Understanding Heart Disease in Patients with Muscular Dystrophy

by

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Abstract

Background

Muscular dystrophy (MD) is a group of inherited neuromuscular disorders with heart disease as a leading cause of morbidity and mortality. Dystrophinopathies such as Duchenne and Becker MD, limb-girdle MD, type 1 myotonic dystrophy (DM1), and facioscapulohumeral MD are most associated with heart disease. Patients present with a broad variety of neuromuscular systems, which complicates prognosis and challenges effective clinical management. Cardiac assessment as well as the impact of cardiac medical and device therapies on patient outcomes has remained an underrepresented facet of clinical research in patients with MD.

Objectives

To assess the value of cardiac monitoring and management on the clinical outcomes of adult patients with MD. This thesis aimed to further understand heart disease secondary to MD from a clinical perspective through a variety of cardiac assessment modalities while evaluating diagnostic and prognostic utility. Furthermore, the association between cardiac assessment, use of cardiac therapies, and MD patient prognosis was analyzed.

Methods and Results

In Chapter 3, the impact of cardiac medical and device therapies as a core component of a collaborative multidisciplinary care pathway for the care of patients with MD was explored. Cardiac intervention can markedly improve all-cause and cardiac-related clinical outcomes over

a sustained period while also improving left ventricular (LV) systolic function. In Chapter 4, the utility of cardiac plasma biomarkers for the management of heart disease in patients with MD was explored. B-type natriuretic peptide and high-sensitive troponin I were able to effectively diagnose heart disease and prognosticate adverse events using novel cutoff values. In Chapter 5, the utility and morphology of 12-lead electrocardiogram (ECG) in patients with MD was explored. The identification of left bundle branch block (LBBB) and QRS fragmentation was associated with a diagnosis of cardiomyopathy, and LV hypertrophy by contemporary ECG criteria was determined to be of limited utility. In Chapter 6, the incidence of ventricular tachycardia (VT) in patients with DM1 was explored. There was a high prevalence of VT in the patient cohort, which highlighted the risk of sudden cardiac death to attention in patients with DM1. In Chapter 7, the value of cardiac resynchronization therapy (CRT) in patients with DM1 with LBBB was explored. Device intervention by CRT was shown to markedly reduce QRS complex duration and improve LV systolic function. In Chapter 8, the utility of transthoracic echocardiogram-derived left ventricular ejection fraction (LVEF) in a mixed adult MD cohort was explored. Baseline LVEF and trajectory by serial measures showed important diagnostic and prognostic utility for heart disease and major adverse cardiac events (MACE), respectively. In Chapter 9, the utility of cardiac magnetic resonance including advanced imaging techniques in a mixed adult MD cohort was explored. Longitudinal, circumferential, and radial strain amplitudes had important prognostic utility for MACE.

Conclusions

Heart disease is characterized by adverse remodeling and reduced systolic function and conduction abnormalities in patients with MD. Importantly, heart disease can be effectively

monitored and managed through the use of medical and device therapies facilitated by various modalities of cardiac assessment within a multidisciplinary care model. Moreover, the use of novel methods as outlined in this thesis are feasible and accessible in modern clinical practice. Active monitoring and management can reduce the burden of disease to improved clinical outcomes in patients with MD.

Preface

This thesis includes writing and data from published and unpublished manuscripts. Specific contributions to the listed projects are outlined below.

Published manuscript adapted for Chapter 3

Nikhanj A, Yogasundaram H, Miskew Nichols B, Richman-Eisenstat J, Phan C, Siddiqi ZA, Oudit GY. Cardiac intervention improves heart disease and clinical outcomes in patients with muscular dystrophy in a multidisciplinary care setting. J Am Heart Assoc. 2020;9:e014004. **Role of A.N.:** Obtained and analyzed clinical data, performed statistical analysis, drafted the manuscript, and integrated contributions and edits from coauthors.

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Published manuscript adapted for Chapter 5

Nikhanj A, Yogasundaram H, Kimber S, Siddiqi ZA, Oudit GY. Clinical utility of 12-lead electrocardiogram in evaluating heart disease in patients with muscular dystrophy: Assessment of left ventricular hypertrophy, conduction disease, and cardiomyopathy. Ann Noninvasive Electrocardiol. 2021;26:e12876. **Role of A.N.:** Obtained and analyzed clinical data, performed statistical analysis, drafted the manuscript, and integrated contributions and edits from coauthors.

Published manuscript adapted for Chapter 6

Nikhanj A, Sivakumaran S, Miskew-Nichols B, Siddiqi ZA, Oudit GY. Ventricular tachycardia in patients with type 1 myotonic dystrophy: a case series. Eur Hear J Case Rep. 2019;3:1-5. **Role of A.N.:** Obtained and analyzed clinical data, performed statistical analysis, drafted the manuscript, and integrated contributions and edits from coauthors.

Published manuscript adapted for Chapter 7

Nikhanj A, Sivakumaran S, Yogasundaram, H, Becher H, Kimber S, Siddiqi ZA, Oudit GY. Comparison of usefulness of cardiac resynchronization therapy in patients with type 1 myotonic dystrophy with versus without left bundle branch block. Am J Cardiol. 2019;124:1770-1774. **Role of A.N.:** Obtained and analyzed clinical data, performed statistical analysis, drafted the manuscript, and integrated contributions and edits from coauthors.

Unpublished data and text adapted for Chapter 8

Nikhanj A, Kashyap N, Wang K, Phan CL, Siddiqi ZA, Becher H, Oudit GY. Trajectory of Left Ventricular Ejection Fraction in Response to Therapies in Patients with Muscular Dystrophy. **Role of A.N.:** Obtained and analyzed clinical data, performed statistical analysis, drafted the manuscript, and integrated contributions and edits from coauthors.

Unpublished data and text adapted for Chapter 9

Nikhanj A, Kashyap N, Pfeffer G, Siddiqi ZA, Thompson R, Paterson I, White JA, Oudit GY. Evaluating the Clinical Utility of Cardiac Magnetic Resonance in Patients with Muscular Dystrophy: A Provincial Study. **Role of A.N.:** Obtained and analyzed clinical data, performed statistical analysis, and drafted the manuscript for the Edmonton cohort. Advanced imaging analysis was performed by Dr. James A. White's research group.

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1. Introduction

1.1. Background

Muscular dystrophy (MD) refers to a group of inherited neuromuscular disorders commonly associated with progressive muscle weakening and wasting as well as various comorbidities.[1, 2] Patient condition and clinical management is often complicated by cardiac, respiratory, neurological, and metabolic manifestations, as well as physical limitations which affect both quality of life and patient lifespan. Heart disease is recognized as the most common cause of mortality and morbidity in patients with MD and respiratory illness as an important comorbidity.[3-6] Of the 9 types and more than 30 subtypes of MD, dystrophinopathies including Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD), limb-girdle muscular dystrophy (LGMD), type 1 myotonic dystrophy (DM1), and facioscapulohumeral muscular dystrophy (FSHD) are documented to have a high burden of heart disease.[1, 3, 7-9]

Muscular dystrophies are most recognized for their signs of muscle wasting and symptoms of muscle weakness. Various mutations cause changes to muscle fibre support proteins, leading to degeneration and unregulated regeneration of muscle fibres, which is replaced by fibrotic tissue.[10, 11] Fibrosis is detectable before patients show clinical symptoms of muscle wasting and signs of fibrosis on initial skeletal muscle biopsy strongly correlate with decreased muscle strength and a decreased ability to ambulate.[10-12] Early detection of fibrosis could therefore predict patient outcomes and help monitor disease progression, especially in those diagnosed with MD. Progression of disease and degree of muscle damage is dependent on the type of MD and the proteins associated. In cases of DMD, loss of functional dystrophin

proteins leads to loss of stability at the Dystroglycan Complex (DGC) of the sarcolemma. As a result, the muscle has a reduced ability to withstand mechanical forces.[10, 11] This is associated with increased muscle cell permeability and changes in sodium, potassium and calcium gradients. Gradient changes ultimately cause the production of reactive oxygen species, thus accelerating muscle fibre necrosis and activating inflammatory responses.[10, 11, 13] Additionally, the activation of growth factors combined with muscle's limited regeneration, leads to excessive deposition of collagen, fibronectin, and fat cells.[14, 15] The variable impact to different locales of skeletal muscle is not fully understood but has been theorized to be due to varying microenvironments of various muscles at a mechanical, humoral, and cellular level.[10, 11, 16-18]

Cardiac manifestations of MD are evident in patients directly affected by the disease and for recessive carriers. The severity of cardiac involvement varies in patients between the different types of MD.[19, 20] We have characterized heart disease across various types of MD and have found impaired left ventricular (LV) systolic function, ascertained by tracking of left ventricular ejection fraction (LVEF), to be common.[3, 21] Patients with dystrophinopathies and LGMD also show LV chamber dilation as part of the adverse remodelling process associated with MD.[3] Cardiac fibrosis has also been detected in patients using cardiovascular magnetic resonance (CMR).[3, 22-24] Conduction defects may also be present without overt adverse remodeling as seen in patients with DM1, however left bundle branch block (LBBB) can impair LV systolic function.[25-28] Arrhythmias and conduction delays in MD patients include atrial flutter, atrial fibrillation, atrioventricular block, LBBB and right bundle branch block (RBBB), and sustained as well as non-sustained ventricular tachycardia (VT).[3, 19, 26-30] Importantly, patients with DMD and DM1 are at risk of sudden cardiac death (SCD) associated with the incidence of VT.[3, 27, 30] Reduced life expectancy is of high concern for patients with MD, namely

in DMD, where most patients die in their late 20s due to heart disease.[31] Further understanding of the progression of heart disease in patients with MD could markedly improve life expectancy. Effective patient screening, monitoring, and management in the context of each type of MD could standardize patient care and improve clinical outcomes.[3, 19] With advancements in genetic testing, mutations can be detected before clinical symptoms present and it is important to recognize that these genetic diseases of MD may present themselves in patients despite an apparent absence of family history.[29]

There is evidence that early pharmacological treatments for MD patients can improve cardiac outcomes and therefore timely diagnosis and management is critical to improve prognosis.[3, 19, 21, 25] At this time, there is no formal, standardized care process for patients with MD, which is a challenge from a cardiac perspective given the complexity of these vulnerable patients. Furthermore, diagnoses are complicated by the overall presentation of the disease since patients are often wheelchair bound. Lack of ambulation may also conceal symptoms and limit their identification at an earlier age, thus challenging the proposed prognostication. In the clinical population, it is highly likely that subclinical heart disease presents itself as the simple shortness of breath, and unfortunately, such symptoms are misattributed to respiratory ailments, without physicians recognizing the multi-system impact of heart disease, leading to incomplete management. Given the progressive nature of these diseases, it is imperative that clinicians consider standard techniques for the detection and monitoring of heart disease in the context of MD. This thesis demonstrates the accessible and feasible use of common clinical modalities with important diagnostic and prognostic value in MD patients to augment the care process and improve clinical outcomes.

1.2. Genetic Basis, Pathophysiology and Clinical Presentation

Dystrophinopathies

Dystrophinopathies, namely DMD and BMD, are the result of an X-linked recessive disorder affecting males, with documented symptomatic female carriers.[1, 25, 32-35] The most prominent signs of Dystrophinopathy are visible in young children whereby severe motor deficits often lead to wheelchair usage in their early teens. Furthermore, life expectancy is low and the oldest patients survive only into their early 40s.[22-24, 36, 37] Patients diagnosed with DMD exhibit proximal muscle weakness and wasting accompanied by impaired respiratory and cardiovascular function, while patients with BMD as well as carriers present with a milder phenotype.[1, 38] Progressive muscle weakness is also noted in patients diagnosed with BMD, though the phenotype is milder and has a later age of onset compared with DMD.[19, 34]

The genetic mutation associated with dystrophinopathies is a nonsense mutation in the *DMD* gene leading to absent dystrophin protein formation (i.e. DMD) or the formation of a truncated form of dystrophin (i.e. BMD).[24, 25] As a result, the protein dystrophin may not be expressed, or a truncated form may be evident in a patient.[24] There currently is no definitive understanding as to how the absence of Dystrophin causes disease.[19] Dystrophin maintains cell membrane stability by facilitating the transduction of mechanical forces across the extracellular matrix in skeletal and cardiac muscle. Dystrophin links actin filaments, via f-actin, to the sarcolemma of muscle fibers, via Dystroglycan, thereby creating an indirect connection from the cytoskeleton to the sarcolemma, as shown in Figure 1.1 and defined in Table 1.1.[39, 40] Dystrophin allows for the transduction of mechanical force across the extracellular matrix in skeletal and cardiac muscle, but in its absence, it can be understood why this distribution is severely interrupted.[24] We can elucidate that a lack of dystrophin will leave cells vulnerable to mechanical

damage.[24] On a cellular level, increased ionic flux has been observed across membranes in DMD patients, leading to an increase in intracellular sodium and calcium levels.[24] This facilitates the creation of a more oxidation-dominant state within the cytosol, promoting the activity of damaging cellular pathways, thus resulting in necrotic and apoptotic muscle cells.[24] Additionally, the rise in calcium allows for the activation of calcium-dependent mechanisms downstream, such as protease and caspase activity.[24] It is therefore understood that the integrity of the skeletal and cardiac muscle fibers has been compromised, thus compromising the vital system of which they are apart.[41] This has downstream effects on both mechanical and electrical properties of the heart (Figure 1.2).

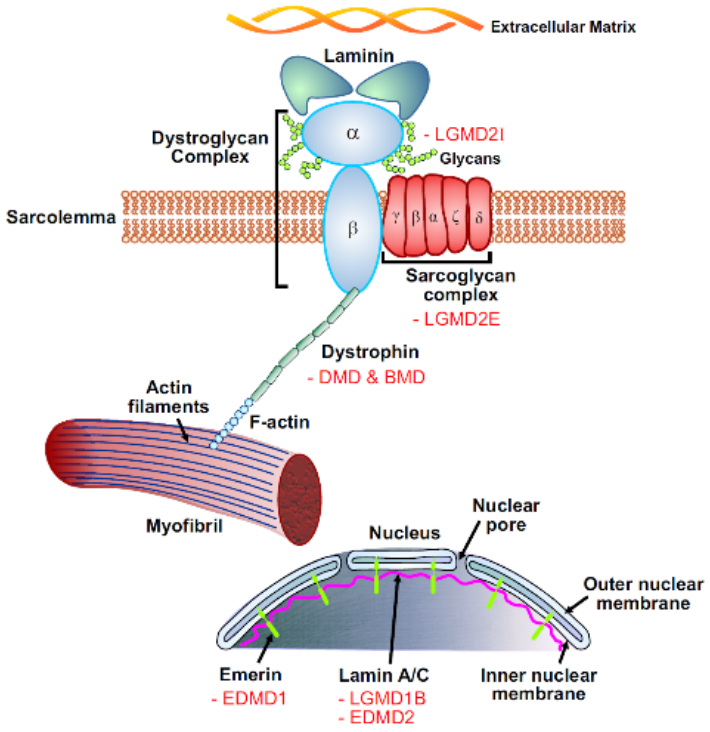


Figure 1.1. A schematic illustrating the close proximity and structural relations between MDs affecting cardiac muscle. Both structural and support proteins are illustrated, alongside involved sarcolemma-based complexes. Absent or truncated Dystrophin proteins phenotypically manifest themselves into a diagnosis of DMD, or less severe BMD. A compromised α-subunit of the DGC shows the phenotype of LGMD2I. A compromised β-subunit of the Sarcoglycan complex shows the phenotype of LGMD2E. Absence of functional Lamin A/C proteins is common in diagnosis of both LGMD1B and EDMD2. Absence of functional Emerin in the nuclear membrane manifests into EDMD1.

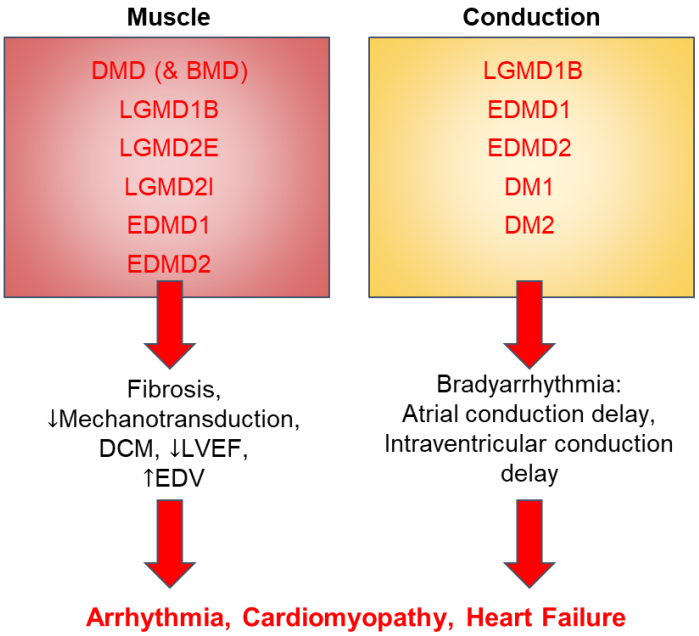


Figure 1.2. A flowchart demonstrating muscle and conduction-dominant pathophysiologies of absent or truncated structural and support proteins in cardiac muscle, related to subtypes of Dystrophinopathies, LGMD, EDMD, and DM. Some subtypes pertain to both musculature and conduction abnormalities; all MDs involved share arrhythmia, cardiomyopathy and heart failure as highly probable downstream clinical phenotypes.

Limb-Girdle Muscular Dystrophies

Limb-girdle muscular dystrophies are a group of MDs with muscle weakness and wasting in the shoulder and pelvic regions as hallmark features.[42] Limb-girdle muscular dystrophy type 1B is a rare class of autosomal dominant-inherited mutations affecting the *LMNA* gene, responsible for producing Lamin A/C proteins, which are intermediate filaments responsible for the nuclear lamina of cells in striated muscle (Figure 1.1).[43, 44] Limb-girdle muscular dystrophy 2A is known as a calpainopathy (calpain is a modulatory protease for structure and function of cellular proteins) due to loss-of-function mutations in the *CAPN3* gene.[45] Limb-girdle muscular

dystrophy type 2E is also the result of a loss-of-function mutation to the gene encoding for the β-subunit of the sarcoglycan complex (Figure 2).[43] Limb-girdle muscular dystrophy type 2I on the other hand is the more prevalent subtype, inherited through autosomal recessive mutations, affecting genes responsible for deriving fukutin-related proteins (FKRPs).[42] Inappropriate expression of FKRPs leads to defective glycosylation and therefore membrane targeting of the α-subunit of the dystroglycan complex resulting in cytoskeletal instability in straited muscle (Figure 1.1).[7]

From a cardiac perspective, LGMD2 resembles dystrophinopathies in presenting a reduced systolic function accompanied by chamber dilation, and in more advanced cases LV hypertrophy.[20] It should be noted that specific subtypes of LGMD2, namely LGMD2I and LGMD2E, are generally regarded as the subtypes of concern from a cardiac perspective.[19] With regards to LGMD2I, there is an observed dissociation between subendocardial and subepicardial fiber shortening, related to alpha-dystroglycan subunit discrepancies, which ultimately leads to a reduced systolic function.[20] Interestingly, LGMD2I is prevalent among the Hutterite population in North America, and has been coined “Hutterite LGMD”. This patient population provides a quality opportunity for research on inheritance patterns and cardiac manifestations. The Hutterites of North American originated from a small group of Europeans; the group remains isolated with high rates of consanguinity and trend of large families. Conveniently, the group keeps detailed information on ancestry, have nutritious diets, quality health care, remain geologically static and have well-defined members.[46] These traits provide a unique opportunity to further the knowledge on MD and its cardiac manifestations. Limb-girdle muscular dystrophy type 2E patients see similar fiber phenotypes, with beta-sarcoglycan subunit discrepancies, which in turn results in the overall dysfunction of the sarcoglycan complex, translating to greater

risk of heart disease in patients.[19, 47, 48] Reduced muscle strength is a physiological characteristic of both types of LGMD but should not be assumed to be correlated with a displayed reduced cardiac function.[19] Numerous other subtypes of LGMD exist, however there is currently limited evidence to suggest cardiac involvement associated with their pathologies.[48-51]

Myotonic Dystrophies

Type 1 myotonic dystrophy, also known as Steinert's disease, is a form of MD with a large variability in timing and severity of symptoms. Type 1 myotonic dystrophy is the most common form of myotonic dystrophy in the adult population.[30, 52] Type 1 myotonic dystrophy can be differentiated from type 2 myotonic dystrophy (DM2) in considering cardiac symptoms, which tends to show left ventricular dysfunction and hypertension, with minor symptoms.[19, 53] Patients with DM1 tend to show distal muscle weakness, while DM2 patients share the DMD phenotype of proximal muscle weakness. The two are similar in that they both show autosomal dominant patterns of inheritance.[54] Myotonic dystrophy is a conduction disorder, associated with ECG abnormalities and left ventricular dysfunction, both of which are associated with mortality[53], and it is commonly referred to as a repeat expansion disease. Type 1 myotonic dystrophy is associated with a trinucleotide repeat expansion (i.e. repeat expansion disease) in the *DMPK* gene thereby limiting the function of its associated serine-threonine kinase protein, which regulates muscle cell proliferation and differentiation, and has been proposed to be involved in the function of calcium channels in muscle.[55-57] On the other hand, DM2 is associated with a tetranucleotide repeat in the ZNF9 gene.[54] Both DM1 and DM2 are said to interfere with RNA function, leading to downstream cellular abnormalities (Table 1.1).[54] These abnormalities present themselves as dysregulated cardiac conduction phenotypes. DM1 tends to

be associated with both atrial conduction delays and intraventricular conduction delays, visualized by lengthened PR and QRS complexes respectively, on an ECG.[53] Type 2 myotonic dystrophy patients tend to only show delayed intraventricular conduction, but show a higher prevalence of left ventricular dysfunction than DM1 patients.[53] It should also be noted that DM2 patients have a higher risk of hypertension as a comorbidity.[53]

Facioscapulohumeral Muscular Dystrophy

Facioscapulohumeral muscular dystrophy (FSHD), an autosomal dominant form of MD, is the result of the ectopic expression of the toxic germline transcription factor *DUX4* gene in muscle cells, due to the hypomethylation of the D4Z4 region on chromosome 4, allowing the formation of the DUX4 protein.[58] This results in asymmetrical muscle wasting and weakening in the face, scapula region, and upper limbs.[59, 60] There is currently no conclusive evidence of cardiac manifestations directly connected to the genetic origins of FSHD. A clinical study by Trevisan et al. describes cardiac involvement of an arrhythmic nature in 12% of the FSHD patients.[61] On the other hand, a more recent study by Statland and Tawil describes cardiac muscle as a group that is spared from the genetic disease.[62] Our research has also shown a relatively lower degree of cardiac impact compared to other types of MD, however there are indications of RV involvement.

Emery-Dreifuss Muscular Dystrophy

Emery-Dreifuss muscular dystrophy is a rare genetic disease with both skeletal and cardiac musculature phenotypes. Emery-Dreifuss muscular dystrophy is commonly associated with a phenotypic triad: humeroperoneal muscular dystrophy, joint contractures, and heart

disease with conduction defects.[63] Emery-Dreifuss muscular dystrophy is unique in that it has been shown to display both X-linked and autosomal patterns of inheritance. X-linked recessive inherited EDMD, classified as EDMD1, as well as both autosomal dominant and recessive inheritance as EDMD2 and EDMD3, respectively.[64] In all forms of EDMD, localized striated muscle is compromised by weakened nuclear structure due to maldeveloped inner membrane proteins.[64] Emery-Dreifuss muscular dystrophy type 1 is characterized by mutations to the EMD gene, which codes for the membrane protein Emerin.[64] The Emerin protein plays an important role in maintaining the integrity of the nuclear envelope.[64] Autosomal forms, EDMD2 and EDMD3, are characterized by a mutation in the LMNA gene, which is responsible for developing intermediate filaments as part of the inner nuclear membrane.[64] Autosomal recessive EDMD3 is incredibly rare, but is known for its severity of early onset muscle weakness and wasting, and its late onset cardiac involvement.[65] Interestingly, LGMD1B shares a similar genetic origin to autosomal EDMD, allowing them to be classified as laminopathies, sharing similar cardiac phenotypes: arrhythmias and dilated cardiomyopathy.[64] These phenotypes are hypothesized to be the result of mutated Lamin, as displayed in Figure 1.1, which alters the structural interaction between the nucleus and cytoskeleton, thus compromising the ability of the cell to withstand mechanical stress.[64, 65] Mutations to both Emerin and Lamin proteins are said to impact cell proliferation and differentiation pathways through the development of toxic intermediates and inappropriate protein actions, with the potential to activate damaging cellular pathways (Table 1.1).[64, 65]

Table 1.1. Proteins associated with MDs of cardiac concern.

Protein	Location	Function	Type of MD caused by Mutation in the Protein
Dystrophin	Dystrophin-glycoprotein complex (DGC)	Links actin filaments to the sarcolemma to stabilize the muscle membrane [24]	DMD, BMD
Lamin A/C	Nuclear Envelope	Intermediate filaments in the inner nuclear membrane [64]	LGMD1, EDMD2, EDMD3
Calpain3	Skeletal muscles, eye lens, liver, brain, and cardiac muscle	Cleavage of cytoskeletal proteins; Calcium release from muscle [66]	LGMD2A
Dysferlin	Associated with sarcolemma	Muscle membrane repair; Muscle contraction [48]	LGMD2B
Sarcoglycans (α, β, γ, δ)	Adjacent to DGC	Links cytoskeleton to extracellular matrix (ECM) to stabilize the muscle membrane [67]	LGMD2C, D, E, F
TRIM32	Cytoplasmic bodies	E3 ubiquitin ligase in the ubiquitin (Ub)–proteasome pathway [49]	LGMD2H

Fukutin related proteins (FKRPs)	Brain, Cardiac Muscle and Skeletal Muscle	Glycosylation [20]	LGMD2I
Titin	Sarcomere	Assists in assembly of sarcomere [51]	LGMD2J
β-dystroglycan	DGC	Links cytoskeleton to ECM to stabilize the muscle membrane; ECM receptor [19]	LGMD2
Emerin	Inner Nuclear Envelope	Support nuclear structure via interactions with SUN and nesprin proteins [64]	EDMD1
Dystrophia Myotonica Protein Kinase (DMPK)	Brain, Skeletal Muscle, Cardiac Muscle	Unknown [67]	DM1
Zinc Finger Nucleic Acid-Binding Protein	Skeletal and Cardiac Muscle	Unknown [67]	DM1, DM2

DUX4	Skeletal Muscle, Testes	Transcription factor for paired-like homeodomain transcription factor 1 [68]	FSHD
SMCHD1 protein	Unknown	X-chromosome inactivation; Imprinting Double-strand break repair; Development of Eyes and Nose [69]	FSHD

Critical proteins involved in cytoskeletal and membranous structures of striated muscle. Their location and function are noted, alongside the consequences of a mutation to their protein structure; MDs are a result of mutated and truncated cytoskeletal proteins.

1.3. Monitoring Muscular Dystrophy Patients for Cardiac Complications

Heart disease is now recognized as the most common cause of mortality in patients with MD.[3, 7] There are two major forms of heart disease in patients with MD: cardiomyopathies, ranging from structural changes of the heart to heart failure, and arrhythmias.[70] The severity and type of cardiac manifestations vary between the different types of MD. Dystrophinopathies, namely DMD, exhibit heart disease characterized by decreased left ventricular ejection fractions, increased end diastolic volumes, decreased mechanotransduction, and fibrosis.[3] Ventricular arrhythmias are also common.[71, 72] Patients with BMD as well as carriers of dystrophinopathies have similar cardiac manifestations, but to a milder degree.[73, 74] Subtypes of LGMD have similar cardiac manifestations though they are often less severe.[7] Importantly, patients with dystrophinopathies and LGMD are documented to have ventricular arrythmias.[19, 75] Conduction abnormalities are observed in patients with DM1 such as bradyarrhythmias, atrioventricular conduction delays, and left bundle branch blocks.[9, 53] More alarmingly, is the high incidence of

ventricular arrhythmias in DM1 patients and the associated risk of sudden cardiac death, as demonstrated in our research.[3, 8] Facioscapulohumeral muscular dystrophy exhibits both mechanical and conduction abnormalities to a much milder degree.[3] As previously demonstrated by our research, there are no overt concerns regarding diastolic or valvular dysfunction in this cohort of patients.[3] Assessment of cardiac electrophysiology can be conducted at each NMMD clinic visit through 12-lead ECG with the option to incorporate 48-hour Holter monitoring, which is valuable in patients with DM1 given the high burden of conduction disease and arrhythmias. We have previously shown that cardiac biomarkers such as BNP and hsTnI can be used to support the diagnosis of cardiomyopathy in patients with MD as well as prognosticate MACE.[21] In addition, imaging studies such as echocardiograms have proven useful in patients with dystrophinopathies and LGMD given the high prevalence of heart disease, leading to a marked reduction in global systolic function and chamber dilation.[19] Echocardiography is able to adequately provide clinicians with structural and functional information on the cardiac status of patients with MD, which has limited the enthusiasm toward CMR use in these patients. Additionally, there are many cases that challenge the feasibility of CMR use including patients with limited mobility, wheelchair dependence, use of defibrillators and pacemakers incompatible with CMR, spinal stabilization rods with ferromagnetic properties, and mouthpiece ventilation. However, the utility of CMR continues to be explored in patients with MD, particularly with respect to detecting subclinical heart disease and further understanding the adverse remodeling process.[76-78]

In cases of DMD and LGMD, which often share similar skeletal muscle phenotypes, there are shared aspects of reduced LVEF and increased chamber dimensions, which may be monitored through TTE and CMR.[19] It is recommended to perform an TTE upon diagnosis with

MD, with repeat TTE every 2 years for patients under 10, or yearly TTE for those over 10 years of age.[25] Transthoracic echocardiograms are more accessible in clinical practice, however skeletal deformities of the spine and chest wall commonly seen in these patients can limit the diagnostic accuracy of this type of imaging. Ventilation therapy, often used in this population, makes this type of imaging more technically challenging. Cardiac magnetic resonance can easily overcome these challenges and is the most sensitive and accurate imaging modality in this population. Under gadolinium enhancement cardiac MRI has been used to demonstrate cardiac abnormalities in MD patients who presented with normal LV function upon TTE.[79, 80] In considering these facts, CMR is becoming more prevalent as it accurately displays cardiac dimensions, as visualized in Figure 1.3, and function. Cardiac fibrosis, which is indicative of cardiac involvement and LV function, can easily be detected by CMR.[81, 82] The primary value-add of CMR is facilitating earlier detection of cardiac manifestations in patients with MD. Left ventricular circumferential strain is said to be a more sensitive indicator for myocardial damage than reduced systolic function; myocardial damage may already be underway, therefore CMR is the most useful front-line method of detection.[38] Cardiac magnetic resonance is useful for cases of BMD, in which skeletal and cardiac muscle condition is often heterogeneous.[83] Challenges with CMR include the higher cost and decreased number of centers with access in comparison to other imaging modalities. Though earlier detection would be most beneficial to the pediatric population, children may need to be sedated for the CMR to be performed, which introduces potential complications.

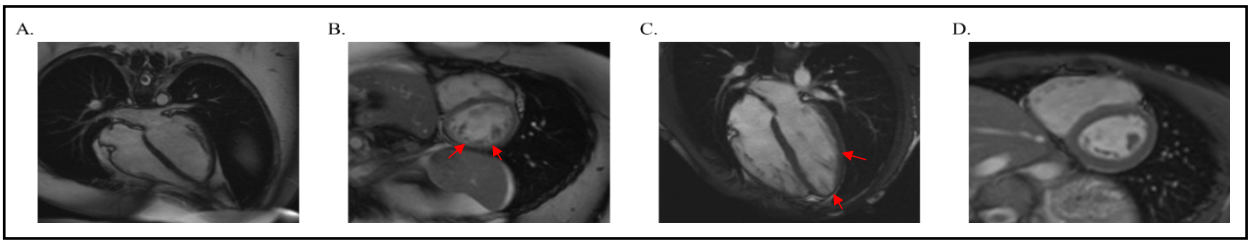


Figure 1.3. Cardiac magnetic resonance imaging of a patient with DM. Fibrosis on the inferior and inferolateral walls, with marked LV dilation (LVEDVi = 111 mL/m2, LVESVi 61 mL/m2, and LV mass indexed = 67 g/m) and reduced LV systolic function (LVEF = 45%; A and B); and CMR of a patient with LGMD, showing fibrosis in the mid and apical regions of the LV, moderate dilation of the LV (LVEDVi = 86 mL/m2, LVESVi = 41 mL/m2, and LV mass indexed = 64 g/m2) and reduced systolic function (LVEF = 53%; C and D). Red arrows indicate location and extent of fibrosis as visualized by late gadolinium enhancement

Plasma biomarkers should be monitored in all relevant cases of MD. Namely CK, which is expected to be at an elevated level in serum due to a described elevated muscle leakage in dystrophinopathies and LGMD, due to muscle wasting.[20, 41] Skeletal muscle phenotypes typically become evident within the first decade of life as contractures appear before muscle weakness and wasting, which is unique to this MD [64]. Skeletal muscle abnormalities also appear to be localized to specific anatomical regions unlike other MDs. Cardiac symptoms are said to follow in the second decade of life, where it would be most appropriate to take a multifaceted approach to monitoring including the use of plasma biomarkers as well as monitoring by ECG or Holter for arrhythmias.[21, 64] We have demonstrated the utility of BNP and hsTnI in our previous research, which has important diagnostic utility for heart disease and prognostic utility for MACE.[3, 21] Monitoring an ECG for sinus bradycardia, and lengthened PR intervals alongside shortened QRS

intervals, is a signature of EDMD1, allowing for further identification and differentiation.[65] Since the nature of EDMD and DM1 is best described with conduction abnormalities, cardiac monitoring through ECG and Holter would be most useful.

The presence of fibrosis in MD patients is of significant clinical importance since it predict patient outcomes, reflect disease progression, and is frequently used as an indicator of therapeutic efficacy. Functional exams, such as the six-minute walking test, may be used to indirectly measure fibrosis. However, there is a large risk of influence from patient confounding variables. The six-minute walking test is also not validated in young children, who would benefit most from fibrosis measurements. Muscle biopsy is a more reliable option, but biopsy location must be chosen with caution, with limitations due to its invasive nature acknowledged [84]. Various imaging modalities including CT, ultrasound and CMR have been used to detect and measure fibrosis in MD patients; CMR is superior for fibrosis detection due to its improved contrast and resolution. [85] Importantly, CMR use has already contributed significant knowledge to the field of MD.[84-87] The use of CMR in MD patients can help to detect cardiac fibrosis early allowing for targeted treatment and improved outcomes.[19, 88, 89] Medical therapies for MD can also use CMR data; applications include determining a start date for medications and individual patient dose adjustments.[88, 89] MD patients benefit most when therapies are started before muscle degeneration begins. Early detection of fibrosis, which occur before muscle degeneration, would allow patients to receive maximum benefit from current MD therapies.[10, 11] Young children are the MD patients that would benefit most from use of CMR for early detection of heart disease but capturing useful images presents a technical challenge.[11, 84, 86]

Electrodiagnostic evaluation brings another degree of confidence towards patient diagnosis, and is described as an extension of the physical examination to help diagnose

muscular disease and relevant myopathy.[90] An Electromyograph (EMG) may be used to analyze motor and sensory neuron conduction at the lower and upper extremities.[90, 91] An increase in polyphasic potentials is commonly found through EMG in muscular degradation, as seen in dystrophinopathies.[91] EMG also presents opportunity for differentiation between MDs: preserved proximal or preserved distal neuron conduction offers a view at which muscle groups are affected, giving both patient and physician insight toward the type of MD in effect.[90]

Additionally, proper renal clearance is important in cases of pharmacological intervention for both current regimens and future drug dosage decisions.[92] A typical biomarker assay of plasma will include a measure of creatinine; since creatinine production is directly dependent on skeletal muscle mass, in cases of MD where muscle wasting or absence of muscular development is evident, measures of creatinine levels are unreliable. This would also mean that if creatinine measures were used for eGFR, then eGFR is also inaccurate. An alternative to the compromised creatinine levels is the cysteine protease inhibitor cystatin C. Cystatin C is yet another useful biomarker for measuring renal function, which has just recently been implemented in cases of MD. Cystatin C is produced by almost all nucleated cells independent of patient age, gender, size, lean muscle mass and metabolism.[93] This biomolecule is an acceptable alternative to understanding kidney function, with the added benefits of being less costly and more convenient for patients than the use of radioisotopes or infusions, practiced today.

1.4. Respiratory Comorbidities

Due to the nature of most forms of MD, a patient may see drastic impairment to their respiratory system and the overall cardiopulmonary relationship.[3] Patients with DMD are of

particular risk, with a large portion of DMD patients dying as a result of muscular respiratory failure.[94, 95] Weakened expiratory muscles and inspiratory muscles, specifically the diaphragm, impede the patient's ability to respire; a condition of nocturnal desaturation may develop due to the diaphragm's inability to overcome increased airway resistance during sleep.[95] This issue may become diagnosed as nocturnal hypoventilation or sleep apnea; Sleep Disordered Breathing (SDOB).[95, 96] These concerns may be monitored through measurements of vital capacity; maximal inspiratory pressure and maximal expiratory pressure.[95, 96] A polysomnogram has been a useful method of monitoring a combination of oxygen content in the blood, heart rate, and breathing among other variables as a patient sleeps.[94] Due to the close relationship between the cardiovascular and pulmonary systems, these respiratory errors may manifest into cardiac consequences: arrhythmias, hypertension, ventricular hypertrophy, and even SCD.[95, 96] Undue mechanical stress on the heart as well as the introduction of reactive oxygen species during hypoxia will further aggravate the pathology.[96]

Noninvasive positive-pressure ventilation techniques such as Bilevel Positive Airway Pressure (BiPAP) and Continuous Positive Airway Pressure (CPAP) have been used as mechanical support to aid sleep, enhance comfort, and ultimately improve patient outcomes.[94] Use of ventilation therapy has reduced the number of fatalities due to respiratory failure, thus leading to a decreased proportion of MD patients succumbing to cardiac failure. Mechanical ventilation positively influences the LV by decreasing transmural effects, thus decreasing afterload.[97] On the other hand, mechanical ventilation may impede venous return to the right atrium through positive via positive intrathoracic pressure, thus increasing afterload; peripheral oedema and ascites may result.[97]

1.5. Use of Medical Therapies for Heart Disease

Due to the predictable nature of MD-related cardiac phenotypes over a patient's life, signs of heart failure may be addressed more directly.[3] Angiotensin converting enzyme inhibitors (ACEi) are the first line of therapy for MD patients with cardiac involvement (Table 1.2).[20, 23, 25, 98] Dystrophinopathy patients should be started on ACEi before the patient becomes symptomatic or shows signs on ECG and imaging.[25, 98-100] Angiotensin converting enzyme inhibitors should be started when symptoms first appear in patients with LGMDs.[20] Early initiation has been shown to slow progression of left sided heart failure in these patients.[19] Additionally, ACEi have been proven to reduce myocardial fibrosis in MD patients.[22] In cases where patients experience intolerable side effects from ACEi, angiotensin receptor blockers (ARB) can be used as a substitute, as they both operate to interrupt the same pathway. If further treatment is required: beta-blockers, diuretics and aldosterone receptor blockers should be used in addition to the ACEi.[20, 25, 33, 101] Addition of a beta-blocker with an ACEi earlier on in Dystrophinopathies has been shown to be beneficial in slowing LV dysfunction in some studies.[19, 102, 103] Beta-blockers on their own are useful in treating cardiac arrhythmias, such as those found in EDMD and DM, as well as clinical hypertension.[101, 102, 104] However, other studies have shown no significant positive effect on MD patient outcomes with the addition of a beta-blocker to ACEi treatment.[104, 105]

Corticosteroids, such as Eplerenone, a cardioprotective aldosterone receptor antagonist, have recently become candidates for pharmaceutical therapy in the treatment of DMD. A trial conducted by Raman et al. in 2015 demonstrated a significant reduction in LV circumferential strain in boys aged 11 to 19 over a 12 month period, and Eplerenone administered in addition to background ACEi or ARB therapy attenuated the progression of LV systolic dysfunction,

compared to ACEi or ARBs.[38] Eplerenone's effectiveness: over an extended period of 24 months, boys who continued Eplerenone therapy saw no significant decline in LV systolic function.[106] The corticosteroid Deflazacort has been used to treat patients with DMD in clinical trials and has been found to preserve skeletal muscle.[107, 108] Other steroidal drugs such as Idebenone, beta blockers such as Losartan, and even exon skipping Etiplersen have seen clinical trials, as presented in Table 1.2.[100, 109, 110] Patients diagnosed with LGMD1B and autosomal EDMD can benefit from Rapamycin therapy. LMNA mutations in both scenarios lead to the downstream creation of farnesylated prelamin A, which is a toxic processing intermediate.[65] Rapamycin will trigger lysosomal degradation of this mutant intermediate, to prevent the activation of harmful cellular pathways which are phenotypically observed in both cases of MD.[65] MD patients with arrhythmias rely mostly on beta-blockers to manage their condition.[102] Anticoagulation agents should be considered in those with particular arrhythmias, such as atrial fibrillation.[25] This reduces the chance of clot formation, thus preventing thrombus. Cardiac manifestations may be exaggerated by the use of glucocorticosteroids, a common medication for treatment of skeletal muscle and ambulatory symptoms.[111] A side effect of glucocorticosteroids is hypertension which will exacerbate heart failure. Steroid dose adjustment may be required to manage this potential side effect.[25]

Table 1.2. Clinical trials of pharmacological therapy for the cardiac manifestations of MD.

Therapy (Max dose)	**Number of Participants**	**Follow-up Time (years)**	**Participant MD Type**	**P-values**	**Clinical Trial Results**
ACE inhibitors					
Perindopril (4 mg/day)	57	5	DMD	p= NS	**Outcome:** LVEF Treatment Group**:** 58.6 +/- 8.1% Control Group: 56.0 +/- 15.5% [99] **Conclusion:** Neutral
Perindopril (4 mg/day)	57	10	DMD	p= 0.02	**Outcome:** Survival status Treatment Group: 26 (92.9%) of 28 patients Control Group: 19 (65.5%) of 29 [98] **Conclusion:** Positive
Lisinopril (5 mg/day)	23	1	DMD	p= 0.02	**Outcome:** EFs Treatment with Lisinopril: 47.5% to 54.6% [100] *Compared to Losartan **Conclusion**: Positive compared to no treatment, neutral compared to losartan
Beta-Blockers					

Carvedilol (1.25 mg/day)	8	0.5	DMD	p= NS	**Outcome:** ANP Treatment Group: 83.8 +/- 17.5 Control Group: 89.5 +/- 44.4 pg/ml **Outcome**: BNP Treatment Group: 169.0 +/- 46.2 Control Group: 186.3 +/- 61.8 pg/ml **Outcome**: EF Treatment Group: 24.0 +/- 2.2 Control Group: 16.5 +/- 1.9% **Outcome:** Heart/Mediastinum ratio of the 123I-MIBG delayed image Treatment Group: 1.65 +/- 0.08 Control Group: 1.6 +/- 0.10 **Outcome:** Washout rate Treatment Group: 46.5 +/- 8.6 Control Group: 41.4 +/- 7.8 [104] **Conclusion**: Negative
Carvedilol (Maximum tolerated dose)	22	0.5	DMD, BMD	p< 0.02 for EF p< 0.05 for pressure rise (dP/dt) during isovolumetric contraction p< 0.01 for myocardial performance index	**Outcome:** MR-derived ejection fraction Treatment Group Change over 6 months: 41% +/- 8.3% to 43% +/- 8%; p < 0.02 **Outcome:** mean rate of pressure rise (dP/dt) during

					isovolumetric contraction Treatment Group change over 6 months: 804 +/- 216 to 951 +/- 282 mmHg/s; $p < 0.05$ **Outcome:** myocardial performance index Treatment Group change over 6 months: 0.55 +/- 0.18 to 0.42 +/- 0.15; $p < 0.01$ [102] **Conclusion:** Positive
Carvedilol (2.80 mg/day)	54	5	DMD	p= 0.002	**Outcome:** Experiencing death, deterioration of heart failure and severe arrhythmia Treatment Group: 9.8 % Control Group: 53.8% [101] **Conclusion**: Positive
Steroids					
Idebenone (450 mg/day)	21	1	DMD	p= 0.067	**Outcome**: Peak systolic radial strain LV inferolateral wall, % Treatment Group:39.74 ± 9.31 Control Group: 7.5 ± 12.0 [109] **Conclusion:** Positive

Eplerenone (25 mg/day)	42	1	DMD	p= 0.02 for Left ventricular strain % p= 0.329 for LVEF %	**Outcome**: Left ventricular strain % Treatment Group: 1.0% (0.3 to -2.2) Control group: 2.2% (1.3 to –3.1) **Outcome:** LVEF % Treatment Group: −1.8% (-2.9 to 6.0) Control Group: −3.7% (−10.8 to 1.0) **Outcome**: EDV, mL Treatment Group: −2.44 (9.82) Control Group: 0.07 (17.32) **Outcome**: ESV, mL Treatment Group:−1.64 (7.89) Contorl Group: 4.07 (8.25) **Outcome**: LGE, % of left ventricular mass Treatment Group: −1% (−6 to 3) Control Group: −3% (−5 to 4) [38] **Conclusion:** Positive
Deflazacort	300	3	DMD	N/A	No Cardiac outcomes [107, 108]
Angiotensin II Receptor Antagonists					
Losartan (25 mg/day)	23	1	DMD	p= 0.02	**Outcome**: EF Treatment with Losartan: 47.7% to 55.2% [100]

					*Compared to Lisinopril **Conclusion:** Positive compared to no treatment, neutral compared to Lisinopril
Phosphorodiamidate Morpholino Antisense Oligonucleotide (PMO)					
Eteplirsen (50 mg//kg)	80	48 weeks	DMD	N/A	No Cardiac outcomes [110]
Combination Pharmacotherapy					
Carvedilol and ACE Inhibitors Combination (Varying doses)	28	3-Feb	DMD & EDMD	p< 0.05	**Outcome**: LV fractional shortening (FS) ACE inhibitor Alone: 0.18±0.06 to 0.16±0.06 ACE inhibitor + Carvediliol: 0.16±0.06 to 0.21±0.05 [103] **Conclusion:** Positive
ACE inhibitor and Beta-Blocker Combination (Varying doses)	42	4	DMD	p= NS	**Outcome:** EF ACE inhibitor alone: mean EF increased from 47 ± 6.1% to 52 ± 8.4% ACE inhibitor + Beta Blocker combination: mean EF increased from 46 ± 10% to 50 ± 11% [105] **Conclusion:** Neutral

Renin-angiotensin-aldosterone system (RAS) antagonists and Steroid Combination (Varying doses)	86	15	DMD	p = 0.0005 for survival rates p = 0.0351 for mortality rate p = 0.0025 for annual rates of decline in left ventricular ejection fraction p= 0.0105 for increase in left ventricular end-diastolic dimension	**Outcome:** Patient Death RAS antagonist alone: 43% RAS antagonist + steroid therapy: 11% **Outcome:** Survival Rates RAS antagonist alone: 27.9% RAS antagonist + steroid therapy: 72.1%, **Outcome**: Mortality rate RAS antagonist + steroid therapy: 76% lower mortality rate (hazard ratio: 0.24) **Outcome**: Annual LVEF change rate RAS antagonist alone: -1.09%, RAS antagonist + steroid therapy: -0.43% **Outcome**: shortening fraction RAS antagonist alone: -0.65%, RAS antagonist + steroid therapy -0.32% **Outcome**: LV end-diastolic dimension RAS antagonist alone: 0.92 mm RAS antagonist + steroid therapy +0.47 mm [111] **Conclusion:** Positive

Number of participants, MD diagnosis of participants and the maximum follow up time are reported. Outcomes are also provided. ACE inhibitors, perindopril and Lisinopril were tested. Early use of perindopril was found to delay cardiac progression and decrease mortality in MD patients. Lisinopril was tested against Losartan; both Lisinopril and Losartan improved cardiac manifestations, with no significant differences. Carvedilol has mixed results on its effectiveness in treating DCM and arrhythmias. Steroid use has reduced cardiac impact on MD patients. PMO pharmaceuticals have been approved by the FDA, and therefore their trials have been included in this review. Combination therapy has shown to be more effective than use of ACE inhibitors and other RAS antagonists alone.

1.6. Use of Device Therapy for Heart Disease

For the general heart failure population, there is strong support for surgical strategies such as left ventricular assist devices (LVAD), pacemakers, and heart transplantation. A case study by Stoller et al. demonstrates the significant benefits of a centrifugal continuous flow LVAD in an 18 year old DMD patient with advanced heart failure, when medical therapy was unable to stabilize his deteriorating condition.[112] Thirty-eight months post-LVAD insertion, the patient has been able to live free of nutritional and ventilatory support.[112] This case study demonstrates the effectiveness of LVAD insertion as a destination therapy. Surgical interventions may be used in cases where medical therapy is insufficient to manage symptoms, or sustain life, in MD patients. There are two main groups of MD patients to consider for surgical interventions: those with arrhythmias and those with heart failure. Unfortunately, surgical interventions may be accompanied by significant complications.[113, 114] The risk to benefit ratio, as well as the cost, should be carefully considered before referring a patient for surgery. While surgical approaches may have a larger role in the future, they are generally considered only in cases of severe cardiac disease.

Muscular dystrophy patients may be considered for valve surgery, either replacement or repair, to improve symptoms of valvar regurgitation. This may prevent further progression of

DCM and allow for partial regression of dilation, with the hope of improving heart function.[115] An example is a 35-year-old female carrier of one of the DMD mutations, with a LVEF of 13% and dilated LV. The patient had previously been diagnosed with DCM and presented with worsening symptoms of heart failure. Tricuspid annuloplasty and a tissue mitral valve replacement were performed. The surgery was successful, but the outcomes were poor: there was no improvement in symptoms, LVEF, or chamber dimensions.[33]

The use of implantable defibrillators in MD patients has seen mixed success rates. To address conductive concerns in patients with EDMD and DM1, surgical methods have been proposed, and in some cases implemented, as a means of primary and secondary prevention. Use of an implantable defibrillator has been recommended for patients diagnosed with EDMD to prevent SCD in situations of ventricular dysrhythmia.[64] Unfortunately, treatment for EDMD is regarded as symptomatic, meaning that due to the current limits of medicine, symptoms are only managed rather than resolved entirely.[65] For patients diagnosed with DM1, cardiac resynchronization therapy (CRT) pacemakers have been implemented to resolve atrial and ventricular conduction abnormalities. One case study with a 32-year-old male DMD patient was promising and providing some evidence for the use of implantable defibrillators in Dystrophinopathies. This patient had a 3rd degree atrioventricular block and LVEF of 45%. After implantation with a cardiac resynchronization therapy defibrillator, he showed symptomatic improvement.[116] On the other hand, a case study looking at defibrillator use in LGMD patient was less successful. The studied outlined a 35-year-old female patient with symptomatic left sided heart failure (LVEF of 30%) and a series of conductive abnormalities because of DCM. These issues included atrial flutter, atrial fibrillation, atrioventricular block, supraventricular tachycardia and later ventricular arrhythmias. The patient showed progressive

heart failure even after surgical implantation of a cardioverter-defibrillator.[29] The patient discussed went on receive a heart transplant and was successful 5-years post-op.[29]

Case studies looking at the use of valve replacement/repair and implantable defibrillators in MD patients have shown limited success, with 2 of 3 case study patients continuing to show signs of progressive heart failure.[29, 33, 116] However there has been little research into the use of these surgical interventions in this population. As the two failed patients had severe heart failure before surgical interventions were performed, it is possible that better outcomes could have been achieved had the surgeries been performed earlier. There is much debate within the medical community as to if and when to use surgical interventions in the MD population.[30] More information is needed on the applicability and ideal timing of these types of surgical interventions in MD patients. A left ventricular assist device (LVAD) has shown to be successful in MD patients with heart failure. As organ donation is limited and there are many contraindications to transplantation, LVADs can be used as an alternative to heart transplantation in end-stage heart failure patients. Amodeo et al. published case studies on two teen MD patients who successfully underwent implantation of the Jarvik 2000 LVAD.[117] A following publication with older patients, ages 23 and 29, was published providing further support for the use of LVADs in late stage heart failure patients with MD.[118] Cardiac transplantation is a viable option for MD patients with severe heart failure and has been shown to be successful in this population with various case reports, including patients with DCM, showing a quality postoperative course comparable to the general population.[29, 83, 119-121]

1.7. Potential and Proposed Use of Genetic Therapies

When considering dystrophinopathies, there does not appear to be any treatment capable of rescuing skeletal, cardiac, and respiratory muscle in full. Current therapies, such as steroid treatment, only target symptoms rather than the underlying cause. There are currently genomic techniques being studied with the aim of modifying problem genes in MDs to improve muscle function by targeting the root cause. In the future genomic editing and exon skipping could improve muscle integrity, lengthen lifespan and reduce cardiac complications.[34, 37, 122]

Recently, the concept of genomic editing of germline DNA has been introduced to resolve DMD at its origin. The experimental process, utilized on various mice models, serves to remove the dysfunctional copy of the gene encoding for Dystrophin.[41] The CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 system has proved efficacious in genomic editing.[123] A Cas9 nuclease is guided by single-guide RNA to a genomic locus.[41] Cas9 will then generate a double-stranded break, prompting repair via non-homologous end joining or homology-directed repair guided by an exogenous template.[41] This method has been successfully implemented on mice models possessing a nonsense mutation at exon 23 of the DMD gene.[41] Various muscle types were examined in wild-type mice, DMD mice, and corrected mice. The corrected mice receive this treatment through an established adenosine-associated virus delivery system, enacted into the mice when they were in their zygote stage of development.[41] In comparison to the poor developmental phenotype of the DMD mice, corrected mice displayed no significant signs of dystrophin-absence.[41] In comparing serum CK, corrected mice showed a substantial decrease compared to DMD mice, implying less muscle wasting in the corrected mice.[41] This concept can also be applied to the expanded repeat in the DMPK gene to treat DM1.[123] Although many question the feasibility of genomic germline editing, this genetic

process displays the potential for innovative regimens to resolve MD and associated cardiac manifestations.

Another experimental approach to dealing with DMD is the concept an Exon Skipping Strategy (ESS). ESS uses antisense oligonucleotides (AOs) to interfere at the level of mutant pre-mRNA. Antisense oligonucleotides are used to allow for the recovery of a disrupted open reading frame, to produce a shortened, but functional dystrophin.[37, 124, 125] The antisense oligonucleotides recognize specific pre-mRNA sequences prompting a modified splicing pattern to produce a restored open reading frame.[125] The result is a much milder phenotype due to the truncated form of dystrophin produced, in contrast to the severe DMD phenotype observed in total dystrophin absence.[126] Use of ESS at exon 51 is claimed to be able to assist as many as 15% of DMD patients.[125] As many as 60% of the DMD patient population could see benefit from the use of ESS at numerous loci from exons 40 through 55.[125, 126] This experimental approach has made use of the high-dosage drug AVI4658, which showed no adverse drug-related effects.[125] Both safety and results of the treatment were visualized and verified through the use of immunostaining, physical examination as well as hematological and urinary parameters; a variety of tests showed appreciable results.[126] Though the lab results show efficacy, the effectiveness is yet to be demonstrated in a clinical trial setting.

Implementation of ESS has also been proposed for patients with autosomal EDMD, specifically at exon 5 of the LMNA gene.[65] The issue with utilizing ESS in treating EDMD, is the low frequency of nonsense mutations present in this MD, making targeting of specific loci a difficult and taxing process. An alternative genetic treatment known as RNA Interference (RNAi) has been proposed to address autosomal EDMD. RNAi uses short-hairpin RNA or synthetic oligonucleotides to destroy mutant LMNA mRNA.[65] Targeting mutant mRNA with

RNAi would prevent the creation of abnormal proteins, thus circumventing the issue of compromised cell integrity.

Multi-exon skipping therapy uses the same principal as ESS, however a cocktail of multi-AOs are used to modify a larger section of the gene thus widening the potential target population. Single exon skipping is mutation specific whereas multi-exon skipping can be used on a variety of mutation types and locations in the same gene.[37] Data from cellular and animal model experiments indicate that the use of multi-exon skipping therapy has significant potential for the treatment of DMD and other forms of MDs in the future.[37] While exon skipping has shown promise for recovery of skeletal muscle in DMD animal models, one challenge has been improving cardiac function. Cardiac muscle has proven more difficult for the AOs to penetrate, thus reducing the effect of the therapy on the heart. Changes in AO design have been implemented such as changing the backbone of the oligonucleotides to improve cardiac muscle penetration. These changes have shown an increase in functional dystrophin fibers in both mouse and dog models.[37, 127-130] Recently it has been demonstrated that use of a particular form of AOs called cell-penetrating peptide-conjugated Phosphorodiamidate morpholino oligomer (PPMOs) can be used to improve conduction deficiencies in DMD dog models.[127] While evidence for the use of multi-exon skipping as a potential therapy is strong, clinical trials still need to be conducted to ensure safe and effective use in humans.

Recent research in treating LGMD by Roudaut et al., has yielded innovative genetic intervention to be used to treat the most invasive form of LGMD, which is autosomal-recessive LGMD2. Limb-girdle muscular dystrophy type 2I is most commonly associated with the overexpression of the cysteine protease calpain3, caused by a mutation at the CAP3N gene.[66] Interestingly, there have been no observed pathological effects to the heart when calpain3 is in

excess in skeletal muscle.[66] Interestingly, in cases of LGMD, there is an observed state of calpain3 deficiency in proximal skeletal muscle, making this strategy ideal for resolving two issues at once.[66] Using this principle, a molecular strategy was derived: the construction of a microRNA-regulated vector aimed at modifying localization of the calpain3 protein.[66] This vector would exclusively block the derivation of calpain3 in cardiac muscle, in an effort to preserve physiological function.[66] In essence, modification of the promoter on a CAP3N transgene, will restrict the expression of calpain3 to skeletal muscle; this method aims to redirect the cysteine protease expression away from cardiac muscle towards the deficient skeletal muscle.[66]

1.8. Importance of Collaborative Multidisciplinary Care

Given the need for comprehensive clinical care for these vulnerable patients, the Neuromuscular Multidisciplinary (NMMD) clinic was founded in 2012 at the Kaye Edmonton Clinic at the University of Alberta, in collaboration with healthcare professionals at the Mazankowski Alberta Heart Institute to treat patients with neuromuscular diseases. All patients referred to the clinic receive multidisciplinary care from specialist physicians in neurology, cardiology, pulmonary medicine, and physiatry supported by allied healthcare professionals for efficient triage and effective treatment and management of their complex clinical conditions.[3] The disease can affect the muscular, cardiac, respiratory, gastrointestinal, nervous and endocrine systems; thus involving many medical specialists.[25, 52] Some forms of muscular dystrophies can also affect cognitive function and learning ability to varying degrees.[39] Muscular dystrophy patients may require social workers, occupational therapists, specialized assistants at school for

diagnosed children, and other social supports to overcome cognitive deficits. Pharmacists, physiotherapist, speech and language pathologists, dietitians, genetic counselors and psychologists are also heavily involved in medical and symptomatic care of patients.[25, 52]

Application of conceptual Integrated Care Pathways, which serve to combine the expertise of multiple disciplines, brings a higher standard of solutions to clinical issues such as MD.[131] Collaboration by medical professionals allows for a more effective and efficient means of diagnosis and treatment planning, to reach preferred clinical outcomes.[131] From a cardiac perspective, relevant clinical history is reviewed, and patients are closely followed. Patients receive electrophysiology studies through 12-lead ECG and Holter monitoring as well as cardiac imaging studies primarily conducted using echocardiography. This multifaceted approach has facilitated the optimization of medical therapies and timely use of device intervention, which we have previously shown to result in improved all-cause and cardiac-related clinical outcomes.[3] The clinic consistently supports important research initiatives, including a recent investigation analyzing patient plasma biomarkers. The application of traditional biomarker assays and reference values for heart disease has been shown to be ineffective in this unique cohort of patients.[3] We have recently demonstrated the importance of attentive monitoring towards cardiac biomarkers such as BNP and hsTnI as they were shown to support the clinical diagnosis of DCM and prognosticate MACE in patients with MD.[21] Up until this point, we have had the opportunity to implement translational and clinical methods derived from similar research initiatives to improve patient care and prognosis though there is room for improvement. We continue to be presented with opportunities to clarify the many ambiguities of cardiac pathophysiology in this unique patient cohort.

Strong communication between the family practitioner, medical specialists, other healthcare providers, and social services is vital for achieving the best outcomes in MD patients. MD requires careful follow up over many years to achieve good medical and social outcomes. Poor use of the interdisciplinary approach decreases the likelihood of sufficient patient follow-up. An interdisciplinary approach can improve health outcomes, patient satisfaction and the patient's ability to integrate into the community.[3, 25] Through the collection of past and present research of MD and clinical patient outcomes, it has become evident that the medical community is still a great distance from primary prevention or complete resolution of these diseases in patients. Although significant medical advancements have afforded diagnosed patients with longer lifespans and a greater degree of day-to-day comfort, patients are still directed toward an early death or admission to palliative care; current research and clinical practice does not have the capacity to fully address these genetic diseases. By better understanding the many variations of MDs and their implications on organ systems in the body, such as what is covered in this thesis, we strategically approach the diagnosis of heart disease and prognostication of adverse outcomes in this adult patient population.

2. Methods

2.1 Patient Cohort

In coordination with the NMMD clinic established in 2012 at the Kaye Edmonton Clinic, 303 patients were recruited into our patient registry over a period of 7.5 years from November 5, 2014, to May 6, 2022. This thesis covers investigations from May 8, 2017, to May 6, 2022.

Following neurological assessments, muscle biopsy, and genetic testing to confirm diagnosis, patients were categorized into the various cohorts of MD including: dystrophinopathies (DMD, BMD, and dystrophinopathy carriers [56 patients]), LGMD (47 patients), DM1 (87 patients), and FSHD (32 patients) in our electronic study database on the REDCap ("Research Electronic Data Capture)" platform. We also recruited a negative control cohort, which included patients with myositis, mitochondrial myopathies, spinal muscular atrophy, and undefined congenital myopathies (62 patients). These patients had moderately impaired ambulation and respiratory disease, similar to our MD patients, but did not have heart disease. Patients with DM2 (10 patients), Emery-Dreifuss MD (4 patients) and Friedreich's ataxia (5 patients) were also recruited as seen for future investigation. Patients were referred to the NMMD clinic with no bias towards patients with overt cardiac or respiratory symptoms to receive multidisciplinary care.

2.2 Patient Assessment and Data Obtainment

Patients received collaborative multidisciplinary assessment and care following their recruitment to the NMMD clinic (Figure 2.1). In addition to demographic and clinical parameters, biochemical, 12-lead electrocardiogram (ECG), transthoracic echocardiogram (TTE) and cardiac magnetic resonance (CMR) imaging data were collected to create detailed patient profiles. Patient plasma was also collected for analysis. Heart rate and blood pressure were recorded at each visit, and the presence of cardiomyopathy and comorbidities, such as anemia, diabetes, ambulatory status, respiratory disease and sleep disordered breathing (SDOB), defined as obstructive sleep apnea or nocturnal hypoventilation, were documented. All patients suspected of SDOB underwent polysomnography for further evaluation. Respiratory disease was defined as patients with COPD,

asthma, recurrent aspiration pneumonia, respiratory muscle weakness, or restrictive lung disease. Appropriate patients were trained in lung volume recruitment, the use of a mechanical insufflator-exsufflator, and provided with ventilatory assist devices such as continuous positive airway pressure or bi-level positive airway pressure, when appropriate. Overall ambulatory function was evaluated by physiatrists, and interventions in the form of different mobility aids such as cane or walker, and wheelchair use, manual or powered, were instituted where needed. Guideline-based medical therapy was implemented, and appropriate device implantations and follow-up care was carried out.

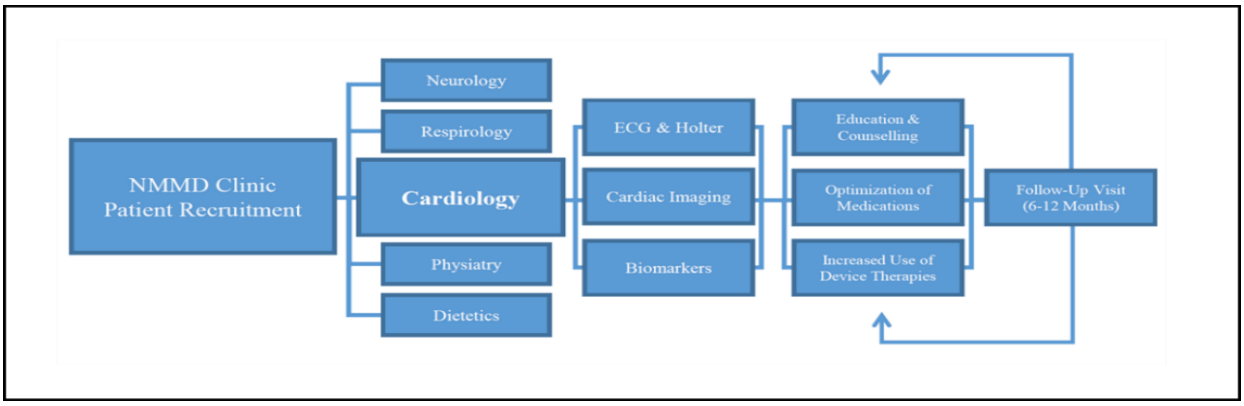


Figure 2.1. Cardiac assessment of patients with muscular dystrophy (MD) in the Neuromuscular Multidisciplinary (NMMD) clinic. ECG, electrocardiogram

Serum biochemistry, fasting lipid profile, hemoglobin, plasma B-type natriuretic peptide (BNP) and creatine kinase (CK) were monitored. Anemia was defined as per the hemoglobin level cutoffs established by the World Health Organization. Dyslipidemia was defined in accordance with the 2016 Canadian Cardiovascular Society guidelines.[132] Serial 12-lead resting ECGs and Holter data collected over either 24- or 48-hour periods were evaluated. Subsequent Holter studies

were completed after follow-up visits for patients who required further electrophysiological studies due to suspected risk of arrhythmias. For all investigations, cardiomyopathy was defined as LVEF < 55% or a left ventricular end-diastolic volume index (LVEDVi) > 105 mL/m^2,[133] as determined by TTE and CMR, when available. Serial CMR and TTE were used to detect and follow cardiac structure and function as applicable. Incident heart failure (HF) and arrhythmias were also documented. Heart failure was diagnosed following a comprehensive cardiac assessment, which considered symptoms and signs such as dyspnea, orthopnea, poor appetite, elevated jugular venous pressure, peripheral edema, and abdominal distention. Medical therapy by maximum tolerated dose[134], use and type of device therapy, and use of continuous positive airway pressure or bi-level positive airway pressure ventilation were documented at each clinic visit.

Clinical outcome data, such as the number of unplanned, all-cause outpatient clinic visits, the duration of hospitalization, and the number of cardiac-related hospitalizations was collected by the Data Integration and Management Repository (DIMR) analytics branch of Alberta Health Services using our provincial electronic health records. The investigations covered in this thesis conform to the principles outlined by our locally appointed ethics committee, the Health Research Ethics Board, at the University of Alberta. All patients provided informed and written consent prior to being recruited into our study cohorts.

2.3 Statistical Analysis

The investigations outlined in this thesis leveraged an objective statistical approach from which to draw conclusions from a broad variety of datapoints. Datasets included both continuous and categorical variables that were assessed independently and by association using non-

parametric statistics. Methodologies for respective investigations have been outlined in this thesis. A P-value < 0.05 was considered significant through all statistical analysis. Statistical analyses were conducted using SPSS Statistics Version 25 (IBM, NY, USA) and R version 4.0.3.

3. Cardiac Intervention Improves Heart Disease and Clinical Outcomes in Patients with Muscular Dystrophy in a Multidisciplinary Care Setting

3.1. Abstract

Background: Muscular dystrophy (MD) patients represent a vulnerable patient population with no clearly defined care model in modern-day clinical practice to manage a high burden of heart disease and comorbidities. We demonstrate the effectiveness of cardiac interventions, namely the initiation and optimization of medical and device therapies, as part of a multidisciplinary care approach to improve clinical outcomes in patients with MD.

Methods and Results: We conducted a prospective cohort study at the Neuromuscular Multidisciplinary clinic following patients with dystrophinopathies, limb-girdle MD, type 1 myotonic dystrophy (DM1), and facioscapulohumeral MD. A negative control group classified as non-MD myopathies without heart disease, was also tracked. Our cohort of 185 patients (median age: 42 years; 79 [42.7%] women), included 145 patients with MD: cardiomyopathy was present in 65.6% of the dystrophinopathies (21 of 32) and 27.3% of the limb-girdle MD (9 of 33) patients. Conduction abnormalities were common in DM1 (33.3% [20 of 60] patients). Cardiac intervention reversed systolic dysfunction left ventricular ejection fraction improved

from 43% to 50.0% over a three-year period. A sustained reduction in healthcare utilization was also observed: the number of outpatient clinic visits decreased from 3.0 to 1.5 visits/year; the duration of hospitalizations was reduced from 14.2 to 0.9 days/year; and the number of cardiac-related hospitalizations decreased from 0.4 to 0.1 hospitalizations/year associated with low mortality.

Conclusions: Our study demonstrates that cardiac intervention as part of a comprehensive multidisciplinary care approach to treating patients with MD leads to a sustained improvement in clinical outcomes.

3.2. Clinical Perspective

What Is New?

- Cardiac interventions as part of a multidisciplinary care approach can markedly improve all-cause clinical outcomes in patients with muscular dystrophy.
- Use of medical and device therapies improved systolic dysfunction in different cohorts of patients with muscular dystrophy.

What Are the Clinical Implications?

- Design and implementation of multidisciplinary care that includes cardiology should be undertaken to provide optimal care to patients with muscular dystrophy.
- Cardiomyopathy and arrhythmias are frequent comorbidities in patients with muscular dystrophy, which requires expedient diagnosis and management with frequent monitoring.

3.3. Introduction

Muscular dystrophies (MD) are inherited neuromuscular diseases with a wide range of systemic manifestations that are often life-threatening. The multisystem involvement of MD leads to significant disability ranging from limited ambulation to heart and lung disease, resulting in poor quality of life, and increased morbidity and mortality.[2, 135, 136]

Heart disease, characterized by cardiomyopathy and arrhythmias, is now recognized as the primary cause of mortality in patients with MD.[4, 7, 137-139] Of the nine main types of MD, dystrophinopathies, namely Duchenne and Becker MD (DMD; BMD), exhibit dilated cardiomyopathy (DCM), characterized by decreased left ventricular ejection fraction (LVEF), and increased end-diastolic volumes.[135, 140] Many subtypes of limb-girdle muscular dystrophy (LGMD), specifically subtypes 1B, 2E, and 2I have similar cardiac manifestations.[7, 141] Conduction abnormalities are observed in patients with type 1 myotonic muscular dystrophy (DM1) such as atrioventricular block (AVB) and left bundle branch block (LBBB). Importantly, ventricular arrhythmias are a serious complication of patients with DMD, BMD, LGMD and DM1.[7, 27, 142] Facioscapulohumeral muscular dystrophy (FSHD) exhibits cardiac conduction abnormalities to a milder degree.[61]

Given the inherent complexities of MD, the ideal care model for these patients has not been established.[135, 143, 144] Our Neuromuscular Multidisciplinary (NMMD) clinic was established to provide multifaceted care by specialist physicians and allied health care professionals to patients with MD in a single visit. Our prospective cohort study was designed to determine the impact of specialist cardiology care as part of a novel multidisciplinary care pathway for patients with MD.

3.4. Methods

In coordination with the NMMD care clinic established in 2014 at the Kaye Edmonton Clinic, 185 patients were recruited into our prospective study, after providing written consent, over a four-year period from November 5, 2014, to December 1, 2018. Following neurological assessments, muscle biopsy, and genetic testing to confirm diagnosis, patients were categorized into the various cohorts of MD including: dystrophinopathies (32 patients), LGMD (33 patients), DM1 (60 patients), and FSHD (20 patients). We included a negative control group classified as non-MD myopathies, which included patients with myositis, mitochondrial myopathies, spinal muscular atrophy, and undefined congenital myopathies (40 patients). These patients had moderately impaired ambulation and respiratory disease, similar to our MD patients, but did not have heart disease. Patients were referred to the NMMD clinic with no bias towards patients with overt cardiac or respiratory symptoms to receive specialist care as well as interventions from allied health care professionals. Guideline-based medical therapy was implemented, and appropriate device implantations and follow-up care was carried out. The investigation conforms to the principles outlined by our locally appointed ethics committee, the Health Research Ethics Board, at the University of Alberta. All patients provided informed and written consent prior to being recruited into our study.

Risk Assessment

In addition to demographic and clinical parameters, biochemical, electrocardiogram (ECG), transthoracic echocardiogram and cardiac magnetic resonance imaging (MRI) data were collected to create detailed patient profiles. Heart rate and blood pressure were recorded at each visit, and the presence of cardiomyopathy and comorbidities, such as anemia, diabetes, ambulatory status,

respiratory disease and sleep disordered breathing (SDOB), defined as obstructive sleep apnea or nocturnal hypoventilation, were documented. All patients suspected of SDOB underwent polysomnography for further evaluation. Respiratory disease was defined as patients with COPD, asthma, recurrent aspiration pneumonia, respiratory muscle weakness, or restrictive lung disease. Appropriate patients were trained in lung volume recruitment, the use of a mechanical insufflator-exsufflator, and provided with ventilatory assist devices such as continuous positive airway pressure or bi-level positive airway pressure, when appropriate. Overall ambulatory function was evaluated by physiatrists, and interventions in the form of different mobility aids such as cane or walker, and wheelchair use, manual or powered, were instituted where needed.

Serum biochemistry, fasting lipid profile, hemoglobin, plasma B-type natriuretic peptide (BNP) and creatine kinase (CK) were monitored. Anemia was defined as per the hemoglobin level cutoffs established by the World Health Organization. Dyslipidemia was defined in accordance with the 2016 Canadian Cardiovascular Society guidelines.[132] Serial 12-lead resting ECGs and Holter data collected over either 24- or 48-hour periods were evaluated. All DM1 patients received a 48-hour Holter monitor following their initial NMMD clinic visit. Subsequent Holter studies were completed after follow-up visits for patients who required further electrophysiological studies due to suspected risk of arrhythmias. Serial transthoracic echocardiograms and cardiac MRI were used to detect and follow cardiac structure and function. Cardiomyopathy was defined as LVEF < 55% or a left ventricular end-diastolic volume index (LVEDVi) > 105 mL/m^2,[133] as determined by cardiac MRI. Incident heart failure (HF) and arrhythmias were also documented. Heart failure was diagnosed following a comprehensive cardiac assessment, which considered symptoms and signs such as dyspnea, orthopnea, poor appetite, elevated jugular venous pressure, peripheral edema, and abdominal distention. Medical therapy by maximum tolerated dose[134], use and type of device

therapy, and use of continuous positive airway pressure or bi-level positive airway pressure ventilation were documented at each clinic visit.

Outcome Data Assessment

Cardiac systolic function was tracked through the monitoring of LVEF, obtained by cardiac MRI and/or echocardiogram, from 57 patients who had a cardiomyopathy and had imaging data available for the full three-year period, spanning from their initial NMMD clinic visit to their three-year follow-up visit. This includes 23 dystrophinopathies, 11 LGMD, 20 DM1, and 3 FSHD patients. Outcome data, such as the number of unplanned, all-cause outpatient clinic visits, the duration of hospitalization, and the number of cardiac-related hospitalizations was collected by the Data Integration and Management Repository (DIMR) analytics branch of Alberta Health Services using our provincial electronic health records. All 185 patients had DIMR data available over a three-year period, beginning from November 1, 2015, until December 1, 2018, to capture clinical outcomes. In order to account for the ongoing enrolment process and variable lengths of follow-up, outpatient clinic visits and duration of hospitalization were standardized as rates for the patients in each cohort. Outcome rates of the MD patients and non-MD myopathies cohort were plotted in six-month intervals as time series graphs to quantify the change of healthcare utilization rates, in days per year, following initial intervention and optimization of medical therapies six months after their initial clinic visit.

Data Analysis

Continuous variables analyzed were compared using a Mann-Whitney U test or Kruskal-Wallis test where appropriate, and all categorical data were compared using Pearson's Chi-square

tests. Patient LVEF at the point of clinic enrollment was compared to data collected at the end of the study using a paired t-test. Time series plots were used to illustrate the rates associated with clinical outcome data and accompanied by linear regression analysis. A P value < 0.05 was considered significant through all statistical analysis. Statistical analyses were conducted using SPSS Statistics Version 25 (IBM, NY, USA).

3.5. Results

Dystrophinopathies

Patients were recruited from our multidisciplinary care clinic where they received concurrent care from specialists in cardiology, neurology, respirology, and physiatry (Figure 3.1A). The dystrophinopathies cohort is comprised of young male patients (27 DMD patients and five BMD patients) (Table 3.1). The majority of these patients exhibited severe skeletal muscle wasting and motor difficulties at their initial clinic visit, with 27 (84.4%) of patients being wheelchair-bound (Table 3.1). DCM is highly prevalent with 21 (65.6%) of patients affected based on echocardiogram and cardiac MRI (Figure 3.1B), illustrated by biventricular remodeling with a moderately reduced LVEF (Table 3.2), with 6 (42.9%) patients who received a cardiac MRI showing evidence of myocardial fibrosis (Figure 3.2). In this cohort, echocardiogram confirmed LV dilation and dysfunction (Table 3.2) and showed no valvular abnormalities (Table 3.3). The high prevalence of cardiomyopathy resulted in a high rate of incident HF diagnosed following the initial NMMD clinic visit (Figure 3.1C).

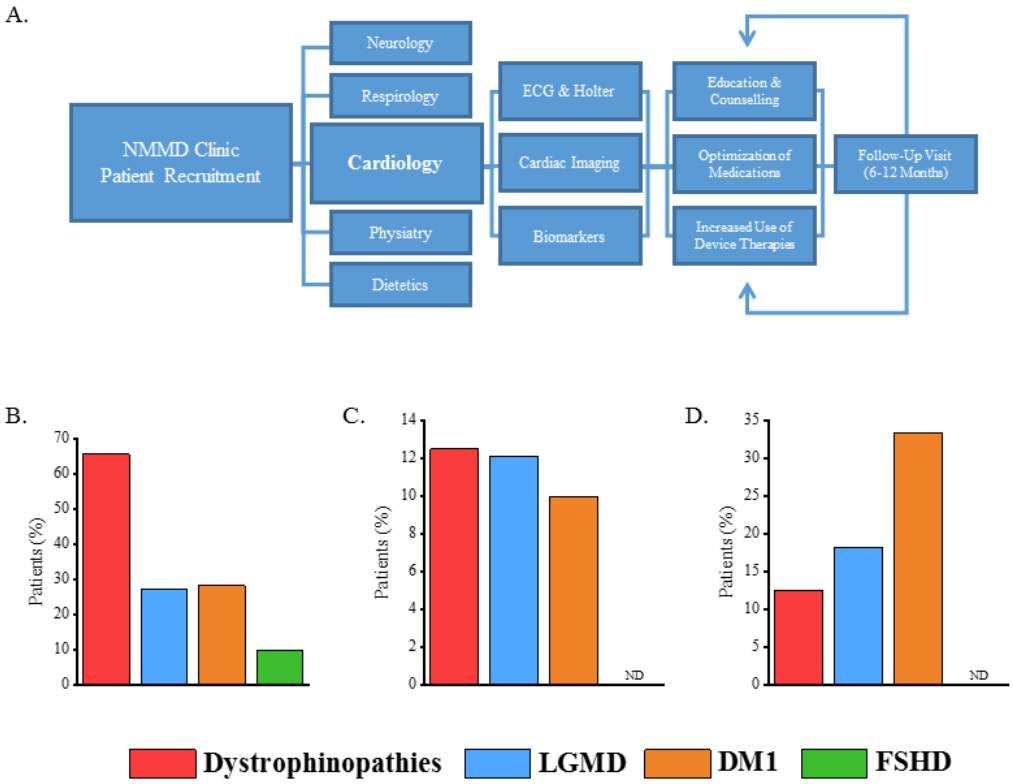


Figure 3.1. Cardiac assessment of patients with muscular dystrophy (MD) in the Neuromuscular Multidisciplinary (NMMD) clinic. Cardiac assessment and intervention applied through the NMMD clinic care pathway (A). Prevalence of cardiomyopathy in patients with MD (B). Heart failure diagnosed in patients at their initial NMMD clinic visit (C). Arrhythmia burden as captured by ECG, Holter monitoring, and device interrogation (D). DM1 indicates type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; LGMD, limb-girdle muscular dystrophy; ND, not detected.

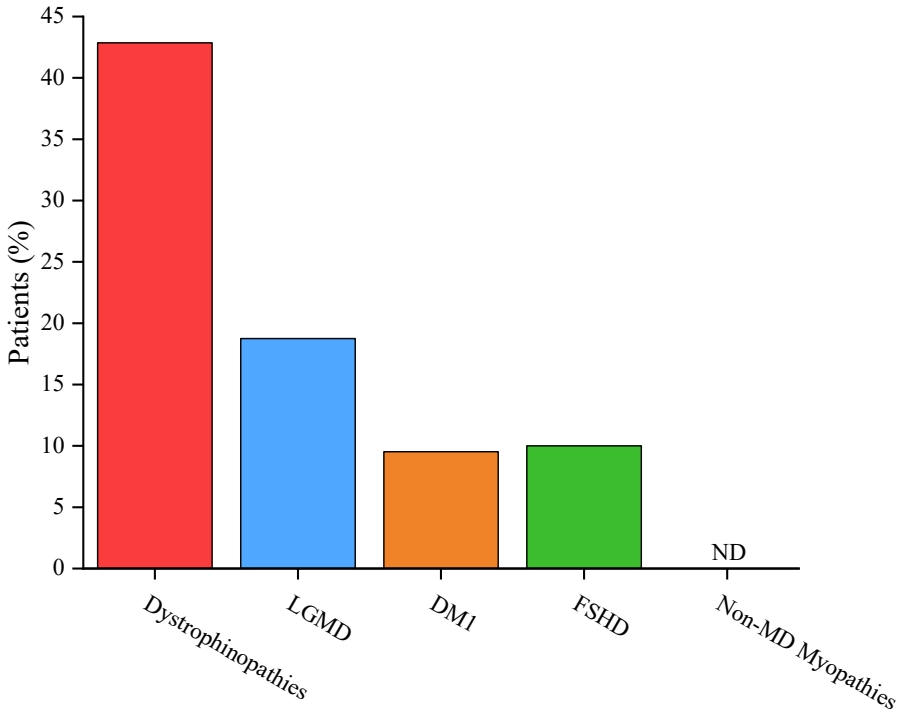


Figure 3.2. Prevalence of myocardial fibrosis based on cardiac magnetic resonance imaging (MRI). Patients screened by cardiac MRI that showed fibrosis as visualized by late gadolinium enhancement. DM1 indicates type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; LGMD, limb-girdle muscular dystrophy; and ND, not detected.

Table 3.1. Baseline Characteristics of Patients Referred to the Neuromuscular Multidisciplinary Clinic.

Characteristic	All Patients (n=185)	Dystrophinopathies (n=32)	LGMD (n=33)	DM1 (n=60)	FSHD (n=20)	Non-MD Myopathies (n=40)	*P* Value
Men/Women, No.	106 (57.3)/ 79 (42.7)	32 (100)	14 (42.4)/ 19 (57.6)	30 (50)/ 30 (50)	11 (55)/ 9 (45)	19 (47.5)/ 21 (52.5)	<0.001
Median Age, Yrs	42 (25-54)	22 (18-28.8)	44 (27-57)	42 (34.8-50)	45 (24.8-52.5)	54 (33.5-60)	<0.001
Current/ Former Smokers, No.	22 (11.9)	0	4 (12.1)	11 (18.3)	3 (15)	4 (10)	0.14
Ambulation, No.							
Cane/Walker	16 (8.6)	1 (3.1)	3 (9.1)	7 (11.7)	2 (10)	3 (7.5)	0.70
mWC/pWC	49 (26.5)	27 (84.4)	12 (36.4)	4 (6.7)	3 (15)	3 (7.5)	<0.001
Comorbidities, No.							
Diabetes	18 (9.7)	0	8 (24.2)	5 (8.3)	2 (10)	3 (7.5)	0.03
Dyslipidemia	24 (13.0)	2 (6.3)	4 (12.1)	7 (11.7)	4 (20)	7 (17.5)	0.60
Hypertension	27 (14.6)	2 (6.3)	6 (18.2)	4 (6.7)	6 (30)	9 (22.5)	0.045
Respiratory Disease	37 (20)	12 (37.5)	5 (15.2)	13 (21.7)	2 (10)	5 (12.5)	0.006
SDOB	47 (25.4)	14 (43.8)	5 (15.2)	16 (36.7)	6 (30)	6 (15)	0.07
Anemia	5 (2.7)	3 (9.4)	2 (6.1)	0	0	0	0.022
Non-Invasive Ventilation, No.	36 (19.5)	12 (37.5)	5 (15.2)	8 (13.3)	5 (25)	6 (15)	0.05
Vitals, median							
HR, bpm	76 (67.5-86.5)	86 (78.8-100)	76 (70-83.5)	70 (62.8-80)	77.5 (71-80)	73.5 (61-80)	0.009
sBP, mmHg	120 (110-132)	108 (100-121)	122 (112.5-131)	114 (106-126.8)	130 (120.5-133.8)	126 (119.5-140)	<0.001
dBP, mmHg	75 (70-82)	71 (64-78)	80 (70.5-84.5)	74 (68.5-78)	83.5 (76.5-87.5)	76 (72-84)	0.001
Biomarkers, median							
BNP, pg/mL	20 (12.5-42.5)	20 (12-75)	14.5 (10.5-47.5)	21 (15-40)	21 (14.5-35.5)	20 (13-37)	0.67
CK, U/L	274 (126-569.8)	1277 (377.8-2380.8)	526 (302.5-1759.5)	237 (127.5-309)	174.5 (159-395.3)	86 (53-211)	<0.001
Creatinine, µmol/L	50.5 (29.3-67)	20 (14.8-25)	37 (25.3-56)	57.5 (52.5-69.5)	48 (39-60)	58 (44.5-68.5)	<0.001
Potassium, mmol/L	4.3 (3.8-4.5)	4.3 (4.0-4.5)	4.1 (3.8-4.4)	4.3 (3.9-4.5)	4.3 (3.9-4.7)	4.2 (4.0-4.4)	0.86

Data presented as median (interquartile range) or n (%). BiPAP indicates bilevel positive airway pressure; BNP, B-Type natriuretic peptide; CK, creatine kinase; CPAP, continuous positive airway pressure; dBP, diastolic blood pressure; DM1, type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; HR, heart rate; LGMD, limb-girdle muscular dystrophy; MD, muscular dystrophy; mWC, manual wheelchair; pWC, power wheelchair; sBP, systolic blood pressure; and SDOB, sleep disordered breathing.

Table 3.2. Cardiac Evaluation of Muscular Dystrophy Patient Cohorts.

Modality	**Dystrophinopathies**	**LGMD**	**DM1**	**FSHD**	***P* Value**
12-Lead ECG	**(n=32)**	**(n=33)**	**(n=60)**	**(n=20)**	
Heart Rate, bpm	83 (71-98)	75 (60-81)	68 (63.3-75)	75 (69.5-85)	0.005
PR Interval, ms	132 (121.5-144)	157.5 (140.5-164)	193 (178.3-223)	162 (148-170)	<0.001
QRS Duration, ms	97 (87.5-109.3)	94 (86-102)	107 (96-118)	91 (85.5-97.8)	<0.001
QT_c Interval, ms	377 (359.5-399)	394 (374.8-426)	412 (396-440)	378 (352-399.5)	<0.001
1° AVB	0	0	18 (30)	0	<0.001
LAFB	2 (6.3)	0	6 (10)	1 (5)	0.29
LBBB	3 (9.4)	1 (3)	11 (18.3)	0	0.039
RBBB	2 (6.3)	2 (6.1)	2 (3.3)	2 (10)	0.71
Echocardiogram	**(n=23)**	**(n=21)**	**(n=50)**	**(n=12)**	
LVIDd, cm	4.7 (4.2-5.3)	4.7 (4.4-5.1)	4.4 (4.0-4.9)	4.4 (4.3-4.7)	0.16
LVIDs, cm	3.5 (3-4.6)	3 (2.8-3.6)	2.8 (2.6-3.1)	2.9 (2.5-3)	0.003
LVPWd, cm	0.7 (0.6-0.8)	0.9 (0.8-0.9)	0.9 (0.7-1)	0.8 (0.7-0.9)	0.003
LVEF, %	38.1 (26.3-52.3)	55 (52.8-60)	55 (50-60)	60 (56.3-60)	<0.001
LVMI, g/m²	63.2 (56-71.1)	57.1 (52.1-64.5)	46.7 (38.2-49.9)	49.1 (41.5-55.7)	0.003

Cardiac MRI	**(n=14)**	**(n=16)**	**(n=21)**	**(n=10)**	
LA Vol Index, mL/m²	39.8 (35.5-43.5)	36 (31.3-38.2)	29 (23.8-31.3)	29 (27.7-33)	0.06
LVEDVi, mL/m²	91 (76.8-110.8)	74 (64.5-92.3)	61 (55-72)	68 (56.5-79)	0.001
LVESVi, mL/m²	50 (31-61)	31 (25-44)	29 (20.5-36.3)	23.5 (20.5-32.5)	0.015
LVEF, %	45 (40-56.5)	55.5 (52-58.3)	56 (50-63)	58.5 (53.5-66)	0.08
LVMI, g/m²	61 (44-67)	45 (39-54)	42.5 (39.5-49.8)	42 (37.5-49.3)	0.07
RVEDVi, mL/m²	76 (68-84)	67 (60.5-78.5)	60 (56-75)	73 (58-94)	0.09
RVESVi, mL/m²	38.5 (35.3-43.3)	32 (28.5-41.5)	31 (26.8-35)	27 (26-41)	0.13
RVEF, %	49 (44-53)	52 (48.5-54)	50 (47-55)	53 (49-55)	0.66

Data presented as median (interquartile range) or n (%). 1° AVB indicates first-degree atrioventricular block; DM1, type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; LA Vol Index, left atrial volume index; LAFB, left anterior fascicular block; LBBB, left bundle branch block; LGMD, limb-girdle muscular dystrophy; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVESVi, left ventricular end-systolic volume index; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; LVMI, left ventricular mass index; LVPWd, left ventricular posterior wall thickness at end-diastole; PR Interval, duration of atrial depolarization; QRS Duration, duration of ventricular depolarization; QT_c Interval, corrected duration between ventricular depolarization and repolarization; RBBB, right bundle branch block; RVEDVi, right ventricular end-diastolic volume index; RVEF, right ventricular ejection fraction; and RVESVi, right ventricular end-systolic volume index.

Table 3.3. Structural Evaluation of the Mitral, Tricuspid, Aortic, and Pulmonic Valves by Cohort.

	Dystrophinopathies (n=23)	**LGMD (n=21)**	**DM1 (n=50)**	**FSHD (n=12)**
Mitral Valve	1 Mild Thickening, 2 Mild Prolapse	1 Mild Thickening	Normal	1 Mild Thickening
Tricuspid Valve	Normal	Normal	Normal	Normal
Aortic Valve	Normal	Normal	Normal	Normal
Pulmonic Valve	Normal	Normal	Normal	Normal

DM1 indicates type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; and LGMD, limb-girdle muscular dystrophy. *Data obtained from the 106 patients that received an echocardiogram study at baseline

For the majority of the patients in this cohort, ECG parameters were within normal limits (Table 3.2) and concordant with these findings, there was a low incidence of atrial and ventricular arrhythmias in this cohort (Figure 3.1D). Respiratory disease, including SDOB, was prevalent in this population with 27 (84.4%) patients affected; lung volume recruitment and mechanical insufflation-exsufflation were implemented in 26 (81.3%) and 13 (40.6%) of patients, respectively (Table 3.1).

Limb-Girdle Muscular Dystrophy

Patients with LGMD presented with a similar degree of muscle weakness as the dystrophinopathies patients, with muscle wasting more localized to the shoulder and pelvic regions (Table 3.1). The LGMD cohort demonstrated a substantial prevalence of DCM based on echocardiogram and cardiac MRI (Figure 3.1B) (Table 3.2) with delayed enhancement present in 3 (18.8%) patients that received a cardiac MRI (Figure 3.2). The mild cardiomyopathy of LGMD patients was not detected by routine transthoracic echocardiography (Table 3.2). 12-lead ECGs and Holter monitoring demonstrated three patients with atrial fibrillation and two patients having ventricular tachycardia (Figure 3.1D), with a low prevalence of conduction disease (Table 3.2). Respiratory disease accompanied by SDOB was prevalent in this cohort with five (15.2%) patients requiring pressure ventilation devices (Table 3.1).

Type 1 Myotonic Dystrophy

Based on echocardiogram and cardiac MRI studies of the DM1 cohort, 17 (28.3%) patients had a cardiomyopathy, as indicated by reduced LVEFs (Figure 3.1B and Table 3.2) with two patients also displaying myocardial fibrosis (Figure 3.2). DM1 patients exhibited a high incidence of conduction abnormalities, with PR intervals and QRS durations prolonged (Table 3.2). Indeed, first-degree AVB and LBBB were common abnormalities occurring in 18 (30%) and 11 (18.3%) patients, respectively, while six patients had LAFB (Table 3.2). Atrial fibrillation or flutter and ventricular tachycardia was detected in fourteen and six patients, respectively. Respiratory disease was accompanied by SDOB in 16 of the DM1 patients, with eight patients requiring ventilator devices. In addition to DM1, seven patients with Type 2 myotonic dystrophy (DM2) were

evaluated; all DM2 patients exhibited normal 12-lead ECGs, echocardiograms and cardiac MRIs (Table 3.4).

Table 3.4. Comparing 12-Lead Electrocardiogram and Systolic Function Data of Type 1 Myotonic Dystrophy Patients with Type 2 Myotonic Dystrophy Patients.

	DM1 (n=60)	DM2 (n=7)	*P* Value
Heart Rate, bpm	68 (63.3-75)	69.5 (66-74.5)	0.61
PR Interval, ms	193 (178.3-223)	152 (144.5-173.5)	0.01
QRS Duration, ms	107 (96-118)	92 (86-95.5)	0.02
QT_c Interval, ms	412 (396-440)	399.5 (393-409.8)	0.37
1° AVB	18 (30)	0	
LAFB	11 (18.3)	0	
LBBB	6 (10)	0	
LVEF, %	55 (50-60)	60 (51.3-60)	0.52

1° AVB indicates first-degree atrioventricular block; DM1, type 1 myotonic dystrophy; DM2, type 2 myotonic dystrophy; LAFB, left anterior fascicular block; LBBB, left bundle branch block; LVEF, left ventricular ejection fraction; PR Interval, duration of atrial depolarization; QRS Duration, duration of ventricular depolarization; and QT_c Interval, corrected duration between ventricular depolarization and repolarization. *12-lead electrocardiogram data was obtained from all 67 patients. Echocardiogram data was obtained from 54 patients (50 DM1 and four DM2 patients).

Fascioscapulohumeral Muscular Dystrophy

Two FSHD patients had a cardiomyopathy as indicated through echocardiogram and cardiac MRI studies (Figure 3.1B), though the degree of cardiomyopathy was milder relative to the other cohorts; both patients had mildly thickened right ventricles (Table 3.2; Table 3.3) and only one showed myocardial fibrosis (Figure 3.2). Electrocardiographic abnormalities were

minimal and associated with a low prevalence of atrial and ventricular arrhythmias (Figure 3.1D and Table 3.2). Respiratory disease and SDOB were observed in six and six patients respectively, with five patients receiving non-invasive pressure ventilation.

Non-MD Myopathies

The non-MD myopathies cohort included 40 patients with a significant burden of respiratory disease and moderate degree of limited ambulation (Table 3.1). These patients were included as a negative control cohort for appropriate comparisons to the broader MD cohort, which had a similar distribution of age and gender. The non-MD myopathies patients had normal cardiac structure and function with no indication of a cardiomyopathy or ECG abnormalities (Table 3.5).

Table 3.5. Cardiac Evaluation of the Non-Muscular Dystrophy (MD) Myopathy Patient Cohort.

Modality	**Non-MD Myopathies**
12-lead ECG (n = 40)	
Heart Rate, bpm	72 (62-83.5)
PR Interval, ms	152 (140-172)
QRS Duration, ms	90 (82-98)
QT_c Interval, ms	396 (382-416)
1° AVB	2 (5)
LAFB	2 (5)
LBBB	1 (2.5)

RBBB	2 (5)
Echocardiogram (n = 33)	
LVIDd, cm	4.5 (4.1-5)
LVIDs, cm	2.8 (2.6-3.3)
LVPWd, cm	0.8 (0.7-1)
LVEF, %	60 (55-60)
LVMI, g/m²	51.8 (43.9-54)
Cardiac MRI (n = 16)	
LA Vol Index, mL/m²	36.8 (24-40.9)
LVEDVi, mL/m²	71 (62.5-78.5)
LVESVi, mL/m²	27 (21.5-31)
LVEF, %	62 (57-66)
LVMI, g/m²	43 (32.5-52)
RVEDVi, mL/m²	74 (65-80)
RVESVi, mL/m²	31 (26-37)
RVEF, %	57 (53-59.5)

Values are median (interquartile range) or n (%). 1° AVB = first-degree atrioventricular block; LAFB = left anterior fascicular block; LA Vol Index = left atrial volume index; LBBB = left bundle branch block; LGMD = limb-girdle muscular dystrophy; LVEF = left ventricular ejection fraction; LVEDVi = left ventricular end-diastolic volume index; LVESVi = left ventricular end-systolic volume index; LVIDd = left ventricular internal dimension at end-diastole; LVIDs = left

ventricular internal dimension at end-systole; LVMI = left ventricular mass index; LVPWd = left ventricular posterior wall thickness at end-diastole; PR Interval = duration of atrial depolarization; QRS Duration = duration of ventricular depolarization; QTc Interval = corrected duration between ventricular depolarization and repolarization; RBBB = right bundle branch block; RVEDVi = right ventricular end-diastolic volume index; RVEF = right ventricular ejection fraction; RVESVi = right ventricular end-systolic volume index.

Biomarkers

Plasma CK (normal < 250 U/L) was markedly elevated in the dystrophinopathies and LGMD cohorts (Table 3.1). In contrast, BNP values (normal range: 100-500 pg/mL) were within the normal range in both cohorts (Table 3.1). There was no substantial elevation of plasma BNP or CK in the DM1 cohort with CK being increased in only three patients; similarly, FSHD patients showed normal BNP while two patients exhibited elevated CK values; all non-MD myopathies patients had normal BNP levels (Table 3.1). There was no evidence of hyperkalemia and serum creatinine remained within the low-normal range (Table 3.1).

Optimization of Medical and Device Therapies

Following enrollment in the NMMD clinic, the initiation and optimization of pharmacological therapies were implemented to better manage and improve heart disease (Figure 3.3). The use of angiotensin converting enzyme inhibitors (ACEi)/ angiotensin receptor blockers (ARB) (P=0.039), beta-blockers (P=0.018), and mineralocorticoid receptor antagonists (MRA) (P=0.001) was increased for eligible patients (Figure 3.4A) and the dose of ACEi/ ARB was uptitrated (P=0.004) and evaluated relative to the maximum tolerated dose as defined by the 2016

American Heart Association Guidelines (Figure 3.4B; Table 3.6).[134] Diuretics and statin therapies were uptitrated in 21 (14.5%) and 25 (17.2%) of patients, respectively. Corticosteroid use, with either deflazacort or prednisone, was used in nine out of 27 DMD patients. Based on clinical guidelines and on a clinical basis of primary and secondary prophylaxis,[145] device therapy was used in patients with more severe cardiomyopathies, namely in the DMD and DM1 cohorts (Figure 3.4C). These included implantable cardiac defibrillators (ICDs) and pacemakers, such as cardiac resynchronization therapy (CRT) devices. Four patients received an ICD and nine patients received a CRT device.

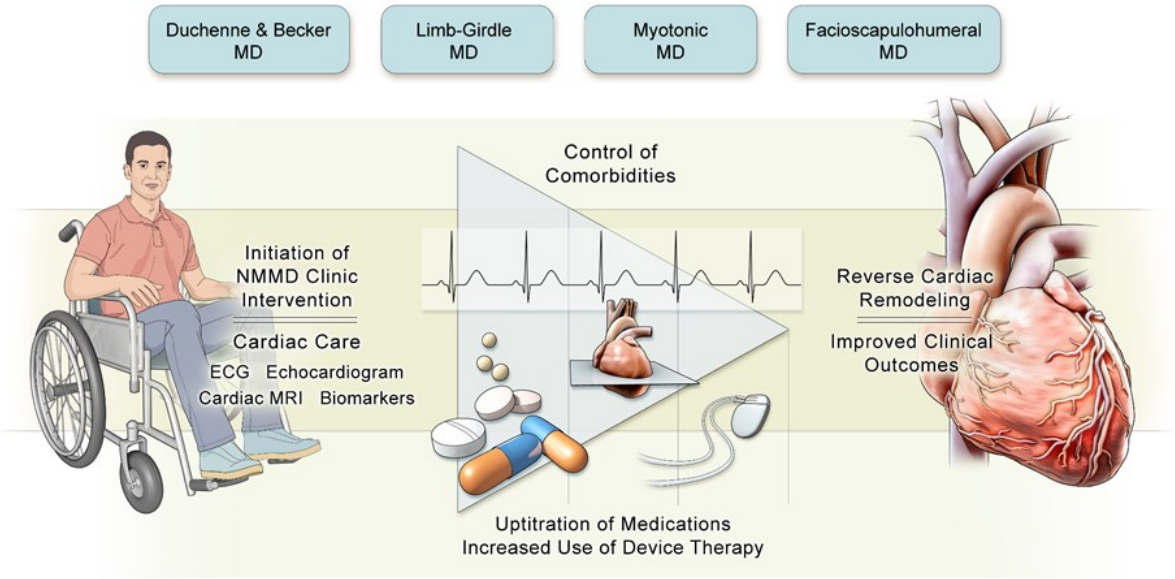


Figure 3.3. Cardiac assessment and management in a multidisciplinary setting improves outcomes in patients with muscular dystrophy (MD). MRI indicates magnetic resonance imaging; NMMD, Neuromuscular Multidisciplinary.

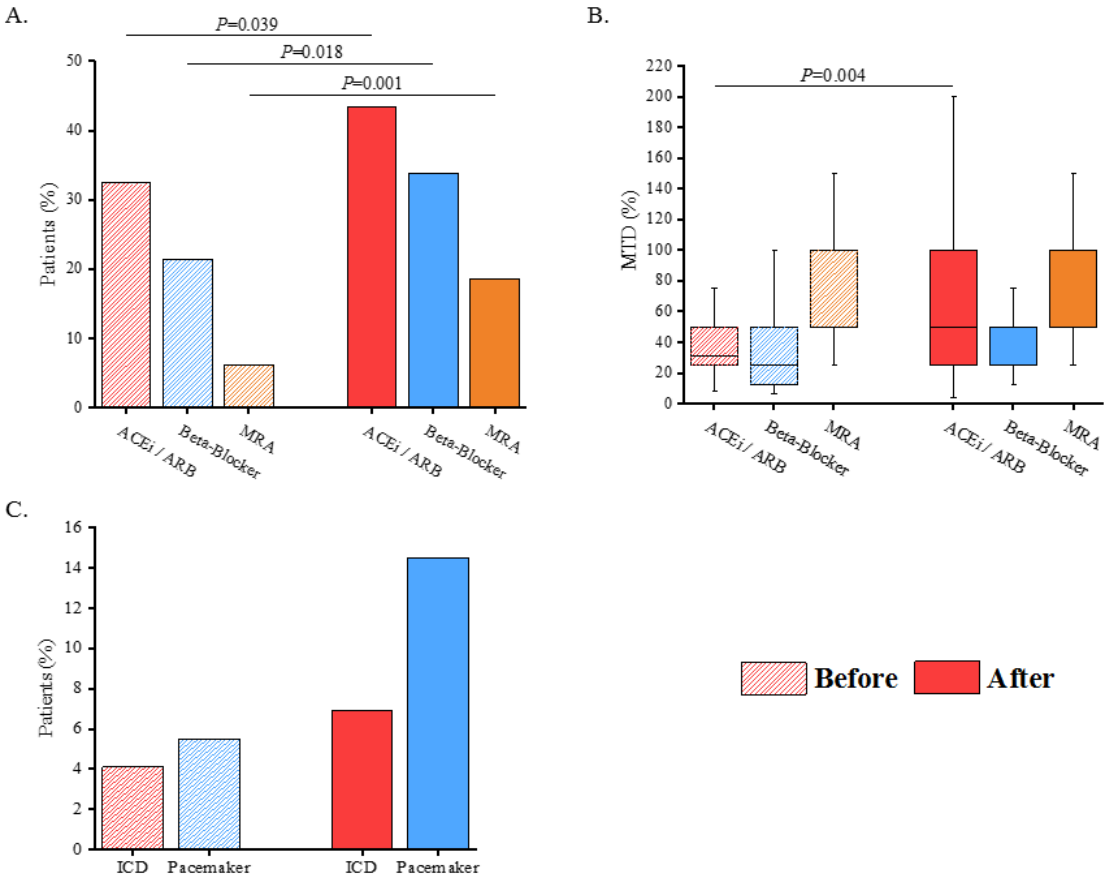


Figure 3.4. Uptitration of medical therapy and increase in device implantation following the initial clinic visit in patients with muscular dystrophy (MD). Baseline use of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), β-blockers, mineralocorticoid receptor antagonists (MRAs) (before), and their initiation at the initial Neuromuscular Multidisciplinary clinic visit (after) (A); and their uptitration following the initial visit (B). Implantable cardioverter-defibrillator (ICD) and pacemaker (including cardiac resynchronization therapy) implantation (C). Maximum tolerated dose (MTD) as defined by 2016 American Heart Association guidelines for the diagnosis and treatment of acute and chronic heart failure

Table 3.6. Defined Maximum Tolerated Dose of Medications.

Drug	Maximal Dose (mg)		Drug	Maximal Dose (mg)
ACEi			**Beta-Blockers**	
Enalapril	20		Bisoprolol	10
Lisinopril	40		Carvedilol	50
Perindopril	8		Metoprolol	200
Ramipril	10			
ARB			**MRA**	
Candesartan	32		Spironolactone	50
Irbesartan	300			
Telmisartan	80			
Valsartan	320			

ACEi indicates angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; and MRA, mineralocorticoid receptor antagonist. *Maximal dose defined as per the 2016 American Heart Association Guidelines.

Clinical Outcomes

We assessed the impact of cardiac intervention as part of a multidisciplinary care approach on the clinical outcomes of our MD patient cohort. The median LVEF of patients tracked with a cardiomyopathy in dystrophinopathies, LGMD, DM1, and FSHD cohorts was 39%, 44%, 45%, and 49%, respectively at the initial NMMD clinic visit which improved to 50%, 57%, 50%, and 55%, respectively (Figure 3.5). Overall, the 57 MD patients tracked with a cardiomyopathy

showed a marked improvement in their median LVEF from baseline of 43% to 50% at the end of the three-year period (P<0.001) (Figure 3.5).

The rate of unplanned, all-cause outpatient clinic visits was reduced from 3.0 visits/year to 1.5 visits/year among all cohorts of MD. Over the first 18 months, there was minimal difference between the rates of all-cause outpatient clinic visits between the MD and non-MD myopathies cohorts (β=0.06 [95% confidence interval (CI): -0.93 to 1.05]; P=0.86), however, from 18 months onwards, there was a marked lowering in all-cause outpatient clinic visits for the MD cohort (β=-1.09 [95% CI: -2.17 to -0.01]; P=0.048) (Figure 3.6A). The dystrophinopathies and DM1 groups showed the most substantial reduction in annual unplanned, all-cause outpatient clinic visits, from 6.5 visits/year to 1.6 visits/year, and 3.2 visits/year to 0.5 visits/year, respectively. A smaller improvement in the rate of outpatient clinic visits was noted in the LGMD group (2.8 visits/year to 1.3 visits/year) and FSHD group (2.5 visits/year to 2.2 visits/year). The rate of hospitalization duration reduced from 14.2 days/year to 0.9 days/year among all cohorts of MD. Over the first 18 months, there was no difference between the rates of hospitalization between the MD and non-MD myopathies cohorts (β=0.65 [95% CI: -6.22 to 7.51]; P=0.78), however, from 18 months onwards, there was a clear divergence in rates illustrating a marked reduction in hospitalizations for the MD cohort (β=-7.72 [95% CI: -13.73 to -1.71; P=0.021) (Figure 3.6B). Both the LGMD and DM1 groups had a large decline in the rates of hospitalization duration from 27.0 days/year to 1.2 days/year, and 14.9 days/year to 0.5 days/year, respectively. The dystrophinopathies group showed a moderate decline in the rates of hospitalization duration from 13.4 days/year to 4.8 days/year. The FSHD group maintained a low rate of hospitalization duration at 0.4 days/year to 0 days/year. The rate of cardiac-related hospitalizations was reduced from 0.4 hospitalizations/year to 0.1 hospitalizations/year, while there were no cardiac-related hospitalizations in the non-MD

myopathies cohort (β=0.21 [95% CI: 0.14-0.28]; *P*<0.001) (Figure 3.6C). At the six-month period following the optimization of medical therapies, outcome rates of the MD patient cohorts were higher than the non-MD myopathies cohort (Figure 3.6). Overall, all MD cohorts showed a marked and sustained reduction in cumulative rates of unplanned, all-cause outpatient clinic visits, the duration of hospitalization, and the number of cardiac-related hospitalizations over the three-year period (Figure 3.6). Over the three-year period, one DMD patient died of cardiac cause and one DMD patient died of non-cardiac cause (2 of 32 patients over three years [2.1%/year]). Two LGMD patients died of non-cardiac causes (mortality rate of 2.0%/year) while three DM1 patients died of non-cardiac causes, yielding a mortality rate of 1.7%/year.

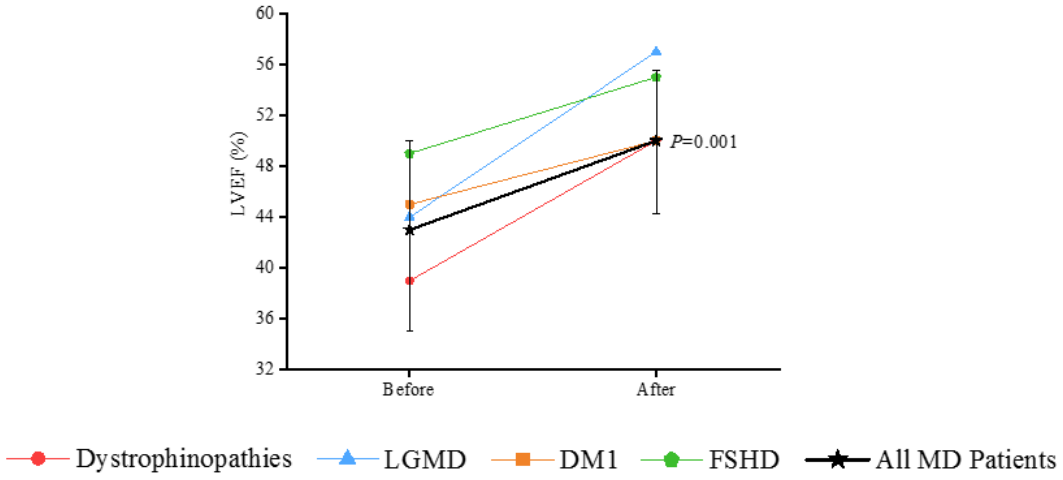


Figure 3.5. Improvement in left ventricular ejection fraction (LVEF) in the various muscular dystrophy (MD) cohorts in response to multidisciplinary care. LVEF obtained by cardiac magnetic resonance imaging and/or echocardiogram for 57 patients with cardiomyopathy, with 3 years of imaging data, shown as the median LVEF at the time of their initial clinic visit and at their 3-year follow-up. DM1 indicates type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; LGMD, limb-girdle muscular dystrophy.

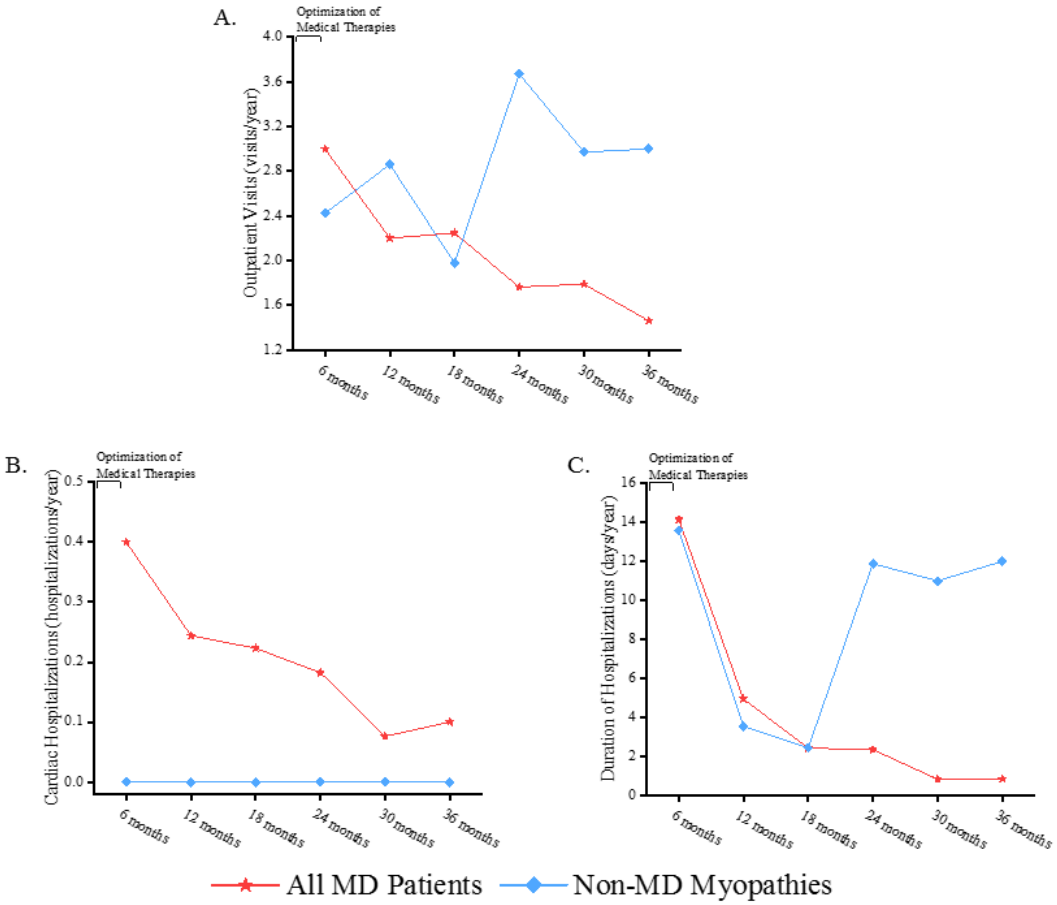


Figure 3.6. Marked improvement in clinical outcomes in the various muscular dystrophy (MD) cohorts in response to multidisciplinary care. Comparing the rates of unplanned, all-cause outpatient clinic visits (A), duration of hospitalizations (B), and incidence of cardiac hospitalizations (C) following a 6-month period of patient assessment and care and within 3 years of the initial Neuromuscular Multidisciplinary clinic visit.

3.6. Discussion

Patients with MD represent a vulnerable population due to the complex syndrome of progressive muscle weakness along with cardiac and respiratory comorbidities. Our cohort clearly demonstrates a significant burden of heart disease, and our care model facilitates a prompt and careful assessment, followed by the initiation and optimization of medical and device therapy with effective follow-up care. We included a cohort of non-MD patients, without heart disease, but with moderately impaired ambulation and respiratory disease concomitant with relatively low adverse outcomes treated in the same multidisciplinary care environment. Our outcome data demonstrated a high burden of healthcare use in the dystrophinopathies, LGMD, and DM1 cohorts. The incidence of unplanned, all-cause outpatient clinic visits; the duration of hospitalizations; as well as the incidence of cardiac-related hospitalizations are indicative of the severity of patient condition. Cardiac intervention had a direct effect on the rate of cardiac-related hospitalizations. Additionally, all-cause outcomes were progressively improved in our MD patients, through optimized medications, illustrated by the divergence of all-cause outcome rates following 18 months of treatment. Furthermore, the implantation of ICDs and pacemaker devices usually occurs within the first 18 months of NMMD clinic enrollment and cardiac assessment. With regards to the non-MD myopathies cohort, these patients show comparable complex neuromuscular disease phenotypes to the MD cohort and receive the same multidisciplinary care, including a similar degree of respiratory intervention, with the exception of cardiac intervention in the form of medical and device therapy. Unlike the MD cohort, these patients do not have heart disease. We can therefore elucidate that heart disease is an important driver of MD patient outcomes by contrasting them with non-MD myopathies patient outcomes. This emphasizes the necessity for cardiac intervention and management in the MD patient population, effectively provided through our

multidisciplinary care model, in which confounding comorbidities may be treated and stabilized. Importantly, in our dystrophinopathies, LGMD, and DM1 cohorts, mortality rates were markedly lower (1.7% to 2.1%) compared with historical cohort data collected from patient cohorts in the absence of multidisciplinary care (2.0% to 3.3%).[6, 146-148]

The demographics of all of the MD patient cohorts appear uniform, except for the dystrophinopathies cohort, which was comprised of younger males due to the X-linked inheritance pattern.[24, 135, 149] We used a combination of transthoracic echocardiography and cardiac MRI at the initial clinic visit and during follow-up to evaluate the degree of cardiomyopathy and the response to therapy. Although cardiac MRI is the preferred imaging modality for patients with MD,[24] its feasibility in patients with advanced disease can be challenging due to limited mobility, wheelchair dependence, use of defibrillators and pacemakers, spinal stabilization rods, and mouth piece ventilation. Therefore, the use of transthoracic echocardiography was an important imaging modality for our patients who were unable to perform a cardiac MRI. We detected a high prevalence of cardiomyopathy in the dystrophinopathies and LGMD cohorts characterized by reduced systolic function as well as LV chamber dilation. Cardiac imaging is particularly important since plasma BNP, a well-known biomarker of pathological cardiac remodeling[150], was not markedly elevated, illustrating the limitation of BNP in predicting heart disease in our cohort.

Patients with DM1 presented with a high burden of atrial and ventricular arrhythmias. Notably, there was a high incidence of 1°AVB and LBBB[151]; the presence of LBBB likely aggravated the cardiomyopathy in patients with DM1 due to the associated electro-mechanical ventricular dyssynchrony leading to progressive left ventricular dysfunction.[152] The demonstrated occurrence of ventricular tachyarrhythmias in our DM1 patient cohort is consistent with the increased risk of sudden cardiac death in this patient population.[27, 138, 153] Of our entire MD cohort,

there were ten cardiac defibrillators (6.9% [10 of 145] patients) and 20 pacemakers (13.8% [20 of 145] patients) implanted. As such, the use of pacemakers, including cardiac resynchronization therapy (CRT), is particularly important while allowing the initiation of beta-blocker therapy. Nine CRT devices were implanted in our DM1 patients and we have recently shown that CRT improves LVEF in DM1 patients with LBBB.[26]

The use of medical therapies in our patients with dystrophinopathies is supported by clinical trial evidence. Angiotensin-converting enzyme inhibition[99], beta-blockade[103, 105], and mineralocorticoid receptor antagonism[34] improves LV systolic function and delays the progression of cardiomyopathy in DMD. Importantly, we extrapolated these findings to other MD cohorts with cardiomyopathies, which was associated with similar improvement in cardiac and clinical outcomes, thereby supporting the widespread use of these therapies in this vulnerable patient population. Our data shows that active cardiac care by way of optimized medical and device therapies, prescribed to MD patients but not non-MD myopathies patients, improved clinical outcomes. However, respiratory care, through the assessment and management of respiratory comorbidities, likely also contributed to the improved outcomes in our patients; SDOB and limited airway clearance in our patients can be attributed to muscle weakness affecting the chest wall, diaphragm and upper airways. The initiation of assisted non-invasive positive pressure ventilation alongside the use of lung volume recruitment strategies and mechanical insufflation-exsufflation likely contributed to improved clinical outcomes, though they were prescribed to a similar degree across all cohorts. The involvement of social workers in our clinic allowed patients to receive financial assistance, counseling for psychosocial distress, and enable adherence to medical therapy. Our dietician provided healthy dietary recommendations and adjusted potassium intake to minimize hyperkalemia, which is particularly important since medications such as ACEi may

cause electrolyte imbalances and there is no accurate measure of renal function in our patients. Taken together, relieved heart disease burden combined with comorbidity control, facilitated by our novel multidisciplinary care model provided at the NMMD clinic, improved outcomes of patients with MD.

Limitations

Due to the systemic manifestations of this disease, MD patients in our region are generally referred to the NMMD clinic, and it is therefore not feasible to recruit MD patients exclusively receiving cardiac care. With regards to cardiac imaging for patients with MD, though echocardiography may be the only feasible cardiac imaging modality, there are limitations to the reliability of data collected due to obstructed acoustic windows as a result of scoliosis, obesity, and lung disease. Another limitation to our study is our modest cohort size. Given that these conditions are relatively uncommon, we believe that our group sizes are reasonable. Our non-MD myopathies cohort was older, which introduces limitations for using these patients as a directly comparable negative control cohort. Our outcome data was provided over a three-year period but given the prospective nature of our study, we will continue to recruit patients and obtain additional outcome data over a longer duration.

3.7. Conclusions

Patients with MD suffer from a considerable burden of heart disease with multiple comorbidities. The treatment of patients using a multidisciplinary care model, such as the one initiated at the NMMD clinic, provides patients with comprehensive care including cardiac assessment and

prompt management of their heart disease. Our prospective cohort study demonstrates the effectiveness of cardiac intervention facilitated through a multidisciplinary care pathway to improve health outcomes of MD patients.

4. Evaluating the Diagnostic and Prognostic Value of Biomarkers for Heart Disease and Major Adverse Cardiac Events in Patients with Muscular Dystrophy

4.1. Abstract

Aims: Heart disease is recognized as the leading cause of morbidity and mortality in patients with muscular dystrophy (MD). Our study demonstrates the clinical utility of cardiac biomarkers to improve the diagnosis of cardiomyopathy and prognostication of major adverse cardiac events (MACE) in these vulnerable patients.

Methods and Results: We prospectively followed 117 patients (median age, 42 (interquartile range [IQR], 26-50) years; 49 [41.9%] women) at the Neuromuscular Multidisciplinary clinic diagnosed with a dystrophinopathy, limb-girdle MD, type 1 myotonic dystrophy, or facioscapulohumeral MD. We determined that B-type natriuretic peptide (BNP) and high-sensitive troponin I (hsTnI) were effective diagnostic markers of cardiomyopathy (area under the curve [AUC], 0.64; *P*=0.017; and AUC, 0.69; *P*=0.001, respectively). Patient risk stratification for MACE was based on cutoff values of BNP and hsTnI defined a priori as 30.5000 pg/mL and 7.6050 ng/L, respectively. Over a median follow-up period of 2.09 (IQR, 1.17-2.81) years there

were 36 confirmed MACE. Multivariate regression analyses showed that patients with BNP and hsTnI levels above the respective cutoff values had a 3.70-fold (P=0.001) and 3.24-fold (P=0.002) greater risk of MACE, respectively, compared to patients with biomarker levels below. Furthermore, patients with biomarker levels above both cutoff values had a 4.08-fold (P=0.001) greater risk of MACE. Inflammatory biomarkers did not show clinical utility for heart disease in these patients.

Conclusion: Our study demonstrates important diagnostic and prognostic value of BNP and hsTnI as part of a comprehensive cardiac assessment to augment the management and treatment of heart disease in patients with MD.

4.2. Introduction

Muscular dystrophy (MD) is a diverse group of hereditary neuromuscular disorders characterized by progressive debilitating muscle weakness and wasting. Skeletal deformities, neurological complications, respiratory disease, and heart disease are common manifestations of MD. While improved therapies and multidisciplinary care have markedly improved clinical outcomes in patients with MD, heart disease continues to have a high burden in these patients.[4, 5, 154-156] Heart disease is recognized as a leading cause of mortality and morbidity in patients with MD.[4-6, 138, 139, 154, 155] Unfortunately, cardiac screening procedures available in common clinical practice, generally applied to traditional patients, are time consuming and often only effective in later stages of disease, thus limiting our ability to prevent or delay further progression of the disease. Muscle breakdown leading to elevated serum creatine kinase (CK) can be markedly

elevated in these patients although it is neither specific to the heart nor predictive of adverse clinical outcomes.[156]

We therefore investigated the clinical utility of cardiac-specific biomarkers, B-type natriuretic peptide (BNP) and high-sensitive troponin I (hsTnI), in patients with MD. We also analyzed plasma inflammatory biomarkers which are known to be elevated in patients with MD.[157, 158] Timely diagnosis and implementation of appropriate therapies improve clinical outcomes in this vulnerable cohort of patients and therefore the investigation of biomarkers as an effective screening method for heart disease is of high priority.

4.3. Methods

In coordination with the Neuromuscular Multidisciplinary (NMMD) clinic at the Kaye Edmonton Clinic (Canada), 117 recruited MD patients voluntarily provided blood samples for our prospective study, which was conducted over a 5-year period from November 5, 2014, to November 6, 2019. We simultaneously collected blood from 43 age and gender-matched healthy controls (HC). Following neurological assessments, muscle biopsy, and genetic testing we confirmed the following diagnoses of MD in our study cohort: dystrophinopathies (Duchenne muscular dystrophy [DMD] and Becker's MD) including DMD carriers (25 patients), limb-girdle muscular dystrophy (LGMD) (25 patients), type 1 myotonic dystrophy (DM1) (52 patients), and facioscapulohumeral muscular dystrophy (FSHD) (15 patients). Patients were referred to the NMMD clinic at various stages of their disease with no bias towards patients with overt cardiac symptoms. All patients received multifaceted care from specialist physicians in neurology, cardiology, pulmonary medicine, and physiatry, with support from allied health care

professionals, as part of the same multidisciplinary care pathway. Physicians implemented guideline-based medical therapy, including device intervention when appropriate, and actively managed treatment during follow-up. The investigation complies with the Declaration of Helsinki and conforms to the principles outlined by our locally appointed ethics committee, the Health Research Ethics Board, at the University of Alberta. All patients provided informed and written consent at study enrollment.

Risk Assessment

Demographic, clinical, and biochemical parameters, in addition to 12-lead electrocardiogram (ECG), transthoracic echocardiogram (TTE) and cardiac magnetic resonance imaging (MRI) data were collected at baseline through review of electronic medical records. Heart rate, blood pressure, and a 12-lead ECG along with a comprehensive cardiac assessment were recorded at each visit and the presence of cardiomyopathy and comorbidities such as anemia, diabetes, ambulatory status, respiratory disease and sleep disordered breathing (SDOB), defined as obstructive sleep apnea or nocturnal hypoventilation, were documented. All patients suspected of SDOB underwent polysomnography for further evaluation. Respiratory disease was defined as patients with chronic obstructive pulmonary disorder, asthma, recurrent aspiration pneumonia, respiratory muscle weakness, or restrictive lung disease. Respiratory strategies such as lung volume recruitment and therapies such as the use of a mechanical insufflator-exsufflator and noninvasive positive pressure ventilation were prescribed when necessary. Patient ambulatory function was evaluated and interventions in the form of different mobility aids such as a cane, walker, and wheelchair use (manual or powered) were implemented when needed.

Serum biochemistry, fasting lipid profile, hemoglobin, plasma B-type natriuretic peptide (BNP), high-sensitive C-reactive protein (CRP), high-sensitive troponin I (hsTnI), high-sensitive troponin T (hsTnT), and creatine kinase (CK) were monitored. We also monitored levels of inflammatory biomarkers such as tumor necrosis factor alpha (TNF-alpha), tumor necrosis factor receptor I (TNFRI), tumor necrosis factor receptor II (TNFRII), interleukin 6 (IL-6), interleukin 1 beta (IL-1 beta), matrix metallopeptidase 2 (MMP-2), and matrix metallopeptidase 9 (MMP-9). Anemia was defined as per the hemoglobin level cutoffs established by the World Health Organization. Dyslipidemia was defined in accordance with the 2016 Canadian Cardiovascular Society guidelines.[132] Baseline 12-lead resting ECGs, TTE and cardiac MRI were used to assess cardiac function and structure. Patients were categorized based on the diagnosis of cardiomyopathy, which was defined as left ventricular ejection fraction (LVEF) < 55% or a left ventricular end-diastolic volume index (LVEDVi) > 105 mL/m^2,[133] as determined by both TTE and cardiac MRI, given their concordance in our cohort.[156] Medical therapy by maximum tolerated dose[159] and the use and type of device therapy was documented at each clinic visit.

Plasma Biomarker Quantification

Plasma samples were obtained upon patient recruitment at the initial NMMD clinic visit, using heparin as an anticoagulant, and immediately processed and stored at –80°C. Healthy control samples previously obtained were stored in the same facility. One hundred and sixty samples (117 MD and 43 HC) were collected. Plasma BNP levels were assessed using a Quidel Triage reagent pack (Quidel, San Diego, CA)[160] and troponin I levels were assessed using an Access High Sensitive Troponin I Assay (limit of detection, 2.3 ng/L; Beckman-Coulter, Fullerton, CA),[161-163] both of which were analyzed using an automated Unicel DxI 800

immunoanalyzer (Beckman-Coulter, Fullerton, CA). Troponin T levels were assessed using an Elecsys Troponin T-high sensitive assay (limit of detection, 5 ng/L; Roche, Basel, Switzerland),[164-166] analyzed using a cobas e601 instrument (Roche, Basel, Switzerland). B-type natriuretic peptide, hsTnI, and hsTnT analyses were conducted at provincial health laboratories in Alberta, Canada. Plasma high-sensitive CRP and CK were obtained through subsequent electronic chart review. Plasma TNF-alpha, TNFRI, TNFRII, IL-1 beta, IL-6, MMP-2, and MMP-9 levels were determined using commercially available human ELISA kits from R&D Systems (HSTA00E, DRT100, DRT200, HSLB00D, HS600C, MMP200, and DMP900, respectively; Minneapolis, MN)[167, 168] and the absorbance was measured using a SpectraMax microplate reader (Molecular Devices, San Jose, CA).

Study Endpoints

Patients were followed over a median follow-up period of 2.1 (interquartile range [IQR], 1.2-2.8) years, over which time endpoints such as the diagnosis of cardiomyopathy and the incidence of MACE were tracked. Major adverse cardiac events were defined as a composite of arrhythmia, device implantation, cardiac-related hospitalization, incident heart failure (HF), and cardiac-mortality. Arrhythmias were captured by 12-lead ECG and Holter monitoring. Incident HF was diagnosed following a comprehensive cardiac assessment, which considered symptoms and signs such as dyspnea, orthopnea, poor appetite, elevated jugular venous pressure, peripheral edema, and abdominal distention. Outcome data such as hospitalizations, mortality, and associated diagnoses were collected by the Data Integration and Management Repository analytics branch of Alberta Health Services using our provincial electronic health records.

Statistical Analysis

Continuous variables were compared using a Mann-Whitney U test or Kruskal-Wallis test where appropriate, and all categorical data were compared using Pearson's Chi-square tests. Receiver-operator characteristic (ROC) curves were used to evaluate the diagnostic ability of the biomarkers for cardiomyopathy. Select biomarkers were combined and standardized using binary logistic regression to create a ROC curve to evaluate their combined use to diagnose cardiomyopathy. Biomarker cutoff values for the dichotomous risk stratification of MACE were established a priori using ROC curves and corresponding Youden's Indices.[169, 170] Multivariate Cox regression models (adjusted for age, gender, diagnosis of cardiomyopathy and respiratory disease, and the use of cardiac medications and respiratory therapies) were used to derive hazard ratios (HR) to evaluate the independent prognostic value of elevated biomarker levels for MACE in our MD patient cohort. A P-value < 0.05 was considered significant through all statistical analyses. Statistical analyses were conducted using SPSS Statistics Version 26 (IBM, NY, USA).

4.4. Results

Clinical Characteristics

The median age of the broader MD cohort was 42 (interquartile range (IQR), 26.0-50.0) years, which included 49 (41.9%) females. Our HC cohort had a median age of 50 (IQR, 39.0-56.5) years, which included 19 (44.2%) females. Cardiomyopathy was prevalent in 35 (29.9%) of the MD patients. The cardiomyopathy cohort was characterized by a predominance of males, (P=0.002; Table 4.1), and primarily comprised of patients with a dystrophinopathy (16 [45.7%] patients, including 3 carriers) and DM1 (12 [34.3%] patients). Additionally, this cohort included

5 LGMD and 2 FSHD patients. There was a relatively low number of female patients in the cardiomyopathy cohort, which represented the diagnoses of dystrophinopathy carriers, DM1, and FSHD. Comorbidities such as dyslipidemia, respiratory disease, and impaired ambulation were highly prevalent in both patients with and without cardiomyopathy (Table 4.1). The prevalence of comorbidities was evenly distributed between males and females in the cohort without cardiomyopathy. However, in the cohort with cardiomyopathy, the prevalence of comorbidities was markedly lower in female patients, likely due to their type of MD. Only 2 of 7 (28.6%) of the female patients required ambulatory aid as opposed to 14 of 28 (50%) males in the cohort with cardiomyopathy. Sleep-disordered breathing was more prevalent in MD patients with cardiomyopathy and diagnosed in 14 (40%) patients, which was reflected in the use of noninvasive positive pressure ventilation, prescribed to 13 (37.1%) patients (Table 4.1).

Table 4.1. Baseline Characteristics of Patients with Biomarker Analysis.

Characteristic	**MD Without CM (n=82)**	**MD With CM (n=35)**	***P* value**
Males/Females, No.	40 (48.8)/42 (51.2)	28 (80)/7 (20)	0.002
Median Age, Yrs	41.0 (26.3-49.8)	43.0 (22.5-52)	0.99
Current/Former Smoker, No.	10 (12.2)	4 (11.4)	0.91
Ambulation, No.			
Cane/Walker	5 (6.1)	6 (17.1)	0.06
mWC/pWC	17 (20.7)	9 (25.7)	0.55

Comorbidities, No.			
Anemia	2 (2.4)	3 (8.6)	0.13
Dyslipidemia	12 (14.6)	6 (17.1)	0.73
Diabetes	9 (11.0)	3 (8.6)	0.69
Hypertension	11 (13.4)	3 (8.6)	0.46
Respiratory Disease	20 (24.4)	11 (31.4)	0.43
SDOB	18 (22.0)	14 (40)	0.04
Cardiac Medications, No.			
ACEi/ARB	15 (18.3)	17 (48.6)	<0.001
Beta-Blocker	3 (3.7)	1 (2.9)	0.83
MRA	6 (7.3)	1 (2.9)	0.35
Respiratory Therapies, No.			
LVR	24 (29.3)	17 (48.6)	0.05
Mechanical Insufflator-Exsufflator	6 (7.3)	1 (2.9)	0.35
Noninvasive Positive Pressure Ventilation	15 (8.3)	13 (37.1)	0.03
Corticosteroids, No.	0	3 (8.6)	

Vitals, median			
HR, bpm	76.0 (67.0-87.0)	76.5 (63.0-80.0)	0.73
sBP, mmHg	120.0 (110.0-131.0)	115.0 (104.5-125.0)	0.09
dBP, mmHg	77.0 (70.0-82.0)	70.5 (62.5-76.0)	0.003
Serum Chemistry, median			
Creatinine, μmol/L	54.0 (38.8-70.5)	42.0 (22.0-60.0)	0.14
Potassium, mmol/L	4.3 (3.8-4.8)	4.3 (4.1-4.5)	0.91

Data presented as median (interquartile range) or n (%). ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BNP, B-Type natriuretic peptide; CK, creatine kinase; CM, cardiomyopathy; dBP, diastolic blood pressure; DM1, type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; HR, heart rate; LGMD, limb-girdle muscular dystrophy; LVR, lung volume recruitment; MD, muscular dystrophy; MRA. Mineralocorticoid receptor antagonist; mWC, manual wheelchair; pWC, power wheelchair; sBP, systolic blood pressure; SDOB, sleep disordered breathing.

At the time of patient plasma collection, a number of patients had already been prescribed cardiac medications prior to NMMD clinic referral such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers (ARB), beta-blockers, and mineralocorticoid receptor antagonists (Table 4.1 and Figure 4.1). Angiotensin-converting enzyme inhibitor and ARB use was markedly greater in MD patients with a cardiomyopathy (Table 4.1). Corticosteroids were only prescribed to 3 dystrophinopathy patients at the time of plasma collection. At the time of plasma collection, cardiac device intervention was minimal with only 3 implantable cardiac defibrillators and 8 pacemakers implanted in the entire study group.

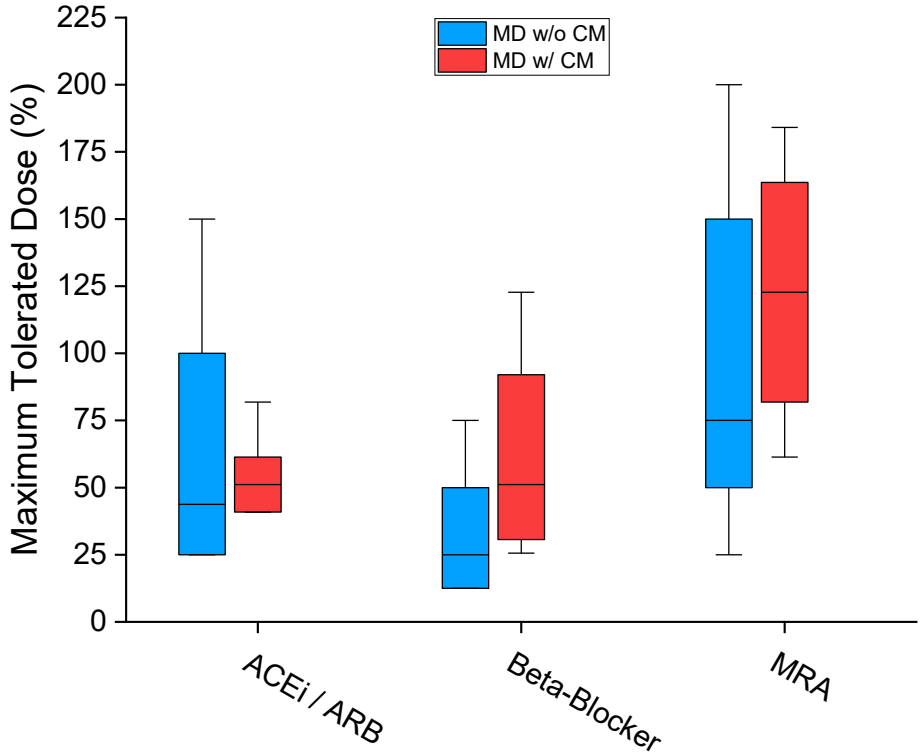


Figure 4.1. Baseline use of cardiac medications. Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), beta-blockers, mineralocorticoid receptor antagonists (MRAs) at the time of plasma draw in muscular dystrophy (MD) patients without and with cardiomyopathy (CM) by maximum tolerated dose.[1,2]

Cardiac assessment of our cohort showed a high burden of arrhythmias across both cohorts of MD patients. Atrial fibrillation or flutter was reported in 10 (12.2%) MD patients without a cardiomyopathy and in 8 (22.9%) patients with a cardiomyopathy. Ventricular tachycardia (VT) was less common in MD patients without cardiomyopathy (3 [3.7%] patients), than MD patients with a cardiomyopathy (10 [28.6%] patients). There was also a high prevalence of conduction delays across both cohorts such as first-degree atrioventricular block (Table 4.2). On the other hand, there was a high prevalence of left bundle branch block (LBBB) in MD

patients with cardiomyopathy, but this was not present in MD patients without cardiomyopathy (Table 4.2). Transthoracic echocardiogram (TTE) showed left ventricular (LV) dilation, as indicated by increased dimensions, increased LV mass, and markedly reduced LV and systolic function in MD patients with cardiomyopathy (Table 4.2). Doppler-derived parameters from TTE indicated a mild elevation of LV filling pressures in the cohort with cardiomyopathy but did not indicate overt diastolic dysfunction (Table 4.2). Cardiac MRI findings were concordant and showed increased volumes, LV systolic dysfunction, but also indicated reduced right ventricular systolic function (Table 4.2). Three out of the 30 (10%) patients without cardiomyopathy that received a cardiac MRI had fibrosis, as visualized by gadolinium contrast, while six out of the 23 (26.1%) patients with a cardiomyopathy showed myocardial fibrosis. Echocardiogram and cardiac MRI findings were concordant when assessing LV structure and function for both cohorts.

Table 4.2. Cardiac Evaluation of Patients with Biomarker Analysis.

Modality	**MD Without CM**	**MD With CM**	***P* value**
12-Lead ECG	**(n=82)**	**(n=35)**	
Heart Rate, bpm	70.0 (62.3-82.0)	71.0 (62.5-81.0)	0.83
PR Interval, ms	165.0 (144.0-192.0)	164.0 (136.0-197.0)	0.76
QRS Duration, ms	98.0 (89.3-108.8)	109.0 (94.5-135.5)	0.004
QTc Interval, ms	404.0 (369.0-432.0)	408.0 (381.5-435.5)	0.34
1° AVB	16 (19.5)	5 (14.3)	0.67
LAFB	6 (7.3)	2 (5.7)	0.75

LBBB	0	11 (31.4)	
RBBB	6 (7.3)	1 (2.9)	0.35
Echocardiogram	**(n=52)**	**(n=32)**	
LVIDd, cm	4.4 (4.1-4.7)	4.9 (4.5-5.7)	<0.001
LVIDs, cm	2.8 (2.6-3.0)	3.8 (3.0-4.6)	<0.001
LVPWd, cm	0.80 (0.70-1.0)	0.87 (0.69-0.94)	0.96
LVEF, %	60.0 (57.8-60.0)	40.0 (30.5-45.0)	<0.001
LVMI, g/m^2	63.5 (55.1-74.1)	83.0 (70.0-99.1)	<0.001
MV E/A	1.4 (1.1-1.9)	1.3 (0.9-1.7)	0.25
MV Deceleration Time, ms	6.3 (5.1-7.7)	6.9 (5.6-7.6)	0.60
E/e’	0.16 (0.14-0.20)	0.27 (0.20-0.31)	0.01
TAPSE, mm	2.1 (1.9-2.5)	2.1 (1.8-2.3)	0.30
RVSP, mmHg	24.5 (20.2-29.9)	26.5 (25.0-29.0)	0.62
Cardiac MRI	**(n=30)**	**(n=23)**	
LA Vol Index, mL/m^2	32.7 (28.1-36.7)	41.4 (28.8-46.3)	0.16
LVEDVi, mL/m^2	64.0 (56.3-77.3)	91.0 (71.3-108.5)	<0.001
LVESVI, mL/m^2	25.0 (22.0-35.5)	49.0 (34.5-54.8)	<0.001
LVEF, %	60.5 (56.0-66.5)	44.0 (39.5-47.0)	<0.001
LVMI, g/m^2	43.0 (38.0-51.0)	49.0 (42.0-62.0)	0.05
RVEDVi, mL/m^2	67.0 (59.0-78.0)	72.0 (61.5-80.0)	0.37
RVESVi, mL/m^2	31.0 (25.5-37.5)	39.5 (33.8-42.8)	0.003

RVEF, %	54.0 (52.0-57.0)	44.5 (41.5-48.5)	<0.001

Data presented as median (interquartile range) or n (%). 1° AVB, first-degree atrioventricular block; CM, cardiomyopathy; DM1, type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; LA Vol Index, left atrial volume index; LAFB, left anterior fascicular block; LBBB, left bundle branch block; LGMD, limb-girdle muscular dystrophy; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVESVi, left ventricular end-systolic volume index; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; LVMI, left ventricular mass index; LVPWd, left ventricular posterior wall thickness at end-diastole; MV, mitral valve; PR Interval, duration of atrial depolarization; QRS Duration, duration of ventricular depolarization; QTc Interval, corrected duration between ventricular depolarization and repolarization; RBBB, right bundle branch block; RVEDVi, right ventricular end-diastolic volume index; RVEF, right ventricular ejection fraction; RVESVi, right ventricular end-systolic volume index; RVSP, right ventricular systolic pressure; TAPSE, tricuspid annular plane systolic excursion.

Plasma Biomarker Analysis

Our analysis identified BNP and hsTnI as biomarkers that could be used to differentiate between our HC and MD cohorts (P=0.032 and P<0.001, respectively; Figure 4.2). Importantly, median levels of BNP were markedly elevated in MD patients with cardiomyopathy at 32 (IQR, 14.5-81.0) pg/mL compared to MD patients without cardiomyopathy at 20 (IQR, 11.9-33.0) pg/mL (P=0.017). Similarly, hsTnI was elevated in MD patients with cardiomyopathy at 7.2 (IQR, 3.9-17.5) ng/L compared to MD patients without cardiomyopathy at 3.9 (IQR, 2.6-6.2) ng/L (P=0.001). Conventional troponin I assays failed to detect elevated troponin I across our entire patient cohort (data not shown). Although CK and hsTnT levels could be used to differentiate between the HC and MD cohorts, there was no major difference between their respective levels in MD patients with and without cardiomyopathy (Figure 4.3). Interleukin-1 beta, IL-6, MMP-2, TNF-alpha, TNFRI, and TNFRII levels were higher in the MD cohort compared to the HC cohort, while high-sensitive CRP and MMP-9 levels did not differ between the cohorts (Figure 4.4). However, all inflammatory biomarkers were unable to discriminate between MD cohorts with and without cardiomyopathy (Figure 4.4). ROC curve analysis was

used to evaluate the diagnostic ability of BNP and hsTnI (Figure 4.5). B-type natriuretic peptide was an effective diagnostic marker of cardiomyopathy (area under the curve (AUC), 0.64 [95% CI, 0.52-0.76]; *P*=0.017) as was hsTnI (AUC, 0.69 [95% CI, 0.58-0.80]; *P*=0.001). When used in combination, both biomarkers showed impressive diagnostic ability with an AUC of 0.72 (95% CI, 0.61-0.83; *P*<0.001).

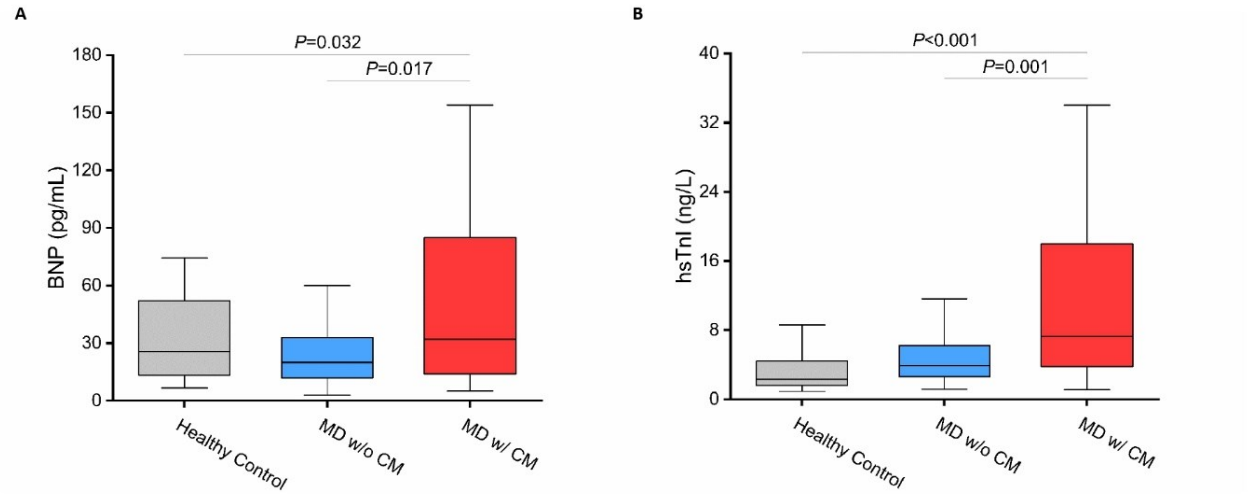


Figure 4.2. Baseline B-type natriuretic peptide (A) and high-sensitive troponin I (B) levels in healthy control and muscular dystrophy groups. CM, cardiomyopathy

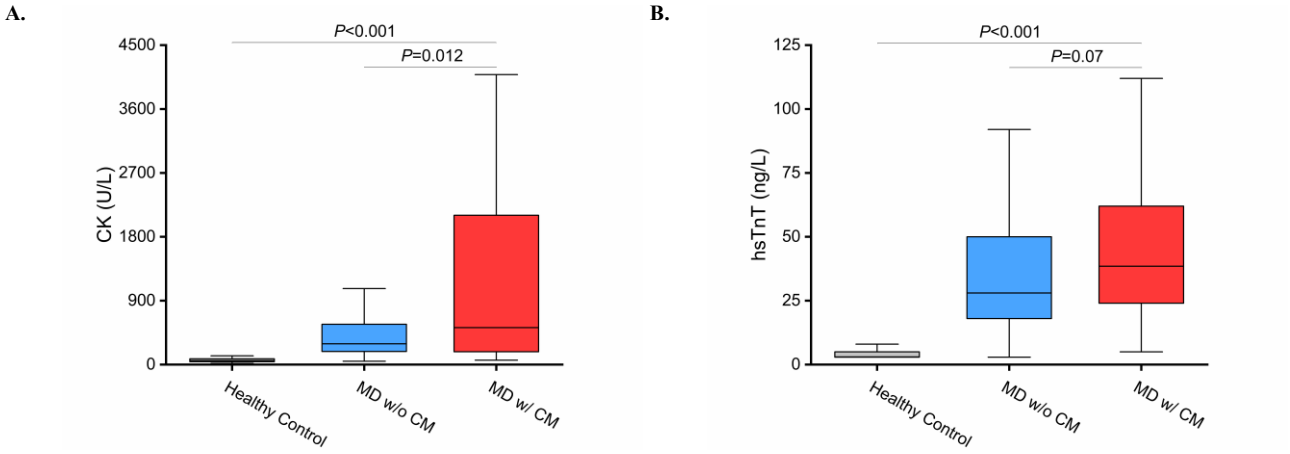
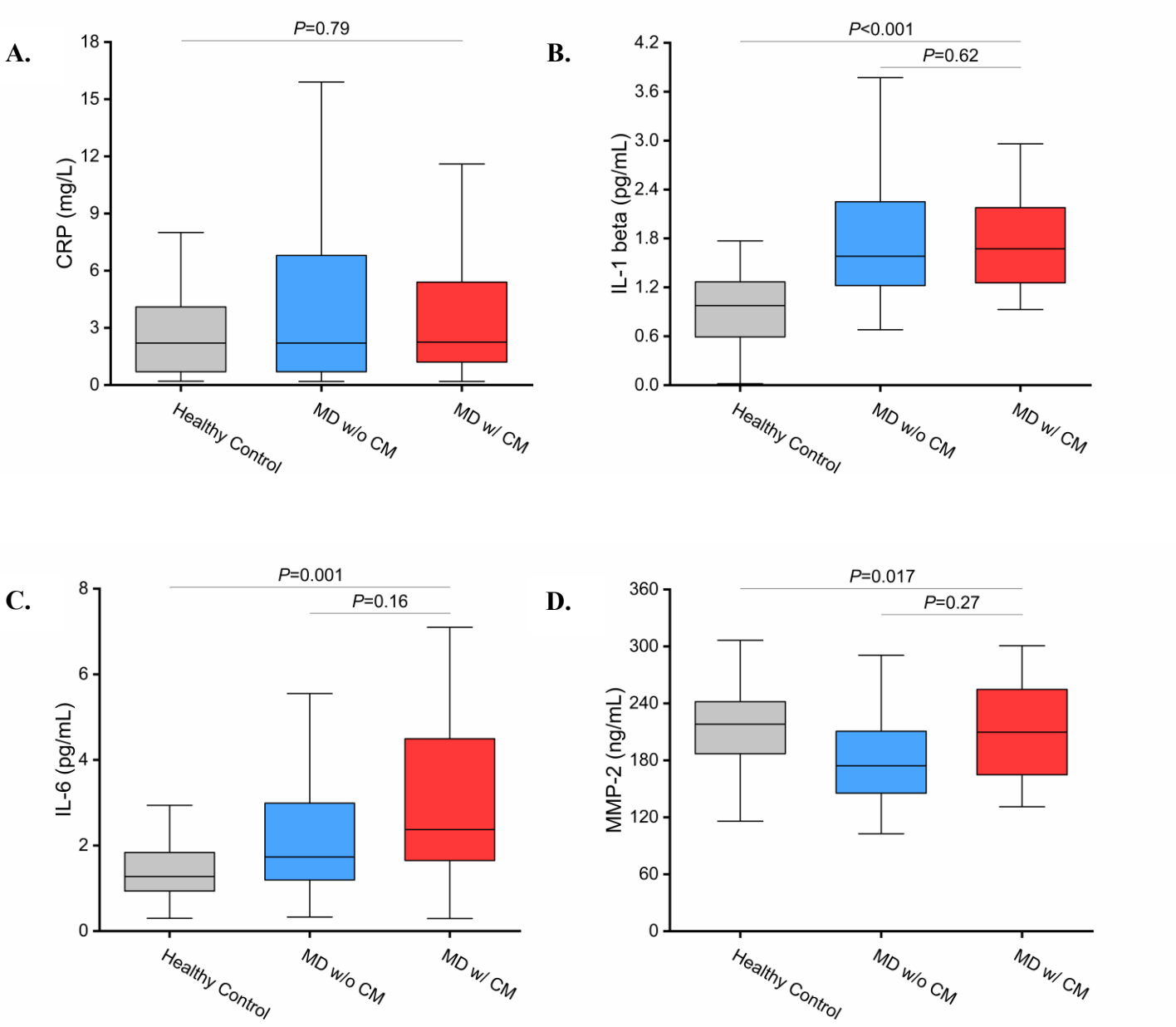
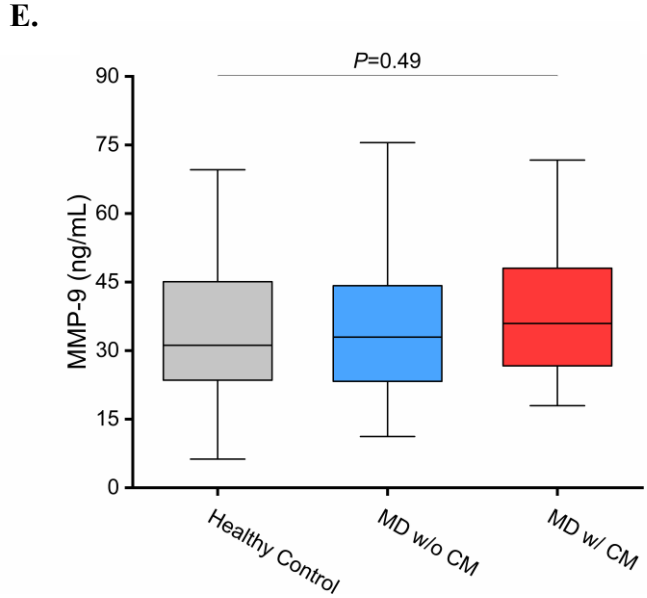


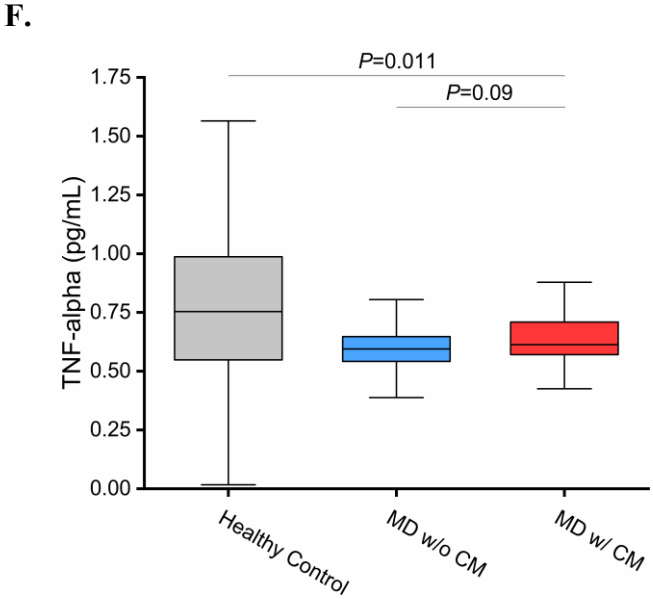
Figure 4.3. Baseline creatine kinase (CK; A) and high-sensitive troponin T (hsTnT; B) levels in healthy control (HC) and muscular dystrophy (MD) groups with and without cardiomyopathy (CM).


A.
P=0.79
CRP (mg/L)
18
15
12
9
6
3
0
Healthy Control
MD w/o CM
MD w/ CM
B.
P<0.001
P=0.62
IL-1 beta (pg/mL)
4.2
3.6
3.0
2.4
1.8
1.2
0.6
0.0
Healthy Control
MD w/o CM
MD w/ CM
C.
P=0.001
P=0.16
IL-6 (pg/mL)
8
6
4
2
0
Healthy Control
MD w/o CM
MD w/ CM
D.
P=0.017
P=0.27
MMP-2 (ng/mL)
360
300
240
180
120
60
0
Healthy Control
MD w/o CM
MD w/ CM

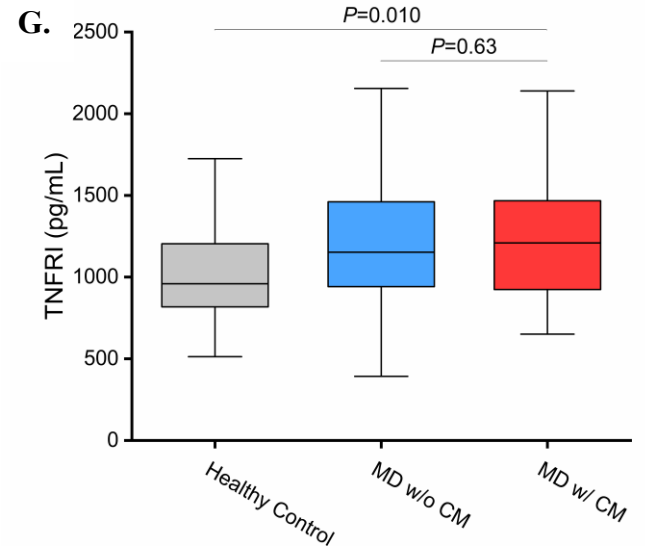
E.


P=0.49
MMP-9 (ng/mL)
90
75
60
45
30
15
0
Healthy Control
MD w/o CM
MD w/ CM

F.


P=0.011
P=0.09
TNF-alpha (pg/mL)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Healthy Control
MD w/o CM
MD w/ CM

G.


P=0.010
P=0.63
TNFRI (pg/mL)
2500
2000
1500
1000
500
0
Healthy Control
MD w/o CM
MD w/ CM

H.

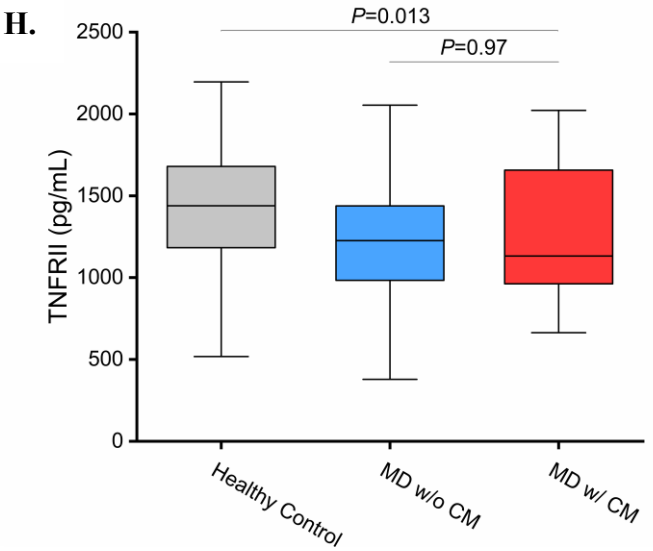

P=0.013
P=0.97
TNFRII (pg/mL)
2500
2000
1500
1000
500
0
Healthy Control
MD w/o CM
MD w/ CM

Figure 4.4. Baseline inflammatory biomarkers. High-sensitive C-reactive protein (CRP; A), interleukin 1 beta (IL-1 beta; B), interleukin 6 (IL-6; C), matrix metallopeptidase 2 (MMP-2; D), matrix metallopeptidase 9 (MMP-9; E), tumor necrosis factor alpha (TNF-alpha; F), tumor necrosis factor receptor I (TNFRI; G), and tumor necrosis factor receptor II (TNFRII; H) levels in healthy control (HC) and muscular dystrophy (MD) groups with and without cardiomyopathy (CM).

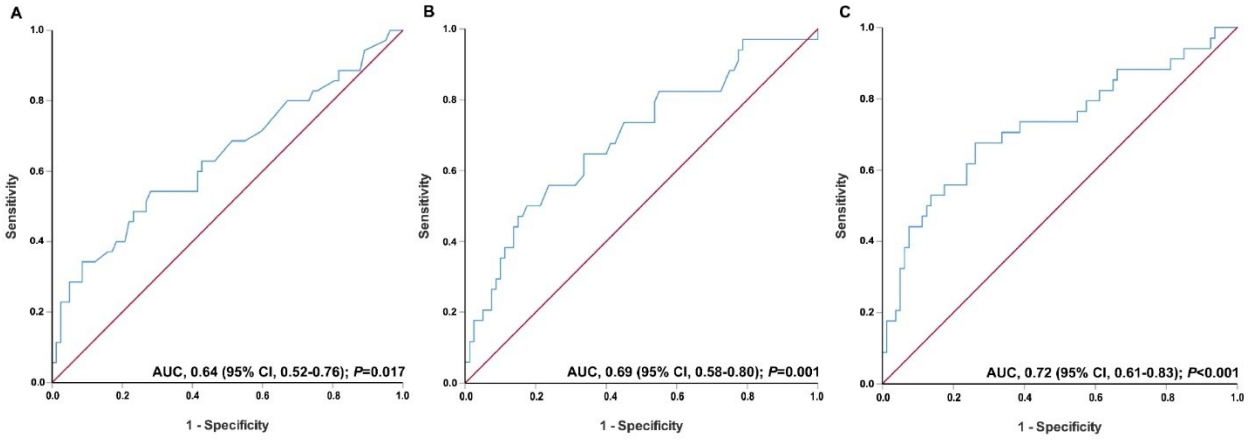


Figure 4.5. Receiver operating characteristic curve analysis of B-type natriuretic peptide (A), high-sensitive troponin I (B), and a combination of biomarkers (C) to assess diagnostic ability of cardiomyopathy. AUC, area under the curve; CI, confidence interval.

Given the diagnostic abilities of both biomarkers for cardiomyopathy, we proceeded to investigate their respective prognostic abilities for MACE. Prior to analysis, we used our ROC curves and corresponding Youden's indices to dichotomously stratify patient risk of MACE. Cutoff values were calculated as 30.5000 pg/mL for BNP (sensitivity=0.54; specificity=0.72) and 7.6050 ng/L for hsTnI (sensitivity=0.50; specificity=0.83). Of the entire MD cohort, 42 (35.9%) patients were determined to have BNP levels above the respective cutoff value and 32 (27.4%) patients were determined to have hsTnI levels above the respective cutoff value. Seventeen (14.5%) patients simultaneously had BNP and hsTnI levels above both respective cutoff values. Classification in this manner allowed for multivariate Cox regression analysis to stratify the risk of MACE (Figure 4.6). Patients with BNP levels above the cutoff were at greater risk of MACE than patients with BNP levels below (HR, 3.70; 95% CI, 1.65-8.28; *P*=0.001) (Table 4.3). Similarly, patients with hsTnI levels above the cutoff value had a greater risk of MACE than patients with hsTnI levels below (HR, 3.24; 95% CI, 1.54-6.81; *P*=0.002) (Table 4.3). Importantly, patients with both biomarker levels above cutoff values had a substantially greater risk of MACE than patients with biomarker levels below both cutoffs (HR, 4.08; 95% CI, 1.72-9.70; *P*=0.001) (Table 4.3).

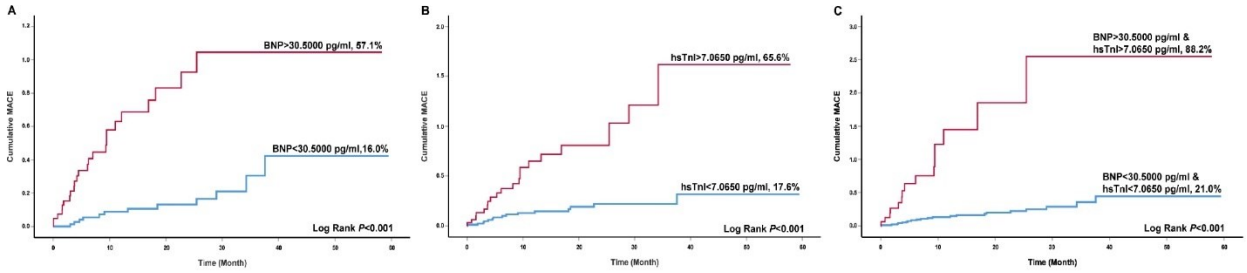


Figure 4.6. Kaplan–Meier analysis of B-type natriuretic peptide (A), high-sensitive troponin I (B), and a combination of biomarkers (C) to assess prognostic ability for major adverse cardiac events. Cut-offs were established using corresponding Youden's indices. CI, confidence interval; HR, hazard ratio; MACE, major adverse cardiac events.

Table 4.3. Cox regression analysis to assess prognostic utility. B-type natriuretic peptide (BNP), high sensitivity troponin I (hsTnI), and a combination of BNP and hsTnI were analyzed for major adverse cardiac **e**vents (n=117).

	Univariate Analysis		**Multivariate Analysis**	
Clinical Variables	**HR (95% CI)**	***P* Value**	**HR (95% CI)**	***P* Value**
B-type Natriuretic Peptide (BNP)				
BNP	4.95 (2.47-9.92)	<0.001	3.70 (1.65-8.28)	0.001
Cardiomyopathy	5.10 (2.58-10.08)	<0.001	4.47 (1.98-10.08)	<0.001
Age	1.02 (1.00-1.05)	0.041	1.01 (0.99-1.04)	0.31
Gender	1.52 (0.76-3.04)	0.24	0.58 (0.22-1.53)	0.27
Respiratory Disease	1.33 (0.65-2.70)	0.43	0.96 (0.43-2.15)	0.92
ACEI/ARB	2.16 (1.12-4.18)	0.022	1.44 (0.60-3.46)	0.41
Beta-Blocker	3.24 (1.65-6.37)	0.001	2.22 (1.01-4.88)	0.048
MRA	1.31 (0.40-4.28)	0.66	1.56 (0.42-5.77)	0.51
Respiratory Therapy	1.40 (0.73-2.71)	0.31	1.63 (0.75-3.51)	0.22
High Sensitivity Troponin I (hsTnI)				
hsTnI	4.66 (2.40-9.06)	<0.001	3.24 (1.54-6.81)	0.002
Cardiomyopathy	5.10 (2.58-10.08)	<0.001	4.23 (1.83-9.75)	0.001
Age	1.02 (1.00-1.05)	0.041	1.04 (1.01-1.06)	0.004
Gender	1.52 (0.76-3.04)	0.24	0.77 (0.29-2.08)	0.61
Respiratory Disease	1.33 (0.65-2.70)	0.43	1.00 (0.44-2.26)	0.99
ACEI/ARB	2.16 (1.12-4.18)	0.022	1.01 (0.41-2.48)	0.98
Beta-Blocker	3.24 (1.65-6.37)	0.001	2.35 (1.12-4.91)	0.024

MRA	1.31 (0.40-4.28)	0.66	1.29 (0.35-4.73)	0.70
Respiratory Therapy	1.40 (0.73-2.71)	0.31	1.34 (0.64-2.78)	0.44
Combined BNP and hsTnI				
hsTnI and BNP	7.82 (3.98-15.39)	<0.001	4.08 (1.72-9.70)	0.001
Cardiomyopathy	5.10 (2.58-10.08)	<0.001	3.66 (1.55-8.63)	0.003
Age	1.02 (1.00-1.05)	0.041	1.02 (1.00-1.05)	0.039
Gender	1.52 (0.76-3.04)	0.24	0.76 (0.29-2.00)	0.58
Respiratory Disease	1.33 (0.65-2.70)	0.43	1.20 (0.54-2.66)	0.66
ACEI/ARB	2.16 (1.12-4.18)	0.022	1.19 (0.51-2.82)	0.69
Beta-Blocker	3.24 (1.65-6.37)	0.001	1.85 (0.83-4.12)	0.13
MRA	1.31 (0.40-4.28)	0.66	1.30 (0.36-4.72)	0.69
Respiratory Therapy	1.40 (0.73-2.71)	0.31	1.76 (0.80-3.87)	0.16

CI, confidence interval; HR, hazard ratio; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; MRA, mineralocorticoid receptor antagonist.

4.5. Discussion

Our investigation of plasma biomarkers in patients with MD demonstrated the clinical utility of BNP and hsTnI as diagnostic markers of cardiomyopathy and prognostic markers of MACE in a unique cohort of patients with MD. Clinical guidelines recommend the use of BNP levels as part of a comprehensive cardiac assessment to guide heart failure management. However it must be recognized that they are not tailored to patients with rare diseases such as MD[159] and the levels of BNP detected in the majority of our patients with cardiomyopathy were markedly lower than levels seen in traditional HF cohorts.[171] Our MD patients with

cardiomyopathy, characterized by marked LV dilation and reduced systolic function had a median BNP level of 32 pg/mL, well below the guideline defined cutoff of 100 pg/mL for patients with heart failure with reduced ejection fraction (HFrEF).[172] Our findings are further supported by the observation that the median level of BNP in MD patients with overt HF was one-third of that seen in typical HFrEF patients.[171, 173] Previous clinical studies have demonstrated the diagnostic use of hsTnI for HF in clinical practice.[174-176] We were able to adapt this concept and demonstrate the diagnostic potential of hsTnI for cardiomyopathy in our rare disease cohort. The demonstrated use of these biomarkers as a complimentary tool for cardiac assessment are clinically relevant given the physical limitations associated with the use of cardiac imaging modalities in patients with MD.[156, 177] BNP and hsTnI can therefore be used prior to the development of overt HF (Stage B, AHA-ACC classification) and be used as a guide for preventative treatment.[178] The use of cardiac imaging modalities to detect early signs of cardiomyopathy is of particular importance given the rapid progression of heart disease in these patients. Myocardial strain assessment through speckle-tracking echocardiography and cardiac MRI have been shown to have prognostic value in MD patients.[179, 180]

We implemented a statistical approach to impartially dichotomize MD patients for risk stratification of MACE. Using Youden's statistical method, we determined cutoff values of BNP and hsTnI with maximized sensitivity and specificity, as the basis for patient risk stratification.[162, 163, 181, 182] Our regression analysis found that patient groups that reflected biomarker levels above the set cutoff values were at greater risk of MACE compared to patients with biomarkers level below the cutoff values. In combination, patients that had BNP and hsTnI levels above both cutoff values could be categorized as being at a higher risk of MACE, compared to cohort peers. By translating these findings to clinical practice, patients with

biomarker levels in excess of the cutoff levels could be monitored more closely; cardiac imaging would be justified in these instances. We specifically included gender as a covariate as patients diagnosed with a dystrophinopathy are exclusively males due to the X-linked recessive mode of inheritance.[23] Additionally, it was necessary to account for the demographic difference of disproportionally fewer females in the cohort with cardiomyopathy, which incidentally had a lower prevalence of comorbidities. We also included the diagnosis of respiratory disease as a covariate given its documented burden on MD patients.[183-185] At the time of patient plasma collection, a number of patients were prescribed cardiac and respiratory therapies, which potentially affected their clinical outcomes and therefore these variables were included as covariates in our regression analysis. Though previous studies have discussed the use of BNP and troponin to identify patients at risk of future adverse cardiac events[176, 186-188], no studies have formally stratified the risk of MACE in patients with MD using these biomarkers. Identifying patients at risk of MACE is particularly important in the MD patient population. Patients with dystrophinopathies, including DMD carrier patients, LGMD, and FSHD are at a high risk of arrhythmias, development of HF, cardiac-related hospitalization, and mortality due to disease progression.[5, 73, 156] Notably, patients with DM1 have a high burden of ventricular tachyarrhythmias and are at risk of sudden cardiac death.[8] The variable stage of disease in these patients upon referral reflects the real-world challenges of clinical care given their complex condition. By recognizing MD patients at risk of MACE we can appropriately implement cardiac intervention such as device implantation, namely the use of implantable cardiac defibrillators and cardiac resynchronization therapy devices,[9] supported by optimized pharmacological therapies, which we have previously demonstrated to improve cardiac and all-cause patient outcomes.[156]

We have taken a novel approach to utilizing clinically available BNP and hsTnI assays to augment the MD patient care. B-type natriuretic peptide is commonly used as a marker of cardiac wall stress, and is thus indicative of adverse cardiac remodelling, and impaired cardiac function.[159, 189] We demonstrated the clinical utility of BNP in MD patients which can be used in clinical practice to guide management prior to the progression to symptomatic HF and adverse cardiac events. We also showed the clinical utility of hsTnI in patients with MD, which revealed diagnostic and prognostic value, beyond its traditional use of identifying acute myocardial injury.[159] We were able to identify the value of elevated troponin levels in our patients through the use of high-sensitivity assays; a previous study was unable to effectively quantify differences in patient levels using conventional assays.[190] Importantly, the use of high-sensitive troponin I assays in these patients saves critical time in patient assessment thereby augmenting the clinical care process, as previously validated in emergent patients with myocardial infarction.[186, 191] The use of clinical data and genetic analysis improves the predictive ability for clinical outcomes in MD patients.[192] By including plasma biomarker analysis into this model we have created a more comprehensive assessment to further improve management and outcomes of MD patients.

Our findings demonstrate the unique pathological remodelling pathways associated with MD. Muscular dystrophies differ from other diseases given that the primary manifestations of the disease are largely evident in striated muscle. Dilated cardiomyopathy is the resulting pathophysiology in the heart, offering a clear explanation for BNP and troponin release. Our findings validate studies that previously described elevations in inflammatory biomarker levels in patients with MD.[157, 158] However, unlike other types of heart disease, which show elevated markers of inflammation,[167, 168] cardiomyopathy secondary to MD is not associated with increased systemic inflammation. We recognize the limitations in our current study. Due to the

small number of patients, we were unable to complete appropriate sub-group analyses to elucidate differences in outcomes between different types of MD. Additionally, our investigation did not involve the serial collection of patient plasma; future studies could implement the use serial biomarker data in MD patients for better prognostic utility.

In conclusion, our prospective study identified a critical role for BNP and hsTnI as diagnostic markers of cardiomyopathy and prognostic markers of MACE in this vulnerable patient cohort. These findings demonstrate the clinical utility of these biomarkers beyond a traditional heart disease cohort.[193] These cardiac-specific biomarkers clearly improve the prognostication of MACE in these patients and plasma BNP and hsTnI analysis should be integrated into the assessment and management of patients with MD.

5. Clinical Utility of 12-Lead Electrocardiogram in Evaluating Heart Disease in Patients with Muscular Dystrophy: Assessment of Left Ventricular Hypertrophy, Conduction Disease, and Cardiomyopathy

5.1. Abstract

Introduction: Heart disease remains a leading cause of mortality in patients with muscular dystrophy (MD) and cardiac assessment by standard imaging modalities is challenging due to the prominence of physical limitations.

Methods: In this prospective cohort study of 169 MD patients and 34 negative control patients, we demonstrate the clinical utility of 12-lead electrocardiogram (ECG) as an effective modality for the assessment of cardiac status in patients with MD. We assessed the utility of conventional criteria for electrocardiogram-indicated left ventricular hypertrophy (ECG-LVH) as well as ECG morphologies.

Results: Cornell voltage, Cornell voltage-duration, Sokolow-Lyon voltage, and Romhilt-Estes point score criteria demonstrated low sensitivity and minimal positive predictive value for ECG-LVH when compared with cardiac imaging. Patients with LBBB had a high probability of a cardiomyopathy (relative risk [RR], 2.75; 95% confidence interval [CI], 2.14-3.53; $p < .001$), requiring cardiac medications (RR, 1.86; 95% CI, 1.17-2.96; $p = .008$), and cardiac device intervention (RR, 12.29; 95% CI, 5.75-26.30; $p < .001$). Patients with QRS fragmentation (fQRS) had a high probability of a cardiomyopathy (RR, 1.76; 95% CI, 1.20-2.59; $p = .004$), requiring cardiac medications (RR, 1.74; 95% CI, 1.10-2.77; $p = .018$) and cardiac device intervention (RR, 4.35; 95% CI, 1.94-9.74; $p < .001$). We found that an R/S ratio > 1 in V1 and V2 is highly specific (specificity, 0.89; negative predictive value [NPV], 0.89 and specificity, 0.82; NPV, 0.89, respectively) for patients with dystrophinopathies compared to other types of MD.

Conclusion: The identification of LBBB and fQRS was linked to cardiomyopathy in patients with MD, while ECG-LVH was of limited utility. Importantly, these findings can be applied to

effectively screen a broad cohort of MD patients for structural heart disease and prompt further evaluation and therapeutic intervention.

5.2. Introduction

Heart disease remains a leading cause of mortality in patients with muscular dystrophy (MD).[4-6, 142, 156] Patient condition and management is often complicated by respiratory, neurological, and metabolic comorbidities, and clinical assessment is challenged by progressive muscle weakening and wasting, obesity, wheelchair dependence, and respiratory aids. Patients with dystrophinopathies including Duchenne muscular dystrophy (DMD) and Becker's muscular dystrophy (BMD), limb-girdle muscular dystrophy (LGMD), type 1 myotonic dystrophy (DM1), and facioscapulohumeral muscular dystrophy (FSHD) are distinguished by their burden of heart disease.[7, 8, 156, 194] 12-lead electrocardiogram (ECG) is an accessible and practical modality for cardiac assessment. Morphology such as electrocardiogram-indicated left ventricular hypertrophy (ECG-LVH) defined by specific criteria has been shown to be associated with heart disease, heart failure, and adverse clinical outcomes including mortality.[195-197] Left bundle branch block (LBBB) is a predictor of mortality and is linked to LV systolic dysfunction.[198, 199] Additionally, QRS fragmentation (fQRS), has been associated with major adverse cardiac events (MACE) including life-threatening arrhythmias and mortality in patients with heart disease,[200, 201] as well as an association with systolic dysfunction in patients with DMD.[202, 203] We identified ECG-LVH, LBBB, and fQRS as common and recurrent ECG features in our heterogenous MD patient cohort and we investigated their clinical utility for the front-line cardiac assessment of patients with MD.

5.3. Methods

Study Population

One hundred and sixty-nine patients with MD were recruited from the Neuromuscular Multidisciplinary (NMMD) clinic at the Kaye Edmonton Clinic, University of Alberta (Edmonton, Canada). All patients received a baseline 12-lead ECG study with a subsequent transthoracic echocardiogram (TTE) or cardiac magnetic resonance (CMR) imaging study within 6 months. Thirty-four age- and gender-matched patients with non-MD myopathies were recruited to serve as a negative control cohort for heart disease, as previously described.[156] Patients were prospectively tracked over a median follow-up period of 1.88 (interquartile range [IQR], 1.21-2.25) years between November 5, 2014, and November 9, 2020. Our cohort included patients with a dystrophinopathy (26 DMD and 10 BMD patients), LGMD (36 patients), DM1 (74 patients), and FSHD (23 patients), as confirmed by genetic testing. All clinical data including use of medical therapy and device intervention was obtained by electronic chart review. Patients were referred to the NMMD clinic and recruited to our study at various stages of their disease and all patients provided informed and written consent at study enrollment. The investigation was approved by the Health Research Ethics Board at the University of Alberta.

12-Lead Electrocardiogram

All patients were assessed using a Philips PageWriter TC70 Cardiograph (Philips Healthcare, Amsterdam, Netherlands) ECG system as part of routine clinical care. Patients with mild and moderate ambulatory status were assessed in a supine position, while wheelchair-bound patients remained sitting. All ECGs were interpreted by the attending cardiologist as part of

patient clinical care and followed by a blinded analysis of ECG morphology and intervals using Cardio Calipers version 3.3 digital caliper software (Iconico Inc., New York, USA). Standard interval measurements were captured and corrected QT intervals were acquired using Bazett[204], Fridericia[205], Framingham[206], and Hodges[207] formulae. We also applied the recently proposed corrective QT interval formula by Tang & Rabkin to patients with LBBB.[208] On the basis of unique R-wave progression documented in patients with dystrophinopathies, namely DMD,[209] we assessed the performance of R-wave patterns such as R wave amplitude in V1 > V2, R/S in V1 > 1.00 and > 1.50, and R/S in V2 > 1.00 and 1.50, for their ability to differentiate patients diagnosed with a dystrophinopathy from the broader cohort. Electrocardiogram axis and segmental variants such as J-point elevation were noted and atrioventricular block (AVB), left anterior fascicular block (LAFB), left posterior fascicular block (LPFB), LBBB, right bundle branch block (RBBB), and nonspecific intraventicular conduction delay (IVCD) was also captured.[159, 210] All incidences of atrial and ventricular tachyarrhythmias were documented.

We defined ECG-LVH using the Cornell voltage criteria (CV), Cornell voltage-duration product criteria (CP), Sokolow-Lyon voltage criteria (SL), and the Romhilt-Estes point score system (RE), which are conventional criteria used in clinical practice (Table 5.1). We defined LBBB as a QRS duration > 120 ms, accompanied by an absence of Q waves in the lateral leads, slurred R waves in leads I and aVL, and RSR' pattern in V5 and V6 with R peak time greater than 60 ms.[210] We defined fQRS as a RSR' pattern in 2 contiguous anterior, lateral, or inferior leads; or notches in the nadir of R or S waves in 2 contiguous leads.[202] For patients with bundle branch block, fQRS was defined as RSR' patterns in more than 2 contiguous leads or notches in the nadir of R or S waves in more than 2 contiguous leads.[200] Electrocardiogram-indicated left ventricular hypertrophy was compared to anatomical measures of left ventricular (LV) mass

obtained from subsequent TTE or CMR, from which LA volume was also obtained, and both were evaluated relative to guideline-defined reference ranges.[133, 211] Cardiomyopathy was defined as left ventricular ejection fraction (LVEF) < 55% or a left ventricular end-diastolic volume index (LVEDVi) > 105 mL/m²,[133] and LVEF could be obtained from either TTE or CMR given the previously demonstrated concordance.[156]

Table 5.1. ECG Criteria used to define electrocardiogram-indicated left ventricular hypertrophy (LVH).

Criteria	**Parameters**
Cornell Voltage	RaVL + SV3 > 28 mm (men); > 20 mm (women)[212]
Cornell Voltage-Duration Product	(RaVL + SV3) × QRS duration > 2440 mm x ms (men); (RaVL + SV3 + 80 mm) × QRS duration > 2440 mm x ms (women)[213, 214]
Sokolow-Lyon	SV1 + RV5 or RV6 ≥ 35 mm[215]
Romhilt-Estes Point Score	A. R or S wave in limb leads ≥ 20 mm or SV1 or V2 ≥ 30 mm or RV5 or V6 ≥ 30 mm (3 points), B. ST-T segment pattern without digitalis (3 points) or with digitalis (1 point), C. Left atrial involvement (3 points),

	D. Left axis deviation more than -30° (2 points), E. QRS duration ≥ 90 ms (1 point), F. Intrinsicoid deflection ≥ 50 sec in V5 or V6 (1 point); A + B + C + D + E + F > 4 points[216]

Serial tracking of ECG parameters occurred from baseline (n=203), follow-up 1 (n=153; median follow-up of 1.08 [IQR, 0.86-1.51] years from baseline), and follow-up 2 (n=89; median follow up of 1.02 [IQR, 0.85-1.22] years from follow-up 1). The trailing number of patients at each follow-up period reflected ongoing patient enrollment and the prospective nature of the study. Serial data tracking facilitated the analysis of ECG parameter changes over time among MD patients with cardiomyopathy, MD patients without cardiomyopathy, and patients with non-MD myopathies.

Statistical Methods

Continuous variables were compared using a Mann-Whitney U test or Kruskal-Wallis test, and all categorical data were compared using Pearson's Chi-square tests. Criteria used to qualify ECG-LVH were assessed relative to corresponding cardiac imaging studies and compared using performance metrics such as sensitivity and specificity to evaluate criteria accuracy, as well as positive predictive value (PPV) and negative predictive value (NPV) in consideration of the prevalence of left ventricular hypertrophy (LVH). A relative risk (RR) assessment with a 95% confidence interval (CI), was used to compare the probabilities of cardiac outcomes between groups defined by the presence or absence of LBBB and fQRS. Multivariate fixed effect models (adjusted for age, gender, cardiac medication use, and cardiac device intervention, with consideration for variable follow-up periods) were used to compare serial

changes in ECG parameters among defined groups. All statistical analyses were performed in R version 4.0.3 and a p-value < 0.05 was considered significant.

5.4. Results

Clinical Characteristics of Cohorts

The median age of our composite MD cohort was 36 (interquartile range [IQR], 23.5-49.5) years, which included 64 (37.9%) females (Figure 5.1A). The dystrophinopathies cohort was comprised of patients that were exclusively male and notably young (Table 5.2). These patients exhibited profound skeletal muscle weakness and wasting as well as a high prevalence of respiratory disease and sleep disordered breathing (SDOB) (Table 5.2). The LGMD and DM1 cohorts were evenly comprised of males and females with a high prevalence of comorbidities such as diabetes, dyslipidemia, hypertension, and respiratory disease, as seen in patients with FSHD (Table 5.2). Our non-MD myopathies cohort had a median age of 47 (IQR, 29.0-60.0), which included 16 (47.1%) females, with a notable prevalence of comorbidities comparable to those documented in the MD cohorts (Table 5.2). Similarly, respiratory disease was diagnosed in 18 (52.9%) patients and SDOB was diagnosed in 7 (20.6%) patients.

Table 5.2. Baseline characteristics.

Characteristic	Dystrophinopathies (n=36)	LGMD (n=36)	DM1 (n=74)	FSHD (n=23)	Non-MD Myopathies (n=34)	*p*-value[a]	*p*-value[b]
Males/Females, No.	36 (100.0)	18 (50.0)/18 (50.0)	37 (50.0)/37 (50.0)	14 (60.9)/9 (39.1)	18 (52.9)/16 (47.1)	< .001	.32
Median Age, Yrs	22.0 (18.0-28.8)	39.0 (23.0-56.3)	42.0 (33.0-50.0)	45.0 (25.8-53.5)	47.0 (29.0-60.0)	< .001	.08
Current/Former Smoker, No.	1 (2.78)	5 (13.9)	16 (21.6)	3 (13.0)	4 (11.8)	.12	.65
Comorbidities, No.							
Diabetes	0	8 (22.2)	6 (8.11)	3 (13.0)	5 (14.7)	.03	.43
Dyslipidemia	1 (2.78)	4 (11.1)	10 (13.5)	4 (17.4)	8 (23.5)	.13	.05
Hypertension	2 (5.56)	7 (19.4)	4 (5.41)	6 (26.1)	7 (20.6)	.016	.14
Respiratory Disease	26 (72.2)	7 (19.4)	40 (54.1)	6 (26.1)	18 (52.9)	< .001	.51
SDOB	14 (38.9)	4 (11.1)	22 (29.7)	7 (30.4)	7 (20.6)	.08	.38
Anemia	3 (8.33)	2 (5.56)	0	0	0	.04	--
Vitals, median							
HR, bpm	82.0 (75.0-100.0)	72.0 (70.0-82.0)	70.0 (64.0-80.0)	78.0 (66.5-80.0)	75.0 (70.0-80.0)	.021	.84
sBP, mmHg	107.5 (100.8-121.8)	125.0 (114.0-137.0)	114.0 (106.0-122.5)	132.0 (121.0-139.5)	126.0 (120.0-137.0)	< .001	.002
dBP, mmHg	71.0 (64.3-78.0)	80.0 (71.0-87.0)	74.0 (67.5-78.0)	84.0 (77.0-88.0)	76.0 (72.0-84.0)	< .001	.18
Serum Chemistry, median							
BNP, pg/mL	20.0 (9.50-68.0)	24.0 (11.0-45.0)	22.0 (14.0-35.0)	16.5 (10.0-22.5)	19.0 (13.0-27.0)	.71	.98
CK, U/L	1277.0 (377.8-2380.8)	708.5 (322.0-2353.8)	237.0 (144.8-308.3)	174.5 (146.0-452.3)	195.0 (45.8-408.3)	< .001	.005
Creatinine, µmol/L	20.0 (15.8-31.0)	41.0 (29.0-59.0)	57.0 (50.8-69.5)	48.0 (39.0-70.0)	55.5 (29.5-66.0)	< .001	.73
Potassium, mmol/L	4.30 (4.05-4.50)	4.10 (3.80-4.35)	4.30 (3.90-4.50)	4.30 (4.00-4.60)	4.10 (3.90-4.30)	.57	.23

Note: Values are presented as median (interquartile range) or n (%). [a]Indicates statistical analysis comparing all patients. [b]Indicates statistical analysis comparing the cohorts of patients with muscular dystrophy (MD) and non-MD myopathies. BNP, B-Type natriuretic peptide; CK, creatine kinase; dBP, diastolic blood pressure; DM1, type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; HR, heart rate; LGMD, limb-girdle muscular dystrophy; sBP, systolic blood pressure; SDOB, sleep disordered breathing.

Burden of Cardiomyopathy and Arrhythmias

The broader MD cohort had a high prevalence of cardiomyopathy, which was diagnosed in 68 (40.2%) of the MD patients (Figure 5.1B). Patients with dystrophinopathies showed LV and right ventricular (RV) dilation, elevated LV mass, and a marked reduction in biventricular systolic function (Table 5.3), 28 (77.8%) patients were diagnosed with cardiomyopathy. Twelve out of 22 (54.5%) patients that received CMR had evidence of myocardial fibrosis. Patients with LGMD exhibited a comparable prevalence of structural heart disease as well as a reduction in LVEF (Table 5.3), and 13 (36.1%) patients were diagnosed with a cardiomyopathy. Seven out of 22 (31.8%) patients that received CMR had evidence of myocardial fibrosis. Patients with DM1 exhibited normal cardiac structure and reduced median LVEF (Table 5.3), and cardiomyopathy was diagnosed in 22 (29.7%) patients. One out of 22 (4.55%) patients that received CMR had evidence of myocardial fibrosis. Patients with FSHD showed normal LV size and biventricular systolic function, though RV diastolic volumes were elevated (Table 5.3), and accordingly 5 (14.7%) patients were diagnosed with cardiomyopathy. One out of 14 (7.14%) patients that received CMR had evidence of myocardial fibrosis.

Table 5.3. Baseline cardiac assessment.

Modality	Dystrophinopathies	LGMD	DM1	FSHD	Non-MD Myopathies	*p*-value[a]	*p*-value[b]
12-Lead ECG	**(n=36)**	**(n=36)**	**(n=74)**	**(n=23)**	**(n=34)**		
Heart Rate, bpm	79.5 (71.0-97.3)	70.5 (60.0-82.0)	69.0 (62.0-80.0)	70.0 (59.5-83.5)	74.0 (67.3-80.8)	.025	.68
RR Interval, ms	790.0 (647.0-895.0)	910.5 (750.0-1025.5)	889.0 (763.0-1024.0)	856.0 (716.0-1004.0)	826.5 (734.3-896.0)	.07	.32
PR Interval, ms	132.0 (120.0-137.0)	148.0 (133.0-160.0)	190.0 (172.0-221.0)	164.0 (144.5-172.5)	155.0 (140.0-180.0)	< .001	.73
QRS Duration, ms	95.5 (89.5-109.3)	97.0 (90.5-109.5)	107.5 (96.3-121.5)	92.0 (86.0-96.0)	95.5 (86.0-108.0)	< .001	.18
QT Interval, ms	378.5 (360.5-396.0)	394.0 (372.5-426.0)	411.5 (390.3-436.0)	378.0 (357.5-407.0)	388.0 (380.0-419.0)	< .001	.80
Corrected QT Interval (Bazett), ms	430.9 (410.9-451.4)	426.6 (401.5-451.6)	438.0 (417.1-453.0)	424.0 (408.5-433.3)	437.7 (419.8-453.2)	.17	.40
Corrected QT Interval (Framingham), ms	378.5 (360.5-395.9)	394.0 (372.5-426.0)	411.5 (390.3-436.0)	378.0 (357.6-407.0)	388.0 (380.0-419.0)	< .001	.79

Corrected QT Interval (Fridericia), ms	412.1 (388.9-428.2)	409.6 (399.6-437.8)	429.4 (410.6-442.0)	409.6 (395.2-422.6)	424.4 (405.7-435.8)	.007	.54
Corrected QT Interval (Hodges), ms	415.0 (401.7-441.7)	415.9 (402.9-434.6)	429.1 (414.3-444.7)	407.8 (393.9-421.9)	420.9 (402.9-436.7)	.001	.88
QRS Axis	64.0 (11.3-100.3)	46.0 (18.0-82.8)	13.0 (-28.0-50.0)	48.0 (16.0-67.5)	32.0 (-4.75-65.3)	< .001	.84
T wave Axis	47.0 (15.0-75.0)	47.5 (29.3-70.5)	51.0 (37.0-61.3)	49.0 (26.8-63.8)	34.0 (25.5-50.8)	.12	.012
QRS Fragmentation	6 (16.7)	2 (5.56)	11 (23.4)	1 (4.35)	0	.06	--
J-Point Elevation	6 (16.7)	6 (16.7)	7 (9.46)	2 (8.70)	3 (8.82)	.64	.55
1° AVB	0	2 (5.56)	19 (25.7)	0	2 (5.88)	< .001	.27
LAFB	2 (5.56)	0	7 (9.46)	2 (8.70)	1 (2.94)	.31	.42
LPFB	0	0	0	1 (4.35)	0	--	--
LBBB	2 (5.56)	0	15 (20.3)	0	0	< .001	--
RBBB	1 (2.78)	0	1 (1.35)	0	1 (2.94)	.77	.44
IVCD	4 (11.1)	3 (8.33)	7 (9.46)	2 (8.70)	3 (8.82)	.99	.91
Echocardiogram	**(n=31)**	**(n=29)**	**(n=71)**	**(n=20)**	**(n=28)**		

LA Vol Index, mL/m^2	22.4 (15.5-34.1)	20.8 (14.7-25.1)	18.6 (14.9-23.9)	19.1 (14.4-21.6)	17.6 (15.1-27.9)	.78	.78
LVIDd, cm	4.64 (4.14-5.41)	4.77 (4.41-5.20)	4.40 (4.06-4.85)	4.40 (4.00-4.80)	4.52 (3.80-5.00)	.06	.96
LVIDs, cm	3.49 (2.87-4.42)	3.36 (2.84-3.95)	2.80 (2.60-3.10)	2.83 (2.50-3.05)	2.80 (2.61-3.30)	< .001	.28
LVPWd, cm	0.72 (0.66-0.76)	0.87 (0.73-0.98)	0.81 (0.73-1.00)	0.80 (0.73-0.88)	0.82 (0.77-0.97)	.014	.32
LVEF, %	40.0 (25.0-53.9)	55.0 (54.4-60.0)	55.0 (55.0-60.0)	60.0 (55.0-60.0)	55.0 (55.0-60.0)	< .001	.71
LVMI, g/m^2	80.4 (63.0-93.3)	74.9 (63.3-84.0)	65.3 (54.9-80.5)	67.3 (57.5-76.2)	67.4 (58.7-77.3)	.11	.64
Cardiac MRI	**(n=18)**	**(n=22)**	**(n=22)**	**(n=13)**	**(n=14)**		
LA Vol Index, mL/m^2	38.0 (33.0-43.0)	33.4 (28.2-38.2)	29.0 (24.8-33.2)	31.6 (28.0-34.4)	39.8 (31.2-50.4)	.13	.06
LVEDVi, mL/m^2	94.0 (82.3-110.8)	77.5 (65.3-96.8)	62.5 (55.5-72.8)	74.0 (56.0-85.0)	77.0 (64.5-83.3)	< .001	.88

LVESVI, mL/m^2	50.0 (36.0-63.0)	35.0 (26.0-46.0)	29.0 (21.0-37.0)	26.5 (21.3-43.5)	27.0 (22.5-37.3)	.005	.15
LVEF, %	45.0 (39.3-56.5)	55.0 (47.5-58.0)	56.5 (50.3-62.8)	58.0 (53.0-68.0)	61.5 (57.0-65.0)	.004	.012
LVMI, g/m^2	59.0 (45.0-67.0)	48.0 (40.0-60.0)	42.0 (38.0-49.0)	42.0 (37.0-50.0)	52.5 (44.3-61.5)	.035	.27
RVEDVi, mL/m^2	78.0 (68.0-93.0)	68.0 (62.0-79.0)	61.0 (56.5-75.0)	79.5 (57.3-92.5)	74.5 (69.0-80.0)	.10	.53
RVESVi, mL/m^2	38.5 (35.3-47.8)	33.0 (28.0-42.0)	31.0 (27.0-35.0)	39.0 (26.0-45.0)	34.0 (29.5-39.3)	.19	.69
RVEF, %	49.0 (44.0-54.0)	52.0 (49.0-54.0)	51.0 (47.0-55.0)	53.0 (51.3-55.5)	56.5 (49.0-58.5)	.14	.031

Note: Values are presented as median (interquartile range) or n (%). [a]Indicates statistical analysis comparing all patients. [b]Indicates statistical analysis comparing the cohorts of patients with muscular dystrophy (MD) and non-MD myopathies. 1° AVB, first-degree atrioventricular block; DM1, type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; IVCD, intraventricular conduction delay; LA Vol Index, left atrial volume index; LAFB, left anterior fascicular block; LBBB, left bundle branch block; LGMD, limb-girdle muscular dystrophy; LPFB, left posterior fascicular block; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVESVi, left ventricular end-systolic volume index; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; LVMI, left ventricular mass index; LVPWd, left ventricular posterior wall thickness at end-diastole; RBBB, right bundle branch block; RVEDVi, right ventricular end-diastolic volume index; RVEF, right ventricular ejection fraction; RVESVi, right ventricular end-systolic volume index.

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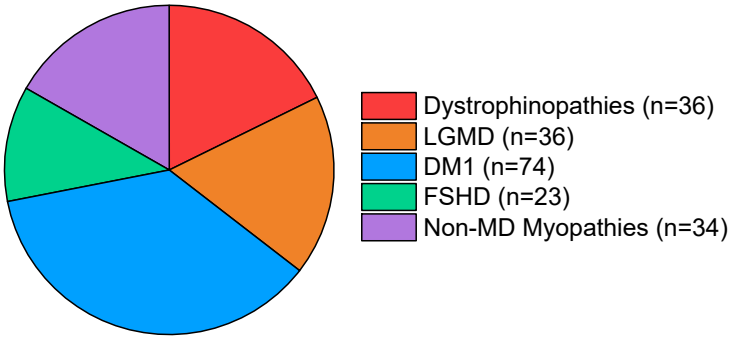
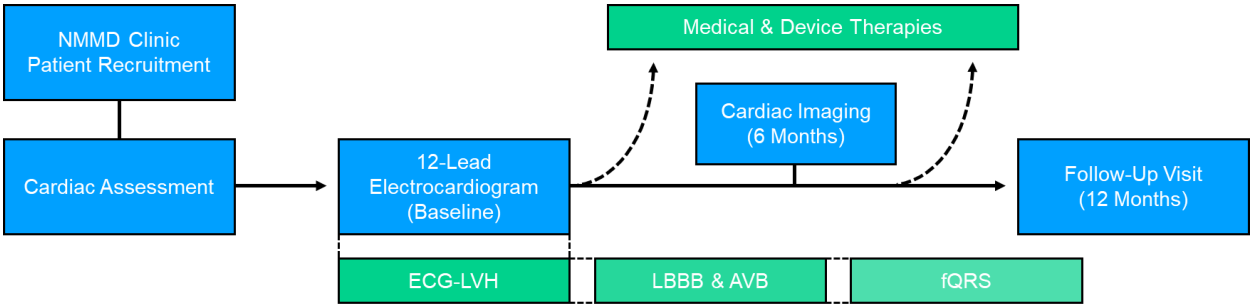


Figure 5.1. Study cohort and management. (A) Distribution of muscular dystrophy cohort patient diagnoses as confirmed by genetic testing. DM1, type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; LGMD, limb-girdle muscular dystrophy.



(B) Timeline of cardiac assessment under the neuromuscular multidisciplinary clinic care pathway. AVB, atrioventricular block; ECG-LVH, electrocardiogram-indicated left ventricular hypertrophy; fQRS, QRS fragmentation; LBBB, left bundle branch block; NMMD, neuromuscular multidisciplinary.

Arrhythmias were documented in patients with DMD including atrial flutter in 1 patient and ventricular tachycardia (VT) in 5 patients. Four patients with LGMD had atrial fibrillation or flutter and VT was reported in 2 patients. Patients with DM1 had a high incidence of arrhythmias as atrial fibrillation or flutter was reported in 16 patients and VT reported in 8 patients. There were no arrhythmias reported in patients with FSHD. Seven patients from our MD cohort received an implantable cardiac defibrillator and 19 patients received pacemaker therapies, including 13 patients receiving cardiac resynchronization therapy (CRT) devices for LBBB.

Differences in 12-Lead Electrocardiogram Features

Patients with dystrophinopathies showed parameters within normal limits though PR intervals were relatively shortened at 132.0 (IQR, 120.0-137.0) ms (Table 5.3). J-point elevation was a common finding and there was a notable prevalence of fQRS in 6 (16.7%) patients (Figure 5.2) and ventricular conduction delays including LBBB in 2 (5.56%) patients (Table 5.3, Figure 5.3). Patients with LGMD exhibited similar ECG findings, though there was a markedly low prevalence of conduction delays in these patients (Table 5.3). Patients with FSHD had parameters within normal limits with a low prevalence of conduction delays (Table 5.3).

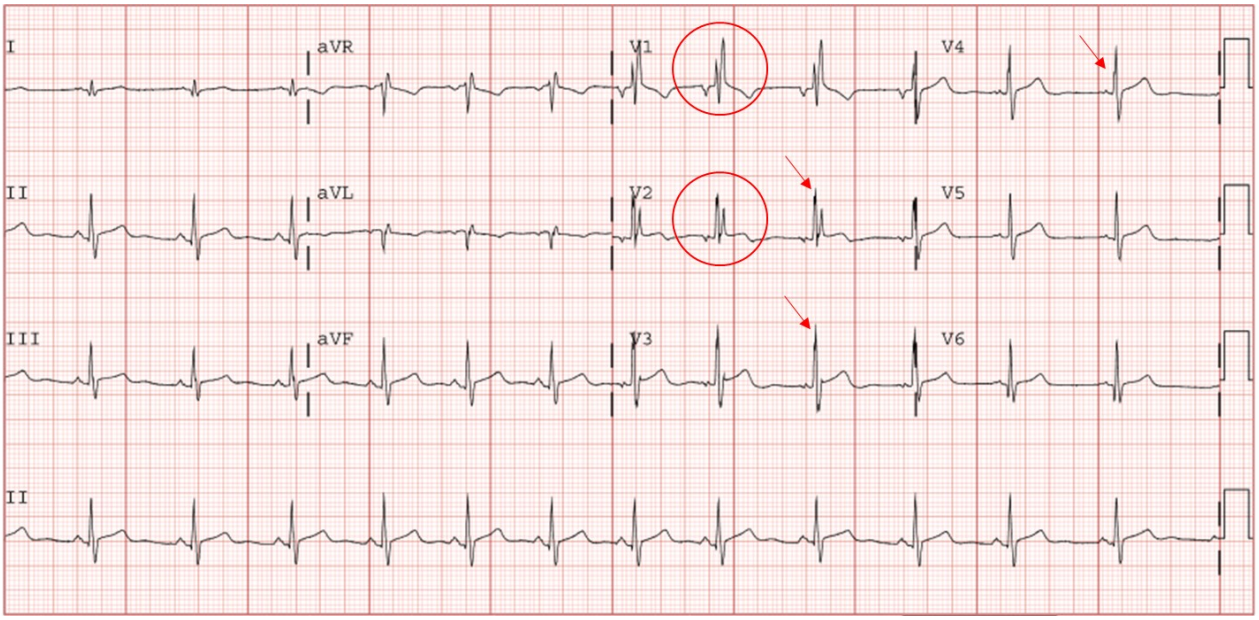


Figure 5.2. 12-lead electrocardiogram of a 32-year-old male patients with Duchenne muscular dystrophy presenting with sinus rhythm, normal intervals, and QRS fragmentation in the anterior leads. Electrocardiogram indicates a heart rate of 80 bpm, PR interval of 120ms, and QRS duration of 96ms. Circles indicate RSR' pattern shown in two contiguous anterior leads and arrows indicate notches in the nadir of R waves in three contiguous anterior leads, concordant with our definition of QRS fragmentation.

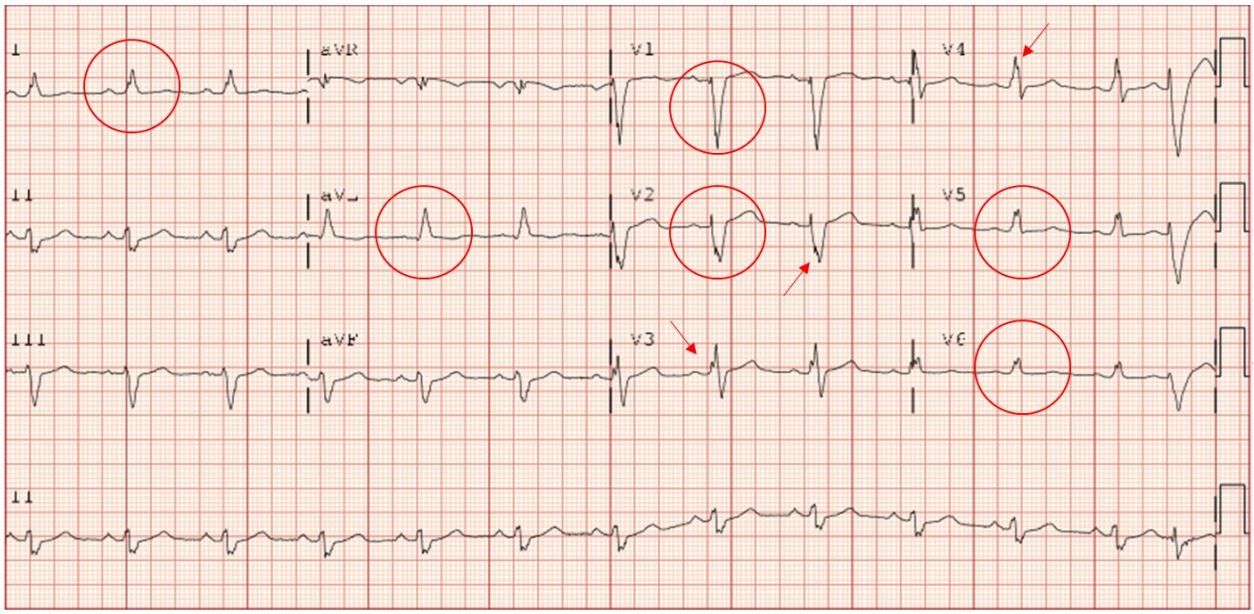


Figure 5.3. 12-lead electrocardiogram of a 25-year-old male with Duchenne muscular dystrophy presenting with sinus rhythm, left bundle branch block, and QRS fragmentation in the anterior leads. Electrocardiogram indicates a heart rate of 74 bpm, PR interval of 168ms, and QRS duration of 140ms. Circles indicate slurred R waves in leads I and aVL, and RSR' patterns in V5 and V6 with peak time greater than 60ms, concordant with our definition of left bundle branch block. Arrows indicate notches in the nadir of S and R waves in three contiguous anterior leads, concordant with our definition of QRS fragmentation in the presence of a bundle branch block.

Patients with DM1 were distinguished by their abnormal ECG studies, which showed prolonged PR intervals at 190.0 (IQR, 172.0-221.0) ms, QRS duration at 107.5 (IQR, 96.3-121.5) ms, as well as a QRS axis at 13.0 (IQR, -28.0-50.0) degrees (Table 5.3). Standard QT intervals and corrected QT intervals, not including the Bazett-formula corrected QT interval, were prolonged (Table 5.3). QRS fragmentation was visualized in 11 (23.4%) patients (Figure 5.4) and conduction delays were prevalent as first-degree AVB in 19 (25.7%) patients, LAFB in 7 (9.46%) patients, LBBB in 15 (20.3%) patients (Figure 5.5), and nonspecific IVCD in 7 (9.46%) patients. The non-MD myopathy cohort had no evidence of structural heart disease and showed normal ECG studies (Table 5.3), making them an appropriate age and gender-matched (p = .08 and p = .32, respectively) negative control cohort. Tracked serial ECG data found no statistical difference in the change of parameters among MD patients with cardiomyopathy, MD patients without cardiomyopathy, and patients with non-MD myopathies (Figure 5.6).

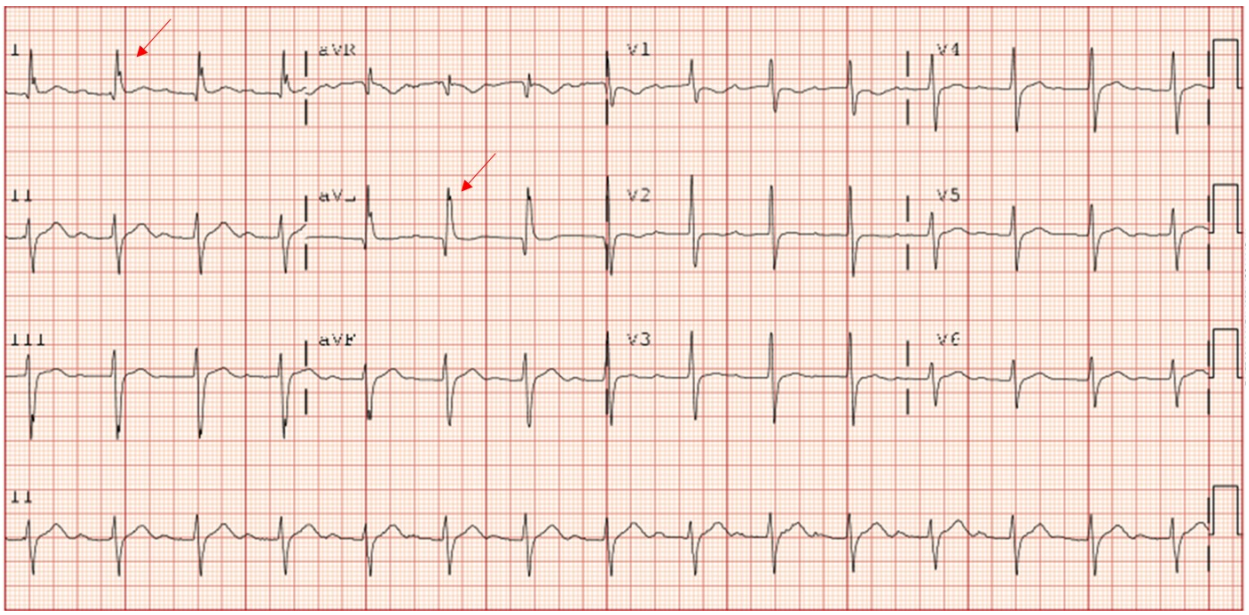


Figure 5.4. 12-lead electrocardiogram of a 46-year-old male with type 1 myotonic dystrophy presenting with sinus rhythm, first-degree atrioventricular block, and QRS fragmentation in the lateral leads. Electrocardiogram indicates a heart rate of 88 bpm, PR interval of 294 ms, and QRS duration of 109 ms. Arrows indicate notches in the nadir of R waves in two contiguous lateral leads, concordant with our definition of QRS fragmentation.

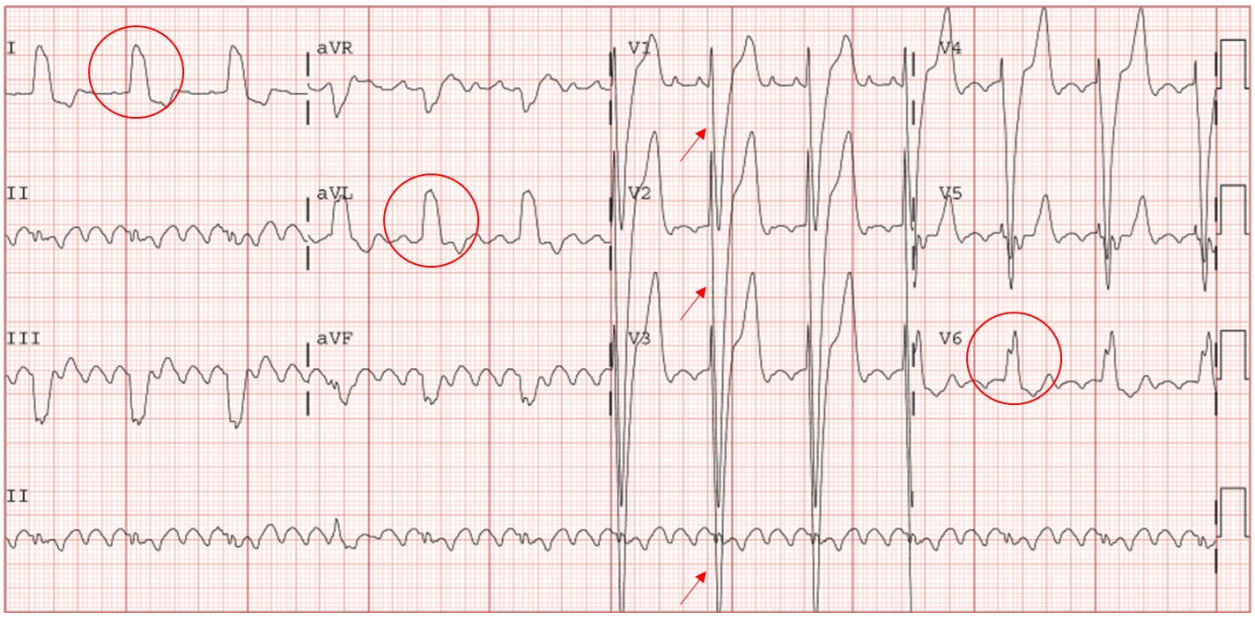
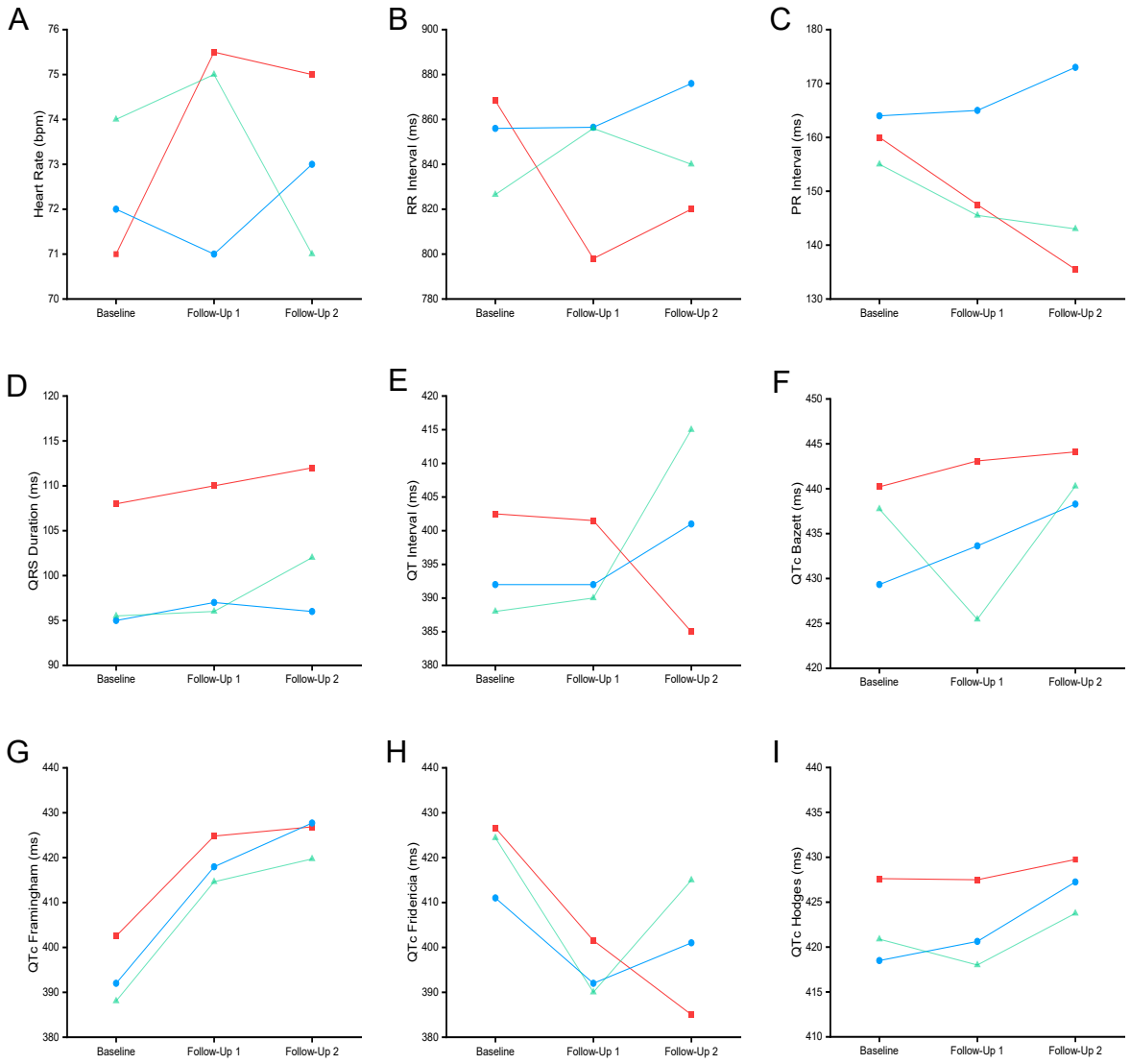


Figure 5.5. 12-lead electrocardiogram of a 45-year-old male with type 1 myotonic dystrophy presenting with atrial flutter with 3-to-1 conduction and left bundle branch block. Electrocardiogram indicates a ventricular rate of 75 bpm. Circles indicate slurred R waves in leads I and aVL, and RSR' patterns in V5 and V6 with peak time greater than 60ms, concordant with our definition of left bundle branch block (LBBB), and arrows indicate deep S waves in V1-V3 as a common identifier of LBBB.



		Baseline	Follow-Up 1	Follow-Up 2
MD With Cardiomyopathy	Patients (n)	68	54	37
	Median Follow-Up (Days)	-	382	364
MD Without Cardiomyopathy	Patients (n)	101	72	39
	Median Follow-Up (Days)	-	419	366
Non-MD Myopathies	Patients (n)	34	27	13
	Median Follow-Up (Days)	-	354	441

Figure 5.6. Comparison of serial median 12-lead electrocardiogram parameter changes among muscular dystrophy (MD) patients with cardiomyopathy, MD patients without cardiomyopathy, and patients with non-MD myopathies.

Assessment of Electrocardiogram Morphologies

In our study cohort of 203 patients, there were 28 (13.8%) indications of ECG-LVH by CV (Figure 5.7A) and 61 (30.0%) indications by CP (Figure 5.7B), where all indications by CV were found in the presence of CP. Additionally, there were 23 (11.3%) indications by SL (Figure 5.7C), and 21 (10.3%) indications by RE (Figure 5.7D). Anatomical LVH was only indicated in 15 (7.39%) patients by cardiac imaging (Figure 5.8A), and therefore all criteria demonstrated low sensitivity, while demonstrating high specificity (Figure 5.8B). Furthermore, all criteria demonstrated markedly low PPV, while demonstrating high NPV (Figure 5.8C).

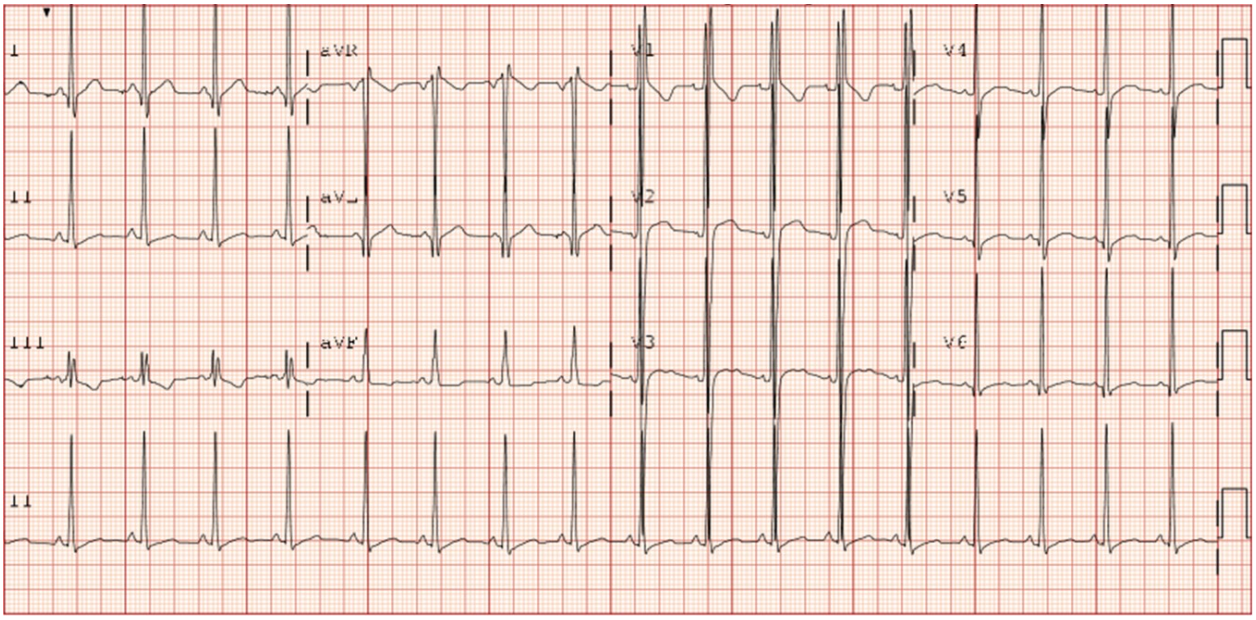
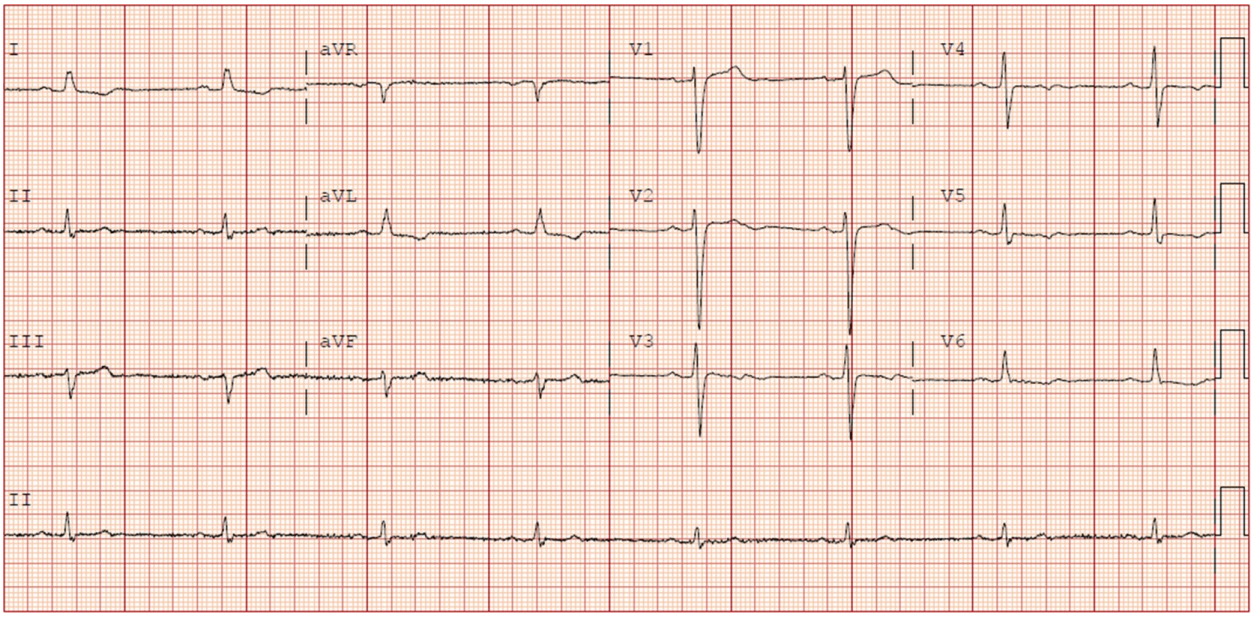
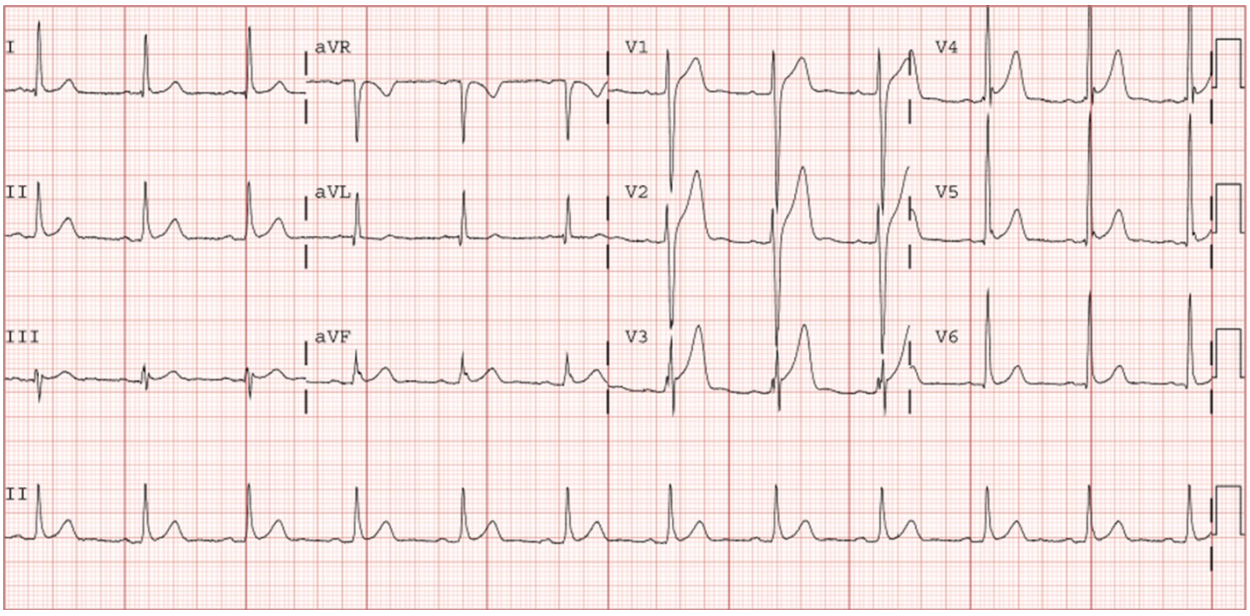


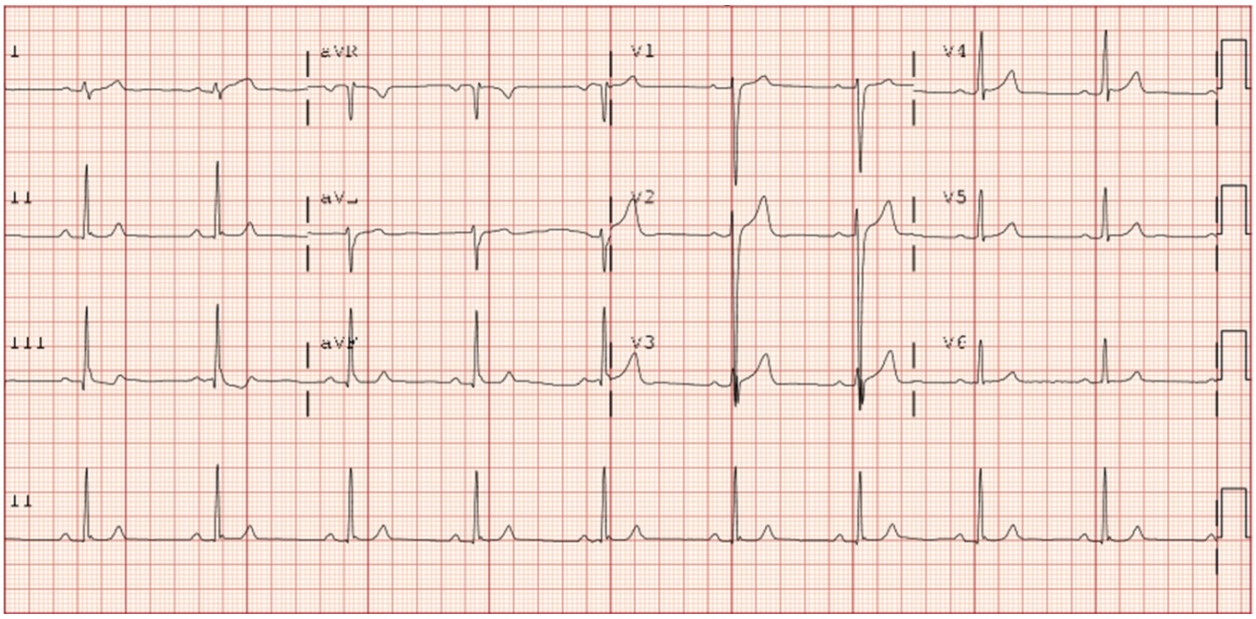
Figure 5.7. (A) 12-lead electrocardiogram of an 18-year-old male with Duchenne muscular dystrophy presenting with sinus tachycardia and left ventricular hypertrophy indicated by Cornell voltage and Cornell voltage-duration product criteria. Electrocardiogram indicates a heart rate of 106 bpm, PR interval of 106 ms, and QRS duration of 94 ms. Left ventricular mass index was 63 g/m^2 by transthoracic echocardiogram.



(B) 12-lead electrocardiogram of a 79-year-old female with limb-girdle muscular dystrophy presenting with sinus rhythm, a first-degree atrioventricular block and left ventricular hypertrophy indicated by Cornell voltage-duration product criteria. Electrocardiogram indicates a heart rate of 47 bpm, PR interval of 217 ms, and QRS duration of 126 ms. Left ventricular mass index was 48 g/m^2 by cardiac magnetic resonance.



(C) 12-lead electrocardiogram of a 30-year-old male with type 1 myotonic dystrophy presenting with sinus rhythm and left ventricular hypertrophy indicated by Sokolow-Lyon criteria. Electrocardiogram indicates a heart rate of 69 bpm, PR interval of 184 ms, and QRS duration of 108 ms. Left ventricular mass index was 62 g/m^2 by cardiac magnetic resonance.



(D) 12-lead electrocardiogram of a 24-year-old male with facioscapulohumeral muscular dystrophy presenting with sinus rhythm and left ventricular hypertrophy indicated by Romhilt-Estes point score criteria. Electrocardiogram indicates a heart rate of 57 bpm, PR interval of 173 ms, and QRS duration of 103 ms. Left ventricular mass index was 44 g/m^2 by cardiac magnetic resonance.

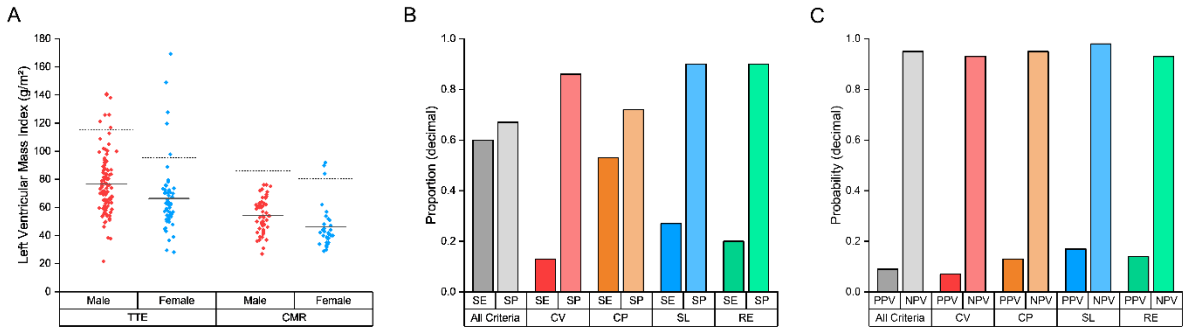


Figure 5.8. Indexed left ventricular mass of patients by sex and imaging modality with corresponding cutoffs for left ventricular hypertrophy (a), and comparison of conventional criteria for 12-lead electrocardiogram-indicated LVH using sensitivity (SE) and specificity (SP) (b), as well as positive predictive value (PPV) and negative predictive value (NPV) (c) in patients with muscular dystrophy. CMR, cardiac magnetic resonance; CP, Cornell voltage-duration product criteria; CV, Cornell voltage criteria; RE, Romhilt-Estes point score system; SL, Sokolow–Lyon voltage criteria; TTE, transthoracic echocardiogram

Left bundle branch block was indicated in the 15 (20.3%) DM1 patients and 2 (5.56%) dystrophinopathies patients. In comparing the Bazett formula versus the Tang and Rabkin formula for QT interval correction, we found intervals of 475.3 (IQR, 438.2-504.1) ms versus 386.0 (IQR, 370.9-430.4) ms ($p < .001$), respectively, in patients with LBBB. Patients with LBBB had a markedly lower LVEF than patients without LBBB (39.6 [IQR, 35.0-46.6] % versus 55.0 [IQR, 50.0-60.0] %, respectively; $p < .001$; Figure 5.9A) and were more likely to have a

cardiomyopathy (RR, 2.75 [95% CI, 2.14-3.53]; $p < .001$). Patients with LBBB were also more likely to require cardiac medical therapies (RR, 1.86 [95% CI, 1.17-2.96]; $p = .008$) and cardiac device intervention (RR, 12.29 [95% CI, 5.75-26.30]; $p < .001$) than patients without LBBB. There was no discernible difference between patients with or without LBBB and the presence of fibrosis or the incidence of arrhythmias.

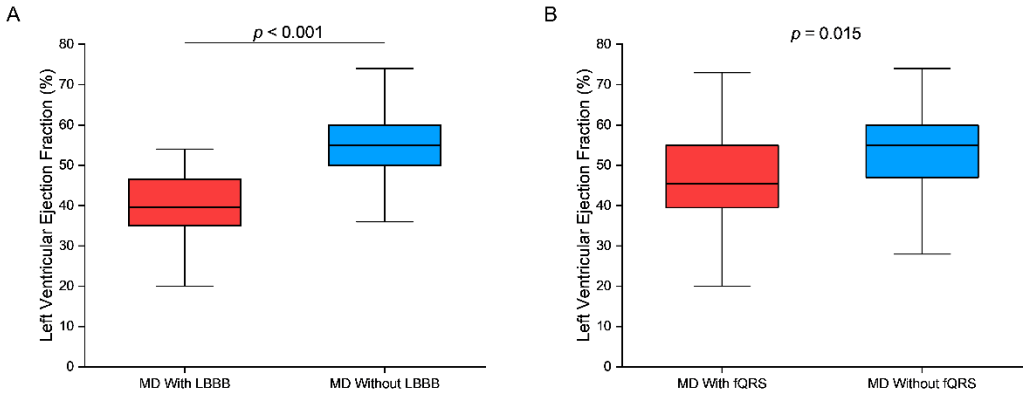


Figure 5.9. Comparison of left ventricular ejection fraction (LVEF) of muscular dystrophy patients with versus without left bundle branch block (LBBB) (a), and in patients with versus without QRS fragmentation (fQRS) (b).

QRS fragmentation was indicated in 6 (16.7%) dystrophinopathies patients, 2 (5.56%) LGMD patients, 11 (14.9%) DM1 patients, and 1 (4.35%) FSHD patient. Patients with fQRS had a lower LVEF than patients without fQRS (45.5 [IQR, 39.6-55.0] % versus 55 [IQR, 47.5-60.0] %, respectively; $p = .015$; Figure 5.9B) and were more likely to have a cardiomyopathy (RR, 1.76 [95% CI, 1.20-2.59]; $p = .004$). Patients with fQRS were also more likely to require cardiac medical therapies (RR, 1.74 [95% CI, 1.10-2.77]; $p = .018$) and cardiac device intervention (RR, 4.35 [95% CI, 1.94-9.74]; $p < .001$) than patients without fQRS. There was no discernible difference between patients with or without fQRS and the presence of fibrosis or the incidence of arrhythmias.

Patterns of R-wave progression were assessed for their ability to differentiate between patients with or without a dystrophinopathy. Presentation of an R wave of greater amplitude in V1 than V2, an R/S ratio greater than 1.00 and 1.50 in V1, or an R/S ratio greater than 1.00 and 1.50 in V2 demonstrated low sensitivity and high specificity, with low PPV and high NPV (Figure 5.10A-E).

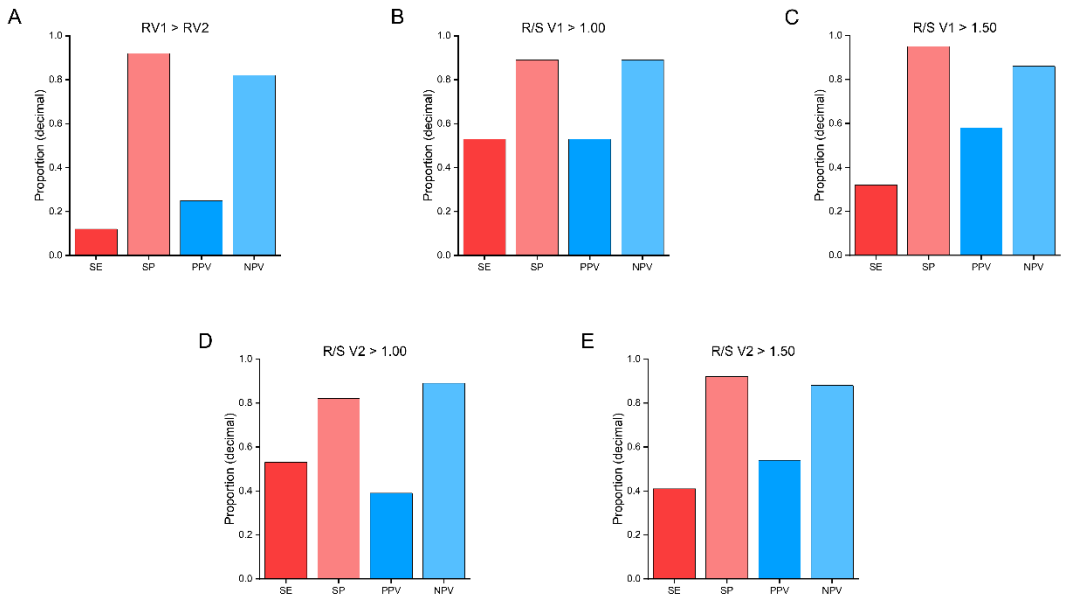


Figure 5.10. Assessment of variations of R-wave progression to differentiate dystrophinopathies from other types of muscular dystrophy using sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) of 12-lead electrocardiogram morphology such as a RV1 greater than RV2 (a), an R/S ratio in V1 greater than 1.00 (b), an R/S ratio in V1 greater than 1.50 (c), an R/S ratio in V2 greater than 1.00 (d), and an R/S ratio in V2 greater than 1.50 (e)

5.5. Discussion

Heart disease is highly prevalent in patients with MD and is a major determinant of their clinical outcomes.[21, 156] 12-lead electrocardiogram assessment is easily accessible, requires minimal training, and is of negligible burden on healthcare resources, making it a convenient and feasible method of assessing heart disease. Our investigation evaluated the use of conventional ECG-LVH criteria in patients with MD. The CV, CP, SL, and RE criteria are conventional methodologies for the identification of LVH through the analysis of voltages and wavelengths, with consideration for gender. We determined that these criteria demonstrated minimal clinical utility in these patients when compared to anatomical measures of LV mass illustrated by markedly low sensitivity and positive predictive value. The high specificity and NPV of the criteria can be useful for the confirmation of LVH indicated by cardiac imaging but would not be independently useful from a diagnostic perspective. Qualification of cardiac hypertrophy in patients with DMD is a greater challenge due to high precordial voltages, namely R waves in V1, though the exact cause remains unknown.[217]

Discordance between ECG-LVH and anatomical LVH is not uncommon in patients with heart disease and has been well-documented in large cohort studies.[196, 218] Our investigation demonstrates a greater degree of disagreement in patients with MD. We noted that the SL criteria, which exclusively considers precordial voltages for the classification of ECG-LVH, did not have an advantage over the other criteria that consider a composite of limb and precordial voltages among other properties. This supports our clinical findings of global muscle wasting in our cohort, which is an obstacle to the clinical utility of these conventional criteria in patients with MD. Although we were not able to capture the association between cardiac biomarkers and anatomical LVH in this cohort due the low prevalence, we support the proposed prognostic value

of comparable B-type natriuretic peptide and hsTnI for MACE in these patients, independent of LVH.[21] We acknowledge that ECG-LVH could be representative of interstitial and ion channel remodeling in the absence of myocyte hypertrophy.[195] With regards to the indication of LA enlargement by ECG, previous studies have shown that the level of specificity could prove useful for confirmation of indications by imaging but not for diagnosis, similar to our findings with ECG-LVH.[219, 220]

Left bundle branch block was an important ECG morphology identified in patients with DMD and DM1 given the high probability of a subsequent diagnosis of cardiomyopathy. Though LBBB was primarily indicated in patients with DM1, patients with DMD were likely also represented due to their advanced progression of heart disease.[156] Patients with DM1 were distinguishable given their high prevalence of conduction disease and incidence of arrhythmias, as shown in previous research.[8, 9, 194] Given that QRS interval progression has been shown to correlate with reduced LV systolic function,[40] CRT remains an important therapy to restore ventricular synchrony in the setting of LBBB, which can be supported with beta-blocker therapies in patients with ventricular tachyarrhythmias, as we have previously investigated in patients with DM1.[9] The severity and progression of conduction disease in patients with DM1 correlate with age and with the quantity CTG repeats, which supports the supplementation of demographics and genetic testing into the risk stratification of patients with DM1 in addition to baseline ECG analysis.[221, 222] As we observed, cardiac conduction abnormalities were less prevalent than structural abnormalities in patients with BMD and LGMD.[223] Patients with dystrophinopathies did present with uniquely abnormal R-wave progression and the various patterns assessed could serve to confirm a diagnosis of a dystrophinopathy given their high specificity. Furthermore, cardiomyopathy secondary to FSHD remains variably reported and any

positive findings have included atrial arrhythmias and atrioventricular conduction delays, though electrophysiological assessment of FSHD patients in our study cohort was unremarkable.[156]

We note that our study presents a MD cohort with a high prevalence of first-degree AVB and LAFB consistent with conduction disease in MD patients.[6] Our clinic has been established and optimized with regional primary care physicians to enroll patients with MD at early stages of their disease course, which is critical to the management of rapidly progressing conduction disease and incidence of arrhythmias. Importantly, VT is common in patients with DMD and DM1 including the risk of sudden cardiac death, which can be mitigated through the use of prophylactic device intervention as provided to our patients.[8, 9, 156]

QRS fragmentation has been described as a marker for cardiac fibrosis and a prognostic indicator of MACE in patients with heart disease.[200, 201, 224] Given that fQRS is representative of heterogenous ventricular activation and structural heart disease in traditional cohorts of heart disease, it is reasonable to conclude that MD patients exhibiting this morphology presented with advanced structural heart disease, as reflected in the high probability of cardiac outcomes in our patients with fQRS.[224] Considerations of fQRS have previously been applied to patients with DMD as a representation of regional wall motion abnormalities, and as an early indicator of adverse cardiac remodelling in these patients.[202, 203] We believe that this concept can be applied to our broader MD cohort considering its prevalence and the associated reduction in LV systolic function. Importantly, fQRS has been shown to be a reliable indicator of adverse cardiac remodelling in the presence of confounding ECG findings, which is relevant to our cohort given the high prevalence of conduction abnormalities.[225] The identification of fQRS in patients with MD upon ECG assessment is therefore important for prompting cardiac imaging, therapeutic intervention, and active monitoring.

Study Limitations

We acknowledge the limitations of our investigation. Due to our modest cohort size, we were unable to complete a thorough sub-group analysis to compare the prevalence of the aforementioned ECG features and serial parameter changes between the different types of MD. We recognize the importance of serial monitoring of cardiac electrophysiology in patients with DM1 due to the progression of conduction abnormalities and associated reduction in LV function, high incidence of arrhythmias, and risk of sudden cardiac death.[8, 221] Taking a rigorous approach to the serial quantification of atypical ECG parameters would be of strong consideration for future studies. Our analysis of ECG morphology provided important insights into cardiomyopathy and the limitations of ECG-LVH in these patients but did not specifically evaluate electrocardiogram-indicated right ventricular hypertrophy (ECG-RVH). Given the high burden of respiratory disease in patients with MD, ECG-RVH may be indicative of pulmonary hypertension or chronic obstructive pulmonary disease in these patients and these indications would be conducive to the multidisciplinary care of these patients.[156, 226]

5.6. Conclusion

Our investigation demonstrates the clinical utility of ECG and the importance of identifying baseline ECG morphologies such as LBBB and fQRS to facilitate active monitoring, further cardiac assessment through imaging modalities, and therapeutic response in patients with various types of MD. We have also demonstrated that ECG-LVH through the use of conventional criteria are of minimal clinical utility in these patients and that serial monitoring of intervals is not an effective methodology for stratifying MD patients for cardiomyopathy.

6. Ventricular Tachycardia in Patients with Type 1 Myotonic Dystrophy: A Case Series

6.1. Abstract

Background

Type 1 myotonic dystrophy (DM1) is associated with a variety of cardiac conduction abnormalities and the frequent need for permanent pacing. However, the role of ventricular tachycardia (VT) and the implied risk of sudden cardiac death (SCD) is poorly understood.

Case summary

This study examined a 56-patient DM1 cohort of men and women and identified five patients (two females and three males) with ventricular arrhythmias (8.9%). Patients were reviewed on a case-by-case basis, with their clinical presentation and management of VT and the associated cardiomyopathy indicated. Patient cardiac function was determined by 12-lead electrocardiogram, 48-h Holter monitor, and transthoracic echocardiography. These patients were therefore suitable candidates for implantable cardioverter-defibrillator implantation and received these devices; four of the five patients also received cardiac resynchronization therapy. Medical therapies included angiotensin converting enzyme inhibition, mineralocorticoid receptor antagonist, and following device implantation, beta-blocker therapy was initiated.

Discussion

Our case series demonstrates the prevalence of VT in patients with DM1 highlighting the associated risks of SCD in this patient population. The burden of ventricular arrhythmias, advanced conduction disease, and cardiomyopathy are best treated with a combination of device and medical therapies.

6.2. Learning points

- Ventricular tachycardia is an important arrhythmia in patients with type 1 myotonic dystrophy.
- Ventricular arrhythmias can account for the increased risk of sudden cardiac death in these patients.
- Appropriate use of device therapy coupled with effective pharmacological therapies is important interventions.

6.3. Introduction

Myotonic muscular dystrophy is the most common form of muscular dystrophy in adults and is characterized by cardiac conduction abnormalities with various other comorbidities.[52, 227, 228] Type 1 myotonic muscular dystrophy (DM1), also known as Steinert's disease, is inherited through an autosomal dominant pattern, presenting with myotonia and distal muscle weakening. DM1 is associated with conduction abnormalities and left ventricular (LV) dysfunction at a greater frequency and prevalence than type 2 myotonic muscular dystrophy.[53] We describe the presentation and outcome of malignant ventricular arrhythmias in a cohort of patients with DM1 as a basis for the increased risk of sudden cardiac death (SCD) in this vulnerable patient population.[229]

Case 1

A 44-year-old female DM1 patient, with a mild dilated cardiomyopathy (DCM) and a left bundle branch block (LBBB), had a pacemaker implanted due to the risk of progression of conduction disease (Table 6.1). This patient had an episode of syncope witnessed by her husband who immediately performed cardiopulmonary resuscitation; she regained consciousness during chest compressions. Subsequent device interrogation demonstrated sustained, rapid, and polymorphic ventricular tachycardia at the time of her event (Figure 6.1A). An echocardiogram indicated global hypokinesis, mild ventricular dilation, and a mild reduction in LV systolic function (Table 6.1). Her device was upgraded to an implantable cardiac defibrillator (ICD) and medical therapy was expanded to include metoprolol and spironolactone, in addition to her previous medical therapy: mexiletine and perindopril.

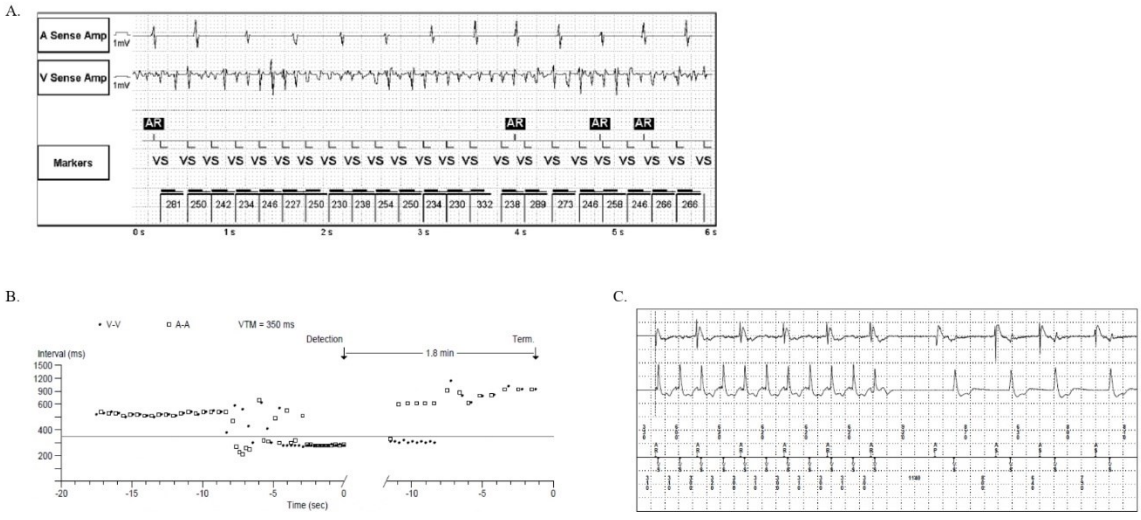


Figure 6.1. Rapid polymorphic ventricular tachycardia resulting in cardiac arrest as illustrated by the electrocardiogram (A), as described for Patient 1. R-R Interval histogram (B)

and electrocardiogram (C) illustrating monomorphic ventricular tachycardia detected during device interrogation of a dual-chamber permanent pacemaker as described for Patient 4

Table 6.1. Clinical characteristics of patients with DM1 presenting with VT.

Patient	Age (yrs)/ Gender	HR (bpm)	ECG Findings (ms)	Echocardiographic Parameters	VT Detection	Presentation	Medications
1	44/F	82	PR: 212; QRS: 105 QTc: 476 Biventricular paced rhythm	LVEF: 46% LVMI: 56.6 g/m^2 LVIDd: 5.0 cm LVIDs: 4.3 cm	CRT-P	Cardiac Arrest	Mexiletine Perindopril
2	36/F	69	PR: 179; QRS: 146 QTc: 388 Atrial paced rhythm, LBBB	LVEF: 46% LVMI: 60.9 g/m^2 LVIDd: 4.6 cm LVIDs: 3.7 cm	DC-PPM	Asymptomatic	Furosemide Ramipril Spironolactone
3	49/M	86	PR: 200; QRS: 107 QTc: 442 LAFB	LVEF: 45% LVMI: 51.5 g/m^2 LVIDd: 4.0 cm LVIDs: 3.3 cm	Tele-monitoring	Syncope	ASA Metoprolol Ramipril Rosuvastatin
4	61/M	64	PR: 272; QRS: 168 QTc: 446 Atrial paced rhythm, LBBB	LVEF: 45% LVMI: 69.7 g/m^2 LVIDd: 4.8cm LVIDs: 2.5cm	DC-PPM	Palpitations	Atorvastatin Candesartan Spironolactone
5	40/M	71	QRS: 170; QTc: 474 Atrial fibrillation, biventricular paced rhythm	LVEF: 38% LVMI: 137 g/m^2 LVIDd: 6.6cm LVIDs: 5.1cm	CRT-D	ICD shock	Digoxin Ramipril Warfarin

LVEF, left ventricular ejection fraction; LV, left ventricle; ECG, electrocardiogram; VT, ventricular tachycardia; LVIDd, left ventricle internal diameter end diastole; LVIDs, left ventricle internal diameter end systole; LBBB, left bundle branch block; CRT-P, cardiac resynchronization therapy pacemaker; DC-PPM, dual chamber permanent pacemaker; ASA, acetylsalicylic acid; CRT-D, cardiac resynchronization therapy defibrillator. Refer to Methods section for how values were obtained or calculated.

Case 2

A 36-year-old female DMI patient with a dual-chamber rate-responsive permanent pacemaker was diagnosed with sustained VT upon device interrogation during routine follow-up. The VT recorded was at an average rate of 147 bpm and spontaneously terminated after 32 seconds. Previous device interrogations had shown five episodes of non-sustained VT up to 26 seconds in duration. The patient was asymptomatic for all recorded episodes. Her 12-lead ECG demonstrated an atrial-paced rhythm and LBBB (Table 6.1). An echocardiogram showed mild LV systolic dysfunction (Table 6.1). Her pacemaker was upgraded to an ICD. Her medical therapy, which included ramipril, furosemide, and spironolactone, was expanded to include metoprolol.

Case 3

A 49-year-old male DM1 patient was admitted to the cardiology ward following 2 episodes of syncope. Electrocardiographic tele-monitoring detected asymptomatic episodes of sustained VT. The patient received an echocardiogram which showed global hypokinesis of the LV with an LVEF of 45% (Table 6.1). This patient also received a cardiac magnetic resonance imaging (MRI) study with gadolinium contrast showing normal biventricular volumes and atrial sizes, no hypertrophy, and a LVEF of 45%. The absence of late gadolinium enhancement (LGE) indicates that there is no myocardial scarring. Following VT detection, the patient received an MRI compatible dual chamber

ICD. He was also newly diagnosed with type 2 diabetes. The patient was prescribed acetylsalicylic acid, ramipril, metoprolol, rosuvastatin, and metformin and insulin. He has had subsequent appropriate ICD shocks and his ICD therapy was adjusted to optimize-ATP-termination and to minimize ICD shocks, and his dose of the beta-blocker, metoprolol, increased.

Case 4

A 61-year-old male DM1 patient with a dual-chamber pacemaker was diagnosed with recurrent asymptomatic VT. ECG findings for this patient included first-degree atrioventricular (AV) block and LBBB (Table 6.1). A 48-hour Holter monitor showed an atrial-paced rhythm and a 1% burden of PVCs. During his regular pacemaker clinic visit, device interrogation revealed one episode of rapid VT at an average rate of 186 bpm, lasting one minute and 49 seconds (Figure 6.1B). His echocardiogram showed mild global hypokinesis of the LV with an LVEF of 45%. A cardiac MRI study with gadolinium contrast showed normal biventricular volumes and atrial sizes, no hypertrophy, and a LVEF of 40%. The absence of LGE indicates that there is no myocardial scarring. He was admitted for extraction of his RV pacing lead and an upgrade to an ICD. Prior to the event, his medical therapy included candesartan, atorvastatin and spironolactone. A beta-blocker, bisoprolol, was initiated prior to hospital discharge.

Case 5

A 40-year-old male diagnosed with DM1, permanent atrial fibrillation, and a dilated cardiomyopathy with an ejection fraction of 30-35% on echocardiography, received a single chamber defibrillator for primary prevention. Two years later, as a result of progressive AV block

and predominantly RV pacing, his system was upgraded to a cardiac resynchronization therapy device, with a defibrillator lead (CRT-D). In the following year, he had two separate episodes of VT, one of which resulted in an ICD shock. A concurrent echocardiogram showed moderate LV chamber dilation and global hypokinesis of the LV with a LVEF of 38% (Table 6.1). His initial medical therapy included warfarin, ramipril, and digoxin; perindopril and the beta-blocker, bisoprolol, were added prior to discharge. Given recurrent ventricular arrhythmias in this patient, he was initiated on amiodarone therapy.

6.4. Discussion

Our cohort is comprised of patients with a high burden of arrhythmias and conduction abnormalities including AV block, left anterior fascicular block, and LBBB; with a high prevalence of cardiomyopathy, and all five patients having reduced ejection fraction. However, four of the five patients in this study had mild cardiomyopathies with ejection fractions well above 35%: the recommended level for primary prophylaxis ICD insertion.[145] These results demonstrate that in patients with DM1, adverse cardiac electrical remodeling occurs prior to the progression to advanced cardiomyopathy. LV systolic dysfunction is likely primarily attributed to the left-bundle branch block and the associated electromechanical dyssynchrony (Table 6.1).[230, 231] Two patients also received cardiac MRI with gadolinium contrast, which did not show myocardial scarring (absence of LGE). Pacemaker device use in four of our five patients, prior to the detection of VT, were inserted in response to conduction disease and bradycardia associated with DM1, in accordance with clinical practice guidelines.[145] The overall cohort of 56 DM1 patients had relatively preserved ejection fraction with a much lower prevalence of conduction abnormalities (Table 6.2).

Table 6.2. Clinical characteristics of the Type 1 Myotonic Muscular Dystrophy Cohort.

Men/Women, No.	28 (50)/28 (50)		**Vitals**	
			Heart Rate, bpm	70 (61-80)
Age, Yrs	43.9 (34.4-53.4)		sBP/dBP mmHg	114 (105-128)/ 74 (68-78)
12-lead ECG (n=56)			**Echocardiogram (n=46)**	
PR Interval, ms	192 (178.5-217.5)		LVIDd, cm	4.4 (4.0-4.8)
QRS Duration, ms	106 (93-112)		LVIDs, cm	2.8 (2.6-3.0)
QTc Interval, ms	412 (399-437)		LVPWd, cm	0.9 (0.7-1.0)
			LVEF, %	57.5 (52.5-60)
Conduction Abnormalities, No.			LVMI, g/m²	42 (39-50.5)
1° AVB	15 (26.8)		TAPSE, mm	2.1 (1.8-2.4)
LAFB	5 (8.9)		RVSP, mmHg	24.5 (19.8-27.2)
LBBB	11* (19.6)		RV Size	1 Thickened
RBBB	1 (1.8)		RV Fxn	2 Hypokinetic

ECG, electrocardiogram; PR Interval, duration of atrial depolarization; QRS duration, duration of ventricular depolarization; Corrected QT (QTc) Interval, duration between ventricular depolarization and repolarization; 1° AVB, first-degree atrioventricular block; LAFB, left anterior fascicular block; LBBB, left bundle branch block; RBBB, right bundle branch block; sBP, systolic blood pressure; dBP, diastolic blood pressure; LVMI, left ventricular mass index; LVIDd, left ventricular (LV) internal dimension at end-diastole; LVIDs, LV internal dimension at end-systole; LVPWd; LVEF, left ventricular ejection fraction; LV posterior wall thickness at end-diastole; TAPSE, tricuspid annular plane systolic excursion; RVSP, right ventricular systolic pressure. *1 patient has an incomplete LBBB.

The development of VT is of considerable concern in DM1 patients, as demonstrated by our case series. One of the five patients in this study experienced cardiac arrest requiring resuscitation. Implantable cardioverter-defibrillator and appropriate medical therapy are crucial to prevent SCD in these patients. The implantation of pacemakers or ICD is particularly challenging in patients with muscular dystrophy given the low skeletal muscle mass and respiratory involvement. Three of our five patients had a successful upgrade of their pacemakers to CRT-D device while one patient received a dual chamber ICD without complications. A recent study tracking 1388 patients over a 12 year period reported an incidence of 2.3% (26 of 1388 patients) of sustained VT in patients with DM1.[228] The much higher prevalence of VT in our case series, at an incidence of 8.9% over four years (5 out of 56 patients) suggest that our cohort of contemporary DM1 patients had more advanced heart disease. As such, while it is important that we recognize the severe conduction abnormality, the potential of VT leading to SCD must be appropriately investigated and managed in patients with DM1. An electrophysiological study may be helpful to evaluate and direct the treatment of inducible ventricular arrhythmias and may play an important role in risk stratification of DM1 patients. [232, 233] Although our cohort size is modest, we continue to recruit muscular dystrophy patients in our clinic, thereby expanding the size of our DM1 patient cohort.

Conduction system disease with progression to complete AV block is a well-recognized complication of several neuromuscular disorders, including myotonic dystrophy. The presence of conduction disease characterized by first-degree AV block and underlying LBBB suggest a key pathogenic role of electrical dyssynchrony in mediating the cardiomyopathy associated with DM1.[234] In patients with DM1, implantation of a permanent pacemaker is recommended even in asymptomatic patients with an abnormal resting ECG or with HV interval prolongation during

electrophysiological study.[235] Cardiac resynchronization therapy allows for complete pharmacological therapy use, namely the use of beta-blockers, thereby allowing both anti-arrhythmic effects and therapeutic responses with the concomitant LBBB and cardiomyopathy. Given the inherent risk of SCD in this vulnerable patient population, it is crucial that physicians recognize the risk of ventricular arrhythmias in DM1 patients and consider the use of medical therapy and device intervention in a timely manner.[228]

7. Comparison of Usefulness of Cardiac Resynchronization Therapy in Patients with Type 1 Myotonic Dystrophy with Versus Without Left Bundle Branch Block

7.1. Abstract

Patients with type 1 myotonic dystrophy (MD1) show reduced LV systolic function in the presence of left bundle branch block (LBBB) due to electromechanical dyssynchrony. Our prospective study tracked a cohort of 64 MD1 patients that demonstrated a high burden of atrial and ventricular arrhythmias and conduction delays. Of these patients, 12 (19%) patients had LBBB, which was associated with reduced left ventricular systolic function. Eight of these patients received cardiac resynchronization therapy (CRT) devices resulting in reduction of median QRS complex duration from 173ms to 166ms ($p = 0.04$), and improvement in median left ventricular ejection fraction from 37% to 46% ($p = 0.007$). In conclusion, CRT device therapy is both feasible and effective in treating advanced cardiac disease in this vulnerable group of patients by improving

left ventricular function. In patients without type 1 myotonic dystrophy (MD1), conduction disease including left bundle branch block (LBBB) leading to electromechanical dyssynchrony, has prognostic implications due to its association with systolic dysfunction and adverse outcomes.[152, 234] Use of pacemakers and ICDs in MD1 patients is driven by the high burden from conduction disease and ventricular arrthymias.[53, 136, 138, 227, 228, 236-238] Cardiac resynchronization therapy (CRT) is standard therapy for patients with LBBB and reduced left ventricular ejection fraction (LVEF) ≤ 35%, and a prolonged QRS complex duration, who remain in New York Heart Association (NYHA) functional classes II and III, despite optimal medical therapy.[145, 239, 240] Specialized and unique patient cohorts not included in clinical trials may also benefit from CRT. We performed a prospective cohort study of patients with MD1 to assess the presence of LBBB and systolic dysfunction, and the response to CRT.

7.2. Methods

Patients were seen at the Neuromuscular Multidisciplinary (NMMD) Clinic located at the Kaye Edmonton Clinic in Alberta, Canada, where they received specialized care from a team of cardiologists, neurologist, respirologists, and physiatrists in conjunction with allied health care professionals. A cohort of 64 patients diagnosed with genetically-confirmed MD1 were recruited and followed for approximately 4 years, from May 20, 2015, until April 1, 2019. Patients provided informed, written consent prior to their enrolment into our prospective cohort study. Our study maintains ethical approval and abides by the guidelines of the Health Research Ethics Board at the University of Alberta

LBBB was defined as QRS duration > 120ms in addition to conventional criteria, defined as mid QRS notching or slurring in two of the following leads (I, aVL, V1, V2, V5, V6), QS or rS in V1, and a monophasic R with no q waves in I and V6. MD1 patients were separated into 2 cohorts based on the presence of LBBB (12 patients), or the absence of LBBB (52 patients). Demographic data, clinical profile, biochemical testing, electrocardiogram (ECG), Holter monitoring, and transthoracic echocardiogram were prospectively collected to create detailed patient profiles. Eight patients with LBBB who received CRT devices over the course of the study were evaluated by serial 12-lead electrocardiograms (ECGs) and echocardiograms obtained during the subsequent follow-up visit to evaluate the effectiveness of the device intervention in this patient cohort.

Continuous data are presented as median values with interquartile ranges and categorical data is presented as quantity with a percentage. All continuous variables analyzed were compared using a Mann-Whitney U test, and all categorical data were compared using Pearson's Chi-square tests. A $p < 0.05$ was considered significant through all statistical analysis. Statistical analyses were conducted using SPSS Statistics Version 25 (IBM, NY, USA).

7.3. Results

The LBBB cohort represented 19% of our MD1 patients (Table 7.1; Figure 7.1). Clinical features were comparable between the non-LBBB and LBBB cohorts with the latter group being slightly older (Table 7.1). Respiratory abnormalities, such as sleep-disordered breathing, were common in this group of patients (Table 7.1). In the non-LBBB cohort, 12-lead ECG assessment showed normal QRS duration and minor prolongation of the PR interval (Table 7.2). First-degree

AVB and left anterior fascicular block (LAFB) were common diagnoses (Table 7.2). Echocardiography showed normal LV dimensions and normal systolic function; RV size and function was unremarkable (Table 7.2). Atrial fibrillation or flutter was detected in 14% of patients and by this study's conclusion, 2 patients had received permanent pacemakers for secondary prophylaxis. Additionally, 2 patients had a dual chamber implantable cardiac defibrillator (ICD) inserted due to symptomatic ventricular tachycardia (VT) and cardiac arrest.

Table 7.1. Clinical characteristics our cohort with type 1 myotonic dystrophy (MD1).

Variable	MD1 Without Left Bundle Branch Block (n=52)	MD1 With Left Bundle Branch Block (n=12)	p value
Men/Women	26 (50%)/26 (50%)	7 (58%)/5 (42%)	0.60
Median Age (Years)	42 (33-50)	47 (43-59)	0.049
Dyslipidemia*	7 (14%)	0	
Diabetes	5 (9.6%)	1 (8.3%)	0.89
Respiratory Disease	13 (25%)	2 (17%)	0.54
Sleep Disordered Breathing	12 (23%)	6 (50%)	0.06
Hypertension†	3 (5.8%)	1 (8.3%)	0.74
Mobility Aids	8 (15%)	4 (33%)	0.38

Non-Invasive Ventilation	7 (14%)	3 (25%)	0.32
Heart Rate (bpm)	71 (64-84)	70 (60-75)	0.37
Systolic Blood Pressure (mmHg)	114 (110-123)	108 (101-123)	0.23
Diastolic Blood Pressure (mmHg)	75 (70-80)	70 (61-73)	0.009

*Dyslipidemia defined as low-density lipoprotein cholesterol ≥ 3.5 mmol/L or non-high-density lipoprotein cholesterol ≥ 4.3 mmol/L.†Hypertension defined as systolic blood pressure > 130 mmHg or diastolic blood pressure > 89 mmHg.

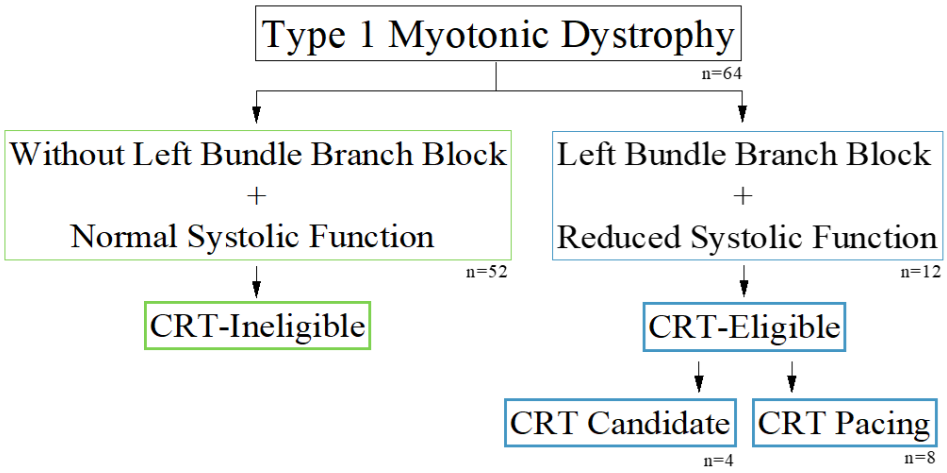


Figure 7.1. Appropriate use of cardiac resynchronization therapy in patients with type 1 myotonic dystrophy.

Table 7.2. Cardiac assessment of our cohort with type 1 myotonic dystrophy (MD1).

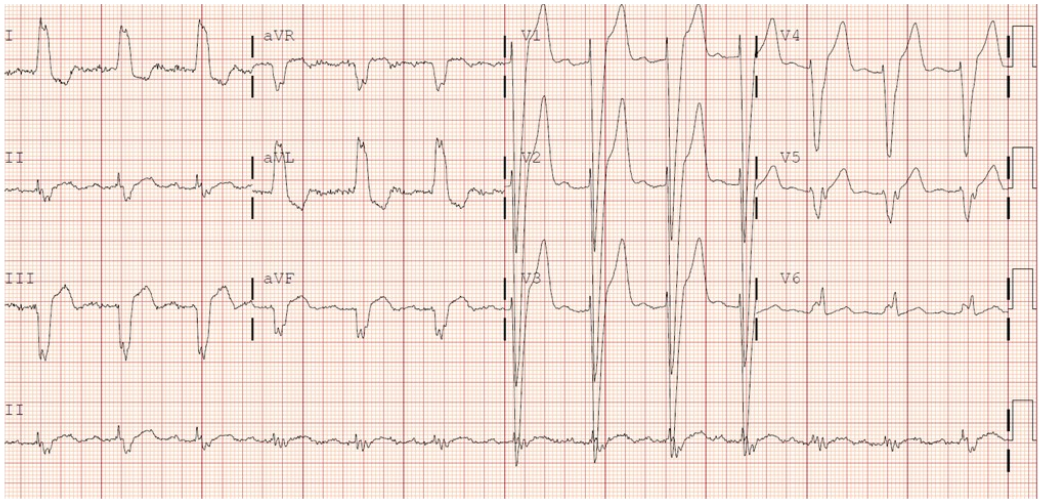
Modality	MD1 Without Left bundle Branch Block (n=52)	DM1 With Left Bundle Branch Block (n=12)	p value
12-Lead ECG			
Heart Rate, bpm	69 (63-81)	64 (63-71)	0.40
PR Interval, ms	192 (176-210)	217.5 (203-235)	0.03
QRS Duration, ms	104 (92-108)	156 (141-171)	<0.001
QTc Interval, ms	410 (391-431)	440 (422-488)	0.004
First-Degree Atrioventricular Block	10 (19%)	5 (42%)	0.10
Left Anterior Fascicular Block	8 (15%)	12 (100%)	
Echocardiogram			
Left Ventricular Internal Dimension at End-Diastole (cm)	4.2 (3.9-4.5)	5.0 (4.7-5.6)	0.001
Left Ventricular Internal Dimension at End-Systole (cm)	2.8 (2.6-2.9)	3.4 (2.9-4.5)	0.003
Left Ventricular Posterior Wall Thickness at End-Diastole (cm)	0.9 (0.7-1.0)	0.9 (0.7-1)	0.51

Left Ventricular Ejection Fraction (%)	60 (58-61)	40 (35-45)	<0.001
Left Ventricular Mass Index (g/m^2)	63 (55-73)	88 (67-118)	0.017
Tricuspid Annular Plane Systolic Excursion (mm)	2.1 (1.8-2.4)	2.2 (1.9-2.4)	0.87
Right Ventricular Systolic Pressure (mmHg)	24 (18-27)	20 (17-23)	0.40
Right Ventricle Size	Normal	3 Hypertrophic	
Right Ventricular Systolic Function	1 Reduced	2 Reduced	

In contrast, 12-lead ECGs of patients in the LBBB cohort showed prolonged PR intervals with a high incidence of first-degree atrioventricular block (AVB) in association with widened QRS duration (Table 7.2). Echocardiogram data of the LBBB cohort showed signs of eccentric hypertrophy with LV dilation with modest involvement of the RV (Table 7.2). LV systolic function was markedly reduced (Table 7.2). Cardiac MRI was performed in 4 patients confirming LV systolic dysfunction with only 1 patient showing myocardial fibrosis as identified by late gadolinium enhancement. Atrial fibrillation or flutter was detected in 3 patients, and VT in 4 patients (4/12, 33%) with reduced systolic function (median LVEF = 46% [IQR 43 to 46%]). At the initiation of this study, one patient had previously had a dual chamber implantable cardiac defibrillator implanted due to a history of recurrent VT, and 3 patients had pacemakers as secondary prophylaxis for bradyarrhythmias.

Five male and 3 female LBBB patients received a CRT device (Figure 7.1). Device implantation was successful in all 8 patients without complications and all patients were discharged on the same day of the surgery. Two patients with existing pacemaker devices, and 2 patients with existing ICDs were upgraded to CRT-defibrillator (CRT-D) devices; 4 patients received *de novo* CRT-pacemaker (CRT-P) devices. Within a 6-month time frame, follow-up 12-lead ECGs showed biventricular paced rhythms in all 8 patients, with a significant reduction in median QRS complex duration from 173ms to 166ms ($p = 0.04$; Figure 7.2 and Figure 7.3A). Follow-up echocardiogram data obtained within 6 months of device implantation showed a median LVEF increase from 37% to 46% ($p = 0.007$; Figure 7.3B). The implantation of a CRT device in these patients allowed for the initiation of beta-blocker therapy, which was supported by angiotensin converting enzyme inhibitor (ACEi)/ angiotensin receptor blocker (ARB) and mineralocorticoid receptor antagonist (MRA) therapies (Figure 7.4A) and uptitration of the doses of these medications (Figure 7.4B), which likely contributed to improved LV function. In contrast, the 4 patients with LBBB who have not received a CRT device, showed a mild decline in LV systolic function (median decrease in LVEF = 4%) over the course of this study.

A.



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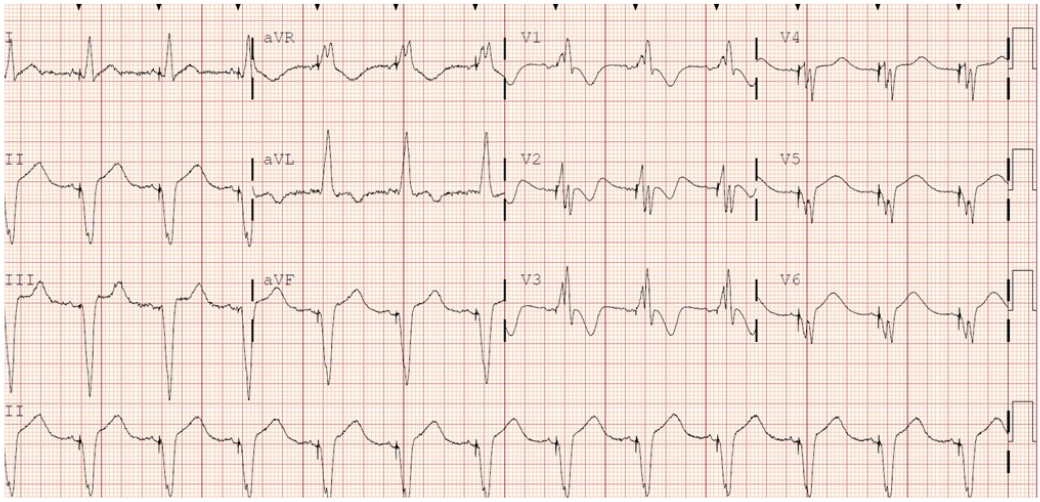


Figure 7.2. A representative case showing the initial 12-lead ECG of a 46-year-old male patient with type 1 myotonic dystrophy with first-degree atrioventricular block and a LBBB with a QRS duration of 194 ms (HR = 78 beats/min, PR = 234 ms) (A) and a follow-up 12-lead ECG after CRT device implantation showing a biventricular paced rhythm with a QRS duration of 166 ms (HR = 76 beats/min, PR = 175 ms) following CRT device implantation (B). CRT = cardiac resynchronization therapy, ECG = electrocardiogram, LBBB = left bundle branch block, QRS duration = duration of ventricular depolarization.

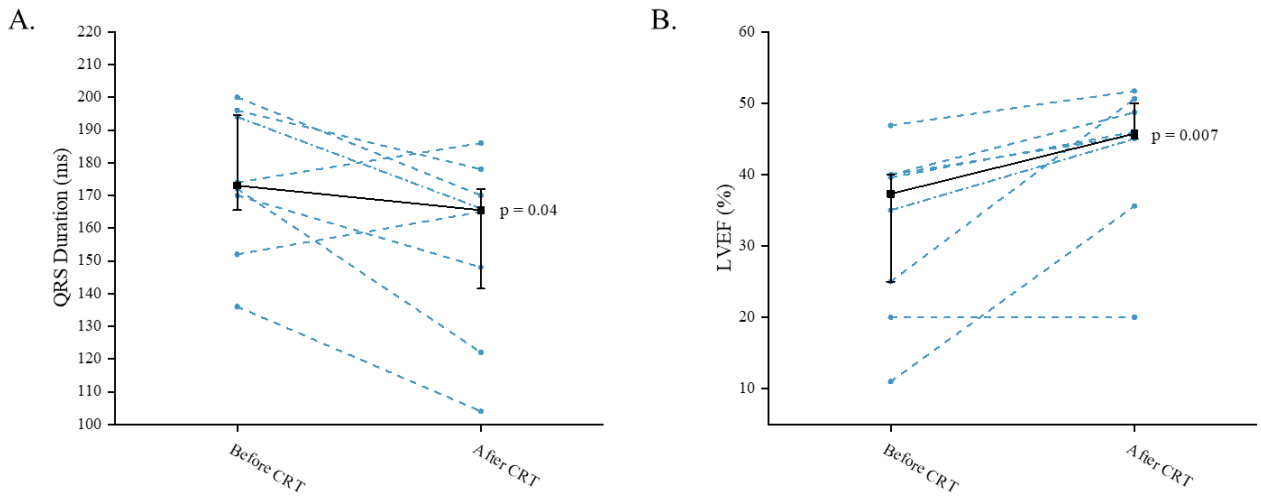


Figure 7.3. Effect of cardiac resynchronization therapy on QRS duration (A) and left ventricular ejection fraction (B) in patients with LBBB. CRT = cardiac resynchronization therapy, LBBB = left bundle branch block, LVEF = left ventricular ejection fraction, QRS duration = duration of ventricular depolarization

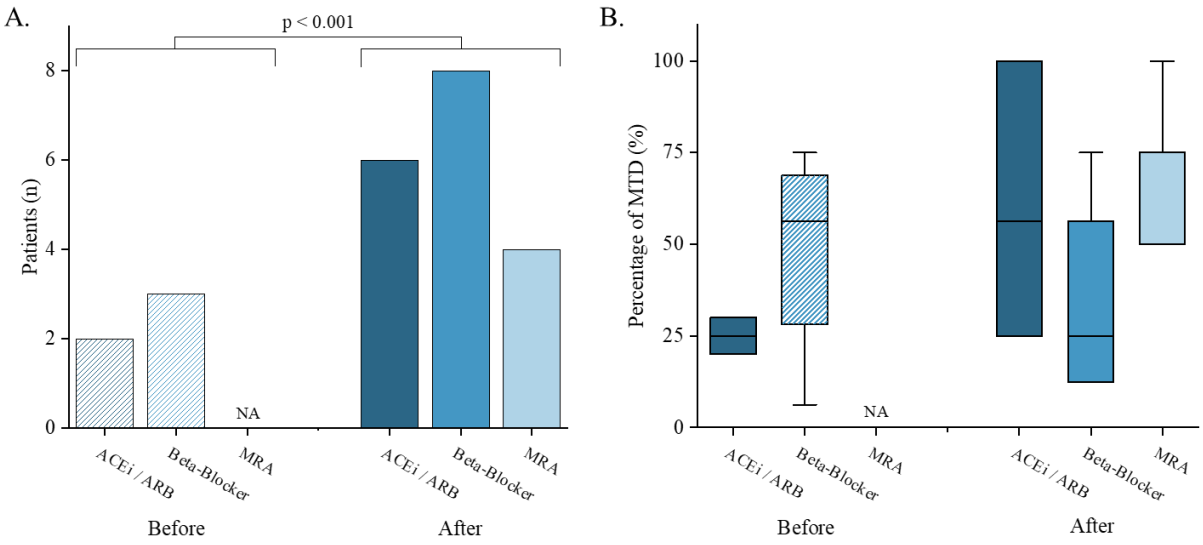


Figure 7.4. Pharmacological therapy use before and after cardiac resynchronization therapy device intervention (A) and uptitration in conjunction with device therapy (B). ACEi = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker, MRA = mineralocorticoid receptor antagonist, MTD = maximum tolerated dose, NA = not applicable.

7.4. Discussion

Our study demonstrates the effectiveness of CRT device therapy in a vulnerable group of patients that demonstrates a high burden of cardiac arrhythmias and conduction abnormalities. LBBB is of particular concern as it leads to early signs of pathological remodeling of the heart due to electromechanical dyssynchrony.[152, 234, 241] Patients with LBBB showed eccentric remodeling with increased LV chamber dimensions and mass associated with moderate LV systolic dysfunction. In contrast, MD1 patients without LBBB did not show any indications of pathological

remodeling and LV systolic function was normal. In addition to LBBB, there was a high prevalence of LAFB in our cohort of patients suggesting early progression of conduction disease and pathology in patients with MD1 and highlights the important for regular monitoring and follow-up care. These findings suggest that the established relationship between systolic dysfunction and LBBB in traditional non-MD1 patients can be applied to MD1 patients.[139, 152, 240, 242]

Studies in the traditional non-MD1 heart failure patients with reduced LVEF (LVEF ≤ 35%) have presented findings on the pathological effects of electromechanical dyssynchrony and the use of CRT devices to improve clinical outcomes.[227, 234, 235, 242] We have demonstrated that these are applicable to the MD1 patient population. Although some of our patients in the LBBB cohort did not have an LVEF ≤ 35%, CRT devices were implanted on a clinical basis, in anticipation of patients developing complete heart block, given the progressive nature of their conduction system disease.[145, 227] In contrast to our 8 patients who have received a CRT device, the 4 LBBB patients who have not received a CRT device showed a progressive decline in LV systolic function, further demonstrating the critical need for CRT intervention in these patients. We believed that the high degree of responsiveness to CRT intervention, as indicated by a marked increase in LVEF, is related to the prolonged QRS duration of our LBBB patient cohort. Optimizing cardiac medications, such as beta blockers, in MD1 patients is particularly challenging due to the high risk of bradyarrhythmias in MD1 patients.[243] CRT device therapy allows the initiation and uptitration of beta-blocker therapy. Uptitration of the doses of ACEi/ARB was facilitated by improved blood pressure likely driven by larger stroke volume in the setting of increased ejection fraction. Additionally, we considered the high risk of ventricular tachyarrhythmias in our MD1 patient cohort, and the associated risk of SCD due to VT.[138, 228, 238]

The use of CRT devices with an ICD (CRT-D) is an effective device therapy in these patients. Four of our patients with LBBB and recurrent VT were upgraded from their standard pacemakers or ICDs to CRT-D devices.

The presence of respiratory disease and inspiratory muscle weakness in patients with MD1 provides an additional challenge for device implantation. Respiratory therapy was involved in monitoring and providing non-invasive ventilation during the procedure. Device implantation was appropriate and safe in all patients. We have demonstrated that LBBB is a marker of advanced cardiac disease in patients with MD1, as is accepted for traditional non-MD1 patients. We have also demonstrated that CRT device use is both feasible and effective in this patient population. Our screening of conduction disease in this vulnerable group of patients now incorporates a routine 12-lead ECG as part of the clinical assessment in the NMMD clinic. We believe CRT device use to be an asset when treating this non-traditional group of patients. While our sample size is modest, we continue to recruit patients with muscular dystrophy to the NMMD clinic, thereby expanding the size of our patient cohort.

8. Trajectory of Left Ventricular Ejection Fraction in Response to Therapies in Patients with Muscular Dystrophy

8.1. Abstract

Background: Patients with muscular dystrophy (MD) are at elevated risk of serious cardiac complications and clinical assessment is limited due to inherent physical limitations. We assessed the utility of left ventricular ejection fraction (LVEF) derived from transthoracic echocardiogram

(TTE) as a prognostic marker for major adverse cardiac events (MACE) in a mixed adult MD cohort.

Methods: One hundred and sixty-five MD patients (median age: 36 (interquartile range [IQR]: 23.0-49.0) years; 65 [39.4%] females) were enrolled in our prospective cohort study. Diagnoses included dystrophinopathies (n = 42), limb-girdle MD (*n* = 31), type 1 myotonic dystrophy (n = 71), and facioscapulohumeral MD (n = 21). Left ventricular ejection fraction, ventricular dimensions at end-diastole and end-systole, and serial measures (n = 124; follow-up period: 2.19 [IQR: 1.05-3.32] years) stratified patients for MACE risk.

Results: Cardiomyopathy was diagnosed in 60 (36.4%) patients of the broader cohort (median LVEF: 45.0 [IQR: 35.0-50.0] %). Ninety-eight MACE occurred over the 7-year study period. At baseline, patients with a LVEF < 55.0% had a high risk of MACE (adjusted odds ratio: 8.30; 95% confidence interval [CI]: 3.18-21.7), concordant with analysis of LV dimensions. Forty-one percent of these patients showed an improvement in LVEF with optimization of medical and device therapies. Relative to patients with preserved LVEF, patients with reduced LVEF were at an elevated risk of MACE (adjusted hazard ratio [aHR]: 7.21; 95% CI: 1.99-26.1), and improved LVEF resulted in comparable outcomes (aHR: 1.84; 95% CI: 0.49-6.91) associated with optimization of medical and device therapies. Reduction in QRS duration by CRT therapy was associated with an improvement in LVEF (average improvement: 12.8 [±2.30] %; *P* = 0.04).

Conclusions: Reduction in LVEF indicates increased risk of cardiovascular events in patients with MD. Baseline and serial LVEF obtained by TTE can prognosticate patients for MACE and guide clinical management.

8.2. Introduction

Heart disease remains a leading cause of morbidity and mortality in patients with muscular dystrophy (MD).[4-6, 70, 142, 244] Patients with dystrophinopathies (Duchenne muscular dystrophy [DMD] and Becker's muscular dystrophy [BMD]), limb-girdle muscular dystrophy (LGMD), type 1 myotonic dystrophy (DM1), and facioscapulohumeral muscular dystrophy (FSHD) have a substantial risk of heart disease and increased risk for adverse outcomes.[244] Despite the high burden of Heart disease has been previously characterized in this mixed adult cohort of MD,[142, 244-249] however the risk of major adverse cardiac events (MACE) and the prognostic value of impaired LV systolic function remains to be defined.

The clinical assessment of patients with MD is complicated by associated muscle weakness and wasting, and the presence of respiratory and ambulatory support devices. Moreover, the use of mouthpiece ventilation, presence of spinal stabilization rods, and wheelchair dependence pose a significant challenge to the use of cardiac magnetic resonance (CMR) in these patients. In this study, we demonstrate that transthoracic echocardiogram (TTE) is an effective method of assessing cardiac status in a large adult MD cohort, with specific attention to the utility of LVEF. Importantly, baseline LVEF measures and LVEF trajectory served as effective prognosticators for the risk of MACE. We also defined the recovery in LVEF following medical and device therapies and highlighted the similarity in clinical outcomes to MD patients with preserved LVEF.

8.3. Methods

Study Cohort and Design

In this prospective cohort study, 165 patients with MD were recruited from the Neuromuscular Multidisciplinary (NMMD) clinic over a 7-year period from November 5, 2014, to December 17, 2021. Neurological assessment, muscle biopsy, and genetic testing confirmed the diagnosis of MD, including dystrophinopathies (34 patients; 24 DMD and 10 BMD patients), carriers of a dystrophinopathy (8 patients; 7 DMD carrier and 1 BMD carrier patients), LGMD (31 patients), DM1 (71 patients), and FSHD (21 patients). For this investigation, patients diagnosed with a dystrophinopathy as well as carriers were grouped together as "dystrophinopathies" given their similarity in underlying pathophysiology, clinical presentations, and management practices.[144] Clinical data was obtained following multidisciplinary assessment and care by electronic chart review. Our study was conducted in accordance with the ethical principles of the Declaration of Helsinki, with approval from the University of Alberta Health Research Ethics Board, and informed consent was obtained from all participants.

Cardiac Imaging by Transthoracic Echocardiogram and Cardiac Magnetic Resonance

Transthoracic echocardiography was performed (Philips iE33; Philips Medical Systems, Andover, MA, USA) by certified sonographers experienced in imaging patients with MD. Obtained images were analyzed using IntelliSpace software (Philips Medical Systems). Wheelchair-bound patients were transferred onto an echocardiography table with the assistance of the echosonographer and care provider. Only four patients remained seated for their TTE study which has been validated to provide accurate echocardiographic assessment.[250] Conventional structural and functional measures were obtained in accordance with current ASE guidelines by

experienced echosonographers.[211] Cardiomyopathy was defined as LVEF < 55%. We established concordance of LVEF measures by TTE with CMR in 84 patients with both studies available at baseline. Cardiac magnetic resonance was performed using a 1.5T Sonata scanner (Siemens Healthcare, Erlangen, Germany) and assessed using image analysis software, Syngo Argus (Siemens Healthcare),[251] by a blinded and experienced CMR interpreter.

Patient Classification and Study Endpoints

Patients were divided into dichotomous groups for each parameter (LVEF, LVIDd, and LVIDs) for the first segment of risk analysis using baseline data. Patients were classified as having a LVEF ≥ 55.0% or < 55.0%. Patients were also classified based on LVIDd or LVIDs below or above the median (4.50 cm and 2.96 cm, respectively). Patients with a LVEF ≥ 55.0%, or LVIDd and LVIDs less than or equal to their respective median value were used as reference groups for risk analyses. Follow-up TTE was performed in 124 patients that were determined to be at higher risk of heart disease progression upon initial NMMD clinic assessment and review of medical history, which included diagnoses of comorbidities, 12-lead ECG findings, and limited ambulation. Serial LVEF measures and the nominal change in LVEF from baseline to the subsequent follow-up TTE assessment was derived to categorize patients into three trajectory-defined groups: a preserved reference group comprised of 27 patients with no functional cardiac impairment, an improved LVEF group (n = 51), and a reduced LVEF group (n = 46). We followed a similar methodology in tracking the nominal dimensional changes of LVIDd and LVIDs.

Major adverse cardiac events were defined as a composite of arrhythmia, device implantation, cardiac-related hospitalization, incident heart failure (HF), and cardiac-mortality. Arrhythmias such as atrial and ventricular tachyarrhythmias were captured by 12-lead ECG and

upon routine device interrogation. Incident HF was diagnosed following a comprehensive cardiac assessment, which considered symptoms and signs such as dyspnea, orthopnea, peripheral edema, and abdominal distention. Outcome data such as hospitalizations, mortality, and associated diagnoses were obtained from provincial electronic health records.

Statistical Analysis

Continuous variables analyzed were compared using a Mann-Whitney U test or Kruskal-Wallis test where appropriate. Categorical variables were compared using Pearson chi-square tests. Baseline LVEF obtained from TTE and CMR were compared to derive a Spearman's correlation coefficient to validate concordance. We incorporated logistic regression models to derive odds ratios for the relationship between LVEF, LVIDd, and LVIDs with the incidence of MACE. Adjusted odds ratios (aOR) were also derived through multivariable regression models, which adjusted for age group, sex, diagnosis of respiratory disease, and the baseline use of cardiac medications and devices. Age was grouped into 5-year intervals and respiratory disease was defined as chronic obstructive pulmonary disease, asthma, recurrent aspiration pneumonia, respiratory muscle weakness, or restrictive lung disease. We also compared the incidence of MACE among groups with differing LVEF trajectories, as previously defined, using Kaplan-Meier curves and a log-rank test over 7 years. Multivariable Cox regression models (adjusted for age group, sex, diagnosis of respiratory disease, and the use of cardiac medications and devices) were used to derive adjusted hazard ratios (aHR) to evaluate the independent prognostic value of LVEF trajectories for MACE in our MD cohort. Averages with standard error, and paired t-tests were used when comparing parameters before and after cardiac resynchronization therapy (CRT) device implantation and binary logistic regression was incorporated to assess the association between

QRS duration reduction and LVEF improvement in a subgroup of 15 patients. All statistical analyses were performed in R version 4.0.3 and a P value < 0.05 was considered significant.

8.4. Results

Clinical Characteristics

The median age of the study cohort was 36 (interquartile range [IQR]: 23.0-49.0) years, comprised of 65 (39.4%) females (Table 8.1 and Figure 8.1A). Within the dystrophinopathies cohort, DMD and BMD patients were exclusively younger males, while carriers were exclusively females. The LGMD, DM1, and FSHD cohorts had a relatively even distribution of males to females (41.9%, 50.7%, and 38.1% females, respectively). Marked muscle weakness and wasting was observed in patients with dystrophinopathies and LGMD that required the use of a manual or powered wheelchair for ambulatory support in 25 (59.5%) and 7 (22.6%) patients, respectively (Table 8.1). Comorbidities including dyslipidemia, diabetes, and hypertension were prevalent across the various types of MD (Table 8.1). Respiratory disease was highly prevalent in patients with dystrophinopathies and DM1 and diagnosed in 24 (57.1%) and 37 (52.1%) patients, respectively. Furthermore, sleep-disordered breathing was diagnosed in 14 (33.3%) and 21 (29.6%) patients, respectively, reflected in the high use of respiratory therapies including lung volume recruitment, mechanical insufflation-exsufflation, and noninvasive ventilation (Table 8.1).

Table 8.1. Baseline Clinical Characteristics.

Characteristic	All Patients (N = 165)	Dystrophinopathies (n = 42)	LGMD (n = 31)	DM1 (n = 71)	FSHD (n = 21)	*P* value
Males/Females, No.	100 (60.6)/ 65 (39.4)	34 (81.0)/ 8 (19.1)	18 (58.1)/ 13 (41.9)	35 (49.3)/ 36 (50.7)	13 (61.9)/ 8 (38.1)	0.01
Age, Yrs	36.0 (23.0-49.0)	24.0 (19.0-35.3)	35.0 (23.0-52.0)	42.0 (32.3-49.8)	45.0 (24.0-54.0)	<0.001
Height	165.8 (157.5-175.3)	160.0 (146.5-165.1)	167.3 (165.0-174.7)	170.0 (158.8-177.9)	172.7 (161.8-176.5)	<0.001
Weight	70.0 (56.9-86.1)	66.1 (49.0-78.4)	73.3 (64.3-95.8)	70.3 (58.0-83.9)	75.8 (60.5-89.1)	0.21
BSA, m^2	1.80 (1.60-2.00)	1.74 (1.40-1.87)	1.89 (1.69-2.07)	1.86 (1.67-2.00)	1.83 (1.67-2.00)	0.10
Current/Former Smoker, No.	26 (15.8)	2 (4.76)	5 (16.1)	16 (22.5)	3 (14.3)	0.10

Ambulation, No.						
Cane/Walker	12 (7.27)	1 (2.38)	1 (3.23)	8 (11.3)	2 (9.52)	0.25
mWC/pWC	40 (24.2)	25 (59.5)	7 (22.6)	5 (7.04)	3 (14.3)	<0.001
Comorbidities, No.						
Anemia	5 (3.03)	3 (7.14)	2 (6.45)	0	0	0.09
Dyslipidemia	28 (17.0)	5 (11.9)	4 (12.9)	13 (18.3)	6 (28.6)	0.36
Diabetes	17 (10.3)	0	8 (25.8)	6 (8.45)	3 (14.3)	0.004
Hypertension	19 (11.5)	4 (9.52)	7 (22.6)	4 (5.63)	4 (19.1)	0.06
Respiratory Disease	71 (43.0)	24 (57.1)	4 (12.9)	37 (52.1)	6 (28.6)	<0.001
SDOB	46 (27.9)	14 (33.3)	3 (9.68)	21 (29.6)	8 (38.1)	0.07
Respiratory Therapies, No.						
Lung Volume Recruitment	42 (25.5)	13 (31.0)	1 (3.23)	25 (35.2)	3 (14.3)	0.003

Mechanical Insufflation-Exsufflation	15 (9.09)	9 (21.4)	0	4 (5.63)	2 (9.52)	0.01
Noninvasive Ventilation	35 (21.2)	14 (33.3)	2 (6.45)	13 (18.3)	6 (28.6)	0.03
Vitals, median						
HR, bpm	75.0 (64.5-82.0)	80.0 (70.8-99.3)	70.0 (66.0-80.0)	70.5 (62.8-80.0)	75.0 (64.8-80.0)	0.02
sBP, mmHg	118.0 (107.3-129.8)	113.0 (103.0-122.0)	123.0 (113.0-133.0)	114.0 (106.0-122.0)	129.0 (120.0-139.8)	<0.001
dBP, mmHg	74.0 (68.0-82.0)	72.0 (66.0-78.0)	76.0 (69.0-90.0)	74.0 (64.3-79.5)	83.0 (76.0-87.5)	0.01

Values are n (%) or median (interquartile range). BSA, body surface area; dBP, diastolic blood pressure; DM1, type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; HR, heart rate; LGMD, limb-girdle muscular dystrophy; mWC, manual wheelchair; pWC, power wheelchair; sBP, systolic blood pressure; SDOB, sleep disordered breathing.

Baseline Cardiac Assessment and Risk Analysis

Despite the challenges of performing TTE in this cohort of patients due to physical limitations, and presence of respiratory and ambulatory support devices, study quality was technically limited in only 7 (4.24%) patients. Cardiomyopathy was diagnosed in 60 (36.4%) patients of the overall MD cohort with a median LVEF of 45.0 (IQR: 35.0-50.0) %. Specifically, LVEF was markedly reduced in the dystrophinopathies cohort ($P < 0.001$; Figure 8.1B). Although the composite DM1 cohort exhibited normal LVEF, patients with DM1 and LBBB had a markedly reduced LVEF compared to patients without LBBB ($P < 0.001$; Figure 8.1C). Importantly, we established a strong concordance between TTE and CMR-derived LVEF ($r = 0.84$; $P < 0.001$) by comparing the imaging of 84 patients, who received studies with both modalities within a median period of 4.08 (IQR: 1.35-13.1) months, in support of our diagnostic methodology (Figure 8.1D). Both the dystrophinopathies and LGMD cohorts showed mildly increased LV dimensions at end-systole (Table 8.2). There was no overt diastolic dysfunction in our MD cohort and right ventricular systolic dysfunction and dilation was minimal, which was supported by the available CMR studies (Table 8.2 and Table 8.3). Valvular heart disease classified as moderate or severe mitral or tricuspid regurgitation was present in 11 patients (6.67%) and was associated with LV systolic dysfunction (LVEF: 20.0 [IQR: 16.3-31.3] %) and LV dilation (LVIDd: 6.60 [IQR: 6.00-6.76] cm) (Table 8.4). 12-lead ECG revealed parameters within normal ranges in the dystrophinopathies, LGMD, and FSHD cohorts (Table 8.5). In contrast, DM1 patients exhibited prolonged PR intervals and QRS duration indicative of a high burden of conduction disease with 22 (31.0%) patients having first-degree AV block, 7 (9.86%) patients with left anterior fascicular block, and 16 (22.5%) patients with left bundle branch block (LBBB).

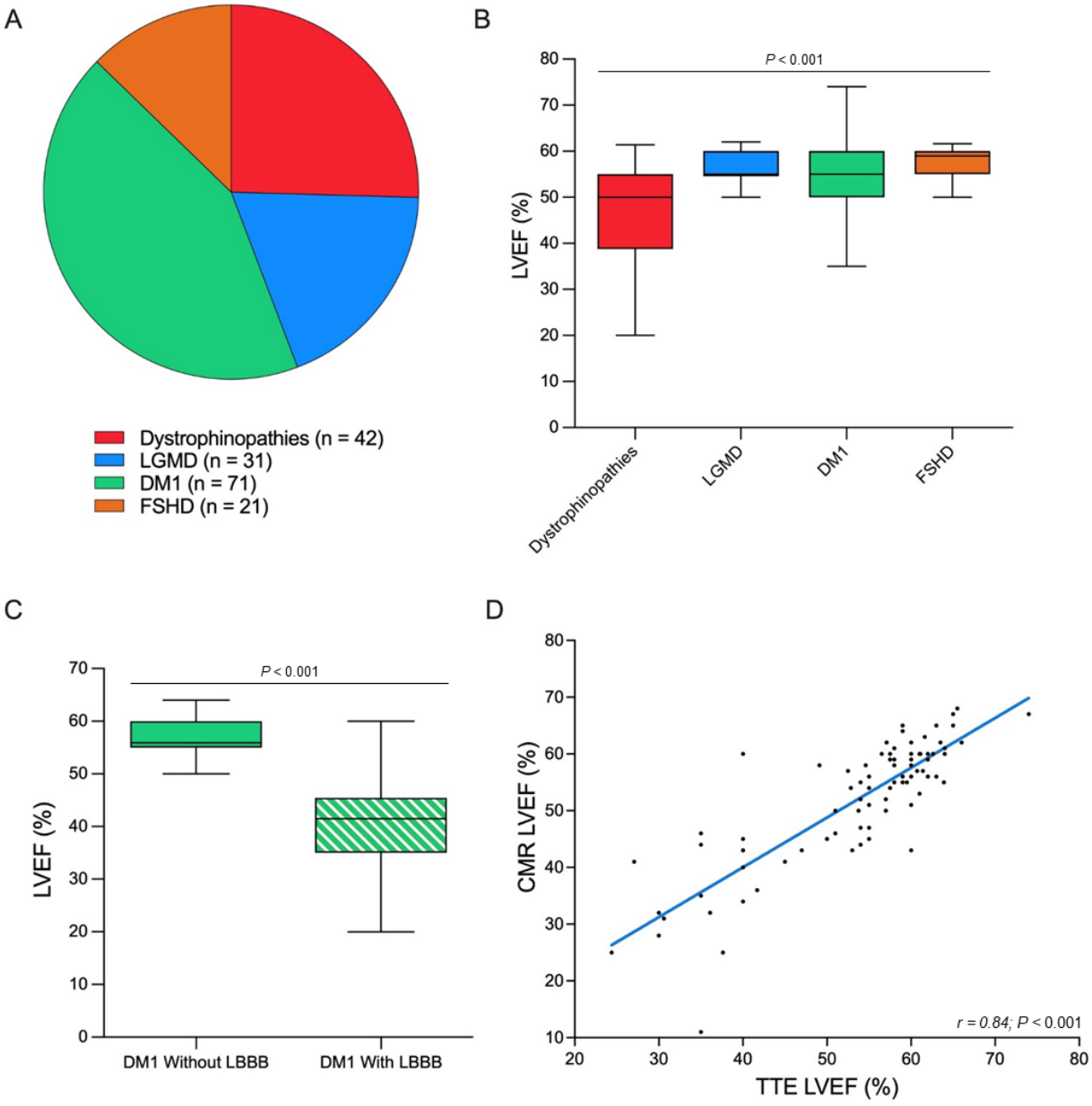


Figure 8.1. Cohort composition and LV systolic function. (A) Diagnoses and respective proportions of the study cohort. (B) Comparison of left ventricular ejection fraction (LVEF) amongst the cohorts of muscular dystrophy. (C) Comparison of LVEF between type 1 myotonic dystrophy (DM1) patients without versus with left bundle branch block (LBBB). (D) Concordance of cardiac magnetic resonance (CMR) and transthoracic echocardiogram (TTE) for the

quantification of LVEF demonstrated in 84 patients with both modalities available at baseline. FSHD indicates facioscapulohumeral muscular dystrophy; and LGMD, limb-girdle muscular dystrophy.

Table 8.2. Baseline Assessment of Left Ventricular Systolic Function and Dimensions.

Parameter	All Patients (n = 165)	Dystrophinopathies (n = 42)	LGMD (n = 31)	DM1 (n = 71)	FSHD (n = 21)	*P* value
IVSd, cm	0.84 (0.73-0.99)	0.80 (0.71-0.90)	0.87 (0.73-0.95)	0.91 (0.80-1.00)	0.82 (0.74-0.91)	0.12
LVPWd, cm	0.78 (0.70-0.92)	0.71 (0.64-0.77)	0.80 (0.71-0.96)	0.83 (0.70-0.98)	0.80 (0.74-	0.01
LVIDd, cm	4.50 (4.10-4.96)	4.52 (4.00-5.28)	4.77 (4.33-5.15)	4.40 (4.08-4.90)	4.45 (4.15-4.80)	0.10
LVIDs, cm	2.91 (2.61-3.49)	3.18 (2.73-4.34)	3.20 (2.81-3.85)	2.80 (2.60-3.08)	2.88 (2.54-3.05)	0.01

LVEF, %	55.0 (50.0-60.0)	50.0 (32.8-58.6)	55.0 (54.6-60.0)	55.0 (50.0-60.0)	57.5 (55.0-60.0)	0.01
LV mass index, g/m^2	69.4 (57.7-83.7)	80.4 (58.9-90.3)	74.2 (63.3-84.8)	64.7 (56.2-80.6)	65.1 (57.6-75.9)	0.14
LA volume index, mL/m^2	19.6 (15.3-25.6)	22.5 (16.1-34.7)	20.8 (15.3-26.0)	18.9 (15.4-23.8)	16.8 (14.6-21.0)	0.21
E-wave velocity, cm/s	78.0 (57.0-95.8)	89.6 (85.9-100.8)	75.4 (47.8-86.9)	73.0 (52.8-91.4)	83.1 (61.1-97.6)	0.11
A-wave velocity, cm/s	55.0 (45.7-64.0)	52.6 (41.6-56.6)	61.8 (55.5-65.3)	46.5 (44.5-56.1)	70.7 (64.7-74.3)	0.03
E/A ratio	1.35 (1.00-1.80)	1.40 (1.20-1.80)	1.28 (0.89-1.61)	1.49 (1.14-2.06)	1.25 (0.96-1.53)	0.15
Deceleration time, ms	196.0 (158.0-230.0)	175.0 (152.5-197.5)	193.0 (182.0-200.0)	215.0 (157.0-250.0)	160.0 (150.0-180.0)	0.55

e' velocity, cm/s	12.0 (9.76-16.9)	10.9 (10.0-11.5)	12.5 (12.0-14.1)	12.8 (9.60-16.9)	12.4 (9.55-11.6)	0.81
E/e' ratio	6.30 (5.0-7.50)	6.75 (5.83-7.83)	6.30 (4.85-7.05)	5.85 (4.63-7.10)	6.30 (4.95-9.40)	0.20
TAPSE. cm	2.10 (1.80-2.30)	1.80 (1.60-2.10)	2.00 (1.75-2.20)	215.0 (157.0-250.0)	2.20 (1.90-2.40)	0.02
RV systolic pressure, mmHg	24.7 (19.3-32.5)	35.3 (25.8-40.8)	29.9 (22.5-34.3)	20.3 (17.2-26.1)	32.9 (26.6-39.3)	0.05
Reduced RV systolic function, %	11 (6.67)	4 (9.52)	2 (6.45)	4 (5.63)	1 (4.76)	0.85
Dilated RV, %	8 (4.85)	1 (2.38)	1 (3.23)	5 (7.04)	1 (4.76)	0.69

Values are n (%) or median (interquartile range). DM1, type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; IVSd, interventricular septal dimension at end-diastole; LA, left atrial; LGMD, limb-girdle muscular dystrophy; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; LVPWd, left ventricular posterior wall at end-diastole; RV, right ventricular; TAPSE, tricuspid annular plane systolic excursion.

Table 8.3. Baseline Imaging by Cardiac Magnetic Resonance.

Parameter	All Patients (n = 84)	Dystrophinopathies (n = 21)	LGMD (n = 18)	DM1 (n = 33)	FSHD (n = 12)	*P* value
LVEDVi, mL/m^2	79.5 (63.3-97.3)	83.5 (80.3-120.0)	89.0 (71.3-101.0)	62.0 (54.5-73.3)	79.0 (61.0-85.0)	<0.001
LVESVi, mL/m^2	36.0 (26.0-48.8)	42.0 (30.5-78.0)	40.0 (31.0-49.0)	29.0 (20.5-36.3)	29.5 (23.3-44.5)	0.01
LVEF, %	55.0 (45.0-59.0)	49.0 (40.0-58.0)	54.5 (46.3-56.0)	55.0 (44.5-63.3)	56.5 (53.0-60.8)	0.18
LV mass index, g/m^2	49.0 (41.0-60.0)	56.0 (44.0-67.0)	50.0 (43.0-60.0)	42.0 (40.0-47.5)	48.5 (41.3-54.8)	0.01
LA volume index, mL/m^2	31.7 (27.8-43.8)	45.0 (39.8-47.1)	33.4 (28.2-46.0)	29.0 (25.7-30.4)	29.0 (27.9-32.5)	0.01
RVEDVi, mL/m^2	73.0 (61.0-85.0)	78.0 (69.0-87.0)	77.0 (63.0-84.0)	60.0 (55.8-72.8)	86.0 (68.0-96.5)	0.006

RVESVi, mL/m^2	36.0 (29.0-42.0)	36.0 (32.5-48.0)	40.0 (32.0-43.0)	31.0 (26.8-35.8)	40.0 (27.0-43.0)	0.09
RVEF, %	50.0 (46.0-55.0)	53.0 (45.0-58.0)	50.0 (47.0-53.0)	49.5 (46.0-55.3)	53.0 (48.0-54.5)	0.93

Values are n (%) or median (interquartile range). LA, left atrial; LV, left ventricle; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVESVi, left ventricular end-systolic volume index; RVEDVi, right ventricular end-diastolic volume index; RVEF, right ventricular ejection fraction; RVESVi, right ventricular end-systolic volume index.

Table 8.4. Baseline Echocardiogram Assessment of Valve Structure and Function.

Heart Valves	**Echocardiogram Assessment (n = 165)**	
	Structure	**Function**
Mitral Valve	2 Mild Sclerotic Thickening	6 Moderate Regurgitation, 1 Severe Regurgitation
Aortic Valve	10 Mild Sclerotic Thickening	1 Severe Regurgitation
Tricuspid Valve	Normal	3 Moderate Regurgitation, 1 Severe Regurgitation
Pulmonic Valve	Normal	Normal

Table 8.5. Baseline 12-Lead Electrocardiogram Assessment.

Parameter	All Patients (N = 165)	Dystrophinopathies (n = 42)	LGMD (n = 31)	DM1 (n = 71)	FSHD (n = 21)	*P* value
Heart Rate, bpm	71.0 (63.0-82.0)	78.0 (70.0-96.0)	70.0 (61.0-82.0)	68.0 (63.0-80.8)	73.0 (59.0-85.5)	0.07
RR Interval, ms	856.0 (732.0-1000.0)	811.0 (652.0-966.7)	882.0 (784.5-1023.5)	872.0 (760.0-988.0)	836.0 (695.5-996.0)	0.45
PR Interval, ms	162.5 (133.8-192.0)	132.0 (122.0-144.0)	154.0 (133.8-176.0)	192.0 (174.0-221.0)	162.0 (144.5-167.5)	<0.001
QRS Duration, ms	100.0 (92.0-112.0)	94.0 (88.0-114.0)	99.0 (92.0-110.0)	107.0 (96.0-126.0)	92.5 (89.0-103.0)	<0.001
QT Interval, ms	396.5 (373.5-432.0)	380.0 (369.0-397.0)	396.0 (383.5-428.0)	412.0 (391.0-455.0)	384.0 (365.5-418.0)	0.001
Corrected QT Interval, ms	438.2 (413.5-456.8)	426.7 (408.9-455.7)	428.4 (407.1-450.2)	449.0 (437.3-489.1)	431.8 (424.2-440.8)	0.01

Conduction Delay, No.						
1° AVB	22 (13.3)	0	0	22 (31.0)	0	-
LAFB	11 (6.67)	3 (7.14)	0	7 (9.86)	1 (4.76)	0.32
LBBB	22 (13.3)	4 (9.52)	2 (6.45)	16 (22.5)	0	0.02
RBBB	8 (4.85)	3 (7.14)	1 (3.23)	3 (4.23)	1 (4.76)	0.87

Values are n (%) or median (interquartile range). 1° AVB, first-degree atrioventricular block; DM1, type 1 myotonic dystrophy; FSHD, facioscapulohumeral muscular dystrophy; LAFB, left anterior fascicular block; LBBB, left bundle branch block; LGMD, limb-girdle muscular dystrophy; RBBB, right bundle branch block

There were 98 incidences of MACE recorded over the 7-year study period, observed in 47 patients. Overall, the group of patients with a LVEF < 55% (n = 60) also exhibited enlarged LV dimensions at end-diastole and end-systole ($P < 0.001$; Figure 8.2A). At baseline, patients with LVEF < 55% were at a substantially higher risk of MACE (aOR: 8.30; 95% confidence interval [CI]: 3.18-21.7; $P < 0.001$; Figure 8.2B and Table 8.6). Additionally, patients with an above median LVIDd > 4.50 cm (aOR: 3.32; 95% CI: 1.35-8.20; $P = 0.01$) and LVIDs > 2.96 cm (aOR: 4.00; 95% CI: 1.60-10.1; $P = 0.003$) were similarly at a higher risk of MACE in the adjusted model (Figure 8.2B and Supplemental Table 8.6).

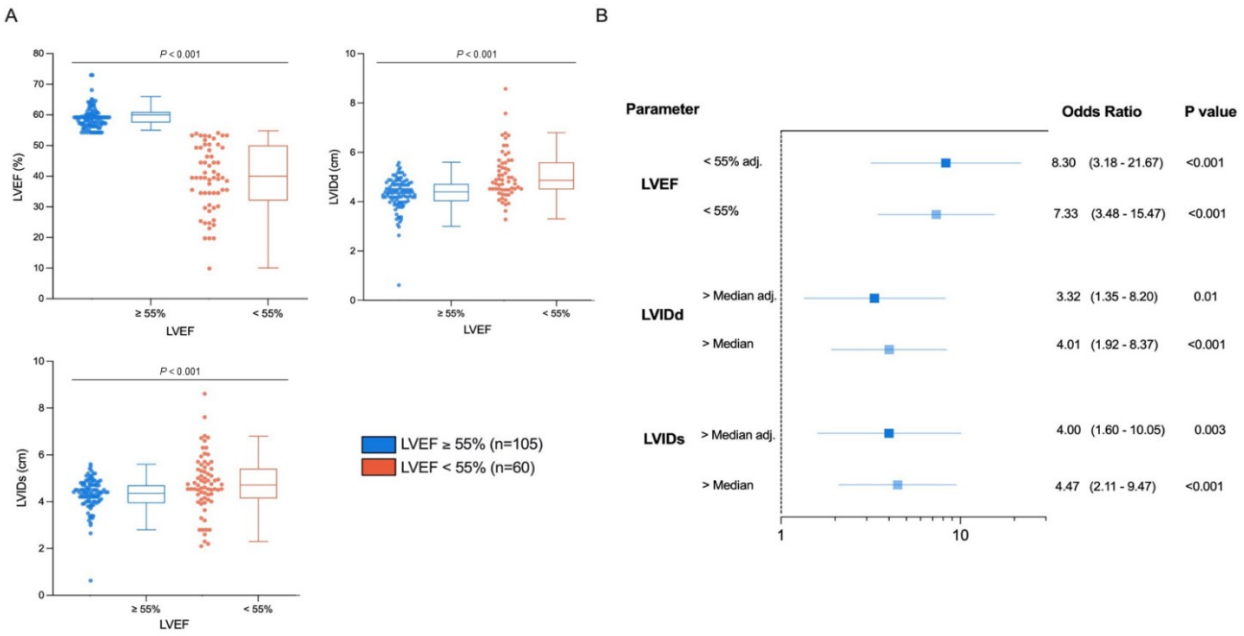


Figure 8.2. Patient classification and risk stratification. (A) Patients classified as having a left ventricular ejection fraction (LVEF) ≥ 55% or < 55% and the associated distributions of LVEF, left ventricular internal dimension at end-diastole (LVIDd), and left ventricular internal dimension at end-systole (LVIDs). (B) Association between LVEF < 55%, LVIDd > cohort median of 4.50 cm, and LVIDs > cohort median of 2.96 cm with major adverse cardiac events. FSHD indicates facioscapulohumeral muscular dystrophy; LGMD, limb-girdle muscular dystrophy.

Table 8.6. Multivariable logistic regression analysis to assess the prognostic ability of baseline measures of left ventricular systolic function and dimensions for major adverse cardiac events.

Parameter	OR (95% CI)	*P* value
LVEF		
Less than 55% (*n* = 60)	8.30 (3.18-21.7)	<0.001
LVIDd		
Greater than median (4.50 cm; *n* = 82)	3.32 (1.35-8.20)	0.01
LVIDs		
Greater than median (2.96 cm; *n* = 83)	4.00 (1.60-10.1)	0.003

Multivariable analysis adjusted for age, sex, and prescribed cardiac medical and device therapies. CI, confidence interval; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; OR, odds ratio.

Clinical Management and Risk Associated with Left Ventricular Ejection Fraction Trajectory

Structural and functional cardiac assessment at baseline by cardiac imaging, 12-lead ECG and Holter monitoring is central to decision making in the therapeutic management of MD patients. There was a marked increase in the proportion of patients prescribed angiotensin converting enzyme inhibitors or angiotensin receptor blockers ($P = 0.003$), beta-blockers ($P = 0.02$), and mineralocorticoid receptor antagonists ($P < 0.001$), along with an uptitration and optimization of dosages relative to guideline-defined maximum tolerated dose (Figure 8.3A and 8.3B and Table 8.7). Over the study period we also tracked the burden of arrhythmias, which included 16 incidences of atrial flutter or fibrillation and 14 incidences of ventricular tachycardia (VT) in our MD cohorts. Specifically, 12 incidences of atrial flutter or fibrillation and 9 incidences of VT were documented in DM1 patients. Cardiac devices including implantable cardiac defibrillator were implanted in 10 patients, single-chamber pacemaker in 10 patients, and CRT devices were implanted in 15 patients (Figure 8.3C).

Table 8.7. Guideline-Defined Maximum Tolerated Dose of Medications.

Drug	Maximal Dose (mg)		Drug	Maximal Dose (mg)
ACEi			**Beta-Blockers**	
Enalapril	20		Bisoprolol	10
Lisinopril	40		Carvedilol	50
Perindopril	8		Metoprolol	200
Ramipril	10			
ARB			**MRA**	
Candesartan	32		Spironolactone	50
Valsartan	320			

ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; and MRA, mineralocorticoid receptor antagonist. Maximal dose defined as per the 2021 American Heart Association Guidelines.

One hundred and twenty-four patients received a follow-up TTE study over a median period of 2.19 (IQR: 1.05-3.32) years. Serial LVEF measures were used to classify patients based on LVEF trajectory and used to assess the association with MACE (Figure 8.3D). Patients with reduced LVEF (median LVEF: 51.5 [IQR: 40.0-58.8] % to 44.0 [IQR: 35.0-49.8] %) were at markedly greater risk of MACE than patients with preserved function (aHR: 7.21; 95% CI: 1.99-26.1; P = 0.003; Table 8.8). Patients with improved function (median LVEF: 46.8 [IQR: 35.8-55.0] % to 55.0 [IQR: 50.0-60.0] %) had a comparable risk of MACE as patients with preserved function (aHR: 1.84; 95% CI, 0.49-6.91; P = 0.37; Table 8.8). Older age was an independent predictor of MACE while the changes in LVIDd and LVIDs was not associated with MACE (Table

8.8 and Supplemental Figure 8.4 and Table 8.9). Patients with follow-up cardiac imaging were at greater risk of heart disease progression compared to the 41 patients in which follow-up TTE was not available as shown by a higher prevalence of dystrophinopathies and DM1 (31.5% and 48.4% of patients, respectively), conduction disease, and use of ambulatory aids. At the end of the study period, the group of patients that received follow-up imaging did indeed have a higher incidence of MACE and all-cause mortality than the group of patients that did not receive follow-up cardiac imaging ($P = 0.002$ and $P = 0.05$, respectively).

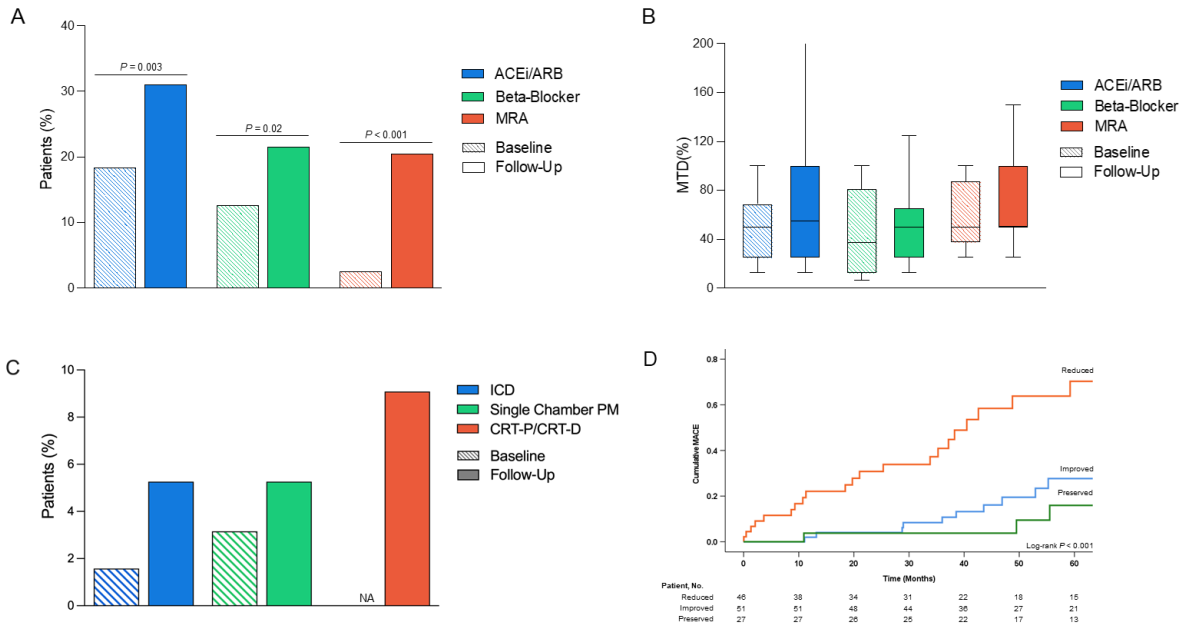


Figure 8.3. Medical and device therapy and associated clinical outcomes. (A) Baseline use of angiotensin converting enzyme inhibitor (ACEi) and angiotensin receptor blocker (ARB), beta-blockers, mineralocorticoid receptor antagonist (MRA) (before), and the initiation at the initial Neuromuscular Multidisciplinary Clinic visit (after). (B) Optimization of cardiac medical therapies following the initial visit. (C) Use of implantable cardiac defibrillator (ICD), single

chamber pacemaker (PM), and cardiac resynchronization therapy (CRT) implantation. (D) Kaplan-Meier analysis of patients stratified by left ventricular systolic function trajectory to assess its prognostic ability for major adverse cardiac events (MACE) in 124 patients that received serial imaging studies. Maximum tolerated dose (MTD) as defined by the 2021 American Heart Association Guidelines for the diagnosis and treatment of acute and chronic heart failure. NA indicates not applicable.

Table 8.8. Association Between LVEF Trajectory and MACE.

	Univariable Analysis		**Multivariable Analysis**	
Clinical Variables	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**
Preserved LVEF	Reference	-	Reference	-
Improved LVEF	1.88 (0.52-6.82)	0.34	1.84 (0.49-6.91)	0.37
Reduced LVEF	5.58 (1.66-18.7)	0.01	7.21 (1.99-26.1)	0.003
Age Group	1.14 (1.02-1.27)	0.02	1.22 (1.09-1.37)	0.001
Sex	1.66 (0.80-3.48)	0.18	1.33 (0.58-3.08)	0.50
Respiratory Disease	0.87 (0.42-1.78)	0.70	0.77 (0.36-1.67)	0.51
Cardiac Medications	2.48 (1.26-4.88)	0.01	2.16 (0.96-4.89)	0.06
Cardiac Devices	3.49 (1.44-8.45)	0.01	1.29 (0.47-3.54)	0.62

Assessment of the prognostic ability of patient categorization by left ventricular ejection fraction trajectory for major adverse cardiac events using Cox regression analysis in 124 patients that received serial cardiac imaging. CI, confidence interval; HR, hazard ratio; LVEF, left ventricular ejection fraction.

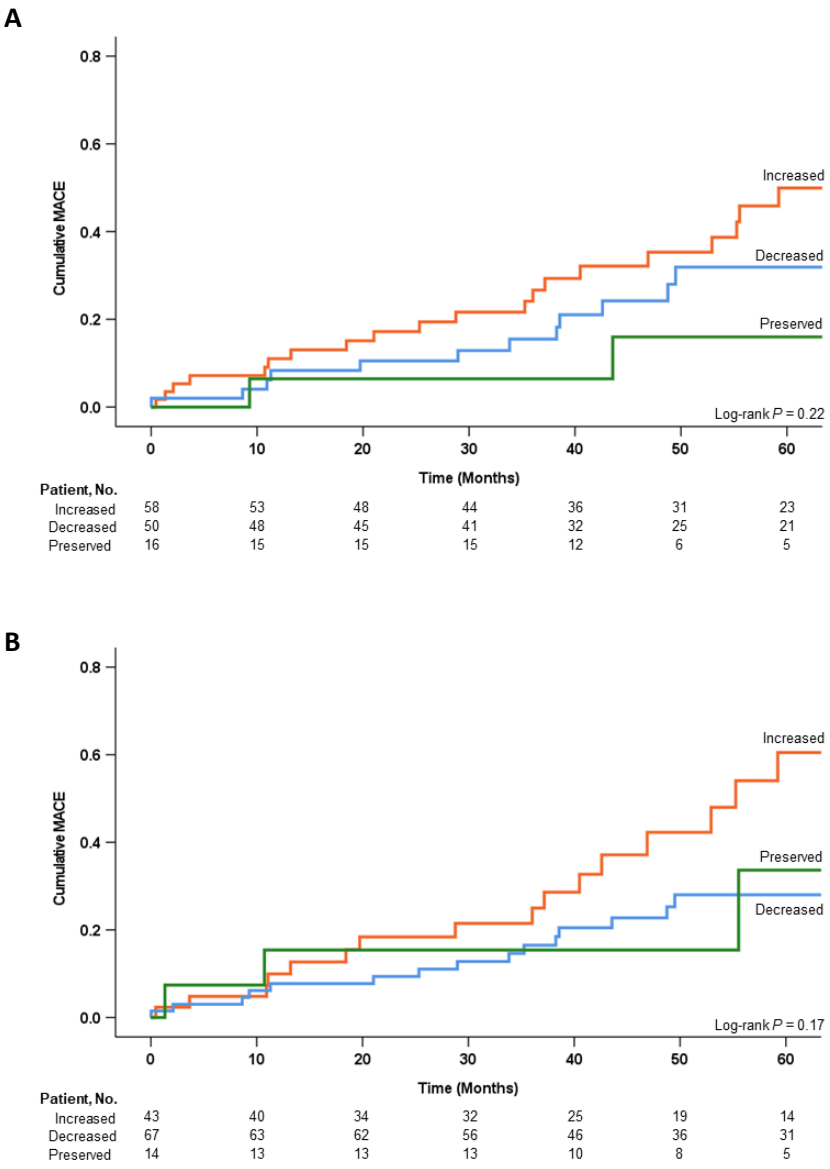


Figure 8.4. Kaplan-Meier analysis of patients stratified by the change in left ventricular dimension at (A) end-diastole and (B) end-systole to assess its prognostic ability for major adverse cardiac events (MACE) in 124 patients that received serial imaging studies.

Table 8.9. Association Between Change in Left Ventricular Dimensions and MACE.

	Univariable Analysis		Multivariable Analysis	
Clinical Variables	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**
Preserved LVIDd	Reference	-	Reference	-
Decreased LVIDd	1.94 (0.43-8.66)	0.39	2.43 (0.52-11.36)	0.26
Increased LVIDd	2.92 (0.68-12.5)	0.15	2.65 (0.60-11.7)	0.20
Age Group	1.14 (1.02-1.27)	0.02	1.15 (1.03-1.29)	0.01
Sex	1.66 (0.80-3.48)	0.18	1.70 (0.74-3.89)	0.21
Respiratory Disease	0.87 (0.42-1.78)	0.70	0.85 (0.39-1.87)	0.69
Cardiac Medications	2.48 (1.26-4.88)	0.01	1.84 (0.82-4.10)	0.14
Cardiac Devices	3.49 (1.44-8.45)	0.01	1.97 (0.72-5.38)	0.19
Preserved LVIDs	Reference	-	Reference	-
Decreased LVIDs	0.96 (0.28-3.31)	0.95	1.21 (0.32-4.67)	0.78
Increased LVIDs	1.82 (0.53-6.25)	0.34	1.59 (0.43-5.92)	0.49
Age Group	1.14 (1.02-1.27)	0.02	1.14 (1.01-1.29)	0.03
Sex	1.66 (0.80-3.48)	0.18	1.50 (0.65-3.51)	0.34
Respiratory Disease	0.87 (0.42-1.78)	0.70	0.88 (0.41-1.91)	0.76
Cardiac Medications	2.48 (1.26-4.88)	0.01	1.87 (0.83-4.20)	0.13
Cardiac Devices	3.49 (1.44-8.45)	0.01	2.01 (0.71-5.69)	0.19

Assessment of the prognostic ability of patient categorization by left ventricular dimensions for major adverse cardiac events using Cox regression analysis in 124 patients that received serial cardiac imaging. CI, confidence interval; HR, hazard ratio; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole.

Patients with LBBB represents a traditional group of patients with a high burden of adverse events as illustrated by the greater incidence of MACE in our cohort of patients (LBBB (n = 22) vs non-LBBB (n = 143); aOR: 24.4; 95% CI, 5.70-104.4; $P < 0.001$). Fifteen out of 22 patients in our cohort with LBBB received a CRT device, which included 13 patients with DM1 and a single DMD and LGMD patient. Baseline QRS duration was 170.3 (± 6.66) ms and subsequently reduced to 160.7 (± 7.99) ms (Figure 8.5A). Baseline LVEF was 36.0% (± 3.31%) which improved to 43.7% (± 3.79%) ($P = 0.02$; Figure 8.5B). Furthermore, a reduction in QRS duration was associated with an increase in LVEF ($\beta = 3.30 \pm 1.56$; $P = 0.04$) and in patients with a reduced QRS duration in response to CRT pacing, the LVEF improved from 37.2 (± 3.32) % to 50.0 (± 2.57) % (n = 11 patients; $P = 0.04$). Illustrative examples of these divergent responses to CRT are further highlighted in a patient with reduced QRS duration resulting in a modest increase in LVEF (Figure 8.6A) while in a patient with more advanced cardiomyopathy, lengthening of the QRS complex post-CRT was associated with a decline in LVEF (Figure 8.6B).

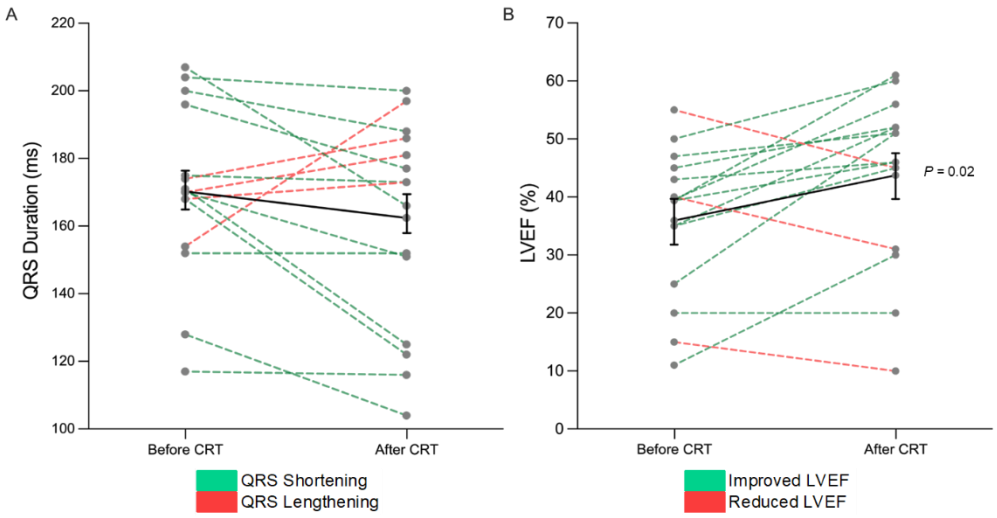
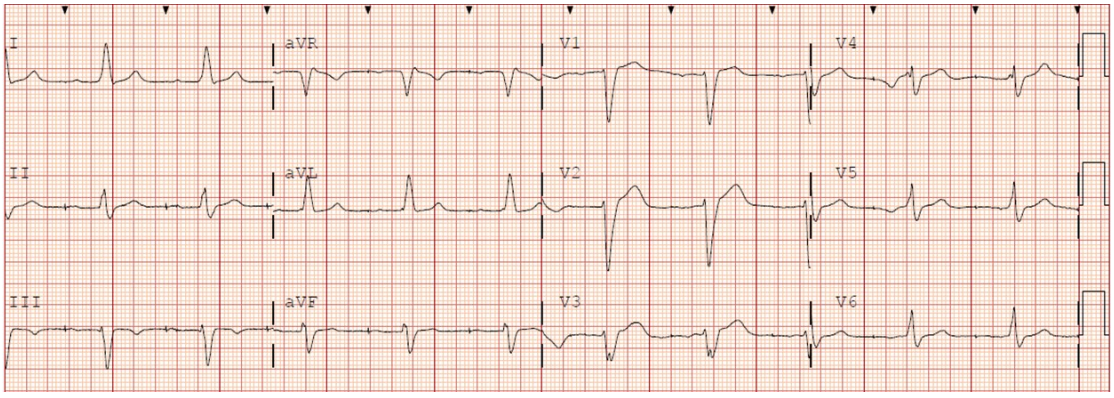


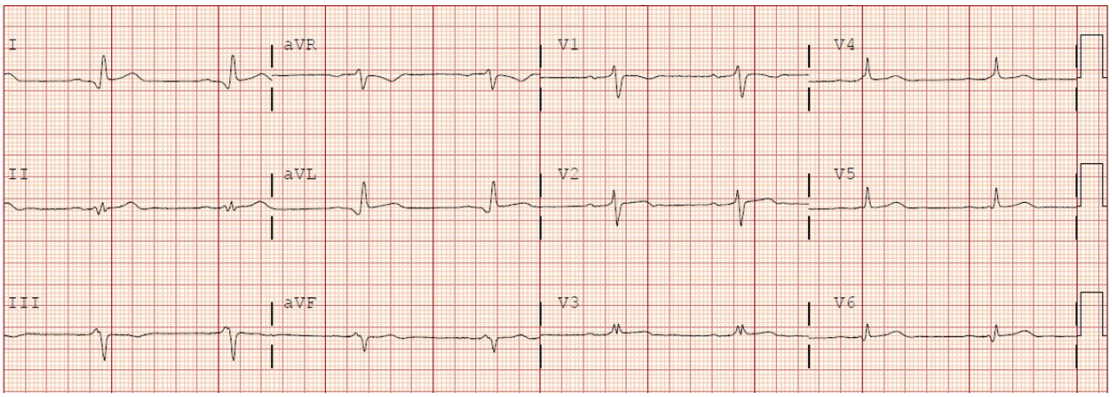
Figure 8.5. Impact of CRT. (A) Change in QRS duration in 15 patients with left bundle branch block that received a cardiac resynchronization therapy (CRT) device. (B) Change in left ventricular ejection fraction (LVEF) following CRT device implantation

A

Before CRT

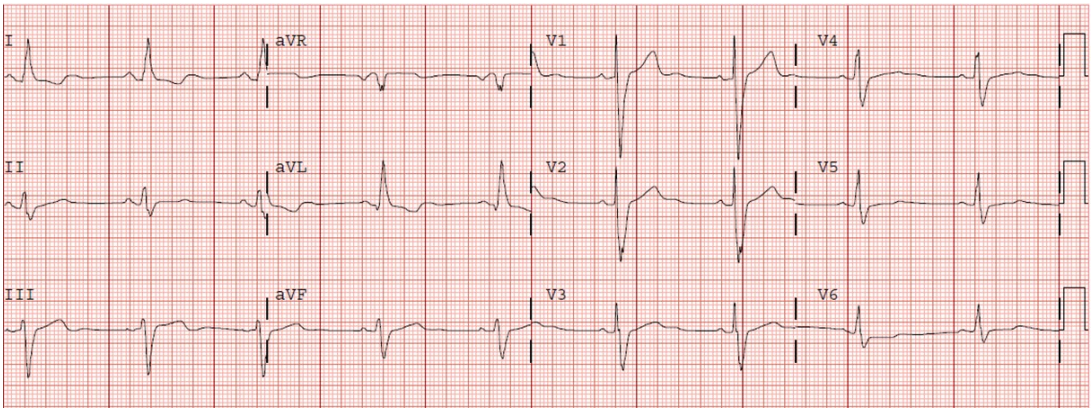


After CRT



B

Before CRT



After CRT

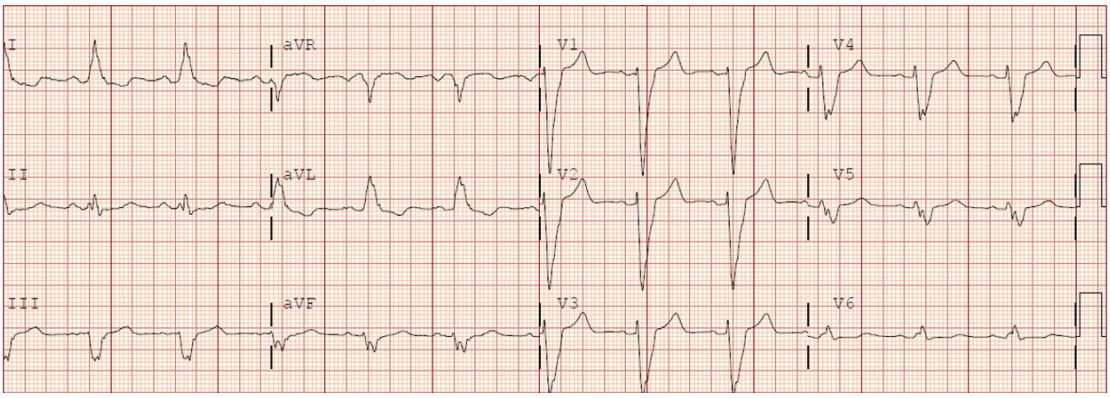


Figure 8.6. Before and after ECG representations of LBBB treated by CRT. (A) The initial 12-lead electrocardiogram (ECG) of a 58-year-old male patient with type 1 myotonic dystrophy with an atrial-paced rhythm, first-degree atrioventricular block, and left bundle branch block (LBBB) with a QRS duration of 168 ms (HR = 64 beats/min, PR = 272 ms, QT = 446 ms, QT corrected [QTc] = 461 ms) and left ventricular ejection fraction (LVEF) of 35.0% and a follow-up 12-lead ECG after cardiac resynchronization therapy (CRT) device implantation showing a biventricular paced rhythm with a QRS duration of 122 ms (HR = 50 beats/min, PR = 240 ms, QT = 455 ms, QTc = 415 ms) and a LVEF which recovered to 52.0%. (B) The initial 12-lead ECG of

a 25-year-old male patient with Duchenne muscular dystrophy with a LBBB with a QRS duration of 154 ms (HR = 53 beats/min, PR = 164 ms, QT = 488 ms, QTc = 459 ms) and left ventricular ejection fraction (LVEF) of 15.0% and a follow-up 12-lead ECG after CRT device implantation showing a biventricular paced rhythm with further QRS complex widening of 197 ms (HR = 70 beats/min, PR = 166 ms, QT = 485 ms, QTc = 524 ms) and a LVEF which declined to 10.0% (B).

8.5. Discussion

We performed the first prospective study investigating the prognostic value of TTE-derived LVEF assessment for MACE prognostication in a mixed adult cohort of MD and illustrated the value of early initiation of medical and device therapies in these patients. Transthoracic echocardiography is feasible and reproducible in a pediatric cohort of patients with DMD, BMD, and LGMD.[252] Given that most pediatric patients with MD now progress into adulthood, our study specifically examined the use of TTE and its prognostic utility in adult MD patients. Firstly, our investigation demonstrated the prognostic value of baseline LVEF and LV dimensions for the incidence of MACE. Secondly, we demonstrated the prognostic value of LVEF trajectory for the incidence of MACE. Additionally, LVEF can be used to distinguish the composite dystrophinopathies cohort, which was characterized by markedly reduced LVEF, from other types of MD. Left ventricular ejection fraction was also effective in distinguishing DM1 patients with LBBB versus those without LBBB, and was a prominent marker of the impact of CRT device intervention.[248] Our cohort showed minimal RV involvement indicating that the RV is functionally spared in heart disease secondary to MD.[253, 254] Importantly, we successfully assessed cardiac structure and function in our MD cohort while encountering technical limitations in only 4.24% of

studies despite a high prevalence of ambulatory limitations and respiratory disease.[255] This was made possible by a high standard of awareness and education at our imaging center with trained echosonographers, care providers, and nursing staff whom are experienced in caring and assessing these patients. Utility of CMR in this cohort remains limited due to the use of spinal stabilization rods, wheelchair dependence, and mouthpiece ventilation, and we demonstrate that TTE is an effective alternative to obtain LVEF.[250] Furthermore, use of TTE allows for an effective allocation of care to patients with MD by identifying patients with reduced LVEF and chamber dilation that require active surveillance to monitor disease progression and associated adverse outcomes.

Transthoracic echocardiography was a central aspect of clinical assessment for our patient cohort, which guided the management of care. Moreover, LVEF was an important indicator of cardiac status and we demonstrated that TTE-derived LVEF is an appropriate alternative to CMR given their strong concordance. We identified 124 out of the 165 patients that required follow-up imaging based on their assessment and diagnosis. Clinical management of our patients included use of medical and device therapies was effective in improving both cardiac and all-cause clinical outcomes in our patients with MD. Low cardio-metabolic requirement of MD patients who ambulate or exercise infrequently results in masking of the clinical signs and symptoms of HF are often classified as pre-HF (stage B HF) and are increasingly recognized as patients needing appropriate early medical and device therapies.[256-258] Furthermore, our study highlights the response to medical therapies in adult MD patients, elucidated through our serial cardiac assessment, which was not defined previously. Specifically, CRT utilization in response to LBBB in patients with DM1 corrects the electromechanical dyssynchrony that led to a marked reduction in LVEF.[248] Accordingly, we demonstrated a direct association between QRS duration reduction and LVEF improvement. Transthoracic echocardiography has been previously demonstrated as a

useful modality for the tracking of LV dysfunction and adverse remodeling in patients with DM1.[259] Adjacent studies have demonstrated the utility of TTE in patients with LBBB to track responsiveness to CRT intervention, which has led to improved clinical outcomes.[260, 261] Patients with LBBB and LV systolic dysfunction with or without a history of ventricular arrhythmias should receive a CRT-D device for secondary or primary prophylaxis, which is particularly relevant for patients with DM1.[247, 248, 262] Cardiac device intervention is an important consideration in these patients as the response to pharmacological therapies is limited given the challenge of prescribing higher doses in patients with MD. Furthermore, the use of CRT earlier in the disease process can substantially improve LV systolic function in patients with LBBB, which has implications on cardiovascular outcomes as described in this study and our previous work.[248]

Baseline cardiac imaging of the composite cohort identified a reduction in LVEF as the defining characteristic of heart disease in MD, which was not accompanied by overt LV dilation, as shown by the assessment of ventricular dimensions and volumes. Our first segment of risk analysis demonstrated that patients with a LVEF <55% were at a higher risk of MACE. Furthermore, patients with above median LV end-diastole and end-systole dimensions also had an elevated risk of MACE. The clinical utility of ventricular dimensions simultaneously provide insight on cardiac remodeling and contractility, while being prognostic for adverse outcomes.[263, 264] However, our cohort only demonstrated mild LV dilation and the majority of our patients did not exhibit ventricular dilation as defined by the cardiac imaging guidelines.[211, 265] In contrast, LVEF is an easily obtained, objective, and conventional parameter of cardiac function, which can augment the clinical care process by guiding management before deterioration to symptomatic HF and experience of adverse cardiac events.[211] In addition, the use of biomarkers such as BNP and

high-sensitive troponin I can be incorporated to support more accurate prognostication of MACE in this patient population.[21, 244]

We also conducted a risk analysis to assess the association between LVEF trajectory and MACE. The utility of serial LVEF evaluation for MACE has been previously demonstrated in traditional HF,[266, 267] but never before in MD. We determined that patients with a reduction in LVEF on follow-up TTE were at greater risk of MACE than patients with preserved LVEF, while dimensional changes lacked independent prognostic value. There were no differences between the proportion of patients prescribed cardiac medications and device therapies among the groups. This implies that despite an optimization of therapies, patients that show a decrease in LVEF trajectory, characterized by their older age and potential genetic susceptibility may require more frequent follow-up given their elevated risk for MACE. Persistent reduction in cardiac function can be driven by adverse myocardial remolding such as the presence of myocardial fibrosis identified using biomarkers in patients with MD.[268] The efficient use of cardiac imaging allows early therapeutic intervention thereby slowly the progression to advanced heart disease. These findings can be applied to various types of MD regardless of sex, prevalence of respiratory disease, or prescription of cardiac therapies and devices, which were the covariates included in our regression analysis. We note that age served as an independent predictor of MACE, which has been demonstrated in traditional HF with reduced ejection fraction,[269] but has not been formally evaluated in MD. Moreover, patients with DMD and DM1 have a high burden of ventricular tachyarrhythmias and are at risk of sudden cardiac death.[247, 270]

We recognize several limitations in our study. Given our modest cohort size, we were unable to complete sub-group analyses to assess and compare the utility of cardiac imaging parameters amongst the different types of MD. Additionally, the use of standardize dimensional

measures by body surface area was limited given the degree of progressive muscle wasting and adipose tissue accumulation in these patients. Tissue characteristics and strain imaging can capture early cardiac involvement in patients with DMD with prognostic implications[271] and it is therefore justifiable to assess these techniques in future studies with a comparable cohort.

8.6. Conclusion

Patients with MD have a high burden of heart disease characterized by a progressive reduction of LV systolic function and a high incidence of MACE. This investigation demonstrated the prognostic utility of baseline and serial measures of LVEF for MACE in a mixed adult cohort of MD. Transthoracic echocardiography remains a feasible and useful modality for obtaining LVEF and should therefore be a central component of the cardiac assessment of patients with MD to facilitate effective clinical management.

9. Evaluating the Clinical Utility of Cardiac Magnetic Resonance in Patients with Muscular Dystrophy

9.1. Abstract

Background: Heart disease remains the leading cause of morbidity and mortality in patients with muscular dystrophy (MD). Given its rapid progression, functional and structural characterizations of cardiac status need to be made in advance of overt heart disease. The utility of cardiac magnetic resonance (CMR) including strain analysis and tissue characterization has not been

comprehensively investigated in adult patients with MD. This investigation evaluated the diagnostic value of CMR to guide the cardiac care of a heterogenous MD patient cohort to detect early heart disease and to determine its prognostic ability for major adverse cardiac events (MACE) and all-cause clinical outcomes.

Methods: Our cohort study prospectively enrolled 87 patients with MD (median age, 35.0 (interquartile range [IQR], 21.0-48.0) years; 34 [39.1%] females) over a 7.5-year period from November 5, 2014, to April 7, 2022. Patients diagnosed with a dystrophinopathy including carriers, limb-girdle MD, and type 1 myotonic dystrophy were recruited at the neuromuscular multidisciplinary clinic (University of Alberta, Canada). We conducted baseline CMR and analyzed conventional parameters as well as global strain, T1 mapping, and late gadolinium enhancement (LGE) for all patients and tracked clinical outcome data including MACE for risk stratification by median values.

Results: Cardiomyopathy was diagnosed in 48 (55.2%) patients of the broader cohort (median LVEF: 45.9 [IQR: 41.2-50.9] %). Cardiac fibrosis was highly prevalent in 24 out of 27 (88.9%) of patients with dystrophinopathies that receive late gadolinium enhancement, which showed differentiating patterns of fibrosis compared to LGMD and DM1 patients. Forty-five MACE occurred over the 7.5-year study period. At baseline, patients with a longitudinal strain amplitude < -13.0% had a high risk of MACE (adjusted odds ratio [aOR]: 6.38; 95% confidence interval [CI]: 1.17-34.7). Similarly, patients with a circumferential strain amplitude < -13.6% were at a

marked risk of MACE (aOR: 14.3; 95% CI: 2.14-95.6). Chamber dilation indicated by indexed left ventricular volumes showed comparable prognostic utility.

Conclusion: Cardiac magnetic resonance has important clinical utility for patients with MD. The use of conventional parameters of function and structure alongside strain analysis and tissue characterization are valuable clinical tools for the diagnosis of heart disease and prognostication of adverse clinical outcomes in patients with MD.

9.2. Introduction

Heart disease is recognized as a common cause of morbidity and mortality in patients with muscular dystrophy (MD).[4-6, 142, 244] Heart disease is prevalent in patients with Duchenne muscular dystrophy (DMD) and Becker's muscular dystrophy (BMD), and limb-girdle muscular dystrophy (LGMD), and is characterized by ventricular dilation, reduced systolic function, and ventricular tachyarrhythmias.[245, 246, 272] Symptomatic heart disease has also been documented in female carriers of DMD and BMD.[73] Conduction disease and arrhythmias are prevalent in patients with type 1 myotonic dystrophy (DM1), and left bundle branch block (LBBB) is associated with a marked reduction in left ventricular (LV) systolic function.[247, 248] A relatively milder cardiac phenotype is observed in patients with facioscapulohumeral muscular dystrophy (FSHD).[244, 273] Given the rapid progression of heart disease and increased risk for major adverse cardiac events (MACE) associated with MD, it is critical that an effective method of cardiac assessment be established so that medical and device therapies can be prescribed well in advance of overt heart disease.

Cardiac magnetic resonance is referred to as the gold standard for cardiac imaging and assessment over echocardiography.[274-277] Its functionality, sensitivity, and accuracy make it an appreciable clinical asset, which is still underutilized in the MD patient population. Imaging quality is also not affected by scoliosis, obesity, or lung disease, which are common comorbidities associated with MD that obstruct acoustic windows in the more commonly used transthoracic echocardiography (TTE). Advanced CMR techniques such as myocardial strain analysis provide a highly granular assessment of contractile function, which can be used to detect subclinical heart disease.[278] Tissue characterization by T1 mapping can assess cardiac structure for fibrosis as a key feature in the adverse remodeling associated with MD.[279, 280] Our study is the first to demonstrate the diagnostic and prognostic utility of CMR, strain analysis, and tissue characterization in a mixed adult cohort of MD. Importantly, our findings can be applied to patients with subclinical heart disease and can be used for the surveillance of disease progression following diagnosis to ensure that medical intervention is administered effectively for improved prognosis.

9.3. Methods

Study Cohort

Our prospective study recruited 87 patients with MD from the Neuromuscular Multidisciplinary (NMMD) clinic at the Kaye Edmonton Clinic, University of Alberta (Edmonton, Canada) over a 7.5-year period from November 5, 2014, to April 7, 2021. Neurological assessment, muscle biopsy, and genetic testing confirmed the diagnosis of MD, including dystrophinopathies (33 patients; 16 DMD, 11 BMD, 4 DMD carrier, and 2 BMD carrier patients), LGMD (28 patients), and DM1 (26 patients). For this investigation, patients

diagnosed with a dystrophinopathy as well as carriers were grouped together as "dystrophinopathies" given their similarity in clinical presentations and management practices.[144] Patients were referred to the NMMD clinic at various stages of their disease and recruited to this study with no bias towards patients with overt cardiac symptoms. All patients received collaborative multidisciplinary care from specialist physicians that implemented guideline-based medical therapy, including device intervention when appropriate, and follow-up care. All clinical data such as clinical assessment and history, 12-lead electrocardiogram (ECG) parameters, medical therapy including pharmacological therapies and device implantation, and clinical outcomes for this investigation were obtained by electronic chart review. The investigation was approved by the Health Research Ethics Board at the University of Alberta and all patients provided informed and written consent at the time of study enrollment.

Cardiac Magnetic Resonance

In collaboration with the Mazankowski Alberta Heart Institute (MAHI) at the University of Alberta, CMR was performed at the MAHI using a 1.5T Sonata scanner (Siemens Healthcare, Erlangen, Germany) and assessed using commercially available image analysis software, Syngo Argus (Siemens Healthcare),[251] by a blinded and experienced CMR interpreter. Conventional structural and functional measures were obtained in accordance with current guidelines.[281] We elected to index volume measures to by both height and body surface area (BSA) to assess the influence of body composition on our data points. Advanced analyses were completed using CVI42 software suite (Circle Cardiovascular Imaging Inc., Calgary, Canada) at a centralized imaging center.

Classification of Patients and Study Endpoints

Patients were divided into dichotomous groups for each parameter of interest (left ventricular ejection fraction [LVEF], left ventricular end-diastolic volume [LVEDV] indexed by BSA and height, left ventricular end-systolic volume [LVESV] indexed by BSA and height, left ventricular (LV) mass indexed by BSA and height, longitudinal strain amplitude, circumferential strain amplitude, radial strain amplitude, and cardiac fibrosis indicated by LGE). Patients were classified as having a LVEF ≥ 55.0% or < 55.0%. Patients were also classified based on indexed LVEDV or LVESV below or above the median (height: 89.6 mL/m and 41.3 mL/m, respectively; BSA: 84.7 mL/m^2 and 42.6 mL/m^2, respectively). We classified patients based on indexed LV mass below or above the median (height: 43.4 g/m and BSA: 45.8 g/m^2). Additionally, patients were classified based on longitudinal strain amplitude, circumferential strain amplitude, and radial strain amplitude below or above the median (-13.0%, -13.6%, and 35.3%, respectively). Twenty-three patients had LGE. Patients with a LVEF ≥ 55.0%; indexed LVEDV, LVESV, and LV mass less than or equal to their respective median value; and longitudinal strain amplitude, circumferential strain amplitude, and radial strain amplitude greater than or equal to their respective median value; and negative for LGE, were used as reference groups for risk analyses.

Major adverse cardiac events were defined as a composite of arrhythmia, device implantation, cardiac-related hospitalization, incident heart failure (HF), and cardiac-mortality. Arrhythmias such as atrial and ventricular tachyarrhythmias were captured by 12-lead ECG and upon routine device interrogation. Incident HF was diagnosed following a comprehensive cardiac assessment, which considered symptoms and signs such as dyspnea, orthopnea, peripheral edema, and abdominal distention. Outcome data such as hospitalizations, mortality, and associated diagnoses were obtained from provincial electronic health records.

Statistical Analysis

Continuous variables analyzed were compared using a Kruskal-Wallis test. Categorical variables were compared using Pearson chi-square tests. We incorporated logistic regression models to derive adjusted odds ratios (aOR) for the relationship between LVEF, LVEDV indexed by BSA and height, LVESV indexed by BSA and height, LV mass indexed by height and weight, longitudinal strain amplitude, circumferential strain amplitude, radial strain amplitude, and cardiac fibrosis identified by LGE with the incidence of MACE. These were multivariable regression models, which adjusted for age group, sex, diagnosis of respiratory disease, and the baseline use of cardiac medications and devices. Age was grouped into 5-year intervals and respiratory disease was defined as chronic obstructive pulmonary disease, asthma, recurrent aspiration pneumonia, respiratory muscle weakness, or restrictive lung disease. All statistical analyses were performed in R version 4.0.3 and a P value < 0.05 was considered significant.

9.4. Results

Clinical Characteristics

We collected baseline data on the 87 MD patients that were recruited to this study (Figure 9.1) The median age of the study cohort was 35 (interquartile range [IQR]: 21.0-48.0) years, comprised of 34 (39.1%) females. Within the dystrophinopathies cohort, DMD and BMD patients were exclusively younger males, while carriers were exclusively females. The LGMD and DM1 cohorts had a relatively even distribution of males to females (50.0%, and 53.8% females, respectively). Marked muscle weakness and wasting was observed in patients with

dystrophinopathies and LGMD that required the use of a manual or powered wheelchair for ambulatory support in 16 (48.5%) and 8 (28.6%) patients, respectively (Table 9.1). Comorbidities including dyslipidemia, diabetes, and hypertension were prevalent across the various types of MD (Table 9.1). Respiratory disease was highly prevalent in patients with dystrophinopathies and DM1 and diagnosed in 19 (57.6%) and 13 (50.0%) patients, respectively. Furthermore, sleep-disordered breathing was diagnosed in 11 (33.3%) and 7 (26.9%) patients, respectively, reflected in the high use of respiratory therapies including lung volume recruitment, mechanical insufflation-exsufflation, and noninvasive ventilation (Table 9.1). By contrast patients with LGMD had a relatively lower prevalence of respiratory comorbidities (Table 9.1).

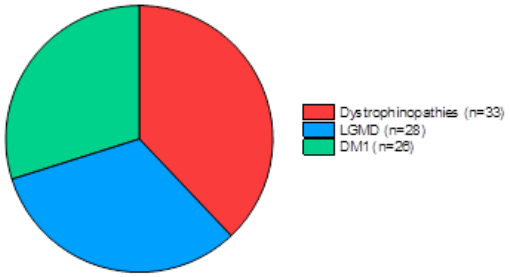


Figure 9.1. Muscular dystrophy patient cohort.

Table 9.1. Baseline Clinical Characteristics.

Characteristic	Dystrophinopathies (n = 33)	LGMD (n = 28)	DM1 (n = 26)	*P* Value
Males/Females, No.	27 (81.8)/ 6 (18.2)	14 (50.0)/ 14 (50.0)	12 (46.2)/ 14 (53.8)	0.01
Age, Yrs	20.0 (18.0-33.0)	36.0 (23.0-49.0)	46.0 (41.0-50.0)	<0.001
Height	157.5 (144.0-171.5)	170.2 (165.0-178.1)	168.0 (162.6-178.0)	0.01
Weight	71.2 (50.6-82.0)	80.0 (67.0-91.2)	77.6 (69.5-91.1)	0.08
BSA, m^2	1.70 (1.38-1.82)	1.91 (1.72-2.03)	1.91 (1.72-2.02)	0.04
Current/Former Smoker, No.	2 (6.06)	3 (10.7)	4 (15.4)	0.50
Ambulation, No.				
Cane/Walker	2 (6.06)	1 (3.57)	2 (7.69)	0.43
mWC/pWC	16 (48.5)	8 (28.6)	1 (3.85)	0.001
Comorbidities, No.				
Dyslipidemia	2 (6.06)	2 (7.14)	4 (15.4)	0.42
Diabetes	0	5 (17.9)	1 (3.85)	0.10
Hypertension	5 (15.2)	3 (10.7)	2 (7.69)	0.66
Respiratory Disease	19 (57.6)	6 (21.4)	13 (50.0)	0.01
SDOB	11 (33.0)	2 (7.14)	7 (26.9)	0.05
Respiratory Therapies, No.				
Lung Volume Recruitment	9 (27.3)	4 (14.3)	11 (42.3)	0.07
Mechanical Insufflation-Exsufflation	5 (15.2)	0	0	-
Noninvasive Ventilation	9 (27.3)	2 (7.14)	4 (15.4)	0.11
Medications, No.				
ACEi/ARB	18 (54.5)	6 (21.4)	3 (11.5)	<0.001
Beta Blocker	10 (30.3)	4 (14.3)	4 (15.4)	0.22
MRA	5 (15.2)	1 (3.57)	0	0.13
Vitals, median				
HR, bpm	80.0 (70.0-90.0)	73.5 (67.5-85.0)	70.0 (61.0-80.0)	0.08
sBP, mmHg	110.5 (103.3-127.5)	120.0 (109.3-127.8)	114.0 (108.0-122.0)	0.16
dBP, mmHg	72.0 (66.5-78.0)	78.0 (69.3-83.3)	73.0 (69.0-81.0)	0.16

Values are n (%) or median (interquartile range). ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BSA, body surface area; dBP, diastolic blood pressure; DM1, type 1 myotonic dystrophy; HR, heart rate; LGMD, limb-girdle muscular dystrophy; MD, mWC, manual wheelchair; pWC, power wheelchair; sBP, systolic blood pressure; SDOB, sleep disordered breathing.

Cardiac Assessment and Diagnostic Utility of Cardiac Magnetic Resonance

Cardiomyopathy was diagnosed in 37 (42.5%) patients of the overall MD cohort with a median LVEF of 55.0 (IQR: 45.0-59.0) %. Left ventricular ejection fraction was markedly reduced in the dystrophinopathies cohort ($P = 0.03$; Table 9.3). Both the dystrophinopathies and LGMD cohorts showed mildly increased LV volumes at end-diastole and end-systole (Table 9.3). Note that indexing by height versus BSA reduced the spread between parameters by cohort. There was no overt diastolic dysfunction in our MD cohort and right ventricular (RV) systolic dysfunction was minimal; we note relative RV dilation in the dystrophinopathies and LGMD cohorts relative to the DM1 cohort (Table 9.2). A gadolinium contrast agent was administered to 27 (81.8%) dystrophinopathies, 25 (89.3%) LGMD, and 24 (92.3%) DM1 patients. Late gadolinium enhancement was highly prevalent in patients with dystrophinopathies, with a modest prevalence in patients with LGMD (Table 9.3). Myocardial fibrosis was indicated in 24 (88.9%) of dystrophinopathies patients, which was markedly more prevalent than the other cohorts ($P < 0.001$; Table 9.3). Patterns of enhancement varied by cohort and dystrophinopathies primarily exhibited patchy mid-wall and subepicardial LGE (Table 9.3). On the other hand, LGMD patients exhibited LGE at the right ventricular insertion point and DM1 patients had a low prevalence of LGE (Table 9.3). 12-lead ECG revealed parameters within normal ranges in the dystrophinopathies and LGMD (Table 9.4). In contrast, DM1 patients exhibited prolonged PR intervals and QRS duration indicative of a high burden of conduction disease with 12 (46.2%) patients having first-degree AV block, 2 (7.69%) patients with left anterior fascicular block, and 8 (30.8%) patients with left bundle branch block (LBBB).

As part of our advanced imaging techniques, we conducted three-dimensional myocardial deformation analysis (3D-MDA) across our cohorts. We determined that 3D-MDA was not able

to differentiate between the dystrophinopathies, LGMD, and DM1 cohorts (Figure 9.2 and Table 9.5). Longitudinal, circumferential, and radial strain amplitudes were of minimal diagnostic utility. Similar observations were made with regards to systolic and diastolic strain rates, however systolic minimum principal strain rate was relatively lower for the dystrophinopathies cohort as supported by the statistical analysis (P = 0.02; Table 9.5). We also conducted segmented T1 mapping, in which we were able to deduce differentiating aspects of cardiac dysfunction among the cohorts. Namely, with native T1 values, in which dystrophinopathies exhibited lower T1 values in the mid-inferoseptal segment (P = 0.03; Table 9.6). Post-contrast T1 values showed differences in patients with DM1, whereby T1 values were relatively lower in the mid-anteroseptal and mid-inferoseptal segments (P = 0.01 and P = 0.04, respectively; Table 9.6). Extracellular volume (ECV) was also analyzed, in which dystrophinopathies exhibited lower ECV (P = 0.01; Table 9.6).

Table 9.2. Standard quantitative cardiac magnetic resonance assessment.

Characteristic	Dystrophinopathies (n = 33)	LGMD (n = 28)	DM1 (n = 26)	*P* value
Chamber Volumes and Mass (Indexed to BSA or Height)				
LVEF, %	48.0 (39.9-53.9)	53.6 (45.9-57.4)	54.5 (48.5-62.3)	0.03
LVESV, mL/m^2	54.3 (43.6-67.2)	42.6 (34.8-56.3)	29.9 (27.1-38.2)	0.001
LVESV, mL/m	51.4 (36.9-70.3)	43.8 (33.2-61.9)	32.7 (25.6-39.5)	0.003
LVEDV, mL/m^2	102.3 (86.2-115.1)	88.7 (71.8-104.7)	66.7 (57.9-79.4)	<0.001
LVEDV, mL/m	96.1 (82.3-115.4)	94.1 (76.5-112.4)	67.3 (62.5-88.8)	0.001
LV Mass, g/m^2	43.5 (39.9-56.0)	51.9 (42.0-56.6)	39.3 (34.7-46.0)	0.01
LV Mass, g/m	45.4 (40.3-57.4)	52.7 (41.1-62.1)	42.0 (36.4-47.4)	0.04

LA Volume, mL/m^2	27.8 (26.3-37.0)	27.3 (22.6-41.5)	26.9 (21.3-29.9)	0.35
LA Volume, mL/m	30.6 (25.3-39.1)	30.9 (25.6-42.3)	27.1 (23.3-32.6)	0.23
RVEF, %	47.3 (42.6-50.1)	49.0 (46.6-53.3)	47.3 (46.1-51.4)	0.10
RVESV, mL/m^2	47.8 (43.3-56.0)	42.9 (37.5-51.4)	36.0 (32.2-42.0)	0.01
RVESV, mL/m	54.0 (38.1-59.6)	44.7 (36.7-56.7)	40.8 (34.6-45.7)	0.08
RVEDV, mL/m^2	93.0 (81.4-102.3)	86.3 (74.6-100.2)	68.5 (63.6-82.7)	0.004
RVEDV, mL/m	99.6 (80.6-108.4)	97.7 (79.7-111.3)	76.8 (65.8-88.3)	0.06
RA Volume, mL/m^2	26.6 (18.8-32.3)	26.7 (20.9-36.9)	21.9 (18.9-27.3)	0.33
RA Volume, mL/m	27.9 (21.2-32.0)	32.1 (20.6-42.6)	25.1 (19.9-32.0)	0.25

DM1, type 1 myotonic dystrophy; LA, left atrial; LGMD, limb-girdle muscular dystrophy; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; RA, right atrial; RVEDV, right ventricular end-diastolic volume; RVEF, right ventricular ejection fraction; RVESV, right ventricular end-systolic volume.

Table 9.3. Patterns of late gadolinium enhancement.

	Dystrophinopathies (n = 33)	LGMD (n = 28)	DM1 (n = 26)	*P* value
Analysis, n	27 (81.8)	25 (89.3)	24 (92.3)	
Scar/Fibrosis	24 (88.9)	16 (64.0)	8 (33.3)	<0.001
Pattern				
Subendocardial	0	0	0	-
MW Striae	1 (3.70)	1 (4.00)	0	0.62
RVI	4 (14.8)	11 (44.0)	6 (25.0)	0.06
MW Patchy	10 (37.0)	2 (8.00)	0	0.001
Subepicardial	16 (59.3)	7 (28.0)	3 (12.5)	0.002
Diffuse	0	0	0	-

DM1, type 1 myotonic dystrophy; LGMD, limb-girdle muscular dystrophy; MW, mid-wall; RVI, right ventricle insertion.

Table 9.4. Baseline 12-Lead Electrocardiogram Characteristics.

Characteristic	Dystrophinopathies (n = 33)	LGMD (n = 28)	DM1 (n = 26)	*P* Value
Heart Rate, bpm	80.0 (72.5-97.8)	75.0 (63.0-82.0)	68.5 (62.8-72.5)	0.003
PR Interval, ms	132.0 (125.5-136.0)	155.0 (131.8-161.0)	205.0 (186.0-232.0)	<0.001
QRS Duration, ms	94.5 (86.5-101.5)	98.0 (92.0-109.0)	107.5 (92.0-131.5)	0.06
QT Interval, ms	371.5 (352.0-384.8)	392.0 (373.0-432.0)	424.5 (400.0-443.8)	<0.001
Corrected QT Interval, ms	423.0 (412.8-435.6)	442.0 (406.2-451.6)	448.0 (424.5-455.6)	0.04
Conduction Delay, No.				
1° AVB	0	1 (3.57)	12 (46.2)	<0.001
LAFB	1 (3.03)	0	2 (7.69)	0.42
LBBB	0	0	8 (30.8)	-
RBBB	0	2 (7.14)	0	-

Values are n (%) or median (interquartile range). 1° AVB, first-degree atrioventricular block; DM1, type 1 myotonic dystrophy; LAFB, left anterior fascicular block; LBBB, left bundle branch block; LGMD, limb-girdle muscular dystrophy; MD, muscular dystrophy; RBBB, right bundle branch block.

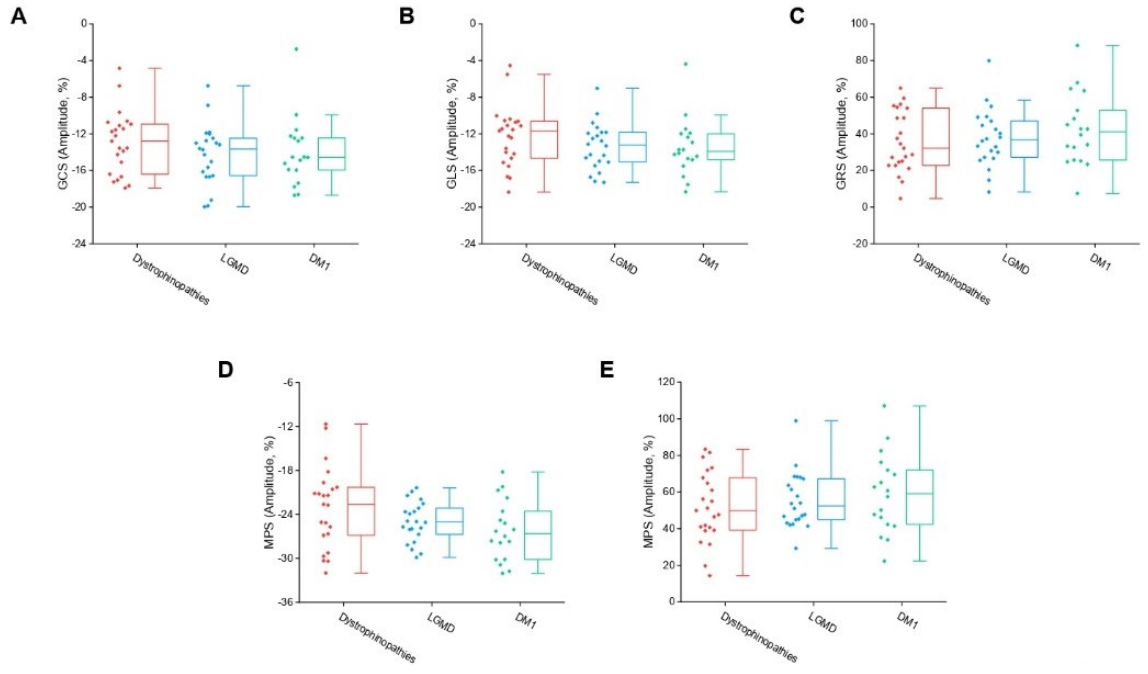


Figure 9.2. Three-dimensional strain analysis by cohort.

Table 9.5. Advanced quantitative cardiac magnetic resonance assessment.

Characteristic	Dystrophinopathies (n = 33)	LGMD (n = 28)	DM1 (n = 26)	*P* value
3D-MDA				
Longitudinal Strain Amplitude, %	-12.3±3.3	-13.4±2.5	-13.5±3.1	0.35
Circumferential Strain Amplitude, %	-12.9±3.4	-14.2±3.3	-14.0±3.7	0.42
Radial Strain Amplitude, %	35.7±16.6	37.6±15.7	42.3±19.7	0.47
Minimal Principal Strain Amplitude, %	-23.1±5.5	-25.0±2.7	-26.2±4.1	0.07
Maximum Principal Strain Amplitude, %	51.7±19.0	55.3±15.2	59.0±21.6	0.46
Systolic Circumferential Strain Rate, s^{-1}	-0.88±0.25	-0.97±0.23	-1.03±0.22	0.13
Systolic Longitudinal Strain Rate, s^{-1}	-0.98±0.30	-1.01±0.23	-1.17±0.30	0.09
Systolic Radial Strain Rate, s^{-1}	2.48±1.06	2.60±0.94	3.06±1.28	0.22

Systolic Minimum Principal Strain Rate, s^{-1}	-1.42±0.36	-1.44±0.28	-1.68±0.33	0.02
Systolic Maximum Principal Strain Rate, s^{-1}	2.94±1.15	3.11±0.90	3.71±1.42	0.10
Diastolic Circumferential Strain Rate, s^{-1}	1.22±0.37	1.15±0.23	1.11±0.27	0.52
Diastolic Longitudinal Strain Rate, s^{-1}	1.17±0.39	1.09±0.23	1.14±0.32	0.75
Diastolic Radial Strain Rate, s^{-1}	-3.97±1.97	-3.68±1.38	-4.07±2.08	0.77
Diastolic Minimum Principal Strain Rate, s^{-1}	1.47±0.47	1.38±0.32	1.46±0.37	0.74
Diastolic Maximum Principal Strain Rate, s^{-1}	-5.12±2.19	-4.75±1.42	-4.89±2.19	0.83

3D-MDA, three-dimensional myocardial deformation analysis; DM1, type 1 myotonic dystrophy; LGMD, limb-girdle muscular dystrophy.

Table 9.6. Cardiac magnetic resonance T1 mapping.

Characteristic	Dystrophinopathies (n = 33)	LGMD (n = 28)	DM1 (n = 26)	*P* value
Native T1 Values				
Mid Anterior, ms	1011.9±74.1	1004.0±42.2	1032.3±50.8	0.28
Mid Anteroseptal, ms	1003.3±31.5	1012.7±44.0	1027.2±33.8	0.19
Mid Inferoseptal, ms	998.8±23.1	1019.3±39.0	1028.2±24.7	0.03
Mid Inferior, ms	1000.8±45.3	1024.4±57.7	1032.9±30.0	0.15
Mid Inferolateral, ms	1011.5±59.4	1044.7±132.4	1022.9±45.6	0.57
Mid Anterolateral, ms	1039.0±94.3	1020.9±56.5	1023.8±31.7	0.69
Blood, ms	1562.4±78.8	1576.8±90.5	1589.9±86.1	0.66
Post-Contrast T1 Values				
Mid Anterior, ms	501.2±89.2	462.1±57.1	437.0±51.6	0.06
Mid Anteroseptal, ms	496.5±85.0	457.2±53.6	421.4±39.5	0.01
Mid Inferoseptal, ms	489.9±85.4	457.0±56.0	424.6±33.2	0.04
Mid Inferior, ms	472.1±85.5	447.7±69.9	428.5±46.3	0.32
Mid Inferolateral, ms	446.8±91.3	451.8±52.8	425.2±46.7	0.53

Mid Anterolateral, ms	467.0±80.8	457.3±58.4	431.0±44.2	0.33
Blood, ms	323.4±82.2	307.3±54.3	270.0±36.6	0.08
ECV Fraction				
Mid Anterior, %	23.6±3.56	25.7±3.33	25.1±3.04	0.26
Mid Anteroseptal, %	23.6±2.51	26.3±2.45	26.4±2.63	0.01
Mid Inferoseptal, %	24.3±2.82	26.6±3.40	26.0±2.52	0.12
Mid Inferior, %	25.4±4.24	28.2±6.36	25.9±2.66	0.27
Mid Inferolateral, %	30.4±10.3	27.6±4.27	26.0±3.12	0.22
Mid Anterolateral, %	27.7±5.65	26.5±3.71	25.3±2.35	0.33

DM1, type 1 myotonic dystrophy; ECV, extracellular volume; LGMD, limb-girdle muscular dystrophy.

Prognostic Utility of Cardiac Magnetic Resonance

Over the study period, there were 45 incidences of MACE, which were recorded in 26 (29.9%) patients. We tracked the burden of arrhythmias, which included 7 incidences of atrial flutter or fibrillation and 10 incidences of ventricular tachycardia (VT) in our MD cohorts. Specifically, 5 incidences of atrial flutter or fibrillation and 4 incidences of VT were documented in DM1 patients. Cardiac devices including implantable cardiac defibrillators, pacemakers and advanced pacemakers (cardiac resynchronization therapy) in 8 patients.

Chamber dilation indicated by indexed LV volumes at end-diastole and end-systole showed prognostic value for MACE in our MD cohort (Table 9.7 and Figure 9.3). Patients with a LVESV

indexed by height > 41.3 mL/m were at a high risk of MACE (aOR: 4.82; 95% CI: 1.14-20.3; *P* = 0.03). Left ventricular end-diastolic volume indexed to both BSA and height had prognostic utility for MACE (aOR: 6.15; 95% CI: 1.04-36.3; *P* = 0.04; aOR: 4.82; 95% CI: 1.14-20.3; *P* = 0.03, respectively). Patients with a longitudinal strain amplitude < -13.0% had a high risk of MACE (aOR: 6.38; 95% CI: 1.17-34.7; *P* = 0.03). Similarly, patients with a circumferential strain amplitude < -13.6% were at a marked risk of MACE (aOR: 14.3; 95% CI: 2.14-95.6; *P* = 0.01). Radial strain amplitude was of marginal utility (radial strain amplitude < 35.3%; aOR: 4.41; 95% CI: 1.00-19.6; *P* = 0.05). Left ventricular ejection fraction, indexed LV mass, and LGE were not effective predictors of MACE (Table 9.7 and Figure 9.3).

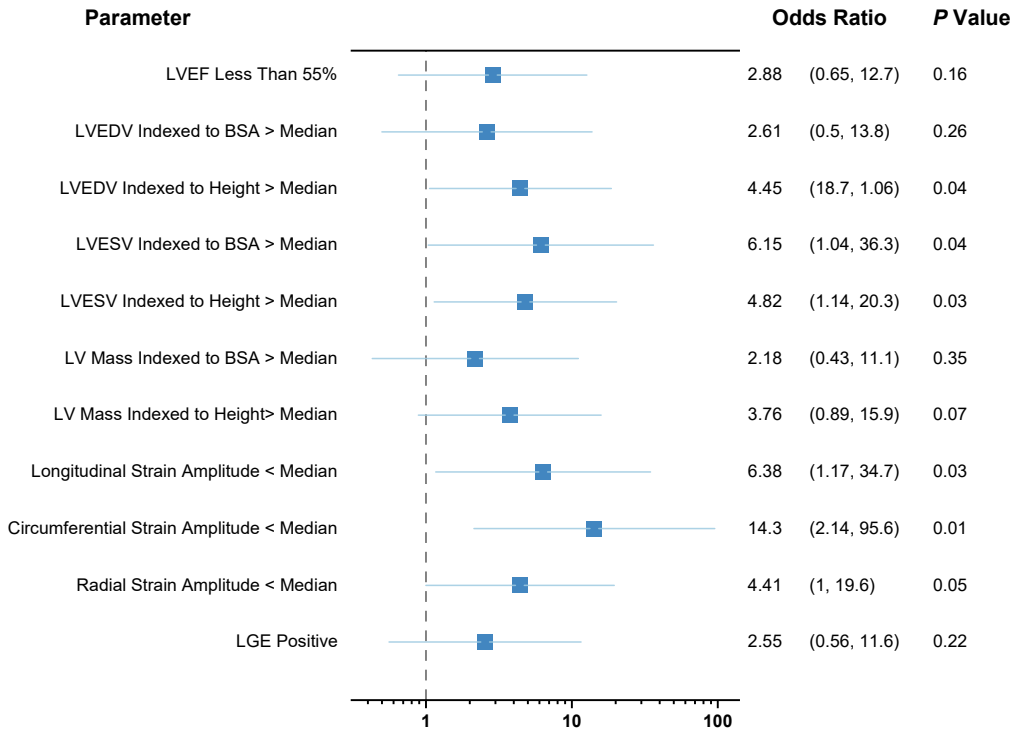


Figure 9.3. Forest Plot of Hazard Ratios for Major Adverse Cardiac Events. LGE, late gadolinium enhancement; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume**.**

Table 9.7. Association between Cardiac Magnetic Resonance and Major Adverse Cardiac Events.

Parameter	OR (95% CI)	*P* value
LVEF		
Less than 55% (*n* = 48)	2.88 (0.65-12.7)	0.16
LVEDV Indexed to BSA		
Greater than median (84.7 mL/m^2; *n* = 27)	2.61 (0.50-13.8)	0.26
LVEDV Indexed to Height		
Greater than median (89.6 mL/m; *n* = 38)	4.45 (1.06-18.7)	0.04
LVESV Indexed to BSA		
Greater than median (42.6 mL/m^2; *n* = 27)	6.15 (1.04-36.3)	0.04
LVESV Indexed to Height		
Greater than median (41.3 mL/m^2; *n* = 38)	4.82 (1.14-20.3)	0.03
LV Mass Indexed to BSA		
Greater than median (43.4 g/m^2; *n* = 27)	2.18 (0.43-11.1)	0.35
LV Mass Indexed to Height		
Greater than median (45.8 g/m; *n* = 38)	3.76 (0.89-15.9)	0.07
Longitudinal Strain Amplitude		
Less than median (-13.0%; *n* = 32)	6.38 (1.17-34.7)	0.03
Circumferential Strain Amplitude		
Less than median (-13.6%; *n* = 32)	14.3 (2.14-95.6)	0.01
Radial Strain Amplitude		
Less than median (35.3%; *n* = 32)	4.41 (1.00-19.6)	0.05
Late Gadolinium Enhancement		
Positive (*n* = 23)	2.55 (0.56-11.6)	0.22

Assessment of the prognostic ability of patient categorization by parameters obtained by cardiac magnetic resonance for major adverse cardiac events. BSA, body surface area; CI, confidence interval; LGE, late gadolinium enhancement; LV, left ventricular; LVEDV, left ventricular end-

diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; OR, odds ratio.

9.5. Discussion

Cardiac magnetic resonance is referred to as the gold standard for cardiac imaging and assessment over echocardiography.[274-277] Its functionality, sensitivity, and accuracy make it an appreciable clinical asset, which is still underutilized in the MD patient population. Importantly, this advanced imaging modality provides high-level granularity for the quantification and qualification of cardiac structure and function. This advantage over echocardiography has previously been described as clinically relevant for patients with DMD.[250, 282, 283] Furthermore, the use of cardiac imaging is particularly important due to limitations of physical examination in these patients including the visualization of jugular venous pressure, which is often not possible due to physical limitations such as wheelchair use and a high prevalence of obesity.

Clinicians can use advanced imaging techniques to assess myocardial deformation (i.e. strain analysis).[278] Strain analysis offers an important advantage over the classic assessment of systolic function as it allows for the quantification of early signs of contractile dysfunction, which is critical for diseases with rapid progression. Myocardial strain assessment is of important clinical relevance as it can be used to detect subclinical signs of cardiomyopathy and stratify patient risk of MACE, thereby altering patient prognosis. Although strain analysis can be conducted through speckle tracking echocardiography, its performance is markedly hindered in MD patients due to obstructed acoustic windows as a result of scoliosis, obesity, and lung disease, and not feasible in patients with arrhythmias due to its analysis confined to a single cardiac cycle.[284, 285] These confounding factors are prevalent in patients with MD.[3, 8] Left

ventricular strain is often described as a more sensitive indicator for reduced contractile function than ejection fraction, which may detect dysfunction later on in the disease process. This critical function makes CMR a useful front-line method for the enhanced detection of heart disease. Identifying patients with reduced peak longitudinal strain amplitude, circumferential strain amplitude, and radial strain amplitude can allow for the identification of early signs of reduced cardiac function when compared to accepted normal values[133, 284, 286] or healthy controls in the same setting.[287] Of the software currently available for strain assessment, cardiac magnetic resonance feature tracking (CMR-FT) is advantageous over other techniques for the assessment of MD patients given its post-processing methodology. Strain analysis with CMR-FT is conducted off-line after patient imaging has been conducted using data acquired during structural assessment with SSFP. Therefore, there is no additional imaging (i.e. acquisition time) required for strain assessment using this technique. Importantly, CMR-FT has been previously used in patients with DMD[288, 289] and has also been shown to be an effective assessor of right ventricle (RV) strain in a variety of patient cohorts.[286, 290] Previous studies with sizable cohorts have also demonstrated the prognostic value of tracking RV function for adverse clinical outcomes.[291, 292] Right ventricular assessment is of critical importance in patients with MD given the associated global adverse remodeling and impairment of function, as well as the high burden of respiratory disease including aspiration pneumonia, restrictive lung disease, sleep disordered breathing, chronic-obstructive pulmonary disorder, and resulting pulmonary hypertension (Group 3).[3]

Cardiac magnetic resonance provides numerous qualitative advantages over echocardiography, which notably includes the ability to characterize tissue. T1 mapping is possible due to the properties of magnetic resonance relaxation following the application of an excitation radiofrequency pulse sequence applied to magnetized protons in a targeted space of

the subject.[293] Fat infiltration of the myocardium has been previously documented in patients with MD.[279, 280] Furthermore, the tracking of fat infiltration is relevant given the high burden of metabolic disease in patients, which is accompanied by various lifestyle-related risk factors for heart disease. In addition, the use of gadolinium-based contrast agents (GBCA) for the visualization of fibrosis has been demonstrated in patients with DMD in previous research.[79] Through T1 mapping we are able to more accurately identify diffuse fibrotic tissue when used in combination with GBCA.[294] This is of particular relevance in the cardiac phenotype of MD, which includes DCM and arrhythmias. Longer T1 values have been recorded in DMD patients with cardiac involvement than in DMD patients without cardiac involvement, suggesting a diffuse cardiac remodeling process throughout the myocardium.[295] In addition to image capturing, these proton relaxation characteristics can be quantified per unit of time for analysis using off-line software and compared to reference values[133] or healthy controls in the same setting.[296, 297]

This investigation evaluated the clinical utility of CMR to determine the added benefit of including it as a standard cardiac assessment tool for the diagnosis of heart disease and to determine its ability to prognosticate adverse clinical outcomes. An important extension to this study would be the tracking of serial CMR to quantitatively and qualitatively track the progression of heart disease in these patients. This is of particular interest since CMR provides an incremental advantage to the assessment of contractile function through the analysis of myocardial strain, which could be tracked over time. Additionally, a focus on tissue changes through serial tracking of T1 images and relaxation times would provide additional insight into the adverse cardiac remodeling that occurs. Over and above cardiac-assessment, CMR provides an opportunity to non-invasively assess pulmonary edema, which is a notable feature of heart

failure. Derived lung water density (LWD) has been shown to have prognostic utility for adverse clinical outcomes.[298] Furthermore, structural assessment of muscles in the chest (i.e. pectoralis major and minor, serratus anterior, and subclavius), shoulder (i.e. anterior, medial, and posterior deltoids), back (i.e. latissimus dorsi, rhomboid major and minor, and trapezius), and abdomen (i.e. external and internal obliques, rectus abdominis, and transverse abdominis) can be conducted. This proposed use builds on previous studies that have tracked fat infiltration and fluid accumulation using magnetic resonance imaging in skeletal muscle in patients with DMD in association with disease progression.[299, 300] This investigation, like future studies, will be limited by the number of MD patients in which cardiac imaging by CMR is feasible. Patients with MD commonly rely on wheelchair use, have spinal stabilization rods, and require ventilatory support, thus limiting the number of patients compatible for CMR study. We will continue to consent MD patients to increase our cohort size to improve the validity of future studies and to ultimately accumulate a cohort size suitable for sub-group analyses. This includes an expansion of this study to include an additional study center to create a Provincial multicentered study.

9.6. Conclusions

Clinicians can leverage the incremental capabilities of CMR over echocardiography to proactively assess the cardiac condition of our MD patient cohort while concurrently evaluating its impact on patient prognosis. Importantly, this advanced imaging modality can better inform clinicians of patient condition, which can facilitate proactive multidisciplinary treatment and

management, including the prophylactic use of medical and device therapies to ultimately improve patient prognosis.

10. Discussion

10.1 General Comments

The work covered in this thesis serves to inform clinicians and researchers alike on the breadth and depth of cardiac assessment that can be feasibly and accessibly performed on the MD patient population. The modalities discussed are well within the realm of standard clinical practice and include patient plasma collection, 12-lead ECG studies, and cardiac imaging studies, which can strategically be used in patients with dystrophinopathies, LGMD, DM1, and FSHD. Importantly, strategic use of these assessment techniques can effectively facilitate the therapeutic management of these diseases through the use of medical and device therapies, which were shown to markedly improve cardiac function as well as prognosticate MACE in our MD patient cohorts. This multifaceted approach can also improve the efficiency of the patient care process given the inherent challenges associated with transporting MD patients to and from various appointments and assessments. Taken together, the impact of heart disease on patient longevity and quality of life can be improved through the dissemination of the cardiac diagnostic and prognostic techniques described in this research for patients with MD.

10.2 Discussion and Impact of Research

The studies completed within the framework of this thesis were conducted at the NMMD clinic at the Kaye Edmonton Clinic, University of Alberta. The collaborative multidisciplinary care model played an important role in stabilizing patients given the high prevalence of respiratory and ambulatory comorbidities, which allowed for a largely unimpeded progression of our studies. We conducted a fundamental assessment of the impact of cardiac care on MD patient outcomes at the NMMD clinic, which adjusted for various clinical characteristics including respiratory disease, which was a prevalent comorbidity. This study serves as the foundation for the thesis, whereby cardiac care is a core component to the multidisciplinary care of patients with MD. Moreover, cardiac intervention markedly improves all-cause clinical outcomes. We built upon these findings by seeking ways in which to optimize the assessment and management of this complex patient population to facilitate this level of cardiac care for providers outside of the NMMD clinic. We leveraged traditional methods of cardiac assessment currently conducted in clinical practice and assessed them in the context of MD using a granular and statistical approach.

Blood collection remains a standard component of patient assessment in clinical practice. Plasma biomarkers such as BNP and troponin I are commonly assessed for indications of adverse cardiac remodeling and acute injury, respectively. However, their utility in patients with MD had not been fully explored in previous literature. From our clinical experience, standard troponin I assays are unable to detect marginally elevated levels in these patients. We therefore utilized hsTnI assays for our analysis, which has been used in HFrEF patients in previous studies.[174-176] We determined that MD patients with cardiomyopathy had markedly elevated levels of both BNP and hsTnI compared to MD patients without cardiomyopathy and HC. Muscular dystrophy

patients with cardiomyopathy had a median BNP level at one-third of the guideline cutoff value for patients with HFrEF.[172] Furthermore, the median level of BNP in MD patients with overt HF was one-third of that seen in traditional HFrEF patients.[171, 173] This is a salient example of how a standard modality for cardiac assessment can be adapted for patients with MD for enhanced diagnostic purposes. We then explored the prognostic value of these biomarkers, which first required the establishment of cutoff values using a rigorous and objective statistical approach. Patients with biomarker levels above the set cutoff values were at a multi-fold greater risk of MACE compared to patients with biomarkers level below the cutoff values. Note that these cutoff values were established within our own study and do not currently exist in clinical guidelines. Importantly, both these diagnostic and prognostic findings can be used to guide the care of patients with MD through standard blood collection.

Another readily accessible modality for cardiac assessment is 12-lead ECG, which can provide valuable insight into the cardiac status of patients and can be conducted during their outpatient clinic visit with minimal physical demand. Routine ECG assessment of patients at the NMMD clinic led to a number of queries on ECG findings including morphology as well as the overall diagnostic utility for cardiomyopathy. 12-lead electrocardiogram morphologies such as LBBB and fQRS are likely indicative of cardiomyopathy in patients with MD with statistically supported diagnostic utility. Recognition of these features by clinicians can facilitate MD patient monitoring and management, such as the use of CRT device intervention in DM1 patients with LBBB as we have shown. Additionally, we tested four conventional criteria for ECG-LVH in our study cohort and found that these are of minimal utility and are often false negatives in patients with MD, particularly in patients with DMD. Serial tracking of ECG parameters was also of minimal clinical utility. We highlighted the importance of considering incidental ECG findings

in the context of MD patients, which can supplement cardiac imaging as it remains an important modality for assessing adverse remodeling.

Heart disease is characterized by reduced cardiac function in patients with MD, predominately affecting the LV, as assessed by cardiac imaging. Given the rapid progression of heart disease in patients with dystrophinopathies and LGMD, it is critical that LV systolic function be monitored, such that therapeutic management can be implemented with brevity. Additionally, patients with DM1 and LBBB are vulnerable to reduced LV function due to the electromechanical dyssynchrony associated with their conduction disease. We found TTE-derived LVEF to be a prominent marker of cardiac status both at baseline and based on trajectory by serial measures for the prognostication of MACE. Both LVIDd and LVIDs were also useful indicators of MACE risk at baseline. Importantly, LVEF is a an easily obtained parameter by TTE and we demonstrated TTE-derived LVEF to be strongly correlated with CMR-derived LVEF in support of our study methodology and findings. Note that MD patients classified as having reduced LVEF were comparable to HF patients with mid-range LVEF, however given the rapid progression of heart disease in these patients and risk of MACE, cardiac intervention must be conducted with greater vigor. In another investigation, we took a more granular approach to understanding the cardiac dysfunction and adverse remodeling process across our MD cohort using CMR. Comparable to our findings with TTE, LVEF is useful in differentiating the different types of MD. Additionally, three-dimensional strain analysis provided insight into subclinical systolic dysfunction in these patients. Late gadolinium enhancement and T1 mapping provided qualitative and quantitative insights into adverse remodeling and cardiac fibrosis, alongside prognostic value for predicting MACE. Taken together, clinicians can calibrate their

use of these imaging modalities for a targeted and structured approach to assessing MD patients for optimal therapeutic management.

Use of cardiac medications and device therapy played a key role in the prognosis of our MD patient cohort. Implementation of the strategies of assessment outlined in this thesis can serve to facilitate the prophylactic use of medical and device therapies in adult patients with MD. This is particularly important in patients faced with aggressive disease progression as seen in DMD. Furthermore, patients with DMD and DM1 are at a high risk of MACE including SCD, therefore early implementation of ICDs for VT is warranted. Effective administration of cardiac care is critical to reduce disease burden, which has implications of patient longevity and quality of life. Active collaboration between primary care and specialist physicians is critical for an effective and efficient patient care process. Importantly, these concepts can be applied more broadly to other institutions providing care to patients with MD. The implementation of the screening methodologies for heart disease used in the investigations outlined in this thesis should be considered in the pediatric MD population given the progressive nature of these diseases and the associated adverse outcomes.

We should be cognizant of the limitations presented in the preceding investigations. All studies primarily considered MD patients as a single composite cohort for which the analyses were applied. Although this methodology can be readily applied in a clinical setting to effectively prognosticate patients, the heterogeneity of the cohort should be considered. The burden on cardiac function and adverse remodeling process are unique to each subtype of MD alongside the corresponding clinical manifestations. This is particularly evident in DMD patients which have a high prevalence of myocardial fibrosis identified by LGE, and DM1 patients which are characterized by conduction disease and arrhythmias at an incidence that is markedly greater

than the other types of MD. Subgroup analyses are an important consideration for future investigations provided they are supported by a larger cohort size for statistically validity. Ultimately, the external validity of these studies can be improved through the expansion of the cohort sizes. Additionally, although the statistical methodology outlined in each study was indeed rigorous and suitable for the timeliness of the investigations, conduction of regression analyses using a stepwise process, as opposed to the standard or hierarchical process used, could assist with the reduction of selection bias for additional statistical validity.

10.3 Future Directions

The work outlined in this thesis serves as the foundation for future research initiatives, relevant to our established MD platform. Firstly, there is the opportunity to further pursue analysis on the utility of plasma biomarkers, including tenascin c for heart disease and cardiac fibrosis, and cystatin c as an alternative to CK for the monitoring of renal function. Both biomarkers can offer care providers additional insight on the status and progression of disease in patients with MD while facilitating management. Secondly, the opportunity to expand our study cohort to build upon the research presented in this thesis through continued patient recruitment and multicentered collaboration. Moreover, this permits the performance of additional analysis including the comparison of subgroups, which was limited given our modest cohort sizes. We intend to pursue collaborations with another care center in Calgary, Alberta for the creation of a Provincial MD study platform. Development of our MD research platform in these ways would increase clinical relevance and research impact through the analysis of larger cohorts with serial datapoints.

Beyond patient monitoring, the assessment of groundbreaking therapeutic interventions remains an under-researched area of study in patients with MD. Given the high prevalence of dyslipidemia in patients with MD and given the challenge of using statin therapy in patients with MD due to side effects including myalgias, the impact of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibition should be studied. Additionally, given the prevalence of diabetes in these patients, the use of sodium-glucose transport protein 2 (SGLT2) inhibition should also be studied. The prevalence and morbidity of dyslipidemia and diabetes are further propagated by lifestyle factors including diet and sedentary lifestyle, which are commonly observed in patients with MD. Importantly, both PCSK9 inhibitors and SGLT2 inhibitors have been shown to be effective in the treatment of heart disease as well as reducing cardiac-related adverse events, and could be assessed in patients with MD.[301-303] These therapies can serve as an asset for the management of patients with MD, which can be appropriately administered in conjunction with the assessment strategies outlined in this thesis.

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