensive processing cycle to avoid duplication. This annual database is then stored in the HES data warehouse within NHS Digital. Hospital coders use the WHO International Classification of Diseases (ICD) to categorise diagnostic information, which is typically divided into a primary diagnosis (main problem treated) and various secondary diagnoses (including comorbidities and complications).

The Office of National Statistics (ONS) was formed in 1966 and represents a well-established non-ministerial department responsible for the collection of national population demographics, including all births, marriages and deaths.

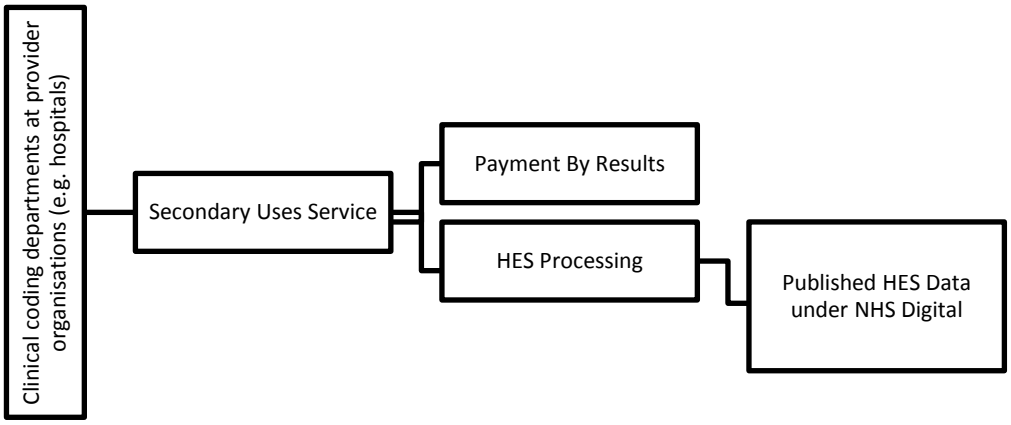


Figure 3-1. Summary diagram of the HES processing cycle and data quality (reproduced from NHS Digital) [163]

DATA APPLICATION REQUEST

In order to access HES and ONS data, a data access request service (DARS) was submitted through the NHS Digital online portal. In this application, we provided scientific justification for exactly what information was required and a clear explanation of the intended outcomes and benefits to patients.

We sought to obtain all available data on hospital admissions specifically due to 'myocarditis' (I40, I41 & I51.4) in all age groups across England in recent years. In accordance with data minimisation principles, we requested 24% (n=270) of the available data fields within the HES admitted patient care (APC) dataset and applied an upper age cut-off of 80 years of age. Data on age, sex, ethnicity, length of admission, method of admission and specific cardiac procedures (including cardiac catheterisation, endomyocardial biopsy, pacemaker/implantable cardioverter defibrillator (ICD)/ ventricular assist device (VAD) implantation and cardiac transplantation) were requested Data on readmission was requested to understand predictors of disease recurrence, and ultimately, linkage to mortality data with cause of death to provide new insights into clinical outcomes in a real-world setting. Geographical data was requested to understand whether social deprivation or pollution levels are implicated.

Following favourable review by the Independent Group Advising on the Release of Data (IGARD), the following requested HES data fields were provided (in some cases, fields may be duplicated or be replaced with updated titles in subsequent years of data collection):

Patient demographics and admission data:
ACTIVAGE Age at activity date,
ADMIAGE Age on admission,
ADMIDATE Date of admission,
ADMIMETH Method of admission,
ADMISORC Source of admission,
AEKEY Record identifier,
BEDYEAR Bed days within the year,

DISDATE Date of discharge,
DISDEST Destination on discharge,
DISMETH Method of discharge,
DOB_CFL Date of birth check flag - patient,
ENDAGE Age at end of episode,
EPIDUR Episode duration,
EPIEND Date episode ended,
EPIKEY Record identifier,
EPIORDER Episode order,
EPISTART Date episode started,
EPISTAT Episode status,
EPITYPE Episode type,
ETHNOS Ethnic category,
ETHRAW Ethnic character (audit version),
ETHRAWL Ethnic category (audit version),
FAE Finished Admission Episode,
FAE_EMERGENCY Finished Admission Episode, emergency classification,
FCE Finished Consultant Episode,
FCEFLAG Finished consultant episode flag,
FDE Finished In-Year Discharge Episode,
FYEAR Financial Year,
MYDOB Date of Birth - month and year,
PARTYEAR Year and month of data,
SEX Sex of patient,
SPELBGIN Beginning of spell,
SPELDUR Duration of spell,
SPELEND End of spell,
STARTAGE Age at start of episode,
STARTAGE_CALC Age of patients at start of episode, babies restated,

Geographical data:
CCG_TREATMENT CCG of Treatment,
CURRWARD Current electoral ward,
CURRWARD_ONS Current electoral ward (ONS),
GORTREAT Government office region of treatment,
GPPRACHA Health Authority area where patient's GP is registered,
GPPRACRO Regional Office area where patient's GP was registered,
GPPRPCT Primary Care Trust area where patient's GP was registered,
GPPRSTHA Strategic Health Authority area where patient's GP was registered,
HATREAT Health Authority of treatment,
PCTNHS Primary care trust of responsibility - NHS,
POSTDIST Postcode district of patient's residence,

PROCODE3 Provider code - 3 character,
PROCODE5 Provider code - 5 character,
PROCODET Provider code,
PROTYPE Provider type,
PROVSPNOPS Pseudonymised hospital provider spell number,
PURCODE Commissioner code,
PURRO Commissioner's Regional Office,
RESCTY County of residence,
RESCTY_ONS County of residence (ONS),
RESGOR Government office region of residence,
RESGOR_ONS Government office region of residence (ONS),
RESPCT Patient's Primary Care Trust of residence,
RESPCT_HIS The primary care trust of residence - mapped according to source year,
RESPCT02 Patient's Primary Care Trust of residence - historic,
RESSTHA Patient's Strategic Health Authority of residence,
RESSTHA02 Patient's Strategic Health Authority of residence - historic,
ROTREAT Region of treatment,
RURURB_IND Rural/Urban Indicator,
SITETRET Site code of treatment,
STHATRET Strategic Health Authority area of treatment,

Augmented care data (ITU):
ACPDISP_N Augmented care period disposal,
ACPEND_N Augmented care period end date,
ACPLOC_N Augmented care location,
ACPN_N Augmented care period number,
ACPOUT_N Augmented care period outcome indicator,
ACPSEQ ACP sequence number,
ACPSOUR_N Augmented care period source,
ACPSPEF_N Augmented care period speciality function code,
ACPSTAR_N Augmented care period start date,
ACSCFLAG Ambulatory Care Sensitive Condition Flag,
DEPDAYS_N High-dependency care level,
INTDAYS_N Intensive care level days,
NUMACP Number of augmented care periods within episode,
ORGSUP_N Number of organ systems supported,

Diagnoses:
ALCDIAG Principal alcohol related diagnosis,
ALCDIAG_4 4 character concatenated alcohol related diagnosis,
ALCFRAC Principal alcohol related fraction,

CAUSE Cause code,
CAUSE_3 Cause code - 3 characters,
CAUSE_4 Cause code - 4 characters,
CHAPTER Primary diagnosis chapter,
DIAG_COUNT Count of diagnoses,
DIAG_NN All Diagnosis codes,
MAINSPEF Main specialty,
WARDSTRT Ward type at start of episode

Procedures:
DOMPROC Trust derived dominant procedure,
OPDATE_NN Date of operation,
OPERSTAT Operation status code,
OPERTN_COUNT Total number of procedures per episode,
OPERTN_NN Primary Operative Procedure Codes,
TRETSPEF Treatment specialty,

Social indicators:
IMD04 IMD Index of Multiple Deprivation,
IMD04_DECILE IMD Decile Group,
IMD04C IMD Crime Domain,
IMD04ED IMD Education Training and Skills Domain,
IMD04EM IMD Employment Deprivation Domain,
IMD04HD IMD Health and Disability Domain,
IMD04HS IMD Barriers to Housing and Service Domain,
IMD04I IMD Income Domain,
IMD04IA IMD Income affecting Adults Domain,
IMD04IC IMD Income affecting Children Domain,
IMD04LE IMD Living Environment Domain,
IMD04RK IMD Overall Rank,

Patient Identifier:
ENCRYPTED_HESID Encrypted HESID,
HESID_ORIG Patient ID - HES generated (original).

To facilitate accurate comparisons with other conditions of overlapping biology (pericarditis), clinical presentation (myocardial infarction) and better understanding of long-term complications (sudden cardiac arrest, heart failure and dilated cardiomyopathy), we requested the same depth of HES and mortality linked data on these linked diagnoses:

I40 Acute myocarditis
I41 Myocarditis in diseases classified elsewhere
I51.4 Myocarditis, unspecified
I30 Acute pericarditis
I32 Pericarditis in diseases classified elsewhere
I42.0 Dilated cardiomyopathy
I50 Heart failure
I46 Cardiac arrest
I21 Acute myocardial infarction

We opted to receive record level data rather than aggregated data in order to allow us to accurately study events and outcomes for individual patients, rather than simple demographics averaged over groups of five patients. This added complexity to our application with regards to the potential identification of an individual from their age, postcode (first 3 characters only) and admitting hospital, but was approved by the IGARD committee.

In order to link HES and ONS data, each patient was given a unique 32-character HES ID, known only to NHS digital. Therefore, all data received by us was non-identifiable. As a result, the study was not deemed to require ethical approval as determined by the online Health Research Authority (HRA) decision tool.

INFORMATION GOVERNANCE

Information Governance is a framework through which NHS organisations are accountable for continually improving the quality of their services and safeguarding high standards of care (Department of Health 1998). The appropriate use and handling of clinical information forms one of the seven key areas. In order to meet information governance eligibility criteria, the Trust was required update its Information Governance Toolkit (IGT) score, which is an online system that allows organisations to assess themselves against information governance policies and standards. A data sharing agreement between NHS digital, ourselves and the Trust's Caldicott Guardian (Dr Jan Lukas Robertus) was signed prior to the release of data via a secure online portal. A letter of support from the Trust's Medical Director was also included to support our application (see appendix).

IMPACT OF GDPR

Following the initial submission of our application in November 2017, the EU General Data Protection Regulation (GDPR) was implemented on 25th May 2018. Superseding the Data Protection Directive 95/46/EC, the new regulation provided a range of measures to give individuals control over their personal data. As a result, our application was placed on hold whilst NHS Digital reviewed and updated its internal policies. We were required to provide further information on the lawful basis of our request to receive personal information, despite being non-identifiable.

DEFINITIONS OF EPISODES, SPELLS AND SUPER SPELLS

A 'hospital provider spell' refers to the total continuous stay of a patient using a hospital bed on premises controlled by a single health care provider. This is typically labelled as an admission. During this time, a patient may be under the care of one of more consultants – each

of these periods refers to a 'finished consultant episode' (FCE), which represent the basic counting unit on the HES database. FCE's are aggregated into a spell to represent a hospital admission. This is essential for the Payment by Results system, but also for epidemiological understanding of the patient's journey. However, an additional layer of data manipulation is required as a patient may be physically transferred to another health care provider for ongoing care. Given that the admission source for the second spell was another health care provider, these two spells are linked and referred to as a 'super spell' to more accurately describe the overall hospital admission. Counting both spells as individual admissions would lead to overestimation of the total number of admissions.

GENERAL DATA MANIPULATION AND FILTERING

Data on all cardiovascular FCEs from 1998-2017 were received from NHS Digital following a 15-month application, data minimisation and review process. The data was transferred via the Secure Electronic File Transfer (SEFT) portal, which provided a secure wrapper around all data content files. The files were received as TXT files delineated by the character | with one line per patient. Each annual HES data file contained over 1.2 million FCEs, which could only be opened with specialised packages such as Microsoft SPSS and R studio. A recoding algorithm was written by myself and applied to the 'diagnosis_1 column' to select appropriate FCEs from the list of all ICD diagnoses (e.g. I40 for acute myocarditis). Additional FCEs were also included with a secondary diagnosis (diagnosis_2 column) of acute myocarditis if diagnosis_1 was completed with an ICD code beginning with the letter R to denote a symptom (e.g. R07 = chest pain). FCE's were filtered and aggregated according to the episode order ('EPIORDER') to create spells for each patient identified by their unique HES ID. Spells with invalid length of stay (DISDATE < ADMIDATE) were excluded. Spells ending in transfer to another NHS hospital (by 'DISDEST' or 'ADMISORC') were linked together into super spells.

DATA ANALYSIS

Once spells and super spells were appropriately aggregated for the relevant conditions, cases were taken forward for further analyses by myself in SPSS. These included the assessment of basic demographics, length of hospital admission, seasonal variation, geographical location, operation codes and mortality status.

3.4. Results

OVERALL TRENDS

Across all age groups, there were 12,927 admissions reported with a primary diagnosis of acute myocarditis in NHS Trusts across England between 1998-2017. Over this 19-year period, there was an 88% increase in myocarditis admissions compared to a 57% increase in cardiology admissions in general (figure 3-2).

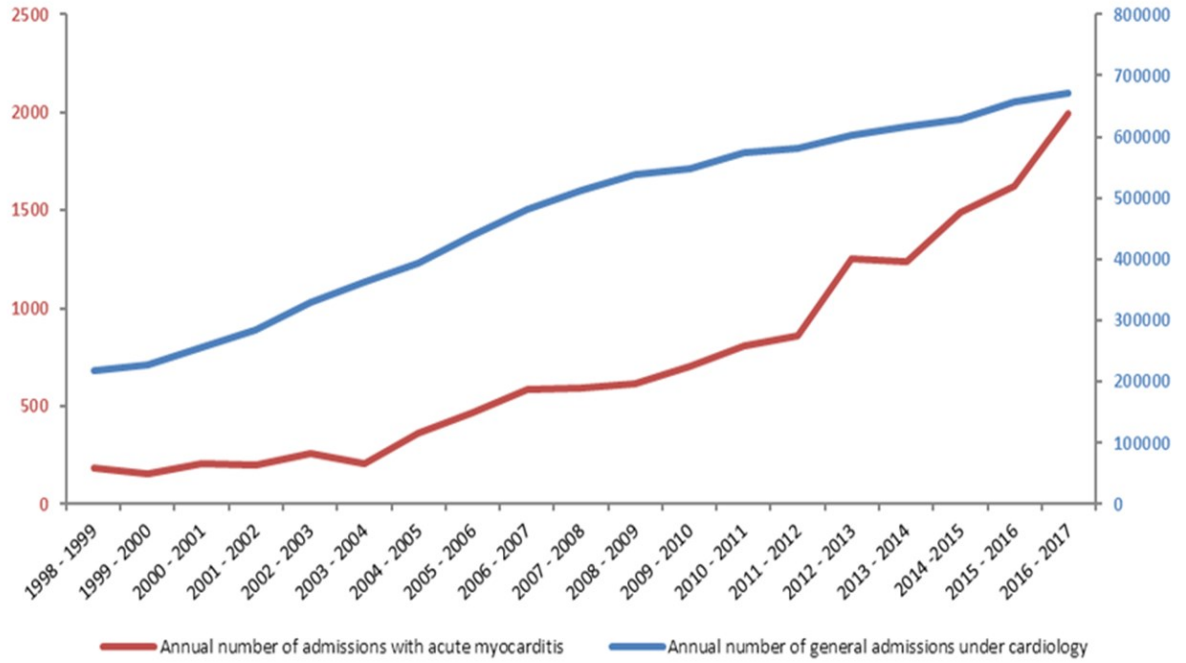


Figure 3-2. Annual number of hospital admissions with a primary diagnosis of acute myocarditis in NHS England from 1998-2017 compared to the overall number of admissions under cardiology as a specialty.

The number of admissions attributed to myocarditis as the secondary diagnosis or more broadly, anywhere in the list of diagnostic codes leading to admission, was far greater. For example, in the most recent complete year of data (2016-2017), the number of admissions with a primary, secondary or 'any' diagnosis of myocarditis was 1103, 1344 and 2146, respectively.

INCIDENCE

The total number of acute admissions in the 2016-2017 for all primary diagnostic codes throughout NHS England was 5,883,234. There was a near exponential increase in the number of admissions with increasing age, likely reflective of the UK's aging population demographic (figure 3-3). Of these admissions, 541,330 were due to a primary diagnostic code relating to diseases of the circulatory system. In total, myocarditis diagnoses accounted for 0.04% of all admissions (36.5 per 100,000), or 0.39% of admissions relating to the circulatory system.

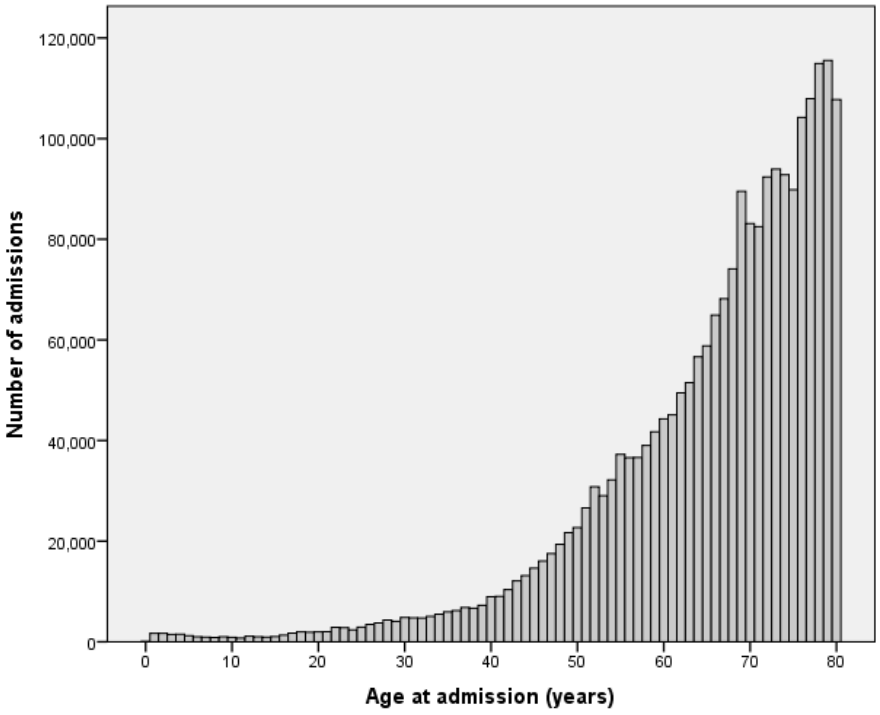


Figure 3-3. Plot to show all hospital admissions (n=5,883,234) in NHS England in the 2016-2017 year. Median age at admission was 69 years.

The population of England in 2016-2017 from published ONS data was 55,268,000, of which 2,673,400 was aged over 80 years. Therefore, the incidence rate of myocarditis admissions amongst the general population under 80 years was 4.1 cases per 100,000 of the general population. Of note, this did not include the speculated large number of myocarditis cases managed in primary care or the emergency department without admission to hospital.

TRENDS BY MAIN DIAGNOSIS

Acute myocardial infarction represented the most common primary diagnostic code across all circulatory admissions. In 2016-2017, there were 75,200 admissions for acute MI. The number of acute MI cases increased steadily over the 19-year period at 2-3% per year. Heart failure represented the second most common diagnosis with 73,411 admissions in 2016-2017. However, heart failure has consistently shown higher growth rates of approximately 5% per annum and may exceed the number of acute MI cases per year in following years (figure 3-4).

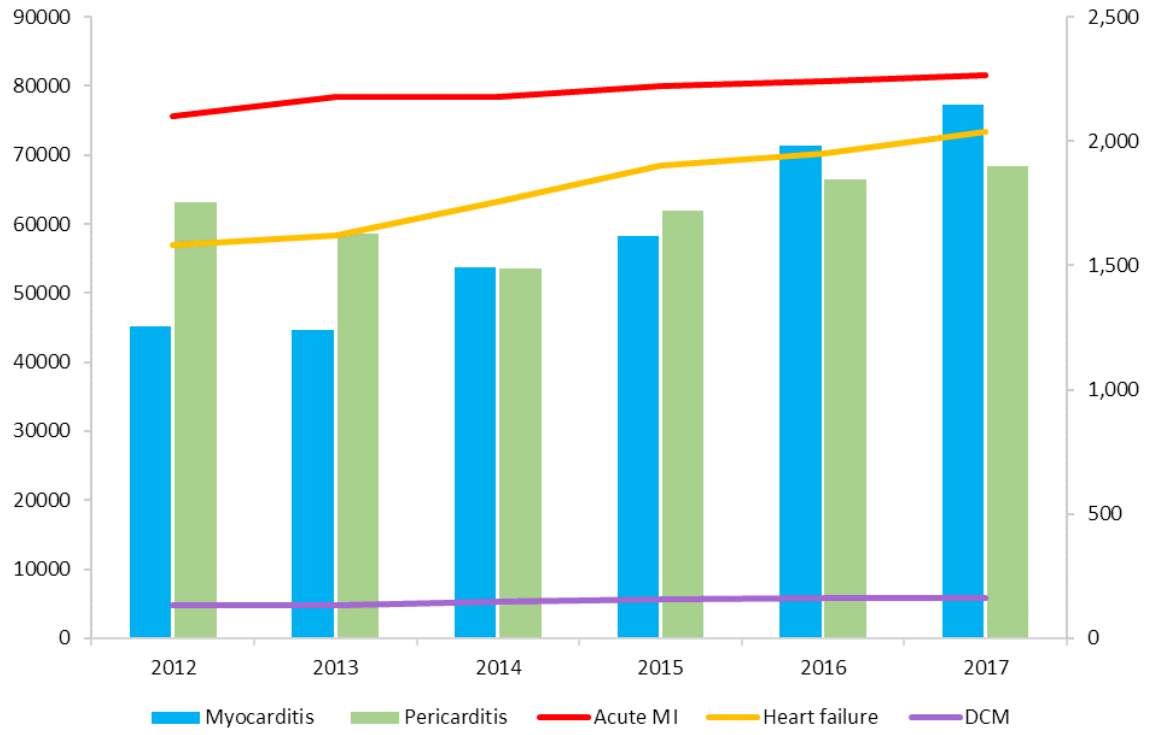


Figure 3-4. Focused analysis on the annual number of hospital admissions due to myocarditis (blue bars) compared primarily to pericarditis (green bars) over the last 5 years. The number of admissions due to acute MI (red line), heart failure (yellow) and DCM (purple) are also shown for comparison.

In contrast, admissions due to a primary diagnosis of myocarditis have risen sharply over the study period, particularly over the last 5 years with the highest annual growth rates of 20% recorded in 2013-2014 and 22% in 2015-2016 (figure 3-5). As a result, there was a transition

point in 2014 when myocarditis admissions became more common than pericarditis admissions for the first time in England.

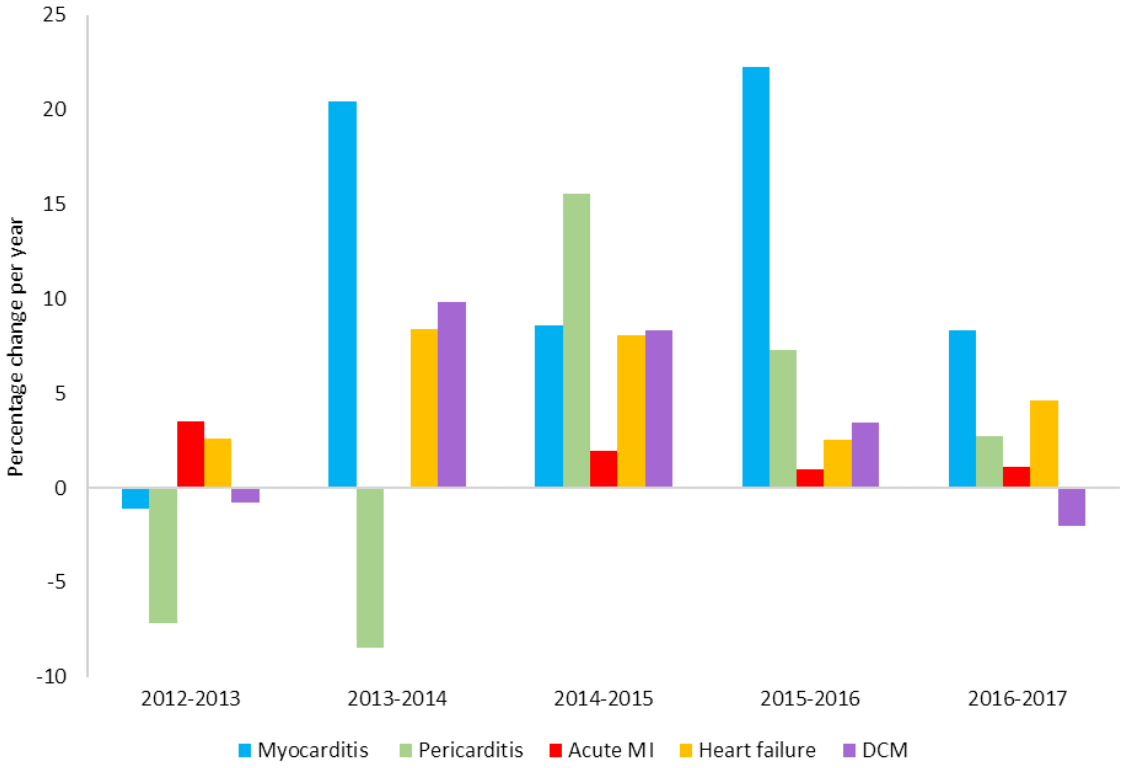


Figure 3-5. Relative percentage change in annual admissions by primary diagnosis in England from 2012-2017

AGE DISTRIBUTION

The median age of all myocarditis patients was 36 years (IQR 25–52 years). There was a small peak in infancy and a linear decline from young adulthood into later life (figure 3-6). Of note, there were very few children between 5-14 years of age with myocarditis.

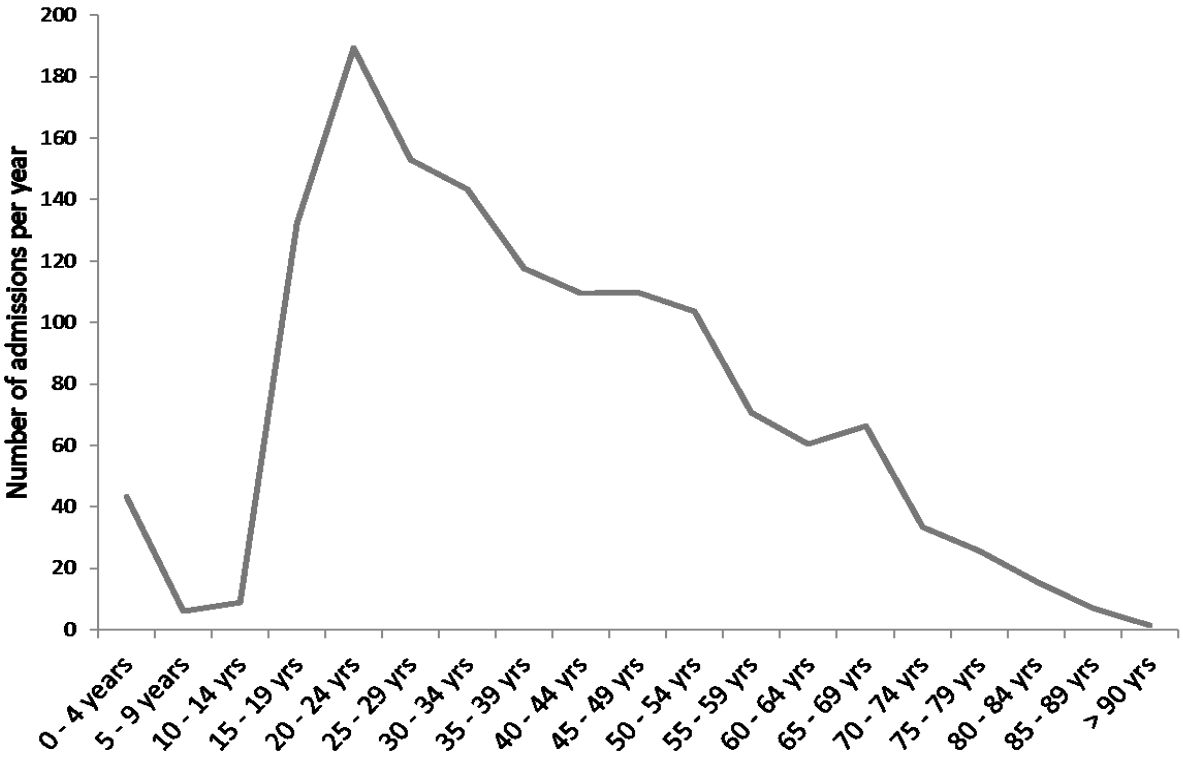


Figure 3-6. Age distribution of hospital admissions with a primary diagnosis of acute myocarditis in England from 1998-2017

LENGTH OF HOSPITAL ADMISSION

The median duration of hospital admission across all age groups was 4.2 days (IQR 3.5 - 4.9 days). This had decreased steadily over the 19-year study period (figure 3-7).

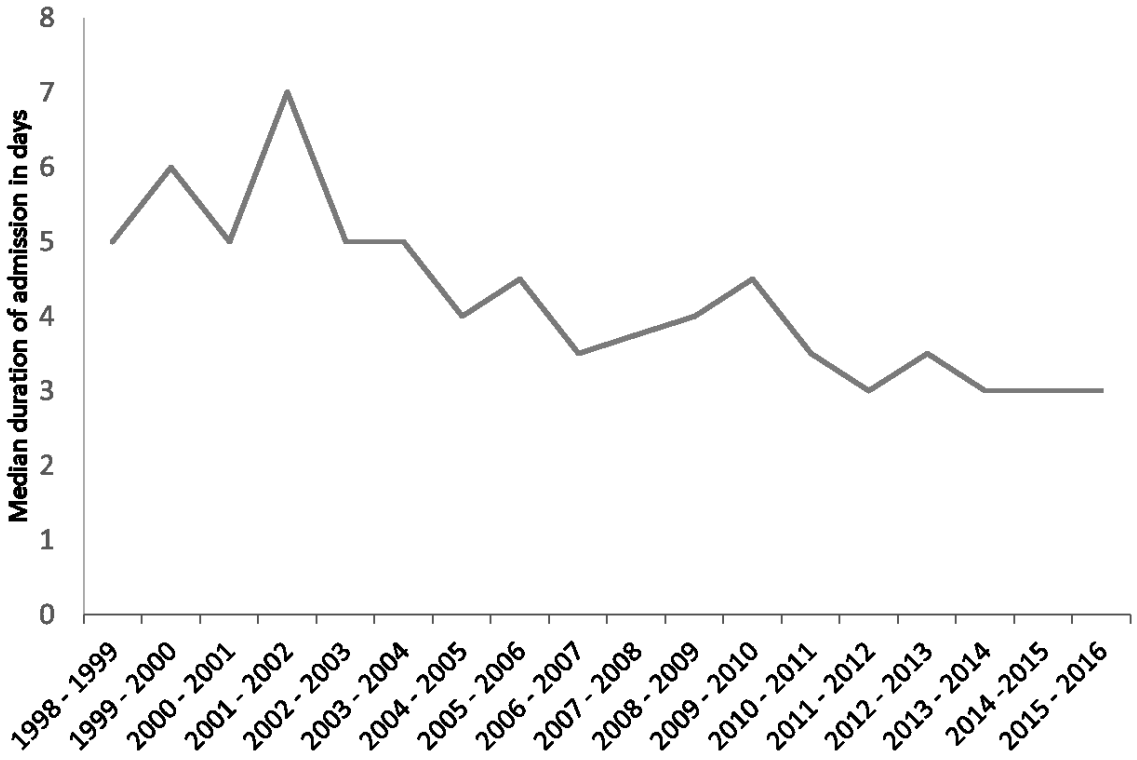


Figure 3-7. Median duration of hospital admission with a primary diagnosis of acute myocarditis in England

There was no signification linear correlation between age at admission and duration of hospital admission across all age groups (figure 3-8; Spearman correlation coefficient of 0.019, p=ns).

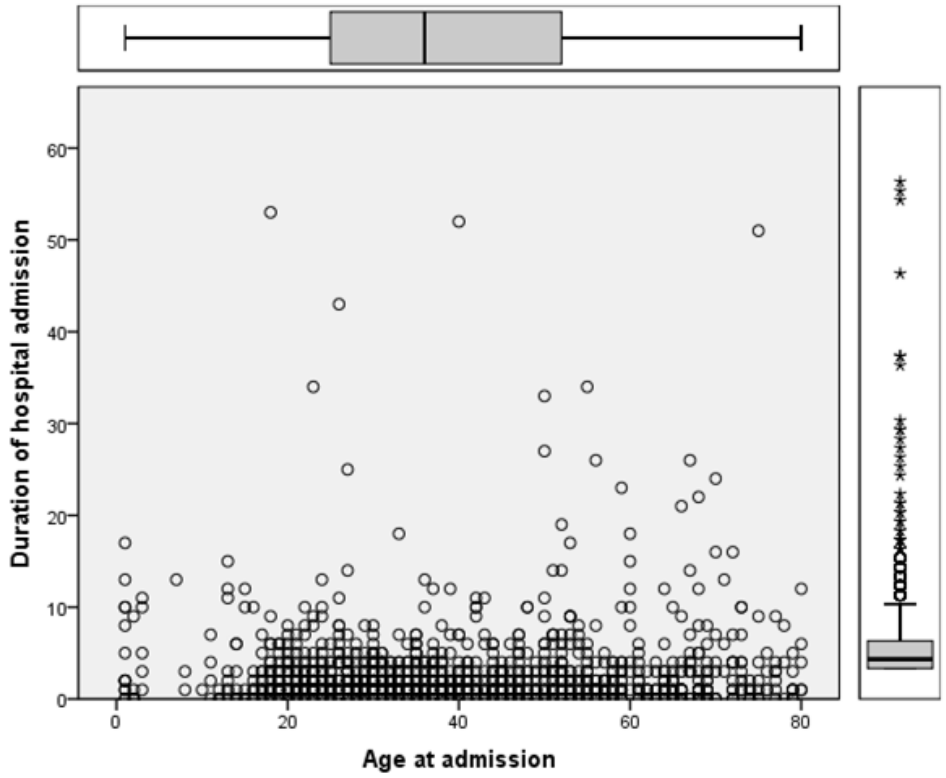


Figure 3-8. Plot to show correlation between duration of hospital admission with a primary or secondary diagnosis of acute myocarditis and age at admission for 2016-2017 data (n=1344). Box and whisker plots are also shown for the variables.

SEX DIFFERENCES

Admission ages of men and women for the 2016-2017 year were compared using the Mann-Whitney test for two independent samples with a non-normal distribution. This showed that men (median age 33 years; IQR 23-47 years) were significantly younger than women (median age 46 years; IQR 32-61 years) on admission to hospital (figure 3-9; Z score -9.633; $p<0.001$).

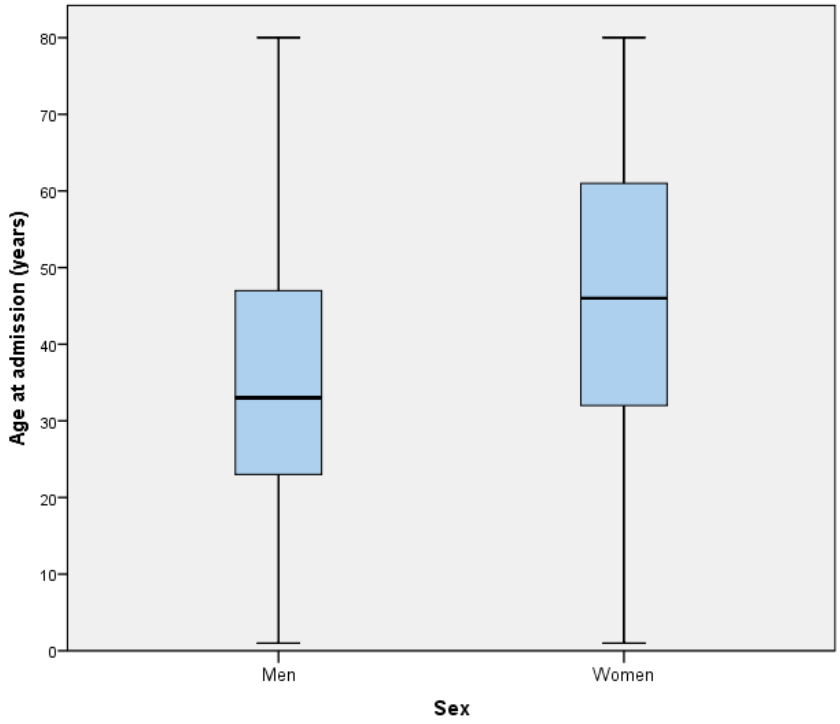


Figure 3-9. Gender differences: box and whiskers plot to show age at admission for men and women.

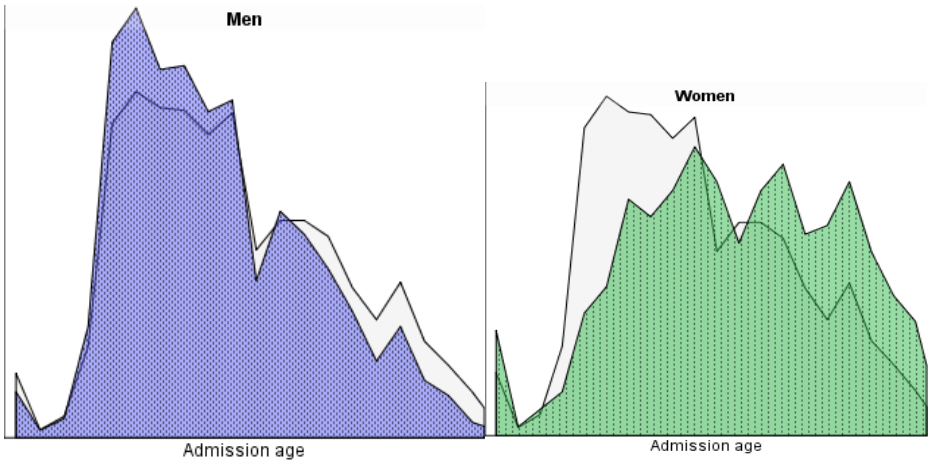


Figure 3-10. Plot to show age distribution of myocarditis admissions for men (blue) and women(green) against the overall mean (dark line – same in both plots).

SEASONAL VARIATION

Hospital admissions due to a primary diagnosis of myocarditis were more common in the Winter months than the Summer months. The greatest number of admissions occurred in the months of March and November each year (figure 3-11).

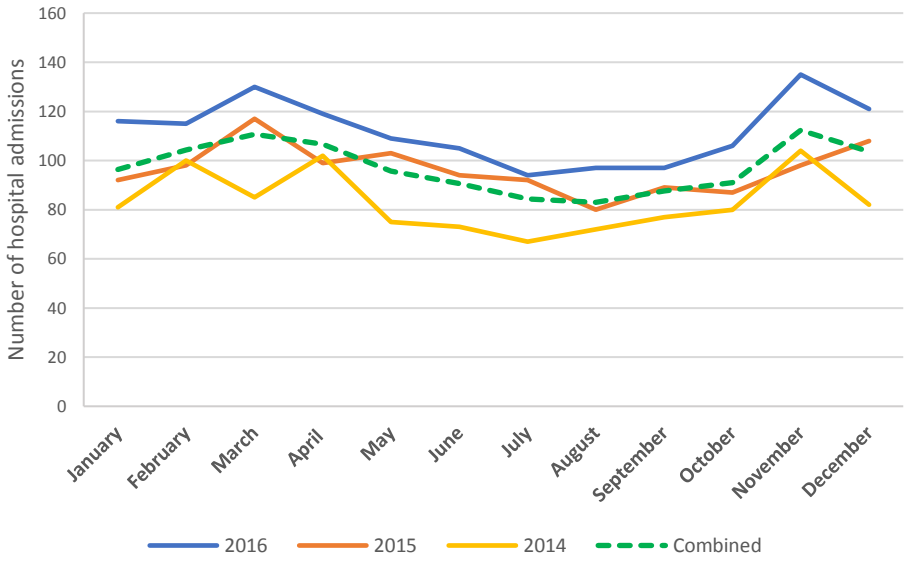


Figure 3-11. Representative line graph showing numbers of myocarditis admissions per month each year

This bimodal distribution was seen for both men and women, reinforcing the observation that men were twice as likely to be admitted with myocarditis (figure 3-12).

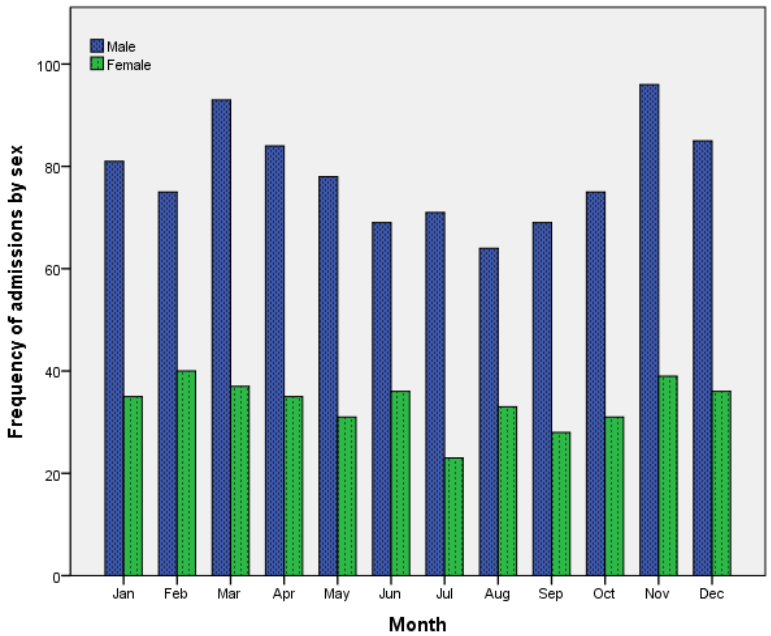


Figure 3-12. Bar graph showing myocarditis admissions per month for men and women from 1997-2016.

GEOGRAPHICAL VARIATION

The number of admissions with a primary diagnosis of myocarditis was compared across the 9 regions defined as the highest tier of sub-national division in England. These 9 regions had statutory delegation of powers from the central government from 1994 to 2011 and continue to be used for administrative purposes.

The greatest number of admissions in 2016-2017 due to a primary diagnosis of myocarditis was recorded in London and the South East (figure 3-11). Whilst these regions also showed the largest regional populations based on the Office of National Statistics, there was still a relative excess in the number of myocarditis cases seen (figure 3-14). For example, 27% of the total number of annual myocarditis admissions occurred in London although the population of London only accounted for only 16% of the total population of England. Conversely, 5% of myocarditis admissions occurred in East Midlands, which accounted for 9% of the total population of England, suggesting a lower prevalence of myocarditis admissions than one would expect for the regional population size.

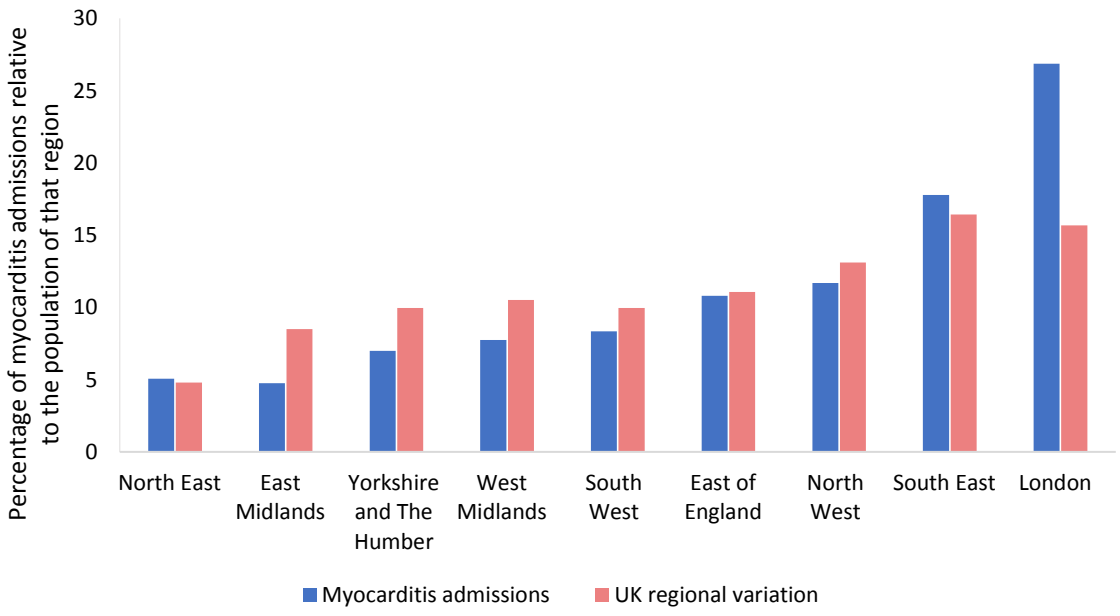
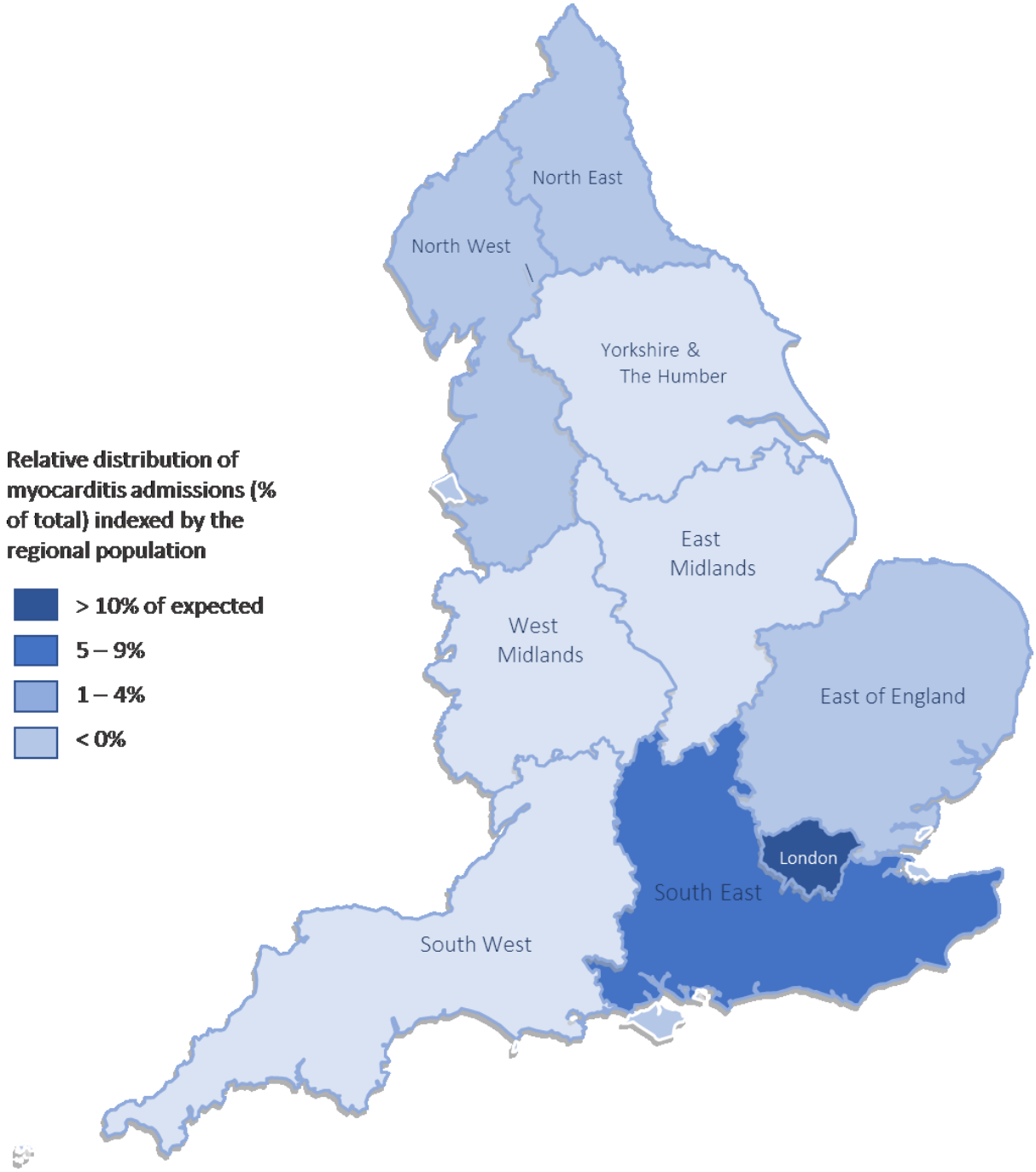


Figure 3-13. Bar chart showing absolute (blue bars) percentage of myocarditis admissions and regional percentage of England's total population (red bars) across the 9 geographical regions in 2016-2017.

Figure 3-14. Heatmap of England showing the burden and distribution of admissions with a primary diagnosis of acute myocarditis relative to the regional population in 2016-2017.



PROCEDURAL CODES

For patients admitted with a primary diagnosis of acute myocarditis in 2016-2017, OPCS-4 procedural codes were extracted and counted (Office of Population Censuses and Surveys Classification of Surgical Operations and Procedures). In this year, a total of 948 invasive coronary angiograms were performed. There were 602 transthoracic echocardiograms, 178 CMR scans and 202 CT scans. Overall, there were 20 left or right ventricular percutaneous biopsy procedures.

OPCS-4 Code	Description	Count
Non-invasive imaging		
U10.2	Cardiac computed tomography angiography	23
U10.3	Cardiac magnetic resonance imaging	178
U20.1	Transthoracic echocardiography	602
U20.2	Transoesophageal echocardiography	6
U35.4	Computed tomography of pulmonary arteries	54
Y97.3	Radiology with post contrast	125
Coronary angiography		
K63.1	Angiocardiography of combination of right and left side of heart	6
K63.3	Angiocardiography of left side of heart NEC	73
K63.4	Coronary arteriography using two catheters	183
K63.5	Coronary arteriography using single catheter	16
K63.6	Coronary arteriography NEC	48
Y53.4	Approach to organ under fluoroscopic control	319
Y98.1	Radiology of one body area (or < 20 minutes)	303
Cardiac Biopsy		
K23.2	Biopsy of lesion of wall of heart	5
K58.2	Percutaneous transluminal right ventricular biopsy	5
K58.4	Percutaneous transluminal left ventricular biopsy	10

Table 3-1. Detailed summary of OPCS-4 procedural codes for hospital admissions with a primary diagnosis of myocarditis in England in 2016-2017.

MORTALITY

Of 1344 admissions due to myocarditis from 1st April 2016 to 31st March 2017, a total of 56 deaths were recorded through ONS, giving an all-cause mortality of 4.16%. Of these, 32 were cardiovascular deaths (57%) and 3 were unknown.

Nine deaths were attributed to acute myocarditis as a primary or secondary cause of death (16%), of which three were specifically due to a cardiac arrest and two due to cardiogenic shock. One additional cardiac arrest was coded as being unspecified but further review of the index admission confirmed acute myocarditis. For the patients with SCD due to myocarditis, the median age was 35 years (IQR 24-48 years) and 2 out of the 4 patients were men. The oldest patient (55years) spent 34 days in hospital at the time of index presentation with myocarditis and had an implantable loop recorder. The other three were admitted for two days each and underwent transthoracic echocardiography (U20.1) and basic investigations such as ECG and chest radiography.

Twelve deaths were attributed to non-ischaemic DCM or heart failure (21%). Of these, one death occurred due to complications following transplantation of a whole organ. Upon further review of the index admissions, a code of T86.2 was recorded, indicating 'heart transplant failure and rejection.' There was one child aged 2 years with DCM as the cause of death following an index presentation with myocarditis. Otherwise, compared with the SCD group, the patients with DCM/HF tended to be older (median age 48 years; IQR 33-64 years; p=0.14) and had longer hospital admissions (median length 5.5 days; IQR 3.3-7.3; P=0.19).

A complete list of the primary and secondary causes of death for all 56 patients is listed below (table 3-2). Further survival analyses are ongoing across the entire dataset of 20 years.

ONS ID	ICD code for primary cause of death		ICD code for secondary cause of death	
1256525	K720	Acute hepatitis	K720	Acute hepatic failure
904813	K720	Acute hepatitis	I839	DVT
26420	I219	Acute MI	R688	Ischaemia heart disease
80886	I219	Acute MI	R688	Ischaemia heart disease
140235	I259	Acute MI	I259	Ischaemia heart disease
322296	I251	Acute MI	I259	Ischaemia heart disease
819523	I514	Acute myocarditis	I514	Acute myocarditis
891362	I514	Acute myocarditis	I514	Acute myocarditis
492369	I519	Acute myocarditis	J988	Respiratory disease
628989	G931	Anoxic brain damage	I059	Mitral valve disease
20568	G931	Anoxic brain damage	I490	Cardiac arrest, unspecified
13275	I350	Aortic stenosis	I350	Aortic stenosis
359675	J180	Bronchopneumonia	I514	Acute myocarditis
622437	J180	Bronchopneumonia	I429	DCM
1383295	J690	Bronchopneumonia, aspiration	E119	Diabetes Mellitus
620330	J690	Bronchopneumonia, aspiration	G35	Multiple sclerosis
9916	I499	Cardiac arrest	I514	Acute myocarditis
629266	I499	Cardiac arrest	I514	Acute myocarditis
1327869	I469	Cardiac arrest	C901	Malignant neoplasm
165408	I499	Cardiac arrest	I499	Acute myocarditis
616203	R570	Cardiogenic shock	I409	Acute myocarditis
6668	R570	Cardiogenic shock	I429	DCM
1072998	R570	Cardiogenic shock	A090	Septicaemia
14495	I420	DCM	I420	DCM
22644	I420	DCM	I420	DCM
267365	S099	Head injury	W130	Fall from, out of or though building or structure
120783	I501	Heart failure	I251	Acute MI
210457	I509	Heart failure	I259	Acute MI
780813	I509	Heart failure	I251	Acute MI
114218	I509	Heart failure	I514	Acute myocarditis
66729	I500	Heart failure	I420	DCM
1164938	I500	Heart failure	I429	DCM
1443592	I509	Heart failure	A169	Septicaemia
111455	C159	Malignant neoplasm	C159	Malignant neoplasm
121376	C920	Malignant neoplasm	C920	Malignant neoplasm
161319	C349	Malignant neoplasm	C349	Malignant neoplasm
184309	C349	Malignant neoplasm	C349	Malignant neoplasm
1482779	C439	Malignant neoplasm	C439	Malignant neoplasm
550688	C509	Malignant neoplasm	C509	Malignant neoplasm
313020	R688	Other specified general symptoms and signs	Y830	Complication following transplantation of whole organ
880507	R688	Other specified general symptoms and signs	I132	Hypertensive heart disease
636258	R688	Other specified general symptoms and signs	I330	Infective endocarditis
876372	R688	Other specified general symptoms and signs	J108	Influenza
5669	R688	Other specified general symptoms and signs	C920	Malignant neoplasm
204448	R688	Other specified general symptoms and signs	C819	Malignant neoplasm
11430	I269	Pulmonary embolism	I802	DVT
45570	I269	Pulmonary embolism	C169	Malignant neoplasm
258325	I269	Pulmonary embolism	M349	Systemic sclerosis
1443335	J969	Respiratory failure	Y830	Complication following transplantation of whole organ
935940	A418	Septicaemia	I420	DCM
78519	A419	Septicaemia	A419	Septicaemia
1248506	B49	Unspecified mycosis	D686	Haematological disorder
5484	T819	Unspecified procedural complication	I251	Acute MI
4579		Not available		
5613		Not available		
709365		Not available		

Table 3-2. Summary of downstream ONS causes of death for patients admitted with a primary diagnosis of acute myocarditis in England in 2016-2017.

3.5. Discussion

Acute myocarditis is a heterogenous condition that accounts for an increasingly prevalent burden of acute hospitalisation, particularly amongst young men. Amongst the 12,927 admissions with a primary diagnosis of acute myocarditis from 1998-2017, there were large variations in clinical diagnosis with limited use of CMR and EMB, and significant regional variation in admission rates. Annual incidence based on the most recent complete year of data was estimated at 36.5 cases per 100,000 admissions, or 4.1 cases per 100,000 of the general population of England. Given the risk of SCD and progression to DCM, there is pressing need to further explore and harness such high-quality, longitudinal national data to guide the development of national clinical standards in acute myocarditis.

RISING INCIDENCE OF MYOCARDITIS

The annual number of patients admitted with acute myocarditis has rapidly increased in recent years. In contrast to background population growth of 0.79% in England from 2004-2014 (from Office of National Statistics) and the relatively slow growth of 2-3% in the number of admissions due to acute MI and HF, acute myocarditis has shown multiple year-on-year increases of 20% in recent years. This unprecedented rise in the number of admissions due to myocarditis is likely to be multifactorial.

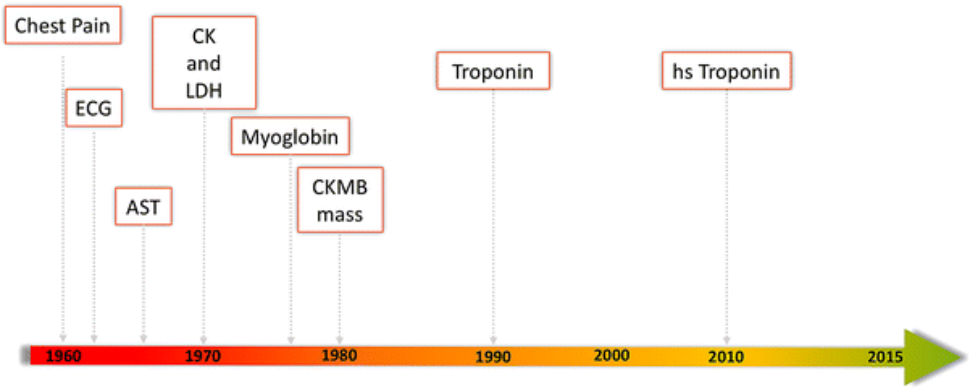


Figure 3-15. Timeline of development of cardiac biomarkers for the diagnosis of acute myocardial infarction.[164]

Cardiac troponin assays were introduced into clinical practice from the mid-1990s and rapidly became the gold-standard biomarker for detecting acute myocardial necrosis, the pathological hallmark of acute MI (figure 3-15).[165] Whereas previous biomarkers, such as CK and myoglobin, lacked specificity and also sensitivity, routine assessment of cardiac troponin became part of standard clinical practice to rule-in or rule-out an acute MI.[166] In this way, many more young individuals were potentially identified with possible myocarditis, rather than being labelled as having musculoskeletal chest pain or pericarditis.

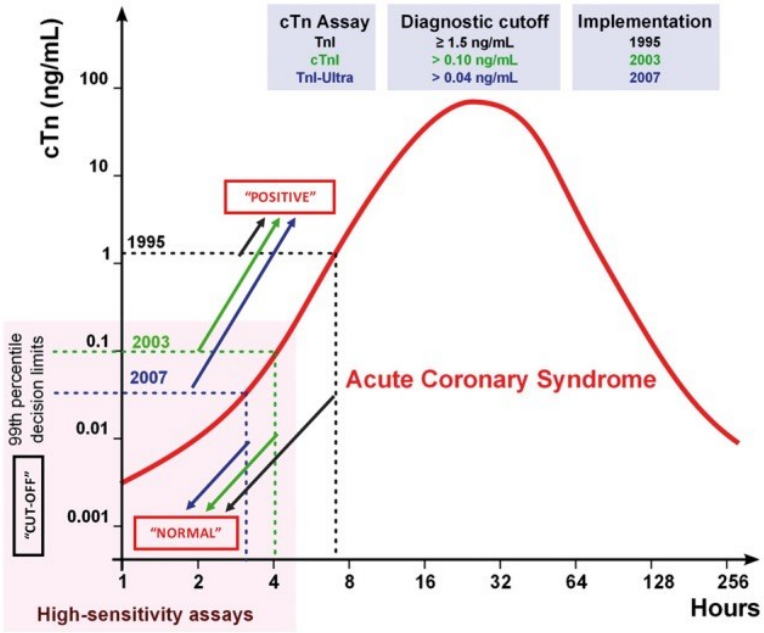


Figure 3-16. The changing 99th percentile diagnostic cut-offs for historical and present troponin assays. Any value which falls outside this decision limit indicates a 'positive' result. (Reproduced with permission)[167]

Diagnostic performance was further enhanced with the introduction of high-sensitivity troponin assays in 2009, which facilitated even greater sensitivity and precision, particularly in patients with more recent onset chest pain (figure 3-16).[168] Many novel rapid rule-in and rule-out diagnostic algorithms have since been evaluated to enable clinicians in emergency

departments to effectively identify patients with much smaller extents of myocardial injury, and these strategies have undoubtedly improved detection of myocarditis.[169] Future biomarkers under development offer even greater sensitivity and kinetics, for example, the 99th centile concentration of cardiac myosin-binding protein C may be exceeded by necrosis of 15 nanograms of myocardium. [170]

The introduction of CMR was also likely to be a key factor in improving the detection of myocarditis. Previous work from our group in 2007 demonstrated the presence of acute myocarditis on CMR by Lake Louise Criteria in 50% of patients with troponin-positive chest pain but unobstructed coronary arteries.[82] Many other CMR based studies subsequently confirmed this finding.[171-173] The visual impact of in-vivo assessment of myocardial oedema and fibrosis in the mid-wall and sub-epicardial layers is likely to have contributed to renewed interest in this condition, as well as the growing recognition of its role in SCD and HF progression.

Aside from better detection and awareness, the underlying incidence of myocarditis may have increased in recent years. Myocarditis aetiologies are diverse. In addition to standard anthracycline-cased chemotherapy with known cardiotoxicity, many new anticancer therapies have emerged, such as immune check-point inhibitors, which have been strongly linked to autoimmune myocarditis.[40, 41] Traditional anti-psychotic agents, particularly clozapine, are also increasingly prescribed with rising patient numbers but uncertain safety profiles of newer agents.[174]

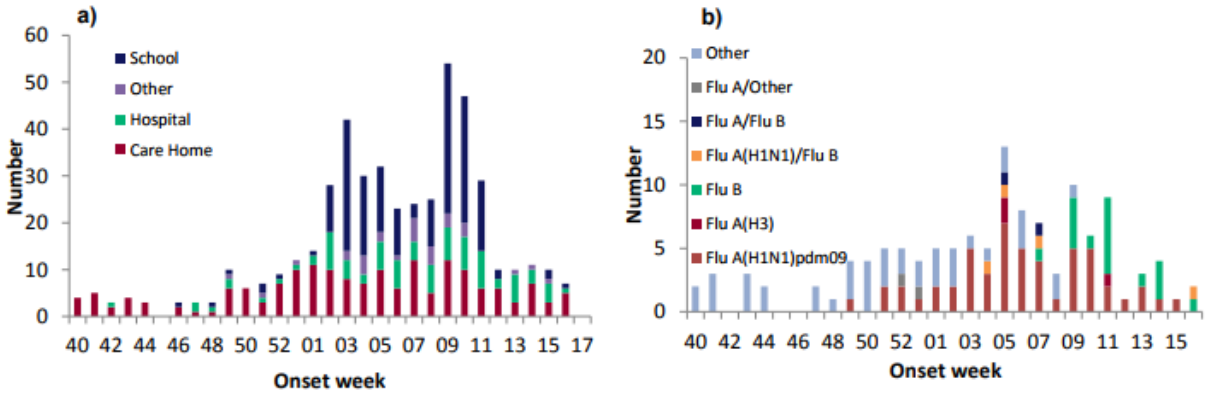


Figure 3-17. Weekly number of influenza outbreaks by (a) institution type and (b) virological test results where available in 2015-2016 in the UK (reproduced from .

Viral myocarditis is the predominant aetiology and annual fluctuations seen in admissions may be related to influenza activity. Public Health England publishes annual reports on influenza surveillance. Peak admission rates of influenza to hospital were higher in 2016-2017 than in the last 5 years.[175] The 2015-2016 outbreak was particularly significant with a large burden of influenza A(H1N1)pdm09, predominantly seen in young adults. This outbreak matched the spike seen in our data for the number of myocarditis cases in 2015-2016. To address this, PHE re-introduced a live attenuated influenza vaccine programme for primary school children from 2017-2018, which will be assessed next year.

EFFECTS OF AGE AND GENDER

Myocarditis was approximately twice as common in men than women in adults across England, and men were approximately 15 years younger than women at first presentation. It is well recognised that men are generally twice as likely to have myocarditis as women. The reasons for this are complex and likely relate to the effect of sex hormones. In murine models, male subjects had increased coxsackie B3–induced myocarditis compared to females due to key

differences in the underlying innate immune response, rather than increased viral replication.[176, 177] In addition, treatment of female mice with exogenous testosterone increased inflammation due to myocarditis within the heart,[178] whereas gonadectomy of male mice had the opposite effect.[179] Mechanistically, male mice had a greater Th-1 response, characterised by the release of pro-inflammatory cytokines such as interferon gamma, whereas female mice had greater protective Th-2 responses with increased B cells and anti-inflammatory interleukin-10.[180, 181] Accordingly, a recent CMR based study showed that young men with myocarditis had greater extents of myocardial inflammation and fibrosis than women.[182]

The later age of presentation of women with myocarditis has only been reported once before in a nationwide registry of 3198 adult patients with myocarditis in Finland.[66] In this study, the mean age of men was 34±15 years compared to 49±18 years for women, which closely matched our findings from a much larger cohort of 12,927 patients. Speculatively, this may arise from the reduction in cardio-protective oestrogen levels in later life, analogous to that seen in patients with Takotsubo cardiomyopathy.[183] However, as highlighted in the latest expert consensus statement, many questions remain regarding the female preponderance of Takotsubo cardiomyopathy.[184]

PAEDIATRIC MYOCARDITIS

We did not investigate gender differences amongst the small number of children (aged 5-14 years) admitted with myocarditis. Definitive diagnosis of myocarditis is challenging in adults and likely to be vastly underestimated in children due to non-specificity of symptoms. In a Finnish study of 213 paediatric patients admitted with myocarditis from 2004-2014, there were no sex differences during the first 6 years of life.[153] However, boys aged 6-10 years (incidence risk ratio 2.46) had significantly higher risk than girls, which increased further in boys aged 11-15 years (incidence risk ratio 3.5). These findings support the idea that rising levels of testosterone may be relevant to pathogenicity.

SEASONAL VARIATION

The seasonal variation seen in our dataset matches that documented with the seasonal influenza virus as discussed above. Peak admissions occurred in November and March. This was also found to be the case in the epidemiological study of 213 paediatric patients with acute myocarditis in Finland.[153] Confirmation of such variability may help guide clinical suspicion at different times of the year.

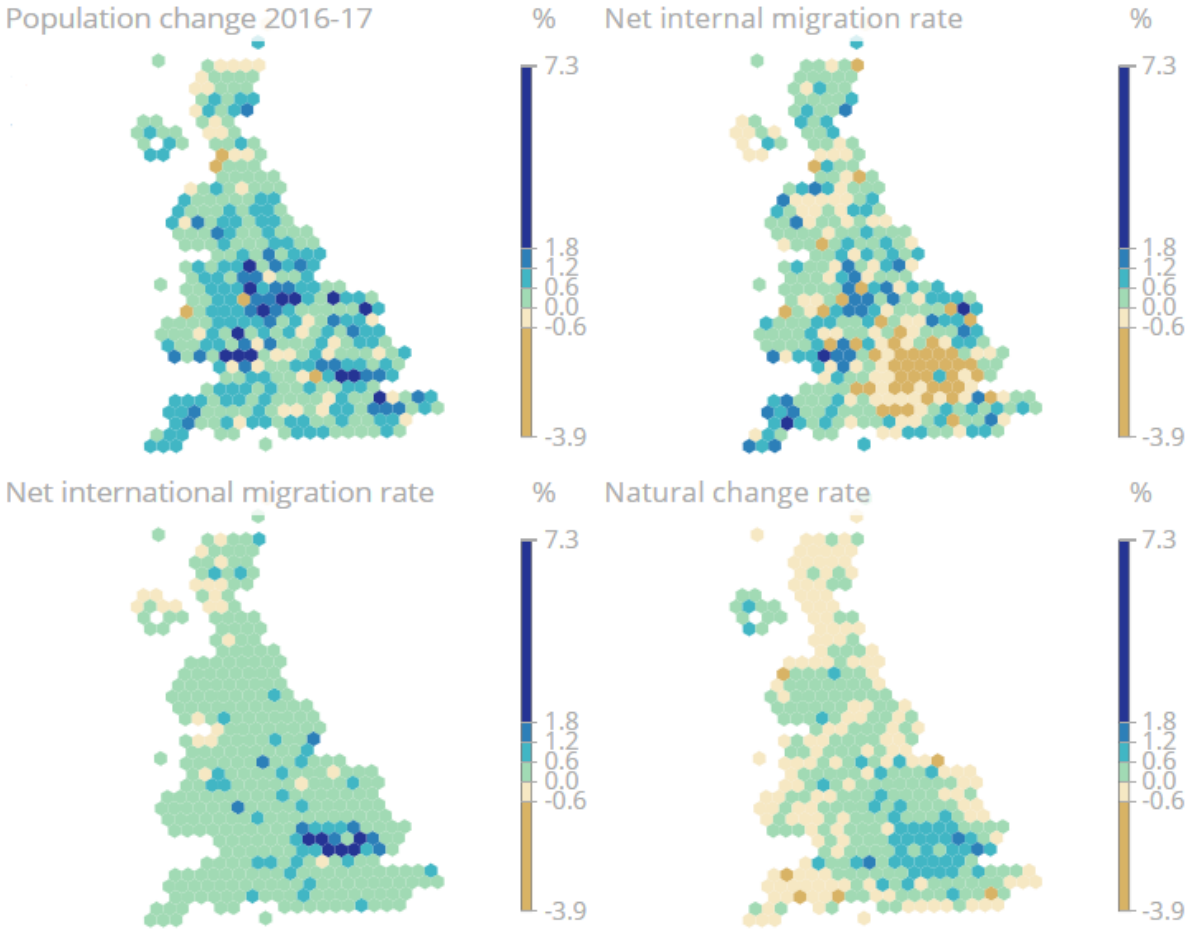


Figure 3-18. UK map of population change for 2016 and main components for change by local authority (reproduced from ONS).

GEOGRAPHICAL VARIATION

The greatest incidence of myocarditis was seen in London and the South East. This finding is novel and likely to be complex and multifactorial. London is the fastest growing English region (1.19% growth in 2016-2017) with changes driven by a combination of international migration, internal migration, births and deaths (figure 3-18). Similarly high rates of growth were also seen in the North East and North West regions of the country. Within these regions of the country, population density is the greatest and such urbanisation has had well-documented effect on the spread of infectious disease since the Industrial revolution.[185] Similarly, patterns and spread of seasonal influenza epidemics have been well mapped to urban areas with highest population densities.[186] However, in addition to the spread to infectious agents, there may also be disparities in access to healthcare among more rural settings.[187] Since the introduction of CMR, there has an exponential rise in the number of scans performed each year. In a survey conducted for the British Society of cardiovascular magnetic resonance, there was an increase of over 85% from 20,597 scans in 2008 and 38,485 in 2010.[188] Twelve high-volume centres performed 66% of all CMR scans nationally, with a strong bias towards London and other main cities. Other lifestyle factors may also contribute. Myocarditis can result from exposure to drugs and toxins, which may be more common in major cities. Similarly, the effects of air pollution are currently under scrutiny. Air pollution has been associated with cardiovascular admissions and mortality.[189, 190] Exact mechanisms remain unknown but recent findings have suggested a direct effect of air pollutants on promoting alveolar oxidative stress, systemic inflammation and endothelial dysfunction in humans.[191]

ROLE OF ENDOMYOCARDIAL BIOPSY

Endomyocardial biopsy is perceived as the gold standard diagnostic test for acute myocarditis according to an ESC consensus statement paper. EMB allows characterisation of the inflammatory infiltrate and assessment of viral load, which may be relevant to ongoing treatment, although the prognostic impact of EMB is yet to be shown.

EMB has several key limitations; (i) peri-procedural risks that include cardiac tamponade (0.7%) and death (0.4%), (ii) high rates of sampling error,[192] and (iii) high levels of inter-observer variability in histopathological interpretation.[75] Despite advances in immunohistochemical characterisation, sampling error continues to result in many false-negative results, reported in up to 55% of cases.[74] This arises from the focal nature of inflammatory infiltrates and involvement of regions inaccessible to the bioptome, for example, within the left heart when accessing the venous system.

Our findings have confirmed that EMB is rarely used as a diagnostic test for myocarditis based on reported data in England. We found that only 20 out of 1344 patients (1.5%) in 2016-2017 underwent a biopsy procedure. This general pragmatic reluctance is anecdotally shared in North America, where the Lake Louise Criteria for myocarditis diagnosis by CMR have recently been updated, incorporating the latest evidence-based T1 and T2 mapping techniques.[89] One of the main challenges in the field is detemining the need, approach and optimal timing for endomyocardial biopsy, recognising that whilst the acquistion of myocardial tissue is helpful within a research setting, it may not always be appropriate for clnical patients with pseudo-infarct presentation in whom there is a greater likelihood of spontaneous recovery.

NEED FOR A NATIONAL AUDIT IN MYOCARDITIS

Parallels can be drawn from the national heart failure and acute MI audits. The Myocardial Ischaemia National Audit Project (MINAP) is a national clinical registry of patients admitted to hospital with acute coronary syndromes. It was established in 1998 in response to the requirements of the National Service Framework for coronary heart disease to improve patient outcomes.[193] Prior to this, it was known that thrombolysis times and resulting patient outcomes were widely variable across the county.[194, 195] By facilitating the prospective collection of detailed care quality and clinical outcome data, MINAP provided a mechanism for hospitals' performance to be benchmarked against national standards. The National Heart Failure Audit was established in 2007 and has similarly transformed our understanding of the trends in symptoms, causes, comorbidities, assessment, place of care, length of stay, treatments and outcomes in HF.[196] For example, it has been shown that patients treated on a cardiology ward rather than a general medical ward were more likely to have greater lengths of stay (median of 9 vs 4 days respectively), but this was also associated with better outcomes likely due to higher rates of implementation of disease modifying therapies and stabilisation prior to discharge. Such insights into patterns and trends in acute MI and HF are lacking in myocarditis patients.

3.6. Future Work

Within the rich dataset received from NHS Digital, there are further opportunities to explore the trends in demographics, diagnostics, interventions and patient outcomes. Additional metrics such as 30-day re-admission rates and real-world risk estimates for SCD and progression to DCM in patient years will form part of our subsequent analyses going forward.

Further studies to enrich the HES data are also planned, drawing upon primary care database (CPRD) to access information on medication usage, including medication type and duration. The advantage of HES was national coverage and longevity, whereas CPRD provides greater granularity but only covers primary care surgeries using a specific IT platform. Data from NHS Scotland is also being explored to provide further understanding of disease heterogeneity.

Given the number of potential data points within each annual data-set from the last 20 years of data collection, we are also exploring machine learning approaches to maximise the outputs from this rich resource beyond our conventional epidemiological approaches.[197]

3.7. Limitations

One of the main limitations inherent to all studies using HES data is the accuracy of clinical data coding. The accuracy of HES data is central to the process of payment-by-results and mortality data is linked directly from the Office of National Statistics. It forms the core dataset from which many metrics and quality indicators within the NHS are derived from. However, poor documentation in medical notes can affect quality, particularly regarding comorbidities.[198] There is also a general lack of clinical engagement between clinical coders and clinicians, which may impact on the quality of data captured.[199] In comparison, existing national registries for acute MI and heart failure rely on the completion of specific data collection tools by the relevant clinical teams, rather than data entered by clinical coders. The accuracy and depth of information is likely to be greater and provides more granularity, but this approach is limited by incomplete coverage and shorter longevity. Indeed, both registries rely on HES data to gauge the level of ascertainment of patient inclusion. For example, in the 2016-2017 year data was provided on 70,086 HF admissions in England out of 81,759 actual HF admissions under HES, indicating case ascertainment of 86%. Therefore, for an epidemiological study looking at historical trends, the HES dataset is preferable, but prospectively a national registry would be advantageous, particular to assess treatment responses. Data on ethnicity is available within the HES dataset and further work is planned to investigate this.

4. CLINICAL OUTCOMES AND OBSERVATIONS IN MYOCARDITIS

Extracts from this chapter are based on my own work which has been published:

Lota AS, Halliday BP and Vassiliou VS. Iatrogenic myocarditis-biomarkers, cardiovascular MRI and the need for early diagnosis. Oxf Med Case Reports. 2018;2018:omx096.[38]

Prasad SK, Lota AS. Improving Risk Stratification by Cardiac Magnetic Resonance Imaging in Heart Failure: Is Strain the Missing Link? JACC Cardiovasc Imaging. 2018 11(10):1430-1432.[200]

Halliday BP, Lota AS, Prasad SK. Sudden death risk markers for patients with left ventricular ejection fractions greater than 40%. Trends Cardiovasc Med. 2018 28(8):516-521.[201]

Baxan N, Papanikolaou A, Salles-Crawley I, Lota A, Chowdhury R, Dubois O, Branca J, Hasham MG, Rosenthal N, Prasad SK, Zhao L, Harding SE and Sattler S. Characterization of acute TLR-7 agonist-induced hemorrhagic myocarditis in mice by multiparametric quantitative cardiac magnetic resonance imaging. Dis Model Mech. 2019;12.[202]

Lota A, Baksi J, Tsao A, Mouy F, Wassall R, Halliday B, Tayal U, Izgi C, Alpendurada F, Nyktari E, Wage R, Gatehouse P, Kilner P, Mohiaddin R, Firmin D, Ware J, Cleland J, Cook S, Pennell D and Prasad S. Cardiovascular Magnetic Resonance in Survivors of Sudden Cardiac Arrest: 14 Year Experience from a Tertiary Referral Centre in the United Kingdom. JACC. 2017;69:491-491.[203]

As the author of these Elsevier articles, I retain the right to include them in a thesis or dissertation, provided this is not published commercially and appropriately referenced

4.1. Aims and Hypotheses

The aims of this chapter are to evaluate the clinical outcomes of patients with myocarditis recruited into a multi-centre prospective cohort study. We investigate the use of both established as well as novel imaging sequences to evaluate the growing role of CMR in myocarditis and provide insights into disease progression and patient risk stratification.

The hypotheses are as outlined:

- Deep phenotyping in acute myocarditis by harnessing recent advances in CMR multiparametric mapping and strain assessment may provide in-depth understanding into disease progression and assessment of treatment response.
- Following index presentation with myocarditis, patients at risk of progression to dilated cardiomyopathy may be identified by subclinical markers of disease characterised by:
    - Persistently elevated T1 & T2 values, as measured by multiparametric mapping.
    - Impaired strain, as measured by DENSE.

4.2. Background

Myocarditis accounts for 12% of all sudden cardiac deaths with at least one death per week of an individual aged <35 years in the UK.[148, 204] Spontaneous recovery of left ventricular (LV) function occurs in two thirds of patients but progressive left ventricular dilatation occurs in the remainder.[6] Dilated cardiomyopathy (DCM) is the second most common cause of heart failure and the leading indication for heart transplantation. Whilst much progress has been made in the non-invasive diagnostic evaluation of suspected acute myocarditis, there is limited understanding of additional mechanistic markers and predictors that identify subsets of high-risk individuals in whom further monitoring and medical therapy are key. Precision medicine

using advanced imaging, genomics and bioinformatics is changing healthcare and may provide new mechanistic and therapeutic insights.[205]

Current diagnostic evaluation of acute myocarditis consists of electrocardiography, echocardiography and cardiac troponin but these investigations have limited sensitivity and/or specificity.[68-70] Endomyocardial biopsy is perceived as the gold standard diagnostic test in current European Society of Cardiology (ESC) guidelines.[1] However, peri-procedural risks, including cardiac tamponade (0.7%) and death (0.4%), combined with sampling errors from insufficient biopsy sites[74] and high inter-observer variability in histopathological interpretation[75] have resulted in wide variation in clinical practice owing to a general pragmatic reluctance. As a result, cardiac biopsy is rarely performed in the UK outside of a cardiac transplant setting, as discussed in the epidemiology chapter.

Continued advances in technology allow three important opportunities; cardiovascular magnetic resonance for detailed in-vivo tissue characterisation, circulating biomarkers hold promise in improving diagnosis, surveillance and possible therapeutic targets, whilst genomic data may improve understanding of the 'at-risk' patient and mechanistic insights into disease development both for acquiring myocarditis and the subsequent development of a DCM phenotype.

CARDIOVASCULAR MAGNETIC RESONANCE

CMR has emerged as an important diagnostic tool for assessing the distribution, nature and severity of myocarditis including; (i) interstitial oedema, (ii) hyperaemia and inflammatory infiltration and (iii) myocyte necrosis and replacement fibrosis.[28] These features form the CMR Lake Louise Criteria, first described in 2009, with a diagnostic accuracy of 78% (sensitivity 67%, specificity 91%) when at least 2 out of 3 are present.[17] Numerous studies have sought to evaluate the diagnostic and prognostic utility of these criteria acute myocarditis. Most recently,

in a study of 627 patients with suspected myocarditis, it was shown that the presence of LGE was associated with a more than doubling risk of MACE (hazard ratio 2.22; 95% CI 1.47-3.35; p<0.001), which remained significant after adjustment for LVEF and other clinical variables.[206]

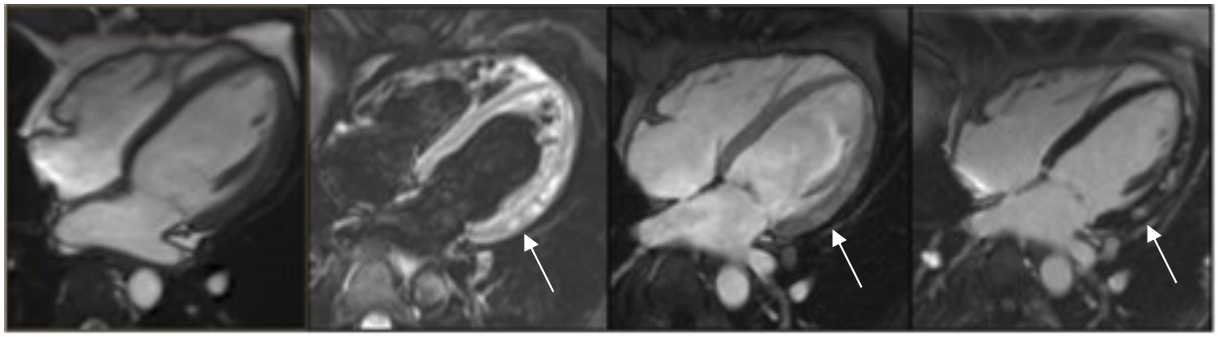


Figure 4-1. Cardiac MRI demonstrating Lake Louise features in acute myocarditis from one of our study participants. Images from left to right; 4 chamber cine, T2-STIR showing myocardial oedema, early gadolinium enhancement showing hyperaemia and late gadolinium enhancement showing focal myocyte necrosis localised in the lateral wall (arrow).

PARAMETRIC (T1/T2) MAPPING

Beyond conventional T2-STIR and LGE imaging, the application of quantitative parametric mapping techniques, such as T1 and T2 mapping, in patients with biopsy-proven myocarditis further increased diagnostic accuracy to 82% and 81% respectively.[83, 207] T1/T2 mapping are CMR techniques that construct a 'parametric map' in which the intensity of each voxel equals the output of an independent calculation incorporating data from a series of different input images for each pixel.[208] Unlike traditional grey-scale images, the value of each pixel (in milliseconds) on the map directly reflects the calculated T1 recovery or T2 relaxation time for that physical region of the myocardium, without the need for gadolinium contrast administration. These techniques allow regional or global quantitative assessment in a range of cardiac diseases.[209]

In myocarditis patients, T1 and T2 mapping have revealed myocardial injury not seen on conventional imaging sequences [84] and offered a means to potentially distinguish between acute and convalescent disease.[85] Numerous studies have evaluated their incremental utility in improving diagnostic accuracy.[210] As a result, the Lake Louise Criteria were formally revised in 2018, providing a diagnostic algorithm that incorporated T1 and T2 mapping for the first time.[89] However, as indicated in an ESC consensus statement, there is an important need for further research into T1 and T2 mapping techniques prior to large-scale application to understand clinical significance.[86]

Although great strides have been made in diagnostic evaluation of myocarditis by CMR there are several important unanswered questions. Firstly, there is widespread variation in sequence parameters, partly driven by the vendor-specific platforms, resulting in a lack of agreed normal reference ranges for health and threshold values for disease in T1 and T2 values.[86] Secondly, there is a need to integrate mapping data with other circulatory and imaging biomarkers to understand biological mechanisms and redefine disease classification. For example, it was recently shown that despite 'normal' iron T2* levels (>20ms) in the myocardium, T1 mapping detected subtle increases in iron loading, as a complementary tool, suggesting a spectrum of subclinical disease.[211] Thirdly, whilst it is established that those with a 'normal' CMR scan have a good prognosis independent of symptoms and other clinical findings,[212] those with impaired LV systolic function[213] or presence of late gadolinium enhancement (LGE)[6] have impaired survival; what is very unclear is how to manage the growing number of patients without overt heart failure or extensive LGE in a so-called 'grey-zone.'

CMR STRAIN ASSESSMENT

The assessment of cardiac function by left ventricular ejection fraction (LVEF) has been the mainstay of risk stratification in heart failure of any aetiology. However, LVEF is limited by load dependency[214] and is increasing recognised to represent a late manifestation of underlying

cardiac disease. Recently, there has been growing interest in the use of myocardial strain as a more sensitive and predictive measure of LV dysfunction.[215] Myocardial strain refers to the degree of displacement (also known as deformation) of a fixed point within the myocardium throughout the cardiac cycle.[216] Assessment of strain by echocardiography has evolved from tissue Doppler imaging of the 1990's, limited by angle-dependency,[217] to speckle-tracking of naturally occurring ultrasound interference patterns within the myocardium.[218] Global longitudinal strain (GLS) by speckle-tracking represents the most reproducible and validated strain parameter, able to detect sub-clinical LV dysfunction prior to reduction in LVEF.[219,220] However, speckle-tracking remains limited by acoustic echo windows and operator dependency, with errors arising from the foreshortening of apical views and off-axis short axis views.

CMR has many advantages over echocardiography in terms of spatial resolution, reproducibility and good quality images in most subjects (free of implantable cardiac devices). Several different methods are available for strain assessment by CMR.[221] Post-processing methods, such a feature-tracking (FT), are similar to speckle-tracking on echocardiography.[222, 223] Whilst the main advantage of FT is the ability to measure strain from routinely acquired cine sequences without additional scanner time, it lacks the sensitivity of dedicated CMR strain acquisition methods. CMR tagging represents the most validated CMR strain technique. Preparatory radiofrequency pre-pulses in two orthogonal directions physically impose a grid pattern of magnetically labelled tag lines on the myocardium, which can then be tracked over time.[224] However, this requires long scanner acquisition times and T1 recovery eventually leads to disappearance of the tags, resulting in reducing insights during the diastolic phase. In contrast, tissue phase velocity provides greater spatial resolution with faster processing but this comes at the expense of temporal resolution.[225] Displacement encoding simulated echoes (DENSE) provides a balance as it preserves temporal resolution and spatial resolution, which

is only limited pixel size, with quick processing speed.[226] To date, no studies have investigated the clinical utility of DENSE in myocarditis or heart failure.

Overall, there is a need to harness the more in-depth insights provided by recent advances in deep phenotyping by CMR to improve risk stratification and the assessment of response to treatment. Genomic data may also play an important role in helping understand if there is a 'vulnerable' patient cohort, and this is discussed further in a later chapter.

4.3. Methods

Work Package 1 – Prospective study

Consecutive clinical patients with suspected acute myocarditis were recruited from three large tertiary centres (Royal Brompton Hospital, Harefield Hospital and Hammersmith Hospital) with specialist services in primary percutaneous coronary intervention (PCI), advanced heart failure and CMR. Additional patients were identified through our established network of collaborating district general hospitals and recruited within the cardiovascular research centre (CRC) at the Royal Brompton Hospital as soon as possible from index presentation and no later than 14 days (figure 4-2).

Screening criteria were defined by current ESC guidance as ≥ 1 clinical presentation and ≥ 1 of the diagnostic criteria for myocarditis (table 4-1). All patients underwent standard clinical evaluation and were included on the basis of confirmed acute myocarditis by CMR (2 out of 3 Lake Louise criteria) or positive immunohistochemistry of myocardial tissue.

Clinical presentation	Diagnostic Criteria
Acute chest pain	ST segment or T wave changes
Breathlessness (<3 months)	Elevated troponin
Palpitations or unexplained syncope	New or unexplained LV or RV dysfunction
Unexplained cardiogenic shock	

Table 4-1. Outline of current ESC guidance on features of clinical presentation and diagnostic criteria supporting a diagnosis of acute myocarditis.

Exclusions included coronary artery disease (>50% luminal stenosis on invasive or CT angiography), pre-existing cardiovascular disease (e.g. primary valvular or congenital heart disease) or estimated glomerular filtration rate eGFR <30mls/min/1.73m^2.

Baseline evaluation consisted of:

- medical history via a standard structured proforma
- family pedigree
- blood pressure
- 12-lead ECG
- venous blood collection for:
    - serum and plasma for biomarker analyses
    - whole blood for DNA extraction
    - whole blood for virology assessment
    - RNA storage
- Research CMR scan at 3-Tesla to assess T1/T2 mapping, DENSE strain & LGE

All EDTA, lithium heparin and SST samples were processed as per our local Biobank sample processing standard operating procedures within a maximum of 2 hours of collection and were stored locally at -80ºC. Patients underwent repeat evaluation at 3 and 12 months to assess ventricular remodelling, changes in clinical status, and circulating biomarkers of inflammation, myocyte injury and fibrosis. An established, bespoke electronic database linked to our Biobank was used to record all clinical data in a study specific proforma. This ensured confidentiality and good data management in accordance with the Data Protection Act, NHS Caldecott Principles, The Research Governance Framework for Health and Social Care, 2nd Edition (2005), GDPR 2018 and the condition of the main REC approval.

All patients gave written informed consent including for the development of CMR sequences in patients and for the use of patient samples in the identification and investigation of genes or biomarkers linked to cardiovascular disease. [Ref: 09/H0504/104+5 South Central, Hampshire REC].

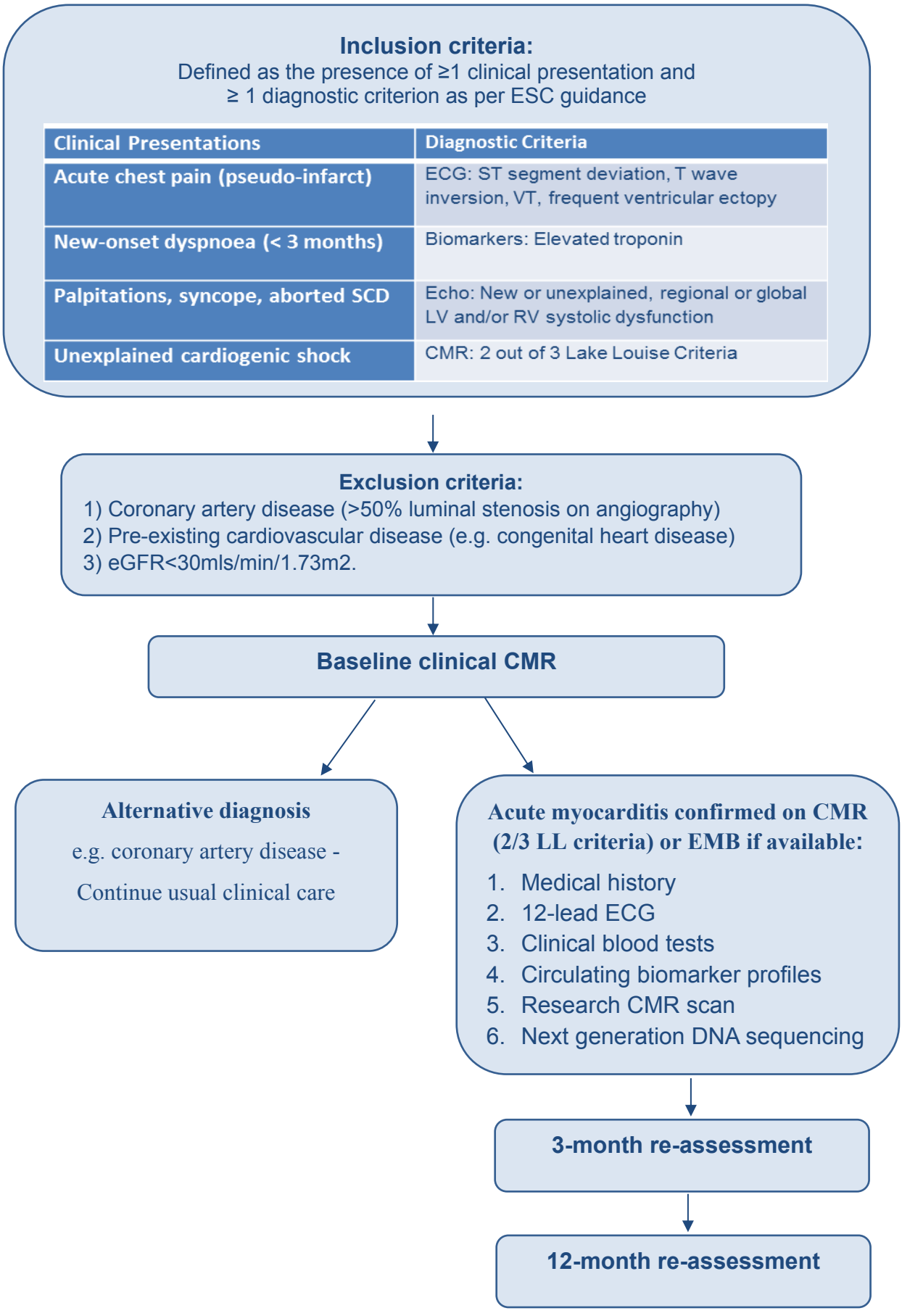
CMR PROTOCOL

Prospectively recruited patients with myocarditis were invited to undergo an enhanced CMR protocol at the 3-Tesla field strength with the inclusion of T2 mapping and DENSE sequences in addition to standard clinical sequences (protocol outlined in the Methods chapter). Images were acquired in identical short- and long-axis views.

COMPARATOR COHORT

A cohort of healthy volunteers with no history of medical illness, not on regular medication and normal cardiac structure and function on detailed CMR assessment was also recruited at the Royal Brompton Hospital by myself and underwent the same research CMR protocol to allow comparisons between health and disease.

Figure 4-2. Flow diagram of recruitment for the prospective study (WP1)



T2 MAPPING PROTOCOL

T2 mapping was performed using 4 single-shot balanced steady-state free precession (SSFP) images acquired at increasing T2 preparation (echo) times (0-75ms) to construct a transverse relaxation curve, as illustrated below (figure 4-3).[122] The following parameters were selected to achieve 1.9x2.2x8mm^3 spatial resolution: TE/TR=1.1/2.5ms, flip-angle=35^o, GRAPPA x2, 6/8ths partial Fourier and 360x285mm^2 field of view (FOV). To ensure maximal T1 longitudinal recovery between shots (to avoid distortion of the calculated T2 time), we used a long repetition time of 2-4 RR intervals.[123] A motion-correction algorithm was used to correct for movement over multiple heart beats during a single breath-hold. Data was acquired on the short axis view at the basal and mid-ventricular level. Long axis views were acquired for visualisation, recognising the partial volume effects of short axis slices at the apical level and predilection of myocarditis for the basal inferolateral wall.[28, 124]

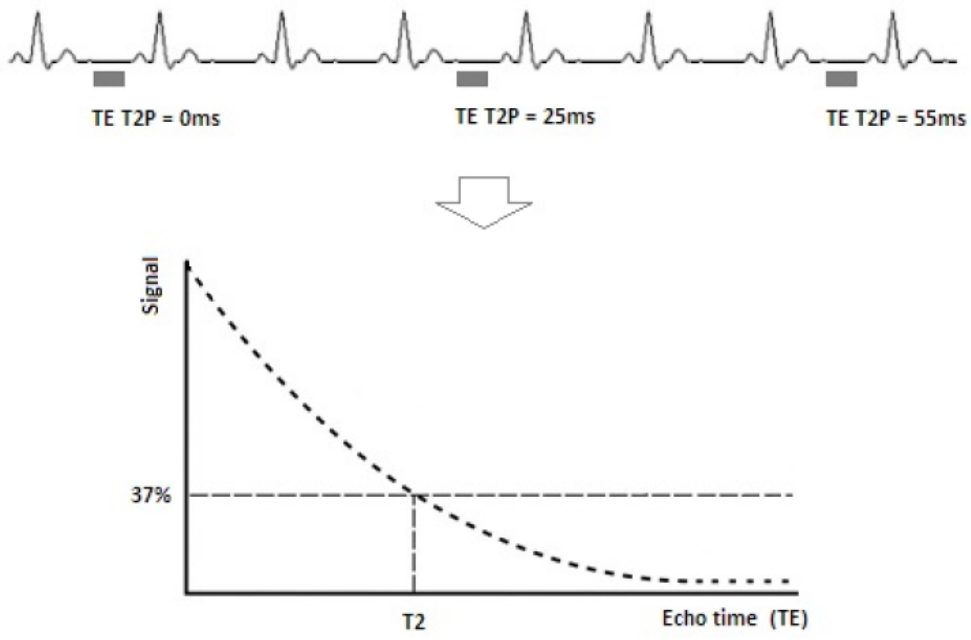


Figure 4-3. Principles of T2 mapping with different T2 preparatory durations with a long repetition time between cardiac cycles to allow as complete T1 recovery as possible, followed by reconstruction of the transverse relaxation curve in each pixel assuming satisfactory registration. T2 is defined as the time in milliseconds for the transverse magnetisation to decay to 37% of the original value.

T2 MAPPING ANALYSIS

T2 maps were analysed using Circle CVI (Calgary, Canada). Raw images from the four different T2 preparation times were initially inspected for artefact secondary to cardiac or respiratory motion (figure 4-4). Those deemed satisfactory were analysed by contouring the epicardium and endocardium, defining the long axis of the heart and the anterior insertion point. The inner 10% of the endocardium and outer 10% of the epicardium were rejected in order to avoid contamination of the myocardial signal with that of the blood pool and pericardium. The software was then used to derive a polar map of the T2 values for each of the 6 segments on the basal and mid-ventricular slice.

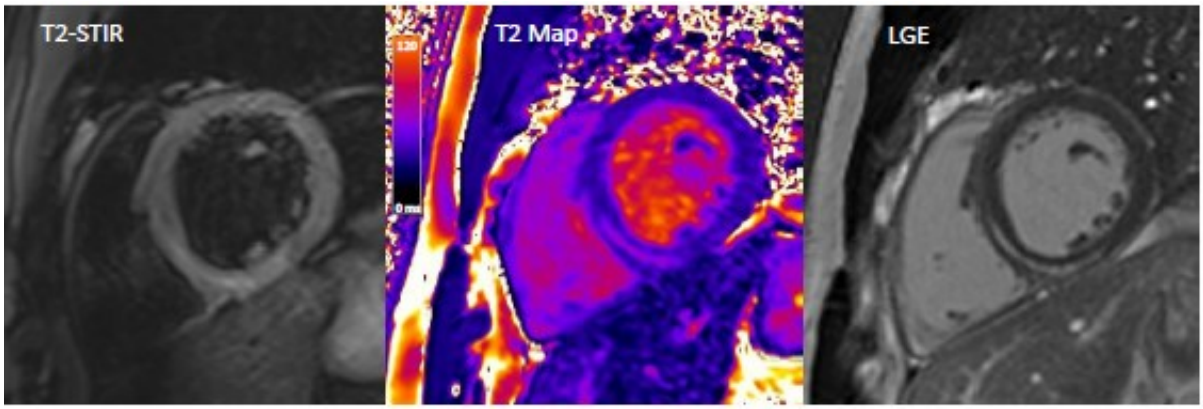


Figure 4-4. Example of a T2 map in the short axis plane (centre) with the standard T2-STIR image to the left, and the post-contrast late gadolinium enhancement image to the right. There is a region of high-signal in the mid-wall layer of the inferior and inferoseptal wall in all images, representing an area of myocardium oedema and corresponding LGE enhancement typical of myocarditis in the acute setting.

DENSE PROTOCOL

Spiral cine displacement encoding with stimulated echoes (DENSE) imaging was acquired to measure circumferential, radial and longitudinal myocardial strain in 2-chamber and 4-chamber planes and in a short-axis plane at basal and mid-ventricular levels.[227, 228] A spatial resolution of 3.3x3.3x8.0mm^3 and temporal resolution of 30ms was achieved through the use of 224x224x8mm^3 FOV and 2-direction encoding at 0.06cycles/mm in mid short-axis (figure 4-5) and 4 chamber planes. Selectively exciting and imaging a reduced FOV, allowed 2D acquisitions to be accelerated and performed with shorter breath-holds.[229]

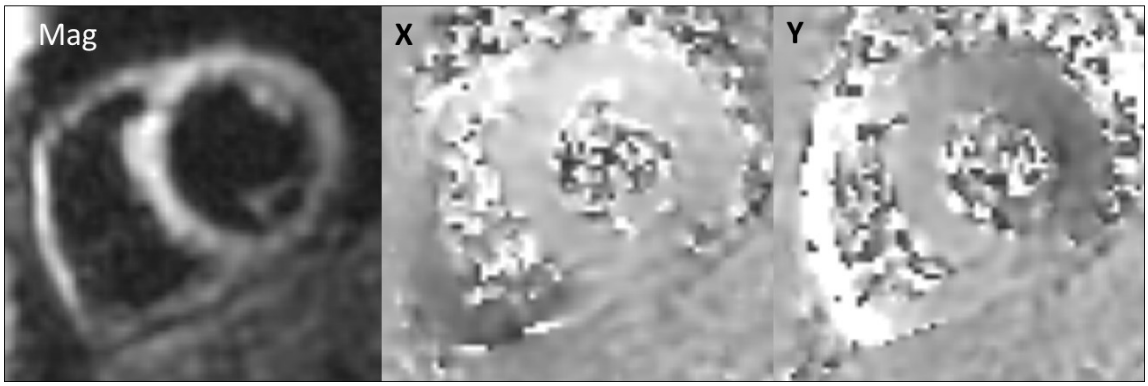


Figure 4-5. Example of DENSE magnitude, X-encoded and Y-encoded images in the short axis plane. These images contain raw data from which strain curves are derived and provide a visual assessment of quality control.

DENSE ANALYSIS

Myocardial strain was calculated from DENSE data using semi-automated software developed by the University of Virginia on Matlab (Mathworks, Natick, USA).[230] For long-axis images, an LV contour was placed in the mid-myocardium in peak systole or diastole, similar to the approach used for speckle-tracking by echocardiography. For short-axis images, LV endocardial and epicardial borders were contoured in the basal and mid-ventricular slices. These contours were then propagated throughout the cardiac cycle using motion-guided segmentation. Any phases with significant artefact were manually adjusted where necessary. Contour strain/time curves were generated for global longitudinal strain and regional polar strain time curves for radial and circumferential strain (figure 4-6).

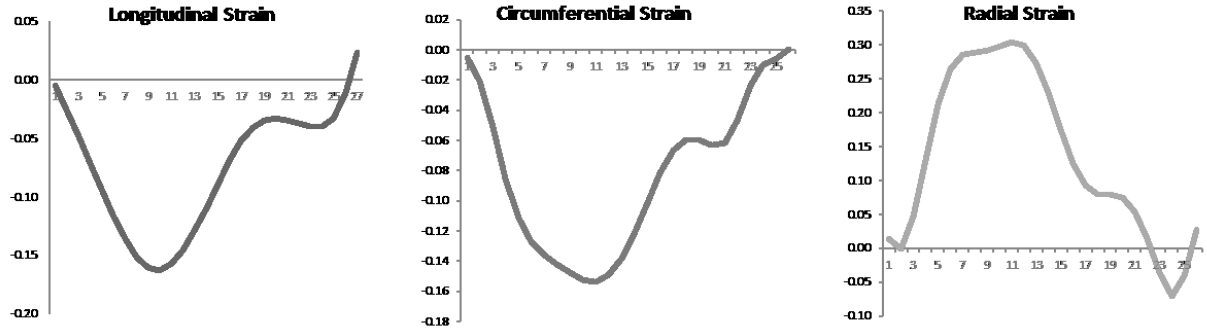


Figure 4-6. Example of global strain-time curves in a patient with acute myocarditis using CMR displacement encoding with stimulated echoes (DENSE) [y-axis absolute strain, x-axis frame number]

ENDOMYOCARDIAL BIOPSY PROTOCOL

If/when a patient was referred for an invasive procedure where myocardial tissue would be available, a member of the research team was available to attend and collect a tissue sample. Invasive procedures included endomyocardial biopsy (EMB) of the left or right ventricle, as well as left-ventricular assist device implantation, where ventricular tissue was routinely removed during cannulation of the cardiac chambers. For patients undergoing a cardiac transplant, any surplus tissue following routine clinical diagnostic use was also collected. Myocardial tissue underwent routine clinical examination by a histopathologist using standard immunohistochemical approaches to confirm a diagnosis of acute myocarditis and underlying aetiology.

Work Package 2: Predictors of Long-Term Remodelling

This was a long-term follow-up study of patients previously diagnosed with acute myocarditis on CMR LLC and/or Dallas criteria on EMB at the Royal Brompton and Harefield Hospitals. The main focus of this work package was to assess long-term LV remodelling and also to increase myocarditis patient recruitment to meet the sample size required for our genetic analyses (discussed in the chapter 5).

We identified a retrospective cohort of 170 consecutive patients (mean age 42±16 years, 84% men) with acute myocarditis confirmed by CMR (two out of three Lake Louise Criteria) or endomyocardial biopsy within our Trust from 2005 onwards.

Patients were invited to return for a single study visit, similar to the baseline evaluation performed in WP1. All patients were recruited with written informed consent.

Evaluation consisted of:

- medical history via a standard structured proforma
- family pedigree
- blood pressure
- 12-lead ECG
- venous blood collection for:
    - serum and plasma for biomarker analyses
    - whole blood for DNA extraction
    - whole blood for virology assessment in future
- Research CMR scan at 3-Tesla to assess T1/T2 mapping, DENSE strain & LGE

Natural history and clinical outcomes were assessed by means of a structured medical proforma with additional information from the patients' primary or secondary care physicians.

POWER CALCULATIONS

Our overarching sample size calculation was based on ensuring adequate power in the study requiring the largest sample size. This was the study assessing the genetic determinants of myocarditis and progression to DCM (discussed in chapter 5).

CMR analysis

We aimed to investigate the role of T1/T2 mapping compared to replacement fibrosis and myocardial oedema as determined by LGE and T2-STIR. Given that our cohorts were defined by CMR LLC rather than validated by EMB, we could not reliably assess diagnostic accuracy but rather we sought to develop the technical aspects of these sequences and to provide mechanistic insights through exploration of the temporal sequence of change with disease progression or recovery. Based on superiority analysis of T2 mapping compared to standard T2-STIR, with an alpha error of 0.05, approximately 88 patients would be required in a study with 90% power.

Genetic analysis

Based on previous work showing that the prevalence of TTN mutations in a healthy population was 1% and in those progressing to DCM was expected to match that reported in DCM and peripartum cardiomyopathy cohorts (~15%), the total number of patients required in this study was 70 patients per group (myocarditis with or without DCM) with 90% power to detect a significant difference in the proportion of patients with a TTN mutation at the 5% significance level. We aimed to actively recruit 210 patients given that one third of patients were expected to progress to DCM. Allowing for 10% drop-out, we aimed for 120 patients recruited prospectively and the remainder would be recruited from our retrospective cohort.

Circulating biomarker analysis

The biomarker analyses were exploratory and pilot data acquired in this study would generate further hypotheses to support subsequent multi-centre studies. We aimed to use our existing Biobank of 1000 healthy volunteers (genotyped/phenotyped) to provide a control group for the biomarker platform.

STATISTICAL ANALYSIS

Baseline characteristics amongst prospective and retrospective patients were compared using the Mann-Whitney U-test for continuous data or Fisher exact test for categorical data. Changes in LV indices were compared across the three study visits using the Kruskal-Wallis test for non-normal data. A p-value of <0.05 was taken as significant. For CMR and strain comparisons, baseline to follow-up measurements were compared using Wilcoxon matched pairs signed-rank test and correlations between changes in variables using Spearman's rank correlation coefficient.

4.4. Results

From 125 patients that were screened, 114 patients were recruited prospectively (figure 4-7). The main exclusions were absence of CMR evidence of acute myocarditis by LLC (7 patients) or the discovery of subendocardial infarction (4 patients). The median delay from first medical contact to baseline CMR was 6 days (IQR 3-12 days). In parallel, from 170 patients identified with a history of CMR or biopsy confirmed myocarditis, 117 patients could be contacted and were recruited retrospectively with a median follow-up of 5.1 years (IQR 2.8-7.3 yrs).

BASELINE DEMOGRAPHICS

The overall median age was 35 years (IQR 27-47 years) and 193 out of 231 (84%) participants were men (table 4-1). Clinical presentation included symptoms of chest pain (85%), breathlessness (42%), palpitations (18%) and syncope (11%). A recent viral illness was reported in 85% of patients, of which coryzal symptoms (37%) and recent gastrointestinal upset (18%) were the main complaints. A history of previous myocarditis was documented in 5% of patients. A family history of sudden cardiac death in a first or second degree relative was reported in 10% of patients, with a family history of myocarditis in 3% of patients. The median interval from symptom onset to first medical contact and subsequent hospital admission was 1 day (IQR 0-3 days) consistent with acute presentation.

PROSPECTIVE VS RETROSPECTIVE PATIENTS

When comparing prospective and retrospective patient groups, ECG changes (82 vs 44%; $p<0.001$), troponin elevation (44 vs 7%; $p<0.001$) and BNP elevation (54 vs 28%; $p<0.001$) were significantly more common in the prospective patients, consistent with recruitment during the active phase of disease. Similarly, LV mass index was greater in the prospective group (75 vs 66g/m^2; $p=0.012$). The median interval from first medical contact to baseline CMR was significantly shorter in the prospective group (median of 6 vs 9 days; $p=0.016$). The median

age of the retrospective group was greater than the prospective group (40 vs 31 years; p=0.258), consistent with recruitment after a median follow-up of 5.1 years (IQR 2.8-7.3 years) from index admission with myocarditis.

Figure 4-7. Identification, inclusion and exclusion of the prospectively recruited study population

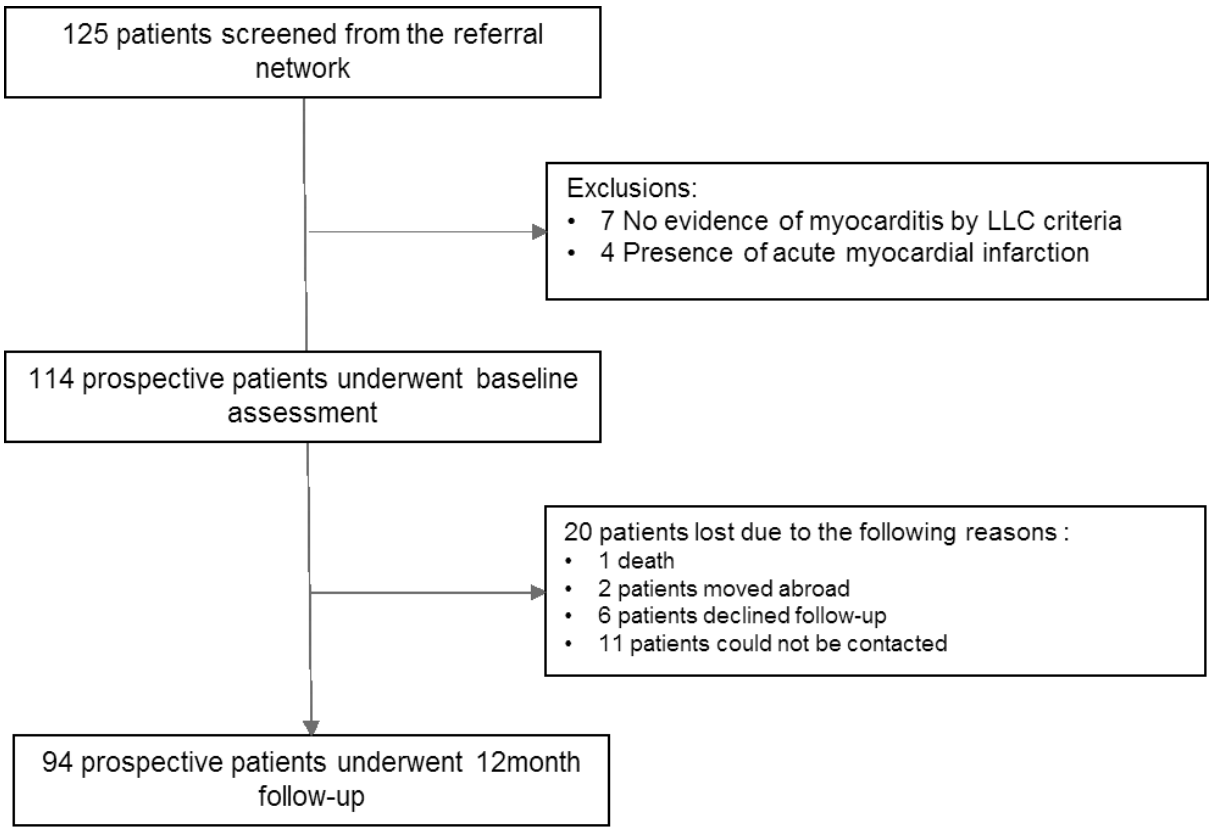


Table 4-1. Baseline demographics of the overall study cohort. Patient numbers are presented with a percentage in parentheses unless otherwise stated as median and IQR.

	All patients (n=231)	**Prospective** (n=114)	**Retrospective** (n=117)	**P value**
Median Age (IQR), years	35 (27-47)	31 (25-40)	40 (28-53)	0.258
Male, n (%)	193 (84)	96 (84)	97 (83)	0.860
Caucasian	203 (88)	96 (84)	107 (91)	0.108
Recent viral illness	197 (85)	93 (81)	104 (89)	0.139
Recent coryzal sx	86 (37)	46 (40)	40 (34)	0.344
Recent gastrointestinal upset	35 (18)	20 (18)	15 (47)	0.361
Previous history of myocarditis	11 (5)	4 (4)	7 (6)	0.539
Family history of myocarditis	8 (3)	3 (3)	5 (4)	0.722
Family history DCM	1 (0)	0 (0)	1 (1)	1
Family history SCD	23 (10)	10 (9)	13 (11)	0.662
Excess alcohol	37 (16)	18 (16)	19 (16)	1
Recreational drug use	40 (17)	26 (23)	14 (12)	0.037
Clinical presentation				
Chest pain	196 (85)	101 (89)	95 (82)	0.104
Breathlessness	97 (42)	49 (43)	48 (41)	0.791
Palpitations	42 (18)	21 (18)	28 (24)	0.337
Syncope	26 (11)	11 (10)	15 (13)	0.533
Median interval symptom onset to FMC (IQR), days	1 (0-3)	1 (0-3)	1 (0-3)	0.797
Median interval symptom onset to admission	1 (0-3)	1 (0-3)	1 (0-4)	0.096
Median duration of index hospital admission	4 (2-6)	4 (2-6)	3 (1-6)	0.275
Investigations				
ECG ST or Tw changes, n (%)	144 (63)	93 (82)	51 (44)	<0.001
ECG arrhythmia	27 (12)	19 (17)	8 (7)	0.024
Troponin elevation	58 (25)	50 (44)	8 (7)	<0.001
BNP elevation	94 (41)	61 (54)	33 (28)	<0.001
Evidence of viral pathogen	35 (22)	35 (31)	not assessed	-
Endocardial biopsy or intraoperative excision	11 (5)	6 (5)	5 (4)	0.767
Giant cell myocarditis	4 (2)	2 (2)	2 (2)	1.000
CMR parameters				
Median interval FMC to baseline CMR (IQR), days	7 (4-15)	6 (3-12)	9 (4-27)	0.016
LVEDVi, ml/m2	87 (74-102)	87 (76-102)	82 (74-102)	0.438
LVESVi, ml/m2	32 (26-40)	32 (27-39)	31 (26-41)	0.583
LVEF, %	63 (57-67)	63 (57-67)	63 (58-67)	0.927
LV mass index, g/m2	72 (62-85)	75 (65-87)	66 (57-58)	0.012
RVEDVi, ml/m2	90 (77-107)	89 (77-106)	96 (80-112)	0.509
RVESVi, ml/m2	38 (33-48)	38 (33-48)	41 (33-49)	0.963
RVEF, %	57 (51-61)	57 (51-61)	58 (52-60)	0.482

DIAGNOSTIC CONFIRMATION

In the prospective cohort, clinical CMR scans were performed in 108 patients (95%) at baseline to confirm a diagnosis of myocarditis. Myocardial tissue was obtained in 7 patients (6%), all within the transplant unit at Harefield Hospital. In the retrospective cohort, clinical CMR scans were performed in 112 patients (96%) at the time of index presentation and myocardial tissue was obtained in 5 patients (4%). Of those cases with a tissue diagnosis (12 out of 231; 5%), the main findings were lymphocytic myocarditis followed by Giant cell and eosinophilic myocarditis (figure 4-8).

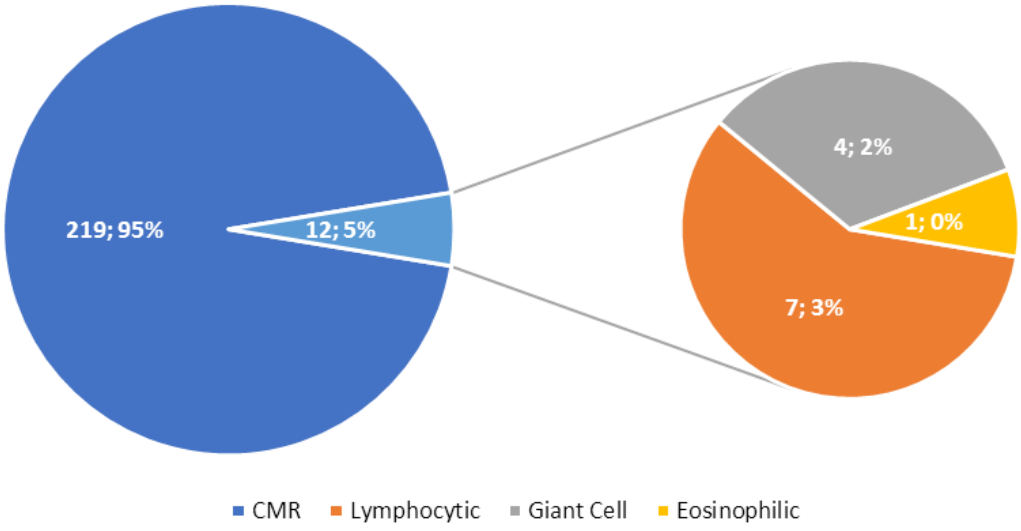


Figure 4-8. Pie chart to show myocarditis aetiology in those patients with a tissue diagnosis (n=12)

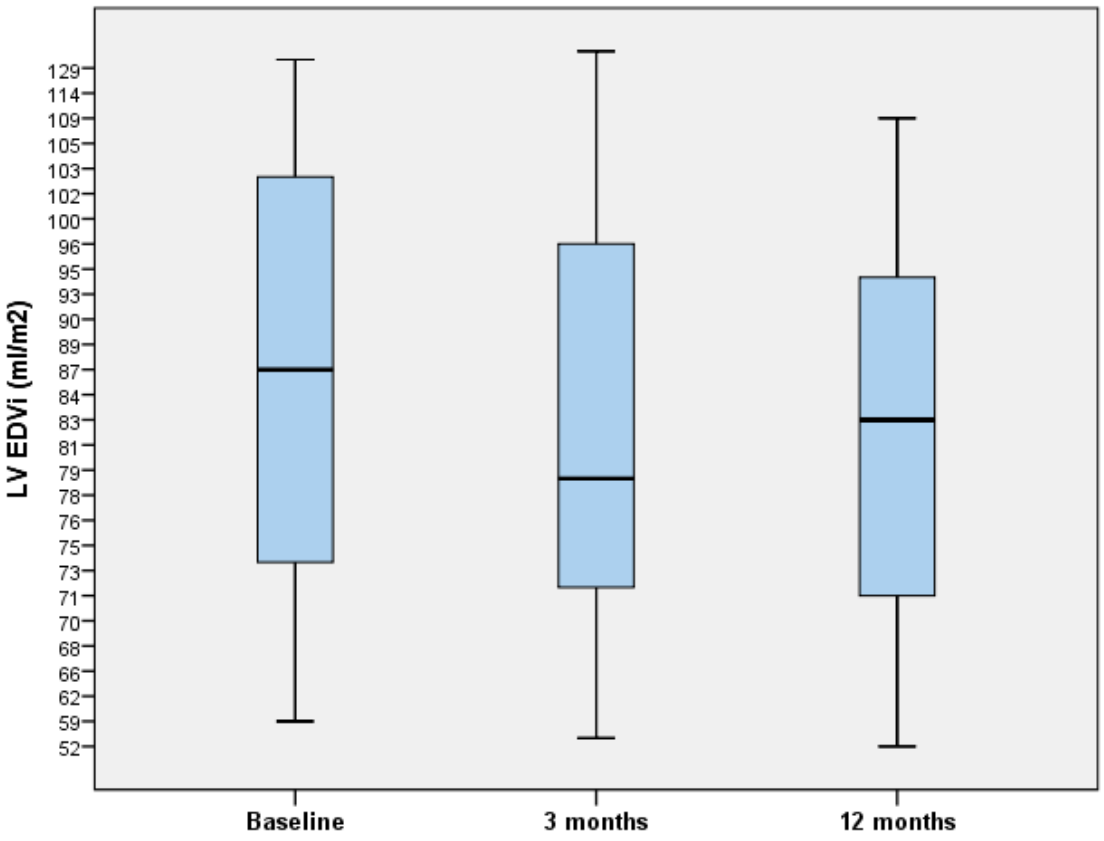
SERIAL CHANGE IN LV PARAMETERS IN PROSPECTIVE PATIENTS

Over the 12-month study period, LV parameters were assessed at 3-time points in those patients that underwent CMR at baseline (table 4-2). There were no significant differences in any LV parameter over this time although the greatest differences were seen in LVEDVi between baseline and 3 months (figure 4-9).

Table 4-2. Summary of LV parameters over the study period

	Baseline			3 months			12 months		
	Median	1st quart	3rd quart	Median	1st quart	3rd quart	Median	1st quart	3rd quart
LV EDVi	**87**	74	102	**79**	72	96	**83**	72	93
LV ESVi	**32**	26	40	**31**	25	35	**29**	25	38
LV EF	**63**	57	67	**62**	59	68	**62**	57	66
LV Mi	**71**	62	85	**68**	60	78	**69**	59	77

Figure 4-9. Boxplot to show serial change in LVEDVi across the 3-time points



T2 MAPPING

In patients with acute myocarditis, mean global T2 at baseline was 43.5±2.2ms compared to 39.9±1.2ms in healthy volunteers (n=9; p<0.001; figure 4-10 & figure 4-11). At 3-months follow-up (mean 82±18 days), there was persistent elevation in global T2 (42.6±2.5ms, p=0.53) with a greater range of values. At 12-month follow-up, mean T2 had returned to the similar levels found in healthy volunteers, although the range of values was greater.

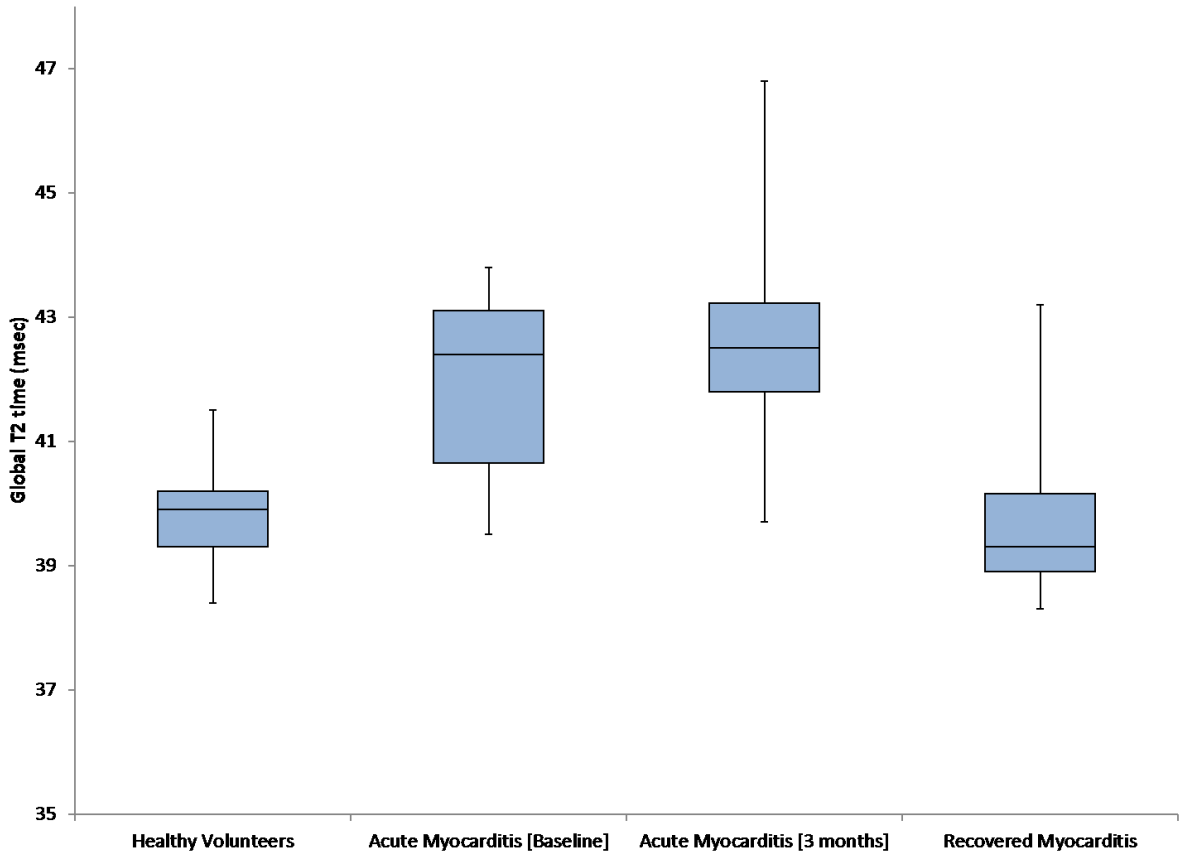


Figure 4-10. Boxplot showing distribution of CMR T2 mapping times representing myocardial oedema at different stages of myocarditis compared with healthy volunteers

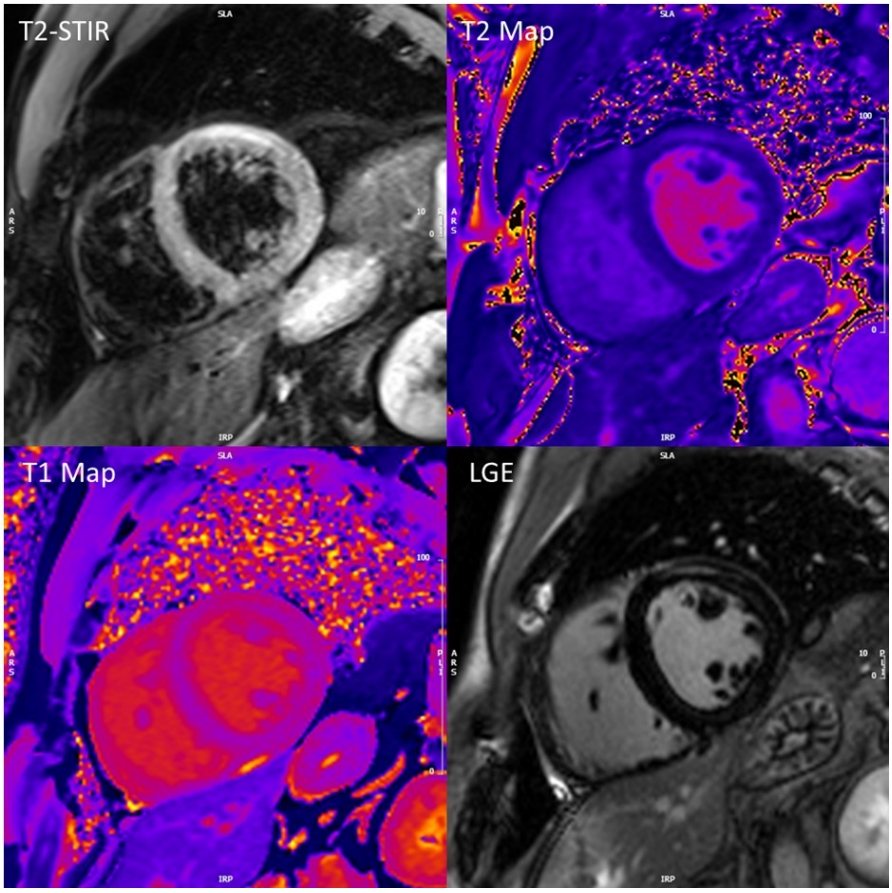


Figure 4-11. Short-axis views of a prospective patient with acute myocarditis affecting the anterior wall as demonstrated by the presence of myocardial oedema on T2-STIR (top left) and T2 mapping (top right), oedema and fibrosis on T1 mapping (bottom left) and scar formation on LGE (bottom right).

MYOCARDIAL STRAIN BY DENSE

Mean LVEF was 63±7.6% at baseline and longitudinal, circumferential and radial strain were -14.9±2.0, -17.6±2.5 and 44.3±11.6% respectively. At 3-months follow-up (mean 82±18 days), there were improvements in all strain parameters. Strain analysis results for the 12-month study visit were still being generated at this time.

ASSOCIATIONS BETWEEN IMAGING AND CIRCULATING BIOMARKERS

Across all observations, there was good correlation between LVEF and all strain parameters (circumferential strain $R=0.60$, $p<0.001$; radial strain $R=0.41$, $p=0.01$; longitudinal strain $R=-0.32$, $p=0.05$; table 4-3). There was also good correlation, (i) between strain parameters, (ii) between measures of T2, including standard deviation (SD) and max T2 as markers of variability, and (iii) between troponin and BNP. Of note, there was no correlation between T2 and LVEF or strain.

Table 4-3. All possible pairwise Spearman Correlation Coefficients and P values between variables at baseline

	Age	Rad. Strain	Circ. Strain	Lon. Strain	T2 Mean	T2 SD	T2 Max	T2 Min	LVEF	Trop	BNP
Age	1.00										
Rad. Strain	0.00 (0.98)	1.00									
Circ. Strain	0.08 (0.63)	-0.39 (0.02)	1.00								
Lon. Strain	-0.16 (0.35)	-0.11 (0.52)	**0.58 (<0.001)**	1.00							
T2 Mean	0.31 (0.08)	0.04 (0.81)	-0.16 (0.40)	-0.05 (0.77)	1.00						
T2 SD	0.03 (0.87)	0.01 (0.97)	-0.11 (0.56)	-0.05 (0.77)	**0.69 (<0.001)**	1.00					
T2 Max	0.47 (0.008)	0.06 (0.74)	0.06 (0.73)	0.03 (0.88)	0.51 (0.003)	0.29 (0.12)	1.00				
T2 Min	-0.01 (0.96)	0.05 (0.77)	0.02 (0.93)	0.02 (0.92)	0.37 (0.04)	0.05 (0.79)	0.34 (0.06)	1.00			
LVEF	0.21 (0.20)	0.41 (0.01)	**-0.60 (<0.001)**	-0.32 (0.05)	-0.03 (0.87)	0.06 (0.75)	-0.01 (0.95)	-0.07 (0.72)	1.00		
Troponin	0.05 (0.78)	0.05 (0.68)	-0.11 (0.51)	0.11 (0.50)	0.18 (0.34)	0.01 (0.94)	0.10 (0.60)	-0.13 (0.49)	0.01 (0.97)	1.00	
BNP	0.45 (0.005)	-0.21 (0.21)	-0.12 (0.49)	-0.20 (0.23)	0.27 (0.14)	0.19 (0.31)	-0.05 (0.79)	-0.18 (0.32)	0.16 (0.32)	0.35 (0.029)	1.00

Highlighted in a medium yellow are any correlations with $p<0.05$. However due to multiple testing, correlations highlighted in darker yellow are those that remain significant after a Bonferroni correction.

Table 4-4. Correlation coefficients between change in variables between baseline & 3-months follow-up

	Baseline Age	Rad. Strain	Circ. Strain	Lon. Strain	T2 Mean	T2 SD	T2 Max	T2 Min	LVEF	Trop	BNP
Baseline Age	1.00										
Rad. Strain	-0.52 (0.02)	1.00									
Circ. Strain	-0.10 (0.68)	-0.32 (0.19)	1.00								
Lon. Strain	-0.23 (0.34)	-0.01 (0.98)	0.26 (0.29)	1.00							
T2 Mean	0.29 (0.37)	0.31 (0.32)	-0.65 (0.02)	-0.04 (0.90)	1.00						
T2 SD	0.07 (0.84)	0.46 (0.13)	**-0.70 (0.01)**	0.05 (0.87)	**0.89 (<0.001)**	1.00					
T2 Max	-0.21 (0.51)	0.12 (0.72)	-0.26 (0.42)	0.07 (0.83)	0.50 (0.10)	0.54 (0.07)	1.00				
T2 Min	0.32 (0.31)	-0.20 (0.54)	-0.03 (0.93)	-0.22 (0.48)	0.62 (0.03)	0.41 (0.19)	0.52 (0.08)	1.00			
LVEF	0.04 (0.86)	0.18 (0.46)	**-0.71 (<0.001)**	-0.17 (0.49)	0.54 (0.07)	0.55 (0.06)	0.40 (0.20)	0.04 (0.90)	1.00		
Troponin	0.02 (0.95)	-0.10 (0.70)	0.10 (0.69)	0.17 (0.48)	0.07 (0.83)	-0.09 (0.77)	-0.20 (0.53)	-0.25 (0.43)	0.30 (0.22)	1.00	
BNP	0.45 (0.05)	0.33 (0.16)	0.13 (0.59)	0.10 (0.67)	-0.14 (0.66)	-0.21 (0.52)	-0.40 (0.19)	-0.49 (0.11)	-0.25 (0.30)	0.35 (0.14)	1.00

Highlighted in a medium yellow are any correlations with $p<0.05$. However due to multiple testing, correlations highlighted in darker yellow are those that remain significant after a Bonferroni correction

ASSOCIATIONS BETWEEN CHANGES IN IMAGING AND BLOOD BIOMARKERS

When assessing correlations in the change from baseline to follow-up, change in circumferential strain showed good correlation with change in LVEF (R=-0.71, p<0.001), change in mean T2 (R=-0.65, p=0.02) and change in T2 standard deviation (R=-0.70, p=0.01; table 4-4; figure 4-12).

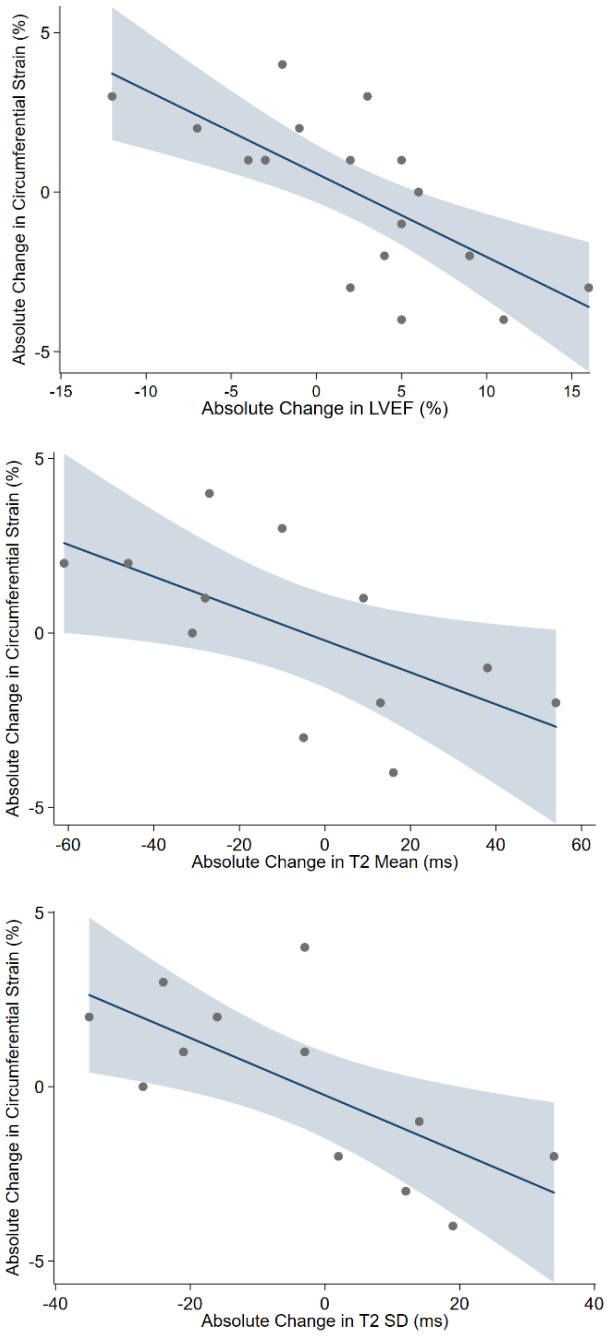


Figure 4-12. Spearman correlation coefficients between change in baseline and follow-up circumferential strain and LVEF (top), mean T2 (bottom left) and standard deviation of T2 (bottom right).

INTEGRATION OF IMAGING AND BLOOD BIOMARKERS

At 3-month follow-up (mean 82±18 days), there was a decrease in troponin (p=0.002) and BNP (p=0.013), despite persistent elevation in global T2 (42.6±2.3ms, p=0.53) and persistent impairment in circumferential strain (-17.6±2.1%, p=0.98). Of note, LV function was normal in these participants able to undergo CMR imaging. Further panels of blood biomarkers are still awaiting analysis.

CLINICAL OUTCOMES

In general, the majority of the cohort that presented with a pseudo-infarct presentation (85%) recovered well with no persistent LV dysfunction. Amongst these patients, there was no risk of aborted or actual sudden cardiac death. Indeed, approximately 6% of the cohort declined returning in person for 12-month follow-up to undergo repeat CMR assessment with the main explanation given as the lack of symptoms or perceived need for repeat imaging.

However, in the minority of patients recruited from the transplant centre, clinical outcomes were very poor. Mechanical circulatory support (MCS) was required in 7 patients, either temporarily in the form of extra-corporeal membrane oxygenation (ECMO; 2 patients), an Impella device (1 patient), or an intra-aortic balloon pump (IABP), or long-term through left ventricular assist device (LVAD) implantation (2 patients) or a total artificial heart (TAH) system (1 patient). Two patients were successfully bridged to cardiac transplantation, of which one died in the peri-operative setting due to hypoxic brain injury. Of note, the 3 patients with temporary MCS showed recovery in LV function following the hyper-acute setting.

In general, outcomes for patients with non-fulminant myocarditis were excellent whereas those with fulminant myocarditis experienced the greatest morbidity and mortality. Quality control and adjudication of clinical events were still in progress at time of writing, precluding multivariable analyses and survival modelling.

4.5. Discussion

Acute myocarditis remains a challenging diagnosis due to heterogeneity in clinical presentation and aetiology with varying clinical outcomes. In this large study of well-characterised myocarditis patients, we demonstrated temporal trends in LV parameters following acute presentation and also provided novel insights into the relationship between myocardial oedema and strain in patients with preserved LV ejection fraction. Clinical outcomes were consistent with published studies and depended on initial presentation with fulminant or non-fulminant myocarditis.

The overall median age of our cohort was 35 years (IQR 27-47 years) and 84% of participants were men. This finding closely matched what we found in our NHS Digital dataset of approximately 13,000 admissions over the last 20 years in England where the median age was 33 years (IQR 23-47 years). Various post-mortem studies have also documented the high burden of myocarditis in individuals less than 35 years of age.[5, 148] Data from Finnish military conscripts similarly showed a relatively young mean age of 20 years (range 17-29 years) although this cohort was derived from a specific age range for conscription. In contrast, many published studies with patients recruited on the basis of biopsy confirmed myocarditis have reported much older median ages, for example, 44 years (IQR 32–56) from a cohort of 129 patients[83] and 52 years (IQR 40–54) from a cohort of 205 patients.[6] It is likely that such studies with biopsy confirmation were biased towards more severe cases with associated LV dysfunction and therefore are not representative of the spectrum of disease. Epidemiological data is more likely to encapsulate real-world distributions amongst all patients meeting the threshold for admission to hospital. Overall, the effects of age and sex are complex but likely relate to imbalances in immune regulation and sex hormones, as discussed in the last chapter.[176]

Clinical presentation for our cohort was predominantly due to chest pain (85%), followed by breathlessness (42%), palpitations (18%) and syncope (11%). ECG ST segment of T wave

changes were observed in 82% of the prospective cohort alongside troponin elevation in 44%. Myocarditis often mimics an acute coronary (ACS) syndrome leading to invasive coronary angiography through standard primary percutaneous coronary intervention (PPCI) pathways.[1] Exceptions are usually young individuals typically aged <35 years where coronary artery disease is unlikely, or individuals with co-existent pericarditis resulting in characteristic pleuritic chest pain, saddle ST elevation and PR depression.[152] Following a normal coronary angiogram, myocarditis has been detected in up to 50% of cases by CMR, highlighting the importance of CMR in this setting.[82, 172, 231] Exact mechanisms of chest pain are unclear but may relate to endothelial dysfunction particularly due to parvovirus,[51] aberrant spasm of microvascular,[232] pro-inflammatory induction of occlusive micro-thrombi,[233] increased vascular permeability leading to myocardial oedema, involvement of the adjacent pericardium and ultimately myocardial ischaemia due to a combination of these effects. In mice with CVB3-induced myocarditis, coronary flow reserve was significantly impaired within one of CVB3 infection and remained reduced for another week, correlating with the severity of myocarditis.[234]

In the setting of pseudo-infarct myocarditis, it has been shown that event rates are low and outcomes are generally favourable. In a landmark study of 128 biopsy-confirmed cases of myocarditis, patients with parvovirus B19 typically presented with severe chest pain, had lateral wall LGE and recovered spontaneously within months.[27] In a similar study of 174 patients of mean age 36±18 years, pseudo-infarct presentation was equally associated with favourable outcome.[235] In most cases of pseudo-infarct presentation, baseline LV ejection fraction was reported to recover or remain preserved in most patients at 6 months following index presentation and failure of this recovery was deemed to be the main determinant of long-term prognosis.[236] This was also observed within our cohort, where the majority of patients had preserved LV ejection fraction and only mildly increased LVEDVi at baseline, which

normalised in most patients by 3 months. One of the strengths of our study design was that baseline CMR was performed within a median of 7 days (IQR 4-15) from first medical contact. For the prospective cohort, this interval was significantly shorter than the retrospective cohort in keeping with active recruitment of consecutive prospective patients scanned close to disease onset to maximise diagnostic yield [6 days (IQR 3-12) vs 9 days (IQR 4-27); p=0.016]. Whilst follow-up questionnaires revealed a high burden of ongoing intermittent chest discomfort and psychological distress (discussed later), no adverse events occurred over the study period in these patients.

In our prospective cohort, T1/T2 mapping and DENSE were assessed by CMR and provided novel insights into disease activity beyond that provided by LV ejection fraction alone. Given that our cohort was scanned within 7 days, we focused our analysis on T2 mapping as diagnostic accuracy has been shown to be superior to T1 mapping in this hyperacute setting.[83] At baseline (patients scanned at a median of 6 days following first medical contact), mean global T2 was significantly elevated at 43.5±2.2 ms compared to 39.9±1.2ms in healthy volunteers ($p<0.001$). At 3-months follow-up, there was persistent elevation in global T2 (42.6±2.5ms, p=0.53) but notably a greater range of values. By 12 months, mean T2 returned to normal, as defined by the healthy cohort. This trend reflects the natural history of myocarditis with the evolution of myocardial oedema in the hyperacute setting. This has been observed in a number of studies over recent years. The Myo-RACER trial included patients with biopsy-confirmed myocarditis with acute and chronic symptoms, separated by a cut-off of 14 days. In the acute group (n=61), chest pain was the main presenting complaint (64% vs 41%; p=0.001) with significantly greater LV ejection fraction than the chronic group (48% vs 27%; $p<0.001$).[83] T2 mapping was reported to be more superior in terms of diagnostic accuracy than T1 mapping in the acute setting, both which were more superior than standard LLC. Several other studies reported similar findings that were recently presented in two large meta-analyses.[87, 88] These

studies collectively led to the incorporation of parametric mapping into updated LLC in December 2018, although implementation into routine clinical myocarditis scan protocols remains variable due to the challenges in non-standardised sequence parameters, partly driven by the vendor-specific platforms, resulting in lack of normal reference ranges for health and threshold values for disease in T1 and T2 values.[89] An innovative approach to potentially obviate the need for normal ranges has been developed using an analytical approach based on one of the observations also seen in our cohort. In a study of 31 patients with myocarditis, T2 maps were analysed globally and segmentally for the absolute T2 relaxation time, but also the maximum pixel-standard deviation. The group focused on the spread or distribution of T2 values rather than the absolute value. In this way, it was demonstrated that pixel-SD within healthy myocardium was much narrower than oedematous myocardium, and that this was a more sensitive marker of disease than reporting a single absolute number averaged over a global or segmental region of interest.[237] This reflects the narrower interquartile range seen in our healthy volunteer group, with the greatest range seen at 3 months, most likely due to the variable rates of recovery, and in some cases progression, of myocardial oedema both within an individuals' myocardium but also between individuals. The optimal method for T2 analysis and reporting remains an active area of investigation but this approach may simplify and accelerate meaningful clinical application.

Integrating parametric mapping with other subclinical indices of myocardial dysfunction in patients with preserved LV ejection fraction represents an area of unmet clinical need across the spectrum of inflammatory cardiomyopathy. In this study, we compared the inter-relationship between T2 times and myocardial strain derived from DENSE at baseline and 3 months to precision phenotype our cohort. As one would expect, there was good correlation between LV ejection fraction and all 3 strain parameters. However, we demonstrated for the first time that there was good correlation between both measures of T2 times (absolute values

and standard deviation) and global circumferential strain (GCS). These findings support earlier discussion regarding the heterogeneity in myocardial oedema, and there was no correlation between T2 times and LVEF. Therefore, despite normalisation of troponin and BNP in most patients by 3 months, there was ongoing and previously unrecognised subclinical myocardial oedema resulting in impaired global circumferential strain. In a small study of 28 patients (mean age 31.9±12.6yrs) with CMR-confirmed acute myocarditis, myocardial strain, specifically GLS, derived from speckle-tracking by echocardiography was shown to correlate with myocardial oedema ($r=0.65$; $p<0.001$). However, oedema was visually assessed by T2-STIR and assessment of GCS and GRS was limited by on 2D echocardiography.[238] Several recent studies have sought to evaluate the role of retrospectively analyses with speckle-tracking[239] or feature-tracking[240] in small patient cohorts with comparable findings. However, such post-processing methods may not be technically feasible in all patients' scans and are subject to bias when performed retrospectively in the research setting with knowledge of patient outcomes. Our findings integrate the latest advances in prospective CMR sequences and highlight the potential utility of a personalised approach using T2 mapping and myocardial strain assessed by DENSE within a single CMR scan in the surveillance of acute myocarditis, but equally relevant to all other forms of inflammatory cardiomyopathy, to detect ongoing subclinical disease.

Insights into the mechanism for the specific effect we observed on GCS rather than GLS or GCS may be drawn from existing work on myocardial fibre orientation. Simplistically, the LV subepicardial layer consists of fibres orientated in a left-handed helical arrangement responsible for torsion, whereas the mid-wall layer contains circumferential fibres responsible for radial thickening and the subendocardial layer has a right-handed helix and longitudinal fibres.[241, 242] Myocarditis is known to have predilection for the mid-wall and subepicardial fibres but spares the subendocardial layer for reasons that remain unclear. Therefore, the

predominant effect seen on GCS, but also GRS to a lesser extent, may reflect involvement of these specific layers of fibres.[243] Taking this further, we are actively investigating correlations between segmental measures of T2 and torsion within our cohort. We also observed ongoing myocardial oedema with impaired strain in the presence of normal troponin highlighting the increased sensitivity provided by these novel imaging tools beyond the acute setting. In myocarditis, troponin release is usually caused by myocyte lysis, similar to acute myocardial infarction, but also massive cytosolic release driven by the inflammatory response rather than ischaemia. Our findings highlight that myocarditis can be present in the absence of detectable rises in troponin level.[244] This notion is supported by the anecdotal understanding that troponin levels do not portend prognostic value in acute myocarditis, unlike in acute coronary syndromes. Again, going forward, our serum samples await evaluation for high-sensitivity troponin, which may provide additional insights and opportunities in the detection of subclinical disease and risk prediction when used in combination with these advanced imaging tools.

In contrast to patients with pseudo-infarct presentation, the smaller number of patients (5% of total cohort) with sub-acute presentation due to breathlessness and new-onset heart failure tended to have worse outcomes. This clinical presentation often represents a diagnostic challenge as conventional disease markers may have normalised by the time of admission and CMR is generally not feasible. In the landmark study described earlier, it was also shown that patients with new onset heart failure most often had human herpes virus 6 with septal wall LGE and frequently progressed to chronic DCM.[27] In our cohort, myocardial tissue was obtained in 5%, predominantly at the time of implantation of mechanical circulatory support, which included the spectrum from Impella, IABP and ECMO to LVAD or TAH. Detailed survival analyses including quality control and adjudication of clinical events were in progress at the time of writing but are likely to highlight the stark contrast in outcomes depending on the initial

level of LV dysfunction and related mode of clinical presentation. One of the key priorities going forward is understanding biological susceptibility and markers of adverse risk amongst these high-risk individuals, particularly those that fall within the grey-zones with moderate LV dysfunction, and those with severe LV dysfunction requiring mechanical circulatory support.

Sudden cardiac arrest represents another often-devastating mode of clinical presentation. Within our Trust, retrospective review of post-mortem findings identified 149 patients with available stored tissue confirming acute myocarditis (98 lymphocytic, 28 giant cell, 14 neutrophilic and 9 eosinophilic). This spectrum of aetiologies is consistent with previous reports.[56] In another separate project not presented as part of this thesis, we also explored the role of CMR in survivors of out-of-hospital sudden cardiac arrest aged <35years.[203, 245] This highlighted the incremental diagnostic value provided by CMR in the identification of potentially arrhythmogenic substrates in a cohort of 89 individuals (<35 years of age) due to the finding of previously unrecognised acute myocarditis and arrhythmogenic right ventricular cardiomyopathy. Rates of survival post sudden cardiac arrest are increasing due primarily to national initiatives promoting bystander cardiopulmonary resuscitation.[246] However, at present, guidelines do not support the routine use of CMR in survivors despite the benefit of in-vivo tissue characterisation directly relevant to patient management.[247]

In summary, using deep CMR phenotyping in this large study of well-characterised myocarditis patients, we demonstrated temporal trends in LV volumetric parameters alongside detailed in-vivo assessment of myocardial oedema and strain following acute presentation to provide novel insights into the inter-relationships between these variables.

4.6. Future Work

The data presented in this chapter represent a preliminary analysis of a rich dataset of prospectively recruited patients with acute myocarditis seen across three time-points, with serial plasma, serum and urine samples in parallel with detailed phenotypic characterisation by CMR. The application of CMR mapping techniques is transforming the non-invasive assessment of all forms of myocarditis in the acute and non-acute settings.[248] Whilst CMR may provide information on disease severity, a combined proteomics approach will provide insight into underlying aetiology and pathobiology.[249] Characterisation of inflammatory and fibrogenic pathways, particularly exploration of the differences between patients showing spontaneous recovery versus progression to DCM, represents an opportunity to improve our understanding. There are also potential cost savings through unnecessary follow-up of the 'recovered' patient, and conversely, more targeted surveillance of the 'at-risk' patient. Using univariable and multivariable linear regression, we will investigate if changes in circulating proteomics and *in vivo* myocardial tissue characteristics (T1/T2 times, extracellular volume, strain and LGE quantity) between baseline and follow-up are associated with adverse ventricular remodelling. Through our collaborators, we hope to validate any potential proteomic signals with myocardial tissue and drive forward the concept of a liquid biopsy through the detection of cell-free genetic material in the circulation due to viral infection as a novel approach over and above serological examination. Ultimately, these findings will inform a larger multi-centre study of the predictors of remodelling and recovery following an episode of acute myocarditis using an integrated approach using CMR, proteomics and genomics.

4.7. Limitations

We believe that our cohort is typical of patients with myocarditis seen broadly across the UK although one of the potential sources of bias included the referral of patients with more severe forms of disease or impaired LV function on echocardiography. To mitigate against this and to ensure we met our targets for recruitment, we recruited all consecutive patients seen within our network of tertiary and collaborating district general hospitals with clinically suspected acute myocarditis:

Site	Local principal investigators
Royal Brompton Hospital	Dr Sanjay Prasad
Harefield Hospital	Dr Nick Banner, Dr Tito Kabir
Hammersmith Hospital	Dr Nilesh Sutaria, Dr Declan O'Regan
Barts Hospital	Dr Sam Mohiddin
Northwick Park Hospital	Dr Nigel Stephens, Dr Jaymin Shah
Chelsea & Westminster	Dr Sam Kaddoura, Dr Julian Collinson
Watford Hospital	Dr Niall Keenan, Dr Amanda Varnava
Wexham Park Hospital	Dr Dinos Missouris, Dr Nav Chalal
Ealing Hospital	Dr Ravi Assomull
North Middlesex Hospital	Dr Amal Muthumala, Dr Roger Rear
Kingston Hospital	Dr Arvind Vasudeva
Basildon Hospital	Dr Jason Dungu
Woolwich, QEH	Dr Carl Shakespeare
West Suffolk Hospital	Dr Vassilis Vassillou

Despite these efforts, our recruited cohort is likely to still only represent the tip of an iceberg. Disease severity ranges widely with many patients having mild symptoms that do not seek medical attention or are managed in the primary care setting and may not be referred for further evaluation in a hospital setting. However, referral or direct presentation to a secondary care hospital is taken as a threshold to identify patients with clinically significant disease, in whom further adverse events are more likely and therefore warrant investigation. At the other end of the spectrum, patients presenting with sudden cardiac death were not recruited. This naturally

represents a more challenging group to study prospectively, but we intend to assess long-term outcomes over extended follow-up in our cohort.

The lack of the gold standard of endomyocardial biopsy (EMB) may be perceived as a potential limitation in this study. However, we proposed to study patients with myocarditis based on ESC guideline criteria as accepted by the community and used in clinical practice.[1] By ESC definition, we did not require an endomyocardial biopsy (EMB) to make a clinical diagnosis of myocarditis that is the entry point for this study. Instead, we included patients with a CMR confirmed diagnosis, reflective of real-world clinical practice in the UK and USA. There is a small but tangible procedural risk from EMB and therefore it was deemed unethical to perform EMB purely as part of a research protocol in young patients with an overall high likelihood of recovery. No UK site currently performs EMB as a clinical routine in all patients with known or suspected myocarditis. Furthermore, there are significant concerns in assuming the biopsy would provide all answers in these patients. The limitations of EMB are many and demonstrated by the lack of concordance of clearly defined inflammation at the myocardial level by CMR, where only two thirds of cases are EMB positive.[250] This arises from the focal nature of inflammatory infiltrates and involvement of regions inaccessible to the bioptome, for example, within the left heart when accessing the venous system. EMB needs to be performed in a biventricular manner to have greatest yield but is still only 'positive' in ~70% of patients meeting diagnostic criteria for myocarditis by guidelines.[76] This does not imply that 30% of patients without a positive EMB do not have myocarditis, indeed by definition they do. Aside from high rates of sampling error,[192] there is also a high level of inter-observer variability in histopathological interpretation.[75] Myocarditis is also caused by many factors (allergen, autoantigen, viral, bacterial, drug etc) and the use of EMB and viral load assessment is not useful in many of these conditions. In EMB positive cases, viral genome types are equally highly variable, and specificity of PCR-based amplification is limited.

Recognising the need for myocardial tissue to validate our future findings in terms of genomics and circulating biomarkers, we have formed collaborations with two international sites where EMB is routinely performed. These collaborations will allow for more definitive analyses of patient cohorts across a broad spectrum of disease severities.

The assessment of viral aetiology was limited in our cohort due to the infrequent use of cardiac biopsy. Whilst serological assays were performed on a variable basis as part of local clinical evaluation, their use remains unsupported due to lack of sensitivity and confidence in causality.[1] Similarly, data on high-sensitivity troponin was not available across the cohort. This will be assessed in future alongside detailed proteomic analyses to understand the role of different biological pathways in patients with different forms and trajectories of disease.

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5. GENETIC DETERMINANTS OF MYOCARDITIS

5.1. Aims and Hypotheses

The primary aim of this chapter is to characterise the underlying genetic basis for myocarditis, with the purpose of informing patient stratification and providing novel insights into disease pathogenesis.

The main hypothesis is:

- Amongst patients with myocarditis progressing to a DCM phenotype, there is a greater prevalence of titin truncating variants (TTN-tv) compared to those without adverse remodeling, suggesting that viral myocarditis acts as an environmental modifier that unmasks the DCM phenotype.

5.2. Background

Myocarditis can arise from a number of causes, including viral infection, toxin exposure or auto-immune disease. Whilst generally considered a self-limiting condition in two thirds of patients, the main challenge is the identification of high-risk individuals that subsequently show adverse remodelling to a dilated cardiomyopathy (DCM) phenotype. Progression to DCM is reported to occur in up to 30% of cases of biopsy-proven acute myocarditis.[6] Amongst these patients, mortality parallels that of patients with idiopathic DCM approaching 50% at 5 years.

PATHOPHYSIOLOGICAL BASIS

Murine models using coxsackievirus B3 (CVB3) to induce acute and chronic forms of myocarditis have informed our understanding of pathogenesis. It is thought that 3 distinct, chronologically successive stages start from the initial myocardial injury (figure 5-1).[251] Despite effective clearance of the initial virus, persistent autoimmune activation, characterised by presence of circulating autoantibodies to cardiac myosin and other heart antigens, drives ongoing low-level cardiac inflammation. This leads to myocardial fibrosis and progressive

ventricular dysfunction. For this reason, numerous clinical trials have evaluated the potential benefit of immunosuppression in virus-negative acute myocarditis with limited results.[112, 113]

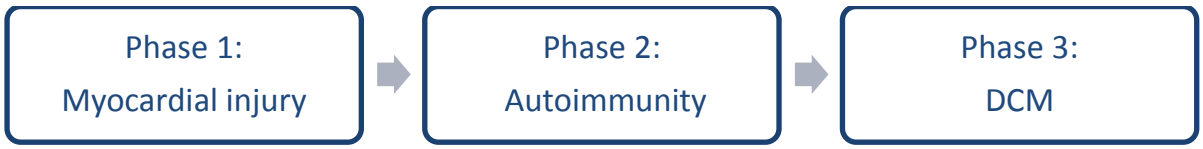


Figure 5-1. Pathophysiological stages of myocarditis progression to DCM

GENETIC SUSCEPTIBILITY

Although well over 95% of the general population experience an acute viral infection in their lifetime, for example, a common cold or influenza virus, only 1-5% of infected individuals develop myocarditis. Of these affected individuals, only a subset progress to DCM. This suggests two levels of underlying genetic susceptibility to; (i) myocarditis itself and (ii) progression to DCM (figure 5-2).

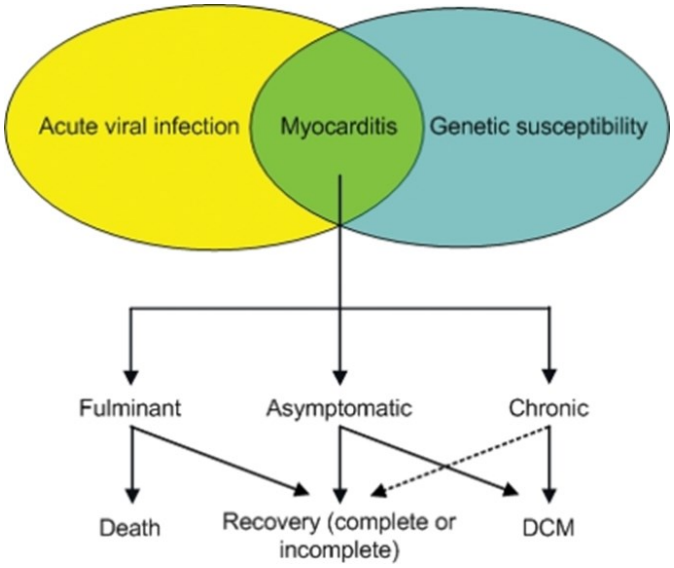


Figure 5-2. Diagram to show the genetic and environmental interaction in acute myocarditis and spectrum of downstream clinical outcomes (reproduced with permission).[252]

Genetic susceptibility to myocarditis is thought to arise from variants in components of the innate immune response, particularly the major histocompatibility complex (MHC) genes, as determined in studies of different inbred strains of mice.[253, 254] It was demonstrated over 30 years ago that only certain strains of mice were susceptible to the development of cardiac autoimmunity following CVB3 viral infection and subsequent DCM suggesting a genetic aetiology.[255] During the first 10 days after CVB3 infection, there are significant differences among strains with respect to prevalence and severity of myocarditis. Recent molecular analyses demonstrated a significant correlation between myocarditis-related DCM and MHC class II antigens, particularly HLA-DR4.[256] Several non-MHC loci have also been shown to modify susceptibility. Toll-like receptors play an important role in the early activation of the innate immune response against viral infections. Toll-like receptor 3 (TLR3) deficient mice showed increased vulnerability to CVB3 infection with disruption of interferon signalling.[257] Many of these deficiencies overlap with other autoimmune disease susceptibility loci, suggesting that although different autoimmune diseases have different clinical manifestations, they are likely to be controlled by a number of shared inherited genetic abnormalities.[258] A genome wide association study of sufficient sample size is required to investigate the complexity and interaction of such polymorphisms in the immune system of human patients with myocarditis, which has not been performed to date.

ROLE OF TITIN TRUNCATING VARIANTS

The predisposition of a subset of myocarditis patients to progress to DCM may be attributed to the potential interaction between the environment and underlying variants in genes linked to DCM. This is supported by reports of viral myocarditis and DCM segregating within families.[259] Previous work from our group has shown that heterozygous mutations that truncate full-length titin (TTN), the most abundant structural, sensory and signalling filament protein in striated-muscle, are found in up to 25% of familial cases of non-ischaemic DCM and 18% of

sporadic cases (figure 5-3).[260] In previously 'healthy' individuals, TTN truncating variants (TTN-tv) appear to render the heart more vulnerable to failure when subject to a 'second hit' which may take the form of pregnancy,[261] atrial fibrillation,[262] excess alcohol consumption[263] or cancer-therapy induced cardiomyopathy.[264] Several isoforms are produced from the multiple (364) exons of the gene due to extensive alternative splicing. Whilst TTN-tv occur in approximately 1% of healthy individuals without overt cardiomyopathy, the clinical significance of TTN-tv is partly predicated by whether the exon is constitutively expressed in all isoforms.[262] Therefore, healthy individuals with specific TTN-tv may have a genetic susceptibility to developing DCM because of an environmental modifier, such as myocarditis.

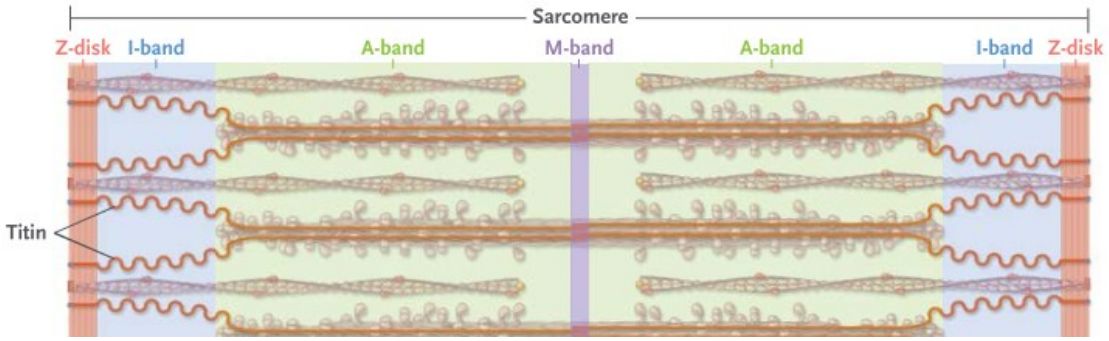


Figure 5-3. Cardiac sarcomere consisting of three major filaments; actin, myosin and titin. (Reproduced with permission from Ware et al,[261] Copyright Massachusetts Medical Society).

PUBLISHED DATA

To date, the prevalence of genetic variants in known cardiomyopathy genes has only been studied in a cohort of 42 children presenting with acute myocarditis.[265] Cases were confirmed by CMR or EMB Dallas criteria. The key finding was that 7 out of 42 patients (16.7%) carried rare homozygous (but not heterozygous) non-synonymous or splice-site variants in 6 cardiomyopathy-associated genes (BAG3, DSP, PKP2, RYR2, SCN5A, or TNNI3). The 2 patients with the most severe mutations (homozygous nonsense in DSP and splicing variants

in TNNI3) died shortly after disease onset. Whereas the other 4 patients with missense variants recovered with treatment. Using a whole-exome sequencing approach, the investigators also looked at variants in over 60 TLR3- or interferon-related genes and found no enrichment, suggesting a lack of underlying 'inborn errors' of the immunity. There is a clear need to investigate these findings in much larger patient cohorts.

MYOCARDITIS OR ARVC

In addition to unmasking or acting in concert to produce DCM, there are also reports of acute myocarditis mimicking the clinical presentation of 'hot phases' of arrhythmogenic right ventricular cardiomyopathy (ARVC).[266] ARVC is an inherited myocardial disease characterised by the presence of defects in cell-to-cell junctions leading to fibro-fatty replacement and high-incidence of ventricular arrhythmia, heart failure and sudden death, particularly in the young.[267] Left-ventricular involvement was found in 76% of ARVC cases on biopsy and was associated with more severe inflammatory infiltrates.[266] In an electroanatomic mapping-guided endocardial biopsy study of 30 patients with ARVC by Task Force Criteria, 15 patients had their diagnosis revised to myocarditis on the basis of biopsy findings.[268] Of note, in subsequent correspondence, the value of genetic screening for desmosomal mutations was highlighted, particularly given that only 5 out of 15 'myocarditis' patients had viral genomes detectable within the myocardial tissue. In a separate study of 131 patients with confirmed AVC by Task Force Criteria, 6 patients presented with acute myocarditis over a median follow-up of 34 months.[269] Four of these patients carried truncating variants in DSP (c.1339C>T and c.5318delT) and were all related family members. The episodes of myocarditis heralded worsening outcomes with increased LV systolic dysfunction and ventricular arrhythmia. Conversely, amongst 64 non-affected mutations carriers, there was only 1 case of myocarditis in a 38 year-old female with a similar DSP variant, which had no

impact on her normal phenotype or outcome. The burden of underlying genotype-positive ARVC amongst patients with a clinical diagnosis of myocarditis is unknown. It remains unclear whether myocarditis represents a superimposed phenomenon during the natural history of ARVC, or a primary event that potentially unmasks the ARVC phenotype and increases morbidity.[100, 270] We sought to also investigate this hypotheses in our large and well-characterised cohort of myocarditis patients.

5.3. Methods

STUDY COHORTS

We recruited and leveraged three separate cohorts for this study (figure 5-4):

1. Myocarditis patients: Consecutive patients with CMR- or biopsy-confirmed myocarditis were prospectively and retrospectively recruited into the research study with a venous blood sample, as described in chapter 4. The main exclusion criterion was coronary artery disease.

2. Healthy volunteers: A existing cohort of healthy volunteers that were recruited following public advertisements with no history of medical illness, no regular medication and normal cardiac structure and function confirmed on detailed CMR assessment (excluding assessment of late gadolinium enhancement).

3. ExAC population reference dataset: The ExAC dataset contains sequencing data for 60,706 unrelated individuals from a number of disease and population genetic studies. It is not specifically enriched for individuals with myocarditis.

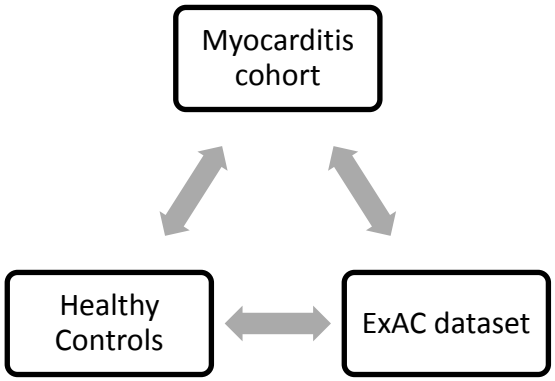


Figure 5-4. Overview of study cohorts used for gene-based burden testing

DNA EXTRACTION AND LIBRARY PREPARATION

DNA extraction for cases was performed from whole blood (single 0.5mL cryovial) using an automated platform (EZ1 Advanced XL, Qiagen) in the RBH Genetics and Genomics

laboratory. DNA quantity and quality was assessed using nanodrop (Thermo Scientific) to ensure 50 nanograms of DNA was available from each blood sample. DNA samples (assessed in batches of 96 individuals) were aliquoted into stock solutions and diluted preparations into 96 well plates. Indexing of the plate was crucial to avoid mixing of different patient samples.

Extracted DNA was then fragmented using a transposase enzyme, which also inserted an adapter sequence (or tag) to the ends of double stranded DNA, in a process known as 'tagmentation.' Magnetic beads attached to the DNA adapters, thereby allowing the use of isopropyl alcohol to wash away the enzyme and any other contaminants. Resuspension buffer was used to remove the magnetic beads. Samples were incubated for index PCR to amplify the samples using primers that contained a unique 8 base pair barcode sequence to enable sample multiplexing. Magnetic beads were used to wash away excess primer A fluorescent probe was added to allow DNA quality and quantity to be assessed using fluorometry (Qubit, Life Technologies). At this stage, purified DNA lengths (or libraries) from the whole genome were contained within each sample well.

For library preparation, specific hybridisation capture probes were used to target and enrich the selected genes implicated in cardiac conditions using the TruSight Cardio kit (Illumina). This included 174 genes associated with inherited cardiac conditions.[143] The biotin/streptavidin pull down method was used, where rapid capture oligos specifically bound to DNA of the target genes were captured with streptavidin coated magnetic beads and then amplified with 12 cycles of PCR with indexing primers. Following elution from the magnetic beads, the targeted DNA sequencing library was then amplified ('solid-phase bridge amplification') again for 16 hours on the Illumina flow cell to generate clusters of primed DNA (figure 5-5).

Healthy volunteer samples were prepared using the same approach by the same team working in the same lab environment.

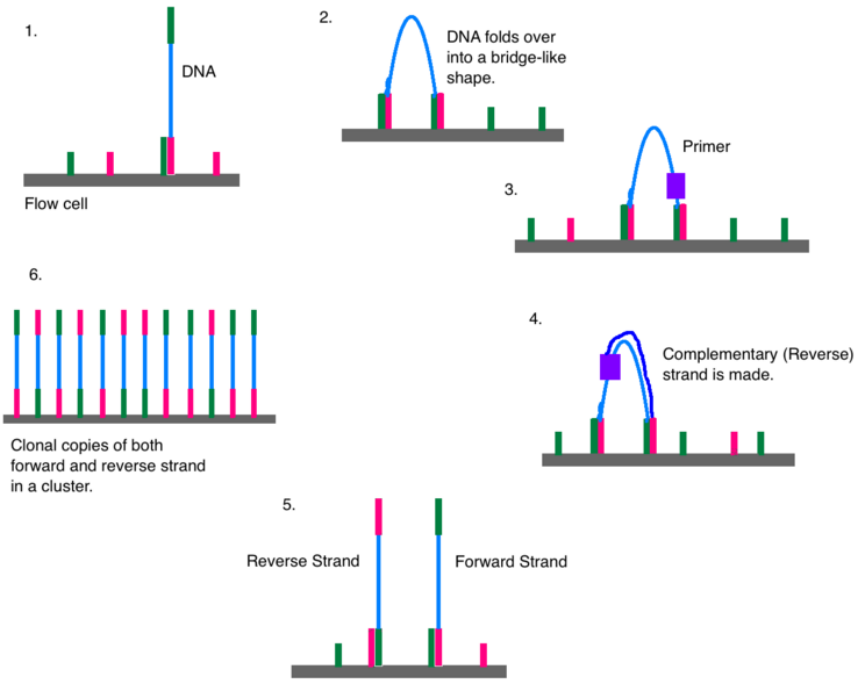


Figure 5-5. Overview of cluster generation. (1) Randomly fragmented genomic DNA attach to specific adapters for target genes on flow cell surface. (2) Each strand bends over and attaches to a second oligo forming a bridge. (3,4) A primer synthesizes the reverse strand (bridge amplification). (5) The two strands are released and straighten. (6) Each new strand forms a new bridge and the process repeats to generate a cluster of double stranded DNA immobilised on the flow cell ready for sequencing (reproduced from Wikimedia Commons)[271]

TARGETED SEQUENCING

DNA samples in this study were sequenced using the Illumina TruSight Cardio Sequencing panel on the Illumina NextSeq platform. In brief, next generation sequencing was performed using a proprietary flow cell surface designed to bind and present immobilised clusters of amplified DNA libraries to enzymes and other reagents. A fluorescently labelled nucleotide was added to each single nucleic acid chain. This label served as a terminator for polymerisation. Fluorescence imaging was then performed to identify the incorporated nucleotide. The terminating nucleotide and fluorescent dye were then cleaved and the process repeated many times, in what is referred to as 'sequencing by synthesis.' This occurred in

parallel across the numerous clusters of amplified DNA. Base calls were made directly from signal intensity measurements during each cycle of SBS, allowing for consensus of fluorescence across all the identical templates in each cluster in that cycle. This process enabled accurate base-by-base sequencing, that eliminated sequence-context specific errors, and generated approximately 100-150bp reads. Automated data collection software aligned the short reads to a reference genome.

GENERAL AND PLATFORM SPECIFIC BIOINFORMATICS

Variants were annotated and filtered according to customised inhouse bioinformatics pipeline run by a team of bioinformaticians. In brief, NGS reads underwent quality control per sample before being aligned against a reference human genome. The resulting sequence alignment map (SAM) file, which could be compressed into a binary alignment map (BAM), underwent pre-processing to identify any duplicates and recalibrate quality scores. The genome analysis toolkit (GATK) was then used to identify (or 'call') any variants.[272] GATK provided a structured programming framework to enable the analysis of this massive data set, including the removal of any other errors resulting from library preparation, base calling, mapping or misalignment. This filtered VCF was subsequently taken forward for quality control and burden testing analysis.

OUTLINE OF VARIANT FILTERING METRICS

Quality control steps were performed on annotated VCF files using scripts to assess coverage, allelic balance, strand bias, quality scores, mapping qualities, and read position (for example, proximity to an indel). Poor quality variants not meeting pre-specified quality filters were excluded to ensure adequate coverage with recommended GATK parameters:

- Read depth >10, meaning that each base had to be sequenced at least 10 times.
- Quality of depth score >4, referred to the variant call confidence normalised by read depth.

- Allelic balance >0.2, referred to the percentage of reads that support a variant.

Following these filtering and quality control steps, a list of variants present was generated, which included all myocarditis cases and all healthy controls.

BURDEN TESTING

Whilst the Illumina TruSight Cardio sequencing kit captures many genes linked to DCM and ARVC, our primary focus was on protein-altering variants in the 11 DCM genes (TTN, MYH7, LMNA, TTNT2, TCAP, DSP, SNC5A, BAG3, TNNC1, VCL and RBM20) and 5 ARVC genes (DSP, PKP2, DSG2, DSC2 and JUP) with the most robust evidence of disease association.[144] For all samples, variants were annotated with the Variant Effect Predictor (VEP) and according to defined transcripts for these 11 DCM and 5 ARVC genes. Truncating variants were defined as those resulting in nonsense, frameshift, or essential splice site mutation. Non-truncating variants were defined as those resulting in missense variants, inframe indels or non-essential splice sites.

Rare variants were defined as having minor allele frequency (MAF) <0.0001 (in other words <0.01%) in the ExAC dataset, and aggregate frequencies (or prevalence) were assessed for each gene in cases and controls. Gene-based burden testing was performed using R (http://www.R-project.org) by comparing the burden of rare variants in each gene between myocarditis cases and HVOLs, and myocarditis cases and ExAC using Fisher's exact test (one-sided for cases vs HVOL/ExAC), with a significance level of $p<0.05$, adjusted with Bonferroni correction for multiple testing.

SAMPLE SIZE CALCULATION

As already discussed in chapter 4, this was based on previous work showing that the prevalence of TTN mutations in a healthy population is 1% and our estimates that in myocarditis patients progressing to DCM, prevalence is expected to match that reported in DCM and peripartum

cardiomyopathy (~15%).[261] Therefore, the total number of patients required in this study was 70 patients per group (myocarditis with or without DCM) with 90% power to detect a significant difference in the proportion of patients with a TTN mutation at the 5% significance level (figure 5-6). We aimed to actively recruit 210 patients given the expectation that one third of patients were likely to progress to DCM. Allowing for 10% drop-out, this was increased to 230 patients. The prevalence of TTN-tv in healthy controls was a critical determinant and recent evaluation of the ExAC reference population reported TTN-tv in exons expressed in all isoforms occurred in 0.36%. This observation was thought to improve our statistical power to detect a significant difference, particularly if TTN-tv were enriched less than 15% amongst myocarditis patients progressing to DCM.

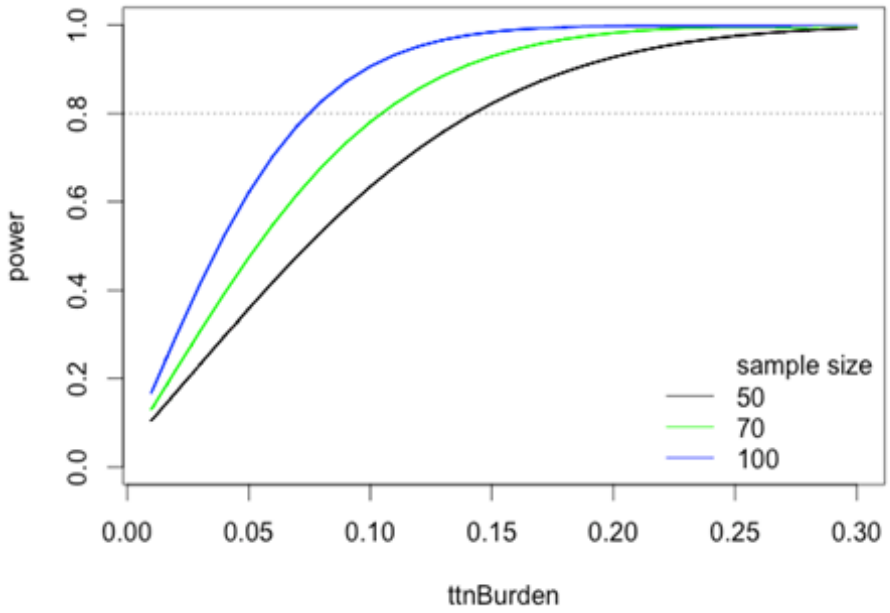


Figure 5-6. Plot to show statistical power as the observed TTN-tv burden in myocarditis progressing to DCM varied from the expected 15% (assuming baseline TTN-tv burden of 1% in those without DCM). Different sample sizes per group are represented by the different curves. The horizontal line represents the threshold for 80% power.

5.4. Results

COHORT REVIEW

The final cohorts for burden testing consisted of 231 myocarditis patients compared to 1054 healthy controls and 68,000 participants in the ExAC dataset. Of the myocarditis study cohort, there were 185 men (80%), median age at recruitment was 30 years (IQR 25-40 years) and the majority of the cohort were NYHA class 1 or 2 (n=220, 95%). Of the healthy volunteers, there were 464 men (44%) with median age at recruitment of 35 years (IQR 27-48 years).

EVALUATION OF VARIANT QUALITY BY TI/TV RATIO

The Ti/Tv ratio is the ratio of the number of transitions to the number of transversions for a single nucleotide polymorphism. There are 2 possible transitions (A<->G and C<->T) but 4 possible transversions (A<->C, A<->T, G<->C or G<->T). If the distribution of transitions and transversions was random, we would expect a ratio of 0.5. However, it is rare to see a methylated cytosine undergo deamination to become thymine. As a result, there is usually a strong lean towards transition mutations. The mean Ti/Tv ratios for the myocarditis and healthy volunteer's cohorts were 2.85 and 2.81, respectively (figure 5-7). These fall within the expected ranges and highlight the good performance of GATK as a tool for variant calling.[273]

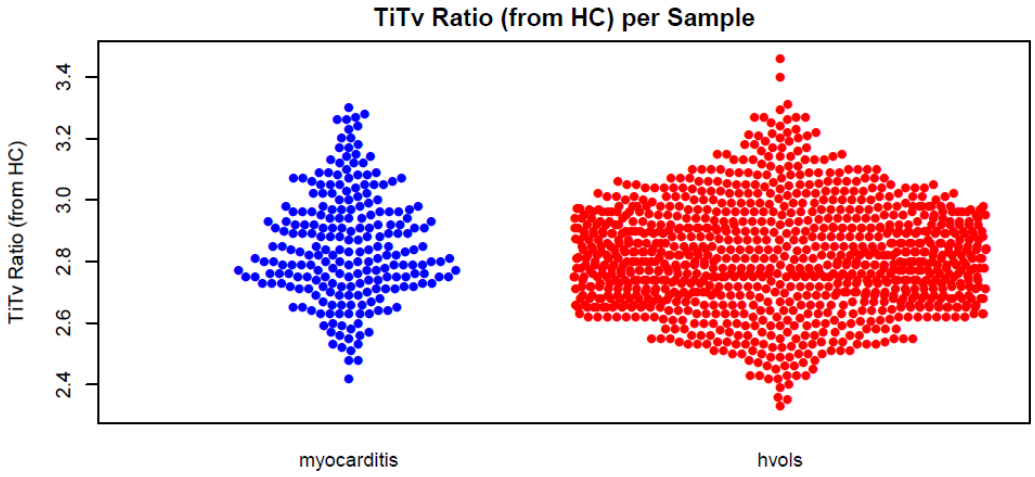


Figure 5-7. Transition-to-transversion ratio of myocarditis (mean 2.85) and healthy control (mean 2.81) cohorts. [HC = Haplotype Caller]

EVALUATION OF DEPTH OF SEQUENCING COVERAGE

The depth of coverage refers to the number of times the bases within a target gene have been sequenced, or 'read.' This is determined by the accuracy of genome alignment algorithms and by the uniqueness or 'mappability' of sequencing reads within a target gene.[274] The median coverage at varying depths across the myocarditis cohort and HVol cohort is shown below (table 5-1). In summary, all samples in the myocarditis cohort were covered at ≥ 30x and 96.5% were covered at ≥ 50x indicating good data quality (figure 5-8). The median coverage was approximately 700x in both cohorts on our platform.

		Bases >=10x	Bases 20x	Bases >=30x	Bases >=50x
Myocarditis	Median	99.98	99.94	99.88	99.69
	1st quartile	99.94	99.88	99.78	99.48
	3rd quartile	99.99	99.96	99.93	99.86
Hvols	Median	99.97	99.93	99.89	99.74
	1st quartile	99.95	99.89	99.80	99.48
	3rd quartile	99.99	99.96	99.93	99.84

Table 5-1. Summary of median (IQR) depth of coverage in the myocarditis and HVol cohorts.

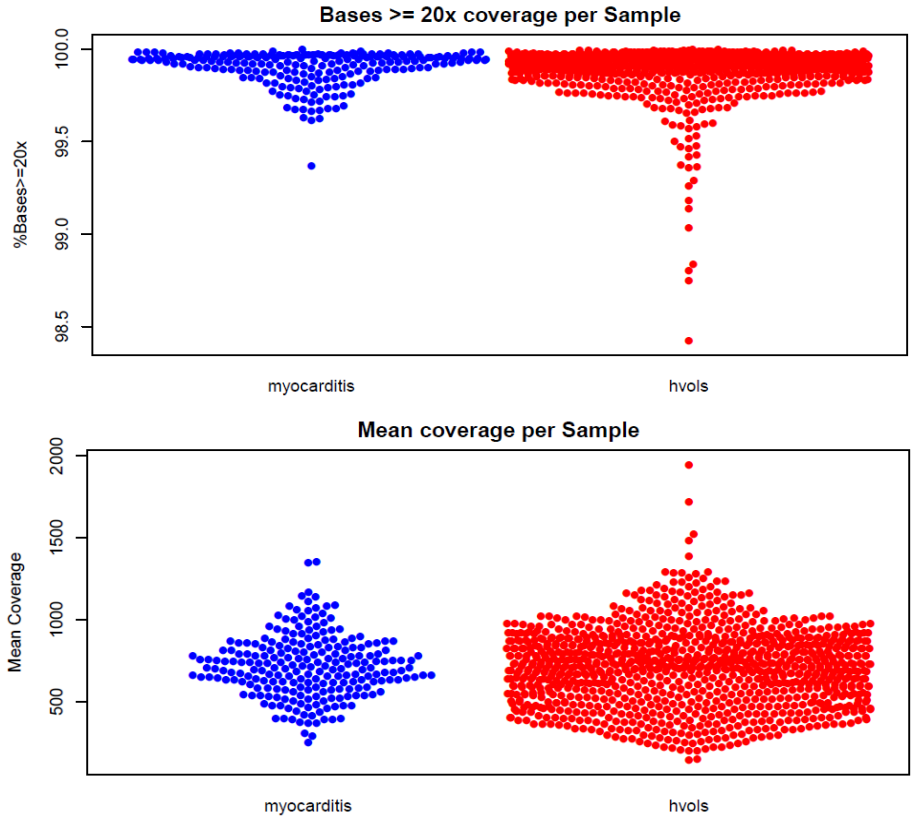


Figure 5-8. Bee swarm plot showing all individual samples with read depth >= 20x (top) and mean coverage for all individual samples (bottom).

OVERALL BURDEN OF RARE GENETIC VARIATION IN MYOCARDITIS PTS VS HVOLS

In the myocarditis cohort, 11 patients (4.8%) had truncating variants and 137 (60.6%) had non-truncating variants in key DCM (figure 5-10) and ARVC (figure 5-9) genes. The overall burden of variants per gene is shown below and was derived from the difference in frequencies of variants seen amongst myocarditis cases versus healthy controls.

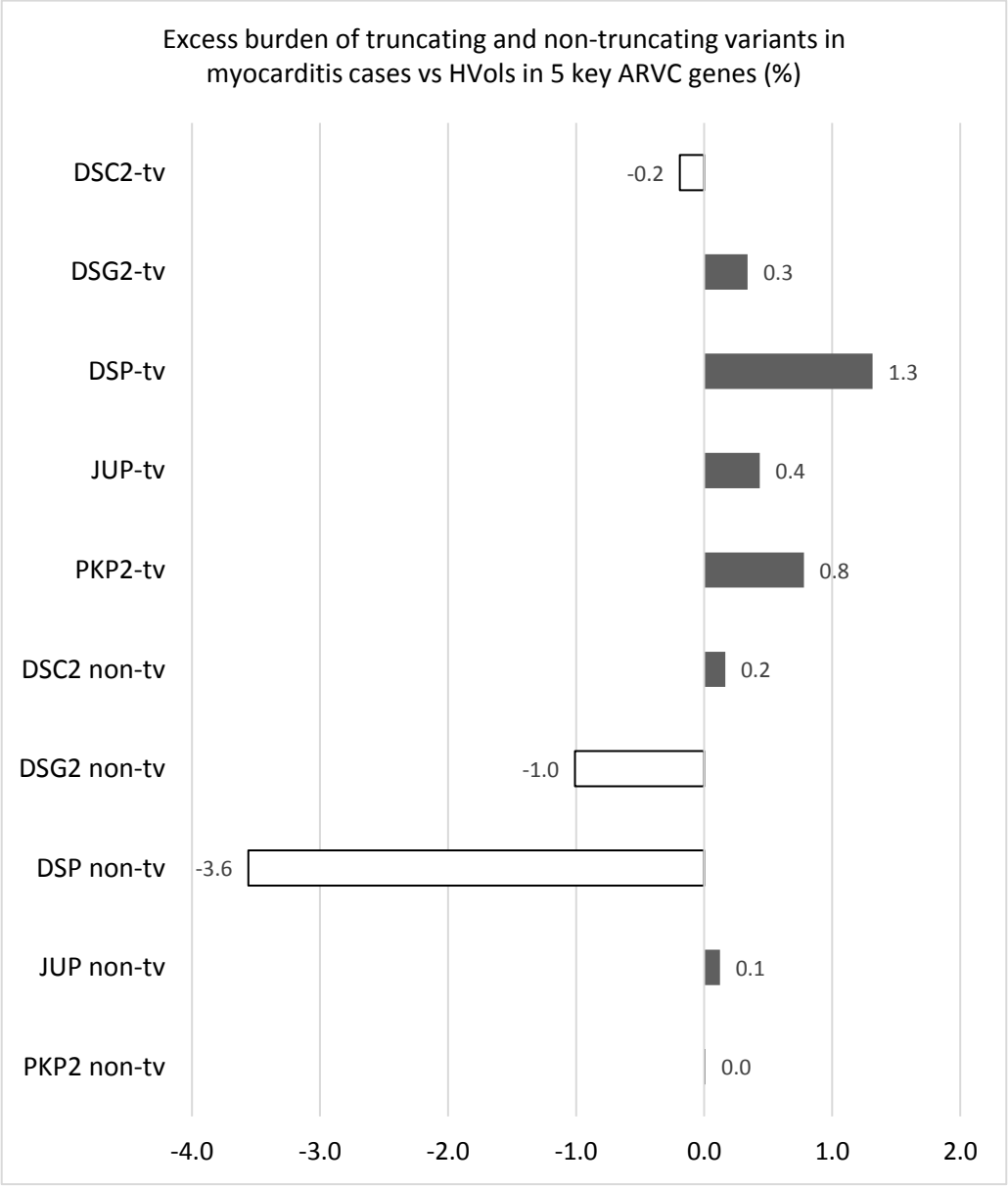


Figure 5-9. Excess burden (in red) of truncating (top) and non-truncating (bottom) variants in myocarditis cases vs healthy volunteers (HVols) in the 5 key ARVC genes.

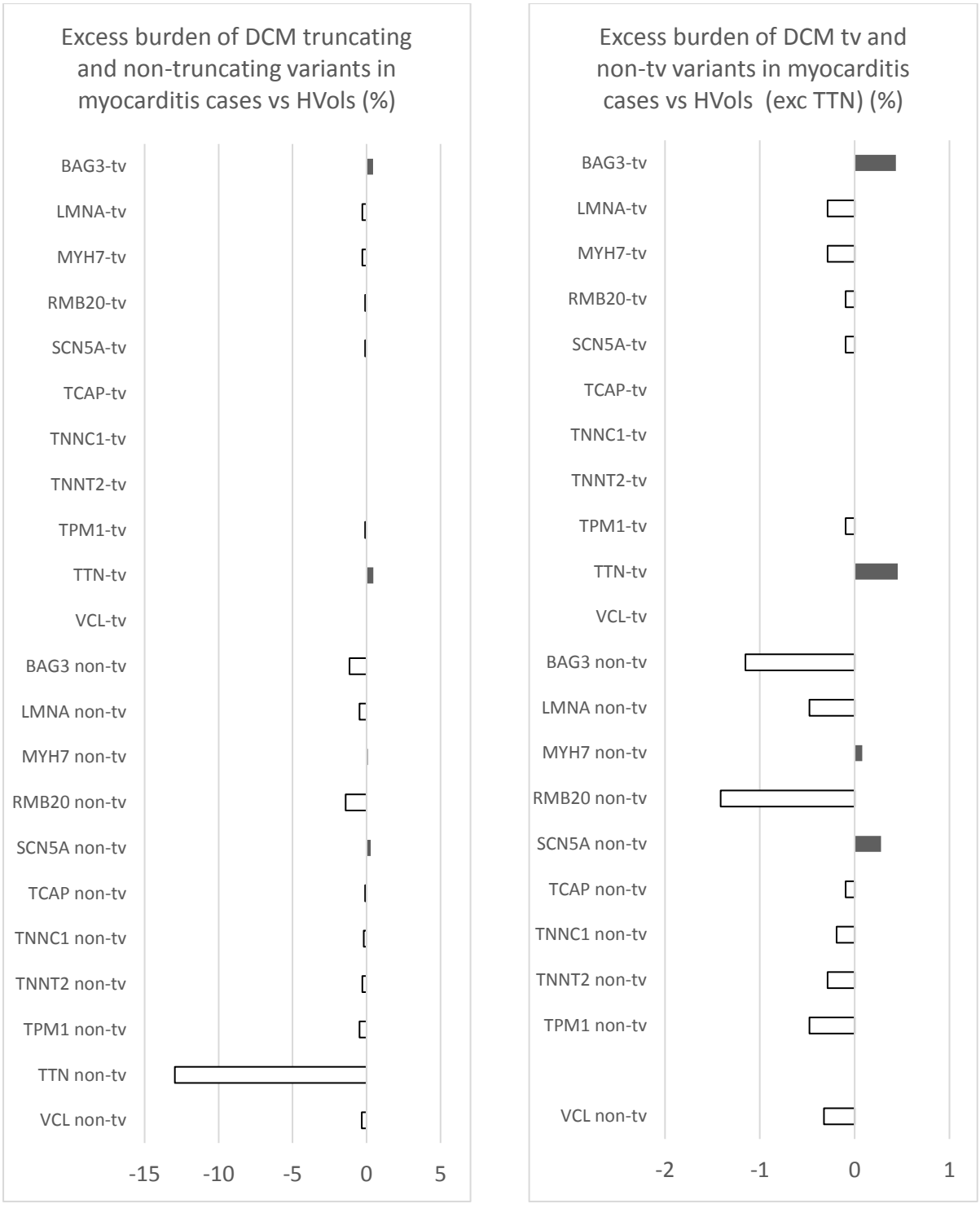


Figure 5-10. Excess burden (red bars) of truncating (top) and non-truncating (bottom) variants in myocarditis cases vs healthy volunteers (HVols) in key DCM genes. The panel to the right shows the same data expanded without TTN non-tv.

We then compared the burden of truncating and non-truncating variants across all ARVC and DCM genes in myocarditis cases versus HVols, as shown below (table 5-2):

Gene	Variant Class	Case freq	Control freq	Case excess	Odds ratio	CI lower	CI upper	Fishers exact
ARVC (all)	Truncating	0.03	0.00	0.03	8.20	2.38	28.26	0.001
	Non-truncating	0.06	0.11	-0.05	0.56	0.31	1.01	0.056
DCM (all)	Truncating	0.03	0.02	0.01	1.80	0.74	4.36	0.175
	Non-truncating	1.22	1.92	-0.70	0.64	0.48	0.85	0.003

Table 5-2. Odds ratios and Fisher's Exact test results for the significance of the excess of rare variation in myocarditis cases versus HVols in key ARVC and DCM genes (Fisher's Exact test 2-sided level of significance = 0.05, with Bonferroni correction for 4 tests = 0.0125).

There was a significant excess burden (3%) of truncating variants in all ARVC genes combined amongst myocarditis cases compared to HVols (odds ratio 8.2; 95% CI 2.4-28.3; p=0.001). Conversely, there was a significant excess burden of non-truncating variants in the DCM genes amongst HVols rather than myocarditis cases (OR 0.6; 95% CI 0.5-0.9; p=0.003). This was primarily driven by the high burden of missense variation in TTN seen in both cohorts, the biological significance of which remains unclear, and is also poorly characterised in population datasets. However, after exclusion of non-truncating variants in TTN, the excess burden of other non-truncating variants in DCM genes amongst HVols rather than myocarditis cases remained present (OR 0.49; 95% CI 0.3-0.8; p=0.003).

GENE-BASED BURDEN OF RARE VARIATION IN MYOCARDITIS PTS COMPARED TO HVOLS

We subsequently compared the burden of truncating and non-truncating variants for the individual ARVC and DCM genes in myocarditis cases versus HVols, as show in the following series of tables (tables 5-3 to 5-6). We found a significant excess burden (1.3%) of truncating variants in the DSP gene in our myocarditis cases compared to our HVols (OR 27.7; 95% CI 1.4-555.6; p=0.0057; table 5-4). This was driven by the presence of 3 truncating DSP variants in 3 separate myocarditis cases compared with 0 such variants in 1054 healthy controls. None of the other ARVC genes reached significance alone. To assess whether the significant enrichment of all truncating ARVC genes in myocarditis was attributed to DSP alone, we combined the truncating variants seen in the 4 other non-DSP genes in myocarditis cases against HVols and confirmed these variants were also significantly enriched in myocarditis cases (OR 4.6; 95% CI 1.1-18.6; p=0.0391).

Gene	Variant Class	Cases +	Cases -	Controls +	Controls -	Case freq	Control freq	Odds ratio	CI lower	CI upper	Fishers exact
BAG3	Truncating	1	230	0	1054	0.0043	0.0000	9.17	0.31	274.03	0.1798
LMNA	Truncating	0	231	3	1051	0.0000	0.0029	0.76	0.04	15.19	1
MYH7	Truncating	0	231	3	1051	0.0000	0.0029	0.76	0.04	15.19	1
RMB20	Truncating	0	231	1	1053	0.0000	0.0009	2.28	0.08	68.15	1
SCN5A	Truncating	0	231	1	1053	0.0000	0.0009	2.28	0.08	68.15	1
TCAP	Truncating	0	231	0	1054	0.0000	0.0000	-			
TNNC1	Truncating	0	231	0	1054	0.0000	0.0000	-			
TNNT2	Truncating	0	231	0	1054	0.0000	0.0000	-			
TPM1	Truncating	0	231	1	1053	0.0000	0.0009	2.28	0.08	68.15	1
TTN	Truncating	3	228	9	1045	0.0132	0.0086	1.53	0.41	5.69	0.4610
VCL	Truncating	0	231	0	1054	0.0000	0.0000	-			

Table 5-3. *Is there an excess burden of rare truncating variants in our myocarditis cases versus our HVols in key **DCM** genes?*

Odds ratios and Fisher's Exact test results testing for significance of the excess of rare truncating variation in myocarditis cases versus HVOLs in DCM genes (Fisher's Exact test 2-sided level of significance = 0.05, with Bonferroni correction for 11 tests = 0.005). For cells with zero values, 0.5 was added to all cells before calculating the odds ratio (lightly shaded).

Gene	Variant Class	Cases +	Cases -	Controls +	Controls -	Case freq	Control freq	Odds ratio	CI lower	CI upper	Fishers exact
DSC2	Truncating	0	231	2	1052	0.0000	0.0019	1.14	0.05	25.33	1
DSG2	Truncating	1	230	1	1053	0.0043	0.0009	4.58	0.29	73.47	0.3273
DSP	Truncating	3	228	0	1054	0.0132	0.0000	27.74	1.38	555.65	0.0057
JUP	Truncating	1	230	0	1054	0.0043	0.0000	9.17	0.31	274.03	0.1798
PKP2	Truncating	2	229	1	1053	0.0087	0.0009	9.20	0.83	101.86	0.0851

Table 5-4. *Is there an excess burden of rare truncating variants in our myocarditis cases versus our HVols in key **ARVC** genes?*

Odds ratios and Fisher's Exact test results testing for significance of the excess of rare truncating variation in myocarditis cases versus HVOLS in ARVC genes (Fisher's Exact test 2-sided level of significance = 0.05, with Bonferroni correction for 5 tests = 0.01). For cells with zero values, 0.5 was added to all cells before calculating the odds ratio (lightly shaded).

Gene	Variant Class	Cases +	Cases -	Controls +	Controls -	Case freq	Control freq	Odds ratio	CI lower	CI upper	Fishers exact
BAG3	Non-truncating	0	231	12	1042	0.0000	0.0115	0.2	0.0	3.2	0.1396
LMNA	Non-truncating	0	231	5	1049	0.0000	0.0048	0.5	0.0	8.3	0.5923
MYH7	Non-truncating	5	226	22	1032	0.0221	0.0213	1.0	0.4	2.8	1
RMB20	Non-truncating	3	228	28	1026	0.0132	0.0273	0.5	0.1	1.6	0.3412
SCN5A	Non-truncating	5	226	20	1034	0.0221	0.0193	1.1	0.4	3.1	0.7924
TCAP	Non-truncating	0	231	1	1053	0.0000	0.0009	2.3	0.1	68.1	1
TNNC1	Non-truncating	0	231	2	1052	0.0000	0.0019	1.1	0.1	25.3	1
TNNT2	Non-truncating	0	231	3	1051	0.0000	0.0029	0.8	0.0	15.2	1
TPM1	Non-truncating	0	231	5	1049	0.0000	0.0048	0.5	0.0	8.3	0.5923
TTN	Non-truncating	108	123	529	525	0.8780	1.0076	0.9	0.7	1.2	0.3462
VCL	Non-truncating	3	228	17	1037	0.0132	0.0164	0.8	0.2	2.8	1

Table 5-5. *Is there an excess burden of rare non-truncating variants in our myocarditis cases versus our HVols in key **DCM** genes?*

Odds ratios and Fisher's Exact test results testing for significance of the excess of rare non-truncating variation in myocarditis cases versus HVOLs in DCM genes (Fisher's Exact test 2-sided level of significance = 0.05, with Bonferroni correction for 11 tests = 0.005). For cells with zero values, 0.5 was added to all cells before calculating the odds ratio (lightly shaded).

Gene	Variant Class	Cases +	Cases -	Controls +	Controls -	Case freq	Control freq	Odds ratio	CI lower	CI upper	Fishers exact
DSC2	Non-truncating	3	228	12	1042	0.0132	0.0115	1.1	0.3	4.1	0.7410
DSG2	Non-truncating	1	230	15	1039	0.0043	0.0144	0.3	0.0	2.3	0.3312
DSP	Non-truncating	3	228	49	1005	0.0132	0.0488	0.3	0.1	0.9	0.0158
JUP	Non-truncating	4	227	17	1037	0.0176	0.0164	1.1	0.4	3.2	0.7806
PKP2	Non-truncating	2	229	9	1045	0.0087	0.0086	1.0	0.2	4.7	1

Table 5-6. *Is there an excess burden of rare non-truncating variants in our myocarditis cases versus our HVols in key **ARVC** genes?*

Odds ratios and Fisher's Exact test results testing for significance of the excess of rare non-truncating variation in myocarditis cases versus HVOLS in ARVC genes (Fisher's Exact test 2-sided level of significance = 0.05, with Bonferroni correction for 5 tests = 0.01).

GENE-BASED BURDEN OF RARE VARIATION IN OUR MYOCARDITIS PATIENTS VS THE EXAC POPULATION DATASET

In addition to our HVol cohort, we also compared the burden of variants between our myocarditis cases and the ExAC population dataset (tables 5-7 to 5-8).

As before, there was a significant excess burden of truncating variants in the gene DSP in myocarditis cases compared to ExAC (odds ratio 18.7; 95% CI 5.7-60.6; p=0.007).

Gene	Variant Class	Cases +	Cases -	Controls +	Controls -	Case freq	Control freq	Odds ratio	CI lower	CI upper	Fishers exact
BAG3	Truncating	1	230	Not assessed		0.0043		-			
LMNA	Truncating	0	231	8	51257	0.0000	0.0002	13.87	0.79	242.18	1
MYH7	Truncating	0	231	29	60441	0.0000	0.0005	4.51	0.27	74.08	1
RMB20	Truncating	0	231	2	10045	0.0000	0.0002	10.87	0.49	241.75	1
SCN5A	Truncating	0	231	34	56381	0.0000	0.0006	3.59	0.22	58.74	1
TCAP	Truncating	0	231	14	55219	0.0000	0.0003	8.54	0.51	143.79	1
TNNC1	Truncating	0	231	2	59154	0.0000	0.0000	64.02	2.88	1423.55	1
TNNT2	Truncating	0	231	17	56906	0.0000	0.0003	7.25	0.43	120.99	1
TPM1	Truncating	0	231	2	58725	0.0000	0.0000	63.56	2.86	1413.23	1
TTN	Truncating	3	228	501	58112	0.0132	0.0086	1.53	0.49	4.78	0.0492
VCL	Truncating	0	231	19	59372	0.0000	0.0003	6.76	0.41	112.46	1

Table 5-7. *Is there an excess burden of rare truncating variants in our myocarditis cases versus the **ExAC** population dataset in **DCM** genes?*

Odds ratios and Fisher's Exact test results testing for significance of the excess of rare variation in myocarditis cases versus ExAC controls in DCM genes (Fisher's Exact test 2-sided level of significance = 0.05, with Bonferroni correction for 11 tests = 0.005). For cells with zero values, 0.5 was added to all cells before calculating the odds ratio (highlighted in green).

Gene	Variant Class	Cases +	Cases -	Controls +	Controls -	Case freq	Control freq	Odds ratio	CI lower	CI upper	Fishers exact
DSC2	Truncating	0	231	16	60046	0.0000	0.0003	8.12	0.49	135.98	1
DSG2	Truncating	1	230	43	59623	0.0043	0.0007	6.03	0.83	43.97	0.1564
DSP	Truncating	3	228	42	59570	0.0132	0.0007	18.66	5.74	60.64	0.0007
JUP	Truncating	1	230	10	55881	0.0043	0.0002	24.30	3.10	190.58	0.0444
PKP2	Truncating	2	229	43	58362	0.0087	0.0007	11.85	2.85	49.22	0.0137

Table 5-8. *Is there an excess burden of rare truncating variants in our myocarditis cases versus the **ExAC** population dataset in **ARVC** genes?*

Odds ratios and Fisher's Exact test results testing for significance of the excess of rare variation in myocarditis cases versus ExAC controls in ARVC genes (Fisher's Exact test 2-sided level of significance = 0.05, with Bonferroni correction for 5 tests = 0.01). For cells with zero values, 0.5 was added to all cells before calculating the odds ratio (highlighted in green).

PATHOGENICITY OF VARIANTS ASSIGNED BY CARDIOCLASSIFIER

Having established the prevalence of DCM and ARVC variants in our myocarditis cohort, we sought to determine the pathogenicity of all identified variants by entering them into a computational decision support tool for inherited cardiac conditions to assign pathogenicity based on current ACMG criteria.[144] Whilst there was a high burden of VUS yet to be identified as disease-causing variants, there were 7 individuals with 'likely pathogenic' variants seen in the DSP (3), PKP2 (2), BAG2 (1) and DSG2 (1) (figure 5-11 & table 5-9). Six out of these 7 variants were seen in genes linked with ARVC, supporting earlier findings from burden testing. Family history was reviewed for these individuals with no symptomatic family members reported, although cascade family screening is required. No patients were found to fulfil modified Task Force Criteria for ARVC after comprehensive review of clinical phenotype (table 5-10, figure 5-13).

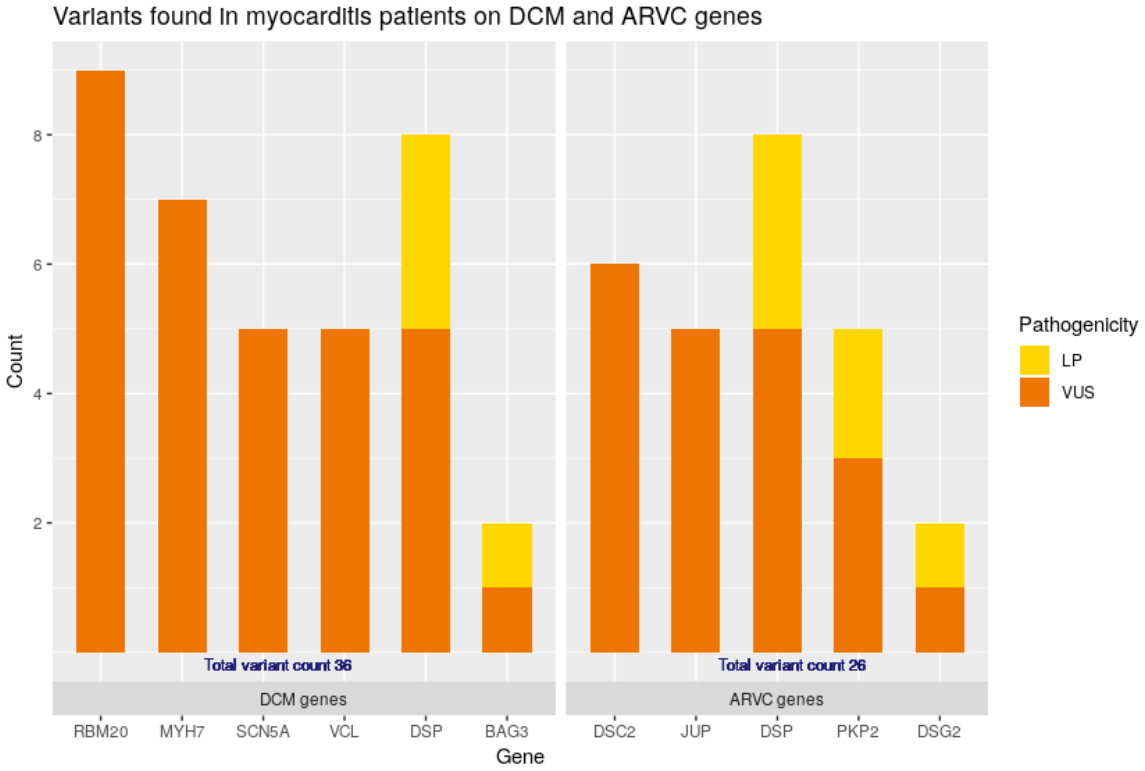


Figure 5-11. Variants found in myocarditis patients assigned as either variants of unknown significance (VUS) or likely pathogenic (LP) based on ACMG criteria applied through a semi-automated computational decision-support tool (CardioClassifier).[144]

Table 5-9. Details of all rare truncating variants (MAF <0.0001) and protein consequences identified in the myocarditis cohort with reference to HVols and ExAC

Disease	Gene	Variant type	Chromo	Position	Variant (HSGV)	Variant (protein)	ACMG class	ACMG rules	Hvol freq	ExAC freq
ARVC	DSP	Frameshift	chr6	7580729	c.4307_4308delCT	p.Thr1436ArgfsTer3	LP	PVS1, PM2	0	0
ARVC	DSP	Frameshift	chr6	7580842	c.4423delA	p.Thr1475ProfsTer9	LP	PVS1, PM2	0	0
ARVC	DSP	Nonsense	chr6	7581479	c.5056C>T	p.Q1686X	LP	PVS1, PM2	0	0
ARVC	PKP2	Frameshift	chr12	33030844	c.968_969delAG	p.Gln323ArgfsTer12	LP	PVS1, PM2	0	4.1E-05
ARVC	PKP2	Essential Splice Site	chr12	33031479	c.337-2A>T		LP	PVS1, PM2	0	0
ARVC	JUP	Frameshift	chr17	39914012	c.1797delC	p.Asn599LysfsTer88	VUS	PM2	0	0
ARVC	DSG2	Essential Splice Site	chr18	29104664	c.829_840delCTTGAAGGGATG	p.Leu277_Met280del	LP	PVS1, PM2	0	8.3E-06
DCM	TTN	Nonsense	chr2	179416939	c.90688G>T	p.G30230X	LP	PVS1 strong, PM2	0	0
DCM	TTN	Frameshift	chr2	179474687	c.51459_51462delTGTA	p.Asp17153GlufsTer11	LP	PVS1 strong, PM2	0	0
DCM	TTN	Nonsense	chr2	179531966	c.35794G>T	p.E11932X	VUS	PM2	0	0
DCM	BAG3	Frameshift	chr10	121429416	c.235delG	p.Ala79LeufsTer132	LP	PVS1, PM2	0	0

5.5. Discussion

The underlying genetic basis of acute myocarditis and progression to DCM remains unknown. In this study of 231 unselected patients with CMR or biopsy confirmed acute myocarditis, we leveraged the power of targeted next generation sequencing and informed variant curation to demonstrate that rare genetic variants linked to ARVC are enriched within this cohort. To the best of our knowledge, this is the largest cohort of patients with myocarditis to be genotyped. We did not find significant enrichment of TTN-tv with myocarditis acting as a second hit unmasking a DCM phenotype and discuss possible reasons for this below. Overall, this data may guide future studies to understand and dissect the genetic susceptibility of acute myocarditis and ARVC.

MYOCARDITIS AS AN ENVIRONMENTAL MODIFIER FOR DCM

The genetic architecture of dilated cardiomyopathy remains complex and the role of environmental factors triggering phenotypic expression in previously healthy genotype-positive individuals formed the basis of this study. This was highly relevant given that 1% of the healthy population were found to harbour a titin truncating variant without overt DCM but subtle forms of eccentric cardiac remodelling, as shown with machine-learning-based 3D mapping studies.[275]

Peripartum cardiomyopathy was previously not considered a genetic disease but was found to share genetic predisposition with familial and idiopathic DCM in a study of 172 patients.[261] More recently, TTN-tv titin truncating variants were similarly found to be enriched amongst patients with alcohol excess and chemotherapy that subsequently develop cardiac impairment.[263, 264] These three studies suggested that genotype-positive individuals may be phenotypically silent until the occurrence of an environmental trigger. The molecular phenotype of TTN-tv is understood to represent a form of chronic cardiac adaptation to heart

failure with a shift away from fatty acid metabolism and activation of the mTORC1 pathway, and limited ability to respond to further stress.[275] This may explain why haemodynamic stress associated with pregnancy may result in altered vascular and hormonal responses leading to a DCM phenotype. We hypothesised that acute myocarditis may pose similar haemodynamic stress due to reduced LV compliance from myocardial oedema and stunning, with an added inflammatory pathway, ultimately leading to DCM in susceptible individuals. Further support came from published reports of families with inflammatory DCM, which pointed towards a genetic basis.[276]

We therefore suspected a similar paradigm with acute myocarditis. However, TTN-tv were not found to be significantly enriched within out cohort of 231 myocarditis patients. The reasons for this are likely to be multifactorial. Firstly, the number of patients experiencing persistent LV dysfunction was only 10%, rather than the expected 30%, consistent with previous biopsy-confirmed patient series.[6] For this reason, our sample size of 230 would not have been sufficient to include 70 patients with inflammatory DCM. Those with LV dysfunction were primarily recruited from our transplant centre and presented with heart failure, as opposed to developing heart failure over the study period.

Secondly, the majority of our patients were indeed recruited following pseudo-infarct presentations rather than heart failure presentations. In this setting, it has been shown that event rates are low and outcomes are generally favourable, in contrast to those with sub-acute presentation arising from ventricular dysfunction and arrhythmia. In a landmark study of 128 biopsy-confirmed cases of myocarditis, patients with parvovirus B19 presented with chest pain, had lateral wall LGE and recovered within months. Whereas, those with human herpes virus 6 presented with new onset heart failure, had septal LGE and frequently progressed to chronic DCM.[28] Assessment of viral aetiology was limited in our cohort due to the infrequent use of cardiac biopsy, and geographical differences in viral agents may a play a role in determining

risk of progression to DCM. Thirdly, such differences in severity determined by viral aetiology suggest that other mechanisms, aside from TTN-tv, may be implicated in the progression of viral myocarditis to DCM. For example, enteroviruses such as Coxsackievirus B3 directly cleaved dystrophin and other dystrophin-associated glycoproteins in infected mice hearts leading to DCM.[277] Lastly, the role of autoimmunity in progression to DCM, which has been studied in murine models, may play a more prominent role in myocarditis, a heterogenous condition, as opposed to a single hit in the form of pregnancy of alcohol.

ROLE OF NON-TRUNCATING DCM VARIANTS

Amongst our healthy volunteer population, there was a significant excess of non-truncating variation in the 9 key DCM genes compared to the myocarditis cohort. Missense variation in TTN (TTN-ms) was the main contributor to this. The titin gene encodes the largest human protein and it plays a key role in passive myocyte stiffness. The biological implications of missense variation in TTN are largely unknown.[278] In a cohort of 147 DCM patients that underwent sequencing for 313 TTN exons covering that two main isoforms, a non-random distribution of 'likely' and 'possibly' disease-causing variants was found suggesting a potential biological role for some TTN-ms.[279] However, our findings suggested a protective effect of missense variation in TTN. To explore this further, we compared the burden of TTN-ms in our HVols to ExAC and found that TTN-ms were significantly enriched in our HVols, which suggests differences could have been driven by sensitivity of panel sequencing. We therefore compared the burden of TTN-ms in myocarditis cases and ExAC and found no significant difference in the burden of TTN-ms.

ARVC MASQUERADING AS MYOCARDITIS

Our main positive finding was the significant enrichment of truncating variants in key ARVC genes (ARVC-tv) in myocarditis cases compared to HVols (OR 8.2; 95% CI 2.4-28.3; P=0.001). When comparing ARVC-tv in myocarditis cases to ExAC, this finding remained

highly significant (OR 11.9; 95% CI 5.5-25.6; P<0.0001). We did not find any significant differences in the burden of non-truncating ARVC variants. Loss-of-function (truncating) variants in these 5 ARVC genes have the most robust evidence of disease association are found in ~46% of ARVC patients.[280]

DESMOPLAKIN

Desmoplakin is a critical structural component in the desmosome (figure 5-12). Two DSP proteins homodimerize to form a single macromolecular complex that anchors plakoglobin/plakophilin at the N-terminal domain to intermediate filaments (desmin) at the C-terminal region.[281] Knockout mice for DSP display embryonic lethality.[282] Truncating variants in DSP account for up to 39% of pathogenic variants found in human cases of ARVC.[280] Aside from one patient carrying a homozygous nonsense DSP variant in a series of 42 paediatric myocarditis patients, no other studies to date have systematically investigated the burden of ARVC variants amongst adults myocarditis patients.[265] The 3 patients identified with DSP-tv all presented with typical pseudo-infarct features and normal LV function. None of these variants were found in our HVol cohort or had been previously reported in ExAC. CMR findings were consistent with acute myocarditis as shown below, with no features to suggest ARVC. There was also no family history of cardiac disease. Therefore, it would seem unlikely that these presentations represented previously undiagnosed ARVC.

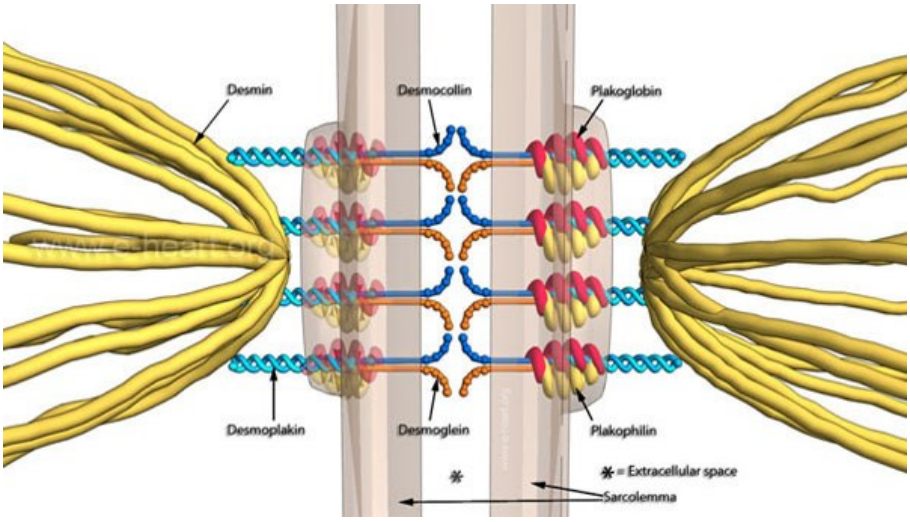


Figure 5-12. Illustration to show the structural proteins within the specialised cell-to-cell junctions of the intercalated disc, known as desmosomes. Desmoglein (DSG2) and desmocollin (DSC2) are members of the cadherin superfamily of transmembrane proteins involved in cell adhesion. Desmoplakin (DSP) interacts with cytoplasmic desmin filaments and the plaque proteins, plakoglobin (JUP) and plakophilin (PKP2). Reproduced with permission (see appendix).[283]

PLAKOPHILIN [PKP2]

Plakophilin is another critical structural component responsible for up to 74% of pathogenic variants found in ARVC.[280] It localises to the desmosome and binds plakoglobin, desmoplakin and the desmosomal cadherins via its N-terminal head domain. Two myocarditis patients were found to have likely pathogenic variants with similar clinical presentations to those with DSP variants.

To date, only one study has examined the underlying prevalence of genetic variants in paediatric myocarditis. This similarly indicated that 16.7% (7 out of 42) of the children carried rare variants in genes associated with inherited cardiomyopathy. Interestingly, pathogenic variants also occurred in the genes DSP and PKP2, although these were homozygous (DSP, n=1, patient died) or compound heterozygous (PKP-2, n=1) variants. In contrast, all the variants seen in our cohort were heterozygous, meaning a mutation occurred on one allele of the affected gene, thus it is assumed that half of the proteins are defective. Of note, most of the

patients with pathogenic variants in DCM and HCM have heterozygous variants.[284, 285] Disease severity is usually linked to the fraction of mutated protein, which is highlighted by the death of the child with a homozygous variant in DSP.

SUMMARY

We have shown that pathological variants associated with ARVC were significantly enriched in our large prospective cohort of well-characterised myocarditis patients. There were no other clinical features to indicate the presence of ARVC by current criteria, suggesting that an acute inflammatory episode labelled as myocarditis may potentially constitute an environmental modifier that subsequently unmasks the underlying myocardial abnormality (table 5-10, figure 5-13). This finding may have clinical implications in the diagnosis and follow-up of myocarditis patients, despite the absence of a family history of cardiomyopathy. A key unanswered question is whether the underlying genetic defect rendered the heart more susceptible to viral infection in the first instance. We did not find significant enrichment of DCM variants, which is likely to be multifactorial, but may suggest the importance of additional mechanistic pathways, such as auto-immunity, in a heterogenous and complex condition such as myocarditis.

Demographics			Genotype				Phenotype							
	Sex	*Age*	*Mutation*	*Zygosity*	*Hvol Freq*	*MAF ExAC*	*Presentation*	*Trop (ng/L)*	*ECG*	*LVEF (%)*	*LGE distribution*	*Trigger*	*ARVC features*	*FHx of ARVC/SCD*
P1	M	29	c.4307_4308delCT	Het	0	0	Chest pain, palpitations	19, 608	Normal	61	Inferolateral	Tonsillitis	None	None
P2	M	28	c.4423delA	Het	0	0	Chest pain, palpitations	762	Normal	67	Inferior	Upper resp tract infection	None	None
P3	M	17	c.5056C>T	Het	0	0	Chest pain, palpitations	40	Normal	66	Inferolateral	Gastrointestinal upset	None	None

Table 5-10. Genotype and phenotype of the 3 patients with truncating variants in DSP.

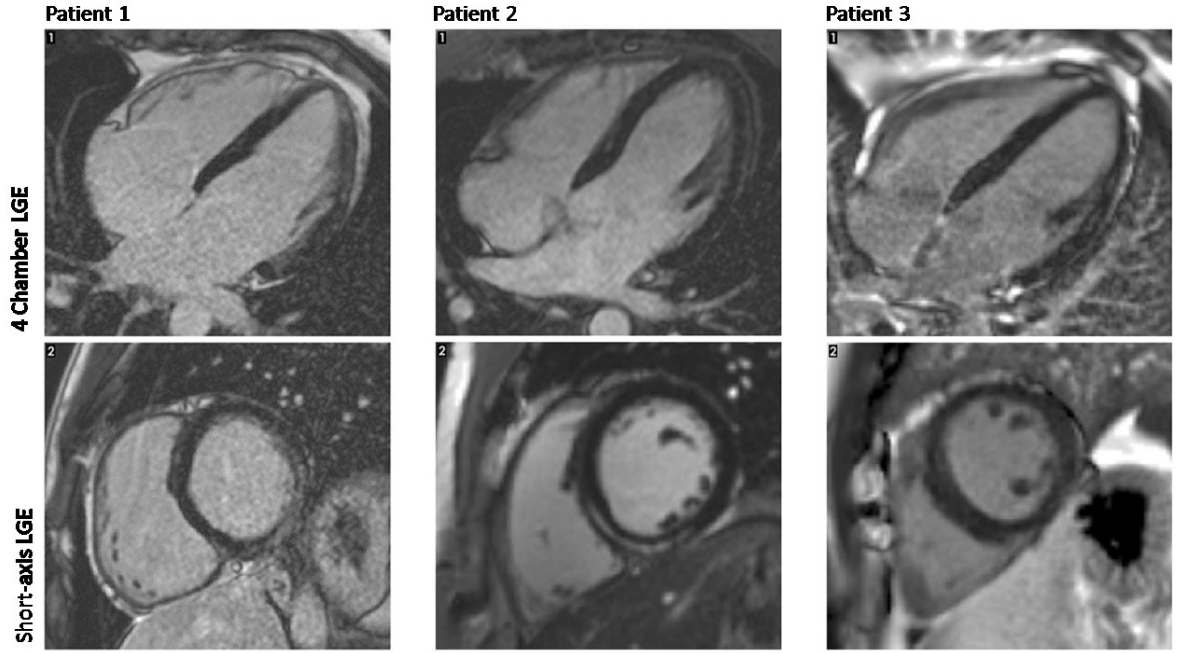


Figure 5-13. CMR late gadolinium enhancement images for these 3 patients.

5.6. Future Work

There are four key areas of future work arising from our findings:

In order to confirm the biological accuracy of our novel genetic findings, it would be important to assess whether the same findings are reproduced in an independent dataset. For this reason, we have identified an external validation cohort of ~250 biopsy-proven myocarditis cases in Maastricht, Prof Stephane Heymans.

To evaluate further genetic determinants that predispose the progression of viral myocarditis to DCM, we aim to increase our sample size given the relatively low burden of DCM within our cohort. In order to achieve this, we have established links with the transplant units at Papworth Hospital and Golden Jubilee National Hospital, as well as a number of other tertiary centres across the UK to set up a prospective national myocarditis study. Using the ICC targeted gene panel, we have data on 174 genes linked to cardiovascular disease. Going forward, we will extend burden testing to these genes, which have been less extensively studied for disease association.

Duchenne Muscular Dystrophy (DMD) is an X-linked disorder that affects 1 in 3600 boys. It is well known that patients have absent or reduced dystrophin resulting in progressive skeletal muscle weakness and cardiomyopathy, which classically results in subepicardial fibrosis of the inferolateral wall similar to the pattern observed in viral myocarditis.[286] However, animal studies have demonstrated that enteroviral protease 2A directly cleaved dystrophin leading to functional dystrophin impairment in cardiac muscle, without skeletal muscle involvement.[277] Additionally, dystrophin-deficient mice were more susceptible to viral mediated cardiomyopathy.[287] Therefore, the presence of underlying dystrophin truncating variants (DMD-tv) may similarly define a genetic susceptibility to DCM (without skeletal muscle

abnormality), which may be latent and only revealed in combination with acute myocarditis. We intend to evaluate this hypothesis going forward.

Finally, to address the underlying genetic susceptibility to viral myocarditis following exposure to a commonly encountered infectious agent, we aim to complete a genome wide association study of the entire cohort. This will allow us to evaluate single-nucleotide polymorphisms across the genome, including key regulatory regions linked to innate immunity. Again, sample size will be key.[288] However, the first GWAS investigating age related macular degeneration identified a risk allele in the complement factor H gene that increased the likelihood of AMD by 7.4 in a study of 96 cases and 50 controls.[289] In this way, it may be possible to assign a genetic risk score for myocarditis, similar to that reported recently for hypertension.[140]

5.7. Limitations

There are several potential limitations to this work.

This was a single-centre study with consecutive patients recruited from our dedicated clinic, CMR and cardiac transplant services. Ascertainment bias may therefore have arisen. As discussed in an earlier chapter, to minimise this recruitment was of consecutive patients referred from a large network of over 12 district general hospitals. We therefore believe that our cohort is typical of those seen more broadly across the UK with this condition.

We recognise that disease severity in acute myocarditis ranges widely with many patients having mild symptoms that do not seek medical attention, and that a proportion that are seen in the primary care setting may not be referred for further evaluation in a hospital setting. However, referral or direct presentation to a secondary care hospital is taken as a threshold to identify patients with clinically significant disease, in whom further adverse events are more likely and therefore warrant investigation.

Variants commonly found in the general population are less likely to be responsible for disease. For this reason, we focused on the 11 DCM and 5 ARVC genes for which evidence of disease-association has been found to be most robust based on ExAC data. As this dataset is derived from exome sequencing, some genomic regions, such as those with high GC content, are not fully covered. To overcome this limitation, our analysis focused on comparison between cases and local healthy volunteers all sequenced on the same TruSight Cardio sequencing panel. This allowed us to identify Illumina platform-specific technical artefacts and thereby distinguish true differences between case and control frequencies from sequencing errors.

Patients of all ethnicities were included in the analyses presented above. As part of the work going forward, we will stratify patients by self-reported ethnicity, supported by principal component analysis of genetic data, to explore differences linked to ethnicity. In this way, we

could use ethnicity as a covariate in a regression model to test for associations, which will form part of our future work.[290]

Healthy volunteers underwent CMR to assess myocardial structure and function but did not undergo late gadolinium assessment to exclude the presence of previously unrecognized replacement fibrosis, which may be attributed to healed myocarditis or other subclinical forms of cardiomyopathy. This limitation is challenging to overcome due to ethical considerations and discussed further in the next chapter.

6. LONG-TERM OUTCOMES IN HEALED MYOCARDITIS

This chapter is currently under journal review with JACC and was presented at ACC 2018:

Lota A, Tsao A, Al-Balah A, et al. Prognostic significance of non-ischaemic myocardial fibrosis in patients with normal LV size and function: a large CMR registry study. JACC 2018;71:436.

6.1. Aims and Hypotheses

The primary aim of this chapter is to evaluate the prognostic significance of non-ischaemic patterns of myocardial fibrosis attributed to healed myocarditis in patients with normal left ventricular size and function.

The main hypothesis is outlined as follows:

- Myocardial fibrosis attributed to healed myocarditis with normal left ventricular size and function portends increased risk of fatal ventricular arrhythmia in the long-term.

6.2. Background

Over the past two decades, there has been rapid growth in the adoption of cardiovascular magnetic resonance (CMR) for diagnostic evaluation, surveillance and assessment of treatment response across the spectrum of cardiovascular (CV) disease.[291] Appropriate use criteria highlight the evolution in complexity and capability of CMR to support clinical decision-making.[292-294] Replacement fibrosis identified by late gadolinium enhancement (LGE) indicates an adverse prognosis in hypertrophic cardiomyopathy, dilated cardiomyopathy (DCM), aortic stenosis, ischaemic, inflammatory and infiltrative heart disease.[6, 295-297] In these conditions, left ventricular (LV) volumes and/or function are typically abnormal. However, myocardial fibrosis remains a powerful independent predictor of sudden cardiac death (SCD) even when the severity of LV dysfunction is only modest.[298]

Increasing numbers of individuals are now being identified with normal LV volumes, wall thickness and ejection fraction but with previously unrecognised myocardial fibrosis. In one series, subendocardial myocardial fibrosis indicative of a silent myocardial infarct was present in 17% of people over 67 years of age and had incremental prognostic utility beyond standard clinical and angiographic predictors -including left ventricular dysfunction.[299-302] However,

there is a paucity of data on the prognostic significance of a non-ischaemic pattern of myocardial fibrosis in the mid-wall and/or subepicardium of patients with normal LV volumes, wall thickness and ejection fraction. A study of 374 patients with acute myocarditis but preserved LV ejection fraction from the ITAMY registry (ITalian multicenter study on Acute Myocarditis) showed that outcomes were worse in the minority of patients with anteroseptal LGE (36%) over a median follow-up of 1,572 days.[303] However, these patients were also noted to also have higher LV end-diastolic volumes and regional abnormalities of wall motion linked to presence of myocardial oedema. Fibrosis represents the final common pathway to organ injury and failure from a diverse range of diseases and insults.[304] Whether non-ischaemic myocardial replacement fibrosis is a risk factor for SCD in the absence of other structural markers of disease such as LV dysfunction and dilatation is unknown. Moreover, the aetiology of mid-wall/subepicardial is usually unclear and often ascribed to remote events. Typically, this finding is attributed to a previous clinically silent episode of myocarditis with the identification of mild residual fibrosis at the site of injury. The uncertainty surrounding the clinical significance and management of such cases may lead to patients receiving conflicting advice and multiple consultations and investigations at considerable expense and some risk that heighten anxiety. Patients may receive treatments and advice, including to refrain from high-intensity exercise lifelong,[305] for which there is no evidence of benefit.

To the best of our knowledge, no study to date has specifically investigated the outcome of people with normal LV structure and function but with non-ischaemic patterns of LGE and no other manifestation of cardiac disease. Given the steady increase in utilisation of contrast-enhanced CMR worldwide, we took the opportunity to review the natural history and outcome of such a cohort identified amongst referrals to our service to define the prognostic significance of non-ischaemic LGE with no documented predisposing aetiology in an otherwise structurally normal heart.

6.3. Methods

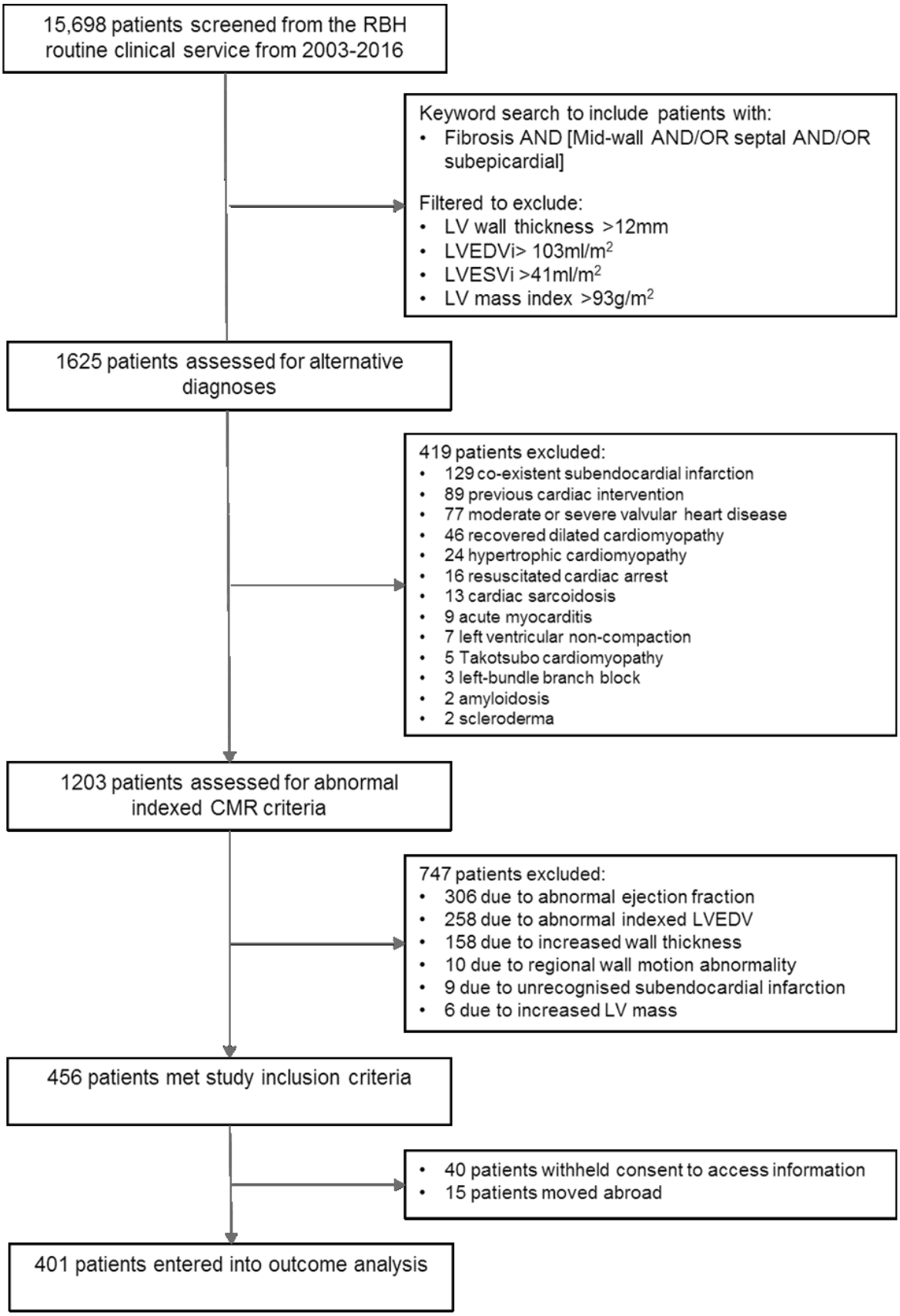
PATIENT COHORT

Patients referred for CMR between 2003 and 2016, who had mid-wall and/or subepicardial LV myocardial fibrosis were identified. Exclusion criteria were applied in a stepwise approach (listed in figure 6-1). Filters were initially used to exclude all patients with left ventricular wall thickness >12mm, indexed end-diastolic volume >103ml/m^2, indexed end-systolic volume >41ml/m^2 and indexed LV mass index >93g/m^2 based on the upper 95% confidence intervals for men aged 20-29 years, consistent with established reference ranges.[306] All men aged greater than 29 years and all women regardless of age would have indexed volumes below these thresholds and therefore any outliers were excluded by this filter. Then, patients with a potential reason for myocardial fibrosis, such as clinically active myocarditis, sarcoidosis, previous chemotherapy, recovered DCM or increased afterload due to aortic stenosis were excluded. Patients with controlled hypertension receiving treatment with one or two antihypertensive agents at the time of CMR were included, but not those with resistant hypertension (defined as BP >140/90 despite treatment with a diuretic and two other antihypertensive drugs).[307] Patients with a stenosis of >50% in a major coronary artery, infarct pattern of LGE, left-bundle branch block, history of coronary bypass grafting or percutaneous intervention were also excluded. Finally, CMR data for remaining individuals were manually curated to exclude indexed LV values above or below the appropriate age- and gender-adjusted normal ranges.[306] In total, 456 patients were met the stringent criteria to define a structurally normal heart.

In addition, a cohort of patients referred for CMR between 2003 and 2016 *without* myocardial fibrosis on LGE imaging (LGE-) provided a control cohort. These individuals were matched to the LGE+ cohort in terms of scan indications. Inclusion and exclusion criteria were otherwise identical.

Patients provided written informed consent for the collection of clinical baseline and follow-up data as approved and directed by the National Research Ethics Service and received Institutional Board Approval by the Royal Brompton Hospital. The data that support the findings of this study are available from the corresponding author upon request.

Figure 6-1. Patient consort flow diagram to show the identification, inclusion/exclusion of the study cohort



CMR IMAGE ACQUISITION AND ANALYSIS

CMR was performed on one of three 1.5 Tesla clinical scanners (Sonata/Avanto, Siemens, Erlangen, Germany) using a standardized protocol with 0.1mmol/kg of gadolinium contrast agent, either Magnevist or Gadovist (Bayer, Germany).[296, 298] Inversion-recovery gradient echo sequences were used with images repeated in two separate phase-encoding directions in multiple orthogonal views to exclude artefacts. Left ventricular volumes, ejection fraction, and mass were measured using dedicated software (CMRtools) and indexed to body surface area.[306] Blood pool thresholding was used to delineate and exclude the papillary muscles from ventricular volumes. The presence, extent and location (septal vs non-septal) of mid-wall and/or subepicardial fibrosis was assessed by two independent expert readers who were blinded to all clinical data (figure 6-2). LGE was considered as present when seen in two orthogonal planes, in both phase-encoding directions and extending beyond the focal ventricular insertion points but excluding right ventricular septo-marginal trabeculae. Patients identified with previously unrecognised subendocardial LGE indicative of silent myocardial infarction were excluded on expert review. The mass of LGE (grams) was quantified by a blinded operator using the full-width at half-maximum technique (FWHM) and indexed as a percentage of left ventricular (LV) mass (CMR42, Circle Cardiovascular Imaging Inc, Calgary, Canada). This method estimates the mass of myocardium with signal intensity >50% of the maximally enhanced myocardium defined by the user (figure 6-3).[308]

FOLLOW-UP AND OUTCOMES

Patient follow-up was done retrospectively at periodic intervals by a combination of postal questionnaires, telephone interviews and retrieval of information from family physicians and hospital records. The presence of a family history of SCD in a first degree relative was sought for all cases. Deaths were identified through the UK Health and Social Care Information Service. The prespecified primary outcome was a composite of actual or aborted SCD. SCD

was defined as unexpected death within one hour of the onset of cardiac symptoms in the absence of progressive cardiac deterioration, during sleep, or ≤24 hours of last being seen alive.[309] Aborted SCD was defined as an appropriate ICD shock for ventricular arrhythmia, a non-fatal episode of ventricular fibrillation, or sustained VT with haemodynamic compromise requiring cardioversion.[310] The principal secondary outcome was all-cause mortality. An additional secondary outcome was a composite of cardiovascular mortality (SCD, heart failure, stroke, or thromboembolism) and unplanned cardiovascular hospitalization. Follow-up duration was calculated from the baseline CMR scan and censored at the first event or date of last confirmed patient contact. All clinical event data was adjudicated by an independent committee of cardiologists blinded to CMR data. Cause of death was established from death certification and post-mortem results, which were also reviewed by the adjudication committee, in line with published guidance.[309]

STATISTICAL ANALYSIS

Baseline characteristics (LGE+ versus LGE-) were compared using the Mann-Whitney U-test for continuous data or Fisher exact test for categorical data. Patients with both septal and non-septal LGE were included in the septal group, given that septal and multiple patterns of LGE were previously recognised as the main drivers of arrhythmic risk.[311] Medication was recorded at the time of baseline scan. Cumulative incidence curves were generated for outcomes with event times measured from the baseline CMR date for up to 10 years. Associations between the location and extent of fibrosis and outcomes were analysed using uni- and multivariable hazard modelling adjusted for recognised prognostic baseline covariates including age, sex, New York Heart Association (NYHA) class and atrial fibrillation. Results are presented as hazard ratios (HRs) with 95% confidence intervals (CIs). Statistical analyses were performed using Stata version 14 (StatCorp). A p-value of <0.05 was taken as significant.

Figure 6-2. CMR late gadolinium enhancement images from 4 patients with normal indexed LV volumes, wall thickness and ejection fraction demonstrating; A) mild sub-epicardial enhancement in the inferolateral wall, B) mild sub-epicardial enhancement in the anterolateral wall, C) linear mid-wall enhancement in the septum, D) mid-wall enhancement of the septum and sub-epicardial enhancement of the anterior, lateral and inferior walls.

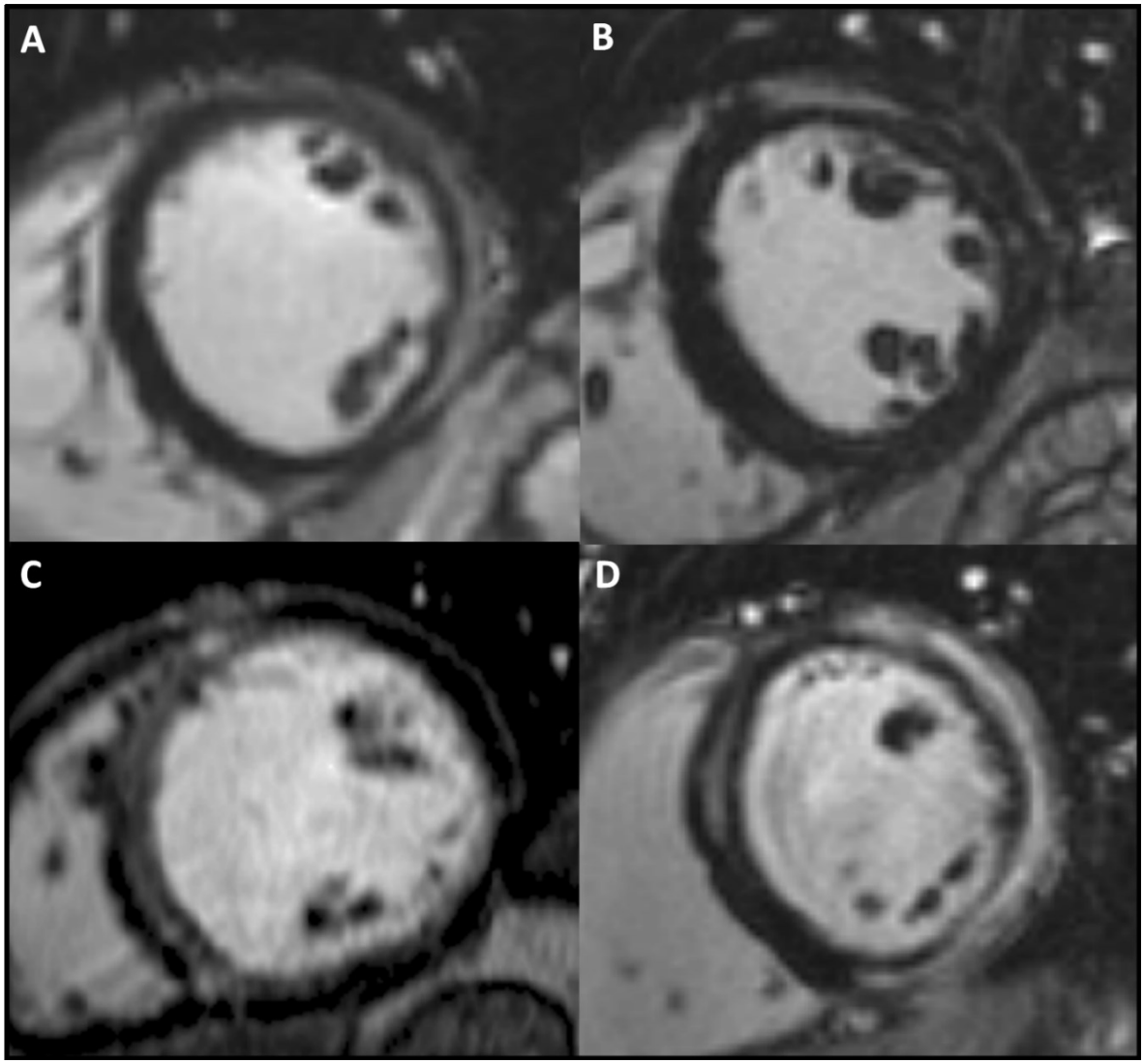
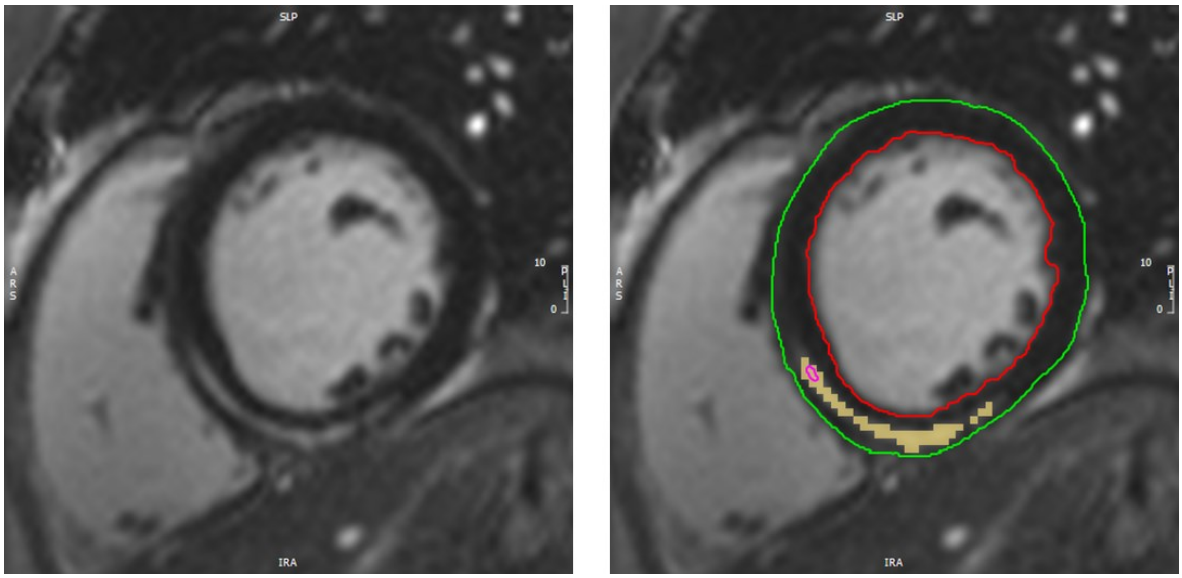


Figure 6-3. Method for late gadolinium enhancement quantification: a mid-ventricular, short-axis, late gadolinium enhancement image with enhancement quantification using the full-width at half maximum method (Circle Cardiovascular Imaging, Calgary, Canada). The green line defines the epicardial border, the red line defines the endocardial border, the pink area outlines a reference region of LGE and the yellow region outlines the area of LGE quantified using this method.



6.4. Results

From 15,698 patients scanned with gadolinium contrast in our institution from 2003-2016, 1,625 patients were identified for further evaluation into the LGE+ group (figure 1). Of these, 422 (26%) were excluded due to co-existent subendocardial LGE (129; 32%) or prior cardiac arrest (16; 4%). Of the remaining 1,203 patients, 747 (62%) were excluded due to abnormalities of indexed LV measurements. As a result, 456 patients met all the inclusion and exclusion criteria (figure 6-1). Of these, 40 withheld consent to access follow-up medical records and 15 had emigrated. Therefore, the LGE+ group consisted of 401 patients. A total of 347 LGE- patients were identified over the same time period with matching scan indications, resulting in an overall cohort of 748 people.

BASELINE CHARACTERISTICS

The median age of the cohort (n=748) was 50 years (IQR 38-61) and 287 (38%) were women (table 6-1; figure 6-4). Scan indications (figure 6-5) consisted of investigation for symptomatic patients with chest pain (40%), palpitation/syncope (33%), or breathlessness (13%), and asymptomatic patients undergoing familial cardiomyopathy screening (11%) and aortic assessment (1%). A family history of SCD in a first degree relative was reported for 35 patients (5%). Most patients were in either NHYA class I (83%) or II (15%). Overall, 25% were on beta-blockers, mostly for management of palpitation or chest pain, and 22% were on ACE-inhibitors or ARB, primarily for hypertension. The median indexed LV end-diastolic volume (LVEDV) was 77 ml/m^2 (IQR 66-85), LV end systolic volume 25 ml/m^2 (IQR 21-31) and LVEF 66% (IQR 62-70). In the LGE+ patients with mid-wall or subepicardial LGE, the patterns seen were septal in 69 (17%) patients, non-septal in 305 (76%) and both septal and non-septal in 27 (7%). The median LGE mass in the LGE+ group as a percentage of overall LV mass was 2.25% (IQR 1.21-4.14).

LGE+ patients were more likely to be men ($p<0.0001$) with a history of controlled hypertension ($p<0.0001$) and treatment with a beta-blocker ($p<0.001$) or angiotensin receptor blocker ($p<0.001$). NYHA class was also more likely to be class II or III ($p<0.0001$). Those who were LGE- were more likely to be women and have lower LVEDVi ($p<0.0001$), lower LVESVi ($p<0.0001$) and lower mass index index ($p<0.0001$) within the normal reference ranges. There were no significant differences between groups in age, comorbidity or scan indication.

In the LGE+ subgroup, those with non-septal LGE were more likely to be men ($p=0.029$) and present with chest pain ($p<0.0001$). Patients with septal LGE were more likely to be women ($p=0.029$), present with breathlessness or for familial cardiomyopathy screening ($p<0.0001$), have atrial fibrillation ($p=0.028$), and prescribed an ACE inhibitor ($p=0.027$). There were no significant differences between groups in age, baseline medical history or medication.

Figure 6-4. Histogram of patient age by presence or absence of LGE at time of baseline CMR ($p=0.11$)

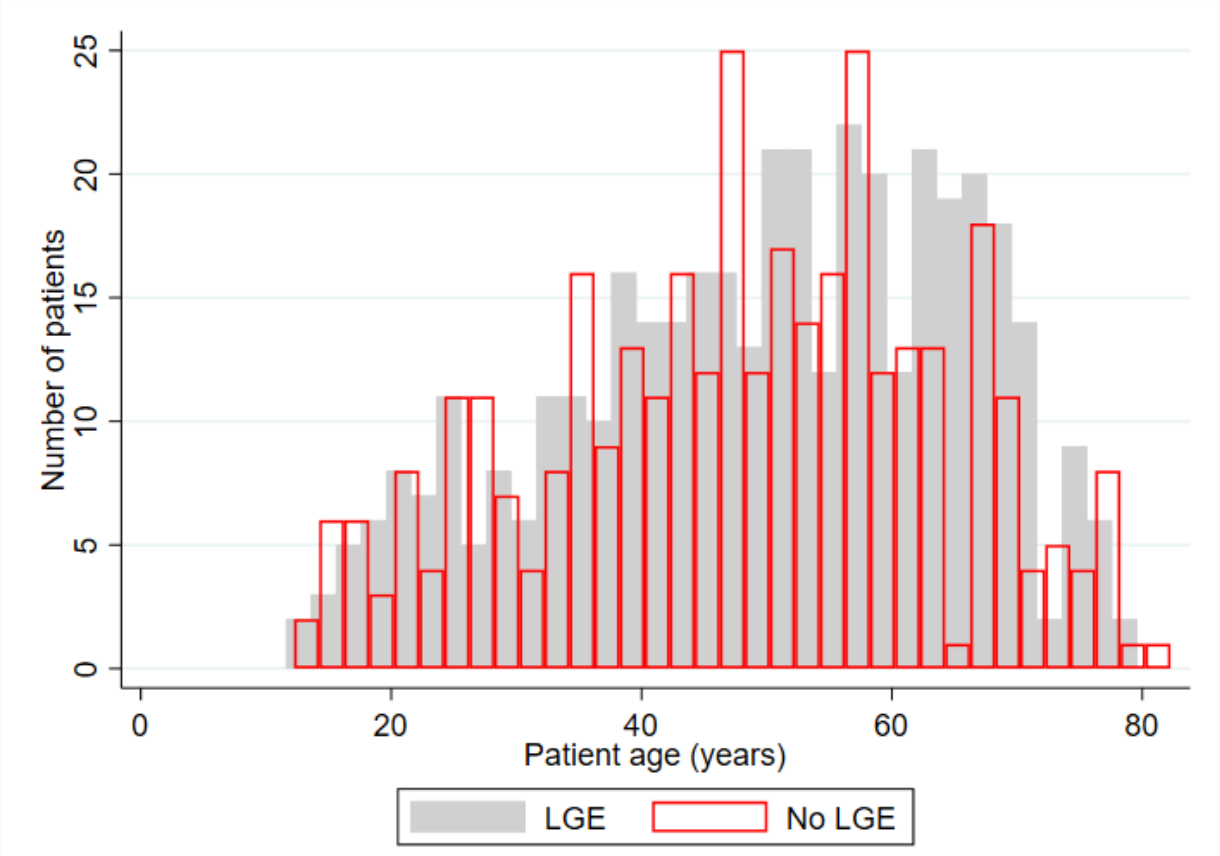


Figure 6-5 CMR scan indications for the total cohort (n=748)

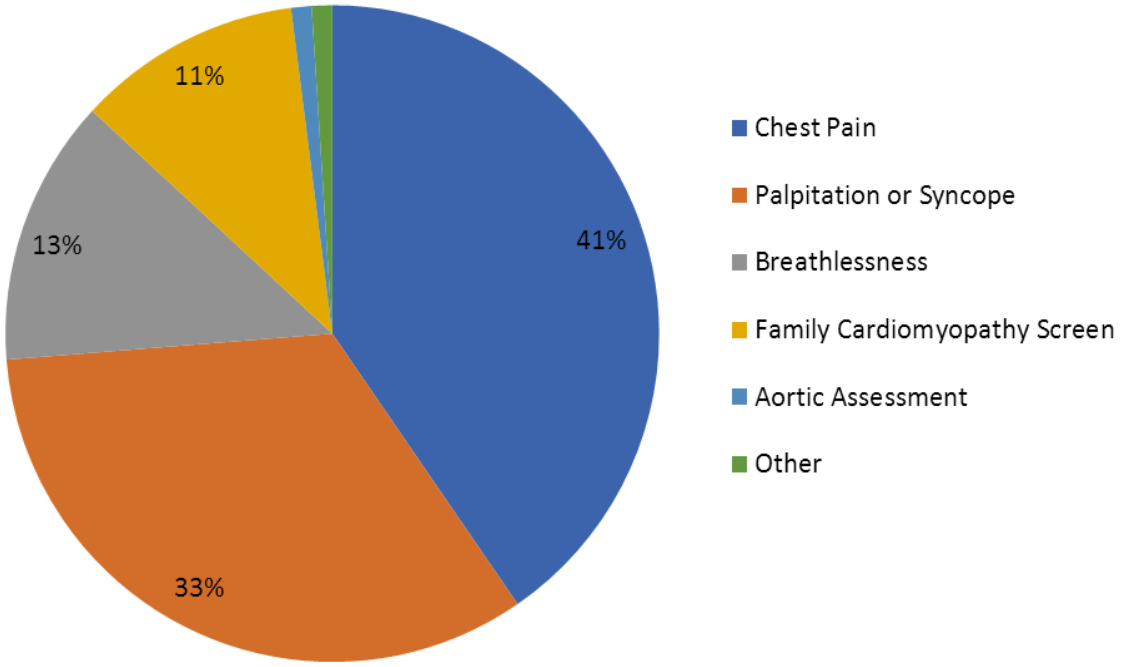


Table 6-1. Baseline demographics

Variable	All patients (N=748)	LGE		P-value
		No (N=347)	Yes (N=401)	
Median Age (IQR), years	50 (38-61)	49 (37-59)	51 (39-62)	0.11
Female, n (%)	287 (38.4)	175 (50.4)	112 (27.9)	<0.0001
Body surface area (m^2)	1.9 (1.7-2.1)	1.9 (1.7-2.0)	1.9 (1.8-2.1)	<0.0001
Atrial fibrillation	42 (5.6)	17 (4.9)	25 (6.2)	0.43
Hypertension	173 (23.1)	53 (15.3)	120 (29.9)	<0.0001
Diabetes mellitus	56 (7.5)	22 (6.3)	34 (8.5)	0.27
Hypercholesterolaemia	127 (17.0)	59 (17.0)	68 (17.0)	0.99
Current smoker	58 (7.8)	21 (6.1)	37 (9.2)	0.11
Cerebrovascular accident	15 (2.0)	2 (0.6)	13 (3.2)	0.010
Excess alcohol	98 (13.1)	50 (14.4)	48 (12.0)	0.32
Family history of sudden cardiac death	35 (4.7)	14 (4.0)	21 (5.2)	0.44
Medication				
ACE inhibitor, n (%)	113 (15.1)	37 (10.7)	76 (19.0)	0.002
Beta blocker	186 (24.9)	66 (19.0)	120 (29.9)	<0.001
ARB	55 (7.4)	13 (3.7)	42 (10.5)	<0.001
Anti-arrhythmia medication	36 (4.8)	15 (4.3)	21 (5.2)	0.56
New York Heart Association				
Class I, n (%)	624 (83.4)	320 (92.2)	304 (75.8)	<0.0001
Class II	110 (14.7)	27 (7.8)	83 (20.7)	
Class III	14 (1.9)	0 (0.0)	14 (3.5)	
Scan indication				
Chest pain, n (%)	300 (40.1)	139 (40.1)	161 (40.1)	0.64
Palpitation or syncope	248 (33.2)	112 (32.3)	136 (33.9)	
Breathlessness	98 (13.1)	43 (12.4)	55 (13.7)	
Asymptotic family screen	80 (10.7)	41 (11.8)	39 (9.7)	
Other	12 (1.6)	5 (1.4)	7 (1.7)	
Aortic assessment	10 (1.3)	7 (2.0)	3 (0.7)	
CMR parameters				
LVEDVi (ml/m^2)	77 (66-85)	74 (64-83)	79 (69-87)	<0.0001
LVESVi (ml/m^2)	25 (21-31)	24 (19-29)	26 (22-32)	<0.0001
LVEF (%)	66 (62-70)	67 (63-72)	66 (62-69)	0.002
LV mass index (g/m^2)	63 (54-71)	59 (51-70)	66 (58-73)	<0.0001
RVEDVi (ml/m^2)	78 (68-89)	75 (64-87)	81 (71-91)	<0.001
RVESVi (ml/m^2)	30 (24-37)	29 (23-37)	31 (25-37)	0.03
RVEF (%)	61 (56-66)	61 (56-66)	61 (56-65)	0.85
LGE (grams)	2.80 (1.50-5.25)	-	2.80 (1.50-5.25)	
LGE (%)	2.24 (1.21-4.14)	-	2.24 (1.21-4.14)	
LGE>2.25%	202 (27.0)	-	202 (50.4)	

PRIMARY OUTCOME

Over a median follow-up of 4.3 years (IQR 2.1-6.5), only one patient (0.13% of the total cohort; 0.2% of the LGE+ group) met the primary outcome, presenting with an aborted SCD. This event occurred during the 11th year of follow-up in a patient who had a primary prevention ICD, basal-septal LGE and no family history of SCD. The incidence rate per 100 patient-years in the LGE+ group was 0.05% (95% CI 0.008-0.39; figure 6-6A).

In the LGE+ group, eleven patients had an ICD implanted during follow-up for primary prevention without LV dilatation or impairment in LVEF and seven individual patients had an elective radiofrequency ablation procedure for non-sustained VT. Thus, 18 LGE+ patients had an intervention for ventricular arrhythmia, of which 5 had septal LGE. In the LGE- group, two patients had a primary prevention ICD and none had an intervention for ventricular arrhythmia.

SECONDARY OUTCOMES:

(i) <u>All-cause mortality</u>

There were 30 deaths during follow-up (4.0% of the total cohort), of which 2 were cardiovascular and 28 non-cardiovascular giving an incidence rate per 100 patient-years of 0.81 (95% CI 0.57-1.16). There was no difference in the overall mortality rate between LGE+ and LGE- patients (3.7% vs 4.3%; p=0.71). All-cause mortality was associated with patient age (HR 2.04 per 10-year increase; 95% CI 1.46 – 2.79; p<0.001) and hypercholesterolaemia (HR 4.13; 95% CI 2.01 – 8.47; p<0.001; figure 6-6B & table 6-2). The aetiology for CV death was worsening heart failure from newly developed ischaemic heart disease (both men aged 70 and 76 years). Causes of non-cardiovascular death included cancer (18 patients, aged 34 to 73 years; 64%), pneumonia with chronic lung disease (7 patients aged 65-69 years; 25%), end-stage renal failure (1 patient, aged 47 years; 4%), leukaemia (1 patient, aged 38 years; 4%), and motor neuron disease (1 patient aged 66 years; 4%). There was no association between LGE location or extent and all-cause mortality

(ii) Cardiovascular death, aborted SCD and unplanned hospitalisation

During follow-up, there were two CV deaths and 73 CV hospitalisations (9.7% of the total cohort), of which 25 (34%) were unplanned. Twenty-one of 401 LGE+ patients (5.2%) experienced this composite outcome compared with 4 of 347 LGE- patients (HR 7.22; 95% CI 4.26 – 21.17; p<0.0001). Indications for unplanned admissions included suspected myocarditis (n=7), symptomatic palpitations arising from documented non-sustained ventricular tachycardia (n=5), cerebrovascular accident (n=4), pacemaker implantation (n=3), palpitations from atrial fibrillation with rapid ventricular response (n=2), non-ST elevation myocardial infarction (n=2) and acute pulmonary embolism (n=2). In patients with LGE and an event, 79% had an LGE mass index above the median of 2.25% (LGE+ HR 11.27; 95% CI 3.73 – 34.07 vs LGE- HR 3.55; 95%CI 0.99 – 12.75; p<0.0001; figure 6-6D). Other variables that showed an association on univariable analysis (table 6-3) included a prior history of cerebrovascular accident (HR 8.26; 95% CI 2.86 – 23.85; p<0.0001) and prescription of beta-blockers (HR 3.09; 95% CI 1.47 – 6.49; p=0.003) or other anti-arrhythmic medication for documented arrhythmia (HR 5.70; 95% CI 2.31 – 14.06; p<0.001).

In multivariable analysis adjusting for LGE mass, atrial fibrillation/flutter, age, sex and NYHA class, the presence of LGE remained associated with this secondary composite outcome (HR 7.16; 95% CI 2.30 – 22.58; p=0.001

GENETIC SEQUENCING

Twelve out of 39 LGE+ patients (31%) with a family history of cardiomyopathy as the initial scan indication, although phenotypically unaffected, were subsequently found to have rare genetic variants in genes associated with cardiomyopathy (table 6-4). Of these patients, four had ICDs implanted for primary prevention but none met the primary outcome and only two met the composite secondary outcome. Sensitivity analysis confirmed that the primary study findings were not altered significantly by the inclusion or exclusion of this group.

Figure 6-6. Cumulative incidence of (A) actual and aborted sudden cardiac death, (B) all-cause mortality, (C) CV death, aborted SCD and CV hospitalisation stratified by presence or absence of LGE, and (D) CV death, aborted SCD and CV hospitalisation stratified by LGE extent above and below the median of 2.25% in this cohort with comparison to LGE- group.

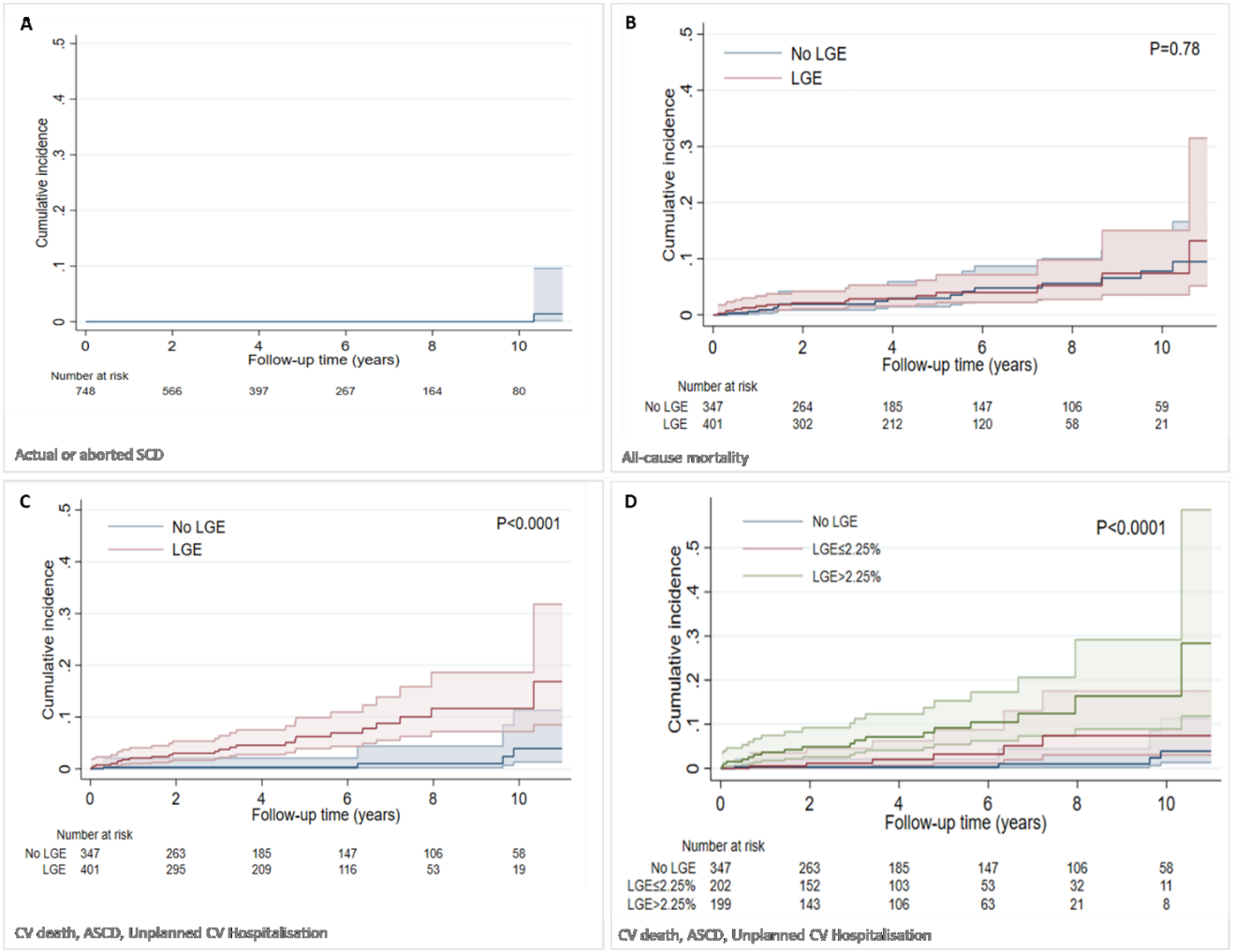


Table 6-2 Univariable predictors of all-cause mortality

Variable	HR (95% CI)	P-value
Age (per 10-year increase)	2.04 (1.49, 2.79)	<0.0001
Female	1.35 (0.66, 2.76)	0.42
Body surface area (m^2)	0.38 (0.08, 1.83)	0.23
Atrial fibrillation	1.46 (0.35, 6.12)	0.61
Hypertension	0.79 (0.32, 1.93)	0.61
Diabetes mellitus	0.38 (0.05, 2.82)	0.35
Hypercholesterolaemia	4.13 (2.01, 8.47)	<0.001
Current smoker	0.38 (0.05, 2.82)	0.35
Cerebrovascular accident	0.00 (0.00, .)	1.00
Excess alcohol	1.77 (0.76, 4.14)	0.18
Family history of sudden cardiac death	0.62 (0.08, 4.55)	0.64
Medication		
ACE inhibitor	1.35 (0.55, 3.29)	0.52
Beta blocker	0.76 (0.31, 1.87)	0.56
ARB	0.40 (0.05, 2.90)	0.36
Anti-arrhythmia medication	0.62 (0.08, 4.56)	0.64
New York Heart Association		
NYHA I	Reference group	
NYHA II/III	2.33 (1.07, 5.10)	0.03
CMR parameters		
LVEDVi (ml/m^2)	0.98 (0.95, 1.00)	0.08
LVESVi (ml/m^2)	0.96 (0.91, 1.01)	0.13
LVEF (%)	1.02 (0.95, 1.08)	0.63
LV mass index (g/m^2)	0.99 (0.96, 1.02)	0.48
RVEDVi (ml/m^2)	0.98 (0.96, 1.00)	0.11
RVESVi (ml/m^2)	0.97 (0.93, 1.01)	0.09
RVEF (%)	1.04 (0.99, 1.09)	0.16
LGE presence	1.11 (0.53, 2.30)	0.78
LGE extent:		
None	Reference group	0.55
≤2.25%	0.88 (0.34, 2.30)	
>2.25%	1.33 (0.58, 3.09)	

Table 6-3. Univariable and multivariable analyses for the composite secondary outcome of CV death, aborted SCD and unplanned CV hospitalisation

	Univariable		Multivariable	
Variable	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
Age (years)	1.07 (0.84, 1.37)	0.58	1.04 (0.81, 1.33)	0.77
Female	0.62 (0.27, 1.42)	0.26	1.00 (0.42, 2.37)	1.00
Body surface area (m^2)	3.48 (0.77, 15.62)	0.10		
Atrial fibrillation	3.48 (1.20, 10.06)	0.02	3.62 (1.20, 10.98)	0.02
Hypertension	1.52 (0.69, 3.36)	0.30		
Diabetes mellitus	1.30 (0.39, 4.31)	0.67		
Hypercholesterolaemia	1.01 (0.38, 2.66)	0.98		
Current smoker	3.77 (1.60, 8.88)	0.002		
Cerebrovascular accident	8.26 (2.86, 23.85)	<0.0001		
Excess alcohol	1.27 (0.48, 3.34)	0.63		
Family history of sudden cardiac death	0.66 (0.09, 4.84)	0.68		
Medication				
ACE inhibitor	1.78 (0.76, 4.19)	0.19		
Beta blocker	3.09 (1.47, 6.49)	0.003		
ARB	0.87 (0.21, 3.66)	0.85		
Anti-arrhythmia medication	5.70 (2.31, 14.06)	<0.001		
New York Heart Association				
NYHA I	Reference group		Reference group	
NYHA II/III	2.26 (0.99, 5.14)	0.05	1.47 (0.61, 3.52)	0.39
CMR parameters				
LVEDVi (ml/m^2)	1.00 (0.97, 1.03)	0.87		
LVESVi (ml/m^2)	1.01 (0.96, 1.06)	0.59		
LVEF (%)	0.96 (0.90, 1.02)	0.19		
LV mass index (g/m^2)	0.99 (0.96, 1.02)	0.36		
RVEDVi (ml/m^2)	1.00 (0.98, 1.03)	0.82		
RVESVi (ml/m^2)	1.00 (0.96, 1.04)	0.95		
RVEF (%)	1.01 (0.96, 1.06)	0.74		
LGE presence	7.22 (2.46, 21.17)	<0.001	7.16 (2.30, 22.28)	0.001
LGE extent:				
None	Reference group			
≤2.25%	3.55 (0.99, 12.75)			
>2.25%	11.27 (3.73, 34.07)	<0.0001		

Table 6-4. List of individual genetic variants detected through routine clinical evaluation

Gene	Transcript	HGVSg	HGVSc	HGVSp	Consequence	gnomAD allele frequency	ACMG class*	ACMG rules
DES	ENST00000373960	chr2: g.[220286086C>T]	c.1048C>T	p.Arg350Trp	missense variant	Seen in 6/251398 alleles	VUS	PM2
FLNC	ENST00000325888	chr7: g.[128496571G>A]	c.7252-1G>A	p.Gln4215ValfsTer16	splice acceptor variant	0	VUS	PM2
HCN4	ENST00000261917	chr15 g.[73615051C>A]	c.4377G>T	p.Gly1128Val	missense variant	0	VUS	PM2
LMNA	ENST00000368300	chr1: [g:156104988C>T]	c.821C>T	p.Ala274Val	missense variant	0	VUS	PM2, PP2, PP3
TTN	ENST00000589042	chr2: g.[179605316-179605317delTG]	c.12643_12644delCA	p.Gln4215ValfsTer16	frameshift variant	0	LP	PM2, PVS1
TTN	ENST00000589042	chr2: g.[179571661delC]	c.29062delG	p.Ala96888GLnfsTer7	frameshift variant	0	VUS	PM2
TTN	ENST00000589042	chr2: g.[179495659T>A]	c44026A>T	p.Lys14676Ter	nonsense variant	0	LP	PM2, PVS1
TTN	ENST00000589042	chr2: g.[179466193-179466199delTCCTGT]	c.55525_55531delGACAGGA	p.Asp18509SerfsTer29	frameshift variant	0	LP	PM2, PVS1
MYBPC 3	ENST00000545968	chr11: g.[47365143C>T]	c.1123G>A	p.Val375Met	missense variant	Seen in 5/278004 alleles	VUS	PM2, PP2
DSP	ENST00000379802	chr6: g.[7584681T>C]	c.7186T>C	p.Y2396H	missense variant	0	VUS	PM2

*ACMG class was determined using CardioClassifier15 followed by manual curation of segregation, de novo and functional data from the literature and ClinVar.
P = pathogenic, LP = likely pathogenic, VUS = variant of uncertain significance. For four further patients, the medical records referred to clinical genetic testing that had reportedly identified rare variants in cardiomyopathy-associated genes, but it was not possible to identify precise details of the variant in the medical record. These were variants in DSG2, DSP, LMNA & DES (1 variant per patient).

6.5. Discussion

Management of patients with mid-wall/subepicardial fibrosis in the setting of normal LV volumes and ejection fraction is a clinical conundrum due to the lack of data on how to manage and advise such patients. This is the first study to investigate the prognostic significance of non-ischaemic patterns of LGE in patients with normal LV size and function. Overall, there was a low burden of major arrhythmic events during the median follow-up period of 4.3 years. All-cause mortality was driven primarily by age-related disease and was not associated with presence or absence of LGE. There was, however, an increase in the burden of unplanned CV hospitalisation amongst patients with LGE, particularly amongst those with a greater volume of LGE, independent of age.

SCD remains a major public health issue with devastating impact. Traditional approaches to risk stratification are imprecise and reliant on LVEF. However, the vast majority of SCDs occur amongst patients either not diagnosed with heart disease (45% of patients) or with a history of heart disease but LVEF >40% (40% of patients).[312] As highlighted in the 2017 AHA guidelines for management of patients with ventricular arrhythmias and the prevention of SCD, there is unmet need to improve the identification of individuals without significant ventricular dysfunction who are at risk of SCD. In our cohort, there was a low overall burden of major arrhythmic events with an incidence rate of actual or aborted SCD of 0.05 per 100 patient years (95%CI 0.008-0.39). The only aborted SCD event that occurred was in a patient with septal LGE. The underlying arrhythmia that triggered an appropriate shock was monomorphic VT initiated by a premature ventricular ectopic couplet occurring during an episode of sinus tachycardia. This suggests that patients with incidental and otherwise unexplained, non-ischaemic patterns of LGE do not require ICD implantation if LV volumes, wall thickness and ejection fraction are all within normal limits. Furthermore, this observation supports the notion that the genesis of ventricular arrhythmias is dependent on the presence of multiple factors, of

which structural substrate is just one component. Our data suggest that myocardial fibrosis in the absence of other risk factors, such as LV dilatation, contractile impairment or a family history of cardiomyopathy, is not a marker of high risk even over extended follow-up.

Our results should be considered in the context of other studies investigating the prevalence and prognostic significance of LGE in patients without known CV disease. In a cohort of 939 patients (median age 76 years) from the Age, Gene/Environment Susceptibility (AGES)–Reykjavik study, incidental infarct-pattern LGE was detected in 17% and this was independently associated with all-cause mortality.[299] Similarly, in a cohort of 310 patients with an LVEF>50% and infarct-pattern LGE, LGE presence predicted cardiac transplantation and all-cause mortality.[302] In a study of 44 patients with myocardial infarction, the presence of even small amounts of LGE (<2% mean LV mass) was associated with a 7-fold increase in the hazard ratio for MACE on univariable analysis, and remained an independent predictor when adjusted for LVEF.[301] In our study mortality was associated with increasing age and a history of hypercholesterolaemia rather than presence or absence of LGE; the low CV mortality in our cohort may reflect the stringent exclusion of patients with coronary artery disease. We confirmed the excellent negative predictive value of an entirely normal CMR scan (structure, function and absence of LGE) reported in a previous study of 225 patients with clinically suspected myocarditis, in which no patient with a normal CMR had a major cardiovascular event over a median follow-up of >4 years.[212]

In a recent sub-group analysis of the AGES–Reykjavik cohort, 54 patients (6%) were identified with 'major' non-ischaemic patterns of LGE.[313] Non-ischaemic patterns were classified as due to myocarditis, infiltrative cardiomyopathy or hypertrophic patterns. LGE was associated with a primary composite endpoint of all-cause mortality and HF hospitalisation (HR 3.2) and indeed was associated with a poorer outcome than infarct-pattern LGE, which was present in 211 (23%) patients (HR 2.3). This study highlights the prognostic significance of aetiologically

heterogeneous, non-ischaemic scar in a relatively small number of individuals with normal LVEF (median 62%), but later in life with greater levels of comorbidity compared to our cohort.

Understanding of the dose-response relationship between LGE extent and SCD in any cardiac disease remains challenging. In our study, patients with a higher volume of LGE were more likely to have a CV hospitalisation, mainly due to concerns about myocarditis or palpitation/arrhythmias. However, there were insufficient numbers of events to evaluate LGE extent as a continuous variable. Nevertheless, abnormal test results are likely to increase surveillance and it is unclear to what extent the test result rather than underlying disease drove hospitalisation rates, including ICD implantation and ablation procedures. The relationship between beta-blocker or anti-arrhythmic medication and the composite secondary outcome may reflect the higher burden of disease for these individuals but prescribing bias should also be considered. Ambulatory ECG monitoring was not done routinely and few patients had loop recorders implanted in this cohort, therefore we do not know the true burden of subclinical arrhythmia.

In our cohort, underlying aetiology of LGE in patients with otherwise normal LV size and function was often uncertain, reflecting real-world clinical practice. Lateral free wall LGE is often ascribed to a previous, potentially silent, episode of myocarditis.[27] The prevalence of myocarditis is not well characterised and likely to be globally underestimated. Myocarditis accounts for 11.6% of all SCD in the young (<35yrs of age),[204] and yet is only detected on 2% of SCD post-mortem studies suggesting widespread under recognition of this potentially arrhythmogenic substrate.[247] Explanations for lateral wall predilection include greater susceptibility of watershed territories to parvovirus B19 mediated endothelial dysfunction and polyserositis from the adjacent pericardial layer.[314] In our cohort, most patients had lateral wall

LGE, and of these, a greater percentage were men and presented with chest pain, all typical of myocarditis, as shown in other CMR studies of myocarditis patients with preserved LVEF.[303]

Lateral wall LGE may also be seen with other pathologies such as lamin cardiomyopathy, early presentations of left-dominant forms of arrhythmogenic cardiomyopathy, Duchenne's muscular dystrophy cardiomyopathy, or Anderson Fabry disease prior to LV hypertrophy. Whilst it is possible that many of our patients had a remote episode of myocarditis, other genetic forms of cardiomyopathy are also a consideration.[304] In our cohort, the main revised diagnosis downstream was of gene carrier status. No other patients developed a new diagnosis on follow-up that may have accounted for their initial presentation.

While genetic cardiomyopathies are generally associated with adverse outcomes, the event rate in the early preclinical phase of disease with normal LV size and function is low, as observed in our cohort. Lamin cardiomyopathy is strongly associated with malignant arrhythmia and characterised by non-ischaemic LGE patterns in both the septum and free wall.[315] Recently, a prognostic model of four independent and cumulative risk factors was proposed for patients with lamin cardiomyopathy (LVEF <45%, non-sustained VT, male gender and a specific LMNA mutation). No malignant ventricular arrhythmia occurred in patients with 0 or 1 risk factor; ICD implantation was recommended for patients with at least 2 risk factors.[316] As discussed in chapter 5, there is emerging evidence of an overlap between myocarditis and ARVC, both in terms of CMR features and genetic variants.[265] However, lamin cardiomyopathy and ARVC are both progressive diseases, associated with poor outcome in the setting of even mild LV dysfunction and therefore require close follow-up. Novel 3D CMR atlas approaches using machine learning algorithms combined with analysis of myocardial strain are able to probe cardiac morphology more deeply than conventional 2D volumetric assessment and may facilitate early detection of maladaptive LV structural change beyond those assessed here.[275]

6.6. Limitations

The patient cohort was enrolled from a single centre and therefore may be susceptible to referral bias. However, our referral base for CMR is broad and includes a range of secondary and tertiary centres. After several levels of filtering, the final LGE+ study cohort comprised 401 patients from a pool of 15,698 patients. This reflects the stringency of our algorithm. Certain cohorts were excluded, for example, patients with mild LV dilatation due to athleticism. Similarly, we actively excluded patients with resuscitated cardiac arrest, where the need for ICD implantation for secondary prevention was already established. Ethnicity is known to influence LV measurements, particularly wall thickness, and the findings of this study are most applicable to Caucasian subjects.

Normal range criteria used in this study were established in a study of 120 healthy people in six age deciles from 20 to 80 years of age.[306] This early study provided comprehensive assessment of LV volumes using a steady-state free precession (SSFP) imaging pulse sequence with breath-holding, which continues to represent the primary technique used to assess ventricular volumes. Normal range distribution charts demonstrated the extensive variation in LV parameters due to age, sex and BSA. More recently, other groups have sought to characterise normal reference ranges in larger cohorts, such as the UK Biobank.[317] In the latter study, after multiple exclusion steps, 802 (16.2%) healthy participants were identified and indexed values were reported in a traffic light format with an upper cut-off of 110ml/m^2 for men and 94ml/m^2 for women, irrespective of age. Specific values stratified by age and sex were similarly provided. Of note, LV papillary muscles were included as part of the LV cavity and therefore whilst ventricular volumes tended to be larger than previously reported by Maceira et al, as would be expected, this did not materially affect interpretation of our data.[318-320]

The full-width at half maximum (FWHM) method was used to quantify LGE.[308] Using the same method, we have previously shown the absolute mean difference between operators in

LGE quantification to be 0.87% (intraclass correlation coefficient: 0.87).[311] Compared to alternative semi-automated techniques that quantify regions of LGE with signal intensity >2 standard deviations above remote reference myocardium, the FWHM method may underestimate LGE quantity but provides the highest intra- and inter-observer reproducibility, both in patients with DCM and HCM. [321, 322]

Follow-up CMR data was not available to evaluate the presence of adverse remodelling in LGE+ patients, particularly those with supra-median LGE, in whom there was a greater burden of unplanned CV hospitalisation. Recent technical developments in CMR have allowed for the assessment of extracellular volume (ECV), a preclinical biomarker of reactive interstitial fibrosis, using T1 mapping.[86] However, this was not available at the beginning of the study. Retrospective CMR assessment of myocardial strain may also provide biologically relevant information prior to overt reduction in LVEF.[219] This may help to confirm whether diastolic dysfunction was a significant contributor to the impaired functional class (NHYA III) identified in 3.5% of LGE+ patients, although left atrial size and, when available, plasma BNP were normal in most cases, suggesting that non-cardiac comorbidities may have contributed to symptoms. Similarly, routine genetic sequencing might have provided additional insight into aetiology.

6.7. Conclusions

Our data provide new information on the prognostic significance of non-ischaemic patterns of LGE in a large, well-characterised cohort of patients with normal LV size and function. We demonstrate, for the first time, that there is a reassuringly low risk of actual or aborted SCD in this setting. All-cause mortality was driven primarily by age-related disease and was not associated with the presence of LGE. These findings do not support aggressive medical management or the routine use of ICD implantation within this cohort.

6.8. Future work

Further studies are needed to verify the generalizability of these observations to other populations and to develop more personalized SCD risk assessment strategies for patients identified with non-ischaemic patterns of myocardial fibrosis.

7. PSYCHOLOGICAL IMPACT OF MYOCARDITIS

7.1. Aims and Hypotheses

The aims of this chapter are to evaluate the psychological outcomes of patients with myocarditis recruited into our multi-centre cohort study. We investigate the use of an established psychological assessment tool to evaluate the impact of receiving a diagnosis of myocarditis in the short and long-term.

The hypothesis is:

- Patients experience high levels of ongoing psychological distress many years after index hospital admission with acute myocarditis.

7.2. Background

Clinical presentation with myocarditis can be heterogenous leading to diagnostic uncertainty alongside variable clinical outcomes that can include life-threatening arrhythmias, persistent or progressive heart failure and death. In young patients with no past medical history, the need for mechanical circulatory support and intensive care unit admission is likely to result in some level of psychological distress in the long-term. In a study of 41 patients with fulminant myocarditis requiring mechanical circulatory support, post-traumatic stress disorder (PTSD) was formally identified in up to 27% of patients.[323] PTSD is characterised by thoughts of re-experiencing the traumatic event, avoiding reminders of the traumatic event and increased anxiety resulting in irritability, difficulty concentrating or trouble falling asleep.

Amongst patients with non-fulminant myocarditis, we have anecdotally observed similar levels of psychological distress owing to the general uncertainty regarding; (i) diagnosis, (ii) lack of specific management or therapeutic interventions, (iii) advice on exercise avoidance to minimise risk of sudden death, and further uncertainties following recovery on (iv) therapy withdrawal, (v) return to exercise, (vi) risk of recurrence and (vii) optimal timing for follow-

up evaluation. This is due to the lack of consensus in all these areas. With all these unanswered questions in myocarditis, the shock of acute hospitalisation in otherwise fit and healthy young individuals may be equally distressing as those with fulminant forms of myocarditis. To date, there are no published studies focusing on the non-fulminant group and access to psychological support is thus limited due to lack of evidence justifying its need.

In stark contrast, many studies have sought to examine the psychological impact of acute myocardial infarction, but it is important to note that patients have tended to be older, have pre-existing comorbidities, receive a definite diagnosis of a 'heart attack' and promptly undergo a definitive interventional procedure along a protocol-based management plan. Nevertheless, a heart attack can be a life-changing event and the prevalence of PTSD has been estimated at 12% of affected individuals based on a recent meta-analysis of 24 studies consisting of 2383 acute myocardial infarction patients.[324] This effectively equates to PTSD in 1 in 8 individuals.

Early discussions with patients in our centre indicated a high degree of long-standing anxiety and stress related to their myocarditis diagnosis, prompting the need for a more in-depth study to understand the prevalence of PTSD across the spectrum of myocarditis disease severity and to guide the need for supportive measures.

7.3. Methods

PATIENT COHORTS

(1) All myocarditis patients recruited into both work packages of our multi-centre study were asked to complete a self-administered psychometric questionnaire at baseline, 3- and 12-month evaluation in addition to the other assessments as outlined in chapter 4 (n=231). Inclusion and exclusion criteria were otherwise identical.

(2) An additional cohort of patients was recruited at Harefield Hospital following acute ST-elevation myocardial infarction (STEMI) with primary percutaneous coronary intervention (PCI) to provide a control cohort. These patients did not undergo CMR scans.

All patients provided written informed consent for the collection of clinical baseline and follow-up data as approved and directed by the National Research Ethics Service.

PSYCHOLOGICAL ASSESSMENT TOOL

All patients were asked to complete a self-administered psychometric questionnaire known as the Impact of Event Score (IES).[325] Written permission was acquired from the authors to utilise the questionnaire (see appendix). This consisted of 22 questions across the psychological domains of intrusion, avoidance and hyperarousal. There were 5 possible responses to each question; 'not at all' (0 points), 'a little bit' (1 point), 'moderately' (2 points), 'quite a bit' (3 points), and 'extremely' (4 points). The maximum possible score was therefore 88 points.

ANALYSIS

Questionnaire scores were calculated by adding the points for all 22 questions. A total score of 24 points or more indicated a clinical concern of PTSD,[326] 33 or move represented the optimal cut-off for a probable diagnosis of PTSD[327] and 37 or more indicated a level of psychological stress reported to have detrimental effects on an individual's immune system.[328]

7.4. Results

For myocarditis patients, the median IES questionnaire score was 21 points (IQR 7-29) at baseline, 14 (IQR 5-26) at 3 months, 10 (IQR 3-19) at 12 months and 8 (IQR 2-18) in the retrospective cohort with healed myocarditis.

When considering the interpretation thresholds, at baseline 39% of myocarditis patients reported scores indicating a clinical concern of PTSD (≥24 points) with approximately 1 in 5 patients reporting scores ≥33 points suggesting probable PTSD (table 7-1).

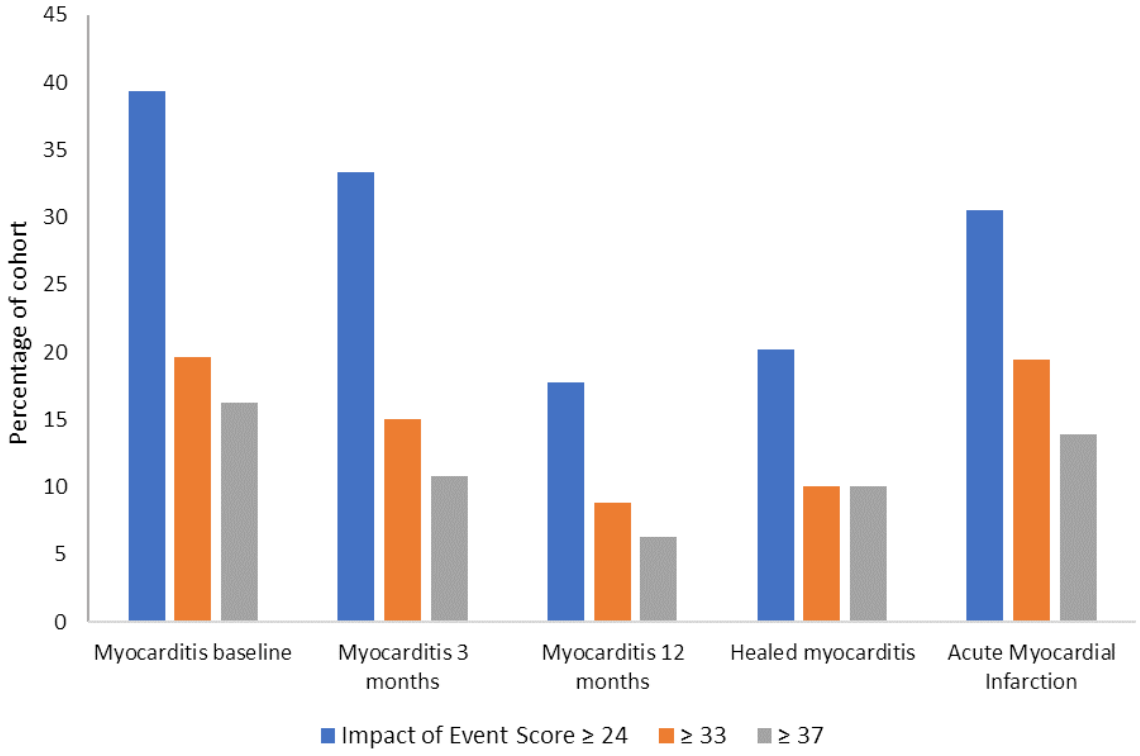
Over the study period, reported scores decreased but remained elevated in the healed myocarditis group which was recruited many years following index admission (figure 7-1). Of note, approximately 1 in 5 patients in the healed myocarditis group continued to meet criteria for a clinical concern of PTSD, with 10% meeting criteria for probable PTSD.

In the setting of acute STEMI, scores were also elevated with 31% of patients indicating a clinical concern of PTSD, compared to 39% of the baseline myocarditis patients, although the median score of 11 (IQR 7-24) did not differ significantly from acute myocarditis (p=0.26).

Table 7-1. Percentage of patients scoring above the defined impact of event scoring thresholds across the different study cohorts and time points also represented in tabular form.

	Myocarditis baseline	Myocarditis 3 months	Myocarditis 12 months	Healed myocarditis	Acute MI
≥ 24 points	39.3	33.3	17.7	20.2	30.6
≥ 33	19.7	15.1	8.9	10.1	19.4
≥ 37	16.2	10.8	6.3	10.1	13.9

Figure 7-1. Bar graph showing the percentage of patients scoring above the defined impact of event scoring thresholds across the different study cohorts and time points.



7.5. Discussion

Acute myocarditis remains a challenging diagnosis due to heterogeneity in clinical presentation and aetiology with varying clinical outcomes. In this large study of well-characterised myocarditis patients with predominantly non-fulminant forms of disease as outlined in chapter 4, we demonstrated for the first time the profound and long-lasting nature of the psychological impact of receiving a diagnosis of myocarditis compared to acute myocardial infarction. These findings highlight the considerable morbidity associated with this condition and the need for supportive measures.

The psychological impact of myocarditis remains vastly underestimated. Whilst patients with fulminant myocarditis have been studied extensively with the expected finding of significantly high levels of anxiety and distress arising from implantation of circulatory support systems, the identification of a clinical concern of PTSD in 39% of our patients at baseline and still 20% in the retrospective cohort suggests an acute onset but long-lasting psychological effect on these patients. Of note, IES scores exceeded 37 points in 16% of the baseline cohort and still 10% of the healed myocarditis cohort. In a study of 1550 Japanese men, it was shown that amongst the 60 men with IES scores above 37 due to previous events such as traffic accidents, violence and fire, the numbers of lymphocytes, NK-cell activity and total amounts of IFN-gamma and IL-4 in peripheral blood samples were all significantly lowered compared to men with no reported PTSD.[328] A similar finding has also been reported in war veterans with combat-related PTSD.[329] In myocarditis patients, such dysregulation of immune function may contribute to initial and recurrence susceptibilities and represents an important area for future research.

Mental health is an area that has received much attention in recent years, and we have identified a clear unmet need to alleviate the potential psychological distress experienced by young individuals diagnosed with acute myocarditis. In light of our findings, strategies to improve patient eduction, access to psychological support and sign-posting of existing patient resources

were promptly adopted and offered to all study participants. As such, we set up an annual myocarditis patient and relatives information evening supported by the Janson Foundation, and later Cardiomyopathy UK, at the Royal Brompton Hospital. This was established in 2016 and continues to expand in attendance each year.

7.6. Limitations

The assessment of an individuals' psychological state of mind is complex and often requires a face-to-face interview by a trained expert. Nevertheless, the IES questionnaire provided a window into the potential psychological distress experienced by these young individuals and was self-administered removing any potential bias from a member of the research team asking the questions verbally and interpreting the responses.

7.7. Future work

As part of our ongoing work, we aim to integrate circulating biomarker signatures with CMR and genetic findings and plan to incorporate these psychological results into the analyses to investigate the potential interaction between psychological distress and immune system dysregulation resulting in more severe forms of disease. We also continue to actively engage with myocarditis and cardiomyopathy patient charities to ensure the ongoing delivery of patient information evenings, including access to online support networks and events. We also plan to harness the recently developed HeartHive online platform developed within our group to extend patient recruitment beyond our local region as we prepare to launch our forthcoming national myocarditis study to build upon the findings presented here.

8. WHAT THIS THESIS ADDS AND FUTURE WORK

Review of aims and objectives

The overall aim of this thesis was to evaluate whether the integration of clinical, advanced imaging and genomic data could inform patient risk stratification and improve mechanistic understanding of myocarditis pathobiology, whilst leveraging electronic healthcare data on a population level to improve our understanding of myocarditis epidemiology and clinical outcomes. Our pertinent findings and future work are summarised as follows:

Epidemiology

To address these aims, the first part of this thesis explored the national numbers of myocarditis admissions across NHS England over the last 20 years. To date, this represents the largest epidemiological study of myocarditis patients on a population level. We demonstrate the rapidly rising incidence and prevalence of myocarditis with significant geographical variation in admission rates. We demonstrate clear age and sex differences, seasonal variation and highlight the limited use of endomyocardial biopsy, contrary to current ESC working group consensus guidelines. Only patients meeting the threshold for hospital admission were included in this study. However, the clinical need is presently greatest amongst these admitted individuals. Given the risk of SCD and progression to DCM, coupled with a clear focus on technology in the latest NHS Long Term Plan, going forward we plan to further explore and harness this high-quality, longitudinal national dataset to guide the development of national clinical standards in acute myocarditis and related conditions, including pericarditis.

Integration of advanced CMR techniques

Whilst much progress has been made in the invasive histopathological evaluation of myocarditis, there is limited understanding of additional non-invasive markers that identify subsets of high-risk individuals in whom further monitoring and medical therapy are key. In the second part of the thesis, we demonstrate the use of a precision approach amongst a prospective cohort of myocarditis scanned at 3 time-points in the first year after presentation to provide novel insights into the relationship between myocardial oedema and strain in patients with preserved LV ejection fraction. Multiparametric mapping has already transformed the assessment of various myocardial disorders and quantification of subtle regions of myocardial oedema and fibrosis holds much promise in the diagnosis and prognosis of myocarditis, as reflected by the recently updated Lake Louise Criteria. In future, the application of diffusion tensor imaging may provide additional understanding of the aberrations in myocardial fibre orientation in the setting of acute myocarditis.

Clinical outcomes

The thesis then reported clinical outcomes within our recruited cohort of myocarditis patients. We demonstrate that outcomes from our cohort are consistent with published studies and are predominantly stratified depending on initial presentation with fulminant or non-fulminant myocarditis. Further work is underway to integrate the in-depth precision phenotyping by CMR with characterisation of inflammatory and fibrogenic pathways by mass spectrometry to explore and dissect the differences between patients showing spontaneous recovery versus progression to DCM. There are also potential cost savings through unnecessary follow-up of the ‘recovered’ patient, and conversely, more targeted surveillance of the ‘at-risk’ patient.

Genetic determinants of myocarditis

In the next part of the thesis, we explored the genetic determinants of myocarditis amongst our recruited cohort of 231 individuals with CMR or biopsy confirmed myocarditis. To date, this work represents the largest study of adult patients with myocarditis. We demonstrate a significant excess burden of loss of function (truncating) variants in key ARVC genes in myocarditis cases compared to our healthy volunteers sequenced on the same platform. Importantly, these individuals did not exhibit other clinical features of ARVC by current criteria, suggesting that an acute inflammatory episode labelled as myocarditis may potentially constitute an environmental modifier that subsequently unmasks the underlying myocardial abnormality. This finding may have important clinical implications in the diagnosis and follow-up of myocarditis patients, despite the absence of a family history of cardiomyopathy. Going forward, extracted DNA will also be evaluated in a genome-wide association study to explore whether any single-nucleotide polymorphisms within the immune system may be linked to an underlying predisposition to myocarditis in the setting of acute viral infection.

Prognostic significance of healed myocarditis

In this chapter, we explored the prognostic significance of non-ischaemic patterns of LGE, typically ascribed to healed myocarditis, in the presence of normal LV volumes and function. Our objective was to address a question that commonly arises in patients with non-ischaemic patterns of LGE and normal LV volumes and function regarding their sudden cardiac death risk. We demonstrate for the first time that the identification of non-ischaemic patterns of myocardial fibrosis in the absence of other risk factors, such as LV dilatation, contractile impairment and a family history of cardiomyopathy, is not a marker of increased SCD risk over an extended period of follow-up, therefore aggressive medical therapy or routine use of ICD implantation are not required in this cohort. Whilst other studies have shown that even small

extents of incidental, subendocardial infarction increase all-cause mortality, non-ischaemic fibrosis does not appear to affect all-cause mortality when LV structure and function are otherwise normal. All-cause mortality was primarily driven by increasing age and not LGE presence, location or extent. However, those with LGE were at increased risk of unplanned CV hospitalization, mainly for suspected myocarditis and symptomatic, non-sustained ventricular tachycardia. This again highlights the morbidity in affected individuals.

Psychological impact

In the final part of the thesis, we explored the psychological impact of receiving a diagnosis of myocarditis. Our data highlights for the first time the profound and long-term psychological morbidity associated with this condition in the form of post-traumatic stress disorder, which typically affects young individuals during the prime of their life. Going forward, we are working with patient experience groups to develop and sign-post strategies for psychological support and improve the mental health of our patients living with myocarditis.

Conclusions

Myocarditis is a heterogeneous disease predominantly affecting young individuals with a significant burden of morbidity and mortality. A disease with such complexity requires contemporary, personalised and precision approaches to guide risk prediction and management. The work in this thesis refines our current understanding in all these areas and provides novel insights in epidemiology, advanced imaging, genomics and long-term outcomes of patients with myocarditis. Ultimately, these findings will inform a larger multi-centre study of the predictors of remodelling and recovery following an episode of acute myocarditis using an integrated approach using CMR, proteomics and genomics.

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10. APPENDIX

Letter of Support to Access NHS Digital HES Data

Royal Brompton & Harefield NHS
NHS Foundation Trust

16th April 2018

Frances Hancox
Data Access Request Service at NHS Digital,
1 Trevelyan Square
Boar Lane
Leeds, West Yorkshire
LS1 6AE

Dear Frances

Re: Epidemiology and Prognosis of Acute Myocarditis
DARS-NIC-144568-D7G6V

I write to support this application to study the epidemiological trends and prognosis in acute myocarditis from one of our clinical fellows, Dr Amrit Lota, supported by his supervisor, Dr Sanjay Prasad, who is also one of the cardiology consultants in our Trust.

This study will greatly enhance our understanding of the incidence and prevalence of myocarditis on a national level, which will ultimately lead to improved disease detection and recognition, risk stratification and clinical outcomes.

Anonymised record level data is requested from the Hospital Episode Statistics dataset on all admissions due to myocarditis from 1997-2017 in addition to other related conditions with similar clinical presentation (pericarditis and acute myocardial infarction) and long-term complications (heart failure, dilated cardiomyopathy and sudden cardiac arrest).

Office of National Statistics mortality data linked to the HES dataset is required in order to: (i) define the prevalence of cardiovascular mortality in these patients who are often young and otherwise healthy, and (ii) correlate HES data with cardiovascular mortality to retrospectively identify high-risk clinical features that may guide the care of patients with myocarditis in the future.

The funds to support this work are held within the Royal Brompton & Harefield NHS Foundation Trust, which will ensure there is no unauthorised use or disclosure of the data. Dr Prasad and Dr Lota are aware that all supplied data must be destroyed after use and that no additional use can be made of the data.

Please do not hesitate to get in contact if any further supporting information is required.

Yours sincerely,

Dr Richard Grocott-Mason
Medical Director and Responsible Officer
Consultant Cardiologist

Living with Myocarditis Support Leaflet

A lifetime of specialist care

Royal Brompton & Harefield NHS
NHS Foundation Trust

Living with Myocarditis - Psychological Support

The experience of new heart symptoms and medical investigations can be a difficult time, involving a lot of worry and uncertainty. Whilst a diagnosis of myocarditis can bring some relief and reduce uncertainty, it can also give rise to new concerns and ongoing stress, this can be a difficult time both for you and your family as everyone adjusts to the new information and learns about myocarditis and how to take care of your health and live well. During these times, it is understandable to experience worry and to feel down at times; if you feel that it would be helpful to talk to someone about your concerns, there are lots of places for information and support:

- **You can talk to your GP** about being referred for counselling or psychological therapy.
- **You can find your local psychology service** – it is called IAPT, which stands for 'Improving access to psychological therapies' – and you can self-refer to this service. Look at this website to find your nearest service and type in your postcode or town: https://www.nhs.uk/Service-Search/Psychological-therapies-(IAPT)/LocationSearch/10008
- **You can call the British Heart Foundation** on 0300 330 3311 and speak to one of their nurses on the heart helpline between 9-5, Monday to Friday.
- **You can join The British Heart Foundation online community platform**, HealthUnlocked. Members include those who have been affected by the same condition: https://healthunlocked.com/bhf
- **You can get information about heart conditions and mental wellbeing on the British Heart Foundation website:** https://www.bhf.org.uk/informationsupport/heart-matters-magazine/wellbeing/mental-health
- **You can call the Samaritans** who provide confidential, non-judgemental emotional support for people experiencing feelings of distress or despair, including those that could lead to suicide. You can phone, email, write a letter or in most cases talk to someone face to face. Telephone: 116 123 (24 hours a day, free to call), Website: www.samaritans.org

There are a number of charities that understand the needs of people living with myocarditis and that are undertaking dedicated, committed work to advance myocarditis research, treatments, knowledge and awareness:

- **Myocarditis Foundation:** https://www.myocarditisfoundation.org/about-myocarditis/
- **Myheart: Cardiac Risk in the Young** (CRY). Support for young people diagnosed with life threatening heart conditions: https://www.myheart.org.uk/
- **Cardiomyopathyuk Emotional Health:** https://www.cardiomyopathy.org/emotional-health/emotional-health
- **Alexander Janson's Fund:** http://alexanderjansonsfund.org/

There are also a number of mindfulness and relaxation apps which you might find helpful:

- **Headspace:** Mindfulness get 10 free sessions. https://www.headspace.com/how-it-works
- **Stop, Breathe and Think:** https://app.stopbreathethink.org/
- **Calm App:** Reduce anxiety, sleep better and feel happier. https://www.calm.com/
- **SAM:** App for anxiety http://sam-app.org.uk/

We hope that you find this helpful

This information has been developed by the Myocarditis team at the Royal Brompton Hospital: Dr Sanjay Prasad (Consultant Physician), Dr Amrit Lota (Clinical research fellow), Sara Saimi (research nurse), Dr Anne-Marie Doyle, Consultant Clinical Psychologist and Anna Bootle, Psychology assistant.

CMR Safety Checklist

Royal Brompton & Harefield NHS
NHS Trust

Cardiovascular Magnetic Resonance Unit
Tel: 020 7351 8800

Royal Brompton Hospital
Sydney Street
London SW3 6NP

Checklist & Information prior to having a Magnetic Resonance Scan

Name:	Date of birth:	Hospital Number:	Height	Weight

As explained in the Patient Information Leaflet, we need to know about any metallic objects or implants in the body, and some other conditions.	Yes	No
Do you have a pacemaker, a defibrillator, or pacing wires in your heart?		
Have you had any operation on your head or spine involving the insertion of clips or implants?		
Do you have any other implants or metal in your body? For example: Hearing aid, ear implant, eye implant, spine implant, others?		
Do you have a hydrocephalus shunt? If so, is it programmable?		
Have you had an injury to an eye which might have left metal in it?		
Have you ever had cardiac surgery?		
Do you suffer from epilepsy, diabetes, asthma or allergies (If yes, please circle which)?		
Are you wearing a drug-releasing patch?		
Are you aware of any problems with your kidneys or are you on kidney dialysis?		
Are you awaiting a liver transplant?		
Have you had recent major surgery or major illness?		
Is there any possibility you could be pregnant?		
Are you breastfeeding?		
Are you able to lie flat? If not, please state why.		
Have you removed your watch, bankcards, spectacles, hearing aids, keys, coins, jewellery, and hairgrips? (Gold rings are not a problem)		
	Please tick	

Please note that this is a teaching hospital, and there may be doctors/staff in training observing the scan.

I have explained the procedure to the patient. In particular, I have explained the intended benefits, serious or frequently occurring risks. I have discussed what the procedure is likely to involve, the benefits and risks of any available alternative treatments (including no treatment) and any particular concerns of those involved.

Consentor's Signature: ..... Date: .....

By signing below you acknowledge that you have read the Patient Information Leaflet, that the procedure has been explained to you by a qualified person, and that you have answered the above questions correctly.

Patient's Signature: ..... Date: .....

Witness' Signature ..... Date: .....

Impact of Events Questionnaire

IMPACT OF EVENTS SCALE-Revised (IES-R)

INSTRUCTIONS: Below is a list of difficulties people sometimes have after stressful life events. Please read each item, and then indicate how distressing each difficulty has been for you DURING THE PAST SEVEN DAYS with respect to the diagnosis of myocarditis (event) that occurred on _____ (date). How much have you been distressed or bothered by these difficulties?

	Not at all	A little bit	Moderately	Quite a bit	Extremely
1. Any reminder brought back feelings about it	0	1	2	3	4
2. I had trouble staying asleep	0	1	2	3	4
3. Other things kept making me think about it.	0	1	2	3	4
4. I felt irritable and angry	0	1	2	3	4
5. I avoided letting myself get upset when I thought about it or was reminded of it	0	1	2	3	4
6. I thought about it when I didn't mean to	0	1	2	3	4
7. I felt as if it hadn't happened or wasn't real.	0	1	2	3	4
8. I stayed away from reminders of it.	0	1	2	3	4
9. Pictures about it popped into my mind.	0	1	2	3	4
10. I was jumpy and easily startled.	0	1	2	3	4
11. I tried not to think about it.	0	1	2	3	4
12. I was aware that I still had a lot of feelings about it, but I didn't deal with them.	0	1	2	3	4
13. My feelings about it were kind of numb.	0	1	2	3	4
14. I found myself acting or feeling like I was back at that time.	0	1	2	3	4
15. I had trouble falling asleep.	0	1	2	3	4
16. I had waves of strong feelings about it.	0	1	2	3	4
17. I tried to remove it from my memory.	0	1	2	3	4
18. I had trouble concentrating.	0	1	2	3	4
19. Reminders of it caused me to have physical reactions, such as sweating, trouble breathing, nausea, or a pounding heart.	0	1	2	3	4
20. I had dreams about it.	0	1	2	3	4
21. I felt watchful and on-guard.	0	1	2	3	4
22. I tried not to talk about it.	0	1	2	3	4

Total IES-R Score:_____

INT: 1, 2, 3, 6, 9, 14, 16, 20
AVD: 5, 7, 8, 11, 12, 13, 17, 22
HYP: 4, 10, 15, 18, 19, 21

Weiss, D.S. (2007). The Impact of Event Scale-Revised. In J.P. Wilson, & T.M. Keane (Eds.) *Assessing psychological trauma and PTSD: a practitioner's handbook* (2nd ed., pp. 168-189). New York: Guilford Press.

AETR2N 22 1/13/2012

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Tue 24/04/2018 01:13

WD

Weiss, Daniel <Daniel.Weiss@ucsf.edu>

Re: Impact of Event Scale

To Lota Amrit

You replied to this message on 30/04/2018 09:47.

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please see attached files

Daniel S. Weiss, Ph.D.
Editor in Chief Emeritus, *Journal of Traumatic Stress*
Professor of Medical Psychology
Department of Psychiatry
University of California San Francisco
San Francisco, CA 94143-0984
P: 415 476 7557
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From: A.Lota@rbht.nhs.uk <A.Lota@rbht.nhs.uk>
Sent: Monday, April 23, 2018 8:45:32 AM
To: Weiss, Daniel
Cc: Sosa, Hugo
Subject: Impact of Event Scale

Dear Dr Weiss,

I am a clinical research fellow based at the National Heart & Lung Institute of Imperial College London.

Our groups is interested in using the revised Impact of Event scale in a research study involving young patients with heart disease in whom we suspect the burden of receiving a life-changing diagnosis is often underestimated.

Please could you let me know about how we may access and obtain permission to use and publish data from the IES scale in our patient cohort?

Many thanks,

Amrit

Dr Amrit Lota
Clinical Research Fellow
Cardiovascular Biomedical Research Unit
Royal Brompton Hospital
Tel: 020 7352 8121 Ext 2920

From: ERR [mailto:rrodriguez@e-heart.org]
Sent: 23 March 2019 14:11
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Many thanks,

Amrit

Dr Amrit Lota
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Sudden death risk markers for patients with left ventricular ejection fractions greater than 40%

Author: Brian P Halliday,Amrit S Lota,Sanjay K Prasad

Publication: Trends in Cardiovascular Medicine

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