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Trabeculated myocardium in healthy adults:
A first step in understanding the diagnosis of non-compaction cardiomyopathy by magnetic resonance

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RÉSUMÉ

La cardiomyopathie non-compacté (NCC) est une maladie rare avec des critères diagnostiques basés sur la détection de l'augmentation du myocarde trabeculé par échocardiographie. L'imagerie par résonance magnétique (IRM) est devenue la méthode de référence pour étudier la fonction et la morphologie du cœur compte tenu de sa meilleure résolution spatiale et temporale. Toutefois, les critères diagnostiques de NCC que nous utilisons en ce moment en IRM ont été tirés d'études en échocardiographie. Ceci pourrait impliquer une augmentation de l'incidence de nouveaux cas de NCC, de même que la positivité chez les adultes en santé. Le présent travail a voulu étudier la "normalité" par IRM en termes de présence et de distribution de myocarde trabeculé chez les adultes en santé et déterminer la présence des associations entre le myocarde trabeculé et les paramètres de fonction cardiaque.

ABSTRACT

Non-compaction cardiomyopathy (NCC) is a rare entity that is currently diagnosed for the most part by echocardiography in cases of an increased layer of trabeculated myocardium. Recently, magnetic resonance imaging (MRI) became the gold standard imaging technique in the study of cardiac function and morphology due to its high spatial and temporal resolution. However, diagnostic NCC criteria developped for echocardiography have been directly applied to MRI in the abscence of a standard definition of trabeculated myocardium. This direct application of echocardiography criteria to MRI may have led to an increased incidence of new NCC cases. The aim of this present work is to clarify clinical practice by determining normality by MRI in terms of presence and distribution of trabeculated myocardium in healthy adults and determine if any association exist between the presence and extension of trabeculated myocardium and cardiac function.

AVANT-PROPOS

Clinical practice should be based on evidence derived from research. That simple and easy statement is what I basically learned through my years of fellowship both in interventional cardiology and cardiac imaging. We become better clinicians and practitioners if we ask ourselves questions and become curious about what we need to understand whether to continue smoothly on without questioning our daily practice. The period of fellowship that I finally finished one year ago just awakened my curiosity about what we think we know and we really do not.

The master in experimental medicine is set in this time of my life after my residency in cardiology. During that time I have become a “cath-lab” woman but also a cardiologist interested in imaging. That mix is what has made me grow as a cardiologist despite preconceived old ideas about what a “cath-lab” staff member should be. During this time I have participated in several research teams, contributed to a variety of research projects, learned from each experience and learned to write and do my own statistics.

This work on trabeculated myocardium rises from daily practice and is a part of the huge and amazing project about youth population of Québec. The whole project aims to describe the current cardiovascular status of more than 500 Québec inhabitants aged 18 to 35 years. The project has recruited not only participants but brilliant contributors that determined the shape of the whole. For this particular paper on trabeculated myocardium, all co-authors have been involved during some of the phases of the still-on study of young inhabitants of Québec. On these work about myocardial compaction all co-authors have been devoted to the success of the project and the paper. Dr. Philippe Pibarot has reviewed the paper several times and gave clarifying ideas. Dr. SwapnilSinha was involved in the serologic analysis at the Institut de recherchescliniques de Montréal. Dr. James Engert was involved in the genetical analysis and was actively involved during the review of the paper. Dr. Christian Deschepper was involved during the data analysis and was actively involved during the statistical revision of the work. Dr. Olivier Bertrand was a reviewer of the paper. Drs. Sergio Pasian and Élizabeth Bédard were actively involved during the tracings and helped with the clinical issues of the paper. Drs. Éric Larose and Tizon discussed several

times about the clinical implications and the hypothesis for this study. Dr. Larose was deeply committed to this work and has participated in the hypothesis, tracings, statistical analysis, writing of the paper, revision of the paper and revision of this work. Dr. Tizon was involved during all the phases of this work: stated the hypothesis, wrote the protocol, drew all tracings several times, did the statistical analysis, wrote the draft of the paper and finally this work. The paper has been submitted to Circulation and is currently under revision.

This project was made known several times in different countries. It was first presentedat the "Journée Scientifique de la Recherche du Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec (CRIUCPQ)" on May the 25th, 2010, as an oral presentation. It was then presented at "Molecular function and imaging Symposium 2010: Imaging Heart Failure" on June 19th, Ottawa, as an oral presentation. It was also presented at the Congress of the Spanish Cardiovascular Society on October 23rd 2010, Valencia, Spain, as an oral presentation. It was also presented as a poster at the Canadian Congress of Cardiology 2010, Montreal, on October the 25th. It was finally presented as an oral presentation at the Scientific Session's of the American Heart Association of 2010 that took place in Chicago on November 17th.

ACKNOWLEDGMENTS

Fellowship is a wonderful time of learning and absorbing every teaching you can. During that period of my life, my family encouraged and supported me. My family in Spain was crucial. However, my family in Québec was also an important piece. Dr. Larose and his research team was part of my québecoise family and I feel deeply grateful for all their support, help and encouragement. They are a model of efficient, tireless and professional work. I would like to emphasise my gratitude to Dr. Larose for his encouragement, teaching and support. Marc Amyot helped me during the first months and became an unforgettable colleague and friend. Marie-Christine Perron and Grabielle Prefontaine were excellent research team colleagues available when needed. Julie Carange and Karine Bibeau have formed an excellent new team and helped me endlessly during my lasts months in Québec.

I would also thank all the cardiologists involved in magnetic resonance from whom I learned clinical cardiac magnetic resonance: Sergio Pasian, Elisabeth Bédard, Bernard Noël and Gérald Barbeau.

I would like to thank Dr. Josepa Mauri for her encouragement to start my fellowship abroad. Dr. Robert De Larochellière supported me during fellowship and was an example for me to follow. Dr. Jean-Pierre Déry encouraged me to continue my training in magnetic resonance imaging. Dr. Philippe Pibarot was an excellent teacher for me during fellowship on cardiovascular imaging. Dr. Paul Poirier encouraged me and helped me during the difficult task of choosing a job. And, to all my colleagues at the office U-4794, especially to Vincent Manguy and Andreas Kumar for their help. Elianne De Larochellière was an excellent team mate and friend and together we enjoyed our final master sprint.

My master inexperimental medicine was funded with a grant obtained in 2010 from the CRIUCPQ.

"Bring me light to see through darkness"

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LIST OF ABBREVIATIONS

AHA : American Heart Association

ANP : Atrial Natriuretic Peptide

BNP : Brain Natriuretic Peptide

CMR : Cardiovascular magnetic resonance

ESC : European Society of Cardiology

INCLV : Isolated non-compaction of the left ventricular myocardium

NCC : Non-compaction cardiomyopathy

LVD : Left ventricular dysfunction

LVEF : Left ventricular ejection fraction

WHO : World Heart Organization

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1. INTRODUCTION

1.1 THE CHALLENGE IN DEFINING A CARDIOMYOPATHY

Cardiomyopathies are a variety of myocardial disorders that have structural and functional phenotypes and are frequently of genetic origin. Great efforts have been made to reach this simple definition since the awareness of heart muscle disease in the mid 1800s.

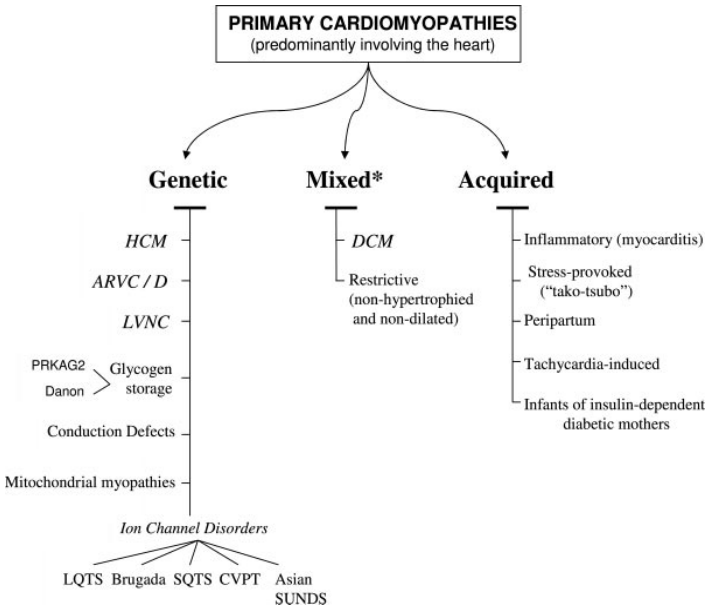
Since 1980 different societies and organizations have worked to first define and secondly classify each different entity. The World Health Organization (WHO) defined cardiomyopathies as "heart muscle disease of unknown origin" to well distinguish cardiomyopathies from those clearly well-known dysfunctions due to recognised cardiovascular problems as hypertension, diabetes mellitus, coronary disease and valvular disease [1]. In 1995 the WHO and the International Society and Federation of Cardiology Task Force defined cardiomyopathies as "diseases of the myocardium associated with cardiac dysfunction" and established the basis for future classifications [2] and for first time included the term "cardiac dysfunction". Different entities distinguish several types depending on the anatomy and physiology: dilated (DCM), hypertrophic (HCM), restrictive (RCM), arrhythmogenic right ventricular dysplasia (ARVD) and the group of unclassified cardiomyopathies. This classification included ischemic, valvular and hypertensive disease as cardiomyopathies. However, several limitations have been identified in these previous classifications. The first is that they blend anatomic with functional designations as one entity may legitimately be included in more than one category. Furthermore, different categories of cardiomyopathies may share similar etiologies and phenotypes. In addition, some categories of cardiomyopathies do not have static manifestations and rather evolve throughout their natural clinical course.

In 2006, the American Heart Association (AHA) updated the definition stating that cardiomyopathies were a group of "heterogeneous" heart muscle diseases with "mechanical and/or electrical dysfunction" and that they were frequently "genetic". Two large categories were included: primary and secondary. Primary cardiomyopathies are those affecting solely

the heart muscle. Secondary cardiomyopathies are those with pathologic involvement of the heart muscle as a part of a systemic disease. Primary cardiomyopathies are divided into genetic (or hereditary), mixed and acquired (Figure 1) [3]. In 2008 the European Society of Cardiology defined cardiomyopathy as a myocardial disorder in which the heart muscle is structurally and functionally abnormal in the absence of coronary artery disease, hypertension, valvular and congenital heart disease sufficient to account for the myocardial abnormality [4]. The main categories are familial or genetic and non-familial and this classification also excludes secondary causes.

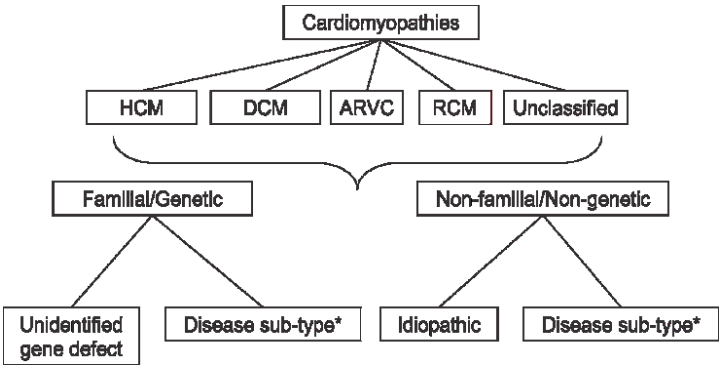
Therefore, the concept that emerges to embrace the four different definitions is that cardiomyopathies are heart muscle disorders with heterogeneous etiologies including genetic/familial and acquired/non-familial causes. Recently, secondary aetiologies such as ischemic, hypertensive, valvular and congenital and also conditions such as cardiac tumours or endocardial diseases have been excluded from the modern concept of cardiomyopathy.

Figure 1. AHA classification of primary cardiomyopathies [3].



HCM: hypertrophic cardiomyopathy, DCM: dilated cardiomyopathy, AVRC/D: arrhythmogenic right ventricular cardiomyopathy/dysplasia, LVNC: Left Ventricular non-compaction, PRKAG2: protein kinase AMP-activated and gamma 2 non-catalytic subunit syndrome, LQTS: long QT syndrome, SQTS: short QT syndrome, CVPT: catecholaminergic polymorphic ventricular tachycardia, SUNDS: Sudden unexplained nocturnal death syndrome.

Figure 2.ESC classification of cardiomyopathies [4].

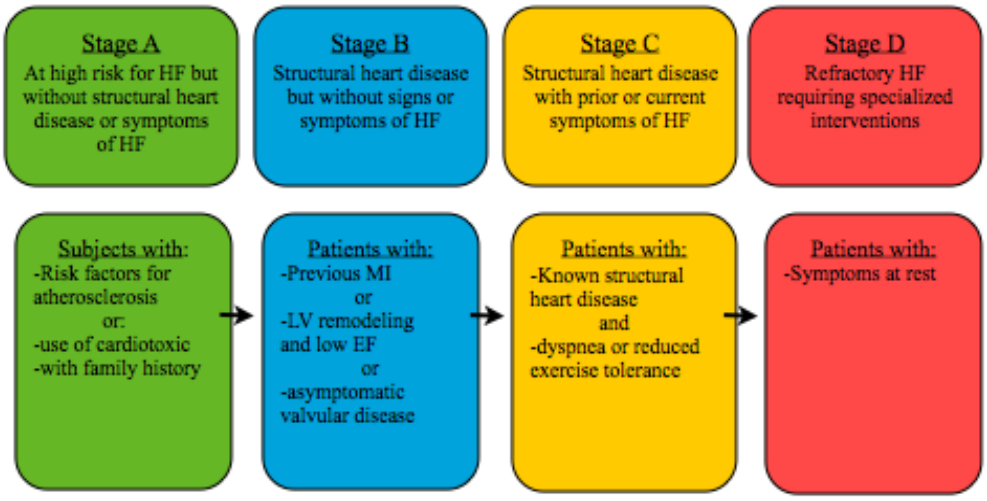


HCM: hypertrophic cardiomyopathy, DCM: dilated cardiomyopathy, AVRC: arrhythmogenic right ventricular cardiomyopathy, RCM: restrictive cardiomyopathy.

1.2 A POORLY DEFINED NATURAL HISTORY

As observed in the previous section, not only is a standard definition of cardiomyopathy still controversial but also the natural history of the whole group of cardiomyopathies is still poorly understood. However, it is thought that there is a progression-line from a "at risk status" to a "clinical state" in a continuum of phases with multiple overlapping mechanisms involved in disease onset and progression (Figure 3).

Figure 3. American College of Cardiology/AHA guidelines for the evaluation of chronic heart failure by stage.



HF: heart failure, MI: myocardial infarction, LV: left ventricle, EF: ejection fraction.

Symptomatic phases of heart failure are well known compared to asymptomatic stages (A and B). Patients with symptomatic heart failure have reduced survival rates. The two year survival rate was between 50 to 63% in two large observational studies conducted in the early 90s [5, 6]. And, despite the advances in current life-prolonging therapies, current data shows that there has been little improvement [7] and the survival is similar to those patients diagnosed with colon cancer. For that reason, several trials supported the hypothesis that early detection of LVD may confer a better survival rate. However, the early detection of myocardial dysfunction is shown to improve prognosis only when adequate pharmacological therapy is started before the initiation of symptoms in selected patients. Only those with previously known heart disease, mostly coronary heart disease, with severe to moderate LV dysfunction (35-40%) [8, 9] are shown to improve survival rates. However there is no data regarding the benefits of treating asymptomatic LVD in other types of cardiomyopathy.

The prevalence of asymptomatic left ventricular dysfunction depends on the population studied and the criteria used to define the degree of dysfunction. Most studies have been done in patients from 50 to 70 years of age and there is no data regarding younger patients. The prevalence of LVD (LVEF≤54%) in that population reaches 12.5% whereas when LV dysfunction is defined by LVEF ≤ 30% it only reaches 2.1% (almost all of them had evidence of coronary heart disease or hypertension). The prevalence of moderate and mild LV dysfunction (LVEF≤50%) is thought to be < 4.7% [10-15]. Several subgroups have been identified with higher rates of asymptomatic dysfunction as men compared to women, older patients compared to young ones and those with heart disease compared to those without. However the odds of having LV asymptomatic dysfunction increases importantly with risk factors. These risk factors were extracted from one epidemiological cross-sectional study that sampled one geographical area and assessed LV function by echocardiography in subjects aged 25 to 74 years old. Subjects who were found to have LVEF ≤ 30% were selected for comparison with those without [15]. Subjects with ischemic heart disease, systemic hypertension, diabetes mellitus, peripheral or cerebrovascular disease and excessive alcohol intake were found to increase the likelihood of having LV dysfunction. This one is the sole cross-sectional trial that studied a younger

population (aged 25 to 34) and the prevalence of severe LV dysfunction in this Scottish cohort was 0% [15]. Assumptions about known risk factors for LVD and its treatment in younger patients need to be done with caution due to the scarcity of available data.

Subjects with asymptomatic LVD have by definition no clinical symptoms. However, even in asymptomatic stages molecular, physiological and metabolic adaptation and maladaptation forces play an important role to mantain the subject without symptoms. Several hormones and pro-hormones may be then detected. Some of these hormones and pro-hormones, as BNP, and ANP, pro-BNP, NT-proBNP, have been largely studied in the general community and also in patients with heart failure due to different conditions. The serum concentration levels of these peptides increases progressively with age and with heart failure progression from A to D stages suggesting worsening fibrosis, dilatation and hypertrophy all driving to a decreasing myocardial function [1] . However, subjects with asymptomatic LVD may have serum concentrations of several hormones that reach intermediate levels between age-matched controls and patients with symptomatic heart failure. And, the degree of neurohumoral activation is predictive of outcome [17, 18]. However, again, these studies were done mostly in patients with left ventricular dysfunction due to ischemic heart disease.

2. HEART FAILURE

2.1 INTRODUCTION

The improvements in treatment in cardiovascular medicine have extended life expectancy and accordingly the prevalence of age-related disease has increaseda as a result of prolonged survival. For these same reasons, the prevalence of cardiovascular diseases is expected to increase in the coming 20 years [19]. The WHO considers cardiovascular diseases as a mixture of coronary heart disease, cerebrovascular, peripheral artery disease, rheumatic heart disease, congenital heart disease and deep vein thrombosis and pulmonary embolism. Although the definition of cardiovascular disease does not include a specific mention of heart failure, main causes of coronary and cerebrovascular diseases are still the main causes of heart failure in industrialized countries [20, 21] and one may infer an increase in heart failure due to these causes.

Most published data on clinical epidemiology of heart failure comes from studies conducted in industrialized countries. With these data, there is no question that heart failure is an important health care issue. Developed countries currently spend 1-2% of the budget for healthcare on patients with this condition. In US hospitals it is not only the single most common diagnostic related group for admission but readmissions are frequent and hospital stays are prolonged [22]. In-hospital mortality of either acute de novo or acute decompensation of chronic heart failure is between 4-7% and mortality at 3 months is high (13%) [23, 24]. Information about developing countries is limited. In 2004 the WHO stated that the incidence of heart failure in 2004 was 5.7 million per year with the highest incidence in East Asia, Europe and Western Pacific [19].

2.2 DEFINITION

The definition of heart failure has also evolved over time. It is a complex clinical syndrome that requires characterization of the syndrome in terms of severity, the underlying cardiac abnormality, its etiology, and also the manner in which the body has adjusted to the dysfunction. In summary, heart failure reflects a structural or functional

cardiac disorder that impairs the ability of the ventricle to fill (diastolic) with or eject blood (systolic) which produces clinical signs and symptoms caused by complex responses to that dysfunction. It is characterized by non-specific symptoms and signs (Figure 4) and as of yet there is no yet single definitive test for its diagnosis. Both European and American guidelines for the diagnosis and management of heart failure state that symptoms should be present at rest or on exercise and evidence of cardiac dysfunction needs to be obtained. Where an element of doubt persists both response to therapy or measurement of plasma concentration of B-type natriuretic peptide is recommended [25]. However, the final diagnosis still remains clinical based on a careful history and physical examination.

Classical clinical criteria have been modified from those initially obtained in the Framingham cohort (Figure 3) [26]. Despite the fact that none of them are either specific or sensitive enough for diagnosing heart failure or for determining the exact etiology [27], these criteria have been applied for several decades and now possible conclusions on trends in incidence and prognosis may be done.

Figure 4. Framingham clinical criteria for the diagnosis of heart failure. The diagnosis of HF requires that 2 major or 1 major and 2 minor criteria cannot be attributed to any other medical condition [28].

Major	Minor
Paroxysmal nocturnal dyspnea and/or orthopnea	Ankle edema
Elevated jugular venous pressure	Hepatomegaly
Bilateral pulmonary rales	Pleural effusion
Third heart sound	Tachycardia (heart rate≥120 beats/minute)
Cardiomegaly on chest x-ray	Weight loss of 4.5 kg or more in five days
Pulmonary edema on chest x-ray	Dyspnea on exertion
Peripheralvenous pressure > 16cm H_20	Decrease in vital capacity (at least 33%)
Weight loss increase of at least 4.5 kg in five days in response to treatment of heart failure	

2.3 EPIDEMIOLOGY

Estimates of the incidence of heart failure have been available due to studies such as the Framingham Heart Study, that observed a cohort of subjects initially free of heart failure every two years, and the Hillingdon and Bromley Heart Failure Studies in England that reviewed all new diagnosis of heart failure within a geographical area [6, 29, 30]. The median age at presentation in recent studies is mid-70s and in all age groups more prevalent in men than in women [31]. The suggested prevalence in industrialized countries is about two percent of the adult population with a steep rise with aging. Heart failure is not a common condition in younger patients and as mentioned before just one study [15] included subjects under 45 years old (from 25 years of age on). On the basis of these studies and newer ones the burden of disease is thought to be 6 million Europeans living with heart failure out of 450 million and 4 million Americans out of 300 million inhabitants of North-America, respectively [12, 32].

2.4 ETIOLOGY OF HEART FAILURE

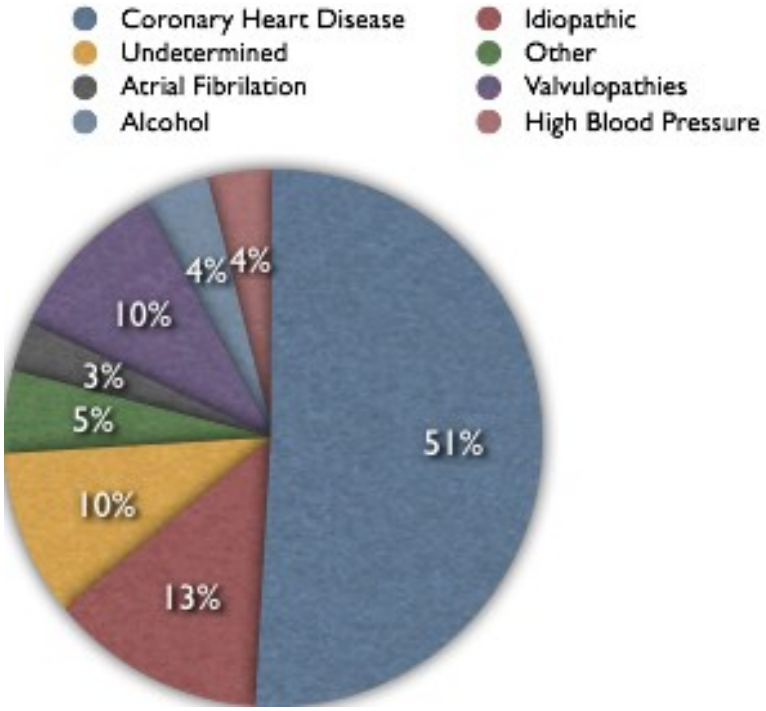
The frequency of each pathology causing the damage to the pumping ability of the heart depends on the population studied and the methods. In most series of industrialized countries, the most common cause of heart failure is coronary heart disease (Figure 5). Hypertension and senile degeneration of the aortic valve are also common causes in the older population of developed countries. Rheumatic valvular heart disease, hypertension and Chagas are common causes of admission to hospital with heart failure in developing countries [29].

2.5 PATHOPHYSIOLOGICAL CONCEPTS OF HEART FAILURE

Any form of heart disease may lead to heart failure and it is thought that there is no single mechanism that accounts for the entire syndrome. The schema of the sequence of events in heart failure is daunting. Multiple subsequent alterations in organ and cellular physiology are thought to contribute at different points in time. In figure 6 there is a summary of possible causes of myocardial failure and the subsequent compensatory

mechanisms. However, distinguishing primary etiological forces from secondary epiphenomena has still to be elucidated.

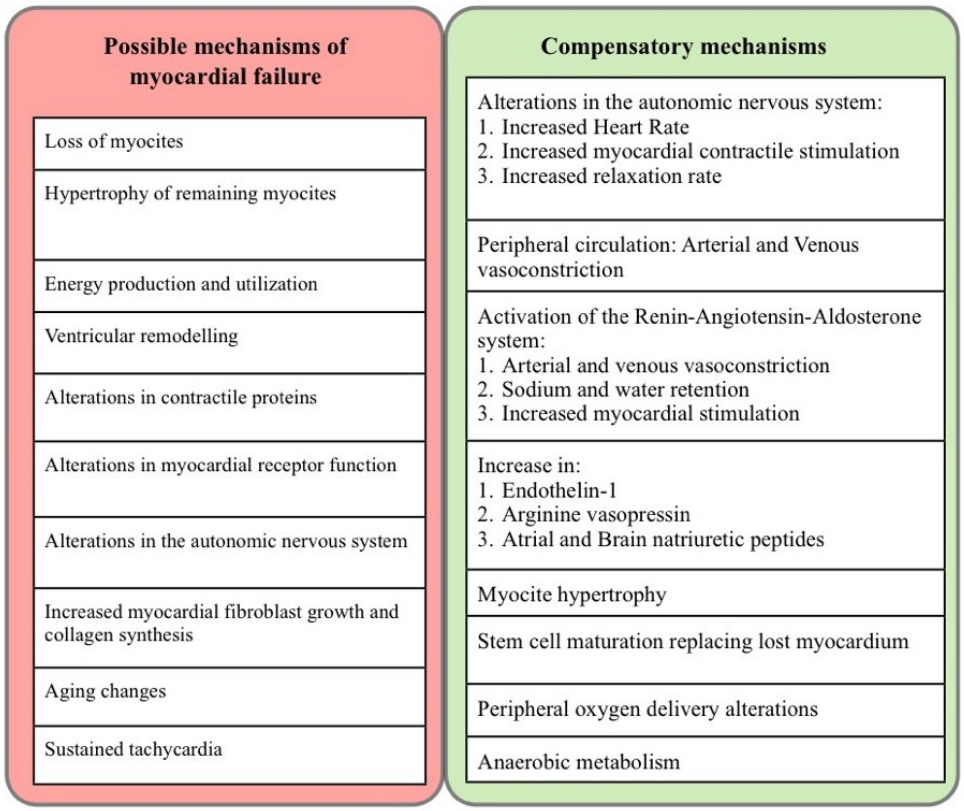
Figure 5. Etiology of new cases of incident heart failure in a population under 75 years of age [29].



Among the known factors that contribute to the pathology of heart failure, genetics and molecular cardiology play a growing role. Sarcomeric and cytoeskeletalproteins are altered in the failing human heart. Aberrant behaviour of both sodium and potassium channels as well as calcium-cycling proteins is also observed in heart failure. Metabolic maladaptations such an abnormal pattern of energy production (lower energy molecule production) and storage can be both the cause or the consequence of heart failure. Increased peripheral vascular resistance, defective parasympathetic control, abnormal response to postural changes and reduced response to a variety of stimuli are physiological adaptations of the autonomic nervous system to heart dysfunction. Hemodynamic perturbations are found when compensatory mechanisms eventually fail in their role. During the period in which the cardiac pumping function and cardiac output can be maintained by compensatory mechanisms, individuals have little or no limitations. Eventually, the myocardium has no

ability to increase the cardiac output in response to increase in oxygen demand and a vicious circle of maladaptation to overload is established. Ultimately, cardiac output decreases even at rest [33].

Figure 6. Possible mechanisms of myocardial failure (left) and compensatory mechanisms (right) [33].



Several mechanisms,including myocite stretch, activate a growth response that initiates myocite hypertrophy but also the same signal may lead to the activation of programmed cellular death. This adaptation leads to structural architectural changes in myocites that initiate remodelling. This remodelling is the increase in intraventricular volume due to increased myocite length and hypertrophy. The excessive stretch of myocites and changes into the cardiac interstitium may lead to interstitial loci of fibrosis that replaces myocites and disrupts the complex maintenance of cardiac homeostasis [34].

Both overload and architectural changes lead to performance causing upregulationof

malcompensatoryneurohormones. Those neurohormones have been found to circulate in abnormal high quantities in heart failure (Figure 7). The predominant consequence of most neurohormone release in heart failure is vasoconstriction and water and salt retention that attempts to maintain perfusion pressure.

Figure 7.Neurohormonal changes in the failing heart. [33]

Increased norepinephrine and epinephrine	Increased atrial and B-type natriuretic peptides
Increased endothelin	Increased dopamine
Increased arginine vasopressin	Increased prostaglandins
Increased renin and angiotensin II	Increased vasodilator peptides
Increased aldosterone	Increased of inflammatory proteins
Increased neuropeptide Y	

By contrast, natriuretic peptides such as atrial and brain peptides are considered to be counterregulatory in signalling high loading pressures. The atrial natriuretic peptide (ANP) is normally synthesized and stored in the atria, and to some extent also in the ventricles. It is released into the circulation during atrial distension. Brain Natriuretic Peptide (BNP) is synthesized mainly in the ventricles and is released in LV dysfunction or early heart failure after cleavage from the pro-peptide to BNP and Nt-proBNP. Those three peptides (ANP, BNP and Nt-proBNP) try to reduce right atrial pressure, systemic vascular resistance, aldosterone secretion, sympathetic stimulation, cell hypertrophy and may enhance sodium excretion [35, 36]. BNP exogenous infusion and drugs designed to inhibit the degradation of natriuretic peptides may be a potential therapy for heart failure [37]. The release of BNP is increased in patients with symptomatic and asymptomatic LVD. Normal plasma values (<100pg/mL) are increased with age and are higher in women than in men. Values below 100 pg/mL have a very high negative predictive value for heart failure as a cause of dyspnea and values above 400pg/mL are associated with high rates of heart failure in patients with dyspnea [38]. ProBNP has also been used to screen general population in the search for asymptomatic LVD. In a study with 2042 selected residents of Minnesota older than 45 years, BNP had a discriminatory value of 0.79 for detecting any significant LV dysfunction [39].

2.6 RISK FACTORS FOR THE DEVELOPMENT OF HEART FAILURE

Risk factors were investigated in the Framingham Heart Study as the development of heart failure increased with age and with the presence of cardiovascular disease. Myocardial infarction and high blood pressure were clearly distinguished [40, 41]. Other population-based studies addressed risk factors: male gender, older age, physical inactivity, overweight, diabetes mellitus, hypertension, valvular heart disease and coronary heart disease are consistently observed as associated with the development of heart failure [21, 42]. These risk factors are of the highest importance in identifying those individuals at increased risk of heart failure. Early modification of risk factors can at least postpone the initiation of heart failure in those individuals in stages A or B.

2.7 PROGNOSIS OF HEART FAILURE

Survival of incident cases is remarkably uniform across all published series: 63, 51 and 35 per cent of survival rate at 1, 2 and 5 years, respectively [43]. Most recent data showed an improvement in prognosis compared to the last 30 years: 60 and 41 per cent of survival at 2 and 5 years respectively [7, 44].

3. NON-COMPACTION CARDIOMYOPATHY

3.1 INTRODUCTION

Isolated non-compaction of the left ventricular myocardium was first described in 1984 [45] as "Identification of a rare congenital anomaly of the myocardium by two-dimensional echocardiography: persistence of isolated myocardial sinusoids". At present it is classified as a primary genetic cardiomyopathy by the American Heart Association [3] and remains as unclassified cardiomyopathy for the European Society of Cardiology [4].

Its main feature is the presence of a thickened myocardium with two persistent layers: the compacted and an unexpected prominent non-compacted layer. The latter is described with prominent trabeculae and deep intertrabecular recesses with continuity with the ventricular cavity (LV). Non-compacted myocardium may also be seen in association with other cardiac abnormalities such as congenital pulmonary atresia, Ebstein's anomaly, bicuspid aortic valve, corrected transposition, isomerism of left atrial appendage and ventricular septal defects [46, 47, 48]. Non-compacted myocardium has also been described in association with neuromuscular disorders - Barth syndrome, Charcot-Marie-Tooth disease 1A, Melnick-Needles syndrome and nail-patella syndrome [49]. In the absence of coexistent congenital defects, this disorder is called "Isolated non-compaction of the left ventricle". And as it is thought that in approximately 50% of all cases of non-compaction, the right ventricle may also be involved, the term IVCLV has evolved to the generic non-compaction cardiomyopathy (NCC).

3.2 ETIOLOGY

The basic mechanism involved in INCLV is thought to be an abnormal arrest in intrauterine myocardial development. During the initial embryogenesis, the two initial myocardial layers (trabecular and epicardial compacted layer) are nourished by blood from the LV cavity that enters the intertrabecular spaces. From the 5^{th} week of human heart development on, the ventricular myocardium undergoes gradual compaction, from the epicardium to the endocardium and from the base to the apex, with the transformation of

the intertrabecular spaces into capillaries [50-52]. Beyond the 8th week of development, the myocardium is nourished by the newly formed coronary artery tree. Despite the fact that the main mechanism is thought to be an arrest in myocardial morphogenesis, there is no actual proof of this theory. Pronounced hypertrabeculation may be the result of various disorders such as myocardium dissection, frustrated attempts of myocardial hypertrophy, myocardial tearing and metabolic defects [49]. The most probable theory may include an altered regulation in cell proliferation, differentiation and maturation of ventricular wall as pathogenetic processes responsible of this anomalous compaction [53].

NCC can be either sporadic or familial. In the case reports series that have addressed familial inheritance, 12-50% of the index cases had a family history of NCC [54, 55]. Autosomal dominant inheritance is more common than recessive or X-linked inheritance [56]. Although differentiation of genotypes is not currently possible for NCC, there is increasing recognition of the genetic loci implicated in the major cardiomyopathies. Hypertrophic and apical hypertrophic cardiomyopathy share molecular etiology with NCC [57] but NCC coexists more frequently with congenital heart disease or Wolff-Parkinson-White syndrome. Several mutations in genes, mainly coding cytoskeleton (alpha-dystrobrevin) and sarcomeric proteins (beta-myosin cheavy chain, alpha-cardiac actin and troponin T2), have been identified as linked to NCC [58, 59]. The mutation in the gene coding for tafazzin protein (a component of cardiolipin at the mitochondrial membrane) in patients with Barth's syndrome is associated with a 53% increase in having left ventricular trabeculations or true NCC [60].

3.3 PREVALENCE AND CLINICAL COURSE

The prevalence of NCC in the general population remains unclear due to two fundamental issues: the unknown preclinical course of this cardiomyopathy and the diagnostic methodology. NCC was diagnosed in 0.014% of all patients undergoing an echocardiogram in Switzerland [61]. In the pediatric population of Australia it is accounted as for 9.2% of all cases of primary cardiomyopathy [62] and represents the 3-4% of patients with overt heart failure [63].

As seen in the introduction section, knowledge of the subclinical course of the known cardiomyopathies is still scarce. Published series included patients who were either symptomatic or undergoing an imaging diagnostic test. For that reason, it may be correct to assume that we are currently detecting the "tip of the iceberg" and actual data may underestimate the real prevalence that should progressively include subclinical individuals. However, owing to the improvement in imaging techniques and the progressive awareness of this condition, its recognised prevalence may be increasing. Besides, prevalence studies nowadays include asymptomatic cases of familial screening [64].

The major clinical manifestations of symptomatic NCC are heart failure, atrial and ventricular arrhythmias and thromboembolic events -stroke- [54, 61, 63] (Figure 8). The mean time from the onset of symptoms to the correct diagnosis was about 3.5 years in the pediatric population [66]. At time of diagnosis the most frequent clinical manifestation is dyspnea. The electrocardiogram, also at time of diagnosis, is usually abnormal and the most frequent findings are ST and T wave abnormalities (41%), tall QRS complex (34%) and left bundle branch block (19%) [67]. However there are no specific or characteristic changes.

The prognosis is thought to be symptom-dependent as asymptomatic patients may be free from cardiovascular events over a mean of 43 months [68]. Early recognition and treatment and also primary sudden death prevention may be supporting a better prognosis in the latest series of cases (Figure 8).

Figure 8. Clinical characteristics of the largest NCC published series.

Characteristics	Pediatric patients		Adult patients						
First author Series Last Name	Chin [80]	Ichida [82]	Oechslin [59]	Sengupta [81]	Murphy [55]	Stöllberger [65]	Kohli [67]	Aras [83]	Lofiego [66]
Number of patients, number	8	27	34	32	45	140	199	67	65
Age at diagnosis, median	7	5	40	49	37 (mean)	53 (mean)	64 (mean)	45	53
Male gender, %	63	56	74	53	62	61	62	56	x
Familial concurrence, %	50	44	18						
Follow-up, maximum in years	5	17	11		15	13		4	16
EKG findings									
Bundle Branch	25	15	56		29	25	24		
Wolff-Parkinson-white	13	15	0		x	3	x		
Ventricular Tachicardia	38	0	41		20	18	7		
Clinical manifestations									
Heart Failure	63	30	68	63	62	45	19	30	32
Thromboembolic events	38	0	21	x	4	x	x	x	x
Associated neuromuscular disorders	x	x	x	x	x	16	x	x	x
Cardiovascular death	38	7	35	x	2	6	x	6	3

EKG: Electrocardiographic

3.4 DIAGNOSTIC CRITERIA

There is no universally accepted definition of NCC at present. The initial suspicion is based on echocardiography. Current diagnostic criteria are based on a relatively "small" number of symptomatic patients (n=104) with echocardiography measurements. Some criteria require double-layered appearance of the myocardium and others require prominent or numerous LV trabeculations (Figure 9).

Figure 9. Current echocardiographic criteria for NCC.

Chin et al. [81]
Ratio of X/Y ≤ 0.5 X= Distance from the epicardial surface to the trough of the trabecular recess Y= Distance from the epicardial surface to the peak of the trabecula *Measurements were taken at LV apex on parasternal short axis and apical views and on LV free wall at end-diastole.*
Jenni et al. [88]
1. A two layered myocardium with a thin compacted (C) layer and a thick non-compacted (NC) with a ratio NC/C > 2. **2. Absence of co-existing cardiac structural abnormalities** **3. Numerous, excessively prominent trabeculations and deep intertrabecular recesses** **4. Recesses supplied by intraventricular blood on colour Doppler** *Measurements were taken at the parasternal and short axis views at end-systole.*
Stollberger et al. [48]
1. More than 3 trabeculations protruding from the left-ventricular wall, apically to the papillary muscles, visible in a single image plane **2. Intertrabecular spaces perfused from the ventricular cavity, visualized on colour Doppler imaging.** *Measurements are valid in a single plane.*

In a recent study by Kohli et al [69], 47 patients fulfilled one of the three criteria of non-compaction: 79% fulfilled Chin criteria, 64% fulfilled Jenni criteria and 53% the criteria of Stöllberger. Depending on the criteria used, the prevalence of NC would have ranged from 12 to 18%. The specificity is observed to be low not only in index cases but also in controls: 24% of patients with LVD (and without NCC) and 8% of the normal controls fulfilled one or more of the three sets of echocardiographic criteria for NCC. However, the presence of all three criteria was rare in cardiomyopathies other than NCC.

Not only is there no gold standard definition but in addition echocardiography posseses important limitations in evaluating the apex of LV where the non-compacted myocardium appears to be more commonly located [70-72]. Additionally, the acquisition may be challenging in patients with poor acoustic windows and interpretation of the images

may vary between echocardiographers (10 to 30% of intra and inter-observer variability) [70-72]. The accuracy of measurements is also limited by geometric assumptions regarding the shape of both left and right ventricles taken in 2D echocardiography. However, advantages of 2D echocardiography are clear in terms of availability, non-invasive and real time examination. New three dimensional echocardiography offers incremental benefits on measurements of LVEF, volumes and structures over the 2D techniques. Measurements taken with 3D echocardiography correlate well with cardiovascular magnetic resonance, the most accurate noninvasive imaging technique for volumes, structures and function [73, 74].

3.5 NCC AND CARDIAC MAGNETIC RESONANCE

New imaging technologies with better spatial and temporal resolution such as magnetic resonance may improve the accuracy in diagnosing NCC.

In the last decade, CMR imaging has changed dramatically as technical and clinical advances have expanded CMR imaging from primarily a tomographic technique to a different one that provides dynamic and high-resolution imaging. Current CMR imaging provides a multifaceted approach to cardiac diagnosis by enabling the assessment of not only morphology and function but also it allows tissue characterization and blood flow during a single comprehensive examination. Medical magnetic resonance imaging is based on the magnetization properties of atomic nuclei of Hydrogen which has a unique magnetic momentum that describes the strength and direction on the microscopic magnetic field that surrounds the nucleus. Each nucleus rotates at a characteristic frequency that is proportional to the strength of the external field. The fraction of protons aligned with the magnetic field can be perturbed by application of radiofrequency energy. The density of protons as well as the rate at which the magnetization returns to equilibrium can be measured by the radiofrequency signal or echo emitted by the protons as they relax. Small differences in the microenvironment of different tissues can be detected by pulse sequences to emphasize tissue differences. This technique affords high quality myocardial definition and contrast, two properties that have been of the outmost importance in the growth of medical literature concerning NCC within recent years.

The initial investigation of NCC with CMR was deceptive. In a series of 19 NCC patients diagnosed by echocardiography using Stöllberg criteria, only 9 met these criteria on CMR [76]. Not only did echocardiographic diagnostic criteria not have enough sensitivity but also a series found non-compacted myocardium in 91% of healthy volunteers at the apex of LV, in 78% within the mid-cavity levels and at 21% in basal levels [76]. And, the two-layered appearance was most common in the apical, anterior and lateral segments in controls and cases. Patients with diagnosed NCC (n=7) were also compared with a group of controls (healthy volunteers and patients with a potentially differential diagnosis for NCC). Patients with NCC had involvement of significantly more myocardial segments and the end-diastolic non-compacted/compacted ratio was, on average, 60% greater than in healthy subjects or patients with other pathologies. A ratio of non-compacted over compacted myocardium above or equal to 2.3, measured in three long-axis views,had a sensitivity of 86%, specificity of 99%, positive and negative predictors of 75% and 99% respectively in distinguishing NCC. From then on, this cut-off ratio was established as diagnostic feature of NCC. Furthermore, data about distribution of the highest amount of trabeculated myocardium in NCC patients was first published: the highest amounts of trabeculated myocardium were found at the apex and at anterior segments.

Figure 10.Current diagnostic NCC criteria by CMR.

Petersen et al. [76]
NC/C Ratio > 2.3 *Measurements done at end-diastole in at least three long-axis planes*
Fazio et al. [77]
NC/C Ratio >2.5 *Measurements done at end-diastole*
Jacquier et al. [78]
LV trabeculated mass > 20% of LV mass *Measurements were done at end-diastole in short axis*

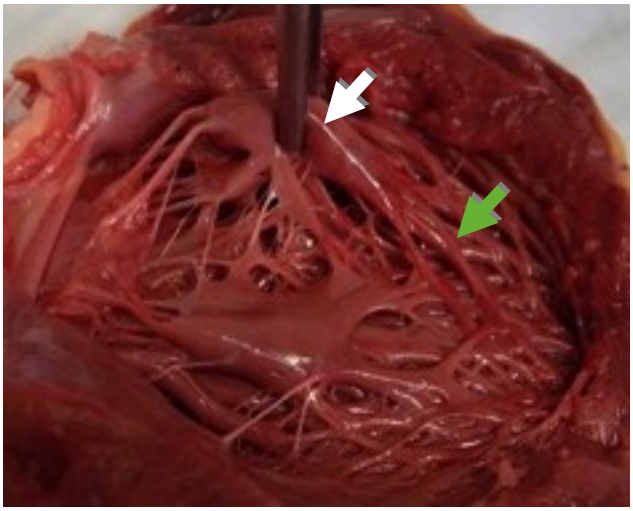
Later, Fazio et al [77] described a mean value ratio of spongeous to compacted

myocardium of 3.1 measured by magnetic resonance in 8 patients diagnosed with NCC. They conclude that due to the higher resolution of magnetic resonance it would be reasonable to increase the previous cut-off to 2.5 (Figure 10).

In 2010, a French group reported a different measure to distinguish NCC patients from those with other cardiomyopathies or healthy controls. A percentage of trabeculated mass above the 20% of the global LV mass predicted NCC with high accuracy and reproducibility [78].

However, there is an important lack of knowledge about the "inside" of the ventricular structure. The progressive awareness of NCC has awakened the curiosity and finally the need to know how the ventricular wall is organized and understand the distribution of the trabeculated myocardium. Autopsy (Figure 11) and daily clinical observations (Figure 12) show that trabeculated myocardium can be seen even in normal hearts and that it does not correspond to what is usually seen in available textbooks or on the Internet (Figure 13). Nevertheless, old case reports are useful to understand that intracavitary structures are frequent and many times underestimated [79, 80].

Figure 11. Postmortem examination of a normal left ventricular myocardium (unpublished). Myocardium hast been cut and retracted to expose inner ventricular wall.



White arrow corresponds to papillary muscles.Green arrow to trabeculations.

Finally during 2011 a description of the distribution of trabeculated myocardium in normal hearts was published [81]. The cohort consisted of 120 subjects (60 females and 60 males) aged from 20 to 80 years old (10 per each decile) that were studied by 1.5T CMR with a similar imaging protocol to that we performed. Trabeculations were more frequently seen at younger ages and gender differences were observed when the whole myocardial thickness was compared. Trabeculated myocardium was a frequent finding that was found to diminish with aging. The highest volume of trabeculated myocardium was found at anterior and apical segments and was most observed during diastole. This study needs to be corroborated by other larger cohorts to obtain an anatomical normal basis to determine what is within normality.

Figure 12. Trabeculations seen in a subject without known cardiovascular disease.

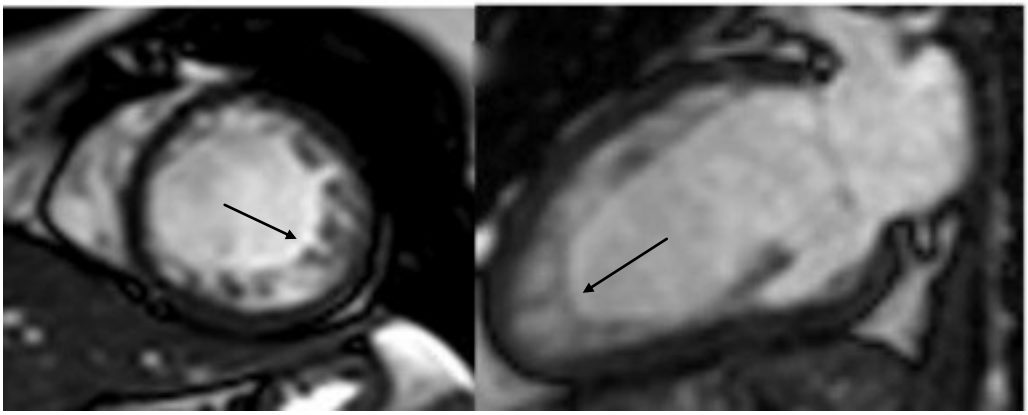
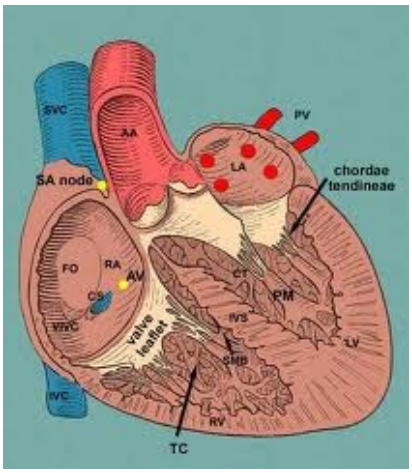


Figure 13. Standard heart anatomy.



4. THE CURRENT UNMANAGEABLE UNCERTAINTY

As scientists and clinicians we understand that CMR is currently the gold standard for visualizing intracavitary LV structures, measuring volumes and obtaining a functional idea of both ventricles. These measurements are vital in our daily practice when we must decide if a patient has a disease or not and take the consequent steps to initiate treatment treat a patient. In addition, modern medical decisions are not focused only on the single patient but also on first-degree relatives in order to identify subclinical disease as current guidelines recommend [82]. These guidelines are supported by the evidence that several cardiomyopathies including NCC frequently have familial inheritance and that affected members are often asymptomatic. Progressive disease may occur within a relatively short period of time in initially asymptomatic family members with abnormal electrocardiographic or echocardiographic findings [83].

However, not only is the diagnosis of NCC difficult at present due to poorly defined "LV normality" by CMR but the screening of first-degree relatives is even more arduous as screening is typically focused on the young population, a population in whom the definition of normal is even more remote. Furthermore, as the natural course of the disease is not known, the follow-up and the treatment of both asymptomatic and symptomatic patients remains uncertain. Therefore, NCC continues to be diagnosed with a high degree of uncertainty underlining the critical importance of determining what is the normal distribution of trabeculations and compacted myocardium in the young and asymptomatic population.

5. HYPOTHESIS AND OBJECTIVES

The objectives of this work were to study the characteristics of the LV myocardium by CMR in a young and asymptomatic population in order to:

1. Quantify trabeculated and compacted LV myocardium;
2. Determine the distribution of trabeculated and compacted LV myocardium;
3. Quantify the percentage of young asymptomatic population that meet the current diagnostic NCC criteria;
4. Determine the reliability of the measurements of the trabeculated and compacted myocardium performed by magnetic resonance imaging
5. Evaluate if trabeculated LV myocardium may be associated with parameters of cardiac function such as global LV ejection fraction, segmental systolic wall thickening and circulating plasma Nt-proBNP.

6. THE PAPER

6.1 TITLE PAGE

Full title: Characteristics of Trabeculated Myocardium Burden in Young and Apparently HealthyAdults

Running title: Tizón-Marcos - Trabeculated Myocardium in Healthy Adults

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Word Count: 6,043

Journal Subject Codes: [6] Cardiac development; [16] Myocardial cardiomyopathy disease; [30]CT and MRI

6.2 RÉSUMÉ

Introduction : La cardiomyopathie non-compactée (NCC) est définie par l'augmentation du myocarde trabeculé. Le progrès de l'imagerie a conduit à l'identification de plus en plus commune de trabéculations chez les adultes en santé avec des conséquences inconnues. Nous avons quantifié et déterminé l'impact de la charge des trabéculations sur la fonction cardiaque chez une cohorte des jeunes adultes en santé.

Méthodes et résultats :Cent adultes âgés entre 18-35 ans (moyenne 28 ± 4 ans, 55% de femmes) sans maladies cardiovasculaires connues ont été étudiés de façon prospective par résonance magnétique cardiovasculaire (CMR). Les volumes ventriculaires gauches, la fonction segmentaire, la fraction d'éjection du ventricule gauche (FEVG), et les volumes auriculaires ont été déterminés. L'épaisseur et la superficie de myocarde trabéculé et dense ont été mesurées pour chaque segment normalisé du ventricule gauche. La fraction N-terminale du pro-peptide natriurétique de type B (Nt-proBNP) a été mesurée. 18% des sujets possèdent au moins un critère traditionnel de NCC. Les ratios du myocarde trabéculé/dense sont uniformément augmentés en fin de la diastole vs.enfin de systole (0,90 ± 0,25 vs. 0,42 ± 0,13, p <0,0001), chez les femmes par rapport aux hommes (moyenne 0,85 ± 0,24 vs. 0,72 ± 0,19, p = 0,006), dans les segments antérieurs vs.non-antérieurs (1,41 ± 0,59 vs. 0,88 ± 0,35, p <0,0001), et dans les segments apicaux vs.non-apicaux (1,31 ± 0,56 vs 0,87 ± 0,38, p <0,0001). Les plus grands ratios de myocarde trabéculé/dense ont été associés à une diminution de la FEVG (57,0 ± 5,3 vs. 62 ± 5,5, p = 0,0001) et à un niveau plus élevé de Nt-proBNP (203 ± 98 vs. 155 ± 103, p = 0,04). La régression multivariée a identifié les ratios de myocarde trabéculé/dense à la fin de systole comme le facteur prédictif indépendant d'avoir une moins bonne FEVG, au-delà de l'âge, du genre, les volumes du ventricule gauche ou de l'oreillette gauche et du niveau de Nt-proBNP (β = -9,9, IC à 95% -15 à 4,9, p <0,001).

Conclusions : Les adultes en santé possèdent des quantités variables de myocarde trabéculé qui ne correspondent pas à la définition actuelle de la NCC. Une quantité importantede trabéculationsest associée à une diminution de la fonction ventriculaire gauche. Apparemment, les jeunes adultes en santé avec une charge augmentée du myocarde trabéculé ont une fonction cardiaque légèrement altérée.

6.3 ABSTRACT

Background: Increased myocardial trabeculations define non-compaction cardiomyopathy (NCC). Imaging advancements have led to increasingly common identification of prominent trabeculations in otherwise healthy adults with unknown implications. We quantified and determined the impact of trabeculations' burden on cardiac function and stretch in otherwise healthy young adults.

Methods and Results: 100 adults aged 18-35 (mean 28±4 years, 55% females) without knowncardiovascular disease were prospectively studied by cardiovascular magnetic resonance (CMR).Left ventricular volumes, segmental function, and ejection fraction (LVEF), and left atrial volumes were determined. Thickness and area of trabeculated and dense myocardium were measured for each standardized LV segment. N-terminal pro-brain natriuretic peptide (Nt-proBNP) was measured. 18% of individuals had ≥1 positive traditional criteria for NCC. Trabeculated/dense myocardium ratios were uniformly greater at end-diastole vs. end-systole (0.90±0.25 vs. 0.42 ±0.13, $p<0.0001$), in women vs. men (mean 0.85±0.24 vs. 0.72±0.19, $p=0.006$), at anterior vs. nonanterior segments (1.41±0.59 vs. 0.88±0.35, $p<0.0001$), and at apical vs. non-apical segments (1.31±0.56 vs. 0.87±0.38, $p<0.0001$). The largest trabeculated/dense ratios were associated with lower LVEF (57.0±5.3 vs 62±5.5, $p=0.0001$) and greater Nt-proBNP (203±98 vs. 155±103, $p=0.04$). Multivariable regression identified greater end-systolic trabeculated/dense ratios as the strongest independent predictor of lower LVEF, beyond age and gender, LA or LV volumes, and Nt-proBNP ($\beta=-9.9$, 95% CI -15-4.9, $p<0.001$).

Conclusions: Healthy adults possess variable amounts of trabeculations that regularly meet criteria for NCC. Greater trabeculations are associated with decreased LV function. Apparently healthy young adults with increased trabecular burden possess evidence of mildly impaired cardiac function.

6.4 INTRODUCTION

Interest in myocardial trabeculations has recently risen due to their importance in non-compaction cardiomyopathy (NCC). This entity - first described in 1984 [1] - is currently classified as a primary genetic cardiomyopathy by the American Heart Association [2] and an unclassified cardiomyopathy by the European Society of Cardiology [3]. Its main feature is the persistence of a double-layered left ventricular myocardium including a prominent inner layer of spongeous or trabeculated myocardium. The etiology of this cardiomyopathy is surmised to be a failure of myocardial compaction that usually occurs by the 5th to 8th weeks of gestation [4]. Normal compaction progresses from the epicardium to the endocardium and from the base to the apex. Although it is commonly held that only a few trabeculae persist at the apex on imaging studies [5-7], autopsy studies revealed that prominent trabeculations may be observed in up to 68% of normal hearts of all ages.These trabeculae course most frequently from the free wall to the ventricular septum, and arisemainly from the apex with a length of 2-4 cm and an average diameter of 5mm [8].

So far, there is no single definition of NCC but the three different diagnostic criteria that have been proposed rely chiefly on the number and extension of trabeculae (Supplemental Table 1) [9-11].

However, echocardiographic studies have observed a wide variation in the prevalence oftrabeculations and false tendons ranging from 1 to 60% [12]. This discrepancy is believed to arisefrom differences in study populations and imaging techniques [13-14]. Although the advent of second iso two harmonic echocardiography has provided significant improvements in imaging quality, large differences persist between the prevalence of trabeculations measured in explanted hearts and those observed in vivo by echocardiography [13]. In the future, contrast media and three dimensional techniques may improve endocardium delineation.

Cardiovascular magnetic resonance (CMR) has recently been adopted as the gold standard imaging modality for left ventricular (LV) morphology and function. Steady-state free precession imaging allows superior LV characterization owing to improved contrast between myocardium and blood leading to more refined delineation of trabeculae and papillary muscles [15]. Using this method, new CMR diagnostic criteria for NCC based on

ratios of non-compacted to compacted myocardium have been proposed after comparing NCC cases - primarily diagnosed by echocardiography - with controls [16-18]. However, when imaged by CMR, trabeculae may constitute up to 13% of LV myocardium in patients with decreased systolic function [19], suggesting that trabeculae may be associated with cardiomyopathies in the absence of NCC. The absence of a gold standard for NCC diagnosis raises the possibility that NCC groups have been contaminated with various other cardiomyopathies in previous trials. At the same time, our daily clinical practice leads us to observe trabeculations in all patients by CMR, with ratios of trabeculated to non-trabeculated myocardium matching diagnostic criteria for NCC routinely observed in patients without suspected cardiomyopathy. We question whether these observations in patients without suspected cardiomyopathy represent a surprising rise in subclinical NCC, an alternate form of subclinical cardiomyopathy, or a normal variant. The implications of increased left ventricular trabeculations in otherwise healthy adults have yet to be examined. We prospectively quantified trabeculations in a large cohort of otherwise healthy adults by CMR, and determined the associations of trabeculae with cardiac function and plasma markers of myocardial stress.

6.5 MATERIAL AND METHODS

Study population

One-hundred (100) consecutive healthy subjects aged 18 to 35 years without known cardiovascular disease or risk factors were prospectively enrolled via email and/or word-of-mouth and provided signed informed consent approved by the institution ethics board. A standardized questionnaire and physical examination were performed. Subjects were excluded if any of the following conditions were present: congenital or acquired cardiovascular disease, hypertension (blood pressure ≥ 135/85 mm Hg or antihypertensive medications), dyslipidemia (plasma total cholesterol > 240 mg/dL or triglycerides > 150 mg/dL; or HDL-cholesterol < 40 mg/dL in men and < 45 mg/dL in women; or lipid-lowering medications), diabetes (fasting blood glucose ≥ 7.0 mmol/L or diabetes medications), or presence of renal, hepatic or blood disorders. Finally, subjects were not included if they possessed contra-indications to CMR, including cardiac pacemaker/defibrillator, non-CMR safe ferromagnetic material in or near to the central nervous system, inner ear, or eyes, or unmanageable claustrophobia. Urine pregnancy tests were performed and pregnant women or those within 1 year of childbirth were excluded because of potential effects on cardiac morphology.

In addition, patients were evaluated with a resting 12-lead electrocardiogram and N-terminal probrain natriuretic peptide (Nt-pro-BNP) (analytical sensitivity 5fmol/mL, proBNP ($^{8-29}$) ELISA,BiomedicaGruppe, Alpco Diagnostics) to further exclude any subclinical cardiac condition. Finally, the CMR images were evaluated for gross congenital anomalies to exclude unknown congenital cardiac conditions. The population therefore consisted of young adults without knowncardiovascular or systemic disease, without cardiovascular risk factors, with a normal resting ECG, with Nt-proBNP within normal limits, and without gross congenital cardiac disease on CMR.

Cardiovascular magnetic resonance

Imaging was performed with a 1.5 Tesla Philips Achieva scanner operating release 2.6 level 3(Philips Healthcare, Best, The Netherlands). Cine imaging of cardiac morphology and function was performed by steady-state free precession (SSFP) technique at 30 phases

per cardiac cycle in held end-expiration. 8-14 contiguous parallel short-axis (8 mm thickness, 0 mm gap) and 3 radial longaxis planes were performed covering the entire cardiac silhouette. Typical parameters included TR/ TE 3.17/1.58 ms, flip angle 60º, and NEX of 1, yielding in-plane spatial resolution of 1.6 x 2mm. For segmental analysis, we also performed myocardial tagging using sensitivity encoding (SENSE) to avoid potential variations due to inclusion/exclusion of trabeculations in assessment of segmental wall function.

Image analysis

Image analysis was performed off-line in an experienced core laboratory (LICA: Laboratoired'imageriecardiovasculaireavancée) using a standardized approach by trained technicianssupervised by an experienced cardiologist (E.L.) following the 16-segment model (CMR Massversion 7.1, Medis, NL) [20-23]. Cardiac volumes and function measurements were performed aspreviously described by our group and others [24]. For left ventricular (LV) volume analysis, theendocardial and epicardial borders were manually determined for all 30 phases of the cardiac cycle and the cardiac phases that demonstrated the largest and smallest ventricular cavity volumes were defined as end-diastole (ED) and end-systole (ES), respectively. Papillary muscles were included in the initial LV wall measurements (equivalent to weighting the LV) and excluded from LV cavity measurements (equivalent to blood pool techniques) [25]. For cardiac volumes and function measurements, the endocardial border was defined as the border between trabeculations and the ventricular blood pool, while still excluding papillary muscles (trabeculations were included in the LV wall and excluded from the blood pool). The LV end-diastolic volume (LVEDV), end-systolic volume (LVESV), stroke volume (LVSV), ejection fraction (LVEF) and LV mass were computed using Simpson's rule. The LVEDV, LVESV and mass were adjusted to body surface area (BSA) calculated by the Dubois Formula [26]. Segmental wall thickness was measured at end-diastole by the centreline method (average of 20-30 chords/segment) and was compared with the average chord thickening at end-systole in each segment to determine segmental wall function. Decreased LV segmental wall function dysfunction was considered present if systolic wall thickening was <30% [27]. As is customary, segment 17 was excluded from functional analysis. We further measured

midwall LV circumferential strain (Ecc) on myocardial tagging images as a measurement of segmental function not influenced by the inclusion/exclusion of trabeculations, contrary to what may be the case when considering only wall thickening. Left atrial (LA) endocardial borders were also determined for all 30 phases and the end-diastole (largest) and end-systole (smallest) volumes and ejection fraction were calculated from Simpson's method [28]. Left atrial appendix volumes were included in the total LA volumes.

Following the preceding standard measurements, LV trabeculated vs. non-trabeculated myocardium was specifically analyzed by two experienced readers (H.T. and E.L.) blinded to each other and to all other variables, followed by resolution of any differences by consensus. Slices from short axis (SA), 4 chamber (4CH) and two chamber (2CH) planes were analyzed. Analysis was performed at end-diastole and end-systole in three planes of SA (basal, mid and apical LV) and in a single plane of 2CH and a single plane of 4CH views (the planes in which the papillary muscles were easier to distinguish from trabeculations) to mirror the approach used for clinical interpretation by most CMR readers. Segmentation followed AHA recommendations by defining mid-ventricular segments as those at the level of the papillary muscles, basal segments as those above the papillary muscles, and apical segments as those below the papillary muscles. We first measured the combined sum of trabeculated and dense (T+D) myocardium by delineating the epicardial border and the trabeculations border. Delineation of the trabeculations border for measurement of T+D was performed by connecting the inner tips (towards the centre of the LV cavity) of all the trabeculations. The full thickness of T+D was then measured by the centreline method along 20-30 chords per segment, providing both 1) maximum and 2) mean thickness and 3) area objectively and avoiding investigator bias associated with individual selection of the measurement site. Afterwards, without altering the delineation of the trabeculations border, the inner border of dense myocardium was delineated to measure trabeculated (T) myocardium thickness and area using the same methodology. Trabeculations were defined per protocol as any muscular structure that moved synchronously with the inner myocardial border (endocardium) over the cardiac cycle and that was not attached to a papillary muscle at end-systole. These two conditions had to be present in at least two orthogonal planes for a muscular structure to be considered trabeculated myocardium. Papillary muscles, false tendons and thrombi were excluded

(Supplemental Table 2) [8, 29, 30]. We thencalculated the thickness and area of dense (D) myocardium for each segment using the formula: D = (T+D) - T. We determined the trabecular burden by each of the three following measurements: 1) thickness of trabeculated over dense myocardium ratio (T/D ratio), 2) thickness of trabeculated myocardium over the total myocardium (%T) and 3) trabeculated area over total myocardium area (%TA). All measurements were performed in ED and ES for all segments in SA, 2CH and 4CH. Taking into account the limits of resolution of the SSFP sequence, we set the analytical sensitivity of our measurements at 1.5 mm.

Statistical methodology

All variables were evaluated by the Shapiro-Wilk test for normality. Categorical variables wereexpressed as percentages and continuous variables as means ± standard deviations (SD). Genderdifferences were verified using chi-square tests for categorical variables and Student t tests forcontinuous variables.When required, logarithmic transformations were performed and linear relationships evaluated byPearson's correlations between global per-subject T/D ratios in ED and ES and 1) LV ejectionfraction, 2) Nt-pro-BNP levels, and 3) Mean segmental function. ANOVA was performed to testassociations between categorical and continuous variables. When segmental analysis wasperformed, repeated observations due to the analysis of 16 myocardial segments per subject weretaken into consideration using generalized estimating equation regression modeling. Univariableand multivariable regression analysis were performed to test associations of T/D ratio with LVEFby including age, gender, body mass index, systolic blood pressure, LA end-diastolic volume, LVend-systolic volume, Nt-ProBNP, and mean T/D ratio at end-systole in the multivariable model.Inter-observer agreement for measurements of trabeculations were evaluated using multipleregression taking into account repeated measures (multiple segments per subject) in a randomsample of 10% of the cohort. Statistical analysis was performed with Stata 11.0 (StataCorp LP,College Station, TX, USA) [31].

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

6.6 RESULTS

General characteristics

The study population consisted of 100 young adults aged 28 ± 4 years, including 55% females(Table 1). No anomalies were present on screening electrocardiograms or plasma Nt-pro-BNPlevels. Blood counts, plasma lipids and blood glucose were within normal limits. Mean leftventricular function, mass and volumes, and LA function and volumes were within normal limitsfor age and gender (Table 2) [32]. On average, females had lower blood pressure and LDL-cholesterol levels, as well as lower LV and LA volumes and LV mass indexed to body surface area (BSA).Loading conditions, as assessed by blood pressure, heart rate, and total body water percentage (bybioelectrical impedance analysis), did not differ between individuals with greater trabecular burden and those without (Supplemental Table 3).

Quantification and distribution of trabeculated myocardium

Trabeculated myocardium was visible by CMR in all subjects. Trabecular burden is reported inTable 3 for the 3 imaging planes in end-systole and end-diastole as 1) T/D ratio, 2) %T (percenttrabeculations relative to total myocardium thickness), and 3) %TA (percent trabeculations relative to total myocardium area). Trabecular burden was not associated to general body morphometrics, defined by BMI (r=0.07, p=1.0). Whether they were quantified by T/D ratio, percent thickness, or percent area, trabeculations were consistently more abundant in SA compared to 2CH plane, and in 2CH compared to 4CH plane. In addition, by all 3 methods of quantification, trabeculations were more abundant in end-diastole compared to end-systole, whether imaging was performed in SA, 2CH or 4CH planes. Females also consistently presented greater proportions of trabeculated myocardium compared to males (mean T/D ratio 0.85 ± 0.24 vs. 0.72 ± 0.19, p=0.006). Ratios of trabeculated vs. dense myocardium per individual segment in the SA plane are reported in Figure 1 for ED and Figure 2 for ES. On average, T/D ratios were greater for anterior wall segments compared to non-anterior wall segments, whether at ED (2.01 ± 1.00 vs. 1.22 ± 0.56, respectively, p<0.0001) or ES (0.81 ± 0.44 vs. 0.52 ± 0.25, p<0.0001). In addition, T/D ratios were greater for apical segments compared to non-apical (basal and mid-

ventricular) segments, either at ED (1.87 ± 0.90 vs. 1.20 ± 0.62, respectively, p<0.0001) or ES (0.73 ± 0.36 vs. 0.53 ± 0.27, p<0.0001). T/D ratio medians and upper limits of normal (90th percentile) are reported in Supplemental Table 4 for each LV segment in each of the 3 imaging planes at end-systole and end-diastole.

Subjects meeting current diagnostic criteria for NCC

In this sample of apparently healthy young adults, 18% met the echocardiographic Jenni criteria for non-compaction (T/D ratio in ES > 2 in at least 1 segment), 13.8% met the Petersen criteria (T/D ratio in ED > 2.3 in at least 1 segment), and 11% met the criteria suggested by Fazio (T/D ratio in ED >2.5 in at least 1 segment) (Supplemental Table 1).

Reliability of trabecular burden measurements

Reproducibility data performed on 10% of the subjects indicated superior inter-observer agreement. Multiple regression taking into account repeated measures performed in each myocardial segment of each subject indicated excellent concordance between T/D ratios measured independently by 2 different observers, whether performed in SA ED (adjusted R2 = 0.99, residual MS = 10.38, p<0.0001), in SA ES (adjusted R2 = 0.99, residual MS = 1.94, p<0.0001), in 2CH ED (adjusted R2 = 1.0, residual MS = 0, p<0.0001), in 2CH ES (adjusted R2 = 0.99, residual MS = 0, p<0.0001), in 4CH ED (adjusted R2 = 0.99, residual MS = 0, p<0.0001), or in 4CH ES (adjusted R2 = 0.99, residual MS = 0, p<0.0001). Correlations between trabecular burden as measured by T/D thickness ratios compared to %T areas were highly positive and significant when measurements were performed in the long axes, whether at end-systole or end-diastole (r>+0.8, p<0.0001) and the short axis (r=+0.86, p<0.0001 at ES, and r=0.62, p=0.01 at ED).

Trabeculated myocardium and Nt-proBNP

On per-subject analysis, greater T/D myocardium ratios were associated with greater Nt-proBNPlevels (per quartile) in the SA plane, whether at ED (F=2.95, p=0.03) or ES (F=6.98, p=0.0003). In addition, the more segments an individual possessed that had an increased T/D ratio (>90thpercentile for the population), the greater was that individual's Nt-proBNP level, whether in SA ED (F=3.2, p=0.03) or SA ES (F=2.66, p=0.05). These

findings were consistent, as the Nt-ProBNP levels of individuals with greater mean T/D ratio (>90th percentile) were significantly greater than Nt-ProBNP levels for the remainder of the study population.

Trabeculated myocardium and LV function

Global LV function

Left ventricular ejection fraction decreased as mean T/D ratios measured at ES increased (Figure 3). Left ventricular ejection fraction also significantly decreased as the number of segments withincreased T/D ratios (>90th percentile) measured at end-systole rose, whether in SA (F=2.50,p=0.02), 2CH (F=2.83, p=0.02) or 4CH (F=4.86, p=0.003) Ejection fraction also decreased astrabeculated area (%TA) measured at ES rose, but only when measured in long axis planes (2CH:r=-0.41, p<0.0001; 4CH: r=-0.47, p<0.0001). These associations between LVEF and trabecularburden were significant only when trabeculations were measured at end-systole, losing significance when measured at end-diastole.

Segmental LV function

Segmental wall function decreased with increasing T/D ratios in long axis planes (Combined 2CH and 4CH: r = -0.37, p<0.0001) (Figure 4) and also with increasing %TA (Combined 2CH and 4CH: r= -0.32, p<0.0001). Decreasing segmental function was significantly associated with increasing T/ D ratios, whether measured in SA (end-diastole coef.= -12.9, p<0.0001; end-systole coef.= -11.8, p<0.0001) or in long axis planes (2CH: end-diastole coef.= -16.6, p<0.0001; end-systole coef.= -24.6, p<0.0001 and 4CH: end-diastole coef.=-18.5, p<0.0001; end-systole coef.= -35.8, p<0.0001).Segmental wall function measured by strain analysis (Ecc) confirmed a statistically significantdecrease occurring with increasing T/D ratio at end-diastole (coef.= -15.7, p<0.0001) and endsystole (coef.= -21.9, p<0.0001).

Multivariate analysis for associations with LVEF

Multivariate linear regression analysis was performed to assess associations with LVEF. A modelincluding age, gender, body mass index, systolic blood pressure, LA end-diastolic volume, LV endsystolic volume, Nt-ProBNP, and mean T/D ratio at end-systole, explained 75% of the LVEFvariance (F=68.12, p<0.001). The variables that remained independently associated to LVEF were: body mass index (β=-0.15, 95% CI -0.29-0.002, p=0.05), LA

volume (β=0.18, 95% CI 0.09-0.27, p<0.001), LV end-systolic volume (β=-0.9, 95% CI -0.98-0.74, p<0.001) and mean T/D ratio at end-systole (β=-9.9, 95% CI -15-4.9, p<0.001). Therefore, even when considering traditional factors explaining variance in LVEF, trabecular burden measured at end-systole maintained an independent association with LVEF.

6.7 DISCUSSION

This is the first report establishing in a prospectively recruited unselected and apparently healthyyoung population that the incidence of non-compaction is greater than anticipated when applyingcurrent non-compaction criteria to CMR. In this, the largest study to date of apparently healthyyoung adults, we identify a greater proportion of trabeculated myocardium at the anterior wall and apex, in females, and at end-diastole. Despite studying apparently healthy adults, increasedrabecular burden is associated with reduced global and segmental left ventricular function, as well as increased plasma Nt-pro-BNP levels. Importantly, these associations of greater trabecular burden remain significant beyond age, gender, and blood pressure.

Myocardial trabeculations have in the past been largely overlooked by clinicians, until recentlywhen non-compacted cardiomyopathy was first described. The diagnosis of NCC is traditionallybased on T/D ratios measured by echocardiography and the subjective appearance of a doublelayered myocardium [9-11]. Newer imaging technologies possessing better tissue contrast, such asCMR, carry the promise of improved accuracy in identifying NCC. In a series of 19 NCC patients previously diagnosed by echocardiography using Stöllberg criteria, only 9 met these same criteria by CMR [33]. Concern rapidly rose when applying these criteria developed for echocardiography led to the identification of non-compacted myocardium in as much as 91% of volunteers by CMR [16]. In our prospectively recruited unselected cohort, 13.8% of all segments met the commonly-used Petersen criteria for NCC (T/D ratio >2.3). Since that time, new diagnostic criteria based on greater ratios or proportions of trabeculated myocardium have been proposed [17, 18]. In our unselected apparently healthy cohort, however, these recently proposed diagnostic criteria are still remarkably prevalent, being met in 7.5% (using the suggested T/D ratio >3 in short axis) to 11% of all segments (using the proposed T/D ratio > 2.5 in any plane).

Previous studies have reported a wide range of trabeculations in healthy subjects. The range varies from 68% in autopsies studies (where trabeculations were defined as >2mm thick) [8] to 8.9% observed with second iso two harmonic echocardiography [13]. CMR studies reported visibletrabeculations involving 6 ± 3 segments of healthy subjects 16 and recent studies indicated thattrabeculations composed up to 12 ±5 % of total myocardial mass [18, 34].

These apparent discrepancies may be explained in several ways. First off, several definitions for trabeculations have been used: from discrete muscle bundles of >2mm [8] to any muscle bundle of >3mm [13] and, in recent CMR studies, as any myocardium protruding from the LV wall into the cavity [18]. Although CMR is an excellent modality to differentiate the different structures of the LV wall and cavity due to its higher spatial resolution in general, and the superior contrast between blood and tissue in dynamic SSFP sequences in particular, there is still no standardized definition of trabeculations. For instance, muscular structures extending from the papillary muscles to the LV cavity are typically difficult to distinguish from trabeculations, and require multiplane dynamic analysis to do so with any degree of certainty (Supplemental Figure 1). Trabeculae delineation in only one axis and one phase may not only be less accurate, by including structures attached to papillary muscles, but also less reproducible. Studies on reproducibility have highlighted the higher inter-observer variability when calculating LV mass and volumes including or excluding trabeculations in short axis [19]. In this study, great effort was made to observe all structures protruding from dense LV myocardium in two orthogonal views throughout the cardiac cycle to precisely differentiate papillary muscles from trabeculations, and measure the latter at both end-systole and end-diastole. While a previous study suggested quantifying trabeculations as a volume (calculated from a sum of areas) to favor a high degree of inter-observer reproducibility [18], we observed superior inter-observer agreement using any of the 3 approaches to measuring trabecular burden (thickness ratio, percent thickness, or percent area). However, it must be taken into consideration that we derived trabeculations thickness by measuring all trabeculations, not by subjectively choosing and measuring only the thickest trabeculae as is commonly performed in clinical practice at this time. For that reason, we found both approaches to be both straightforward and reproducible in quantifying trabeculated myocardium. In addition, our study concludes that whatever the means of accurately measuring trabecular burden (thickness or area), greater trabeculations lead to higher levels of plasma markers of cardiac stretch and lower ejection fraction. However, the strongest predictor of LVEF in our cohort was the mean T/D ratio measured at end-systole. Several factors may explain the repercussions of increased trabeculations on LVEF. The muscular architecture of trabeculated myocardium may not provide contractile function that is as efficient as dense myocardium. Furthermore, the impact of loading

pressures on the cardiac muscle fibers may differ whether they are part of a trabeculated vs. dense architecture, leading to variations in the contractile response to loading described by the Frank-Starling mechanism.

The implications of trabeculations on global and segmental LV function are incompletely understood. Previous reports state that the inclusion of trabeculations in the total LV myocardiumvolume may increase standard LVEF measurements [19] and increase relative systolic wall thickening of the lateral wall when tagging methods are used 35 in patients with systolic dysfunction and controls. Our study of healthy young adults concludes that greater amounts of trabeculations are associated with lower global LVEF, lower segmental function, and increased Nt-ProBNP. It is recognized that asymptomatic left ventricular dysfunction is a precursor of future symptomatic heart failure and is associated with increased mortality [36, 37]. Although the prevalence of asymptomatic left ventricular dysfunction increases with age and risk factors and is thought to be at least 3% in adults older than 40 years old 36, the prevalence of asymptomatic LV dysfunction in younger adults is unknown, and its importance as a precursor of symptomatic heart failure is presumed to be the same than in older adults. In addition, segmental wall dysfunction has also been correlated with a higher incidence of cardiovascular events and death 38 in middle age populations.

Although there are no previous reports of the prevalence and significance of segmental wall dysfunction in younger populations, these findings were associated with lower global LVEF in our cohort. In addition to lower global and segmental function, a greater burden of trabeculated myocardium was associated with higher levels of Nt-proBNP. Nt-proBNP release is increased bycardiomyocyte stretch and commonly used as a marker of greater wall stress. Increased levels arereliable markers of poor cardiovascular prognosis including left ventricular dysfunction, congestive heart failure, acute coronary syndromes, and atrial fibrillation, even when Nt-proBNP levels are only marginally increased or in the diagnostic "gray zone" [39-42], and even in individuals without known cardiovascular disease and LVEF within normal limits [43]. In our apparently healthy population, the consistency of the associations of greater trabeculation burdens with lower ventricular function and greater markers of cardiac stretch underscore a potentially negative role for increased trabeculations. However, although we observe that increasing trabecular burden is

associated with decreasing global and segmental LV function and increasing BNP, which may signal a negative effect on LV physiology, such changes have yet to be associated with increased events in a young and otherwise healthy population.

The distribution of trabeculated myocardium was similar to that reported in healthy controls fromother groups [16, 18], with the greatest burden of trabeculated myocardium located at apical segments and anterior segments. Because of this, the long chamber views may prove to be particularly useful in the measurement of trabecular burden, by simultaneously revealing the anterior wall at full length [44] and facilitating the differentiation of trabeculations from papillary muscles. This may explain why we observed that the associations of trabecular burden and LV function was strongest when trabeculations were measured in the long axis planes. In addition, we also observed a stronger association between trabecular burden and LV function when trabeculations were measured at endsystole.

We attribute this to the observation that the discrimination of trabeculations with papillary muscles is more readily achieved at end-systole when the papillary muscles form a more compactstructure.

We faced several challenges in the accomplishment of this study. To limit the influence of comorbidity and subclinical disease, we recruited young adults without cardiovascular disease orrisk factors. Our findings must therefore be viewed with caution when considering trabeculations in older individuals. To verify our findings in an older population, however, would imply challenging assumptions on "normality" in subjects carrying various amounts of overt and subclinical comorbidities and cardiovascular disease. Furthermore, there is a paucity of data on normal cardiac volumes and function in young adults, leading us to extend markers of risk developed in older populations to this population of younger adults: although global and segmental LV function and Nt-pro-BNP levels carry important prognostic significance in middle-age adults, these factors have yet to be studied in younger adults. On a similar note, these findings relate to an unselected population from North America, and trabeculations distribution/morphology may present variations in specific populations across the globe. Finally, greater delineation of individual trabeculations may have been possible by imaging at higher spatial resolution or with thinner slices, but we felt the tradeoff would have been decreased spatial and temporal

coverage which may have compromised imaging of the entire LV over the entire cardiac cycle.

In conclusion, healthy adults possess variable amounts of trabeculated myocardium, including trabeculated/dense ratios that regularly meet current diagnostic criteria for NCC. Trabeculations are greater at end-diastole, in women, and at the anterior and apical segments. Greater proportions of trabeculated myocardium are associated with a decrease in LV function. Apparently healthy young adults with increased trabecular burden possess evidence of mildly impaired cardiac function.

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6.12 TABLES AND FIGURES

Table 1.Population characteristics.

Characteristics	Total n=100
Age, years	28.0 ± 4.0
Weight, kg	65.7 ± 9.0
BMI, kg/m^2	24.0 ± 4.2
Resting heart rate, bpm	71.0 ± 11.0
Systolic Blood pressure, mmHg	120.0 ± 11.0
Diastolic Blood pressure, mmHg	74.0 ± 8.0
White blood cell count, $10^{12}/L$	6.0 ± 0.8
Platelets, $10^3/mL$	236.0 ± 57.0
Haemoglobin, g/dL	142.0 ±12.0
Total Cholesterol, mmol/L	4.6 ± 0.5
HDL, mmol/L	1.6± 0.5
LDL, mmol/L	2.5 ± 0.8
TG, mmol/L	1.0 ± 0.7
Nt-pro-BNP, pmol/L	168.0 ± 103.0

BMI: Body Mass Index, Nt-proBNP: NT-terminal pro-Brain Natriuretic Peptide, HDL: High Density Cholesterol, LDL: Low Density Cholesterol, TG: Triglycerides.

Table 2.Cardiac morphology and function.

Characteristics	mean ± SD
LV EF, %	60.5 ± 5.8
Cardiac index, L/min/m^2	2.5 ± 0.5
Stroke volume, mL/m^2	35.7 ± 4.9
LV $Mass_i$, g/m^2	46.5 ± 8.5
$LVEDV_i$, mL/m^2	58.2 ± 8.0
$LVESV_i$, mL/m^2	23.1 ± 5.2
LA EF, %	47.0 ± 6.2
$LAEDV_i$, mL/m^2	32.5 ± 7.3
$LAESV_i$, mL/m^2	17.1 ± 4.6

LV: Left Ventricle, LVEDVi: LV End-Diastolic Volume indexed, LVESVi: LV End-Systolic Volume indexed, LAEDVi: Left Atrial End-Diastolic Volume indexed, LAESVi: Left Atrial End-Systolic Volume indexed, LA: Left Atrial.

Table 3. Means and upper limits of normal for trabeculations burden measured by thickness ratio, percent thickness and percent area of myocardium in three standard imaging planes at end-systole and end-diastole.

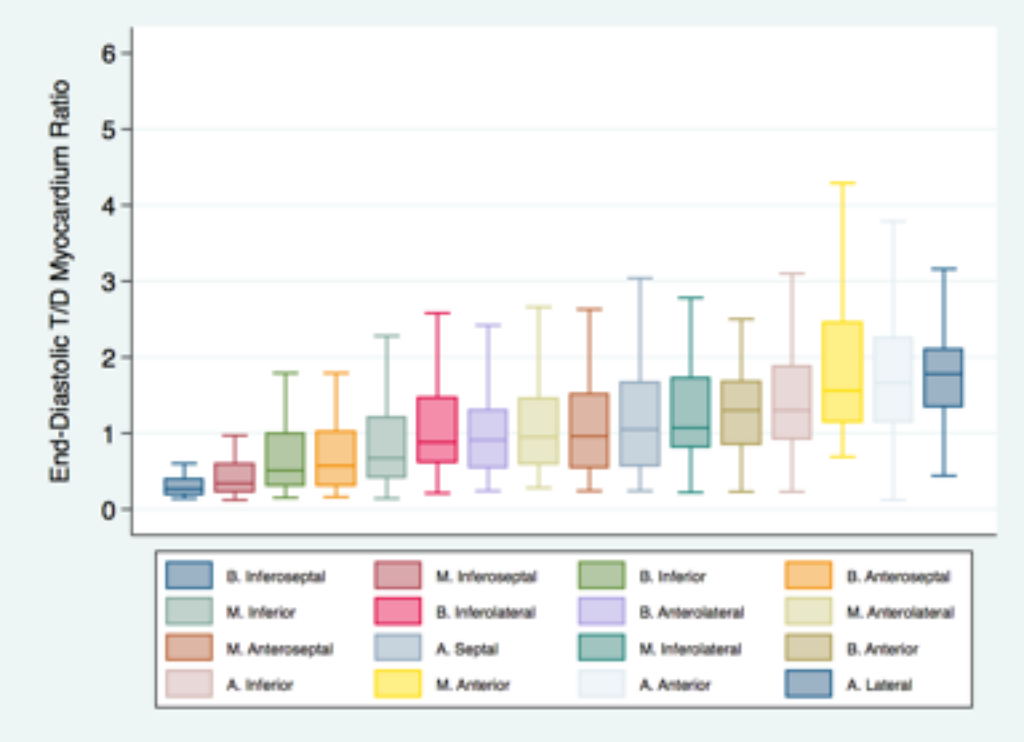
		Trabeculated/dense myocardium thickness ratio		Trabeculated thickness percent of total myocardium		Trabeculated area percent of total myocardium	
		mean ±SD	90th percentile	mean ±SD	90th percentile	mean ±SD	90th percentile
Short-axis plane**	End-diastole*	1.38±0.55	1.90	0.49±0.06	0.56	0.34±0.09	0.38
	End-systole*	0.58±0.25	0.87	0.29±0.06	0.37	0.21±0.45	0.22
2 chamber plane**	End-diastole*	0.74±0.29	1.18	0.38±0.08	0.50	0.30±0.07	0.39
	End-systole*	0.39±0.15	0.60	0.25±0.06	0.33	0.16±0.05	0.22
4 chamber plane**	End-diastole*	0.60±0.21	0.84	0.33±0.06	0.42	0.26±0.06	0.35
	End-systole*	0.30±0.10	0.43	0.21±0.05	0.27	0.13±0.04	0.18

SD: Standard deviation.

* $p<0.05$ End-diastole vs. End-systole for all 3 methods of measuring trabeculations burden

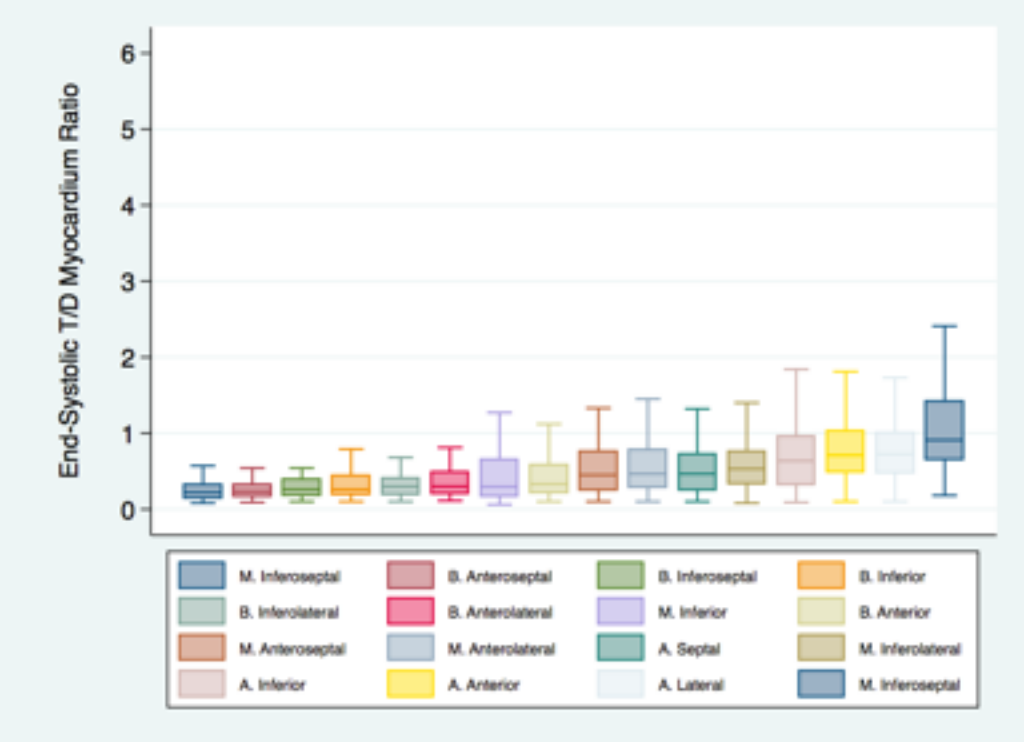
** $p<0.001$ Short-axis plane vs. 2 chamber plane, and 2 chamber plane vs. 4 chamber plane for all 3 methods of measuring trabeculations burden

Figure 1.



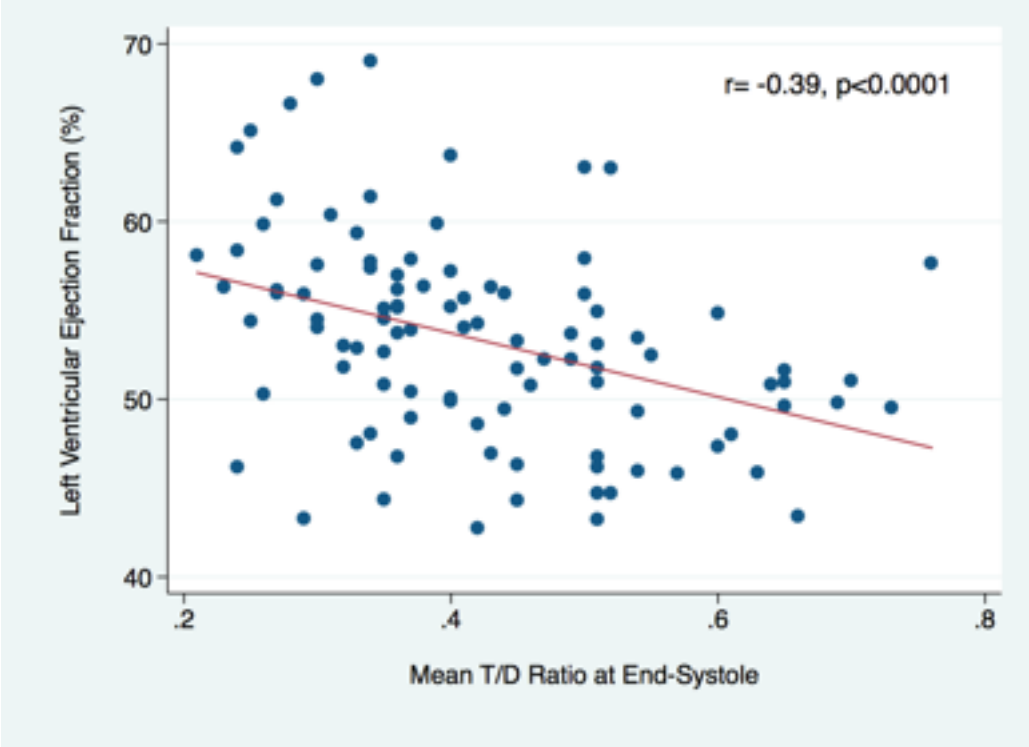
A: apical, B: basal, M: mid-ventricular, T/D myocardium ratio: trabeculated/dense myocardiumratio.

Figure 2.



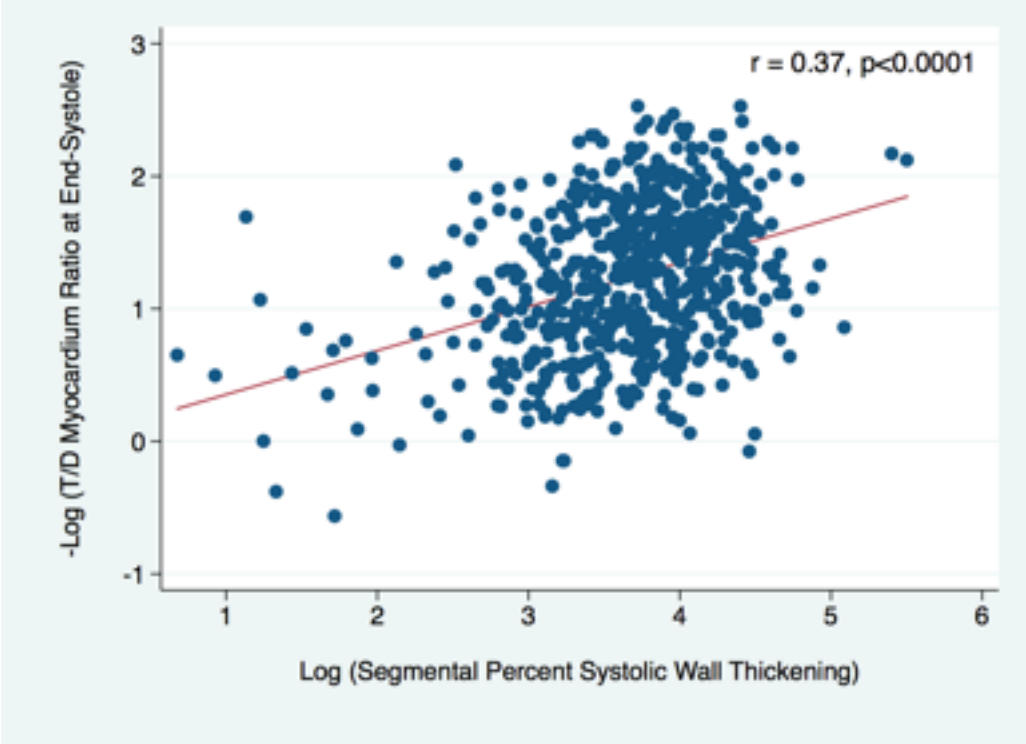
A: apical, B: basal, M: mid-ventricular, T/D myocardium ratio: trabeculated/dense myocardium ratio.

Figure 3.



Mean T/D: mean of the trabeculated/dense ratio per subject.

Figure 4.



T/D: trabeculated/dense ratio.

6.13 FIGURE LEGEND

Figure 1. Box plot of trabeculated/dense myocardium ratios per left ventricular segment in the short axis plane at end-diastole. Segments are listed from left to right in ascending order of T/D myocardium ratio.

Figure 2. Box plot of trabeculated/dense myocardium ratios per left ventricular segment in the shortaxis plane at end-systole. Segments are listed from left to right in ascending order of T/Dmyocardium ratio.

Figure 3. Correlation plot for left ventricular ejection fraction versus mean trabeculated/densemyocardium ratios measured at end-systole (per subject analysis).

Figure 4. Correlation plot for segmental systolic wall thickening versus trabeculated/densemyocardium ratio measured at end-systole in the long-axis plane (per segment analysis).

6.14 SUPPLEMENTAL MATERIAL

Supplemental Table 1.Current diagnostic criteria for left-ventricular non-compaction cardiomyopathy.

Chin et al [10]
Ratio of X/Y ≤ 0.5 X= Distance from the epicardial surface to the trough of the trabecular recess Y= Distance from the epicardial surface to the peak of the trabecular *Measurements were taken at LV apex on parasternal short axis and apical views and on LV free wall at end-diastole.*
Jenni et al [8]
1. A two layered myocardium with a thin compacted (C) layer and a thick non-compacted (NC) with a ratio T/D > 2. 2. Absence of co-existing cardiac structural abnormalities. 3. Numerous, excessively prominent trabeculations and deep intertrabecular recesses. 4. Recesses supplied by intraventricular blood on colour Doppler. *Measurements were taken at the parasternal and short axis views at end-systole.*
Stollberger et al [9]
1. More than 3 trabeculations protruding from the left –ventricular wall, apically to the papillary muscles, visible in a single image plane. 2. Intertrabecular spaces perfused from the ventricular cavity, visualized on color Doppler imaging. *Measurements are valid in a single plane.*
1. More than 3 trabeculations protruding from the left-ventricular wall, apically to the papillary muscles, visible in a single image plane. 2. Intertrabecular spaces perfused from the ventricular cavity, visualized on colour Doppler imaging. *Measurements are valid in a single plane.*
Petersen et al [15]
T/D Ratio in diastole >2.3
Fazio et al [16]
T/D Ratio in diastole >2.5

Supplemental Table 2.Definitions of false tendon, trabeculation and thrombi.

False tendon	Linear cordlike fibromuscular structures that clearly crossed the LV cavity without attachment to mitral valve leaflets
Trabeculations	Muscles bundles that stand out against the background of the ventricular endocardium or coursed like bridges across the ventricle.
Thrombi	Different structures usually attached to LV endocardium with increased signal intensity on T1-weighted image due to oxyhemoglobin or deoxyhemoglobin and lower signal intensity on gradient echo cine images

Supplemental Table 3. Clinical variables of individuals with at least 1 segment possessing ES T/D ratio above the 90th percentile compared to individuals without.

	≥1 segment with ES T/D ratio >90th percentile	No segment with ES T/D ratio >90th percentile	p
Female Gender, %	16	12	0.1
Age, years	28.3 ± 4.5	28.1 ± 3.9	0.8
BMI, kg/m^2	24.3 ± 4.2	24.0 ± 4.3	0.7
Total Body Water, %	55 ± 7	57 ± 8	0.8
Heart Rate, bpm	71 ± 13	71 ± 10	0.8
Systolic BP, mmHg	118 ± 11	120 ± 11	0.2
Diastolic BP, mmHg	74 ± 9	75 ± 8	0.5
Nt-proBNP, pmol/L	203 ± 98	155 ± 103	0.04
LAEDV$_i$, mL/m^2	31.6 ± 6.0	32.9 ± 7.8	0.4
LAESV$_i$, mL/m^2	17.1 ± 3.9	17.0 ± 4.9	0.9
LA Ejection Fraction, %	47.3 ± 5.9	46.4 ± 7.0	0.5
LVEDV$_i$, mL/m^2	58.4 ± 7.9	57.7 ± 8.2	0.7
LVESV$_i$, mL/m^2	24.7 ± 5.3	22.5 ± 5.2	0.05
LV Ejection Fraction, %	57.0 ± 5.3	61.7 ± 5.5	0.0002

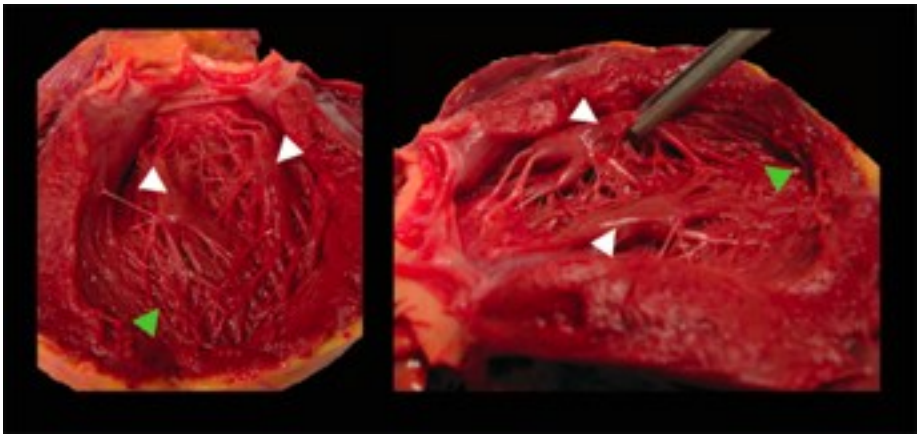
BMI: Body-Mass Index, Nt-proBNP: NT-terminal pro-Brain Natriuretic Peptide, LAEDVi: Left Atrial End-Diastolic Volume indexed, LAESVi: Left Atrial End-Systolic Volume indexed, LA EF: Left Atrial Ejection Fraction, LVEDVi: LV End-Diastolic Volume indexed, LVESVi: LV End-Systolic Volume indexed, LV EF: Left Ventricular ejection fraction, LV: Left Ventricular.

Supplemental Table 4.Means and upper limits of normal for trabecular burden measured bythickness ratio in three standard imaging planes at end-systole and end-diastole.

	SHORT AXIS				2 CHAMBER				4 CHAMBER			
	End-Diastole		End-Systole		End-Diastole		End-Systole		End-Diastole		End-Systole	
Segment	T/D Ratios: mean±SD	T/D Ratios: 90th percentile	T/D Ratios: mean±SD	T/D Ratios: 90th percentile	T/D Ratios: mean±SD	T/D Ratios: 90th percentile	T/D Ratios: mean±SD	T/D Ratios: 90th percentile	T/D Ratios: mean±SD	T/D Ratios: 90th percentile	T/D Ratios: mean±SD	T/D Ratios: 90th percentile
Basal anterior	1.60±1.30	2.52	0.48± 0.45	0.90								
Basal anteroseptal	0.84±0.86	1.57	0.28±0.16	0.45	0.5±0.38	1.02	0.27±0.21	0.52				
Basal inferoseptal	0.37±0.33	0.65	0.32±0.23	0.55					0.36±0.21	0.53	0.20±0.08	0.30
Basal inferior	0.80±0.85	1.44	0.36±0.28	0.72								
Basal inferolateral	1.15±1.10	2.11	0.35±0.3	0.63	0.52 ± 0.32	0.87	0.25±0.18	0.39				
Basal anterolateral	1.17±0.88	2.38	0.44±0.48	0.81					0.36±0.25	0.72	0.14±0.08	0.23
Mid anterior	2.21±1.65	3.90	1.17±0.96	1.99								
Mid anteroseptal	1.48±1.48	2.85	0.57±0.42	1.02	0.75±0.73	1.53	0.34±0.27	0.70				
Mid inferoseptal	0.59±0.99	0.86	0.27±0.18	0.47					0.37±0.25	0.30	0.19±0.08	0.30
Mid inferior	1.03±1.00	1.86	0.62±1.21	1.18								
Mid inferolateral	1.60±1.57	2.75	0.68±0.54	1.32	0.82 ± 0.56	1.47	0.47±0.34	0.91				
Mid anterolateral	1.51±1.78	2.84	0.80±1.39	1.31					0.35±0.22	1.35	0.35±0.22	0.67
Apical anterior	2.23± 1.84	3.79	0.78±0.42	1.31	0.91±0.56	1.66	0.45±0.31	0.78				
Apical septal	1.45± 1.45	2.76	0.57±0.46	0.95					0.84±0.38	1.35	0.48±0.29	0.91
Apical inferior	1.79± 1.56	2.90	0.72±0.5	1.29	0.97 ± 0.72	1.47	0.52±0.41	0.94				
Apical lateral	2.02± 1.36	3.35	0.87±0.64	1.63					0.95±0.71	1.53	0.40±0.25	0.60

T/D Ratios: Trabeculated/Dense myocardium Ratios

Supplemental Figure 1. Example of trabeculated myocardium observed at autopsy of young adultdeceased from traumatic (non-cardiac) cause: direct observation illustrates the widespread presence of trabeculations in the normal heart, and the difficulty in differentiating trabeculations (green arrowheads) from subdivisions of the papillary muscles (white arrowheads).



7. CONCLUSION AND PERSPECTIVES

This paper constitutes a part of the CMR anatomical basis that should help to develop a clinical knowledge about LV morphology and function in young subjects. The cohort study represents the young generation in the Canadian population. Knowledge of the characteristics and distribution of myocardium in the whole LV may help to understand the huge variation of normality in LV in young subjects. As seen in this study, trabeculated myocardium is a frequent and a normal finding in young and healthy subjects. In this regard, the highest volume of this type of myocardium is distributed at anterior and apical segments of LV and its volume is not influenced by body morphometrics but to female gender. Trabeculated myocardium is best observed at end-diastole (higher trabeculated vs. compacted ratios) with high degree of inter-observer reproducibility in any of the three imaging planes studied. Those findings about distribution of trabeculated myocardium were similar to those obtained recently by Dawson [81] and unfortunately the distribution is similar to diagnosed cases with NCC. Therefore the distribution of trabeculated myocardium should not be considered in order to distinguish healthy adults from those cases diagnosed from NCC. That is important because healthy young adults, who undergo screening tests with greater frequency, may be diagnosed with NCC if only current diagnostic criteria are applied.

However, our work adds to the above-mentioned one, which is probably being reviewed at the same time, functional observations focused on a larger cohort of young subjects. Young adults with higher amounts of trabeculated myocardium tend to have lower LV ejection fraction and a trend to have higher Nt-proBNP levels. These two parameters have been largely studied as best markers of cardiac function. And, the relationship between function and morphology in a healthy cohort is first described in this paper. Further studies however are needed to confirm this hypothesis: higher amounts of trabeculated myocardium are associated with worse cardiac function. The understanding of this relationship may establish one of the “most-wanted” descriptions: normality. The determination of a normality in CMR anatomy may shed light in understanding and detecting the asymptomatic stages of NCC and help to prevent the development of overt

heart failure.

Some issues may also arise from this paper. Firstly, there is the consideration of healthy status by medical history, physical examination and electrocardiography. May a subject be considered healthy if these three main pillars are within the normal range but a high Nt-proBNP level is detected? Healthy status has never been described uniformly and its accurate definition is probably more necessary than ever. Secondly, this work has not evaluated right ventricle which is as important as the left ventricle in all cardiovascular diseases as well as in NCC. Growing evidence exists about the dual chamber involvement in NCC. Adding to that, this work has not evaluated diastolic function or the abnormal cardiac response to an effort. These two parameter may point out an incipient cardiomyopathy. As well as the points before mentioned, the progressive technical advances will bring CMR to its maximal development and accuracy. However, reference data of volumes and ejection LV function that are currently the reference of "normality" are obtained from one unique study [32]. This work studied 120 subjects from Great Britain aged 20 to 80, 60 men and 60 women. Only 40 of them corresponds partially to our population by age and gender. That reinforces the challenge of finding the normality in young population. Improved spatial and temporal resolution with 3T and newer sequences will enhance the visualization of LV questioning again previous data. Lastly, this work lacks from genetic analysis that also may support normality or better, the absence of known mutations that are related to NCC in the index subject but also within its family. . However, as seen before, genetical testing on NCC is still to develop. One may think that the long term observation of this cohort may allow us to understand the asymptomatic phases of cardiovascular diseases, as NCC, also it may allow us to determine risk factors to prevent illness and will allow us to correlate genetic factors with the development of signs or symptoms of cardiomyopathies.

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