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CIÊNCIAS MÉDICAS

Role of cardiac imaging in the evaluation of diseases with unclear heart involvement

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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

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ROLE OF CARDIAC IMAGING IN THE EVALUATION OF DISEASES WITH UNCLEAR HEART INVOLVEMENT

PAPEL DA AVALIAÇÃO CARDIO-IMAGIOLÓGICA EM PATOLOGIAS COM ATINGIMENTO CARDÍACO INDETERMINADO

Tese de Candidatura ao grau de Doutor em Ciências Médicas, submetida ao Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto

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This research was done in the following institutions: Centro Hospitalar Universitário do Porto (Department of Cardiology – Medicine) and Barts Heart Center, London, United Kingdom. One of the research projects used the CARDIA (Coronary Artery Risk Development in Young Adults) dataset and was done with approval of the CARDIA Investigators.

This thesis was written in American English, as most of the articles were published in journals with that spelling preference.

The abstract in Portuguese was written in accordance with the “Novo Acordo Ortográfico de 1990”.

References are cited according to the international norm adopted by Instituto de Ciências Biomédicas Abel Salazar.

ABSTRACT

This PhD thesis translates the investigation of underdiagnosed myocardial diseases using non-invasive imaging techniques in the real world – in the context of a cardiac arrest without a defined etiology, alcohol consumption, an autoimmune disease and amyloidosis. Clinical research conducted in those four scenarios used information from transthoracic echocardiography (TTE), cardiovascular magnetic resonance (CMR) or radionuclide imaging, and analyzed possible associations with patients' characteristics, biomarkers and outcomes.

In survivors of sudden cardiac arrest (sCA) or peri-arrest, without coronary artery disease or an established diagnosis, CMR clarified the etiology in 49% and was pivotal in 30% of the cases, particularly for diagnoses such as myocardial infarction (with spontaneous recanalization), myocarditis or arrhythmogenic cardiomyopathy. Major adverse cardiac events were associated with the establishment of a diagnosis by CMR, the presence and extent of late gadolinium enhancement, and with left and right ventricular ejection fractions, the latter being an independent outcome predictor. Therefore, CMR should be incorporated in the investigation of sCA after excluding an acute coronary syndrome.

In a large observational prospective study of healthy young adults with 20 years of follow-up, alcohol intake was associated with an increase in indexed left ventricular mass and end-diastolic volume, indicating cardiac remodeling, although subtle. Wine consumption, in comparison with other beverages, was associated with less deleterious findings.

In patients with rheumatoid arthritis (RA) without known cardiac disease, an echocardiographic screening strategy identified underdiagnosed systolic dysfunction in 4% and diastolic dysfunction in 13% of the patients. Age was the most important and independent predictor of ventricular function. However, determining diastolic dysfunction remains a challenge and the significance of indeterminate diastolic function is still unknown. In older RA patients, TTE screening may be considered, particularly if prognosis-modifying treatment for diastolic dysfunction becomes available.

On the topic of transthyretin-related cardiac amyloidosis, an updated review about treatment options was elaborated. The performance of radionuclide imaging in diagnosing this cardiomyopathy was also analyzed, particularly in patients with early-onset disease and transthyretin mutation Val30Met. In those patients, technetium-99m 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) scintigraphy showed suboptimal sensitivity, significantly associated with the timing of neurologic onset, after adjustment for other cofactors. Therefore, in patients with early-onset of neurologic symptoms and Val30Met mutation, we believe this exam should not be used to rule out cardiac involvement. On the other hand, in patients with

wild-type transthyretin cardiac amyloidosis, radionuclide imaging had a good diagnostic performance.

These studies address different clinical contexts, but they all show how cardiac imaging provides important non-invasive information for the diagnosis and prognosis of patients in scenarios of unclear myocardial involvement. Detecting myocardial disease at an earlier stage, even when symptoms are not evident yet, may bring the benefits of an early intervention and better outcomes.

Key-words: cardiac arrest, alcohol, rheumatoid arthritis, amyloidosis, transthyretin, cardiovascular magnetic resonance, echocardiography, radionuclide imaging

RESUMO

A presente tese de Doutoramento reflete a investigação de patologias do miocárdio subdiagnosticadas, utilizando técnicas de imagiologia não invasiva, no mundo real – em contexto de paragem cardíaca sem etiologia definida, consumo de álcool, doença autoimune e amiloidose. A investigação clínica conduzida nesses quatro cenários utilizou a informação obtida a partir de ecocardiografia transtorácica (ETT), ressonância magnética cardiovascular (RMC) ou imagiologia de radionuclídeos, e analisou possíveis associações com as características dos doentes, biomarcadores e eventos.

Em sobreviventes de paragem cardíaca súbita (PCs) ou peri-paragem, sem doença coronária ou um diagnóstico estabelecido, a RMC esclareceu a etiologia em 49% dos casos e foi crucial em 30%, particularmente para os diagnósticos de enfarte do miocárdio (com recanalização espontânea), miocardite ou miocardiopatia arritmogénica. A ocorrência de eventos cardíacos major associou-se ao estabelecimento de um diagnóstico por RMC, à presença e extensão de realce tardio, e às frações de ejeção ventriculares esquerda e direita, sendo esta última um preditor independente de eventos. Assim, a RMC deve ser incorporada na investigação de PCs após exclusão de uma síndrome coronária aguda.

Num abrangente estudo observacional prospetivo de jovens adultos saudáveis com 20 anos de seguimento clínico, o consumo de álcool associou-se a um aumento da massa e do volume telediastólico indexados do ventrículo esquerdo, indicando remodelagem cardíaca, embora subtil. O consumo de vinho, em comparação com outras bebidas, associou-se a menores efeitos deletérios.

Em doentes com artrite reumatóide (AR) sem doença cardíaca conhecida, uma estratégia de rastreio ecocardiográfica identificou disfunção sistólica subdiagnosticada em 4% e disfunção diastólica em 13% dos doentes. A idade foi o mais importante e independente preditor de função ventricular. Contudo, a determinação de disfunção diastólica permanece um desafio e o significado de uma função diastólica indeterminada ainda não é claro. Em doentes idosos com AR, o rastreio com ETT pode ser considerado, particularmente se surgir algum tratamento modificador de prognóstico para disfunção diastólica.

Relativamente à amiloidose cardíaca relacionada com transtirretina, foi elaborada uma revisão atualizada sobre opções de tratamento. Adicionalmente, foi analisado o papel da imagiologia de radionuclídeos no diagnóstico desta miocardiopatia, particularmente em doentes com a mutação Val30Met no gene da transtirretina e início precoce. Nestes doentes, a cintigrafia com tecnécio-99m e ácido 3,3-difosfono-1,2-propanodicarboxílico (^{99m}Tc-DPD) mostrou sensibilidade reduzida, significativamente associada à idade de aparecimento de

sintomas neurológicos, após ajustamento para outros cofatores. Consequentemente, em doentes com a mutação Val30Met e com início precoce de sintomas neurológicos, julgamos que este exame não deve ser utilizado para excluir envolvimento cardíaco. Por outro lado, em doentes com amiloidose cardíaca por transtirretina selvagem, a imagiologia com radionuclídeos teve um bom desempenho diagnóstico.

Estes estudos debruçam-se sobre diferentes contextos clínicos, mas todos refletem como a imagiologia cardíaca providencia, de forma não invasiva, informação importante para o diagnóstico e prognóstico de doentes em cenários de atingimento miocárdico indeterminado. A deteção de patologia miocárdica num estadio inicial, mesmo quando os sintomas ainda não são evidentes, poderá permitir os benefícios de uma intervenção precoce e melhor prognóstico.

Palavras-chave: paragem cardíaca, álcool, artrite reumatóide, amiloidose, ressonância magnética cardiovascular, ecocardiografia, imagiologia de radionuclídeos

ABBREVIATIONS

ACM – alcoholic cardiomyopathy

AC-TTR – amyloid cardiomyopathy caused by transthyretin, mutated (AC-TTRm) or wild-type (AC-TTRwt)

AVD – any ventricular dysfunction (systolic, diastolic or indeterminate diastolic function)

CMR - cardiovascular magnetic resonance

DD – diastolic dysfunction

DPD - 3,3-diphosphono-1,2-propanodicarboxylic acid

EGCG - epigallocatechin-3-gallate

EMA - European Medicines Agency

FAP - familial amyloid polyneuropathy

FDA - Food and Drug Administration

HF – heart failure

HFpEF – heart failure with preserved ejection fraction

hsTnT - high-sensitivity troponin T

ICD - implantable cardiac defibrillator

IDF - indeterminate diastolic function

LA – left atrium (or atrial)

LGE - late gadolinium enhancement

LT – liver transplantation

LV – left ventricle (or ventricular)

LVEF – left ventricular ejection fraction

MACE - major adverse cardiac events

NYHA - New York Heart Association (functional classification of heart failure)

NT-proBNP – N-terminal prohormone of brain natriuretic peptide

PVF – preserved ventricular function

RA - rheumatoid arthritis

sCA – sudden cardiac arrest (or peri-arrest)

SD – systolic dysfunction

6MWT - six-minute walk test

^{99m}Tc – technetium-99m

TTE – transthoracic echocardiogram

TTR – transthyretin

TUDCA - tauroursodeoxycholic acid

CONTENTS

INTRODUCTION

In Cardiology, as in other areas of Medicine, a correct diagnosis is the cornerstone of any clinical approach. Cardiac imaging became the new stethoscope, allowing an early identification of precursor pathologic states.
However, there is still much to know about how several systemic diseases can affect the heart, how imaging can help us to better understand cardiovascular risk and what is the relevance of those findings in clinical practice.
While access to different imaging techniques is still very heterogeneous, overall they have become much more widespread, efficient, informative and affordable.
However, imaging alone is seldom sufficient. Integration in the clinical context is needed, oftentimes complemented by biomarkers and taking into account comorbidities and demographic characteristics. When we seek to make a diagnosis more upstream in the pathogenic pathway, this is even more so. Subtle changes are frequently hard to interpret and navigating the grey zone between clearly normal and clearly abnormal is quite challenging.

This PhD thesis focuses on the role of non-invasive cardiac imaging in the evaluation of underdiagnosed myocardial involvement in four different clinical settings.
The unclarified issues that led to research papers in the context of this Doctoral Program are: etiologies and prognosis of non-ischemic and potentially fatal ventricular arrhythmias; the impact of alcohol intake in cardiac remodeling; subclinical ventricular dysfunction in a model of autoimmunity; and diagnosis of cardiac changes caused by transthyretin amyloidosis. Different imaging techniques can provide some enlightening into these clinical scenarios. Determining whether there is myocardial damage can have an impact on the treatment and follow-up that is offered to these patients.

Background

Project A)

Survivors of sudden cardiac arrest or a peri-arrest event (sCA) have increased, due to the development of emergency response teams and to the advances in intensive care [1]. Determining the diagnosis underlying a potentially fatal arrhythmia or sudden cardiac arrest is

challenging [2-5], particularly when no significant coronary artery disease is seen in the coronary angiogram, and can have repercussions in clinical management and prognosis.

Although recurrent arrhythmia rates are high following the index sCA and most survivors receive implantable cardiac defibrillators (ICDs), some causes of sCA may be transient and only temporary secondary arrhythmia prevention may be needed [6]. Additionally, inherited cardiac conditions are frequently identified as causes of sCA and an accurate diagnosis is essential for genetic testing and for family counselling and screening [7, 8].

Most studies of sCA have included patients both with and without coronary artery disease. In those excluding coronary disease, the majority are post-mortem studies in non-survivors [2, 9, 10] or focus only on the young and/or athletes.

Cardiovascular magnetic resonance (CMR) is non-invasive, radiation-free and acquires pictures with high spatial resolution. It can detect subtle structural, functional and tissue abnormalities of the cardiac muscle. In combination with other assessments, CMR can increase our ability to diagnose many of diseases affecting the heart muscle that are most commonly associated with sCA [11, 12]. One of the most used CMR sequences detects late gadolinium enhancement (LGE), that usually reflects necrosis or fibrosis of the myocardium, and has been found to have prognostic value across a variety of cardiac diagnoses and clinical settings [13-16].

CMR is therefore likely to have an additional role in determining the substrate of ventricular arrhythmias in sCA survivors, and in identifying those patients at greatest risk of recurrent arrhythmias [12, 17, 18].

Project B)

The relationship between alcohol intake and cardiac remodeling is incompletely understood. Specifically, a threshold for alcohol-induced cardiotoxicity and the impact of alcohol use and its modulators on ventricular dysfunction remain controversial.

Alcohol intake is frequent in Europe and in the United States of America [19, 20]. Its abuse is a known risk factor for heart failure (HF) and for the development of alcoholic cardiomyopathy (ACM) [21, 22]. Previous observational studies have shown that up to one third of those diagnosed with dilated cardiomyopathy report an excessive alcohol intake [23, 24] and that alcohol abstinence can significantly improve both left ventricular (LV) function and symptomatic HF [25]. ACM is usually a presumptive diagnosis, reserved for patients with a heavy drinking history, LV systolic dysfunction and increased volumes and no other known cause to justify their cardiac impairment [26-28]. Alcohol can also have a deleterious cardiac effect by triggering arrhythmias [29] or hypertension [30].

Alcohol consumption is usually quantified using “standardized drinks”, adjusting to the amount

of alcohol in wine, beer or liquor, so that a standardized drink corresponds to 17.24mL or 14g of ethanol. Subjects can be categorized according to a modified version of the National Institute of Alcohol Abuse and Alcoholism (NIAAA) classification [31]: alcohol abstainers (no alcohol consumption); participants who consumed on average >0 and <4 standardized drinks per week (very low risk); ≥4 and <7 (low risk); ≥7 and <14 (at risk) and ≥14 (high risk).

However, the relationship between alcohol intake and cardiac disease does not seem to be linear [32] and, in fact, mild to moderate alcohol consumption (up to 1 standardized drink per day for women and up to 2 drinks per day for men) may even be beneficial, specifically concerning coronary artery disease [33] and even in the development of heart failure [34, 35]. Finally, "idiopathic" non-ischemic dilated cardiomyopathy is often diagnosed in patients who also report a history of alcohol intake, albeit generally mild to moderate [27, 36].

Other studies had shown an association between alcohol and subtle echocardiographic changes in cardiac morphology and function [37, 38], however, most studies were either cross-sectional or performed over a short follow-up period in older individuals. The impact of the drinking pattern and preferred beverage type was also unclear.

Clarifying these issues would not only be important for the prevention and management of alcoholic dilated cardiomyopathy, but could also have a significant impact on public health.

Project C)

Rheumatoid arthritis (RA) is a chronic autoimmune disease, with an estimated prevalence of 1% in adults [39, 40], which has been increasing [41]. Cardiovascular diseases are the main cause of death in patients with RA, who have a shortened life expectancy, by 5 to 10 years, compared to the general population [42, 43].

The increased cardiovascular risk in RA patients (around 50%) [44], that is comparable to the risk imposed by diabetes mellitus, goes beyond traditional risk factors [45]. The mechanisms underlying an increased atherosclerotic burden in AR relate to the dismal effects of a chronic inflammatory state [46].

More recently, a two-fold increased risk of heart failure (HF) has also been documented in these patients [47-49], not entirely explained by coronary artery disease [50]. The fact that the incidence of HF increases after the diagnosis of RA suggests that inflammation plays a part in the pathophysiology. Diastolic dysfunction, more than reduced ejection fraction, appears to be common in RA patients [51].

Heart failure symptoms are difficult to ascertain in these patients, making the diagnosis challenging and contributing to a worse prognosis. Cardiac dysfunction is probably underdiagnosed in these patients.

An echocardiographic assessment may identify those cases, with potential relevance to the

therapeutic approach and, subsequently, to the evolution of the disease.

Moreover, the prevalence of “silent” cardiac dysfunction in RA patients, the correlation with cardiac biomarkers, and its relationship with disease activity and with RA medication are still unclear and there are no recommendations regarding heart failure screening in RA.

Project D)

Cardiac amyloidosis related to transthyretin (TTR) can be caused by mutations in the TTR gene or due to conformational changes in the wild-type protein related to the ageing process (previously addressed as *senile* amyloidosis). In both cases, the tetrameric structure of the transthyretin protein becomes more susceptible to breakdown into monomers, which in turn can form amyloid fibrils that tend to infiltrate the extracellular space. Those fibrils can deposit in the heart, causing an infiltrative cardiomyopathy [52] (amyloid cardiomyopathy due to transthyretin - AC-TTR) and/ or disturbances in the cardiac electric conduction system [53].

Transthyretin is mainly produced by the liver and acts as a transporter of retinol and thyroxine. Several mutations in TTR have been described, with genotype-phenotype correlation and different penetrance [54, 55]. A phenotype of predominant peripheral neuropathy (familial amyloid polyneuropathy - FAP) is estimated to affect 10.000 people worldwide [56]. It is relatively rare, but endemic in Portugal, and usually presents as an irreversible sensorimotor and autonomic neuropathy, particularly frequent in cases of Val30Met mutation (“Portuguese variant”) [57] – recently renamed Val50Met [58] (since the designation Val30Met is still more widespread in the literature, we chose to use it). In the case of early-onset TTR Val30Met disease, the predominant variant in Portugal, it has been described essentially as a progressively disabling peripheral polyneuropathy, that can also affect the autonomous nervous system (with dysautonomia, gastrointestinal and urologic symptoms), the eyes, kidneys and even the central nervous system. The cardiac involvement that has been described in the majority of the “Portuguese variant” cases consists of disturbances in the heart’s electric conduction system, whereas myocardial dysfunction has been rarely reported. However, we have been noticing more cases of late-onset phenotype and the increase in survival may allow late manifestations of heart disease in patients with early-onset FAP. That increase in life expectancy was driven by liver transplantation (LT), that arouse as the first effective therapy in these patients [59]. However, there is evidence that cardiovascular involvement can progress even after LT, particularly in late-onset disease or caused by other TTR variants [60, 61].

Some mutations lead to a predominantly cardiac phenotype, such as Val122Ile, found in 3.4% of African-Americans [62], or Thr60Ala (respectively, Val144Ile and Thr80Ala in the new nomenclature).

On the other hand, wild-type TTR amyloidosis affects predominantly elderly men and is more common worldwide, albeit underdiagnosed [63-68]. It can be present in 13% of the cases of heart failure with preserved ejection fraction (HFpEF) [65] and in 6 [66] to 16% [67] of the patients with aortic stenosis. Its hallmark is amyloid cardiomyopathy. The exact mechanisms related to the ageing process that make some subjects prone to the disease are still unclear.

Recently, tafamidis (a stabilizer of the transthyretin tetramer) became the first drug approved for the treatment of the AC-TTR, mutated or wild-type [69], after having already being approved for FAP in Europe [70]. However, the long-term impact of the drug in the cardiovascular system is still unclear. Several other drugs are being tested for AC-TTR.

The evolution of cardiac changes with different therapies, the threshold for placing a pacemaker [71], as well as the prognosis and the best treatment plan for FAP are still under debate.

Given that Centro Hospitalar Universitário do Porto is a reference center for FAP patients, it is of upmost importance to clarify how to diagnose and treat cardiac amyloidosis caused by mutated TTR.

In clinical practice, amyloid cardiomyopathy caused by TTR (AC-TTR), both mutated (AC-TTRm) or wild-type (AC-TTRwt) is diagnosed essentially the same way: using echocardiography, radionuclide imaging or cardiac magnetic resonance (CMR). A more invasive approach using endomyocardial biopsy is rarely used.

Given the reduced availability of CMR and the fact that many patients have a pacemaker, we have been using essentially echocardiography to identify myocardial involvement. Bone scintigraphy using radioactive 99m-technetium (^{99m}Tc) biphosphonate derivatives, such as 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD), pyrophosphate (^{99m}Tc-PYP) or hydroxymethylenediphosphonate (^{99m}Tc-HMDP), has been increasingly used to diagnose AC-TTR [72, 73], even obviating the need of histologic confirmation of amyloid [73].

^{99m}Tc-DPD scintigraphy uses a qualitative assessment of cardiac uptake relative to the bones, with a grading system 0-3 [74]. Usually, a visual DPD score>1 (moderate or intense cardiac uptake) is considered positive [74, 75]. Mild cardiac uptake (score=1) may also be noted [76], but is not considered diagnostic [75, 77], although it may lead to further investigation.

Most studies that proved a high sensitivity [78, 79] and specificity [80] of the technique were not done in patients with the “Portuguese TTR variant” (Val30Met) and other authors had suggested that different types of fibrils in these patients could account for different results in ^{99m}Tc-DPD scintigraphy [81, 82].

Analyzing the performance of radionuclide imaging in these patients is important, since a negative ^{99m}Tc-DPD scan is interpreted as excluding cardiac involvement, withholding specific treatment.

Aims

The thesis subdivides into four projects, with the following models and objectives:

A)

Consecutive patients admitted at the London Chest or Heart Hospital (now part of Barts Heart Centre), who survived a resuscitated cardiac arrest or sustained ventricular tachycardia with hemodynamic instability requiring emergent cardioversion (a peri-arrest scenario), between 2008 and 2014, were evaluated and followed-up until 2017.

In survivors of cardiac arrest or potentially fatal cardiac arrhythmias, without coronary artery disease or an established diagnosis, the added value of cardiovascular magnetic resonance (CMR) was assessed. We aimed to determine the proportion of patients in whom CMR was determinant for the diagnosis and its value in secondary prevention (in predicting new events during follow-up). Furthermore, another objective of this study was to describe the etiologies underlining these malignant cardiac arrhythmias and to evaluate if the cardiac arrest *per se*, regardless of the mechanism, caused changes in CMR scans.

B)

Our goal was to analyze the relationship between alcohol intake and the development of cardiac remodeling/ dysfunction, as observed by echocardiography, in a healthy cohort of individuals prospectively followed during 20 years – the Coronary Artery Risk Development in Young Adults (CARDIA) study.

The echocardiographic outcomes were left ventricular ejection fraction (LVEF), body surface area indexed LV end-diastolic volume (iLVEDV) and LV mass (iLVmass), and left atrial (LA) diameter. Participants were grouped according to their weighted-average weekly drinking habits. We also performed an analysis using the estimated cumulative alcohol consumption across the 20 years of follow-up.

We hypothesized that alcohol intake would be associated with LV systolic impairment and dilatation. A secondary goal was to assess if this association was modified by gender or race, and if the pattern of drinking and type of predominant beverage had an impact on the occurrence of cardiac changes.

C)

This was a prospective study in rheumatoid arthritis (RA) patients followed in the outpatient clinic of Autoimmune Diseases at Centro Hospitalar Universitário do Porto, without known

heart disease.

Patients were divided into 4 categories of ventricular function, according to transthoracic echocardiogram (TTE) results: preserved ventricular function (**PVF**); systolic (± diastolic) dysfunction (**SD**); (isolated) diastolic dysfunction (**DD**); and indeterminate diastolic function (**IDF**), following the 2016 guidelines for diastolic evaluation [83].

We grouped patients with IDF, DD or SD into one category of "any ventricular dysfunction" (**AVD**), that was compared with PVF.

Finally, using criteria from the 2016 European Heart Failure guidelines [84], we also analyzed a category of potential subclinical heart failure with preserved ejection fraction ("HFpEF risk"), with the following characteristics: LVEF $\geq$50% *and* NT-proBNP (N-terminal prohormone of brain natriuretic peptide) > 125 pg/mL *and* structural echocardiographic changes (left ventricular hypertrophy *or* indexed LA volume *or* diastolic dysfunction as per 2016 echocardiographic guidelines).

Our main goals were to assess the prevalence and type of cardiac dysfunction in RA patients without known heart disease and to identify the predictors of ventricular dysfunction (and the patients that would benefit the most from the screening).

We also wanted to to analyze the factors associated with "HFpEF risk" and validate the value of the identification of subclinical ventricular dysfunction by analysing associations with surrogate prognostic markers.

Finally, we compared the patients' classification of ventricular function using the 2016 echocardiographic guidelines [83] with the categorization using the previous guidelines from 2009 [85].

We also elaborated another research paper **(C')**, currently under review, that is not the main focus of this thesis, but that was also about heart failure in rheumatoid arthritis and will be briefly addressed in the *Discussion*. Its aims were to determine the prevalence, clinical risk factors, and proteomic biomarkers associated with HF in RA patients.

D)

The project on transthyretin cardiac amyloidosis is mainly reflected in 2 papers.

In **D1)**, the current and future treatments for amyloid cardiomyopathy caused by transthyretin (AC-TTR) are reviewed.

Using the Pubmed database, we analyzed original articles focusing on different treatments for AC-TTR, namely: liver and cardiac transplantation, tafamidis, patisiran, inotersen, diflunisal, doxycycline and green tea extract (epigallocatechin-3-gallate - EGCG).

Most of these treatments have been more deeply investigated for the treatment of FAP, but

we focused on the evidence regarding their effectiveness and safety in AC-TTR.

D2) was an observational retrospective study that included patients followed at the Cardiology outpatient clinic, affiliated with the Corino de Andrade Unit of Centro Hospitalar Universitário do Porto.

We evaluated the results of ^{99m}Tc-DPD scintigraphy – the bone scan most commonly used in Europe – in a sample of patients with suspicion of AC-TTRm (all with Val30Met mutation), and compared them with Val30Met carriers without signs of cardiomyopathy, and with AC-TTRwt patients. Our aim was to estimate the sensitivity of ^{99m}Tc-DPD scintigraphy for AC-TTR diagnosis in different settings, particularly in patients with the Val30Met TTR variant. We investigated the influence of certain factors, such as the etiology, age of onset and the type of treatment (specifically, liver transplant or tafamidis treatment).

A secondary goal was to describe the type of cardiac changes found in this sample of early-onset Val30Met TTR patients, in terms of myocardial disease and conduction/ arrhythmic disorders, particularly after liver transplantation.

This subject of cardiac amyloidosis was also explored in smaller articles published as abstracts and presented in scientific meetings (CV annexes).

PAPERS (METHODS AND RESULTS)

This thesis contains materials and results from the following papers, which were published, accepted or submitted for publication. The author of this dissertation has contributed actively in their conceptualization, execution, analysis, interpretation and writing.

A) Rodrigues P, Joshi A, Williams H, Westwood M, Petersen SE, Zemrak F, Schilling RJ, Kirkby C, Wragg A, Manisty C, Mohiddin S. ***Diagnosis and prognosis in sudden cardiac arrest survivors without coronary artery disease: utility of a clinical approach using cardiac magnetic resonance imaging.*** Circ Cardiovasc Imaging 2017; 10(12): e006709. doi: 10.1161/CIRCIMAGING.117.006709.

B) Rodrigues P, Santos-Ribeiro S, Teodoro T, Gomes FV, Leal I, Reis JP, Goff DC Jr, Gonçalves A, Lima JA. ***Association between alcohol intake and cardiac remodeling.*** J Am Coll Cardiol 2018; 72(13): 1452-1462. doi: 10.1016/j.jacc.2018.07.050.

C) Rodrigues P, Ferreira B, Fonseca T, Quelhas-Costa R, Cabral S, Loureiro-Pinto J, Saraiva F, Marinho A, Huttin O, Girerd N, Bozec E, Cyrne Carvalho H, Ferreira JP. ***Subclinical ventricular dysfunction in rheumatoid arthritis*** --- submitted, under review

D1) Rodrigues P, Simões S, Reis H. ***Tratamento da miocardiopatia amiloidótica por transtirretina (Treatment of transthyretin amyloid cardiomyopathy).*** --- submitted, under review

D2) Rodrigues P, Frias AD, Gouveia P, Amorim I, Reis H, Trêpa M, Costa R, Oliveira MF, Palma P, Cyrne Carvalho H, Torres S. ***Radionuclide imaging in the diagnosis of transthyretin cardiac amyloidosis: different sensitivity in early-onset Val30Met mutation?*** --- submitted, under review

Addendum: By the time this thesis was presented, paper C) had already been published in International Journal of Cardiovascular Imaging and project D2) had been accepted for publication in JACC Cardiovascular Imaging.

A)

Rodrigues P, Joshi A, Williams H, Westwood M, Petersen SE, Zemrak F, Schilling RJ, Kirkby C, Wragg A, Manisty C, Mohiddin S. ***Diagnosis and prognosis in sudden cardiac arrest survivors without coronary artery disease: utility of a clinical approach using cardiac magnetic resonance imaging.*** Circ Cardiovasc Imaging 2017; 10(12): e006709. doi: 10.1161/CIRCIMAGING.117.006709.

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Diagnosis and Prognosis in Sudden Cardiac Arrest Survivors Without Coronary Artery Disease: Utility of a Clinical Approach Using Cardiac Magnetic Resonance Imaging

Patricia Rodrigues, Abhishek Joshi, Howell Williams, Mark Westwood, Steffen E. Petersen, Filip Zemrak, Richard J. Schilling, Claire Kirkby, Andrew Wragg, Charlotte Manisty and Saidi Mohiddin

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Diagnosis and Prognosis in Sudden Cardiac Arrest Survivors Without Coronary Artery Disease

Utility of a Clinical Approach Using Cardiac Magnetic Resonance Imaging

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Background—Determining the pathogenesis of sudden cardiac arrest or periarrest without significant coronary artery disease is crucial for management and prognosis. Cardiovascular magnetic resonance (CMR) can detect morphological, functional, or tissue abnormalities, and we sought to evaluate the role of CMR in determining sudden cardiac arrest pathogenesis and prognosis in survivors.

Methods and Results—We retrospectively reviewed cardiac investigations and clinical outcomes in consecutive survivors of potentially fatal arrhythmias without coronary artery disease admitted to our institutions from 2008 to 2014. After coronary angiography and echocardiography, all underwent CMR and, when indicated, electrophysiology studies. Major adverse cardiac events (MACE), comprising significant nonfatal ventricular arrhythmia or death, was the primary outcome. Of 164 included subjects (65% men; mean age 48 [18–80] years), CMR contributed to the diagnosis in 80 (49%) and was decisive in 50 cases (30%). Dilated cardiomyopathy (n=27), myocarditis or sarcoidosis (n=22), occult myocardial infarction (n=13), and hypertrophic cardiomyopathy (n=9) were most frequent. Arrhythmic causes were found in 14% while no cause was identified in 36%. MACE occurred in 31% of subjects during a median follow-up of 32 months. MACE associated with presence of a CMR diagnosis, extent of late gadolinium enhancement, and left and right ventricular ejection fractions. Right ventricular ejection fraction was an independent predictor of MACE.

Conclusions—CMR identified a likely pathogenesis for sudden cardiac arrest in nearly half of survivors in whom coronary artery disease had been excluded. One in 3 subjects had MACE; risk doubled in those with a CMR diagnosis and some CMR parameters—late gadolinium enhancement, left ventricular ejection fraction, and especially right ventricular ejection fraction—associated with prognosis. (***Circ Cardiovasc Imaging*. 2017;10:e006709. DOI: 10.1161/CIRCIMAGING.117.006709.**)

Key Words: arrhythmias, cardiac ■ coronary artery disease ■ death, sudden, cardiac ■ heart arrest ■ magnetic resonance imaging ■ prognosis

Surviving sudden cardiac arrest (sCA) or a periarrest event is increasingly likely in societies investing in emergency response capabilities.[1] In the absence of coronary artery disease (CAD), determining the pathogenesis of sCA is often challenging.[2–5]

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Although most sCA survivors receive implantable cardiac defibrillators (ICDs), other aspects of their management and prognosis will depend on the underlying cause.

In addition, although recurrent arrhythmia rates are high after the index sCA, some causes of sCA may be transient and only temporary secondary arrhythmia prevention may be needed—as indicated in the case of cardiac arrest immediately after an acute myocardial infarction.[6] Finally, inherited cardiac conditions are frequently identified as causes of sCA, and an accurate diagnosis is essential for genetic testing and for family counseling and screening.[7,8]

Notably, most studies of sCA include patients both with and without CAD. In those excluding coronary disease, the majority are postmortem studies in nonsurvivors[2,9,10] or focus only on the young and athletes. For example, diagnoses based on electrophysiology studies and dynamic ECG changes can be missed,[11] as well as those sensitive to sampling errors.[12]

Cardiovascular magnetic resonance (CMR) can detect subtle structural, functional, and tissue abnormalities of the cardiac muscle; in combination with other assessments, CMR

can increase our ability to diagnose many of diseases affecting the heart muscle that are most commonly associated with sCA.[13,14] Alongside, late gadolinium enhancement (LGE; both the presence and extent) has been found to be prognostic across a variety of cardiac diagnoses[15–18] and clinical settings, including cardiac arrest survivors.[13]

CMR is, therefore, likely to have an additional role in determining the substrate for the ventricular arrhythmias in sCA survivors and for identifying those patients at greatest risk of recurrent arrhythmias.[14,19,20]

Our aims were therefore to

1. Provide a contemporary description of the noncoronary causes of sCA, their relative frequency in an adult population, and estimate the frequency with which this carries implications for patient and family management.
2. Describe the diagnostic use of a clinical strategy based on CMR for the evaluation of adult sCA survivors without coronary disease.
3. Assess the prognostic use of CMR findings (LGE in particular) in this population of adult sCA survivors.

Methods

The data that support the findings of this study are available from the corresponding authors on reasonable request.

Study Population

We retrospectively studied consecutive patients admitted at the London Chest and University College London Hospitals National Health Service Trusts between 2008 and 2014 (now part of Barts Heart Centre, Barts Health NHS Trust), who survived a resuscitated cardiac arrest or sustained ventricular tachycardia with hemodynamic instability requiring emergent cardioversion (a periarrest scenario) between 2008 and 2014. All subjects underwent coronary angiography and those without significant CAD (luminal obstruction <30%) and that underwent CMR were included in this study. If an underlying cause was not identified from the CMR or if the ECG raised concern on a primarily arrhythmic cause, electrophysiology assessment was made, including ECG analysis, sodium channel blockade, catecholamine infusion, treadmill test, and electrophysiology studies, where deemed clinically indicated.

The final clinical diagnosis (considered the cause of the sCA) was made during the index admission by the cardiology team managing the patient, using all available data (clinical, imaging, and electrophysiological).

Consent and Ethical Approval

This study was conducted as audit (Clinical Management of the Inherited and Acquired Heart Muscle Diseases, Barts Health NHS Trust audit No. 5298). All clinical data were collected as part of standard care, and all patient identifiable fields were removed before analysis.

CMR Protocol

All CMR studies were undertaken during the index admission after cardiac arrest. Scanning was performed on Philips and Siemens 1.5 Tesla scanners. All protocols included cine imaging and LGE imaging. T1-weighted imaging pre- and post-contrast and T2-weighted triple inversion recovery images (short tau inversion recovery) were acquired where deemed clinically indicated.

Left ventricular dimensions, mass, and systolic function were assessed using steady-state free precession cine imaging. LGE images were acquired 10 minutes post-injection of 0.1 mmol/kg gadoterate meglumine (Dotarem Guerbet S.A. France) with an inversion recovery–segmented gradient echo (Turbo-Flash) sequence, with slice orientations corresponding to the cine images. In patients with limited breath-holding ability or frequent ectopy, a single-shot steady-state free precession–based inversion recovery sequence was used.

A quantitative evaluation of edema was obtained from T2-weighted short tau inversion recovery images by measurement of myocardial signal intensity normalized to skeletal muscle in the same slice.[21]

Quantitative evaluation of the early gadolinium enhancement ratio, a marker of hyperemia, analyzed from T1-weighted spin echo images, was obtained by measuring the signal intensity before and after contrast administration and normalized to the skeletal muscle.[21]

All studies were analyzed by 2 investigators blinded to the original CMR results, final clinical diagnosis, and outcomes in a central core laboratory using CVI42 software (Circle Cardiovascular Imaging, Calgary, AB, Canada). Standard volumetric analysis was performed (papillary muscles included as myocardium) and measurements indexed to body surface area. LGE was automatically quantified using a threshold of 5 SDs above the mean signal intensity of the remote myocardium.[22] The site of enhancement was classified as subendocardial/transmural (ischemic) or as epicardial/midwall/patchy (nonischemic). Small or poorly defined patches of LGE (≤1% of total myocardium) were considered nonspecific.

Final CMR diagnosis was obtained by consensus between at least 2 investigators.

We followed the current criteria for defining arrhythmogenic right ventricular dysplasia,[23] dilated cardiomyopathy,[24] hypertrophic cardiomyopathy,[25] and myocarditis,[21] as published elsewhere.

Final Clinical Diagnosis

We defined final clinical diagnosis as the cause of the sCA deemed responsible for the episode adjudicated by the medical team treating the patient based on all clinical information available (imaging, electrophysiological, biopsy, genetic). This did not need to coincide with the final CMR diagnosis.

Outcome Assessment

The primary end point was the occurrence of major adverse cardiac events (MACE), defined as a composite of significant nonfatal ventricular arrhythmia (appropriate antitachycardia pacing or ICD shock, sustained ventricular tachycardia, or ventricular fibrillation) and death.

Clinical outcome information was obtained from electronic health records, with mortality data from linked national databases, including NHS Spine. Follow-up arrhythmia data were obtained from ICD interrogation and from medical records.

Statistical Analyses

Continuous data are described as mean±SD or median (25–75 interquartile range) for non-Gaussian distributions. Shapiro–Wilk test was used to assess normality. Categorical data are presented as absolute frequencies (n) and percentages.

Baseline differences between patients with and without MACE were assessed using univariable Cox regression analysis.

The hazard ratio for the prediction of the events was calculated for MACE using Cox regression models. Multivariable analysis was performed using Cox regression with a stepwise forward selection method. Parameters that were significantly associated with MACE in the univariable analysis ($P<0.1$) and without collinearity were considered. Sex and age were also included because of their known clinical significance and impact on survival.

Survival analysis was completed with event curves, described according to the Kaplan–Meier method, and comparison of event rates was performed using the log-rank test.

We used SPSS software (version 20) for statistical analyses, considering 2-sided tests with $P<0.05$ for statistical significance.

Results

We included 164 patients, 65% (107) were men, with a mean age at presentation of 48±15 years (range, 18–80 years). Baseline characteristics are presented in Table 1; 9 patients

Table 1. Patients' Characteristics According to the Occurrence of MACE During Follow-Up

Patients' Characteristics	Total Cohort (n=164)	No MACE (n=113)	MACE (n=51)	*P* Value
Age (median [IQR]), y	48 (23)	47 (23)	52 (23)	0.100
Gender (% male)	65.2	61.9	72.5	0.265
Hypertension, n (%)	24 (15%)	13 (12%)	11 (22%)	0.100
Diabetes mellitus, n (%)	5 (3%)	1 (0.9%)	4 (8%)	0.082
Smoking history, n (%)	12 (7%)	10 (9%)	2 (4%)	0.595
Dyslipidemia, n (%)	5 (3%)	3 (3%)	2 (4%)	0.157
Obesity,* n (%)	3 (2%)	1 (0.9%)	2 (4%)	0.106
Excessive alcohol intake, n (%)	9 (5%)	6 (5%)	3 (6%)	0.256
Illicit drug use, n (%)	5 (3%)	5 (4%)	0	0.681
Chemotherapy, n (%)	3 (2%)	3 (3%)	0	0.698
Family history of sCD or IHD, n (%)	6 (4%)	5 (4%)	1 (2%)	0.727
Autoimmune disease, n (%)	4 (2%)	4 (4%)	0	0.650
OOHCA, n (%)	122 (74%)	86 (76%)	36 (71%)	0.226
Time to return to spontaneous circulation (median [IQR]), min	15 (10)	15 (10)	15 (24)	0.227
Shockable rhythm identified by first responder, n (%)	155 (94%)	105 (93%)	50 (98%)	0.241
Length of hospital admission (median [IQR]), d	10 (10)	10 (12)	10 (5)	0.370

IHD indicates ischemic heart disease; IQR, interquartile range (quartile 75–quartile 25); MACE, major adverse cardiac events (a composite of significant nonfatal arrhythmia and death); OOHCA, out of hospital cardiac arrest; and sCD, sudden cardiac death.

*Defined as a body mass index >30 kg/m².

had an arrest with a nonshockable rhythm (pulseless electric activity or asystole), 20 had sustained ventricular tachycardia with hemodynamic instability (a periarrest scenario), and the remaining 135 had pulseless ventricular tachycardia or ventricular fibrillation.

An ICD was placed in 70% (n=114). Follow-up data were available in all but one of the subjects.

CMR Findings and Diagnoses

The main parameters analyzed in CMR imaging are described in Table 2.

Indexed cardiac volumes were normal in 71% of subjects, and median left ventricular ejection fraction (LVEF) was 59 (interquartile range, 47–68), with 31.7% of the subjects presenting LVEF ≤50% (17.7% had LVEF 40%–50%; 7.3% had LVEF 30%–40%, and 6.7% had LVEF ≤30%).

Tissue Characterization With LGE

Abnormal LGE was detected in 61 (37%) of subjects—of these, it was predominantly subendocardial or transmural in 34% (n=21), midwall in 13% (n=8), subepicardial in 33% (n=20), in a diffuse discontinuous distribution in 11% (n=7), and 5 patients (8%) had >1 of these patterns. In almost all (n=54) of these subjects, CMR provided a final diagnosis.

There was no LGE in 81 patients (49%). In the remaining 22 patients (13%), subtle and nonspecific LGE findings were present.

Tissue Characterization With T2-Weighted Imaging

T2-short tau inversion recovery imaging was available in 80 patients (49%). On visual assessment, there was evidence of myocardial edema (increased signal intensity in short tau inversion recovery images) in only 10 (6%) of the subjects—in 6 cases, this colocalized with LGE in a nonischemic pattern, in 2 with myocardial infarct pattern, and the remaining 2 had no LGE. In 13% (n=22) of subjects, the calculated ratio of short tau inversion recovery signal intensity normalized to skeletal muscle was ≥2.

Imaging-Derived Diagnosis

In 80 cases (49%), CMR findings contributed to determining the underlying pathogenesis for the sCA (Table 3). The most frequent diagnoses made from CMR were dilated cardiomyopathy in 27 cases (17%), myocarditis or cardiac sarcoidosis in 22 (13%; 7 of those with possible sarcoidosis), occult myocardial infarction in 13 (8%), and hypertrophic cardiomyopathy in 9 (6%).

Minor and nonspecific changes not suggestive of a specific diagnosis were found in 30 CMR scans (18% of the total, 35% of those without a diagnosis), and 55 patients (34%) had a completely normal scan.

To assess the incremental diagnostic value of CMR, a cardiologist performed an adjudicated review of the clinical history and of the exams, blinded to CMR findings and final diagnosis by the medical team.

There were 50 patients in whom the final clinical diagnosis would not have been made without the additional morphological information and tissue characterization derived from the CMR scan. These included myocarditis (n=14), sarcoidosis (n=7), occult myocardial infarction (n=12), arrhythmogenic right ventricular dysplasia (n=3), nonischemic dilated cardiomyopathy (n=11 cases where CMR was required to exclude alternative differential diagnoses), and 3 other cases where the final clinical diagnoses required exclusion of other CMR-detectable pathology.

It was also judged to be relevant to support other diagnosis, for example, hypertrophic cardiomyopathy.

Electrophysiology Findings and Diagnoses

An arrhythmic pathology was found in 26 cases and deemed to be the main cause for the sCA in 23 cases (14%): 13 patients had a channelopathy, 5 an accessory pathway, and 5 an acquired arrhythmia with a reversible cause.

Final Clinical Diagnosis

After combining all clinical, imaging, and electrophysiology findings, the most frequent final diagnoses made by the physicians were dilated cardiomyopathy, myocarditis, and prior myocardial infarction (Table 3). In 59 cases (36%), the clinicians felt that a specific diagnosis could not be determined; 41%

Table 2. CMR Findings in the Total Sample and Divided According to the Occurrence of MACE During Follow-Up

CMR Parameter	Total Cohort (n=164)	No MACE (n=113)	MACE (n=51)	*P* Value
LVEDV, indexed to BSA, mL/m²	83 (31)	80 (28)	91 (45)	0.021*
LVESV, indexed to BSA, mL/m²	33 (30)	30 (26)	42 (38)	0.002*
LV mass, indexed to BSA, g/m²	70 (22)	69 (22)	75 (30)	0.276
LVEF (%)	59 (21)	62 (19)	53 (23)	0.001*
LV RWMA (% of patients)	28	20	46	0.003*
Cardiac output, L/min	5.9 (1.8)	6.0 (1.8)	5.6(1.9)	0.237
Stroke volume, BSA indexed, mL/m²	46 (15)	48 (14)	44 (17)	0.115
RVEDV, indexed to BSA, mL/m²	81 (25)	77 (27)	83 (21)	0.029*
RVESV, indexed to BSA, mL/m²	34 (18)	31 (16)	41 (17)	0.001*
RVEF (%)	57 (10)	59 (11)	54 (12)	<0.001*
RV RWMA (% of patients)	6	4	11	0.735
TAPSE, mm	20 (8)	22 (7)	18 (7)	0.070*
Left atrial area, cm²	21 (7)	21 (7)	24 (8)	0.002*
Right atrial area, cm²	20 (8)	20 (7)	23 (9)	<0.001*
Pericardial abnormalities (% of patients)	2	2	2	0.778
Mean EGEr in T1†	1.0(0.01)	1.0 (0.01)	1.0 (0.3)	0.364
T2 STIR (mean ratio)‡	2.0 (0.6)	2.0 (0.4)	2.3 (1.1)	0.128
STIR visually abnormal (% of patients)§	6	6	6	0.907
LGE present (% of patients)	37	30	51	0.020*
LGE distribution (% of patients)				0.064*
No LGE	49	55	38	
Subendocardial or transmural LGE	13	12	14	
Epicardial, midwall or patchy LGE	24	18	38	
Minor nonspecific LGE‖	13	15	10	
LGE quantification as % of LV mass	0.001 (4)	0.001 (3)	3.0 (11)	0.022*

Results are presented as median (interquartile range) or as a percentage.

BSA indicates body surface area; CMR, cardiovascular magnetic resonance; EGEr, early gadolinium enhancement ratio; LGE, late gadolinium enhancement; LV, left ventricle; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction (measured on short axis stack pictures; using the biplane method, on 4-chamber and 2-chamber views); LVESV, left ventricular end-systolic volume; MACE, major adverse cardiac events (potentially fatal arrhythmia or death); MAPSE, mitral annular plane systolic excursion; RV, right ventricle; RVEDV, right ventricular end-diastolic volume; RVEF, right ventricular ejection fraction; RVESV, right ventricular end-systolic volume; RWMA, regional wall motion abnormalities; STIR, short tau inversion recovery; and TAPSE, tricuspid annular plane systolic excursion.

*Parameters significantly associated with MACE, with *p* value<0.1 (considered for the multivariable analysis).

†Early gadolinium enhancement ratio (EGEr)—normalized to the skeletal muscle.

‡T2-weighted STIR (short tau inversion recovery) ratio of signal intensity normalized to skeletal muscle.

§Increased signal intensity in STIR images clearly seen visually.

‖Small or poorly defined patches of LGE (≤1% of total myocardium).

of these were presumed to be idiopathic ventricular tachycardia/ventricular fibrillation according to the medical records. In 12 patients, >1 cause for the sCA was found, but one was felt to be predominant and was considered the final diagnosis (Table 3).

Follow-Up: Patient Outcomes

Over a median follow-up of 32 months (interquartile range, 17–52), MACE occurred in 51 patients (31%). In 47 patients (27%), a significant nonfatal ventricular arrhythmic event was recorded. A total of 9 patients (5.2%) died; 2 did not have an ICD, unfortunately we could not adjudicate the cause of death based on the available data. In 5 cases with ICD, a significant arrhythmic event had already been treated by the device before their death.

An ICD complication was registered in 15 patients during follow-up (infection in 1, extrusion in 2, inappropriate shocks in 4, and lead displacement in 7 patients; 1 patient had infection, extrusion, and inappropriate shocks). Notably, the 50 patients without an ICD had fewer registered MACE—8%

Table 3. Predominant Diagnosis Identified by CMR and Final Clinical Diagnosis as per the Medical Team, Considering All Clinical Investigation and Features

Diagnosis	Diagnosis by CMR n (%)	Main Final Clinical Diagnosis n (%)	MACE (Within Main Clinical Diagnosis)
Unknown cause	84 (51%)	59 (36%)	14 (24%)
DCM (idiopathic)	27 (17%)	22 (13%)	9 (41%)
Myocarditis	22 (13%)	14 (9%)	7 (50%)
Cardiac sarcoidosis		7 (4%)	2 (29%)
IHD	13 (8%)	12 (7%)	6 (50%)
Long-QT syndrome		11 (7%)	1 (9%)
HCM	9 (6%)	8 (5%)	3 (38%)
Arrhythmia because of drugs or ionic disturbance		5 (3%)	0
Accessory pathway with fast conduction		5 (3%)	0
DCM because of cardiotoxic substance		4 (2%)	2 (50%)
Severe valvular disease	4 (2%)	4 (2%)	1 (25%)
ARVD	3 (2%)	3 (2%)	2 (67%)
Probable coronary vasospasm		3 (2%)	1 (33%)
Undetermined cardiomyopathy/other cardiac disease	1 (1%)	3 (2%)	1 (33%)
Brugada syndrome		2 (1%)	2 (100%)
Takotsubo cardiomyopathy	1 (1%)	1 (1%)	0
Noncardiac cause		1 (1%)	0

In 12 patients, there were multiple diagnoses made; however, all patients were given one final clinical diagnosis thought to be the main cause of the arrhythmic event. ARVD indicates arrhythmogenic right ventricular dysplasia; CMR, cardiovascular magnetic resonance; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; IHD, ischemic heart disease; and MACE, major adverse cardiac events.

versus 41%, P=0.008 (Cox regression). Most cases who did not get an ICD were driven by a conclusion that the underlying cause had resolved or could be managed with appropriate medication or because of patient refusal or loss of follow-up. The final diagnosis and extension of LGE were not significantly different, but LVEF was higher (58% versus 56%; P=0.041) and the presence of LGE was less common (24% versus 44%; P=0.022) in patients without an ICD. As far as we could determine, none of the patients included was totally dependent or in a vegetative state at the time of discharge. However, a standardized evaluation of their neurological status was not available.

Clinical Outcomes

There was no statistically significant relationship between MACE and baseline demographic characteristics, conventional cardiovascular risk factors, or clinical features of the sCA periarrest (Table 1).

MACE were seen in 41% of the 80 patients with a CMR diagnosis, in 13% of the 23 with a predominant electrophysiology diagnosis, and in 25% of the 59 with idiopathic sCA.

There was a nonsignificant trend toward lower MACE in the group with idiopathic sCA compared with those with a final pathogenic diagnosis (24% versus 34%; P=0.338).

Having a CMR-defined diagnosis was significantly associated with more MACE (41% versus 21%; P=0.022) and a shorter event-free survival (Figure 1). Considering the most common diagnoses, MACE occurred in 41% of patients with dilated cardiomyopathy and in 43% of those with myocarditis/sarcoidosis (50% of patients with myocarditis and 29% of those with sarcoidosis), 50% of those with missed myocardial infarct, and 38% with hypertrophic cardiomyopathy (Table 2). In the 24 patients with an arrhythmic pathogenesis, there was significantly lower MACE when compared with all other patients (13% versus 34%; P=0.034).

We did not find a statistically significant association between individual diagnoses and prognosis.

In the cohort as a whole, several CMR parameters were associated with MACE in the univariable analysis (Table 2). These included the presence and extent of LGE (Figure 2), LVEF and right ventricular ejection fraction (RVEF; Figures I and II in the Data Supplement), left and right atrial area, and ventricular volumes.

The pattern of LGE (ischemic versus non ischemic) was not significantly related to the occurrence of MACE.

Two methods of multivariable analysis for predicting MACE were considered. Covariates included LVEF, RVEF, and LGE, all significantly associated with MACE in the univariable analysis. Ventricular volumes were not included in the model (to avoid collinearity because ejection fraction was included and derives from end-diastolic and end-systolic left ventricular volumes). A significant interaction between LVEF and RVEF in predicting MACE was excluded. We also included age and sex in the model (Methods section). We tested 2 models, using LGE as either a binary (clearly present or absent) or a continuous variable (% of myocardial mass).

RVEF was independently associated with MACE in both models (Tables I and II in the Data Supplement).

MACE rates were similar in patients with subtle/nonspecific changes and those with a totally normal scan (respectively, 20% and 22%). This lack of association between outcome and nonspecific CMR changes remained when cases diagnosed with a primary arrhythmic cause were excluded (21% versus 25%; P=0.769) and when studying only cases without a final diagnosis.

Discussion

To date, this is the largest published cohort of cardiac arrest survivors in whom coronary disease had been excluded as the cause by coronary angiogram. By incorporating CMR into the diagnostic algorithm for investigation of sCA, the pathogenesis was determined in nearly two thirds of patients, with the most common causes in this cohort being nonischemic dilated cardiomyopathy, myocarditis, and missed myocardial infarction. The prognosis after sCA, however, remains poor, with MACE in ≈1 in 3 within a median follow-up of <3 years,

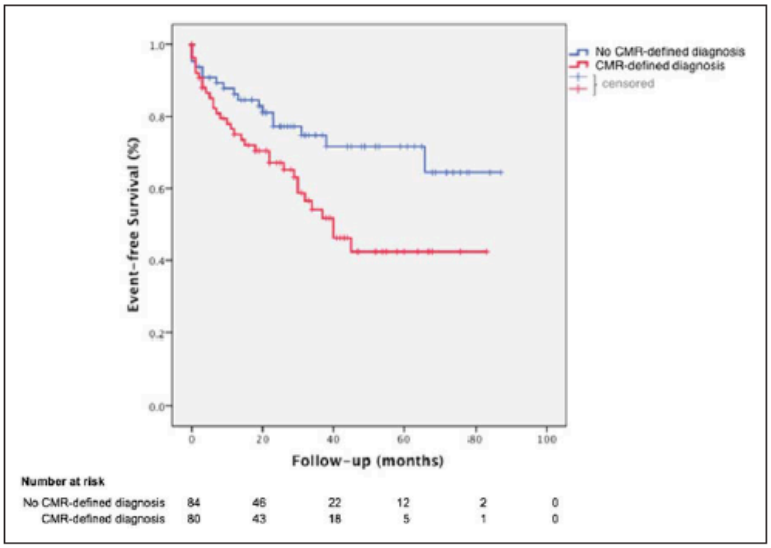

Figure 1. Kaplan–Meier curves displaying the event-free survival according to the findings in cardiovascular magnetic resonance (CMR): patients with a CMR-defined diagnosis had a worse prognosis than those with a normal scan or one with minor nonspecific changes (P=0.018 in the log-rank test; hazard ratio=2; 95% confidence interval=1.1–3.5).

despite defibrillator implantation in 70%. Those with a CMR-defined diagnosis had especially high MACE rates, with the presence of LGE and particularly RVEF as independent predictors of future events.

Worldwide, CAD is the leading cause for sCA,[26] hence coronary angiography is frequently recommended, particularly if an acute coronary syndrome is suspected.[27,28] CMR is not specifically recommended in recent cardiac arrest guidelines although the indication for a thorough investigation to find a pathogenesis was reinforced.[27,28]

Previous studies investigating causes of sCA (without systematic use of CMR) have mainly focused on manifestations of CAD.[29] Dilated cardiomyopathy has been the second most frequent cause of sCA observed in adults.[4] The few studies that extensively explore the nonischemic causes of sCA are mainly in children or young athletes, with hypertrophic cardiomyopathy/left ventricular hypertrophy usually the most common pathogenesis while myocarditis and congenital coronary abnormalities are more common than in older cohorts.[10,30] In those studies, most of them postmortem, between 5% and 18% of the cases had no identifiable pathogenesis,[2,10,30–32] reaching around a third of the patients in studies with survivors.[13,14] This often leaves patients, their families, and clinicians with significant uncertainty on clinical management. A diagnostic approach with routine use of CMR and upstream angiography seems to be a successful method for determining the pathogenesis with CMR alone providing a cause in nearly half of patients. The relatively unique ability of CMR to perform tissue characterization allows detection of myocardial edema and focal scar, enabling identification of many conditions that are difficult to diagnose using other modalities. Our results show that CMR, being able to give information about RVEF and LGE, offers additional prognostic value compared with echocardiographic findings.

CMR was crucial for the main final clinical diagnosis in 50 cases (30%) while it gave an important contribution to all the other cases where it was diagnostic. Cases of myocardial inflammation and specially occult myocardial infarct would not have been detected without CMR's tissue characterization. CMR was also pivotal in the diagnosis of arrhythmogenic right ventricular dysplasia. In fact, CMR can help confirm a diagnosis, suggest a hitherto unexpected diagnosis, and contribute to eliminate differential diagnoses.

These data also highlight the wide range of potential causative underlying diseases in sCA or periarrest cases and illustrate the importance of identifying a diagnosis to facilitate treatment, prognosis, and family screening. Despite all subjects having undergone coronary angiography and echocardiography, the frequency of occult infarcts was high; CMR provides a sensitive method for detection and enables administration of appropriate secondary preventative therapies. A recent study of 137 cardiac arrest survivors[13] without a clear diagnosis before performing CMR reported a higher prevalence of myocardial infarction (58% of patients), possibly related to the application of different angiographic criteria for inclusion (we excluded coronary disease with any stenosis >30%).

From our data, over 1 in 4 subjects were diagnosed with a potential inherited condition and 1 in 5 had an acquired cause, thereby facilitating targeted family screening and genetic testing. This not only helps to diagnose subclinical conditions in family members but also to alleviate concern in family members of those with noninherited conditions.

Patients with an identified pathogenesis for their sCA had a worse prognosis than those labeled as idiopathic ventricular fibrillation, with higher event rates in those with structural or functional cardiac abnormalities identified by CMR. Similar to studies of specific cardiomyopathies, the presence of LGE, lower LVEF, and RVEF were associated with shorter event-free survival. LGE extent was not prognostic in this cohort although this may be because of study power or the method for scar quantification used.[22,33,34]

However, over a third of the cohort received no pathogenic cardiac diagnosis, and these patients remain a difficult

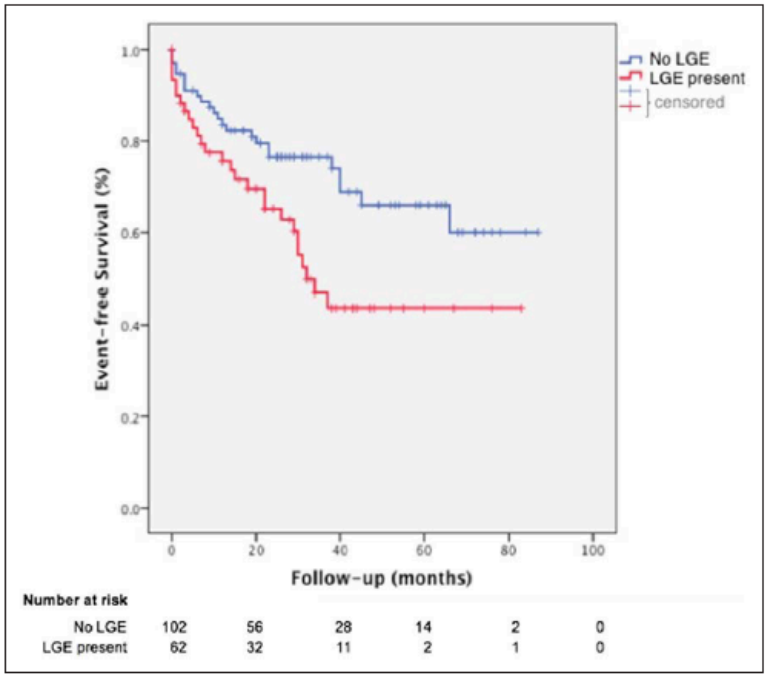

Figure 2. Kaplan–Meier curves of the major adverse cardiac events–free survival according to the presence of late gadolinium enhancement (LGE), which showed a significant relationship (log-rank test P=0.014; hazard ratio=1.9; 95% confidence interval=1.1–3.4 in Cox regression).

group to manage clinically, particularly given the high MACE rates of 1 in 4. In other studies with sCA survivors, the percentages of cases without a final determined diagnosis are identical to ours.[13,14] Therefore, given the high recurrence of events and lack of ability to identify truly low-risk patients, ICD implantation must always be considered in these patients.

Our data also demonstrate that cardiac arrest and resuscitation do not necessarily produce detectable changes by CMR. This means that if changes are seen in a CMR scan in this context, we cannot assume that they are solely because of consequences of the arrest per se. However, subtle nonspecific CMR abnormalities did not influence prognosis.

Limitations and Strengths

This is a retrospective study that used a relatively standardized approach to survivors of cardiac arrests, and CMR exams were blindly reviewed by at least 2 observers.

Given the design of the study, we can only derive association between the CMR findings and sCA; causality can only be hypothesized.

Nonsurvivors were not included, and diseases with a more malignant course may, therefore, be under-represented. Patients with a presumed coronary pathogenesis for their arrests were excluded, and this may have included patients with dual pathology or bystander coronary disease. Patients who could not undergo CMR because of hemodynamic instability, functional deficits that precluded breath-holding, severe renal failure, claustrophobia, or magnetic devices were also excluded.

Validation of CMR tissue characterization is currently incomplete. Notably, endomyocardial biopsy was not performed as a component of this clinical pathway and might have added valuable additional information.

The cohort size prevents more detailed study of pathogenic subgroups, and we must be cautious in interpreting the prognostic value of individual CMR parameters (including ejection fraction and LGE) without first considering them within a diagnostic framework.

Finally, we analyzed all-cause mortality rather than cardiovascular death because the exact cause of death could not be determined in most cases.

Conclusions

By incorporating CMR in clinical pathway for investigation of sCA in the absence of CAD, a cause can be identified in nearly two thirds of patients. Many of the most frequent pathogeneses identified using CMR (idiopathic dilated cardiomyopathy, myocarditis, and occult myocardial infarction) have important implications with regard to specific clinical management, family screening, and prognosis. Although the risk of recurrent ventricular arrhythmias and death is high across this patient population, patients diagnosed with a structural or functional cardiac abnormality by CMR had a 2-fold increased risk. LGE and biventricular systolic function were associated with prognosis, with RVEF being an independent predictor. We, therefore, advocate consideration of CMR for investigation and prognostication of all patients without culprit coronary disease post-sCA.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Finding the diagnosis underlying a potentially fatal arrhythmia or sudden cardiac arrest is challenging, particularly when no significant coronary artery disease is seen in the coronary angiogram, and can have repercussions in clinical management and prognosis. In our study of 164 sudden cardiac arrest survivors (65% men, mean age 48 years), cardiovascular magnetic resonance (CMR) was crucial for the diagnosis in 30% of the cases and contributed to the diagnosis in 49%. Using a standardized clinical pathway for investigation of sudden cardiac arrest, including CMR, a cause can be identified in nearly two thirds of patients. The most frequent sudden cardiac arrest pathogeneses detected by CMR were nonischemic dilated cardiomyopathy (17%), myocarditis or sarcoidosis (13%), occult myocardial infarction (8%), and hypertrophic cardiomyopathy (6%). Primarily arrhythmic causes (caused by channelopathies, accessory pathways with fast conduction, or toxic/ionic disturbances) were found in 14%, while no cause was identified in 36%. Major adverse cardiac events, comprising significant nonfatal ventricular arrhythmia or death, occurred in 31% of subjects during a median follow-up of 32 months. Cardiac arrest and resuscitation per se do not necessarily produce detectable changes by CMR. Nevertheless, subtle nonspecific CMR abnormalities did not influence prognosis. Major adverse cardiac events associated with establishment of a diagnosis by CMR, extent of late gadolinium enhancement, left and right ventricular ejection fractions, with right ventricular ejection fraction as an independent predictor of major adverse cardiac events.

SUPPLEMENTAL MATERIAL

SUPPLEMENTAL TABLES:

Supplementary Table 1 - Multivariable model for MACE, using Cox Regression.

	Hazard ratio	SE	95% CI	p value
RVEF	0.958	0.009	0.936-0.981	<0.001

Covariates: LVEF (left ventricular ejection fraction), RVEF (right ventricular ejection fraction), presence of LGE (late gadolinium enhancement), gender and age. Stepwise forward method, exclusion criteria p>0.1.

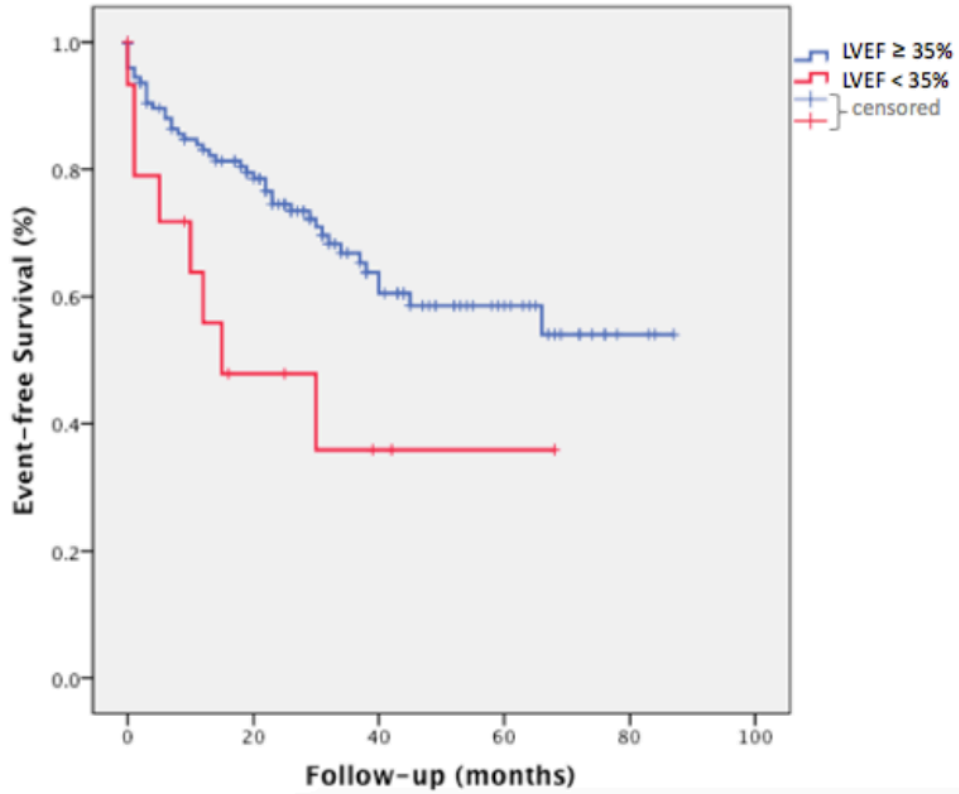
Supplementary Table 2 – Multivariable model for MACE, using Cox Regression.

	Hazard ratio	SE	95% CI	p value
RVEF	0.958	0.012	0.936-0.981	<0.001

Covariates: LVEF, RVEF, LGE quantification, gender and age. Stepwise forward method, exclusion criteria p>0.1.

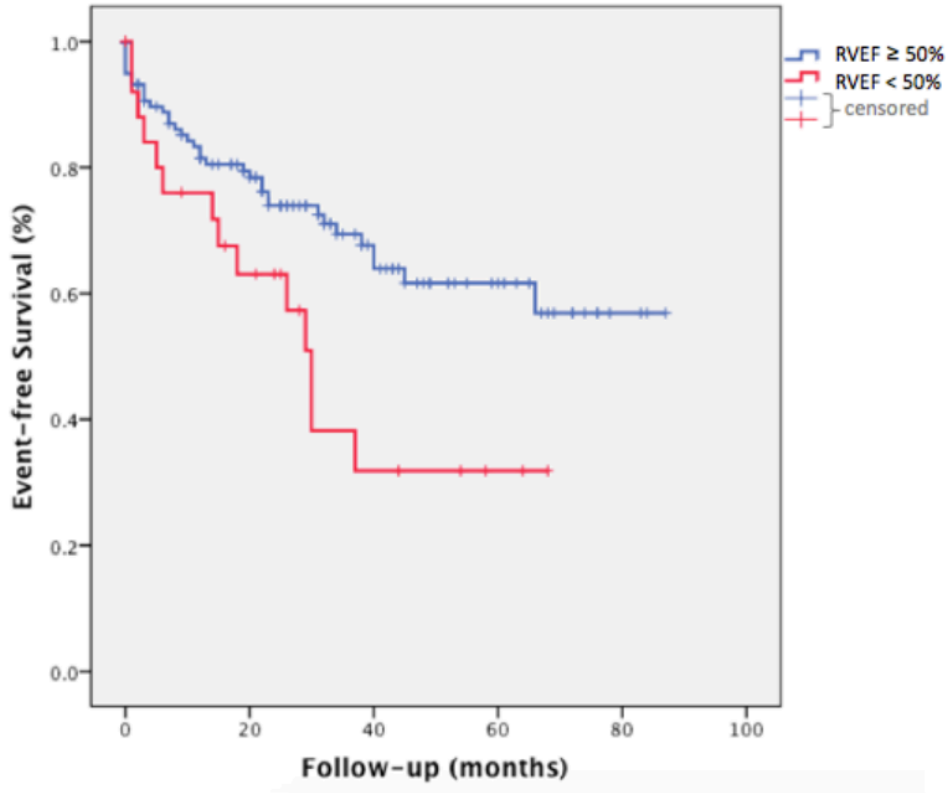
SUPPLEMENTAL FIGURES and FIGURE LEGENDS:

Supplementary Figure 1 – Kaplan-Meier curve showing the MACE-free survival curves according to the left ventricular ejection fraction assessed by CMR (LVEF≥35% versus <35%). Patients with LVEF<35% had less MACE-free survival (Log-rank test p= 0.024; HR= 2.3; CI 95%= 1.1-4.9); the same held true considering LVEF as a continuous variable in a Cox regression model.



Number at risk						
LVEF≥35%	149	84	38	17	3	0
LVEF<35%	15	5	2	1	0	0

Supplementary Figure 2 – Kaplan-Meier curve showing the MACE-free survival curves according to the right ventricular ejection fraction assessed by CMR (RVEF≥50% versus <50%). Patients with LVEF<50% had less MACE-free survival (Log-rank test p=0.018; HR=2.061; CI 95%=1.112-3.820 in Cox regression); RVEF as a continuous variable was also significantly associated with MACE.



Number at risk

RVEF≥50%	137	75	35	16	3	0
RVEF<50%	27	14	5	2	0	0

B)

Rodrigues P, Santos-Ribeiro S, Teodoro T, Gomes FV, Leal I, Reis JP, Goff DC Jr, Gonçalves A, Lima JA. *Association between alcohol intake and cardiac remodeling.* J Am Coll Cardiol 2018; 72(13): 1452-1462. doi: 10.1016/j.jacc.2018.07.050.

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Association Between Alcohol Intake and Cardiac Remodeling

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ABSTRACT

BACKGROUND Alcohol-induced cardiotoxicity is incompletely understood. Specifically, the long-term impact of alcohol use on ventricular remodeling or dysfunction, its modulators, and effect thresholds among young adults remain controversial.

OBJECTIVES The authors sought to evaluate a potential relationship between alcohol intake and cardiac remodeling, assessed by echocardiography, over 20 years of follow-up.

METHODS Among the CARDIA (Coronary Artery Risk Development in Young Adults) study cohort, the authors studied all subjects without baseline heart disorders who provided adequate information on their drinking habits and underwent echocardiographic evaluation at years 5 and 25 of the study. The echocardiographic outcomes were left ventricular (LV) ejection fraction, indexed LV end-diastolic volume and LV mass, and left atrial diameter. Participants were grouped according to their weighted-average weekly drinking habits. An additional analysis used the estimated cumulative alcohol consumption. Regression models and multivariable fractional polynomials were used to evaluate the association between alcohol consumption and the outcomes.

RESULTS Among the 2,368 participants, alcohol consumption was an independent predictor of higher indexed LV mass ($p = 0.014$) and indexed LV end-diastolic volume ($p = 0.037$), regardless of sex. No significant relationship between alcohol intake and LV ejection fraction was found. Drinking predominantly wine was associated with less cardiac remodeling and there was a nonsignificant trend for a harmful effect of binge drinking.

CONCLUSIONS After 20 years of follow-up, alcohol intake was associated with adverse cardiac remodeling, although it was not related with LV systolic dysfunction in this initially healthy young cohort. Our results also suggest that drinking predominantly wine associates with less deleterious findings in cardiac structure. (J Am Coll Cardiol 2018;72:1452–62)

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In 2013, 70% of U.S. adults reported drinking alcoholic beverages in the past year, and 7% had an alcohol use disorder (1). Alcohol abuse is a known risk factor for the development of alcoholic cardiomyopathy (ACM) (2,3), which presents as a dilated cardiomyopathy that can lead to heart failure (4,5).

ACM is usually a presumptive diagnosis reserved for patients with a history of "at risk" drinking (for women, >3 drinks on any single day and >7 drinks per week; for men, >4 drinks on any single day and >14 drinks per week, as per the National Institute of Alcohol Abuse and Alcoholism [NIAAA] classification), left ventricular (LV) systolic dysfunction, and increased LV volumes, without other known cause to justify their cardiac impairment (5–7).

SEE PAGE 1463

"Idiopathic" nonischemic dilated cardiomyopathy is often diagnosed in patients who also report a history of alcohol intake, albeit generally mild to moderate (7,8). Previous observational studies have shown that approximately one-third of those diagnosed with dilated cardiomyopathy report an excessive alcohol intake (9,10) and that alcohol abstinence can significantly improve both LV function and symptomatic heart failure (11). Furthermore, alcohol may also lead to other cardiac diseases besides LV dysfunction, such as arrhythmias (12) or hypertension (13).

However, the relationship between alcohol intake and cardiac disease does not seem to be linear (14) and, in fact, mild-to-moderate alcohol (up to 1 standardized drink per day for women and up to 2 drinks per day for men) consumption may even be beneficial for coronary artery disease (15) and incident heart failure (16,17).

Previous studies have shown an association between alcohol and subtle echocardiographic changes in cardiac morphology and function, systolic and diastolic (18,19). However, most studies are either cross sectional (19) or performed over a short follow-up period in middle-aged and older individuals (18).

We lack information about the long-term effect of alcohol intake in young adults, and there is still controversy about the impact of other patients' characteristics or the pattern of alcohol intake regarding the threshold level for being injurious. Clarifying these issues related to alcohol's cardiotoxicity could have a significant impact in public health.

The main goal of this study was to assess the potential cardiotoxic role of alcohol in cardiac structure and function over 20 years of follow-up during young adulthood into middle age. We hypothesized that alcohol intake would be associated with LV systolic impairment and dilatation. Furthermore, we explored whether particular population subgroups, specific types of alcoholic beverages, and specific drinking patterns modify such associations.

ABBREVIATIONS AND ACRONYMS

ACM = alcoholic cardiomyopathy
BMI = body mass index
BSA = body surface area
FP = fractional polynomials
LA = left atrial
LV = left ventricular
LVEDV = left ventricular end-diastolic volume
LVEF = left ventricular ejection fraction
NIAAA = National Institute of Alcohol Abuse and Alcoholism

METHODS

STUDY SAMPLE. The CARDIA (Coronary Artery Risk Development in Young Adults) cohort study recruited 5,115 apparently healthy black and white individuals between 18 and 30 years of age, stratified by age, race, sex, and educational level. Enrollment was performed between 1985 and 1986 in 4 North American urban centers (Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California). Participants provided written informed consent at each examination, and institutional review boards from each field center and the coordinating center approved the study annually.

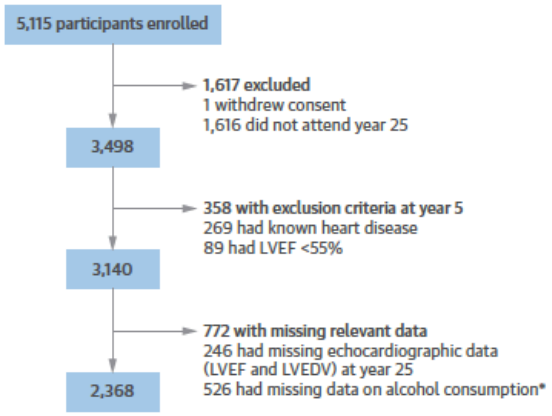
Participants have been followed for >30 years and have undergone a series of questionnaires and examinations at years 0, 2, 5, 7, 10, 15, 20, 25, and 30. Detailed information regarding the design and procedures performed during the study have been published elsewhere (20–22).

We considered year 5 of the CARDIA study as the baseline period of our sample, because it was the first year echocardiographic evaluations were performed. The follow-up period of our study comprised a total of 20 years (i.e., from year 5 until year 25, when a follow-up echocardiogram was obtained).

During this period, 3,498 participants fulfilled attendance criteria. Participants who, at baseline, had either known heart disease (questioned as "has a doctor or a nurse ever said that you have heart problems?") or a left ventricular ejection fraction (LVEF) below 55% were excluded from the analysis (n = 358). Furthermore, we also excluded participants with insufficient information about the echocardiographic outcomes (n = 246) and those who did not provide sufficient information regarding their alcohol consumption habits (i.e., participants who did not respond to the alcohol questionnaires at least at years 5, 15, and 25 of the CARDIA study) (Figure 1). Retention rates were 86% at year 5, 74% at year 15, and 72% at year 25; >90% of initial participants have maintained contact over time.

ALCOHOL CONSUMPTION ASSESSMENT. Data regarding alcohol intake were obtained from a questionnaire filled in by the CARDIA study participants at years 5,

FIGURE 1 Flowchart With Exclusion Criteria and Definition of the Study Sample



*Participants who did not respond to the alcohol questionnaires at least at years 5, 15, and 25 of the CARDIA study. LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction.

15, and 25. At each examination, participants were questioned regarding their past drinking habits and, specifically, the number of drinks of wine, beer, and liquor they typically consumed per week.

Using visual aids for estimating a typical drink, the number of drinks of wine, beer, and liquor typically consumed in a week was assessed. The average alcohol consumption per week was calculated assuming that the amount of ethanol in 1 drink of beer, wine, and liquor was 16.7 ml, 17.0 ml, and 19.2 ml, respectively. This total was then divided by 17.24 ml (the amount of ethanol in an average drink, corresponding to 14 g of alcohol) to obtain the number of standardized drinks per week (20,23). This estimation of standard drinks was used to categorize alcohol intake in all the analyses.

We used a modified version of the NIAAA classification (24) to describe alcohol intake, estimating the average consumption of standard drinks per week (self-reported at years 5, 15, and 25), divided into the 5 following groups: alcohol abstainers (no alcohol consumption); participants who consumed on average >0 and <4 standardized drinks per week (very low risk); ≥4 and <7 (low risk); ≥7 and <14 (at risk), and ≥14 (high risk).

We also estimated the cumulative alcohol consumption during the 20 years of follow-up by calculating the product of the average ethanol intake per year (using the reported intake of alcohol per day at year 5 of the study, year 15, and year 25) and the total time interval (20 years). The effect of sex and race on the exposure outcomes was analyzed, which was facilitated by the stratified enrollment of CARDIA.

In order to be able to evaluate potential differences in the cardiotoxic effect of alcohol according to variations in consumption habits, we also extracted information from those 3 questionnaires equally distributed in time regarding the participants' ingestion of particular types of beverages (namely beer, wine, or liquor) and their drinking patterns (specifically evaluating self-reported binge-drinking, defined as the consumption of more 5 or more standardized drinks on the same occasion, at least once within the last 30 days).

ECHOCARDIOGRAPHIC EVALUATION. Our primary endpoint to assess LV systolic function was LVEF. Secondary endpoints were body surface area (BSA)-indexed LV end-diastolic volume (LVEDV) (a marker of LV dilation and ventricular remodeling with a significant prognostic value [25,26]), BSA-indexed LV mass (another marker of ventricular remodeling [25]), and left atrial (LA) diameter (which has been previously associated with adverse cardiovascular events [27]). LVEDV and LVEF measurements were performed by transthoracic echocardiography, using 2-dimensional apical views, whereas M mode was used to assess both BSA-indexed LV mass and LA diameter (the original values were used).

All studies were digitally recorded using an Artida Cardiac Ultrasound Scanner (Toshiba Medical Systems, Otawara, Japan) and assessed by certified analysts at the Johns Hopkins University Echocardiography Reading Center (Baltimore, Maryland), using standard image analysis software (Digisonics, Houston, Texas). These endpoints were assessed at year 25, but we also considered the individual variation (between year 25 and year 5) of the echocardiographic parameters in one of the models.

COVARIATES AND POTENTIAL CONFOUNDERS. Several variables were considered as potentially confounding factors, namely: sex, age, race, hypertension (in the CARDIA questionnaire: "has a doctor or a nurse ever said you have high blood pressure or hypertension?"), dyslipidemia (according to the CARDIA questionnaire: "has a doctor or a nurse ever said you have high blood cholesterol?"), diabetes (self-reported or taking medication), family history of cardiovascular diseases, body mass index (BMI), self-reported tobacco or illicit drug use, educational level (up to high school or above that), physical activity (using a questionnaire about the duration and intensity of self-reported participation in 13 categories of exercise over the previous 12 months [28]), chronic pulmonary disease, obstructive sleep apnea, thyroid

TABLE 1 Sample Characteristics According to Average Alcohol Consumption

		Standard-Drinks per Week					
	Total (N = 2,368)	None (n = 619)	>0 and <4 (n = 934)	≥4 and <7 (n = 303)	≥7 and <14 (n = 320)	≥14 (n = 192)	p Value
Age, yrs	51 (47-53)	50 (47-53)	51 (47-53)	51 (48-53)	51 (48-53)	51 (47-53)	0.171
Sex							
Men	1,051 (44.4)	192 (31.0)	373 (40.0)	146 (48.2)	185 (57.8)	155 (80.7)	<0.001
Race							
White	1,356 (57.3)	258 (42.7)	560 (60.0)	202 (66.7)	218 (68.1)	118 (61.5)	<0.001
Black	1,006 (42.5)	359 (58.0)	371 (39.7)	101 (33.3)	101 (31.6)	74 (38.5)	
Other	6 (0.2)	2 (0.32)	3 (0.32)	0 (0.0)	1 (0.31)	0 (0.0)	
Educational level*							
High school or above	1,593 (67.3)	384 (62.0)	659 (70.6)	221 (72.9)	223 (69.7)	106 (55.2)	<0.001
Below high school	775 (32.7)	235 (38.0)	275 (29.4)	82 (27.1)	97 (30.3)	86 (44.8)	
Physical activity*							
Low	492 (20.9)	149 (24.2)	212 (22.8)	47 (15.6)	53 (16.6)	31 (16.3)	<0.001
Moderate	1,059 (44.9)	302 (49.0)	399 (43.0)	143 (47.4)	144 (45.1)	71 (37.4)	
High	806 (34.2)	166 (26.9)	318 (34.2)	112 (37.1)	122 (38.2)	88 (46.3)	
Body mass index, kg/m^2							
<18.5	17 (0.7)	5 (0.8)	7 (0.8)	1 (0.33)	3 (0.94)	1 (0.52)	<0.001
18.5-25.0	632 (26.7)	122 (19.7)	258 (27.7)	103 (34.0)	94 (29.5)	55 (28.7)	
25.0-30.0	779 (32.9)	164 (26.5)	304 (32.6)	106 (35.0)	127 (39.8)	78 (40.6)	
>30.0	938 (39.6)	328 (53.0)	364 (39.0)	93 (30.7)	95 (29.8)	58 (30.2)	
Relevant medical history							
Smoking†	386 (43.4)	46 (32.2)	116 (36.9)	58 (42.7)	76 (49.0)	90 (64.8)	<0.001
Illicit drug use‡	959 (40.6)	139 (22.5)	349 (37.5)	146 (48.2)	182 (57.1)	143 (75.3)	<0.001
Cerebrovascular disease	41 (1.7)	14 (2.27)	17 (1.82)	3 (0.99)	4 (1.26)	3 (1.56)	0.711
Peripheral arterial disease	28 (1.2)	10 (1.6)	11 (1.2)	2 (0.7)	2 (0.6)	3 (1.6)	0.636
Hypertension	737 (31.2)	229 (37.1)	258 (27.7)	94 (31.1)	94 (29.6)	62 (32.5)	0.003
Diabetes mellitus	220 (9.3)	85 (13.8)	83 (8.9)	17 (5.6)	23 (7.2)	12 (6.3)	<0.001
Dyslipidemia	685 (29.0)	185 (29.9)	262 (28.1)	98 (32.5)	75 (23.6)	65 (34.0)	0.056
Renal disease	144 (6.1)	43 (7.0)	57 (6.1)	18 (6.0)	19 (6.0)	7 (3.7)	0.586
Liver disease	63 (2.7)	15 (2.4)	22 (2.4)	11 (3.6)	7 (2.2)	8 (4.2)	0.478
Thyroid disease	206 (8.7)	72 (11.7)	87 (9.3)	20 (6.6)	19 (6.0)	8 (4.2)	0.002
Obstructive sleep apnea	222 (9.4)	70 (11.3)	85 (9.1)	31 (10.3)	20 (6.3)	16 (8.3)	0.140
Chronic pulmonary disease§	24 (1.0)	5 (0.8)	8 (0.9)	3 (1.0)	5 (1.6)	3 (1.6)	0.653
Other cardiac diseases‖	151 (6.4)	39 (6.3)	61 (6.6)	23 (7.6)	19 (6.0)	9 (4.7)	0.774
Familiar cardiovascular disease	20 (0.9)	9 (1.5)	5 (0.5)	4 (1.3)	2 (0.6)	0 (0.0)	0.180

Values are median (interquartile range) or n (%). Average alcohol intake per week was estimated using the questionnaires at year 5 of the CARDIA study (baseline), year 15 and year 25, as specified in the Methods section. The p value for trend is represented. *All the characteristics were considered if present in any given time over the 20 years of follow-up, except for educational level, physical activity and body mass index, which are relative to baseline only. †Defined as the consumption of cigarettes, cigars, tobacco pipe or smokeless tobacco regularly for at least 3 months. ‡Including cocaine, heroin, amphetamines and methamphetamines (cannabinoids were not taken into account for this parameter). §Specifically asthma, chronic bronchitis or emphysema. ‖Other than dilated cardiomyopathy, specifically ischemic and valvular heart disease.

disease, liver disease, cerebrovascular disease, peripheral artery disease. or renal disease (Table 1). These variables were assessed at year 25, when we analyzed the echocardiographic outcomes, because the questionnaire asked whether the participants ever had one of these diagnoses or characteristics, and we wanted to adjust for them if they were present at baseline or were detected during follow-up; the exceptions were educational level, physical activity, and BMI, which are relative to baseline only. These possible confounders were selected based on clinical relevance and have been described elsewhere (21); the respective questionnaires are available at the CARDIA website. Furthermore, we also accounted for the development of ischemic or valvular heart disease ("other cardiac diseases," defined as the occurrence of myocardial infarction, angina, rheumatic fever, or valvular disease) during the study period as a potential confounder. We also adjusted for baseline echocardiographic values (at year 5).

STATISTICAL ANALYSES. Descriptive statistics and the prevalence of the baseline covariates were determined for the 5 previously mentioned NIAAA alcohol intake groups. The distribution of covariates was compared among the groups using either the

chi-square test or Fisher's exact test (for categorical variables, wherever adequate), analysis of variance (for continuous variables, followed by pairwise comparison with Bonferroni adjustment for multiple comparisons whenever necessary), or Kruskal-Wallis test (for continuous variables without a normal distribution). Categorical variables were expressed as frequencies. Continuous variables were expressed as mean ± SD if normally distributed, or median and interquartile range if not normally distributed. The normality of distribution was investigated using the Kolmogorov-Smirnov test.

In order to assess the relationship between alcohol consumption and LV dysfunction, the various echocardiographic outcome parameters were analyzed using unadjusted and multivariable regression analysis, namely linear regression (considering the outcomes as continuous variables), accounting for the before-mentioned potential confounders in the multivariable models.

In model 1, alcohol consumption was introduced as a categorical variable (i.e., average number of standard drinks per week, distributed into the 5 NIAAA categories). In model 2, alcohol intake was a continuous variable (i.e., estimated cumulative alcohol consumption in liters during the 20 years of follow-up). Covariates used for multivariable analysis were chosen based on unadjusted analysis ($p < 0.10$) and clinical significance.

As a sensitivity analysis, we also performed inverse probability weighted regression adjustment. We compared each category of alcohol intake with abstainers, which was the reference group. To study a possible nonlinear relationship between average alcohol intake and the echocardiographic parameters, we used multivariable fractional polynomials (FP) as a closed test procedure (29) (Model 3).

To assess whether race or sex modified the relationship between cumulative alcohol consumption and our pre-defined outcomes, we analyzed the potential for an interaction between these variables and alcohol consumption.

Finally, subgroup analysis was performed to evaluate the association of specific types of beverages and binge drinking on our main echocardiographic outcomes. A p value below 0.05 was considered statistically significant. The statistical analyses were performed using Stata Software version 13 (Stata Corp, College Station, Texas).

RESULTS

In total, 2,368 participants were included in the analysis (Figure 1). Their median age at the end of the study was 51 years of age (interquartile range: 47 to 53 years). In total, 44.4% (n = 1,051) were male and 57.3% (n = 1,356) were Caucasian.

The majority of participants either did not consume alcohol or drank <4 standard drinks per week (Table 1). The average daily ethanol intake was 10 ml, and only 8.1% (n = 192) of the participants were "at risk" drinkers, with a weekly alcoholic intake above 14 drinks per week. The estimated mean cumulative alcohol intake was 82 ± 130 l over 20 years (mean of 13 drink-years).

LVEF, END-DIASTOLIC VOLUME AND MASS, AND LA DIAMETER AT THE END OF FOLLOW-UP. The covariates used for adjustment were sex, race, age, educational level, smoking, hypertension, diabetes, BMI, dyslipidemia, illicit drug use, and "other cardiac diseases" (namely, heart attack, angina, mitral valve prolapse, or rheumatic heart disease) at year 25, as well as the echocardiographic values at baseline (year 5). As stated in the Methods section, covariates were chosen based on unadjusted analysis (Table 1) and clinical significance (in the case of age and "other cardiac diseases").

We did not find an overall significant association between cumulative alcohol intake and LVEF, even though the first category of alcohol intake had a subtle increase in LVEF, both in conventional and inverse probability weighted adjustment (Table 2, Online Table 1). Only 76 participants (3.2%) had LVEF <50% at the end of follow-up, and the relationship between alcohol intake and LVEF as a dichotomous variable was similar to that seen as a continuous variable (not significant overall).

There was a progressive and statistically significant increase in BSA-indexed LVEDV with increasing alcohol intake, which remained statistically significant after adjustment (53.1 ± 10.7 ml/m² in non-drinkers vs. 58.8 ± 14.8 ml/m² if >14 drinks/week; $p = 0.037$) (Table 2). Using inverse probability weighted regression adjustment testing a linear model, this lost significance (Online Table 1). However, using an adjusted analysis with FP, this relationship was also significant, but best defined as nonlinear (model 3, in which age was also included as a nonlinear covariate) (Online Table 2). If we analyzed BSA-indexed LVEDV as a dichotomous variable (considering ≥75 ml/m² as abnormal, which is true for both males and females), 131 participants (5.5%) had an increased value at the end of follow-up, and there was a significant association with alcohol intake.

A significant and linear association was found between alcohol intake and BSA-indexed LV mass, which remained after adjustment for covariates

TABLE 2 Echocardiographic Outcomes at Year 25 According to Alcohol Consumption

		Model 1: Standard Drinks per Week Regression Coefficient (95% CI); p Value					
	Total	None (n = 619)	>0 and <4 (n = 934)	≥4 and <7 (n = 303)	≥7 and <14 (n = 320)	≥14 (n = 192)	Model 2: Cumulative Alcohol Intake p Value
LVEF, %	61.6 ± 7.2	61.2 ± 7.4	62.0 ± 6.7	61.4 ± 7.8	61.4 ± 7.2	61.4 ± 7.5	
Unadjusted		Reference	0.81 (0.08 to 1.55); 0.029	0.21 (−0.78 to 1.21); 0.672	0.19 (−0.78 to 1.17); 0.695	0.26 (−0.91 to 1.43); 0.666	0.842
Adjusted		Reference	1.84 (0.08 to 3.60); 0.040	−0.32 (−2.38 to 1.73); 0.758	1.30 (−0.65 to 3.26); 0.192	0.37 (−1.91 to 2.65); 0.752	0.907
BSA-indexed LVEDV, ml/m^2	55.5 ± 12.4	53.1 ± 10.7	55.3 ± 12.2	56.8 ± 13.1	57.0 ± 12.4	58.8 ± 14.8	
Unadjusted		Reference	2.21 (0.96 to 3.45); 0.001	3.68 (2 to 5.37); 0.001	3.92 (2.26 to 5.57); 0.001	5.68 (3.7 to 7.67); 0.001	0.001
Adjusted		Reference	2.94 (0.21 to 5.68); 0.035	4.25 (1.07 to 7.43); 0.009	3.80 (0.76 to 6.84); 0.014	5.69 (2.14 to 9.23); 0.002	0.037
BSA-indexed LV mass, g/m^2	83.6 ± 21.5	81.2 ± 20.9	81.6 ± 20.5	85.8 ± 24.7	87.1 ± 21.5	92.4 ± 19.6	
Unadjusted		Reference	0.43 (−1.83 to 2.71); 0.708	4.59 (1.52 to 7.66); 0.003	5.91 (2.87 to 8.93); 0.001	11.25 (7.55 to 14.94); 0.001	0.001
Adjusted		Reference	0.33 (−4.86 to 5.51); 0.901	4.01 (−2.04 to 10.06); 0.194	6.37 (0.57 to 12.17); 0.031	6.96 (0.19 to 13.73); 0.044	0.014
LA diameter, cm	3.70 ± 0.49	3.67 ± 0.49	3.69 ± 0.49	3.71 ± 0.48	3.70 ± 0.48	3.76 ± 0.47	
Unadjusted		Reference	0.02 (−0.04 to 0.07); 0.562	0.04 (−0.03 to 0.10); 0.311	0.03 (−0.03 to 0.10); 0.335	0.09 (0.01 to 0.17); 0.033	0.062
Adjusted		Reference	0.03 (−0.04 to 0.10); 0.411	0.04 (−0.05 to 0.12); 0.400	−0.01 (−0.09 to 0.08); 0.871	−0.01 (−0.10 to 0.08); 0.877	0.392

Values are mean ± SD unless otherwise indicated. The means for the drinking categories are unadjusted. Adjustment was made for sex, race, age, educational level, smoking, illicit drug use, hypertension, diabetes, body mass index, dyslipidemia, "other cardiac diseases," and for the values of each echocardiographic parameter at baseline (year 5).

BSA = body surface area; CI = confidence interval; LA = left atrial; LV = left ventricular; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction.

(LV mass of 81.2 ± 20.9 g/m^2 in nondrinkers vs. 92.4 ± 19.6 g/m^2 if >14 drinks/week; p = 0.004) (Table 2, Online Table 2).

Finally, there was a significant association of greater LA diameter with greater alcohol consumption in unadjusted analysis. However, this association lost significance in the multivariable analyses.

INDIVIDUAL VARIATION IN LVEF, LVEDV, LV MASS, AND LA DIAMETER DURING FOLLOW-UP. The individual variation (year 25 − year 5) change of each participant, Δ in LVEF, LVEDV, and LV mass was not significantly associated with cumulative alcohol intake in the multiple linear regression analysis (Table 3) and using multivariable FP (Online Table 3). There was an association between the variation in LA diameter and alcohol consumption in unadjusted analyses and using inverse probability weighted adjustment (Online Table 4).

EFFECT MODIFICATION OF SEX AND RACE. We did not find a significant difference between women and men or between white and black participants regarding the relationship between cumulative alcohol intake and the echocardiographic parameters using multiple linear regression. Using multivariable FP, there was a nonlinear association between alcohol and LA diameter in women (p = 0.018), not found in men (p = 0.828) (Central Illustration, panel A, Online Figure 1). An independent nonlinear association between LA diameter and alcohol was also seen in black participants (p = 0.007), but not in whites (Central Illustration, panel B, Online Figure 1).

Regarding the individual variation in echocardiographic parameters, ΔLVEDV in men (p = 0.029) and ΔLA diameter in women (p = 0.007) and in African American participants (p = 0.028) showed an independent linear association with average cumulative alcohol intake.

TYPE OF BEVERAGE. For this analysis, we considered only the predominant beverage (either beer, wine, or liquor—the one with a higher intake in ml of alcohol as assessed by the questionnaires) and excluded the individuals that had never drunk and those who did not show any "type of beverage" preference (n = 1,174). When adjusting for cumulative alcohol intake and covariates that related to the type of predominant beverage taken, drinking wine or liquor was associated with smaller BSA-indexed LVEDV. Drinking predominantly wine was also associated with higher LVEF, lower BSA-indexed LV mass and lower LA diameter than beer or liquor (Table 4).

BINGE-DRINKING. In the univariable analysis, binge-drinking was associated with echocardiographic

TABLE 3 Change in Echocardiographic Parameters (Between Year 25 and Year 5) According to Alcohol Consumption

		Model 1: Standard Drinks per Week Regression Coefficient (95% CI); p Value					Model 2: Cumulative Alcohol Intake p Value
	Total	None	>0 and <4	≥4 and <7	≥7 and <14	≥14	
ΔLVEF, % (n = 1,067)	−2.88 ± 15.15	−3.96 ± 26.27	−2.68 ± 8.79	−3.15 ± 7.81	−1.86 ± 8.30	−2.13 ± 7.92	
Unadjusted		Reference	1.28 (−1.10 to 3.65); 0.292	0.81 (−2.46 to 4.08); 0.628	2.10 (−1.08 to 5.27); 0.196	1.82 (−2.0 to 5.66); 0.350	0.309
Adjusted		Reference	0.95 (−1.75 to 3.66); 0.489	−0.72 (−4.01 to 2.57); 0.668	1.64 (−1.52 to 4.78); 0.307	−0.10 (−3.79 to 3.60); 0.959	0.597
ΔLVEDV, ml, (n = 1,073)	−10.27 ± 29.54	−8.03 ± 26.90	−11.46 ± 29.34	−11.06 ± 28.68	−11.13 ± 31.09	−13.33 ± 28.42	
Unadjusted		Reference	−3.43 (−7.85 to 0.99); 0.128	−3.03 (−9.11 to 3.04); 0.328	−3.11 (−9.01 to 2.80); 0.303	−5.30 (−12.45 to 1.84); 0.145	0.134
Adjusted		Reference	−2.21 (−11.39 to 6.98); 0.637	0.13 (−11.94 to 6.98); 0.982	1.78 (−8.89 to12.44); 0.743	0.12 (−12.42 to 12.66); 0.985	0.251
ΔLV mass, g, (n = 2,047)	22.18 ± 46.14	24.54 ± 46.27	20.50 ± 44.96	22.15 ± 49.59	21.67 ± 46.73	23.79 ± 45.03	
Unadjusted		Reference	−4.04 (−9.07 to 0.99); 0.116	−2.39 (−9.23 to 4.45); 0.493	−2.87 (−9.55 to 3.81); 0.399	−0.74 (−8.94 to 7.45); 0.858	0.659
Adjusted		Reference	−0.49 (−12.27 to 11.29); 0.935	2.27 (−11.52 to 16.06); 0.746	5.51 (−7.75 to 18.76); 0.415	−0.14 (−15.51 to 15.21); 0.985	0.600
ΔLA diameter, cm, (n = 2,238)	0.18 ± 0.50	0.21 ± 0.50	0.18 ± 0.50	0.17 ± 0.50	0.14 ± 0.45	0.13 ± 0.52	
Unadjusted		Reference	−0.026 (−0.078 to 0.025); 0.318	−0.033 (−0.104 to 0.036); 0.347	−0.069 (−0.138 to −0.001); 0.048	−0.077 (−0.159 to 0.005); 0.066	0.043
Adjusted		Reference	0.029 (−0.040 to 0.099); 0.409	0.036 (−0.047 to 0.119); 0.395	−0.007 (−0.089 to 0.076); 0.873	−0.007 (−0.099 to 0.085); 0.879	0.392

Values are mean ± SD unless otherwise indicated. The means presented for the drinking categories are unadjusted. Adjustment was made for sex, race, age, educational level, smoking, illicit drug use, hypertension, diabetes, body mass index, dyslipidemia, "other cardiac diseases," and for the values of each parameter at baseline (year 5).

Abbreviations as in Table 2.

parameters of cardiac remodeling (higher LVEDV, LV mass, and LA diameter); however, when adjusting for covariates that were associated with binge-drinking habits, only a borderline association with LA diameter was found (Table 5).

DISCUSSION

We intended to study the effects of alcohol intake on cardiac remodeling and function over time in a healthy sample of young adults up to middle age in the CARDIA cohort. In our study, greater alcohol consumption was associated with higher values of indexed LV mass and LVEDV, suggesting that alcohol may cause LV remodeling, which can be detrimental (25,26,30,31).

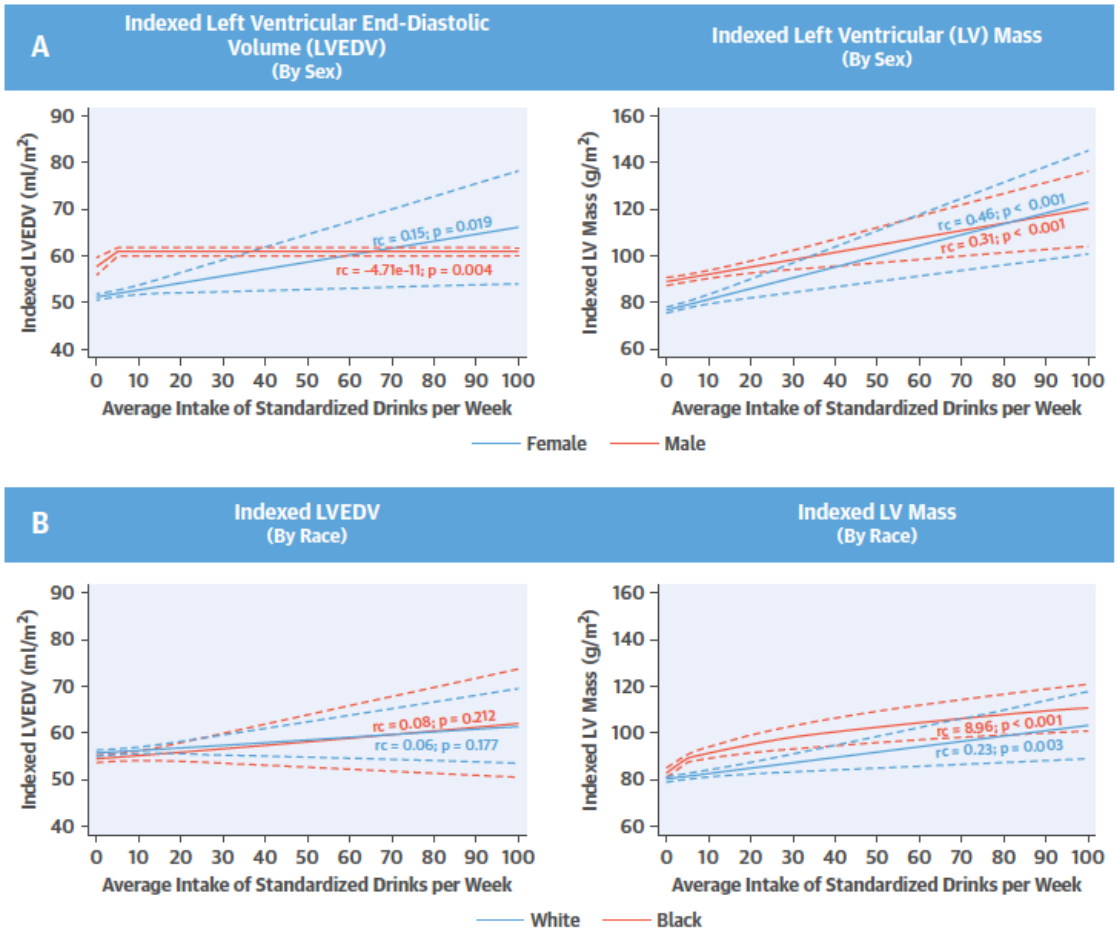
The absolute changes in echocardiographic parameters were small, and overall, the values remained within normal limits. Therefore, our results reinforce the concept that mild alcohol consumption (<7 drinks per week) poses little cardiovascular risk.

The fact that we did not find a significant association between alcohol and the intraindividual variation of the echocardiographic parameters (between year 25 and year 5) is probably due to the fact that we could only calculate the variation in LVED and LVEF in approximately one-half the sample (in 1,073 participants for LVEDV and in 1,067 for LVEF), because the quantification methods were different.

The fact that our sample was relatively young and included few individuals with very high alcohol intake might have contributed to the modest association between alcohol and echocardiographic changes. In our cohort, only 16 patients developed heart failure, and 4 patients had cardiovascular death. Our results also support the idea that the pathogenic role of alcohol in the development of dilated cardiomyopathy may be influenced by other individual factors, for example, genetic predisposition, and may be less easily detected in the general population in the absence of that data.

Another gap in the scientific evidence concerns the existence of a threshold for ethanol cardiotoxicity, whether it is modified by the type of beverage and whether there are specific groups of the population (e.g., in terms of race or sex who may be particularly susceptible to its effects).

CENTRAL ILLUSTRATION Alcohol Intake and Cardiac Remodeling



Rodrigues, P. et al. J Am Coll Cardiol. 2018;72(13):1452-62.

Relationship between average alcohol intake and body surface area-indexed left ventricular (LV) end-diastolic volume (LVEDV) and mass, according to sex **(A)** and race **(B)**. In **A**, males are represented in **orange** and females in **blue**. In **B**, black participants are represented in **orange**, and white participants in **blue**. Regression coefficient and p values for each subgroup are shown; 95% confidence intervals are represented by **dashed lines**. Adjustment was made for age, sex, race, educational level, smoking, illicit drug use, hypertension, diabetes, body mass index, dyslipidemia, and "other cardiac diseases" (multivariable regression using fractional polynomials). Alcohol intake was independently associated with higher body surface area-indexed LVEDV and LV mass, in both sexes and races.

Whereas information regarding the cardiac effects of alcohol in different races is scarce, women have shown to be more susceptible to alcohol toxic effects in some studies (32,33). In our cohort, sex or race did not significantly modify the relationship between alcohol intake and echocardiographic parameters of LV systolic function and remodeling. However, the fact that very few women drank heavily may have compromised our ability to detect a difference.

Knowledge regarding the impact of specific types of alcoholic beverages in ventricular function and remodeling was lacking, and the few studies that have addressed this issue before have failed to find any significant differences in association (34,35).

TABLE 4 Impact of the Type of Beverage Preference on Ventricular Remodeling

	Beer (n = 713)	Wine (n = 295)	Liquor (n = 166)
LVEF, %	61.15 ± 7.33	62.61 ± 6.90	62.19 ± 7.19
Unadjusted	Reference	1.45 (0.47 to 2.43); 0.004	1.04 (−0.18 to 2.26); 0.095
Adjusted*	Reference	1.38 (0.38 to 2.39); 0.007	1.08 (−0.15 to 2.31); 0.085
BSA-indexed LVEDV, ml/m^2	58.57 ± 13.32	53.66 ± 11.50	54.70 ± 12.04
Unadjusted	Reference	−4.91 (−6.64 to −3.18); 0.001	−3.87 (−6.03 to −1.72); 0.001
Adjusted*	Reference	−4.90 (−6.64 to −3.16); 0.001	−3.65 (−5.78 to −1.51); 0.001
BSA-indexed LV mass, g/m^2	87.94 ± 23.44	79.86 ± 18.15	85.85 ± 23.25
Unadjusted	Reference	−8.08 (−11.21 to −4.95); 0.001	−2.09 (−6.02 to 1.84); 0.297
Adjusted*	Reference	−5.78 (−8.84 to −2.71); 0.001	− 3.75 (−7.56 to 0.05); 0.053
LA diameter, cm	3.751 ± 0.491	3.616 ± 0.434	3.813 ± 0.495
Unadjusted	Reference	−0.135 (−0.201 to −0.069); 0.001	0.062 (−0.020 to 0.069); 0.140
Adjusted*	Reference	−0.071 (−0.131 to −0.011); 0.019	0.005 (−0.068 to 0.079); 0.888

Values are mean ± SD or regression coefficient (95% CI); p value. The predominant drink at baseline was chosen (the beverage with the intake corresponding to more milliliters of alcohol). *Adjusted for cumulative alcohol intake and the covariates that were associated with a predominant type of drink in univariate analysis (hypertension, race, body mass index, educational level, sleep apnea, and thyroid disease).

Abbreviations as in Table 2.

However, in our study, drinking predominantly wine was associated with less LV and LA dilation and higher LVEF, an interesting finding that warrants further confirmation. Dietary factors can also have a confounding factor that we could not account for.

Binge drinking, a behavior previously correlated primarily with arrhythmias, specifically atrial fibrillation (36), was also associated with adverse cardiac remodeling, but only in the crude analysis. That association lost significance when adjustment was performed, which could be related to confounding but also to the limited number of participants with binge-drinking habits.

STUDY STRENGTHS AND LIMITATIONS. The major strength of the current study was that it analyzed a large race- and sex-balanced sample with a long follow-up. Besides utilizing this large and very complete dataset, we also performed a robust analysis with multiple adjustments for covariates, in order to minimize the likelihood of confounding.

However, we must also highlight the significant limitations of our study and why the study should be interpreted with caution. Firstly, because alcohol intake was assessed using questionnaires, recall bias may have led to an inaccurate estimation. Secondly, in this cohort, alcohol consumption was relatively low, with 78% of the participants drinking only up to 7 standard drinks per week. Because alcohol cardiotoxicity is probably more striking at greater doses (9,10), the low prevalence of at-risk drinkers (>14 drinks per week) in our sample may have limited our power to adequately assess the risks related to heavy alcohol consumption. A selection bias must also be considered, because using participants who attended the study and performed all the required evaluations may have overrepresented certain characteristics of the population. Finally, as with other observational studies, the potential for unmeasured confounding limits our ability to establish a causal relationship. However, because long-term randomized trials assessing the effect of alcohol consumption on LV function would be impractical and unethical, we consider prospective longitudinal studies such as this one to be the most realistic study designs available to assess the cardiac effect of alcohol intake in populations.

The mechanisms behind the relationship between alcohol consumption and cardiac function and structure still need to be better understood. We still cannot predict which patients will develop ACM and,

TABLE 5 Effect of Binge-Drinking on Ventricular Remodeling

	Non-Binge Drinkers (n = 1,449)	Binge Drinkers (n = 300)
LVEF, %	61.83 ± 7.08	61.32 ± 7.32
Unadjusted	Reference	−0.51 (−1.39 to 0.38); 0.263
Adjusted*	Reference	−1.37 (−2.79 to 0.06); 0.060
BSA-indexed LVEDV, ml/m^2	55.81 ± 12.25	58.83 ± 14.89
Unadjusted	Reference	3.01 (1.43 to 4.60); 0.001
Adjusted*	Reference	1.93 (−0.47 to 4.34); 0.114
BSA-indexed LV mass, g/m^2	83.22 ± 21.64	91.32 ± 20.73
Unadjusted	Reference	8.10 (5.20 to 10.99); 0.001
Adjusted*	Reference	1.92 (−2.40 to 6.24); 0.383
LA diameter, cm	3.684 ± 0.491	3.808 ± 0.483
Unadjusted	Reference	0.124 (0.062 to 0.186); 0.001
Adjusted*	Reference	0.085 (0.001 to 0.170); 0.050

Values are mean ± SD or regression coefficient (95% CI); p value. *Adjusted for cumulative alcohol intake and the covariates that were associated with binge drinking in univariable analysis (illicit drug use, smoking, body mass index, educational level and thyroid disease).

Abbreviations as in Table 2.

beyond sex and race, there are probably genetic factors that need to be taken into consideration. Plus, the potential for reversibility has not been adequately studied (5). Further studies of individuals with greater alcohol intake may help us define better prevention strategies to reduce the deleterious effects of alcohol toxicity in populations.

CONCLUSIONS

Greater alcohol intake had an independent adverse association with ventricular structure (greater indexed LV mass and LVEDV) after 20 years of follow-up. This relationship was not significantly modified by sex or race. Moreover, there was also an association between alcohol intake and LA diameter in women and among African American CARDIA participants.

Alcohol consumption was not significantly associated with LV systolic dysfunction measured by LVEF in this cohort of young adults with mild-to-moderate alcohol consumption. There was a nonsignificant trend for a deleterious effect of binge drinking and drinking predominantly wine was associated with less cardiac remodeling.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: In most middle-aged Americans, moderate alcohol intake has no major deleterious effects on cardiac structure and function. There is an association between alcohol intake and left ventricular dilatation that could be an early form of dilated cardiomyopathy and that is more pronounced with liquor and beer than with wine consumption and with binge drinking.

TRANSLATIONAL OUTLOOK: Prospective trials of alcohol abstinence in patients with dilated cardiomyopathy are needed to assess the potential reversibility of alcohol toxicity, and long-term follow-up studies of heavy drinkers could clarify modulators of the effect of alcohol on the heart.

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KEY WORDS alcohol, alcoholic cardiomyopathy, cardiac remodeling, heart failure, ventricular dilatation, ventricular function

APPENDIX For details on data availability as well as supplemental tables, please see the online version of this paper.

DATA AVAILABILITY

Data are available from the CARDIA Coordinating Center: http://www.cardia.dopm.uab.edu/contact-cardia. A description of the NHLBI policies governing the data and describing access to the data can be found at the following website: http://www.cardia.dopm.uab.edu/study-information/nhlbi-data-repository-data.

SUPPLEMENTARY TABLES

Online Table 1. Echocardiographic outcomes at year 25 according to average alcohol consumption, using inverse probability weighted regression adjustment.

	Standard-drinks per week [regression coefficient (95% CI), p-value]				
	None	**>0 and <4**	**≥4 and <7**	**≥7 and <14**	**≥14**
LVEF, %	Reference	1.58 (0.47; 2.65), p=0.005	1.07 (-0.27; 2.41), p=0.116	1.08 (-0.26; 2.42), p=0.115	1.77 (0.11; 3.43), p=0.036
BSA-indexed LVEDV, mL/m²	Reference	0.71 (-1.09; 2.51), p=0.440	1.21 (-0.82; 3.25), p=0.242	1.06 (-1.08; 3.21), p=0.331	2.47 (-0.79; 5.72), p=0.137
BSA-indexed LV Mass, g/m²	Reference	2.12 (1.19; 5.42); p=0.209	4.05 (-0.30; 8.13), p=0.052	5.07 (1.02; 9.11), p=0.014	4.51 (-0.15; 9.18), p=0.058
LA diameter, cm	Reference	0.003 (-0.068;0.075), p=0.926	0.010 (-0.071; 0.092), p=0.804	-0.010 (-0.095;0.076), p=0.825	-0.037 (-0.158;0.084), p=0.547

Adjustment was made for age, sex race, educational level, smoking, illicit drug use, hypertension, diabetes, body mass index, dyslipidemia and "other cardiac diseases".

Legend: BSA- body surface area; CI- confidence interval; LA- left atrial; LV- left ventricular; LVEDV- left ventricular end diastolic volume; LVEF- left ventricular ejection fraction.

Online Table 2. Echocardiographic outcomes at year 25 according to average alcohol consumption, using multivariable fractional polynomials (model 3).

Echocardiographic outcomes	BEST-FIT (FP POWERS)	ADJUSTED REGRESSION COEFICIENT (95% CI)	P-VALUE
LVEF, %	Linear	0.026 (-0.017;0.069)	0.240
BSA-indexed LVEDV, mL/m^2	FP1 (0)*	0.185 (0.071;0.299)	0.001
BSA-indexed LV mass, g/m^2	Linear	0.329 (0.198;0.461)	<0.001
LA diameter, cm	Linear	-0.0002 (-0.0029;0.0024)	0.872

Adjustment was made for age, sex, race, educational level, smoking, illicit drug use, hypertension, diabetes, body mass index, dyslipidemia and "other cardiac diseases".
*If 4 outlier individuals with >70 drinks per week were excluded, this association remained significant, but linear.

Legend: BSA- body surface area; CI- confidence interval; FP- fractional polynomial; FP1- first-degree fractional polynomial; FP2- second degree fractional polynomial; LA- left atrial; LV- left ventricular; LVEDV- left ventricular end diastolic volume; LVEF- left ventricular ejection fraction.

Online Table 3. Relationship between average alcohol consumption and change in echocardiographic parameters (between year 25 and year 5), using multivariable fractional polynomials.

Echocardiographic outcomes	BEST-FIT (FP POWERS)	ADJUSTED REGRESSION COEFICIENT (95% CI)	P-VALUE
Δ ***LVEF (%)***	Linear	0.083 (-0.059;0.226)	0.253
Δ ***LVEDV (mL)***	Linear	-0.260 (-0.525;0.006)	0.055
Δ ***LV Mass (g)***	Linear	0.225 (-0.086;0.537)	0.156
Δ ***LA diameter (cm)***	Linear	-0.0028 (-0.0059;0.0003)	0.077

Adjustment was made for age, sex, race, educational level, smoking, illicit drug use, hypertension, diabetes, body mass index, dyslipidemia and "other cardiac diseases".

Legend: BSA- body surface area; CI- confidence interval; FP- fractional polynomial; LA- left atrial; LV- left ventricular; LVEDV- left ventricular end diastolic volume; LVEF- left ventricular ejection fraction.
Δ - intraindividual variation (difference between year 25 and year 5).

Online Table 4. Relationship between average alcohol consumption and change in echocardiographic parameters (between year 25 and year 5), using inverse probability weighted regression adjustment.

	Standard-drinks per week [regression coefficient (95% CI), p-value]				
	None	**>0 and <4**	**≥4 and <7**	**≥7 and <14**	**≥14**
Δ ***LVEF (%)***	Reference	4.93 (-2.25; 12.13), p=0.178	3.51 (-3.74; 10.76), p=0.343	5.05 (-2.23; 12.32), p=0.174	5-50 (-2.11; 13.12), p=0.157
Δ ***LVEDV (mL)***	Reference	-0.37 (-7.15; 6.41), p=0.915	-2.00 (-10.39; 6.38), p=0.639	0.57 (-7.09; 8.23), p=0.884	6.55 (-3.42; 16.53), p=0.198
Δ ***LV Mass (g)***	Reference	-1.45 (-9.64; 6.74), p=0.728	-0.55 (-10.24; 9.14), p=0.911	0.11 (-9.72; 9.93), p=0.983	-7.35 (-20.40; 5.69), p=0.269
Δ ***LA diameter (cm)***	Reference	-0.050 (-0.138; 0.037), p=0.258	-0.043 (-0.139; 0.054), p=0.387	-0.098 (-0.192; -0.004), p=0.041	-0.128 (-0.254; -0.003), p=0.044

Adjustment was made for age, sex race, educational level, smoking, illicit drug use, hypertension, diabetes, body mass index, dyslipidemia and "other cardiac diseases".

Legend: BSA- body surface area; CI- confidence interval; FP- fractional polynomial; LA- left atrial; LV- left ventricular; LVEDV- left ventricular end diastolic volume; LVEF- left ventricular ejection fraction.
Δ - intraindividual variation (difference between year 25 and year 5).

Online Figure 1. Relationship between average alcohol intake and BSA-indexed LVEDV, BSA-indexed LV mass, LVEF and LA diameter, according to sex (panel A) and race (panel B).

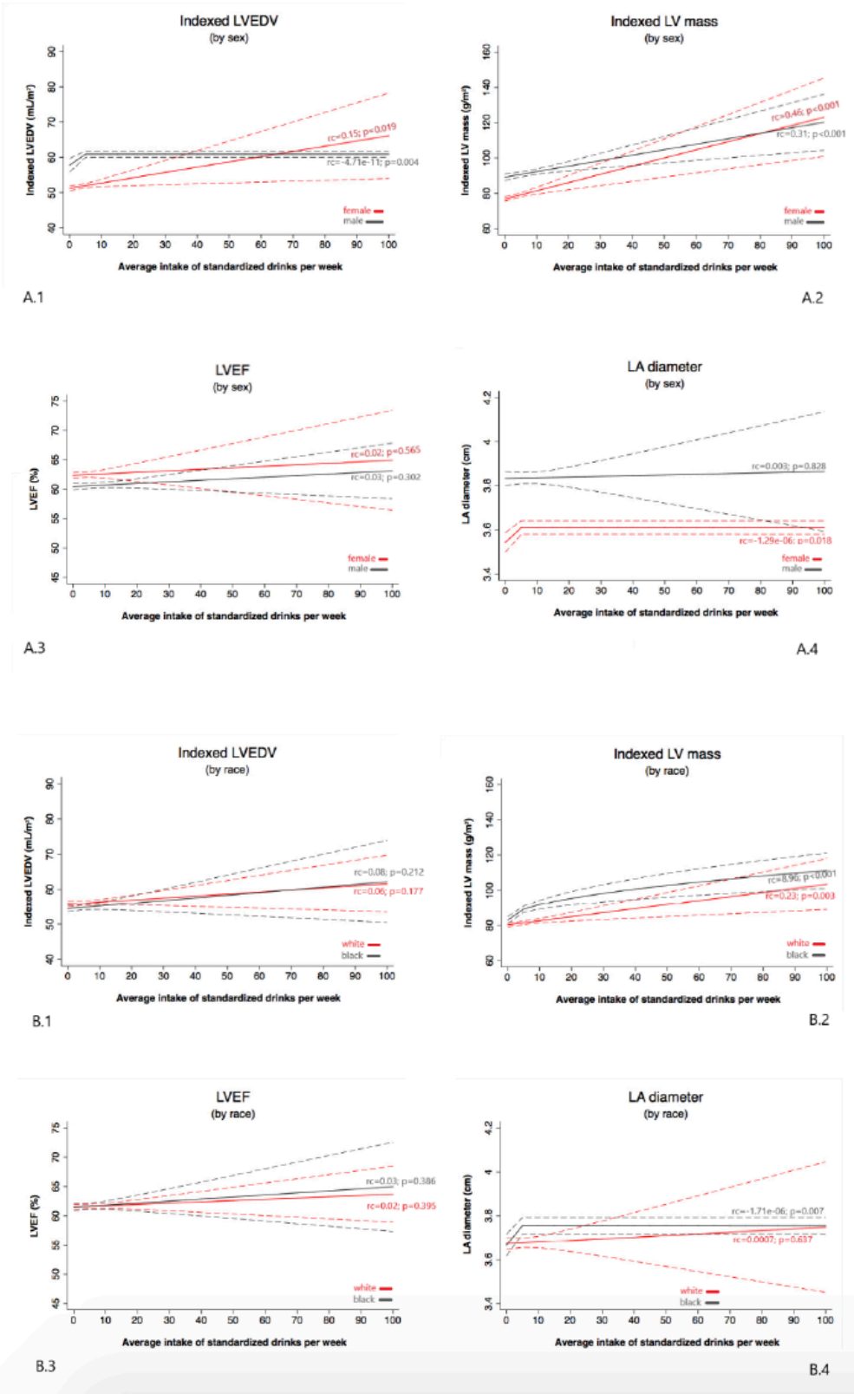

Figure 1 - legend:

In panel A, males are represented in grey and females in red. In panel B, black participants represented in grey and white participants in red. Regression coefficient and p values for each subgroup are shown; 95% confidence intervals are represented by dashed lines.

Adjustment was made for age, sex, race, educational level, smoking, illicit drug use, hypertension, diabetes, body mass index, dyslipidemia and "other cardiac diseases" (Model 3, multivariable regression using fractional polynomials).

In panel A (A.1-A.4), alcohol intake was significantly associated with indexed LVEDV, in both males (p=0.004) and females (p=0.019), and alcohol was also an independent predictor of indexed LV mass (p<0.001 in both groups). We did not find a relationship between LVEF and alcohol in men (p=0.30) or women (p=0.56). In women, there was an association between alcohol and LA diameter (p=0.018), not found in men (p=0.828).

In panel B (B.1-B.4), in what concerns black participants, there was an association between alcohol and indexed LV mass (p<0.001) or LA diameter (p=0.007); we did not find an association with indexed LVEDV (p=0.192) or LVEF (p=0.326). In white participants, there was also an association between alcohol intake and indexed LV mass; no significant association was found between alcohol and indexed LVEDV, LVEF and LA diameter.

C)

Rodrigues P, Ferreira B, Fonseca T, Quelhas-Costa R, Cabral S, Loureiro-Pinto J, Saraiva F, Marinho A, Huttin O, Girerd N, Bozec E, Cyrne Carvalho H, Ferreira JP. ***Subclinical ventricular dysfunction in rheumatoid arthritis*** --- submitted, under review

Subclinical ventricular dysfunction in rheumatoid arthritis

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DECLARATIONS

Authorship: All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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Conflicts of interest: None declared.

Ethics approval: This study was approved by the Ethics Committee of Centro Hospitalar Universitário do Porto (number 2016-023; 020-DEFI/020-CES).

Consent: All participants gave written informed consent.

ABSTRACT

Purpose

Patients with rheumatoid arthritis (RA) are at higher risk for having underdiagnosed heart failure, however there are no recommendations regarding echocardiographic screening. We aimed to determine the prevalence of subclinical ventricular dysfunction in RA applying current echocardiographic guidelines, its association with patients' characteristics, biomarkers and prognostic parameters and compare the 2016 guidelines to the recommendations from 2009.

Methods

Prospective study of RA patients without known heart disease, categorized as preserved ventricular function (PVF), systolic dysfunction (SD), isolated diastolic dysfunction (DD) or indeterminate diastolic function (IDF) as per the 2016 echocardiography guidelines - or any ventricular dysfunction (AVD) comprehending the last 3.

Results

The median age was 58 years and 78% were females. The majority had PVF (73%), followed by DD (13%), IDF (11%) and SD (4%). Concordance with the 2009 echocardiographic guidelines was low. Compared with PVF, AVD patients were older (65 vs 55 years, $p<0.001$), had a higher prevalence of hypertension and dyslipidaemia (56% vs 38%, $p=0.003$ and 60% vs 41%, $p=0.002$, respectively). In multivariable analysis, age (particularly >57 years) was the only independent predictor of AVD or DD. AVD was significantly associated with higher NT-proBNP and lower distance in 6-minute walk test. There were no significant independent associations between characteristics of RA disease and ventricular function.

Conclusion

A total of 17% of RA patients without known cardiovascular disease presented subclinical systolic or diastolic dysfunction, which was associated with older age. The echocardiographic screening may have clinical value in identifying subclinical ventricular dysfunction, especially in older RA patients.

Key-words : rheumatoid arthritis; echocardiogram; systolic dysfunction; diastolic dysfunction; biomarkers

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease, involving autoimmune mechanisms, characterized by a symmetric peripheral polyarthritis; however, extra-articular involvement can occur. RA has been associated with heart disease, the pericardium being frequently affected, while coronary artery disease (CAD) and heart failure (HF) (both ischemic and non-ischemic) are also more common than in the general population [1, 2].

Aiming to better predict the cardiovascular (CV) risk in RA patients, the European League Against Rheumatism (EULAR) suggested applying a 1.5 multiplication factor to algorithms conceived for the general population [3] since patients with RA seem to have a two-fold higher incidence of HF and an increased mortality risk [4, 5]. Although HF with reduced ejection fraction is not particularly frequent, diastolic impairment and left ventricular hypertrophy seem to be more common in RA [6, 7]. Underlying HF aetiologies include atherosclerotic CAD, dilated cardiomyopathy, myocarditis and vasculitis. The role of anti-inflammatory therapies or disease-modifying anti-rheumatic drugs (DMARDs) in heart disease remains unclear [8].

Patients with RA present some specificities and the discrimination of their symptoms is often difficult because symptoms may be non-specific or conditioned by articular limitations [9]. HF may be underdiagnosed in RA patients and there are no available recommendations for HF screening specific for patients with RA. Subclinical ventricular dysfunction can be quickly identified by echocardiography, while surrogate biomarkers can be easily studied. Other studies have already suggested the prognostic utility of N-terminal prohormone of brain natriuretic peptide (NT-proBNP), C-reactive protein (CRP) and rheumatoid factor (RF), but the value of anti-cyclic citrullinated peptide (anti-CCP) antibodies, erythrocyte sedimentation rate and troponin is still more uncertain[10, 11].

An earlier diagnosis could improve treatment decisions and consequently prognosis. The non-invasive diagnosis of diastolic dysfunction – which can be linked to HF with preserved ejection fraction [12] - is particularly challenging. A change in echocardiographic guidelines in 2016 reformulated the previous algorithm, with the expectation of a better performance in clinical practice [13, 14]. Prior studies have not focused on subclinical disease following the 2016 guidelines, with a rigorous state-of-the-art homogeneous echocardiographic analysis (most used the 2009 guidelines, or only one or two parameters and did not the complete echocardiographic algorithm).

In the present study, we assess a relatively large sample of RA patients, adjust for covariates that were systematically investigated, and complement our analysis with biomarker evaluation. Our main objectives were: 1) to assess the prevalence and characterize the type of cardiac dysfunction in RA patients without known heart disease; 2) compare the characteristics of patients with RA according to their ventricular function classification, aiming to identify the

predictors of ventricular dysfunction (and the patients that would benefit the most from the screening); and 3) validate the value of the identification of subclinical ventricular dysfunction by analysing associations with surrogate prognostic markers. The secondary objectives were to determine the prevalence of a possible precursor stage of HF with preserved ejection fraction (HFpEF), using a combination of echocardiographic findings and biomarkers, and compare diastolic dysfunction definitions using the actual 2016 echocardiographic guidelines [14] and the previous guidelines from 2009 [13].

METHODS

Study Design and Ethics

This is a prospective study that enrolled patients followed in the outpatient clinic of Autoimmune Disorders at Centro Hospitalar Universitário do Porto, previously diagnosed with RA, between June 2016 and June 2018. Follow-up was updated in August 2019. The protocol for this study was a priori registered at ClinicalTrials.gov under the number NCT03960515.
This study was approved by the Hospital's Ethics Committee and was conducted in accordance to the principles of the 1975 Declaration of Helsinki. All participants provided written informed consent before enrollment in the study.

Patient selection

The diagnosis of RA was made according to 2010 ACR/EULAR Classification Criteria [15]. Patients with an active neoplasm or severe comorbidity, an expected survival of less than 6 months, dementia, inability to walk or totally dependent in their daily life activities were excluded. For the present analysis, patients with previously known heart disease (HF, CAD, previous percutaneous or surgical coronary revascularization, previous cardiac surgery, at least moderate valve disease, or atrial fibrillation at the time of the echocardiogram) or those that did not perform an echocardiogram were also excluded.

Patients were divided into 4 categories of ventricular function, according to transthoracic echocardiogram (TTE) results: preserved ventricular function (PVF); systolic (± diastolic) dysfunction (SD); (isolated) diastolic dysfunction (DD); indeterminate diastolic function (IDF). The definition of groups was made according the 2016 European Heart Failure guidelines [12], 2016 guidelines for diastolic evaluation [14] and the 2009 recommendations of the American Society of Echocardiography [13], as depicted in Figure 1. If half (or more) of the variables needed for each classification were missing, the diastolic classification was registered as

missing. In the main analyses, the diastolic function categorization followed the 2016 guidelines. We grouped patients with IDF, DD or SD, as per the 2016 guidelines, into one category of "any ventricular dysfunction" (AVD), that was compared to PVF.

Using the 2016 HF European guidelines [12], we also analysed a category of potential subclinical heart failure with preserved ejection fraction ("HFpEF risk"), with the following characteristics: LVEF ≥50% *and* NT-proBNP > 125 pg/mL *and* structural echocardiographic changes (left ventricular hypertrophy *or* indexed LA volume *or* diastolic dysfunction as per 2016 echocardiographic guidelines). We use the term "risk" because the presence of signs or symptoms of HF was not necessary.

Our sample of RA patients was compared with a cohort of the general population (from the EPIPorto study [16], n=1000) that has similar demographic characteristics and risk factors (except for the presence of RA), which was also evaluated using the 2016 European/American guidelines.

Data collection and variables

Clinical Data

Biometric and clinical data was collected and included age, gender, body mass index (BMI), comorbidities, year of diagnosis of RA, Disease Activity Score 28 (DAS28) assessment - that measures disease activity by counting the number of tender or swollen joints [17], combined with the value of erythrocyte sedimentation rate (ESR) - and medication (all the medication used for RA and cardiovascular medication).

Transthoracic Echocardiogram (TTE)

Echocardiographic evaluation was analysed by one cardiologist, that was blinded to the clinical information or the results of any other exams, using Philips® iE33 ultrasound machine. To certify the external validity of the echocardiographic measurements, a random sample of 25 anonymized exams was evaluated at the Institut Lorrain du Coeur et des Vaisseaux Louis Mathieu at Nancy, France, showing good correlation of the measurements (intra-class correlation coefficient >0.75 for variables that are used in diastolic evaluation).

The evaluated parameters included the dimensions of the cardiac chambers (left atrial and end-diastolic ventricular volumes calculated using Simpson's rule and indexed to body surface area), left ventricular wall thickness and mass, left ventricular ejection fraction (using modified Simpson's biplane method), valvular disease, pericardial disease and diastolic function as per the 2016 American and European guidelines [14]. The Chamber Quantification European guidelines [18] were followed for all cardiac chambers analyses.

We assessed the relationship between echocardiographic categories and surrogate prognostic markers (biomarkers, physical exercise capacity and cardiovascular events) because the echocardiographic identification of ventricular dysfunction is more relevant if it is associated with prognosis.

Biomarkers

We analysed several biomarkers in the hospital lab that included the NT-proBNP, high-sensitivity troponin T (hsTnT), CRP and erythrocyte sedimentation rate (ESR). We also checked if patients tested positive for anti-CCP antibodies or rheumatoid factor (RF) and their estimated glomerular filtration rate (eGFR).

Physical Exercise Capacity

Patients performed a 6-minute walk test (6MWT) as recommended by the American Thoracic Society [19].

Statistical analyses

Statistical analyses were conducted using the STATA version 13 and SPSS ® version 23 software. A two-sided p-value of <0.05 was used as statistical significance for all statistical tests. Continuous data were described as mean ± standard deviation for gaussian distribuition or median (IQR - interquartile range between the 25^{th} and 75^{th} quartiles) for non-gaussian distributions. The Shapiro-Wilk test was used to test the normality of distribution. Categorical data were presented as absolute frequencies (n) and percentages (%). We analysed the relationship between two categories of ventricular function and patients' characteristics using independent-samples *t* test or Mann-Whitney U test for continuous variables, and Chi-squared or Fisher exact test for categorical variables, as appropriate. The four groups of LV function were compared using ANOVA, Kruskal-Wallis tests or multinomial logistic regression.

Linear regression was used to analyse the associations between continuous variables. Multivariable logistic regression, using categories of ventricular function as dependent variable (taking PVF as reference) was performed to estimate independent predictors of ventricular function (covariates with a significant association in the univariable analysis were used in the model), obtaining odds ratio (OR) and the respective 95% confidence intervals (CI). Hosmer-Lemeshow test was used to determine goodness of fit of model and area under the Receiver Operating Characteristic curve (AUC ROC) to determine its discriminative power. Logistic regression was also used to compare the prevalence of diastolic dysfunction in our sample with a cohort from the general population (EPIPorto study [16]).

We computed the Cohen's Kappa coefficient (κ) to test the concordance between 2009 and 2016 classifications of diastolic function.

RESULTS

Baseline characteristics

We included a total of 319 RA patients without known heart disease (Figure 2). The median age was 58 years (IQR 19) and 78% (n=249) were females. Patients' characteristics are presented in Table 1.

Patients with any ventricular dysfunction (AVD)

Eighty-seven patients (27%) had AVD (Table 1). These patients were older, presented more frequently hypertension, dyslipidaemia, used corticosteroids more often, reported a lower dosage of cardiovascular medications (ACEi or ARB and statin), had increased NT-proBNP and lower eGFR concentrations, and showed poorer physical performance in the 6MWT test. In the multivariable analysis (model adjusted for age, hypertension, dyslipidaemia, corticosteroids and eGFR), age was the only independent predictor of AVD (OR 1.079; 95% CI, 1.045 to 1.114). The AUC ROC was 0.71 (95% CI, 0.65 to 0.77) with the best cut-off point (best sum of sensitivity and specificity) of 57 years old (sensitivity of 78% and specificity of 58%).

Comparison between categories of ventricular function (using 2016 echocardiographic guidelines)

Systolic dysfunction was found in 4% (n=13), being mild (LVEF between 40 and 50%) in 11 patients and moderate (LVEF between 40% and 30%) in 2 patients.

Patients with LVEF≥50%, were classified according to the 2016 guidelines.

Isolated DD was documented in 40 patients (13%) and 34 patients (11%) had IDF. Tricuspid regurgitation (TR) velocity could be adequately determined in only 151 patients (47%).

Age, hypertension and dyslipidaemia were expressed significantly different amongst categories of ventricular function (Table 2). The RA variables, which included the RA duration, anti-CCP/RF positivity, DAS28-ESR, ESR and RA medication were not associated with TTE results, however corticosteroids were more frequently used in patients with DD and CRP was higher in SD, as compared to reference category (preserved ventricular function). In the multivariable analysis (adjusted for age, hypertension, dyslipidaemia, corticosteroids and

CRP), age was the only independent factor associated with categories of ventricular function, with an OR for DD (PVF as reference) of 1.097 (95% CI, 1.055 to 1.141; p<0.001).
Comparing DD category to PVF, there were significant differences in age (66.5 (IQR 12) vs 54.5 (IQR 19); OR 5.826; 95% CI, 3.704 to 7.048; p<0.001), dyslipidaemia (65% vs 41%; OR 1.645; 95% CI, 1.158 to 2.336; p=0.005), corticosteroid use (58% vs 39%; OR 1.457; 95% CI, 1.036 to 2.048; p=0.031), eGFR (86.5 (IQR 22.2) vs 95.0 (IQR 28.0); OR 2.214; 95% CI, 0.639 to 12.566; p=0.041) and family history of ischaemic heart disease (2.5% vs 17.3%; OR 0.351; 95% CI, 0.128 to 0.960; p=0.041). In multivariable analysis, age, eGFR and family history were independently related with DD.
The prevalence of DD was significantly higher in patients with RA as compared to the general population from the EPIPorto cohort (13% vs 1.4%; p<0.001).

"HFpEF risk": combined strategy of echocardiographic evaluation and NT-proBNP

Excluding those with systolic dysfunction or without NT-proBNP analysis (n=298 of 319), we identified 40 patients (13%) at "HFpEF risk" (LVEF ≥50% *and* NT-proBNP > 125 pg/mL *and* structural echocardiographic changes [12]). After analysis of all covariates in Table 1, age (OR 1.078; p<0.001), CKD (OR 0.178; p= 0.013), eGFR (OR 0.977; p=0.005) and RA duration (OR 1.031; p= 0.037) were associated with "HFpEF risk". On multivariable analysis, age was the only independent predictor of "HFpEP risk".

The "HFpEF risk" was significantly associated with ventricular function categories, with 82% of those without "HFpEF risk" showing preserved ventricular function (vs 35% with risk). However, patients with "HFpEF risk" were equally distributed among preserved ventricular function (14/40), indeterminate diastolic function (13/40) and diastolic dysfunction (13/40).

Comparison with 2009 guidelines for diastolic dysfunction classification

A total of 311 patients (97%) had enough data to compare the 2009 and 2016 classifications. When we applied a simplified model of the 2009 recommendations [13] to classify diastolic dysfunction, more patients would fulfil criteria for DD (23% versus 13% using the 2016 criteria) – Figure 3 and Supplementary Table 1. The percentage of IDF would also be much higher (47% vs 11%). The concordance between both classifications was low (Cohen's Kappa = 0.25).

The specific echocardiographic parameters that can represent structural or functional changes related to HF were on average within normal limits (Supplementary Table 2). Most of these echocardiographic parameters were associated with several cofactors, namely age, hypertension and diabetes, and correlated with NT-proBNP and 6MWT.

Cardiovascular surrogate markers

NT-proBNP

The NT-proBNP levels were associated with "any ventricular dysfunction" and with the categorization of ventricular function using either 2009 or 2016 guidelines (but were not significantly different between DD and PVF). NT-proBNP levels were also significantly associated with most echocardiographic parameters (shown in Supplementary Table 3). Overall, 87 of the 311 patients (28%) presented an NT-proBNP $\geq$125 pg/mL (cut-off generally set for chronic HF), corresponding to 34% of DD and 46% of SD patients.

hsTnT

The hsTnT levels were associated with categories of ventricular function (using 2009 or 2016 classification), but were not significantly associated with having "any ventricular dysfunction" or "HFpEF risk". The log(hsTnT) was associated with having DD versus PVF. Overall, 25 of 311 patients (8%) had a hsTnT above normal (>0.014 pg/mL), but only 2 patients had a value above the cut-off value considered at our laboratory for acute coronary syndrome (>0.054 pg/mL).

6MWT

All the major echocardiographic parameters were significantly associated with 6MWT performance. The total distance walked in 6MWT was also significantly associated with ventricular function as per the 2016 or 2009 classifications (and specifically with DD versus PVF), with "any ventricular dysfunction" and "HFpEF risk".

Cardiovascular events

During a mean follow-up time of 2.8±0.6 years (54 to 1095 days), only 9 cardiovascular events (cardiovascular death, heart failure or other cardiovascular driven hospitalization) were recorded, without association with ventricular function or with "HFpEF risk".

DISCUSSION

The main finding of our study is that RA patients without known cardiac disease showed a 4% prevalence of subclinical systolic function and 13% of diastolic dysfunction. An increasing age

was the most important independent predictor of ventricular function.

Most previous studies used the 2009 guidelines [13], but their application is complex and different authors applied distinct algorithms; in most cases the presence of one DD parameter was sufficient [20-22]. Our findings also support the belief that the 2009 guidelines have a poor agreement with newer 2016 classification [16, 23-25]. Using the 2009 guidelines instead of the 2016 recommendations, the proportion of DD would have risen from 13% to 23%, while IDF would be found in 47% of patients instead of 11%. Invasive studies suggested that the 2016 guidelines are more specific [26] and that both guidelines cannot be used interchangeably. Even when using the same 2016 guidelines, the application of those recommendations and the number of parameters taken into consideration differs amongst studies, which can result in a significant discrepancy of the reported prevalence of DD [27]. Using the latest 2016 guidelines, the diastolic function can be considered normal or abnormal if >50% of the available variables (and not necessarily 3 out of 4) are normal or abnormal, respectively. Therefore, we considered that 3 parameters were enough to classify diastolic function, but patients with less than 3 parameters had to be excluded.

In our study, that reflects real world circumstances, the TR velocity could not be properly assessed in more than half of the patients, which played an important role in the determination of ventricular function. In such cases, other parameters can be used - such as pulmonary vein flow, S' velocity, E/A with Valsalva, atrial longitudinal strain, global left ventricular longitudinal strain and stress echocardiography - but when applied to a large-scale screening program it is unrealistic to use a non-standardized classification. Therefore, only participants with 4 measurable parameters could be labelled with IDF (none of the patients with only 3 available parameters could be classified with IDF since it is impossible to have 50% of abnormal parameters when only 3 are considered). Patients with 3 abnormal parameters out of 3 available parameters were classified with DD and patients with only 2 abnormal parameters out of 3 were also classified with DD, since they had >50% positive criteria (albeit in patients without significant tricuspid regurgitation it is unlikely that the pulmonary pressures are increased). If we had considered that 3 *abnormal* parameters were mandatory to classify DD, then the number of DD would be much lower and IDF much higher. Unfortunately, most authors using 2016 guidelines do not specify how they classified participants with missing parameters. We could also have applied a different algorithm for patients with some ventricular hypertrophy that was proposed for myocardial disease in 2016 guidelines [14], but this was not done in other studies, namely in the main study we used for comparison [16]. The normal echocardiographic parameters in the elderly are different from a younger population and both the 2009 and 2016 classifications do not contemplate the age factor.

Acknowledging all these precautions needed when comparing studies, we compared our results to the EPIPorto cohort [16], that used the same classification for DD and was conducted in the general population of the same city, sharing similar biometric and sociodemographic features and that excluded patients with systolic dysfunction [16]. The study comprised a sample of 1,000 individuals and presented a 1.4% prevalence of DD, which is substantially smaller than in our cohort with RA patients (13%). Similarly, the STANLISLAS cohort [23] included a sample of 1,485 participants and also found a smaller overall prevalence of DD (1.3%) - even when comparing to their cohort with over 60 years of age, the prevalence of DD was only 3.1%. In opposition to other studies, we focused on subclinical ventricular dysfunction in patients RA - older studies [6, 28] have reported a DD prevalence of 30-50% in RA patients versus 25-30% in the general population, but these studies have considered patients who already had cardiovascular events and used different definitions of DD.

The terms DD and HFpEF are often used as interchangeable terms, but in fact their overlap is limited. We found that 13% of the patients had HFpEF features according to the 2016 HF European criteria [12], but interestingly the correspondence with ventricular dysfunction categories was weak. The echocardiographic parameters most frequently used to assess diastolic function or structural changes that are related to HFpEF were associated with traditional cardiovascular risk factors and showed a generally good correlation with prognostic surrogate markers, such as 6MWT, NT-proBNP and even hsTnT.

Given the short follow-up and small number of cardiovascular events, and albeit the association between ventricular function and surrogate prognostic markers such as 6MWT, NT-proBNP and hsTnT, our sample was probably underpowered to detect an association between echocardiographic classification and events. Most patients with ventricular dysfunction presented NT-proBNP and hsTnT levels that were within the normal range, and it is therefore difficult to use these biomarkers in clinical practice to detect subclinical ventricular impairment. The NT-proBNP and hsTnT levels were also changed in IDF and not specifically in DD or SD.

Overall, we did not find significant associations between characteristics of RA disease and echocardiographic parameters. Nonetheless, inflammatory markers, particularly ESR, showed a significant association with most echocardiographic findings (and also with other outcomes), suggesting that the inflammatory pathways may play a role in the development of cardiovascular diseases in patients with RA.

Compared to the general population, the prevalence of DD was significantly higher in our cohort of RA patients, particularly in older patients, who can benefit the most from echocardiographic screening. An early diagnosis would allow for a close follow-up and can improve effectiveness of treatment strategies to decrease the risk of HF. Even though RA and

other autoimmune diseases have been identified as increasing the HF risk, there are no recommendations to date on how to follow-up and diagnose these patients.

Limitations

We acknowledge that our study has some limitations in addition to those inherent to the definition of DD that are previously discussed. Despite being a prospective study, the follow-up duration was short. Due to its observational nature, we cannot infer causality, but only associations. Considering the relatively low prevalence of systolic and diastolic dysfunction, our sample size was probably underpowered to detect other independent predictors of SD and DD.

We did not explore different grades of diastolic dysfunction, given the reduced group size. We used the most common operational definition for systolic impairment, but it is possible that patients with LVEF>50% may also have some systolic dysfunction. We did not evaluate other echocardiographic parameters, such as global longitudinal strain, that can detect early systolic ventricular dysfunction – however, this would make screening much more cumbersome and there are no recommendations on how to manage changes in ventricular mechanics in the absence of systolic or diastolic dysfunction.

When analyzing NT-proBNP levels and particularly for the classification of "HFpEF risk", one should be aware that age-stratified cut-offs may be more appropriate. Moreover, this biomarker is affected by renal function and weight. Our goal was to assess current guidelines, but we believe that future guidelines may take stratified thresholds into consideration.

In future studies, our intention is to continue the follow-up of these patients, particularly those with indeterminate diastolic function, to ascertain what is their evolution and prognosis. We believe that our ongoing search of the optimal echocardiographic identification of diastolic dysfunction must be guided by clinical outcomes.

CONCLUSION

Patients with RA without any known cardiac disease showed a 4% prevalence of subclinical systolic function and 13% of diastolic dysfunction. The prevalence of diastolic dysfunction was higher than a comparable general population. Older age (particularly >57 years) stood out as the most important independent predictor of ventricular dysfunction in patients with RA. A screening strategy using TTE may therefore be useful in older RA patients.

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TABLES

Table 1 – Patients' characteristics according to the presence of any signs of ventricular dysfunction

	Total (n= 319)	Preserved ventricular function (n=232)	Any ventricular dysfunction (n=87)	p value
Age, years	58 (19)	55 (19)	65 (12)	**<0.001**
Sex (male), n(%)	70 (22%)	51 (22%)	19 (27%)	0.978
Diabetes mellitus, n(%)	37 (12%)	28 (12%)	9 (24%)	0.669
Hypertension, n(%)	136 (43%)	87 (38%)	49 (56%)	**0.003**
Systolic blood pressure, mmHg	130 (23)	128 (24)	135 (22)	**<0.001**
Diastolic blood pressure, mmHg	76 ± 10	75 ± 11	78 ± 8	**0.050**
Heart rate, bpm	78 (18)	78 (19)	75 (77)	0.477
Smoking[#], n(%)	111 (35%)	83 (36%)	28 (32%)	0.549
Dyslipidaemia, n(%)	146 (46%)	94 (41%)	52 (60%)	**0.002**
BMI, kg/m^2	26 (6)	26 (6)	27 (6)	0.272
Obesity (BMI ≥30), n(%)	63 (20%)	47 (20%)	16 (18%)	0.709
Chronic kidney failure, n(%)	10 (3)	6 (3%)	4 (5%)	0.365
COPD, n (%)	16 (5%)	14 (6%)	2 (2%)	0.190
Family history of ischemic cardiovascular disease, n(%)	48 (15%)	39 (17%)	9 (10%)	0.154
Cardiovascular medication, n (%)				
ACEi or ARB	111 (35%)	69 (30%)	42 (48%)	**0.002**
Beta blocker	19 (6%)	11 (5%)	8 (9%)	0.141
Thiazide	49 (15%)	30 (13%)	19 (22%)	0.052
Loop diuretic	5 (2%)	5 (2%)	0	-
MRA	1 (0.3%)	0	0	-
CCB	30 (9%)	19 (8%)	11 (13%)	0.228
Statin	114 (36%)	71 (31%)	43 (49%)	**0.002**
RA duration, years	8 (13)	7 (13)	9 (12)	0.325
DAS28-ESR	2.6 (1.5)	2.5 (1.6)	2.8 (1.3)	0.482
RA medication[§], n(%)				

NSAIDS	85 (27%)	58 (25%)	27 (31%)	0.279
Corticosteroids	136 (43%)	91 (39%)	45 (52%)	**0.045**
Methotrexate	197 (62%)	146 (63%)	51 (59%)	0.554
Biologics	61 (19%)	50 (22%)	11 (13%)	0.075
Other drugs	129 (40%)	93 (41%)	34 (39%)	0.762
Biomarkers				
NT-proBNP, pg/mL	75 (94)	68 (84)	103 (141)	**0.008**
hs-troponin T, ng/mL	0.004 (0.005)	0.003 (0.004)	0.005 (0.004)	0.236
ESR, mm	20 (25)	20 (22)	21 (28)	0.260
CRP, mg/dl	3.0 (6.4)	3.0 (6.4)	3.3 (6.9)	0.361
eGFR, mL/min/1.73m^2	93 (27)	95 (28)	90 (26)	**0.010**
Anti-CCP or RF, n(%)	249 (78%)	185 (80%)	64 (74%)	0.236
Distance in 6MWT, m	390 (105)	390 (90)	360 (86)	**0.001**

Legend: ACEi – angiotensin-converting enzyme inhibitor. Anti-CCP + – positive for anti-cyclic citrullinated peptide antibodies. ARB – angiotensin receptor blocker. BMI – body mass index. CCB – calcium channel blocker. COPD – chronic obstructive pulmonary disease. CRP – C-reactive protein. DAS28 – Disease Activity Score 28; eGFR – estimated glomerular filtration rate; ESR – erythrocyte sedimentation rate. MRA – mineralocorticoid receptor antagonist. RA – rheumatoid arthritis. RF + – positive for rheumatoid factor. 6MWT – 6-minute walk test (distance in meters).

Ventricular function was classified as per 2016 guidelines. Patients in any category of ventricular dysfunction (systolic, diastolic or indeterminate) were categorized as "any ventricular dysfunction" and all the others as "preserved ventricular function".

Continuous variables are represented as mean ± standard deviation or as median (75-25 interquartile range), according to their gaussian or non-gaussian distribution, respectively.

[#]Current or previous smoking habits were considered.
[§] More than one medication per patient was allowed.
Values of blood pressure and heart rate were obtained when patients were enrolled.

Table 2 – Patients' characteristics according to categories of ventricular function

	Total (n=319)	Preserved ventricular function (n=232)	Indeterminate diastolic function (n= 34)	Isolated diastolic dysfunction (n= 40)	Systolic (± diastolic) dysfunction (n=13)	p value
Age, years	58 (19)	55 (19)	63 (12) **p<0.001**	67 (12) **p<0.001**	59 (16) p=0.263	**<0.001**
Sex (male), n(%)	70 (22%)	51 (22%)	5 (15%) p=0.328	8 (20%) p=0.764	6 (46%) p=0.056	0.140
Diabetes mellitus, n (%)	37 (12%)	28 (12%)	3 (9%) p=0.597	4 (10%) p=0.724	2 (15%) p=0.713	0.900
Hypertension, n (%)	136 (43%)	87 (38%)	23 (68%) **p=0.001**	20 (50%) p=0.115	6 (46%) p=0.496	**0.005**
Systolic blood pressure, mmHg	130 (23)	128 (24)	136 (21) **p=0.010**	134 (23) **p=0.003**	136 (23) p=0.219	**0.004**
Diastolic blood pressure, mmHg	76 ± 10	75 ± 11	76 ± 7 p=0.595	78 ± 8 p=0.096	81 ± 11 **p=0.045**	0.067
Heart rate, bpm	78 (18)	78 (19)	75 (11) p=0.394	76 (17) p=0.270	79 (30) p=0.156	0.340
Smoking[#], n (%)	111 (35%)	83 (36%)	12 (35%) p=0.991	9 (22%) p=0.115	7 (54%) p=0.188	0.190
Dyslipidaemia, n (%)	146 (46%)	94 (41%)	19 (56%) p=0.099	26 (65%) **p=0.005**	7 (54%) p=0.354	**0.018**
BMI, kg/m^2	26 (6)	26 (6)	26 (5) p=0.622	28 (6) p=0.120	26 (5) p=0.874	0.289
Obesity (BMI ≥30), n(%)	63 (20%)	47 (20%)	4 (12%) p=0.310	10 (25%) p=0.385	2 (15%) p=0.745	0.530
Chronic kidney failure, n(%)	10 (3)	6 (3%)	1 (3%) p=0.923	2 (5%) p=0.431	1 (8%) p=0.319	0.680
COPD, n(%)	16 (5%)	14 (6%)	0	2 (5%) p=0.770	0	0.380
Family history of ischemic cardiovascular disease, n(%)	48 (15%)	39 (17%)	6 (18%) p=0.955	1 (3%) p=0.041	2 (15%) p=0.862	0.120
Cardiovascular medication, n (%)						
ACEi or ARB	111 (35%)	69 (30%)	21 (62%) **p<0.001**	16 (40%) p=0.195	5 (39%) p=0.503	**0.003**
Beta blocker	19 (6%)	11 (5%)	4 (12%) p=0.120	4 (10%) p=0.204	0	0.220
Thiazide	49 (15%)	30 (13%)	11 (32%) **p=0.003**	6 (15%) p=0.590	2 (15%) p=0.713	**0.020**
Loop diuretic	5 (2%)	5 (2%)	0	0	0	0.580
MRA	1 (0.3%)	0	0	0	0	-
CCB	30 (9%)	19 (8%)	5 (15%) p=0.204	6 (15%) p=0.159	0	0.210

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Statin	114 (36%)	71 (31%)	15 (44%) p=0.118	21 (52%) **p=0.008**	7 (54%) p=0.089	**0.015**
RA duration, years	8 (13)	7 (13)	9 (14) p=0.210	11 (12) p=0.591	6 (17) p=0.936	0.361
DAS28-ESR	2.6 (1.5)	2.5 (1.6)	2.6 (1.1) p=0.829	3.0 (1.2) p=0.482	2.9 (1.4) p=0.805	0.590
RA medication[§], n(%)						
NSAIDS	85 (27%)	58 (25%)	11 (32%) p=0.319	10 (25%) p=0.928	6 (46%) p=0.090	0.290
Corticosteroids	136 (43%)	91 (39%)	14 (41%) p=0.803	23 (58%) **p=0.031**	8 (62%) p=0.117	0.078
Methotrexate	197 (62%)	146 (63%)	17 (50%) p=0.141	28 (70%) p=0.414	6 (50%) p=0.359	0.260
Biologics	61 (19%)	50 (22%)	5 (15%) p=0.411	5 (12%) p=0.228	1 (8%) p=0.276	0.380
Other drugs	129 (40%)	93 (41%)	16 (47%) p=0.516	13 (32%) p=0.305	5 (38%) p=0.848	0.630
Biomarkers						
NT-proBNP, pg/mL	75 (94)	68 (84)	120 (139) **p=0.011**	90 (140) p=0.280	107 (550) **p=0.007**	**0.002**
hs-troponin T, ng/mL	0.004 (0.005)	0.003 (0.004)	0.005 (0.004) p=0.702	0.005 (0.006) p=0.355	0.006 (0.002) p=0.183	**<0.001**
ESR, mm	20 (25)	20 (22)	21 (21) p=0.777	27 (28) p=0.100	16 (41) p=0.355	0.377
CRP, mg/dl	3.0 (6.4)	3.0 (6.4)	3.2 (4.2) p=0.550	2.8 (6.8) p=0.505	11.0 (34.1) **p=0.029**	0.098
eGFR, mL/min/1.73m^2	93 (27)	95 (28)	90 (30) p=0.059	87 (22) p=0.051	95 (76) p=0.304	0.078
Anti-CCP or RF, n(%)	249 (78%)	185 (80%)	26 (77%) p=0.671	28 (70%) p=0.177	10 (77%) p=0.813	0.590
Distance in 6MWT, m	390 (105)	390 (90)	360 (75) **p=0.006**	360 (90) **p=0.007**	360 (98) p=0.153	**<0.001**

Legend: ACEi – angiotensin-converting enzyme inhibitor. Anti-CCP + – positive for anti-cyclic citrullinated peptide antibodies. ARB – angiotensin receptor blocker. BMI – body mass index. CCB – calcium channel blocker. COPD – chronic obstructive pulmonary disease. CRP – C-reactive protein. DAS28 – Disease Activity Score 28; eGFR – estimated glomerular filtration rate; ESR – erythrocyte sedimentation rate. MRA – mineralocorticoid receptor antagonist. RA – rheumatoid arthritis. RF + – positive for rheumatoid factor. 6MWT – 6-minute walk test (distance in meters).

Ventricular function was classified as per 2016 guidelines.

Continuous variables are represented as mean ± standard deviation or as median (75-25 interquartile range), according to their gaussian or non-gaussian distribution, respectively.

The p value in the right-handside column represents the comparison between the 4 categories of ventricular function; the p value presented under each category refers to the comparison with the reference category (preserved ventricular function).

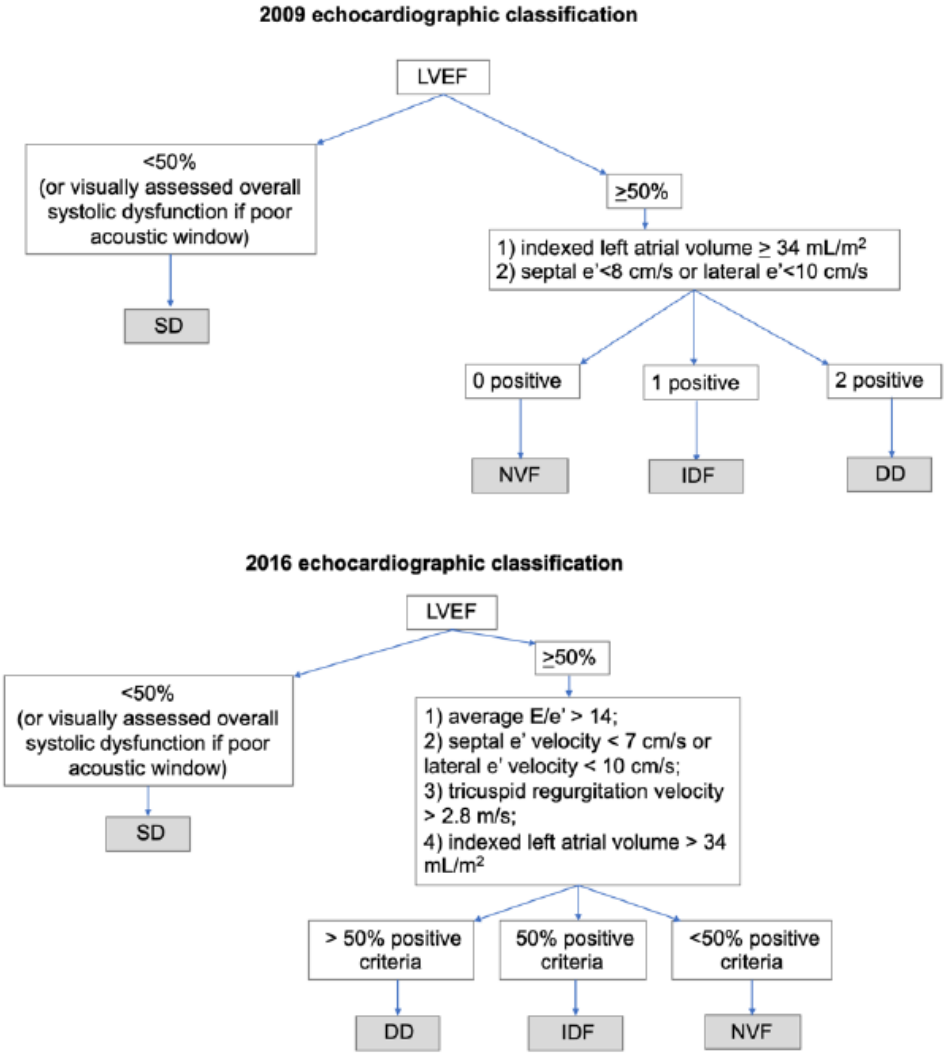
[#]Current or previous smoking habits were considered.

[§] More than one medication per patient was allowed.

Values of blood pressure and heart rate were obtained when patients were enrolled.

FIGURES

Figure 1 – Simplified 2009 and 2016 echocardiographic guidelines for ventricular function classification.



Legend: DD – diastolic dysfunction; IDF – indeterminate diastolic function; NVF – normal ventricular function; SD – systolic dysfunction.

Using 2016 guidelines, if a patient had only 3 parameters available and 2 or 3 were abnormal, he/she was categorized as having DD, if 2 were normal, as having normal diastolic function. If only 2 or less variables were available, diastolic classification was recorded as missing.

Using 2009 guidelines, it was mandatory that a patient had at least one e' value and indexed LA volume; if one of those variables was missing, diastolic classification was recorded as missing.

Figure 2 – Study flowchart, summarizing participants' selection.

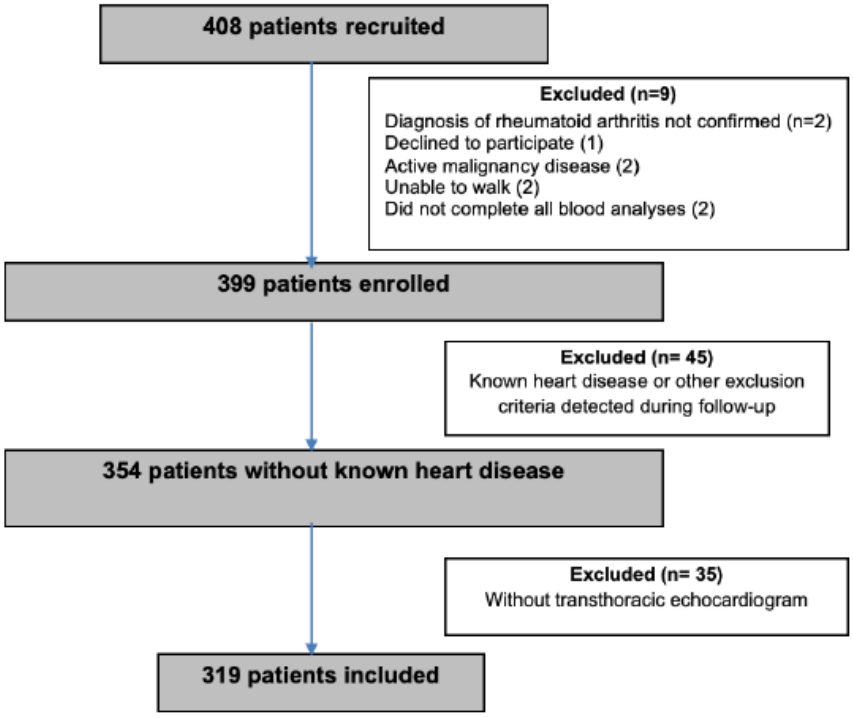
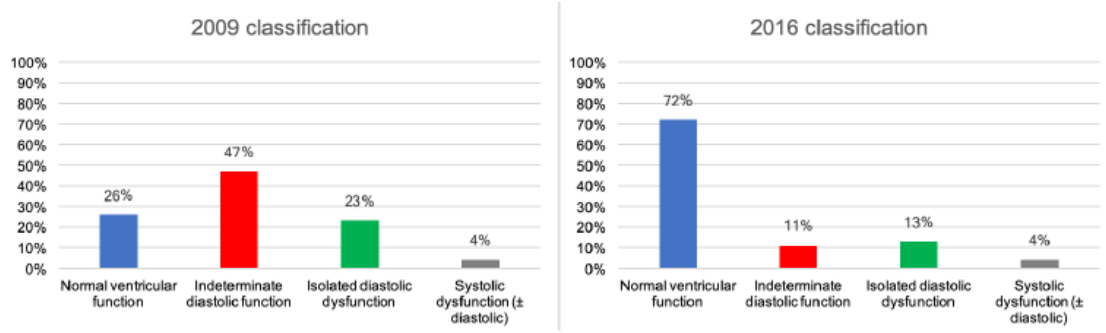


Figure 3 – Patients' distribution according to echocardiographic ventricular function categories, according to 2016 and 2009 criteria of diastolic function.



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SUPPLEMENTARY TABLES

Supplementary Table 1 – Distribution of the sample's ventricular function according to 2016 classification versus 2009 criteria

			2009 criteria			
		Total	Normal ventricular function	Indeterminate diastolic function	Diastolic dysfunction (isolated)	Systolic dysfunction (± diastolic)
2016 criteria	Normal ventricular function	224*	81	136	7	0
	Indeterminate diastolic function	34	0	8	26	0
	Diastolic dysfunction (isolated)	40	0	2	38	0
	Systolic dysfunction (± diastolic)	13	0	0	0	13
		Total	81	146	71	13

A total of 311 patients were considered in this comparison (patients that lacked the criteria needed for the 2009 classification were not considered).

Supplementary Table 2 – Echocardiographic parameters (total sample)

	Mean ± SD or median (IQR)
Interventricular septum diameter, mm	8.0 (3.0)
LV posterior wall diameter, mm	8.0 (2.0)
LV telediastolic diameter, mm	43.0 (6.0)
LV telesystolic diameter, mm	28.0 (6.0)
RV dilatation, n(%)	4 (1%)
RV free wall diameter, mm	2.6 (1.3)
Mitral E wave velocity, cm/s	71.2 (27.0)
E/A	0.90 (0.46)
E/A with Valsalva	0.80 (0.35)
E wave deceleration time, ms	145 ± 34
Mitral A wave duration, ms	120.0 (20)
Pulmonary Ar duration, ms	110.0 (18)
Ar-A duration, ms	-10.0 (29)
IVRT, ms	90.0 (20.0)
IVRT/$T_{E-e'}$	5.0 (5.2)
LV mass (2D, BSA-indexed), g/m^2	64.3 ± 16.3
LVEF (biplane), %	61.1 ± 6.0
LVEDV (biplane, BSA-indexed), ml/m^2	44.4 (13.5)
LVESV (biplane, BSA-indexed), ml/m^2	17.1 (6.9)
Stroke volume (BSA-indexed), ml/m^2	37.0 (9.1)
Cardiac output, L/min	4.4 (1.3)
RAt area, cm^2	13.5 (3.0)
LA area, cm^2	18.0 (5.8)
LA volume (biplane, BSA-indexed), ml/m^2	32.4 ± 8.6
Septal E', cm/s	7.2 (4.4)
Septal S', cm/s	8.4 ± 1.9
Lateral E', cm/s	11.0 (6.3)
Lateral S', cm/s	9.9 ± 2.8
E/E' (average)	8.3 (3.4)
RV S' (tricuspid), cm/s	13.5 (3.7)
TAPSE, mm	24.1 ± 3.5
TR peak velocity, m/s	2.2 (0.4)
PASP, mmHg	23.0 (6.8)
MR, n(%)	5 (2%)
MS, n(%)	0
AoR, n(%)	2 (1%)
AoS, n(%)	4 (1%)

IVC diameter, mm	15.5 ± 2.5
IVC decreased respiratory variability, n(%)	3 (1%)
Pericardial effusion, n(%)	4 (1%)

Legend: AoR – aortic regurgitation (more than mild). AoS – aortic stenosis (more than mild). Ar-A – difference between Ar and A wave duration. IVC – inferior vena cava. IVRT – isovolumic relaxation time. LV – left ventricle. LA – left atrial. LVEDV – left ventricular end-diastolic volume. LVEF – left ventricular ejection fraction (2D Simpson's biplane method). LVESV – left ventricle end-diastolic volume. MR – mitral regurgitation (more than mild). MS – mitral stenosis (more than mild). PASP – pulmonary arterial systolic pressure. RAt – right atrial. RV – right ventricle. TAPSE – tricuspid annular plane systolic excursion. TR – tricuspid regurgitation.

Continuous variables are represented as mean ± standard deviation or as median (25; 75 interquartile range), according to their gaussian or non-gaussian distribution, respectively.

Supplementary Table 3 – Association between specific echocardiographic parameters and clinical or laboratory data

Echocardiographic parameter	Covariates with significant association
Interventricular septum diameter	age, gender, diabetes, hypertension, dyslipidaemia, obesity, CKD, eGFR, RA duration, ESR, NT-proBNP, hsTnT, 6MWT
LV mass (BSA-indexed)	age, gender, hypertension, COPD, CKD, eGFR, corticosteroids, ESR, NT-proBNP, hsTnT, 6MWT; borderline with CRP and smoking history (p=0.055)
Average E/e' ratio	age, gender, diabetes, hypertension, dyslipidaemia, smoking, eGFR, corticosteroids, ESR, hsTnT, NT-proBNP> 125 pg/mL (but no linear association with NT-proBNP), 6MWT
Lateral e'	age, diabetes, hypertension, dyslipidaemia, smoking history, obesity, BMI, eGFR, corticosteroids, ESR, NT-proBNP, hsTnT, 6MWT
LA volume (BSA-indexed)	age, hypertension, RF or anti-CCP positive, ESR, NT-proBNP, 6MWT
Tricuspid regurgitation velocity	age, hypertension, RA duration, corticosteroids, 6MWT, eGFR, NT-proBNP, hsTnT; borderline with diabetes (p=0.058) and DAS28-ESR (p=0.054)
E/A ratio	age, hypertension, dyslipidaemia, BMI, eGFR, anti-CCP or RF positivity, corticosteroids, ESR, hsTnT, 6MWT, NT-proBNP>125 pg/mL (no linear association with NT-proBNP)
LVEF	gender, diabetes, smoking history, CRP, NT-proBNP

Legend: Anti-CCP – positive for anti-cyclic citrullinated peptide antibodies. BMI – body mass index. BSA – body surface area. COPD – chronic obstructive pulmonary disease. CRP – C-reactive protein. DAS28 – Disease Activity Score 28; ESR – erythrocyte sedimentation rate. hsTnT – high sensitivity troponin T. LA – left auricular. LV- left ventricular. LVEF – left ventricular ejection fraction. RA – rheumatoid arthritis. RF – positive for rheumatoid factor. 6MWT – distance in 6-minute walk test.

D1)

Rodrigues P, Simões S, Reis H. ***Tratamento da miocardiopatia amiloidótica por transtirretina (Treatment of transthyretin amyloid cardiomyopathy).*** --- submitted, under review

Tratamento da miocardiopatia amiloidótica por transtirretina

Artigo de revisão

Treatment of transthyretin amyloid cardiomyopathy

Review article

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Os autores não têm conflito de interesses a declarar.

Tratamento da miocardiopatia amiloidótica por transtirretina

RESUMO

Introdução: A amiloidose cardíaca causada pela deposição de transtirretina é uma patologia provavelmente subdiagnosticada, que se manifesta como perturbações na condução elétrica cardíaca ou miocardiopatia infiltrativa. Pode ser causada por mutações no gene da transtirretina ou adquirida – por desestabilização dos tetrâmeros de transtirretina selvagem, associada ao envelhecimento.

Até 2018, não existia ainda nenhuma terapia específica comprovadamente eficaz para tratar a amiloidose cardíaca por transtirretina. Contudo, têm havido progressos nesta área e antevê-se uma mudança no tratamento desta doença.

Materiais e métodos: Revisão sobre o tratamento da amiloidose cardíaca por transtirretina (mutada ou ***wild-type***). Foram analisados ensaios clínicos e estudos observacionais publicados como texto completo, da base de dados Pubmed.

Resultados: Foram considerados 11 artigos referentes ao tratamento com tafamidis, 5 ao diflunisal, 5 à doxiciclina, 3 ao patisiran, 3 ao inotersen, 3 ao extrato de chá verde, 5 artigos sobre outros fármacos e 14 sobre transplante hepático e/ou cardíaco.

Discussão: O tafamidis é o único fármaco com ensaio clinico randomizado de fase 3 que mostrou benefício significativo em termos de mortalidade e hospitalizações, particularmente em doentes ***wild-type*** em estadios iniciais de insuficiência cardíaca. O patisiran mostrou efeitos ecocardiográficos promissores, mas um estudo maior focado nos eventos cardíacos é mandatório. O mesmo se aguarda para o inotersen. O diflunisal, doxiciclina e extrato de chá verde também necessitam ensaios clínicos randomizados.

Conclusões: Atualmente os doentes com amiloidose cardíaca por transtirretina podem ser candidatos a tafamidis, embora a custo-efetividade seja problemática. Outros fármacos também se revelam promissores, mas precisam de estudos mais aprofundados.

Palavras-chave: Transtirretina; amiloidose; miocardiopatia.

Treatment of transthyretin amyloid cardiomyopathy

ABSTRACT

Introduction

Cardiac amyloidosis caused by transthyretin deposition is an underdiagnosed disease, that presents as cardiac electrical disturbances or infiltrative cardiomyopathy. It can be due to mutations in the transthyretin gene or acquired – after destabilization of the wild-type transthyretin tetramers, related with ageing.

Until 2018, there was no specific therapy proven effective to treat transthyretin-related cardiac amyloidosis. However, progress has been made in this field and a paradigm shift in the treatment of this condition is coming.

Materials & Methods

Review on the treatment of cardiac amyloidosis caused by transthyretin (mutated or wild-type). Clinical trials and observational studies published as complete text were analysed from the Pubmed database.

Results

We found 11 articles focused on tafamidis treatment, 5 on diflunisal, 5 on doxycycline, 3 on patisiran, 3 on inotersen, 3 on green tea extract, 5 about other drugs and 14 about liver and/or heart transplantation.

Discussion

Tafamidis is the only drug with a phase 3 randomized clinical trial that showed significant improvement in mortality and hospitalizations, particularly in wild-type and in early stages of heart failure. Patisiran showed promising echocardiographic results, however a larger study focused on cardiac events is crucial. Such a study is also waited for inotersen. Diflunisal, doxycycline and green tea extract also need randomized clinical trials.

Conclusions

Currently, patients with transthyretin-related cardiac amyloidosis can be candidates to tafamidis, even though the cost-effectiveness is problematic. Other therapies are also promising, but need further studies.

Key-words: Transthyretin; amyloidosis; cardiomyopathy.

ABREVIATURAS

AINE: anti-inflamatório não esteróide

AL: amiloidose por cadeias leves livres de imunoglobulinas

ARN: ácido ribonucleico

ATTR: amiloidose por transtirretina

ATTRm: amiloidose por transtirretina mutada

ATTRwt: amiloidose por transtirretina ***wild-type*** (selvagem)

ATUDC: ácido tauro-ursodesoxicólico

BAV: bloqueio auriculo-ventricular

BNP: peptídeo natriurético tipo B

proBNP: pró-hormona do peptídeo natriurético tipo B

EMA: European Medicines Agency

FA: fibrilhação auricular

FDA: Food and Drug Administration

FEVE: fração de ejeção do ventrículo esquerdo

HVE: hipertrofia ventricular esquerda (padrão de aumento da espessura das paredes do ventrículo esquerdo)

IC: insuficiência cardíaca

MA-ATTR: miocardiopatia amiloidótica por transtirretina

NIS-LL: Neuropathy Impairment Score – Lower Limbs (score que quantifica as funções motora, sensorial e reflexa dos membros inferiores)

NIS+7: Neuropathy Impairment Score (NIS) mais 7 estudos neurológicos (condução de 5 nervos dos membros inferiores, limiar de deteção vibratória e variabilidade da frequência cardíaca durante respiração profunda)

NT-proBNP: porção N-terminal da pró-hormona do peptídeo natriurético tipo B

NYHA: New York Heart Association (classificação funcional de insuficiência cardíaca)

OAS: oligonucleotídeo antisense

PAF: polineuropatia amiloidótica familiar

RMC: ressonância magnética cardíaca

SIV: septo interventricular

TH: transplante hepático

TTR: transtirretina

VE: ventrículo esquerdo

INTRODUÇÃO

A amiloidose cardíaca caracteriza-se pela deposição extracelular de fibrilas de amilóide no miocárdio, causando miocardiopatia infiltrativa, e no tecido de condução elétrica, provocando perturbações do automatismo e condução. Estas fibrilas são geralmente formadas a partir de alterações conformacionais das cadeias leves livres de imunoglobulinas (amiloidose AL) ou de transtirretina (amiloidose TTR).

A amiloidose AL pode surgir quando há uma produção monoclonal de cadeias livres de imunoglobulinas [1] e tem um tratamento específico, com base em quimioterapia[2].

A amiloidose por transtirretina (ATTR) pode ser hereditária ou ocorrer na presença da proteína selvagem ou *wild-type* (ATTRwt), anteriormente designada amiloidose *senil*[3].

A transtirretina é produzida maioritariamente no fígado, circula como um tetrâmero e atua como transportadora do retinol e da tiroxina. A sua desagregação em monómeros, por instabilidade relacionada com o envelhecimento ou mutações, pode levar à formação de fibrilas de amilóide.

Estima-se que a ATTRm tenha uma prevalência global de aproximadamente 10.000 pessoas, mas em Portugal pode ultrapassar os 200 por milhão de habitantes[4]. Apresenta hereditariedade autossómica dominante e penetrância variável, que no nosso país se estima ser de 91% aos 70 anos[5].

Mais de 120 mutações diferentes foram identificadas no gene da transtirretina (TTR), localizado no cromossoma 18. A mutação genética mais comum, particularmente prevalente em Portugal, Suécia e Japão, é a substituição de valina por metionina na posição 30 da cadeia de aminoácidos (V30M ou Val30Met) – p.TTRV50M na nomenclatura mais recente[6, 7] – e caracteriza-se por atingimento predominantemente neurológico (daí ser habitualmente designada como PAF - polineuropatia amiloidótica familiar). Geralmente, as mutações não-Val30Met, de que se destaca a Val122Ile, estão associadas a maior envolvimento cardíaco, idade de apresentação mais tardia e menor taxa de sobrevida. Existe uma correlação genótipo-fenótipo, com mutações relacionadas com fenótipos predominantemente cardíacos e outras com atingimento predominantemente neurológico, embora com todo um espectro intermédio e variabilidade individual. Curiosamente, a mutação Val30Met quando dá sintomas precoces tem afetação predominantemente neurológica, mas quando se apresenta tardiamente tem atingimento cardíaco mais pronunciado.

Com o aumento da esperança de vida e desenvolvimento de técnicas de diagnóstico tem-se apurado que a amiloidose *wild-type* (ou selvagem), apesar de raramente diagnosticada, pode ser relativamente frequente, sobretudo em homens idosos: em

estudos de autópsia, estima-se que afete 25% da população com mais de 85 anos[8], ou 5% se não considerarmos esse limiar de idade[9]. Pode corresponder a 13% dos casos de insuficiência cardíaca com fração de ejeção preservada[10] e ocorrer em 6%[11] a 16%[12, 13] dos doentes com estenose aórtica.

Relativamente à PAF, o primeiro tratamento eficaz foi o transplante hepático (TH)[14-18]. Porém, o envolvimento cardíaco progride frequentemente após TH [9, 19-28].

O tafamidis foi o primeiro fármaco oral aprovado pela European Medicines Agency (EMA) para a PAF em estadio 1, tendo sido comercializado no nosso país em 2012 e não tendo sido aprovado pela Food and Drug Administration (FDA) para esta indicação.

Tratamentos para o atingimento cardíaco da amiloidose por transtirretina têm sido apenas baseados em terapêuticas de suporte. Acresce que os medicamentos habitualmente utilizados na insuficiência cardíaca com fração de ejeção reduzida (como beta-bloqueadores ou inibidores do sistema renina-angiotensina) se revelam muitas vezes ineficazes e até prejudiciais nestes doentes.

Só em 2018 foi publicado o primeiro ensaio clínico randomizado sobre a utilização de tafamidis na miocardiopatia amiloidótica por transtirretina (MA-ATTR).

Recentemente, tem crescido a investigação sobre o tratamento da amiloidose cardíaca por transtirretina (hereditária ou *wild-type*), tornando pertinente esta revisão.

METODOLOGIA

Pesquisa bibliográfica de artigos originais completos publicados, utilizando a Pubmed/Medline com as seguintes palavras-chave e critérios: "cardiac" AND "amyloidosis" AND "transthyretin" AND ("treatment" OR "transplant"), abrangendo artigos publicados até abril de 2019. Foi adicionada ainda a pesquisa com o nome específico de cada fármaco. Foram considerados ensaios clínicos, ensaios clínicos pragmáticos, estudos observacionais e ensaios randomizados controlados e só foram incluídos artigos que se referissem especificamente ao tratamento das manifestações cardíacas, que incluíssem nos *outcomes* uma avaliação ecocardiográfica, de biomarcadores ou de eventos cardiovasculares.

RESULTADOS

Da pesquisa bibliográfica inicial (*Metodologia*) resultaram 15 artigos, sendo incluídos 13; foram excluídos 2 artigos que não se referiam ao tratamento da miocardiopatia amiloidótica. Dos 13 artigos selecionados, 5 referiam-se ao tratamento com tafamidis, 3 ao extrato de chá verde, 1 ao diflunisal, 1 à doxiciclina e ácido tauro-ursodexoxicólico, 1 ao inotersen, 1 ao transplante hepático e 1 ao transplante cardíaco.

Da pesquisa bibliográfica com o nome específico de cada tratamento, resultaram adicionalmente: 151 artigos sobre o tratamento com tafamidis, tendo sido selecionados 6 que cumpriam os critérios de inclusão; 17 sobre inotersen, com seleção de 2; 52 sobre patisiran, sendo selecionados 3; 778 sobre diflunisal, com seleção de 4; 18 sobre doxiciclina , sendo 4 selecionados; e foram considerados 5 artigos sobre outros fármacos. Em relação ao transplante hepático ou cardíaco combinado resultaram 12 artigos relevantes.

Os diferentes tratamentos atuam em locais diferentes da amiloidogénese (Figura 1) e os achados dos principais estudos encontram-se sintetizados na Tabela 1.

Com exceção do transplante hepático, que se destina apenas a casos de TTR mutada, todos os outros tratamentos poderão teoricamente ser aplicáveis a casos com mutação ou *wild-type*.

Tafamidis

Tafamidis é um fármaco oral que atua como estabilizador dos tetrâmeros de transtirretina (Figura 1).

Merlini et al[29], num ensaio de fase 2, concluíram que tafamidis 20mg estabilizava a transtirretina em doentes com PAF e mutações não-Val30Met e não-Val122Ile [29]. Damy et al[30] verificaram que na amostra do estudo anterior ocorreu agravamento ecocardiográfico em 63% e eletrocardiográfico em 43%, mas as médias dos parâmetros não se alteraram significativamente.

Outro pequeno estudo de segurança com sobredosagem única de tafamidis não demonstrou efeitos no intervalo QTc[31].

Um ensaio clínico aberto de fase 2 avaliou a segurança e a eficácia de tafamidis relativamente à progressão da miocardiopatia por ATTR (*wild-type* ou com mutação Val122Ile)[32] e não detetou alterações clinicamente significativas nos parâmetros ecocardiográficos nem efeitos adversos graves, embora 48% tenham tido progressão clínica cardíaca.

Um estudo observacional[33] mostrou que 15% dos doentes apresentaram progressão da

doença cardíaca e em 35% registou-se início de miocardiopatia.

Recentemente, o ensaio clínico randomizado ATTR-ACT, de fase 3, multicêntrico e duplamente cego[34] foi desenvolvido para determinar a eficácia e a segurança de tafamidis em doentes com MA-ATTR hereditária ou *wild-type* (excluindo doentes em classe IV NYHA). Englobou 441 participantes, com seguimento de 30 meses. Menos de ¼ tinham TTR mutada e a maioria eram mutações não-Val30Met; idade mediana 75 anos; 90% homens. A mortalidade foi mais baixa nos doentes a receber tafamidis em relação aos doentes a receber placebo (29,5% vs 42,9%), com efeito a partir dos 18 meses, e a taxa de hospitalizações por eventos cardiovasculares também foi menor. Adicionalmente, tafamidis associou-se a uma redução significativa do declínio da capacidade funcional e da qualidade de vida. Não houve diferenças significativas entre as duas doses do fármaco, embora com tendência para maior eficácia na dose mais elevada (80 vs 20 mg). No subgrupo em classe III NYHA não se verificou um efeito benéfico do tafamidis, sendo que relativamente às hospitalizações houve um acréscimo em relação ao grupo placebo. No subgrupo de doentes com TTR mutada também não houve vantagem estatisticamente significativa do tafamidis, mas neste parâmetro (ATTRm versus ATTRwt) já não se verificou interação significativa do tratamento, pelo que a ausência de significado estatístico pode dever-se ao tamanho reduzido do subgrupo ATTRm. Também observaram um aumento menor dos níveis de NT-proBNP nos pacientes a receber tafamidis. Os parâmetros ecocardiográficos não foram significativamente diferentes.

Inotersen

O inotersen é um oligonucleotídeo antisense (OAS), causando a destruição do ARN mensageiro da TTR, e impedindo a tradução de TTR (Figura 1), mutada ou selvagem, sendo administrado por via subcutânea.

O ensaio clínico NEURO-TTR[35] (112 doentes sob inotersen e 60 com placebo, com seguimento de 15 meses) mostrou eficácia do inotersen no tratamento da polineuropatia relacionada com ATTRm em estadio 1 e 2. Na subanálise de alterações cardíacas não se verificaram diferenças significativas entre o fármaco e placebo relativamente a parâmetros ecocardiográficos, embora o estudo não tivesse sido desenhado com esse propósito e tenham sido excluídos doentes em classe III ou IV NYHA. Houve uma tendência para mais mortes e efeitos adversos cardíacos nos doentes sob tratamento, mas não significativa.

Anteriormente, um ensaio clínico aberto [36] tinha mostrado que a maioria dos pacientes obteve uma rápida redução dos níveis de TTR, juntamente com uma ausência da progressão da doença cardíaca. A função sistólica global e a espessura do septo interventricular melhoraram na maioria dos doentes, bem como a capacidade funcional. Num estudo posterior[37] com seguimento de 2 anos, voltou a haver sinal para estabilização ou discreta melhoria cardíaca estrutural nos doentes tratados com inotersen, embora sem comparação com placebo em ambos os estudos.

Patisiran

O patisiran é um ácido ribonucleico (ARN) de interferência que leva à degradação do ARN mensageiro da TTR e, consequentemente, reduz a sua produção[38].
O estudo APOLLO mostrou eficácia do fármaco, administrado por via endovenosa a cada 3 semanas, no tratamento de polineuropatia hereditária por TTR em estadio 1 ou 2[39]. Contou com 148 participantes no grupo tratamento e 77 no grupo placebo, com seguimento de 18 meses. O subestudo[40] relativo a alterações cardíacas teve bons resultados no que respeita à espessura das paredes ventriculares, *strain* longitudinal global e proBNP, com idêntica taxa de eventos cardiovasculares em relação ao placebo (Tabela 1). Foram excluídos doentes em classe III ou IV NYHA. Embora não tenha havido acréscimo de mortalidade global, as mortes no grupo tratamento foram por insuficiência cardíaca e mais doentes precisaram de implantar pacemaker (embora de forma não significativa), o que levou as autoridades a exigir vigilância cardiovascular. Os efeitos adversos mais comuns foram de reação alérgica.

Diflunisal

O diflunisal é um anti-inflamatório não esteróide (AINE) que estabiliza a transtirretina (Figura 1). O risco de desencadear descompensação de IC e hemorragias obriga a monitorização apertada, sendo desaconselhado na insuficiência renal.
Um ensaio clínico randomizado com 130 doentes com PAF mostrou eficácia do diflunisal na progressão neurológica[41], com bom perfil de segurança.
Castaño et al[42] desenvolveram um estudo aberto, de braço único com tratamento de diflunisal, durante 1 ano, em 13 pacientes com MA-ATTR hereditária ou *wild-type*. Não houve uma mudança significativa da estrutura e função cardíaca e foi bem tolerado.

Sekijima et al[43] verificaram que, em doentes com ATTRm, as taxas de deterioração dos parâmetros ecocardiográficos foram menores após o período de tratamento, mas não estatisticamente significativas, tendo o fármaco sido bem tolerado.
Ikram et al[44] avaliaram a segurança do uso crónico de diflunisal numa população com MA-ATTR e concluíram que pode ser usado em segurança, com monotorização clínica, renal e hematológica apropriada.

Extrato de chá verde

A epigalocatequina-3-galato (EGCG), a catequina mais abundante no chá verde, inibe a formação de fibrilas de amilóide, o que poderá interromper a progressão da amiloidose cardíaca por transtirretina.
Neste sentido, um estudo observacional sobre os efeitos do consumo de chá verde em pacientes com amiloidose cardíaca por transtirretina[45] detetou uma diminuição ou estabilização da espessura das paredes ventriculares (no septo interventricular ou parede posterior, respetivamente).
Outro grupo[46, 47] também verificou estabilização ecocardiográfica em pacientes com MA-ATTRwt após o consumo de extrato de chá verde.

Doxiciclina + ácido tauro-ursodesoxicólico (ATUDC)

Cardoso et al[48], num modelo de rato com PAF, relataram que o tratamento combinado de doxiciclina com ATUDC tem um efeito sinérgico na remoção dos depósitos de amilóide de TTR. Para além disso, parece ser também um tratamento benéfico noutros tipos de amiloidose, nomeadamente AL[49].
Obici et al[50] realizaram um ensaio clínico de fase 2 com 20 participantes (com ATTRm, ou ATTRwt), em que a neuropatia e miocardiopatia se mantiveram estáveis ao longo dos 12 meses de tratamento, com boa tolerância.
Recentemente, um estudo observacional[51] com 53 pacientes com MA-ATTR tratados com doxiciclina e ATUDC mostrou que, durante o acompanhamento, não foram observadas mudanças significativas nos biomarcadores cardíacos, nos parâmetros ecocardiográficos e na classe de NYHA, sugerindo estabilização da doença.

Outros fármacos

A imunoterapia tem sido testada com resultados encorajadores, nomeadamente um anticorpo dirigido ao componente P do amilóide sérico, com potencial para desagregar

e remover as fibrilas de amilóide nos tecidos, atuando a jusante das restantes terapêuticas e para diferentes tipos de amiloidose[52]. Outros anticorpos dirigidos especificamente para amiloidose AL[53, 54] têm sido testados e estão em curso estudos dirigidos especificamente para a transtirretina. No entanto, aguardam-se ainda ensaios clínicos randomizados com robustez necessária para levar estas terapêuticas para a prática clínica.

O AG10 é um fármaco oral estabilizador da transtirretina que mostrou repor níveis de transtirretina sérica "estabilizada" em doentes com ATTRm e ATTRwt [55], aguardando-se um ensaio de fase 3.

A curcuma mostrou também resultados interessantes em modelos animais [56].

Transplante cardíaco ± hepático

O transplante hepático (TH), iniciado em 1990, foi o primeiro tratamento comprovadamente eficaz para o tratamento da ATTRm[8]. Como mais de 95% da transtirretina é produzida pelo fígado, a sua substituição possibilita a remoção quase total de transtirretina mutada em circulação. O TH mostrou travar a progressão neurológica em 76% dos doentes com PAF com mutação Val30Met em estadio 1 e aumentou significativamente a esperança de vida destes doentes, para mais de 50% 20 anos depois do TH[57]. No entanto, não está tão bem validado para as outras mutações e não é uma solução para ATTRwt.

Em doentes com PAF submetidos a TH verificou-se progressão do atingimento cardíaco apesar de melhoria em termos neurológicos[58, 17, 23].

Alguns autores verificaram que em doentes com PAF, após TH, havia deposição cardíaca com maior teor em proteína *wild-type*[59, 60] explicando possivelmente a maior taxa de mortalidade após TH em homens com doença de início tardio[61], tal como acontece na ATTRwt. Isto deve-se provavelmente a mecanismos de *seeding*, isto é, uma vez presentes fibrilas de amilóide nos tecidos, elas promovem a acumulação de mais amilóide, nomeadamente *wild-type*[62].

Assim, sabemos que o TH não evita a progressão da doença cardíaca, pelo menos em absoluto, mas não foi efetuada uma comparação especificamente sobre o atingimento cardíaco dos doentes transplantados *versus* não-tratamento ou *versus* tratamentos farmacológicos, isto é, não sabemos se previne parcialmente o atingimento cardíaco.

No caso de já existir miocardiopatia amiloidótica estabelecida, não faz sentido equacionar o TH isoladamente, mas poderá ponderar-se a substituição de órgão – tal como no caso da disfunção renal por amiloidose.

O transplante cardíaco nestes doentes foi relatado pela primeira vez em 1988[63], mas permanece pontual devido à menor sobrevivência em comparação com pacientes submetidos a este transplante para outras formas de patologia cardíaca. Além da escassez de órgãos, da exigência de imunossupressão vitalícia, dos riscos associados à cirurgia e custos elevados, a natureza sistémica da amiloidose, com potencial deposição de amilóide no enxerto, a idade avançada no momento do diagnóstico e comorbilidades, como insuficiência renal, neuropatia e enteropatia, fazem com que a maioria destes doentes não sejam candidatos a transplante cardíaco[64]. Contudo, indivíduos mais jovens com miocardiopatia amiloidótica por TTR *wild-type*, sem envolvimento extracardíaco da doença, poderão ser candidatos a transplante cardíaco isolado[65-67].

O transplante hepático e cardíaco combinado mostrou ser eficaz em doentes com algumas mutações[68], mas será raramente uma opção pelas razões já mencionadas.

DISCUSSÃO

Todos os estudos sobre MA-ATTR têm sido unânimes na importância de iniciar tratamento precocemente, pelo que o diagnóstico atempado e sensibilização de clínicos para reconhecerem esta patologia é essencial, particularmente agora que se perspetivam tratamentos específicos.

É de referir que a maioria dos estudos se tem focado na miocardiopatia e não tanto nas perturbações da condução e de ritmo, possivelmente porque são menos específicas e na sua maioria conseguem ser controladas com a implantação de pacemaker.

O TH e o tratamento com tafamidis alteraram muito o curso natural da PAF e aumentaram significativamente a esperança de vida[69].

O papel do TH no futuro é incerto, mas não se aplica no tratamento de amiloidose cardíaca estabelecida. Poderá ser utilizado sobretudo como terapêutica de resgate em doentes com progressão neurológica sob tafamidis e sem envolvimento cardíaco relevante, com mutação Val30Met de apresentação precoce, devendo o doente ter um papel na tomada de decisão. Ainda não sabemos exatamente que fatores tornam alguns doentes suscetíveis de desenvolverem posteriormente miocardiopatia e se isso se relaciona com o *timing* do transplante. Apesar da doença poder progredir a longo prazo, não sabemos se o mesmo não acontecerá com outras terapêuticas que ainda não têm um seguimento tão longo.

O tafamidis ganhou recentemente um grande destaque atendendo ao ensaio ATTR-ACT[34], motivando aprovação americana para MA-ATTR e, mais recentemente, parecer europeu favorável. Apresenta um bom perfil de segurança e facilidade de administração. Apesar de não haver um claro efeito dose-resposta, foi aprovada a dose mais elevada para a miocardiopatia, que parece ser mais eficaz. A ausência de diferenças ecocardiográficas significativas ainda não têm explicação clara. Os dados sugerem que os doentes em classe NYHA III provavelmente já não beneficiam muito com o tratamento.

De referir que o tafamidis – e outros estabilizadores do tetrâmero de transtirretina - pode não ser eficaz para travar a produção de fibrilas que surjam por proteólise da transtirretina. Efetivamente, para além da via amiloidogénica da desagregação do tetrâmero de transtirretina em monómeros instáveis que podem formar fibrilas, há alguma evidência de que esta é uma via alternativa para a ocorrência de amilioidose[70].

O patisiran e o inotersen foram aprovados pela FDA e EMA para tratamento de PAF em estadio 1 e 2. O modo de administração do inotersen é mais fácil do que o do patisiran, mas apresentou efeitos adversos mais preocupantes. Em termos de eficácia, embora não haja nenhum estudo comparativo entre fármacos, o patisiran destacou-se por ter

desencadeado não só desaceleração da progressão, mas também melhoria nos parâmetros neurológicos e a mesma tendência a nível cardíaco. Para além disso, vão decorrer estudos com patisiran administrado por via subcutânea. Aguardam-se ensaios clínicos randomizados destes dois fármacos inibidores da produção de TTR, focando-se na miocardiopatia. Será ainda necessária vigilância a longo prazo para comprovar se a supressão de transtirretina não acarreta efeitos deletérios, nomeadamente cognitivos.
O diflunisal é um "velho" fármaco oral com custo reduzido e que merece consideração no tratamento da amiloidose, mas carece de um ensaio clínico randomizado com enfoque na parte cardíaca e não está disponível em Portugal.
A terapia com doxiciclina e ATUDC, fármacos também conhecidos há muitos anos, orais e de custo reduzido, é igualmente relevante e merece um estudo maior randomizado.
O extrato de chá verde, que pode ser ingerido como chá ou como comprimidos, também carece de um ensaio randomizado nesta população, mas teve resultados preliminares interessantes.
Para além da imunoterapia, aguardam-se resultados do ensaio de fase 3 do AG10, focado no tratamento de amiloidose cardíaca.
Importa referir a relevância de estudar estes fármacos em doentes já submetidos a TH e com aparecimento de miocardiopatia, que têm ficado excluídos de todos estes ensaios. Outras linhas de investigação estão também por explorar, nomeadamente: o tratamento de doentes com insuficiência renal grave; tratamento dirigido para atingimento do sistema nervoso central e oftalmológico; possibilidade de combinação de tratamentos; diferentes *timings* para início de tratamento, bem como resultados a mais longo prazo.
Seria também muito interessante um estudo comparativo entre estas terapêuticas para determinar o seu papel na prevenção de atingimento cardíaco nos doentes com PAF e, por outro lado, no tratamento de doentes já com atingimento cardíaco (excluindo-se neste caso o TH e incluindo-se doentes com ATTRwt).
Importa sublinhar a importância de estudos de custo-eficácia para todos estes fármacos, que têm custos muitíssimo elevados[71].
Assim, ao mesmo tempo que é imperativo identificarmos estes doentes, que têm sido subdiagnosticados e podem beneficiar de uma *medicina de precisão*, também é *preciso* reunir mais evidência sobre estes tratamentos. Ao médico que tem um doente a seu cargo compete-lhe garantir que aquele doente recebe o melhor tratamento possível para a sua situação, contudo, compete aos decisores de políticas de saúde e do medicamento coordenar a sustentabilidade e justiça distributiva de todo o sistema.
Por último, paralelamente a todas estas abordagens terapêuticas – nenhuma delas curativa -, um maior investimento na prevenção, nomeadamente no diagnóstico

genético pré-implantação, atualmente de acesso muito difícil, poderia alterar substancialmente a incidência da forma hereditária da doença, com impacto sustentado para o futuro das famílias afetadas e custo-efetividade para o sistema de saúde. Embora tecnicamente mais complexo do que o diagnóstico pré-natal, não obriga a uma decisão sobre eventual abortamento e tem o potencial de reduzir o número de pessoas afetadas por ATTRm, não devendo passar à margem do investimento público nesta área.

CONCLUSÃO

O tratamento da amiloidose cardíaca por transtirretina tem vindo a evoluir, sobretudo nos últimos anos, assistindo-se a uma mudança radical na história natural destes doentes. Neste momento, o tafamidis é o fármaco já disponível para esta patologia, com um estudo robusto, mas aguardam-se estudos adicionais com patisiran e inotersen. Para além disso, outros compostos como diflunisal, extrato de chá verde, doxiciclina e ATUDC beneficiariam de ensaios maiores randomizados e duplamente cegos.

Seria também muito importante que os *outcomes* dos estudos fossem idênticos, utilizando parâmetros uniformes de avaliação de atingimento cardíaco.

Por último, não podemos ignorar que o custo muito elevado destes fármacos pode efetivamente limitar o acesso e tornar-se uma decisão complexa de política de saúde, sobretudo considerando que o número de ATTRwt tenderá a aumentar.

A criação de protocolos de consenso nacional para o tratamento da MA-ATTR e negociação com a indústria farmacêutica pode ajudar a garantir justiça no acesso ao tratamento e sustentabilidade destes tratamentos.

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Figura 1 – Cascata da amiloidogénese por transtirretina, com os locais de atuação dos diferentes tratamentos.

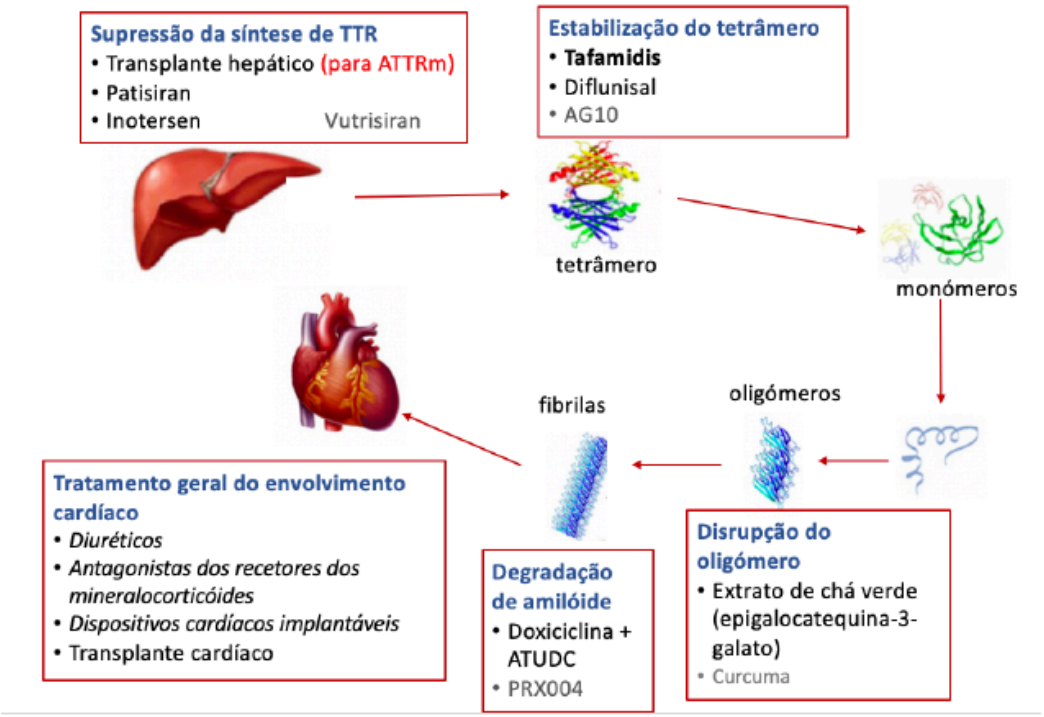

Tabela 1: Resumo dos principais artigos sobre fármacos para o tratamento de amiloidose cardíaca por transtirretina.

Tabela I

Estudos com Tafamidis na Miocardiopatia Amiloidótica

Estudo	Follow-up	Tipo de estudo	Amostra (características basais)	Ecocardiograma	ECG	Biomarcadores	Função neurológica	Eventos clínicos
Merlini et al.[29] e Damy et al.[30]	12 meses	Fase 2 (open-label, só um braço de tratamento com tafamidis 20 mg)	-21 doentes com polineuropatia amiloidótica por mTTR (todos com alguma alteração ecocardiográfica) -Mutações não-Val30Met e não-Val122Ile - Todos com alguma alteração ecocardiográfica (aumento da espessura SIV em 17/19) -13/21 com sintomas de miocardiopatia - Todos em classe I ou II NYHA -HBAE ou BAV 1ºgrau (13/21) - Alterações no Holter (14/21) - 14/21 com NT-proBNP>300 pg/ml - 1 doente com TnI elevada	- No total, sem alterações estatisticamente significativas dos parâmetros. - 4 tiveram aumento da espessura septo ≥ 2mm. - Novas alterações ecocardiográficas: 12/19 (mas os valores médios e % não se alteraram significativamente)	- No global, sem alterações significativas. - Alterações clinicamente significativas de novo: 9/21 no ECG e 9/19 no Holter	- NT-proBNP: elevação significativa apenas num doente - Troponina I: concentrações estáveis, mas 3 doentes ficaram com elevação de TnI.	Agravamento	-18/21 completaram o estudo (1 parou por AIT, 2 por transplante hepático). - Eventos cardiovasculares: 1 doente com AIT, 1 doente com estenose artéria coronária, 1 com BAV)
Maurer et al.[32]	12 meses	Fase 2 (open-label, um só braço com tafamidis 20 mg)	- 31 participantes com ATTRwt com atingimento miocárdico e IC classe I ou II NYHA - 43% com FEVE < 50%	Sem alterações significativas.	-8/18 FA/Flutter auricular. -4/11 TVNS. -5/24 pausas sinusais.	-Troponina I e T: níveis aumentaram moderadamente. -NT-proBNP: níveis não aumentaram significativamente.	----	-3 descontinuaram o estudo -2 mortes (1 AVC e 1 amiloidose AL) -7 hospitalizações por eventos CV -15/31 tiveram progressão clínica (IC agravada, FA ou síncope).

Cortese et al.[33]	36 meses	Observacional e multicêntrico, italiano, só um braço (doentes a iniciar tratamento com tafamidis 20 mg/dia)	-61 pacientes com polineuropatia por ATTRm (69% homens, idade média 62 anos). -72% mutações não-Val30Met -34/57 (60%) com espessura do SIV >12mm. -NT-proBNP elevado em 22/53 (42%). - 27 (44%) com alterações no ECG.	-34 doentes com aumento da espessura do SIV (0.6+/-1,6mm ao 12º mês e 1.05 +/-2mm ao 24º mês). -15% tiveram progressão ecocardiográfica.		3 com aumento de NT-proBNP superior ou igual a 30%	- Agravamento (mais acentuado nos primeiros 6 meses, mas a progressão desacelerou a partir daí); pior naqueles com doença mais avançada. 9 doentes progrediram de estadio. - mIMC estável.	-7 descontinuaram tratamento (1 morte por IC, 1 fez transplante hepático, 3 por progressão da doença; os outros 2 por motivos não relacionados com a doença) -35% (8/23) desenvolveram miocardiopatia de novo. -1 necessitou de pacemaker após 18 meses.
Maurer et al.[34]	30 meses	Ensaio clínico randomizado duplamente cego, fase 3, multicêntrico (tafamidis 80 mg, 20 mg e placebo em 2:1:2)	-441 pacientes com miocardiopatia amiloidótica por transtirretina (76% ATTRwt e 24% ATTRm sobretudo não-Val30Met), excluindo classe IV NYHA -177 placebo; 264 tafamidis (20mg ou 80mg) - Idade mediana 75 anos, 90% homens	- Globalmente sem diferenças significativas (apenas menor decréscimo no volume de ejeção do VE, strain radial e circunferencial nos segmentos médios, nos doentes sob tafamidis)		- Aumento menor de NT-proBNP nos doentes a receber tafamidis vs placebo		-Mortalidade e hospitalizações por eventos CV significativamente mais baixas nos doentes sob tafamidis do que sob placebo (mortalidade 29,5% vs 42,9%; taxa de hospitalizações anuais 0.48 vs 0.70) - Nos doentes em classe III NYHA não se confirmou este benefício (tiveram até mais hospitalizações) - Sem diferenças significativas entre as duas doses de tafamidis. - Sem diferenças nos efeitos adversos entre tafamidis vs placebo.

Estudos com Inotersen na Miocardiopatia Amiloidótica

Estudo	**Follow-up**	**Tipo de estudo**	**Amostra (caracteristicas basais)**	**Ecocardiograma**	**ECG**	**Biomarcadores**	**Função neurológica**	**Eventos clínicos**
Benson et al.[36]	12 meses	Open-label, fase 2 (inotersen 300mg semanalmente por via subcutânea)	-15 pacientes com miocardiopatia (8 com ATTRm e 7 com ATTRwt). Todos com fenótipo restritivo a moderado.	- Espessura da parede do VE e do septo interventricular e função sistólica global estáveis.	---	-BNP diminuiu na maioria dos doentes com ATTRm, mas não na maioria dos doentes com ATTRwt.	---	-7 descontinuaram o estudo. -Bom perfil de segurança, sem alterações na função renal, baixa taxa de trombocitopenia e reações no local de injeção de inotersen.
Benson et al.[35]	15 meses	Ensaio clínico NEURO-TTR, internacional, randomizado, duplamente cego, fase 3 (inotersen 300 mg, 3 injeções subcutâneas na primeira	-172 pacientes com polineuropatia por ATTRm, estadio 1 e estadio 2. 63% com envolvimento cardíaco concomitante.	- No total, sem diferenças estatisticamente significativas dos parâmetros entre os dois grupos.	---	---	-Menor agravamento nos doentes submetidos a inotersen.	-5 mortes com inotersen e 0 com placebo. -Efeitos laterais: glomerulonefrite e trombocitopenia

		semana e posteriormente uma vez por semana, ou placebo, em 2:1)	-Excluídos doentes em classe III ou IV de NYHA.					

Estudos com Patisiran na Miocardiopatia Amiloidótica

Estudo	Follow-up	Tipo de estudo	Amostra (características basais)	Ecocardiograma	ECG	Biomarcadores	Função neurológica	Eventos clínicos
Solomon et al.[40]	18 meses	Fase 3, internacional, randomizado, duplamente-cego, controlado por placebo (randomização 2:1, de 0,3 mg/kg de patisiran ou placebo a cada 3 semanas).	-126 pacientes com miocardiopatia por ATTRm, com espessura basal da parede ventricular esquerda >12 mm, sem HTA ou doença valvular aórtica.	-Redução da espessura das paredes ventriculares e strain longitudinal global com patisiran. -Menor redução do débito cardíaco e aumento de volume diastólico final do VE com patisiran vs placebo.	---	-Redução do proBNP com patisiran.	---	-Com placebo a taxa de hospitalizações e mortes foi 18,7 vs 10.1 por 100 doentes por ano com Patisiran.

Estudos com Diflunisal na Miocardiopatia Amiloidótica

Estudo	Follow-up	Tipo de estudo	Amostra (características basais)	Ecocardiograma	ECG	Biomarcadores	Função neurológica	Eventos clínicos
Castaño et al.[42]	0.9 +/- 0.3 anos	Open-label, um só braço com diflunisal 250 mg oral, 2 vezes por dia	-13 pacientes com miocardiopatia (46% com ATTRm e 54% com ATTRwt).	-Ausência de alteração significativa na massa do VE e na fração de ejeção do VE.	---	Ligeiro aumento	---	-6% com declínio da função renal. -1 descontinuou por sobrecarga de volume.
Sekijima et al.[43]	2 anos	Ensaio clínico só com um braço (diflunisal 250 mg oral, 2 vezes por dia)	-40 pacientes japoneses com ATTRm.	-Alterações não estatisticamente significativas.	---	Estabilização (tendência a melhoria)	-mBMI deteriorou-se ao longo dos 2 anos de tratamento.	-3 pacientes descontinuaram por agravamento da função renal e trombocitopenia.
Ikram et al.[44]	2 anos	Estudo retrospetivo, longitudinal (3 doentes tomaram diflunisal 500 mg 2 vezes por dia e os restantes 250 mg duas vezes por dia).	-23 pacientes com miocardiopatia (10 com ATTRm e 13 com ATTRwt).	---	---	---	---	-1 doente com agravamento transitório e ligeiro da função renal. -13 % dos doentes (3/23) abandonaram o tratamento devido a queixas gastrointestinais. - Não se verificaram mortes nem hospitalizações por agravamento de IC.

Estudos com Extrato de chá verde na Miocardiopatia Amiloidótica

Estudo	Follow-up	Tipo de estudo	Amostra (características basais)	Ecocardiograma	ECG	Biomarcadores	Função neurológica	Eventos clínicos
Kristen et al.[45]	12 meses	Estudo observacional, unicêntrico, um braço. Ingestão diária de 500-700 mg de EGCG.	-19 pacientes com miocardiopatia (10 com ATTRm e 9 com ATTRwt).	-Diminuição da espessura do septo interventricular. -Ausência de aumento da espessura da parede do VE e da massa do miocárdio. -Subgrupo avaliado por RMN (n=9): evidência de redução da massa do miocárdio do VE (-12,5%) em todos os pacientes. -Aumento de cerca de 9% na velocidade do fluxo sistólico mitral.	---	---	---	-Nenhum efeito adverso grave. -2 óbitos, 2 descontinuaram o consumo e 1 submetido a transplante cardíaco.
Aus dem Siepen et al[46]	12 meses	- Estudo observacional, 1 braço. - Consumo diário de 600mg.	-25 pacientes com miocardiopatia por ATTRwt. -Sexo masculino, entre 64 e 80 anos.	-Diminuição da massa do VE em 6%. -Ausência de alterações significativas nos outros parâmetros ecocardiográficos.	---	---	---	---
Estudos com Doxiciclina + ácido tauro-ursodesoxicólico (ATUDC) na Miocardiopatia Amiloidótica								
Estudo	**Follow-up**	**Tipo de estudo**	**Amostra (características basais)**	**Ecocardiograma**	**ECG**	**Biomarcadores**	**Função neurológica**	**Eventos clínicos**
Obici et al[50]	12 meses	-Ensaio clínico de fase 2, 1 braço. -100 mg de doxiciclina 2x por dia e ATUDC 250mg 3x por dia.	-Inicialmente 20 participantes (17 com ATTRm, 2 com ATTRwt, 17 com envolvimento cardíaco). -10% de desistências: 7 doentes com envolvimento cardíaco completaram o estudo.	-Estabilidade dos parâmetros ao longo do estudo.	---	---	-Resultados estáveis (NIS-LL).	-10% desistências por baixa tolerância. -Ausência de eventos adversos graves.
Karlsted t et al[51]	22 meses	-Estudo observacional, 1 braço -100 mg de doxiciclina 2x por dia e ATUDC 250mg 3x por dia.	-53 pacientes com miocardiopatia (42 com ATTRwt e 5 com ATTRm).	-Estabilidade dos parâmetros ao longo do estudo. -Melhoria do strain longitudinal global do VE em 38% (em doentes com doença menos avançada).	---	-Valores permaneceram estáveis.	---	-11% não toleraram o tratamento por efeitos laterais gastrointestinais e dermatológicos. -5 faleceram durante o tratamento.

Tabela I: Resumo dos principais artigos sobre fármacos para o tratamento de amiloidose cardíaca por transtirretina.
Legenda: AIT: acidente isquémico transitório; AL: amiloidose por cadeias leves; ATTRm: amiloidose por transtirretina mutada; ATTRwt: amiloidose por transtirretina *wild-type* (selvage); AVC: acidente vascular cerebral; BAV: bloqueio auricular esquerdo; CV: cardiovascular; ECG: eletrocardiograma; EGCG: epigalocatequina-3-galato; FA: fibrilhação auricular; FEVE: fração de ejeção ventricular esquerda; HBAE: hemibloqueio anterior esquerdo; IC: insuficiência cardíaca; mIMC: Índice de massa corporal modificado; NIS-LL: Neuropathy Impairment Score – Lower Limbs; NT-proBNP: porção N-terminal da pró-hormona do peptídeo natriurético tipo B; NYHA: new York heart association functional classification; RMN: ressonância magnética nuclear; SIV: septo interventricular; TnI: troponina I; TVNS: taquicardia ventricular não sustentada; VE: ventrículo esquerdo.

D2)

Rodrigues P, Frias AD, Gouveia P, Amorim I, Reis H, Trêpa M, Costa R, Oliveira MF, Palma P, Cyrne-Carvalho H, Torres S. ***Radionuclide imaging in the diagnosis of transthyretin cardiac amyloidosis: different sensitivity in early-onset Val30Met mutation?*** --- submitted, under review

Radionuclide imaging in the diagnosis of transthyretin cardiac amyloidosis: different sensitivity in early-onset V30M mutation?

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Brief title: Low sensitivity of bone scintigraphy in V30M

Conflicts of interest: None.

ABSTRACT

Background

Radionuclide imaging using bone-avid radiotracers, such as technetium-99m 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD), has been increasingly used to diagnose amyloid cardiomyopathy due to transthyretin (AC-TTR), due to a hereditary mutation (m) or wild-type (wt). However, distinct types of transthyretin amyloid fibrils can influence the ^{99m}Tc-DPD scintigraphy findings. Data about the accuracy of radionuclide imaging in V30M mutation patients with early-onset disease is scarce.

Objectives

Our main aim was to analyse the performance of bone scintigraphy in diagnosing AC-TTR in patients with V30M mutation and to explore which factors can explain the results.

Methods

Observational study of AC-TTR patients, defined as an echocardiographic ventricular wall thickness $\geq$12 mm, after transthyretin (TTR) gene sequencing and exclusion of light-chain (AL) amyloidosis. All patients with V30M had a positive biopsy for amyloid (of the salivary glands in the majority of cases). A visual ^{99m}Tc-DPD scintigraphy score>1 (moderate or intense cardiac uptake) was considered "DPD positive".

Results

We included 61 patients with AC-TTRV30M; median age 56; 62% males. They were compared with 22 patients with AC-TTRwt and with 12 subjects with TTRV30M amyloidosis without AC.

In AC-TTRV30M patients, ^{99m}Tc-DPD scintigraphy had a sensitivity of only 41% and an accuracy of 47%. Age of neurologic onset stood out as an independent predictor of the

scintigraphy result (median age of 32 in DPD-negative vs 66 years old in DPD-positive; p<0.001).

Conduction disorders were frequent (82%) and in 27 cases development of AC-TTR occurred late after liver transplantation.

Compared with AC-TTRV30M patients, those without AC were significantly younger and a higher proportion was treated with tafamidis, whereas AC-TTRwt patients were older and had more severe myocardial involvement.

Conclusions

Our data shows that radionuclide imaging has limitations in identifying AC-TTR in early-onset patients with V30M TTR mutation. This can have implications in the current diagnostic algorithm.

Key-words: radionuclide imaging; transthyretin; amyloidosis; cardiomyopathy; V30M

INTRODUCTION

Amyloid cardiomyopathy (AC) having transthyretin (TTR) as the precursor protein (AC-TTR) can be caused by mutations in the TTR gene or due to conformational changes in the wild-type protein related to the ageing process.

Several mutations in TTR have been described, with genotype-phenotype correlation and different penetrance. A phenotype of predominant peripheral neuropathy is estimated to affect 10.000 people [1], and the mutation most commonly responsible is V30M – p.V50M in the revised nomenclature [2]. Other mutations lead to a predominantly cardiac phenotype, such as V122I, found in 3.4% of African-Americans [3].

On the other hand, wild-type TTR amyloidosis affects predominantly elderly men and is more common worldwide, albeit underdiagnosed [4-9]. It can be present in 13% of the cases of heart failure with preserved ejection fraction [6] and in 6 [7] to 16% [8] of the patients with aortic stenosis.

AC-TTR now has specific treatment - tafamidis was recently approved [10] and several other drugs are being tested [11, 12] -, making the implications of the diagnosis more relevant.

Bone scintigraphy using technetium-labelled tracers, such as technetium-99m 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD), pyrophosphate (^{99m}Tc-PYP) or hydroxymethylenediphosphonate (^{99m}Tc-HMDP) has been increasingly used to diagnose AC-TTR, both due to a mutation/ variant (AC-TTRm) or wild-type (AC-TTRwt) [13, 14], even obviating the need for a histologic confirmation of amyloid [14].

The mechanism responsible for the myocardial retention of these bone-avid radiotracers is unknown, but has been attributed to microcalcifications [15] and this avidity is more exuberant in TTR than in light-chain (AL) infiltration.

^{99m}Tc-DPD scintigraphy uses a qualitative assessment of cardiac uptake relative to the bones, with a grading system 0-3 [16]. Usually, a visual DPD score>1 (moderate or intense cardiac uptake) is considered positive for the diagnosis of AC-TTR [16, 17]. Mild cardiac uptake (score=1) may also be noted [18], but is not considered diagnostic [17, 19], although further investigation should be considered.

Recent studies reporting a sensitivity reaching 100% [20, 21] and a specificity of 95% [22], allied with its low cost and accessibility, made it pivotal in the diagnosis of AC-TTR. However, performance in patients with the “portuguese variant” (V30M) TTR mutation is scarce. Other authors, in an elegant study but with few subjects, have suggested that different types of fibrils in these patients could account for different results in ^{99m}Tc-DPD scans [23, 24]. A very recent study published in this journal showed that ^{99m}Tc-DPD has a low sensitivity for AC-TTR

detecting caused by P64L mutation [25].

Our primary aim was to estimate the sensitivity of ^{99m}Tc-DPD scintigraphy in patients with AC-TTR with V30M mutation (AC-TTRV30M) and to explore which factors could explain different results in radionuclide imaging. We also compared these results with the performance of ^{99m}Tc-DPD scintigraphy in AC-TTRwt patients and in V30M patients without signs of cardiomyopathy.

As a secondary goal, we described the cardiac changes found in our sample of AC-TTRV30M patients, in terms of myocardial disease and conduction/arrhythmic disorders, many years after the symptom onset, now that these patients have a much longer survival [26].

METHODS

Observational study of adult patients referred to the Cardiology clinic of Centro Hospitalar Universitário do Porto, with suspicion of AC-TTR, from 2016 to 2019. Follow-up was updated in May 2020.

This study is part of a research project that was approved by the Hospital's Ethics Committee and was conducted in accordance with the principles of the Declaration of Helsinki. We only included patients who did a transthoracic echocardiogram and ^{99m}Tc-DPD scintigraphy within that period.

AC-TTR was considered if there was left ventricular thickening (LVT), defined as any ventricular wall thickness $\geq$12 mm in the echocardiogram. All patients underwent genetic testing with TTR sequencing (to differentiate between AC-TTRm and AC-TTRwt). Light-chain (AL) amyloidosis was excluded in patients above 50 years old. All the patients with AC-TTRV30M had a positive biopsy showing amyloid (of the salivary glands in the majority of the cases).

Patients taking more than one drug for hypertension or with moderate to severe aortic stenosis were excluded.

Information about comorbidities, clinical evaluation and cardiac events - death and hospitalizations due to heart failure (HF) - was obtained from electronic medical records.

^{99m}Tc-DPD scintigraphy

For the ^{99m}Tc-DPD scan, patients were given approximately 800 MBq of ^{99m}Tc-DPD intravenously. Static thoracic and whole-body (speed 10 cm/min) images were performed 5 minutes and 2 hours after injection, on a dual-head gamma camera (Siemens ECAM®). They were acquired using low energy, high-resolution parallel-hole collimators. Image processing was performed in TeleHERMES® system. Images were processed by drawing an adjusted

elliptical region of interest (ROI) over the heart and rectangular ones over the whole body. These ROIs were copied and mirrored on posterior images and geometric means of the 2 projections were calculated for each ROI. All ROIs were corrected for background counts.
A qualitative assessment of cardiac ^{99m}Tc-DPD fixation was established using the Perugini score [16]: 0 - there is no cardiac uptake and bone uptake is normal; 1 – there is cardiac uptake but less intense than bone uptake; 2 – cardiac uptake is similar or greater than bone signal; 3 – evident cardiac uptake with attenuated or absent bone signal. Semi-quantitative analysis of heart retention and whole-body retention were analysed using heart to whole body ratio (H/WB), then correlated to visual score.
In the main analysis, patients were considered "DPD positive" if they had a visual ^{99m}Tc-DPD score >1.

Transthoracic echocardiogram

The main parameters that were analysed were the bidimensional measurement of maximal left ventricular (LV) wall thickness diameter in end-diastole, cardiac chambers' dimensions, body surface area (BSA)-indexed LV mass, bi-ventricular systolic function (including LV ejection fraction) and diastolic function, according to the latest guidelines [27, 28]. Pericardial and valvular disease were also recorded.

Statistical analyses

For statistical analyses, SPSS® software was used, considering $p<0.05$ for statistical significance (two-sided p-value).

Continuous data were described as mean ± standard deviation or median (IQR - interquartile range between the 25th and 75th quartiles) for gaussian and non-gaussian distributions, respectively. Shapiro-Wilk test for normality was used. Categorical data were presented as absolute frequencies (n) and percentages.
We used Fisher's exact test to compare proportions between DPD positive and negative patients; independent samples t-test to compare continuous variables with normal distribution and Mann-Whitney U test for non-gaussian distributions.
Logistic regressions were used to test an association between ^{99m}Tc-DPD result and different covariates; in multivariable binary logistic regression analysis, we used a stepwise method.

RESULTS

We enrolled 100 patients: 78 with TTR mutation (all V30M) and 22 wild-type.

Among cases with TTR mutation, 61 had left ventricular thickening and were considered AC-TTRV30M.

AC-TTRV30M

In 61 patients with AC-TTRV30M, median age was 56 years (IQR 25) and 62% were males; median duration of neurologic symptoms was 9 (IQR 15) years.

Only 41% (n=25) were DPD positive (defined as a visual score >1) - Table 1.

Even if we had increased the threshold for echocardiographic diagnosis to wall thickness ≥ 14 mm, only 51% (19/37) would be DPD positive.

The ^{99m}Tc-DPD scintigraphy results were significantly associated with age – DPD positive patients being significantly older (median age at the time of the scan of 72 vs 48; p<0.001) and with late-onset of neurologic symptoms (66 vs 32 years old; p<0.001) and of cardiac symptoms (73 vs 49.5 years old; p<0.001). Moreover, in patients with a positive scan, neurologic symptoms were less frequent (72% versus 97%, p= 0.006) and less severe (assessed using Coutinho's classification [29]), compared to those with a negative scan.

A negative DPD was associated with liver transplantation (LT). Of the 27 patients with AC-TTRV30M submitted to LT, all with early-onset of neurologic symptoms, only 1 was DPD positive. On average, patients had undergone LT 16± 5.6 years earlier.

Tafamidis was taken by 34% of AC-TTRV30M patients, on average for 2.7 ± 1.5 years, and not associated with the ^{99m}Tc-DPD result.

Negative DPD patients showed systemic involvement more frequently, namely, ophthalmologic, urologic and renal changes (microalbuminuria, although creatinine clearance was higher). They had lower wall thickness (median of 14 vs 15 mm; p=0.034); fewer had hypertension, diastolic dysfunction or used loop diuretics (Table 1). Still, 18 patients with negative DPD had at least moderate LVT (wall thickness ≥14 mm). In 4 DPD negative patients, endomyocardial biopsy was performed and it was positive for amyloid in all cases.

In multivariable logistic regression analysis, we considered the factors that were significantly associated with a positive DPD in univariable analysis, that did not have collinearity and that were not related to outcome or manifestations of the cardiomyopathy itself (age at DPD, age at the beginning of neurologic symptoms, age at suspicion of cardiac involvement, liver transplantation, hypertension, maximal wall thickness). Timing of first neurologic symptoms was an independent predictor of the DPD result (OR 1.203; 95% CI (1.098; 1.318); p<0.001).

There was a high proportion of patients with electrical conduction disorders (82%), pacemakers (69%) and atrial fibrillation/ flutter (26%) - Table 1.

None of the patients had severe aortic stenosis or resistant hypertension.

During a median follow-up of 20 (IQR 15) months after radionuclide imaging, cardiac events were rare and without significant differences between groups.

In a secondary analysis, using a lower threshold for the ^{99m}Tc-DPD result and considering any visual ^{99m}Tc-DPD score >0 as "DPD positive" (Supplemental Table 1), 17 patients (28%) would remain negative, 9 of them with wall thickness ≥ 14 mm. A positive DPD was positively associated with age: age at the time of the scan (61.5 (21) vs 45 (15) years old, p<0.001), age at the onset of neurologic symptoms (55 (37) vs 30 (14); p=0.003) and age at the suspicion of cardiac involvement (63 (21) vs 45 (16); p<0.001), with the latter keeping statistical significance in the multivariable model (OR 1.101; 95% CI (1.036; 1.170); p=0.002).

TTRV30M without amyloid cardiomyopathy

There were 17 patients with TTRV30M amyloidosis without LVT; 5 patients had a visual ^{99m}Tc-DPD score ≥1 and were excluded since doubts about possible AC could be raised.

The remaining 12 patients without any signs of AC were compared with those with AC (Table 1). They all had a positive extracardiac biopsy for amyloid.

Compared with AC-TTRV30M, patients without AC were younger (median age 41 (IQR 10); p<0.001) and more frequently treated with tafamidis (83%; p=0.003), on average for 5.8 ± 3.4 years.

AC-TTRwt

Amongst the 22 AC-TTRwt cases, 20 (91%) had a positive DPD (the remaining 2 had a score= 1). Compared with AC-TTRV30M, AC-TTRwt patients were older (median age 81.5 (IQR 9); p<0.001) and myocardial involvement was more exuberant: wall thickness and LV mass were significantly higher, while systolic impairment was more common and more severe. NT-proBNP levels were significantly higher (4058 (IQR 9672) vs 423 (IQR 1093) pg/mL; p<0.001), the use of loop diuretics more frequent and more patients had HF hospitalizations.

Comorbidities such as aortic stenosis and hypertension were also more frequent – Table 1.

Atrial fibrillation was more prevalent (68% vs 26%; p=0.001), whereas conduction disturbances and pacemaker implantation were less frequent (59% vs 82%, p=0.043; 18% vs 69%, p<0.001, respectively).

DISCUSSION

Main findings

In our sample, the sensitivity of a positive DPD (defined as a visual ^{99m}Tc-DPD score >1) was of only 3% in early-onset and 86% in late-onset AC-TTRV30M patients, whereas it reached 91% in AC-TTRwt.

Even increasing the echocardiographic cut-off to a wall thickness ≥14 mm, a positive ^{99m}Tc-DPD scan would only diagnose 51% of these patients.

Radionuclide imaging results were significantly associated with the timing of neurologic symptoms, age at ^{99m}Tc-DPD scan and extra-cardiac involvement. In patients that developed AC-TTR after LT, the sensitivity of ^{99m}Tc-DPD scintigraphy was particularly poor (4%) and we can speculate that these patients are probably being underdiagnosed.

The only independent predictor factor of a false negative ^{99m}Tc-DPD scintigraphy was age at the beginning of neurologic symptoms. Patients with a negative scan were early-onset and presented neurologic symptoms usually in their 30s (a difference of over 30 years compared with DPD positive patients).

Tafamidis was not associated with the ^{99m}Tc-DPD result, but patients were taking the drug for a much shorter period of time compared with LT.

There were few TTRV30M patients without AC and they were younger than cardiomyopathy patients, restricting comparisons between these groups. However, we have to acknowledge that a few patients without LVT were DPD positive (excluded in the main analysis). In those cases (they were older patients without LT), radionuclide imaging may actually be more sensitive than echocardiography or magnetic resonance [30]. However, our study was not designed to detect possible false positive ^{99m}Tc-DPD scans. If those patients were true positive AC-TTRV30M cases, the accuracy of ^{99m}Tc-DPD scintigraphy would increase to 54%.

The estimated sensitivity of ^{99m}Tc-DPD scintigraphy in AC-TTRwt was good, with none of the patients having a score of 0. Compared with AC-TTRV30M, patients with AC-TTRwt were older and myocardial involvement was more pronounced. Even though a significant proportion of the patients had a history of hypertension and some had aortic stenosis, these pathologies probably coexisted – in that case, we used the scintigraphy to validate the diagnosis of AC-TTRwt, since its positive predictive value is high (particularly using the cut-off of a Perugini score ≥2).

Limitations

This study was purely observational, so we cannot exclude selection bias and causality cannot be determined.

We also recognize the limitations of considering bidimensional wall thickness in echocardiography as the gold standard for the diagnosis, even excluding significant aortic stenosis, resistant hypertension or AL amyloidosis. However, other studies used a similar methodology [13, 16, 20, 25]. Some of them considered wall thickness >12 mm as the cut-off. This would mean a wall thickness of 12 mm would be considered normal (those patients would be included in the "no AC" group), in discordance with current echocardiographic guidelines

[28]. We therefore used ≥12 mm as the cut-off, following a recently published paper in this journal [25], but also performed an additional analysis using a higher cut-off point.

We did not exclude all patients with hypertension, since they represent a significant proportion in real world, but adjusted for that variable. Interestingly, hypertension was significantly associated with a positive DPD (making it difficult to argue that DPD negative scans were due to hypertensive cardiomyopathy).

In the few dubious cases that underwent a endomyocardial biopsy, it confirmed amyloid infiltration in all. Unfortunately, most patients did not have information from endomyocardial biopsies, cardiac magnetic resonance or strain, that would complement the diagnosis.

We have no evidence to say that decreasing the cut-off to any visual score>0 (Supplemental Table 1) would be more discriminative – namely, wall thickness was similar in both groups. However, a longer follow-up and validation with clinical events would be important.

We believe that the current algorithm for AC-TTR diagnosis [14] should be changed, as others have recently suggested [25]. Particularly in cases of early-onset V30M disease, a visual score<2 in the ^{99m}Tc-DPD scintigraphy should not exclude the diagnosis and further investigation should be considered, including endomyocardial biopsy, positron emission tomography (PET) or even cardiovascular magnetic resonance (CMR), although the sensitivity of CMR may be relatively lower. Furthermore, patients with a ^{99m}Tc-DPD score of 1, with increased wall thickness and TTR V30M mutation, should be considered for. This is relevant, since a negative ^{99m}Tc-DPD scan is usually interpreted as excluding AC-TTR, for which there is specific treatment.

Other authors have suggested that in early-onset V30M disease there are only full-length amyloid fibrils (type B) [23], that can explain a different behaviour in bone scintigraphy or even in Congo red staining and in the response to tafamidis [24]. Patients with late-onset, other mutations or wild-type disease have type A fibrils (a mixture of full-length and truncated) and this seems to be related with late presentation and lower penetrance [23].

We hope this study can lead to further investigation, from bedside to bench, in order to clarify the interaction of ^{99m}technetium-biphosphonate derivatives with different amyloid fibrils. We also need to confirm that the behaviour of the different bone-avid radiotracers is identical and explore if it is not affected by other factors.

Additionally, we also showed that AC-TTR can develop after LT in early-onset V30M disease – it had already been reported in late-onset scenarios and with other mutations [31-35], but data on myocardial involvement post-LT in early-onset V30M was missing. Development of conduction disturbances was already known to occur after LT [36-38]. AC-TTR occurred more than a decade after transplantation, making us conclude that long term cardiac follow-up of these patients is necessary. Even in our endemic region of V30M mutation traditionally described as having early-onset polyneuropathy, with conduction disorders but rarely with

myocardial involvement, we now observe that the development of cardiomyopathy is not uncommon, probably due to the increased survival achieved in the last decades [26]. Seeding mechanisms and wild-type fibril deposition have been implicated in cardiac changes after LT [34], however, compared with AC-TTRwt, these amyloid deposits seem to have a different behaviour at least in the avidity for bone radiotracers.

CONCLUSIONS

^{99m}Tc-DPD scintigraphy showed a low sensitivity for detecting amyloid cardiomyopathy in early-onset patients. In those patients, further investigation is needed before excluding myocardial involvement, namely comprising other cardiac imaging techniques and endomyocardial biopsy.

In hereditary late-onset and wild-type cases, the performance of radionuclide imaging was good, in line with previous studies.

We have also shown late development of AC-TTR after LT in early-onset V30M patients; therefore, echocardiographic follow-up should be performed and we believe that treatment options should also be investigated in these patients.

Additional research is needed to investigate the formation of different types of amyloid fibrils in patients with transthyretin mutations and to better understand its implications, not only in the interpretation of the visual ^{99m}Tc-DPD score, but in the whole management of cardiac involvement in these patients.

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TABLES

Table 1 - Patients' characteristics, comparing patients with AC-TTRV30M (with and without a positive DPD scintigraphy), versus TTRV30M without AC and AC-TTRwt.

	AC-TTRV30M				TTRV30M without AC		AC-TTRwt	
	Total	DPD+	DPD-	p-value (DPD+ vs DPD-)		p-value (comparison with AC-TTRV30M)		p-value (comparison with AC-TTRV30M)
Patients (n)	61	25	36		12‡		22	
Age at DPD, years; median (IQR)	56 (25)	72 (11)	48 (10)	**p<0.001**	41 (10)	**p<0.001**	81.5 (9)	**p<0.001**
Gender - male, n (%)	38 (62%)	15 (60%)	23 (64%)	p=0.793	5 (42%)	p=0.213	19 (86%)	p=0.059
Aortic stenosis; n (%)	0	0	0		0		4 (18%)	**p=0.016**
Hypertension; n (%)	16 (26%)	11 (44%)	5 (13%)	**p=0.016**	1 (8%)	p=0.273	17 (77%)	**p<0.001**
Echo: Maximal wall thickness in mm; median (IQR)	14 (3)	15 (3)	14 (2)	**p=0.034**	10.5 (1)	**p<0.001**	17 (3)	**p<0.001**
Echo: BSA-indexed LV mass*; g/m2; median (IQR)	128 (56)	131 (65)	124 (52)	p=0.648	85 (35)	**p<0.001**	162 (64)	**p=0.001**
Echo: diastolic dysfunction; n (%)	absent - 5(8%); present - 10 (16%); indeterminate- 46 (75%)	absent- 5(20%); present- 4 (16%); indeterminate - 16 (64%)	absent - 0; present - 6 (17%); indeterminate - 30 (83%)	**p=0.019**	absent-2 (17%); present- 1 (8%); indeterminate- 7(58%)	p=0.478	absent- 0; present- 6 (27%); indeterminate - 16 (73%)	p=0.245
Echo: LV systolic dysfunction; n (%)	4 (7%)	3 (12%)	1 (3%)	p=0.296	3 (25%)	p=0.082	13 (59%)	**p<0.001**
Age at suspicion of cardiac involvement, years; median (IQR)	57 (25)	73 (10)	49.5 (11)	**p<0.001**	NA	NA	81 (8.5)	**p<0.001**
NYHA†	I- 17 (28%); II- 32 (52%); III- 5 (8%); IV-0	I- 6 (27%); II- 13 (59%); III- 3 (14%); IV-0	I- 11 (34%); II- 19 (59%); III- 2(6%); IV- 0	p=0.613	I- 3 (25%); II- 1 (8%); III- 1 (8%); IV-0	p=0.237	I- 2 (9%); II- 12 (54%); III- 8 (36%)	**p=0.007**
Conduction disturbances; n (%)	50 (82%)	19 (76%)	31 (86%)	p=0.333	8 (67%)	p=0.253	13 (59%)	**p=0.043**
Pacemaker; n (%)	42 (69%)	14 (56%)	28 (78%)	p=0.094	8 (67%)	p=1.0	4 (18%)	**p<0.001**
Atrial fibrillation/ flutter; n (%)	16 (26%)	7 (28%)	9 (25%)	p=1.0	1 (8%)	p=0.273	15 (68%)	**p=0.001**
Orthostatic hypotension; n (%)	28 (46%)	10 (40%)	18 (50%)	p=0.602	7 (58%)	p=0.533	1 (4.5%)	**p<0.001**
NT-proBNP, pg/mL; median (IQR)	423 (1093)	535 (1049)	304 (1127)	p=0.420	147 (145)	**p=0.002**	4058 (9672)	**p<0.001**
CrCl, mL/min; mean ± SD	82 ± 36	72 ± 27	91 ± 40	**p=0.037**	105 ± 38	p=0.061	43 ± 15	**p<0.001**
Loop diuretics; n (%)	22 (36%)	15 (60%)	7 (19%)	**p=0.002**	1 (8%)	p=0.089	20 (91%)	**p<0.001**
Treatment - liver transplantation; n (%)	27 (44%)	1 (4%)	26 (72%)	**p<0.001**	2 (17%)	p=0.108	NA	
Treatment - tafamidis; n (%)	21 (34%)	12 (48%)	9 (25%)$	p=0.100	10 (83%)	**p=0.003**	1 (4.5%)	**p=0.005**
Treatment - doxycycline; n (%)	0	0	0		0		7 (32%)	**p<0.001**
Treatment – another drug; n (%)	4 (7%)	1 (3%)	3 (12%)	p=0.296	1 (8%)	p=1.0	0	
Neurologic symptoms – stage§; n (%)	0- 8 (13%); 1- 38 (62%); 2-	0- 7 (28%); 1 - 13 (52%); 2-	0- 1 (3%); 1- 25 (69%); 2-	**p=0.006**	0- 0; 1- 10 (83%); 2- 2 (17%); 3- 0	p=0.432	NA	

	13 (21%); 3- 2 (3%)	3 (12%); 3 - 2 (8%)	10 (28%); 3 - 0					
Age at initial neurologic symptoms, years; median (IQR)	39 (34)	66 (13)	32 (12)	**p<0.001**	30 (8)	**p=0.012**	NA	
Ophtalmologic symptoms; n (%)	21 (34%)	3 (12%)	18 (50%)	**p=0.003**	1 (8%)	p=0.092	NA	
Urologic symptoms; n (%)	24 (39%)	5 (20%)	19 (53%)	**p=0.016**	3 (25%)	p=0.516	NA	
GI symptoms; n (%)	30 (49%)	9 (36%)	21 (58%)	p=0.120	6 (50%)	p=1.0	NA	
Renal involvement; n (%)	13 (21%)	1 (4%)	12 (33%)	**p=0.009**	1 (8%)	p=0.440	NA	
HF hospitalization; n (%)	4 (7%)	3 (12%)	1 (3%)	p=0.296	0	p=1.0	11 (50%)	**p<0.001**
Death; n (%)	6 (10%)	4 (16%)	2 (6%)	p=0.216	0	p=0.581	5 (23%)	p=0.150

Legend: AC- amyloid cardiomyopathy. BSA – body surface area. CrCl – creatinine clearance. GI – gastrointestinal. HF – heart failure. LV – left ventricle. NT-proBNP - N-terminal prohormone of brain natriuretic peptide. NYHA – New York Heart Association functional classification of heart failure symptoms. TTRV30M- transthyretin mutation V30M. TTRwt- wild-type transthyretin.

Continuous variables with a normal distribution are present as mean ± standard deviation (SD) and those with a non parametric distribution as median (IQR – interquartile range, representing the subtraction between quartile 75 and quartile 25).

Categorical variables are described as number of patients (n) and percentage.

* Missing values in 12 patients.

† Not all patients could be classified.

‡ Patients with DPD>0 were excluded.

§ Neurologic Coutinho staging: 0 – no symptoms; 1- mild symptoms, affecting lower limbs but with ability to walk without help; 2- patients require walking with assistance; 3 – patients need a wheelchair.

$ Two patients took tafamidis before liver transplantation.

SUPPLEMENTAL MATERIAL

	AC-TTRm		
	DPD+ (score>0)	**DPD-** (score=0)	p-value
Patients (n)	44	17	
Age at DPD, years; median (IQR)	61.5 (21)	45 (15)	**p<0.001**
Gender - male, n (%)	27 (61%)	11 (65%)	p=1.0
Aortic stenosis; n (%)	1 (2%)	0	p=1.0
Hypertension; n (%)	14 (32%)	2 (12%)	p=0.193
Echo: Maximal wall thickness in mm; median (IQR)	14 (3)	14 (2)	p=0.448
Echo: BSA-indexed LV mass*; in g/m2; median (IQR)	128 (56)	124 (49)	p=0.786
Echo: diastolic dysfunction; n (%)	absent - 5(11%); present - 8(18%); indeterminate- 31(71%)	absent - 0; present - 2 (12%); indeterminate- 15 (88%)	p=0.252
Echo: LV systolic dysfunction; n (%)	4 (9%)	0	p=0.569
Clinical suspicion of HF	very likely- 17 (39%); possible - 25(57%); unlikely- 2 (4%)	very likely- 9 (53%); possible - 6 (35%); unlikely- 2 (12%)	p=0.262
Age at suspicion of cardiac involvement, years; median (IQR)	63 (21)	45 (16)	**p<0.001**
NYHA†	I- 10 (26%); II- 24 (61%); III- 5 (13%); IV-0	I- 7 (47%); II- 8 (53%); III- 0	p=0.168
Positive endomyocardial biopsy; n	3/4	1/4	
Conduction disturbances; n (%)	37 (84%)	13 (76%)	p=0.481
Pacemaker; n (%)	29 (66%)	13 (77%)	p=0.544
Atrial fibrillation or flutter; n (%)	14 (32%)	2 (12%)	p=0.193
Orthostatic hypotension; n (%)	19 (43%)	9 (53%)	p=0.573
NT-proBNP, pg/mL; median (IQR)	529.5 (1358)	298 (551)	p=0.288
CrCl, mL/min; mean ± SD	77 ± 31	100 ± 45	p=0.083
Loop diuretics; n (%)	21 (48%)	1 (6%)	**p=0.002**
Treatment - liver transplantation; n (%)	16 (36%)	11 (65%)	p=0.083
Treatment - tafamidis; n (%)	16 (36%)	5 (29%)	p=0.766
Treatment - doxycycline; n (%)	0	0	
Treatment - another drug; n (%)	4 (9%)	0	p=0.569
Neurologic symptoms - stage‡; n (%)	0- 7(16%); 1- 27(61%); 2- 8 (18%); 3- 2 (5%)	0- 1 (6%); 1- 11(65%); 2- 5 (29%); 3- 0	p=0.482
Age at initial neurologic symptoms, years; median (IQR)	55 (37)	30 (14)	**p=0.003**
Ophtalmologic symptoms; n (%)	17 (39%)	4 (24%)	p=0.371
Urologic symptoms; n (%)	16 (36%)	8 (47%)	p=0.561
GI symptoms; n (%)	20 (45%)	10 (59%)	p=0.402

Renal involvement; n (%)	8 (18%)	5 (29%)	p=0.486
HF hospitalization; n (%)	4 (9%)	0	p=0.569
Death; n (%)	5 (11%)	1 (6%)	p=1.0

Supplemental Table 1 – Characterization of patients with amyloid cardiomyopathy caused by transthyretin with Val30Met mutation (AC-TTRm), comparing those with versus without a positive DPD scintigraphy (visual score>0 for this analysis).

Legend:
AC- amyloid cardiomyopathy. BSA – body surface area. CrCl – creatinine clearance. GI – gastrointestinal. HF – heart failure. LV – left ventricle. NT-proBNP - N-terminal prohormone of brain natriuretic peptide. NYHA – New York Heart Association functional classification of heart failure symptoms. TTRm- mutated transthyretin. TTRwt- wild-type transthyretin.

All patients with TTRm had Val30Met mutation.

* Missing values in 12 patients.

† Not all patients could be classified.

‡ Neurologic Coutinho staging: 0 – no symptoms; 1- mild symptoms, affecting lower limbs but with ability to walk without help; 2- patients require walking with assistance; 3 – patients need a wheelchair.

Continuous variables with a normal distribution are present as mean ± standard deviation (SD) and those with a nonparametric distribution as median (IQR – interquartile range, representing the subtraction between quartile 75 and quartile 25).
Categorical variables are described as number of patients (n) and percentage.

DISCUSSION

A)

Ischemic heart disease has dominated the list of sCA etiologies [86, 87], but the proportion of non-ischemic causes has been increasing, probably due to the success of medication, myocardial revascularization techniques and devices. Our understanding of the causes lying behind non-ischemic sCA in the general population is still limited [10, 88] and it was one of the goals of this project.

In our study of 164 sudden cardiac arrest survivors (65% men, mean age 48 years), CMR was crucial for the diagnosis of 30% of the cases and contributed to the diagnosis in 49%. Using a standardized clinical pathway for the investigation of sudden cardiac arrest, including CMR, a cause can be identified in nearly two thirds of patients.

The most frequent sCA etiologies detected by CMR were dilated cardiomyopathy (17%), myocarditis or sarcoidosis (13%), occult myocardial infarction (8%), and hypertrophic cardiomyopathy (6%).

Another study, including 137 cardiac arrest survivors [11] without a clear diagnosis before performing CMR, reported a higher prevalence of myocardial infarction (58% of patients), possibly related to the application of different angiographic criteria for inclusion (we excluded coronary disease with any stenosis >30%).

Primarily arrhythmic causes (caused by channelopathies, accessory pathways with fast conduction, or toxic/ionic disturbances) were found in 14% of the cases and they had significantly fewer major adverse cardiac events (MACE) - comprising significant nonfatal ventricular arrhythmia or death - compared to other patients.

Minor and nonspecific changes not suggestive of a specific diagnosis were found in 30 CMR scans (18% of the total, 35% of those without a diagnosis), with 22 patients (13%) having subtle and/or non-specific LGE findings – that were not associated with prognosis.

About a third of the patients had a completely normal scan – suggesting that detectable changes in CMR cannot be attributed to cardiac arrest and resuscitation *per se*.

During a median follow-up of 32 months, MACE occurred in 31% of subjects. An ICD was placed in 70%. MACE were associated with the establishment of a diagnosis by CMR, extent and presence of LGE, left and right ventricular ejection fractions. The latter was an independent predictor of MACE.

LGE extent was not an independent prognostic marker in this cohort, although this may be due to the study power or the method used for scar quantification [89-91]. LGE was automatically quantified using a threshold of 5 standard deviations above the mean signal intensity of the

remote myocardium [90], but the best method for quantification is not consensual.

The main limitations of our study are its retrospective design and possible selection bias, with obvious under-representation of patients with a more malignant course that did not survive before reaching the hospital. Follow-up was not homogeneous and not all patients had an ICD allowing continuous rhythm monitoring. Endomyocardial biopsy was rarely performed and genetic testing did not follow any specific protocol; both could add valuable information and should be considered in future studies.

B)

We investigated the effects of alcohol intake on cardiac remodeling and function over time, in a healthy sample of young adults of the CARDIA cohort.

The major strength of the current study was that it analyzed a large race- and sex-balanced sample with a long follow-up. Besides utilizing a very complete dataset, we also performed a robust analysis with multiple adjustments for covariates, in order to minimize the likelihood of confounders.

In our study, greater alcohol consumption was associated with higher values of indexed LV end-diastolic volume (iLVEDV) and LV mass (iLVmass), suggesting that alcohol may cause LV remodeling, which can be detrimental [92-95] and represent the earliest stage of a dilated cardiomyopathy phenotype.

We did not find a significant association with LVEF, although it was mildly increased in very low-risk drinkers.

The absolute changes in echocardiographic parameters were small and overall the values remained within normal limits. Alcohol intake was generally mild (the majority drank <7 standardized drinks per week), which may have limited the conclusions about higher levels of consumption. Therefore, our results reinforce the concept that mild alcohol consumption poses low cardiovascular risk.

Other authors had shown that women are more susceptible to alcohol toxic effects [96, 97], but in our analyses gender and race had little impact on the results. We merely found some interaction regarding LA diameter, that had a significant non-linear association with alcohol intake only in women and black participants.

The relationship between specific types of alcoholic beverages and ventricular function has been unclear [98, 99] and our data suggested that the type of beverage can also play a role, with liquor and beer appearing to be more detrimental than wine. However, more studies focused on the type of beverages are needed to extrapolate a robust conclusion.

Binge-drinking, a behavior previously correlated primarily with arrhythmias, specifically atrial fibrillation [100], was also associated with adverse cardiac remodeling, but only in the crude

analysis, possibly due to the small sample of binge-drinkers.

Some factors, such as male gender, Caucasian race, smoking, illicit drug use and high physical activity, were related to higher alcohol intake and this can help to target public health campaigns.

Our research, as well as other studies that focused on drinking or eating habits, has several limitations: it is observational, the patterns change over time, there can be sampling bias and recall bias. Adjusting for confounding factors that are hard to determine, such as genetics or diet, could not be made.

Even though the best dependent variable was probably the intra-individual variation of each of the echocardiographic parameters, we could only calculate the variation in iLVEDV and LVEF in approximately half the sample, since the quantification methods were different. This may explain the absence of significant associations between alcohol intake and echocardiographic findings in that analysis. Therefore, as most studies about alcohol intake, our main analysis focused on the echocardiographic measurements at the end of follow-up, excluding those with signs of dilated cardiomyopathy or other heart disease at baseline.

Further studies with long term follow-up of heavy drinkers could help us to determinate the modulators of alcohol effect in the heart. Prospectively studying the effects of alcohol abstinence in a population of dilated cardiomyopathy could also enlighten the potential reversibility of alcohol toxicity.

C)

In our sample of RA patients without known cardiac disease, echocardiographic screening detected SD in 4% and DD in 13%.

Age, hypertension, dyslipidemia, corticosteroids and eGFR were associated with ventricular dysfunction, but age stood out as the most important independent predictor. The best cut-off point in terms of the best sum of sensitivity and specificity was 57 years old.

Even though SD prevalence was similar to what had been described before, DD was less common. Previous studies reported a prevalence of 30-50% in RA patients versus 25-30% in the general population [101, 102]. However, previous studies considered patients who already had cardiovascular events and different definitions of DD were used across studies. Most previous studies used the 2009 European guidelines for diastolic classification [85], that in our study had a poor agreement with the 2016 European/ American guidelines, as others had shown [103]. Invasive studies suggested that the 2016 guidelines are more specific [104] and, comparing with the 2009 classification, we found fewer cases of DD (13% instead of 23%) and IDF (11% instead of 47%). The prevalence of DD in the general population is also much lower in other studies that used the 2016 guidelines (around 1.3%) [105].

Additionally, in many studies, the presence of one DD parameter was sufficient for labelling patients with DD and the majority did not specify exactly how they coded patients, particularly if some parameters were missing. In our study, TR velocity could not be properly assessed in more than half of the patients, which seems to reflect what happens in the real world. This meant that in those patients with only 3 available parameters according to the 2016 algorithm, none could be classified with IDF and patients with only 2 abnormal parameters out of 3 were classified with DD (even though it is unlikely that the pulmonary pressures were significantly increased in patients without significant tricuspid regurgitation).

One could also argue that in patients with ventricular hypertrophy a different algorithm could have been applied, proposed for myocardial disease in the 2016 guidelines [83], but this was not done in other studies either.

Additionally, in the elderly, normal echocardiographic parameters are different from a younger population and neither the 2009 nor the 2016 classifications contemplate that.

In fact, the absence of a gold-standard and consensual definition of DD across studies makes the comparison difficult [106]. We used a particular study for comparison, that described a cohort of the general population, with the same characteristics as our sample except for RA (conducted in the same city and with similar distribution of age, gender and cardiovascular risk factors), also using the 2016 European/ American echocardiographic guidelines for diastolic classification [103]. Using it as our "control" group, we can infer that DD prevalence was significantly higher in RA patients (13% vs 1.4%; $p<0.001$).

According to the 2016 European HF criteria [84], HFpEF features were found in 13%, but interestingly they did not correspond well to ventricular dysfunction categories. The terms DD and HFpEF are often used interchangeably, but their overlap seems limited.

There were few cardiovascular events, so we considered it underpowered to detect an association between echocardiographic classification and events. There was a significant association between surrogate prognostic markers - such as the distance in the 6-minute walk test (6MWT), NT-proBNP and high-sensitivity troponin T (hsTnT) - and ventricular function. Those biomarkers and the functional capacity estimated by the 6MWT were also associated with the echocardiographic parameters most frequently used to assess diastolic function (interventricular septum diameter, LV mass, average E/e' ratio, lateral e', LA volume, tricuspid regurgitation velocity, E/A ratio). However, those surrogate markers were also higher in IDF and still within the normal range in RA patients, making it challenging to apply them in clinical practice.

Contrary to what we had hypothesized, we did not find significant independent associations between characteristics of RA disease and subclinical ventricular dysfunction, but we should be aware that our sample had particularly low disease activity in this modern era of treatment.

Our study has several limitations: it is observational; the sample size and low prevalence of systolic and diastolic dysfunction could have limited the detection of independent predictors of SD and DD; follow-up is short; there is no control group (although we used a cohort from the general population [103] for comparison).

We intend to reduce these limitations in future studies, having planned a long-term follow-up of these patients, particularly those with indeterminate diastolic function, to ascertain what is their evolution and prognosis. We also want to investigate if there is an added value of longitudinal global strain analysis (that can represent a precursor stage of systolic impairment) in this context.

C') Related article under review:

Ferreira MB, Fonseca T, Costa R, Marinho A, Cyrne-Carvalho H, Zannad F, Rossignol P, Gottenberg JE, Saraiva F, Rodrigues P, Barros A, Ferreira JP. ***Prevalence, risk factors and proteomic bioprofiles associated with heart failure in rheumatoid arthritis: the RA-HF study.***

Using the same initial cohort of RA patients, but not excluding those with known heart diseases, we found that 115/355 (32%) had "HF" - defined as NT-proBNP >125 pg/mL and at least one structural or functional echocardiographic change (i.e, left ventricular hypertrophy, left atrial enlargement or left ventricular systolic or diastolic dysfunction) or use of loop diuretics or HF history.

Only 7% had a previously established HF diagnosis. Age, classic cardiovascular risk factors (diabetes, hypertension, obesity, dyslipidemia, atrial fibrillation and arterial ischemic disease) and RA duration increased the HF odds.

Several protein-biomarkers remained independently associated with HF, namely adrenomedullin, placenta-growth-factor, tumor necrosis factor (TNF)-receptor-11A, and angiotensin-converting-enzyme-2. Similar HF-associated biomarker pathways were externally replicated in patients without RA.

Having RA plus HF greatly increased the risk of cardiovascular events after an average of 4 years of follow-up (hazard ratio= 3,5; 95% confidence interval (1.04-12.1); p =0.044).

Overall, we found that HF in RA patients largely shares the features of non-RA patients. Therefore, RA patients are probably responsive to the same treatment options used in the general population, while HF seems to be underdiagnosed in this population.

D)

D1)

Liver transplantation (LT) was the first effective treatment for FAP and significantly increased

the survival of these patients, particularly in early-onset Val30Met TTR cases [107]. However, the reduced organ availability, peri-procedural morbi-mortality and the risks associated with long term immunosuppressive therapy are important limitations [108]. Moreover, others have shown that cardiac disturbances [61] (and eye involvement) can progress after LT, and we have also shown that cardiomyopathy can develop many years after LT in our population of Val30Met mutation (study D2). This has been attributed to *seeding* phenomena and to the deposition of wild-type TTR after LT [109]. Still, LT seems to be a good option for early-onset Val30Met FAP patients [110] and we do not have such a long follow-up with any of the drugs. However, LT is not applicable in cases of established cardiomyopathy nor obviously in wild-type TTR amyloidosis.

In the past few years, several drugs have been tested that act in different parts of the amyloidogenesis cascade.

Tafamidis is the only drug with a robust randomized clinical trial (ATTR-ACT) [69] focused on AC-TTR treatment, that will be discussed with more detail. Tafamidis has a good safety profile, without frequent serious adverse events reported, and has oral administration. The ATTR-ACT trial had a follow-up of 30 months and tafamidis showed a 31% relative reduction in the occurrence of hospitalizations or death. This was in line with a lower increase in NT-proBNP levels and a smoother decline in functional capacity and quality of life. The reduction in all-cause mortality was seen after 18 months of treatment. Approximately ¾ of the patients had AC-TTRwt and amongst the participants with TTR mutation, very few had Val30Met. In the subgroup of ATTRm, there was not a significant improvement in the treatment arm, but this might have been due to the small group size.

Patients in New York Heart Association classification (NYHA) class IV were excluded. In patients with NYHA class III there was not a significant benefit of the treatment. In that subgroup, the hospitalization rate was higher with tafamidis versus placebo, while survival was not statistically different.

The absence of significant echocardiographic changes between tafamidis and placebo is also not clear.

Two doses of the drug were tested, but there were no significant differences between them, even though there was a trend towards greater efficacy with 80mg versus 20mg of tafamidis meglumine. The 20mg dosage of tafamidis had been used for the treatment of FAP [70, 111]. Prior studies, with fewer patients and open-label, had also used the 20mg dose and had shown modestly positive results [112-115]. However, some small studies suggested that a higher dose would provide maximal kinetic stabilization of TTR [116].

Another formulation of 61mg of tafamidis (free acid, not meglumine) is also available and

considered equivalent to the 80mg dose of tafamidis meglumine – and these 2 doses were the ones approved by the FDA (Food and Drug Administration) and EMA (European Medicines Agency). This can have implications in cost-effectiveness balance.
Finally, in the main article, one of the exclusion criteria is "an implanted cardiac device", which can be misleading. In fact, only an "implanted cardiac *mechanical assist* device" was an exclusion criteria, as well as having an indication for pacemaker but not having it placed. While "implanted cardiac *mechanical assist* device" concerns advanced ventricular assistance, "an implanted cardiac device" may be referring to a pacemaker, cardiac resynchronization therapy (CRT) or implantable cardiac defibrillator (ICD). Since conduction disorders are frequent in amyloidosis, if carriers of any cardiac device were excluded, this would limit the use of the drug significantly. In the trial appendix, it is clear that some participants had a pacemaker or ICD, and we confirmed this with the authors.

Diflunisal is a known non-steroidal anti-inflammatory drug, which is also a TTR stabilizer. Concerns have been raised about possible hemodynamic decompensation, worsening of renal function and bleeding risk, but the very small studies available did not show major safety issues [117-119]. It has a very reduced cost but lacks substantial data showing efficacy in AC-TTR.
AG10 is another TTR stabilizer, that is currently being tested in AC-TTR.

In comparison with TTR stabilizers, the suppression of TTR altogether may have the advantage of also inhibiting the production of fibrils due to TTR proteolysis [81]. However, we will need data about the long-term effects of TTR suppression.
Patisiran and inotersen both block the production of TTR and have been approved for FAP. We still await studies focusing on AC-TTR and for now only have subanalyses of the FAP trials using these two drugs, that in both cases excluded patients with NYHA class III or IV. The subanalysis of the APOLLO trial [120], patisiran showed promising results in terms of echocardiographic parameters and NT-proBNP. However, events were not significantly different and the few deaths that occurred in the treatment arm were HF-related.
In the inotersen substudy [121], no significant changes were noted with inotersen versus placebo regarding echocardiographic parameters or events (although there was a trend towards more adverse events in the treatment arm). The major adverse events were thrombocytopenia and renal failure.
Studies with other small interfering ribonucleic acids (siRNA) or antisense oligonucleotides are also ongoing.

Epigallocatechin-3-gallate (EGCG) – green tea extract – has shown some promising results in

small observational studies, but without a control group and with only 1 year of follow-up [122, 123].

Doxycycline and tauroursodeoxycholic acid (TUDCA) have been used for many years with other purposes, but can also promote amyloid degradation. Small studies using this combination, without a control arm, have shown stabilization of echocardiographic parameters [124, 125], and it is an inexpensive treatment. However, approximately 10% of the patients do not tolerate it.

Even though it transcends the focus of our review, in order to ensure justice and equitable distribution of resources in healthcare, these innovative drugs need to be affordable [126], particularly if we want to use them in AC-TTRwt, that is a not so rare disease.

D2)

In our sample of AC-TTRm (all with Val30Met mutation), the sensitivity of a positive ^{99m}Tc-DPD scan (defined as a visual score >1) was only 41%. Age at neurologic onset stood out as an independent predictor of the scintigraphy result (median age of 32 in DPD-negative vs 66 years old in DPD-positive; $p<0.001$).

Even increasing the echocardiographic cut-off to $\geq$14 mm (moderate left ventricular "hypertrophy"), we would diagnose only 51% of those patients with the ^{99m}Tc-DPD scan.

We have no evidence to say that decreasing the cut-off to any visual score>0 would be more accurate.

Colleagues from Lisbon had already suggested that radionuclide imaging could have worse performance in diagnosing Val30Met patients with familial amyloid polyneuropathy [127].

The ^{99m}Tc-DPD scintigraphy results were significantly associated with age and with the timing of presentation – negative DPD patients being significantly younger and with early-onset of neurologic and cardiac symptoms. Additionally, DPD negative patients had more frequent and more severe neurologic symptoms; involvement of other organs was also more common.

In patients that underwent liver transplantation (LT) - that took place on average 16± 5.6 years before - the sensitivity of DPD scintigraphy was particularly poor (4%). We can deduce that patients that develop AC-TTRm after LT are probably underdiagnosed. Moreover, treatment for AC-TTR in LT recipients is lacking, since drug trials exclude these patients, that therefore are not entitled to any specific therapy in clinical practice, not even patients that developed the disease after domino liver transplantation. Therefore, studies testing drugs approved for AC-TTR in patients after LT would be important and this is one of our objectives for the future.

Tafamidis was not associated with the ^{99m}Tc-DPD scintigraphy result, but patients were taking the drug for a relatively short period of time.

There were few patients with Val30Met mutation without cardiomyopathy, so we cannot properly compare them with AC-TTRm patients. However, we observed that they were younger and were more frequently medicated with tafamidis. We excluded 5 patients that might have cardiomyopathy not identified using ventricular wall thickness criteria – in fact, in older patients without LT, radionuclide imaging may be more sensitive than echocardiography.

Our study confirmed that the estimated sensitivity of ^{99m}Tc-DPD scintigraphy in cases of AC-TTRwt is good. Compared with AC-TTRm, those patients were older and myocardial involvement was more exuberant: wall thickness and LV mass were higher, LV systolic dysfunction was more common, NT-proBNP levels were higher, the use of loop diuretics more frequent and more patients had HF hospitalizations. Even though a significant proportion of the patients had a history of hypertension and some had aortic stenosis, these pathologies probably coexisted.

We recognize the limitations of considering bidimensional wall thickness in echocardiography as the gold standard for the diagnosis, even in patients without resistant hypertension, aortic stenosis or AL amyloidosis. However, we followed the same criteria as other studies [72, 74, 78], so that results can be more easily compared.

In the few dubious cases that underwent a endomyocardial biopsy, the histological examination confirmed amyloid infiltration in all. Unfortunately, most patients did not have information from endomyocardial biopsies, cardiac magnetic resonance or strain, that would complement the diagnosis.

Nevertheless, a longer follow-up and validation with clinical events will be important and it is another of our goals for the future.

CONCLUSIONS

This is the era of multimodality imaging in Cardiovascular Medicine. Non-invasive techniques such as echocardiography, CMR and radionuclide imaging provide a particularly important pathophysiological insight in the area of cardiomyopathies and heart failure. They are fundamental in the diagnostic pathway, but also provide prognostic information and may even help us to monitor the response to treatment.

Computed tomography (CT) is also extremely valuable to identify coronary artery disease and to guide valvular or aortic interventions, but not so much for diagnosing cardiomyopathies, therefore, it was not the focus of this work.

Each imaging modality has some limitations (i.e., a poor acoustic window limits echocardiographic evaluation, a claustrophobic patient may not undergo CMR and there is exposure to radiation using nuclear imaging or CT). However, each modality also has several strengths. Echocardiography is widely available, affordable, can be performed virtually anywhere and carries no side effects. CMR provides outstanding spatial resolution and tissue characterization. Nuclear imaging can detect biological processes, namely inflammation and infiltration, making it particularly important in the setting of cardiomyopathies, and it is relatively inexpensive.

In recent years, imaging modalities have become more sophisticated and more sensitive to detect the precursor stages of different myocardial pathologies. Detecting myocardial disease at an earlier stage, when symptoms are not evident yet, can provide the benefit of an early intervention and better outcomes.

This thesis explored the role of non-invasive cardiac imaging in four real-world settings of unclear myocardial involvement.

In study **A)**, CMR provided important information regarding the diagnosis and prognosis of patients with sCA without a previously known etiology. A correct diagnosis can also have therapeutic implications. One could argue that dilated cardiomyopathy and hypertrophic cardiomyopathy could also be diagnosed with echocardiography; however, in almost a third of the cases, diagnoses such as myocardial infarction with spontaneous recanalization, myocarditis or arrhythmogenic cardiomyopathy would not have been established otherwise. Tissue characterization given by CMR allows detection of myocardial edema and focal scar, and more precise information about right ventricular systolic function, that carries prognostic implications. Therefore, after upstream ECG evaluation, blood analyses and angiography, CMR should be considered in the investigation of such patients.

In study **B)**, alcohol intake was positively associated with indexed left ventricular mass and end-diastolic volume, suggesting cardiac remodeling. However, on average, these values were still within the normal range, so in our sample of predominantly low to moderate consumption, alcohol intake posed limited cardiovascular risk.
No significant association between drinking alcohol and left ventricular ejection fraction was found. Left atrial diameter was associated with alcohol intake only in women and in black participants. However, overall there were no major differences in the relationship between alcohol consumption and echocardiographic parameters according to sex or race.
Drinking predominantly wine associated with less cardiac remodeling compared to beer or liquor, and there was a non-significant trend for a deleterious effect of binge drinking.

Study **C)** focused on RA patients without known cardiac disease, where we found a prevalence of subclinical ventricular dysfunction in 17% (SD in 4% and DD in 13%). In the contemporary era of RA treatment, diastolic dysfunction was significantly more frequent than in the general population.
Even though the 2016 guidelines simplified and homogenized diastolic classification, it remains a challenge in the real world: it could not be predicted using NT-proBNP dicothomically, oftentimes at least a parameter could not be determined and indeterminate diastolic function was frequent. Moreover, concordance between 2016 and 2009 guidelines for diastolic classification was poor.
Still, patients with any ventricular dysfunction had significantly higher NT-proBNP levels and lower functional capacity measured by the 6MWT.
Age was the most important and independent predictor of ventricular function in RA patients, particularly above 57 years old. Therefore, a screening strategy using TTE may be considered in older RA patients, particularly if a disease-modifying treatment for diastolic dysfunction or HFpEF becomes available.

Project **D)**
After reviewing treatment options for AC-TTR in study **D1**), at the time of this thesis redaction, only tafamidis has a double-blinded phase 3 randomized controlled trial that showed efficacy and safety in clinical cardiovascular outcomes. A significant improvement in mortality and hospitalizations was shown, particularly in early stages of HF. It is the only drug approved by the FDA and EMA for the treatment of AC-TTR.
However, a variety of options will probably become possible in the future and this field has experienced intense innovation in the last years. Patisiran showed promising

echocardiographic results, however a larger study focused on cardiac events is crucial. Such a study is also pending for inotersen. Diflunisal, doxycycline and green tea extract also need randomized clinical trials focused on AC-TTR.

When treating AC-TTR, we also need to acknowledge that these patients have a higher risk of developing rhythm disturbances or hypotension and some drugs that are used for other causes of heart failure should be avoided. Moreover, if a TTR mutation is found, that also carries implications to the other family members. Therefore, a correct diagnosis of AC-TTR is definitely important and the cornerstone for a correct treatment.

With that in mind, in study **D2**), we explored the role of radionuclide imaging in AC-TTR diagnosis. In our analysis, ^{99m}Tc-DPD scintigraphy seems insufficiently sensitive in early-onset AC-TTRm patients with Val30Met mutation. In those patients, further investigation is needed before excluding myocardial involvement.

In our study, ^{99m}Tc-DPD scintigraphy had a much better performance in late-onset or in wild-type AC-TTR, that represented the majority of the cases analyzed in previous studies.

Additional research is needed to explore what makes patients with Val30Met produce type A or B fibrils and what are the possible consequences in what concerns treatment.

We believe that the current algorithm for AC-TTR diagnosis [73] should take these findings into consideration and, in cases of early-onset Val30Met disease, grade 0 or 1 in ^{99m}Tc-DPD scintigraphy should not exclude the diagnosis. If there is ventricular wall thickening, further investigation should be pursued.

Additionally, we also showed that AC-TTR can develop after liver transplantation in early-onset Val30Met disease, usually more than a decade after transplantation, and that these patients should be carefully followed.

These studies address different clinical contexts, but they all show how cardiac imaging provides important non-invasive information for the diagnosis and prognosis of patients in scenarios of unclear myocardial involvement.

There are still several evidence gaps and we plan to continue our research on the identification of myocardial disease using cardiac imaging.

In the future, artificial intelligence can represent a paradigm shift in imaging research. The use of big data and machine learning can help us to interpret the exams and to identify the changes that better predict clinical outcomes.

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The important thing is not to stop questioning."

Albert Einstein

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