

Expert Opinion on Orphan Drugs

ISSN: (Print) (Online) Journal homepage: <https://www.tandfonline.com/loi/ieod20>

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To cite this article: Tamer Rezk, Marianna Fontana & Julian D. Gillmore (2021): A review of the criteria for non-invasive diagnosis of cardiac transthyretin amyloidosis, *Expert Opinion on Orphan Drugs*, DOI: [10.1080/21678707.2021.1898371](https://doi.org/10.1080/21678707.2021.1898371)

To link to this article: <https://doi.org/10.1080/21678707.2021.1898371>



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Published online: 12 Apr 2021.



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A review of the criteria for non-invasive diagnosis of cardiac transthyretin amyloidosis

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ABSTRACT

Introduction: Cardiac transthyretin (ATTR) amyloidosis is a progressive and fatal infiltrative cardiomyopathy (ATTR-CM) characterized by congestive cardiac failure, often with preserved left ventricular ejection fraction, and significant risk of conduction disease. Diagnosis is often delayed or missed due to poor specificity of echocardiography and the historical requirement for a histological diagnosis, frequently an endomyocardial biopsy.

Areas covered: Following a detailed literature review focusing on peer reviewed articles (Pubmed, Cochrane Library, Google Scholar), from 1995 to 2020, alongside international diagnostic guidelines and expert opinion in the field, this article will explore the current non-invasive diagnostic criteria for ATTR-CM including the role of transthoracic echocardiography, cardiac MRI, bone scintigraphy, and assessment for exclusion of a clonal dyscrasia.

Expert opinion: ATTR-CM is an emerging and increasingly diagnosed cause of heart failure, particularly in the elderly. Promising novel therapies make accurate and swift diagnosis of the disease vital. With the increasing use of cardiac MRI to investigate cardiomyopathy and repurposing of technetium-labeled-bone scintigraphy, clinicians are now often able to diagnose ATTR-CM without recourse to an endomyocardial biopsy.

ARTICLE HISTORY

Received 27 November 2020
Accepted 1 March 2021

KEYWORDS

Cardiac attr amyloidosis;
ATTR-CM; transthyretin; TTR;
noninvasive/non-invasive
diagnosis; bone scintigraphy

1. Introduction

Systemic amyloidosis is a rare disease caused by the extracellular deposition of amyloid, a fibrillar material, derived from a variety of precursor proteins, that aggregate with a highly abnormal cross B sheet confirmation [1,2]. Amyloid deposition is associated with disruption of tissue structure and disturbance of organ function, which if left unchecked, invariably leads to organ failure [3,4]. Cardiac amyloidosis (CA) classically manifests with restrictive cardiomyopathy, is a fatal condition, associated with significant morbidity and is often challenging to diagnose. In the majority of cases the amyloid fibril precursor protein is either a monoclonal immunoglobulin light chain (AL) [5] or transthyretin (ATTR); the latter unmutated, wild-type transthyretin amyloidosis (wtATTR), or mutated, variant transthyretin amyloidosis (vATTR) [6–8].

The cardiac phenotype typically includes excessive ventricular wall thickening leading to diastolic dysfunction and subsequent congestive cardiac failure, often complicated by syncope and arrhythmias. Extra-cardiac manifestations are common in systemic AL amyloidosis due to infiltration of other visceral organs by amyloid. In patients with vATTR the clinical phenotype can be dominated by cardiomyopathy (vATTR-CM), polyneuropathy (vATTR-PN) or may include both cardiomyopathy and polyneuropathy to varying degrees (vATTR-mixed).

Until recently, diagnosis of systemic amyloidosis was focused upon biopsy of a clinically affected or ‘target’ organ and staining

with Congo red dye demonstrating pathognomonic green birefringence when visualised under cross-polarized light. Nonetheless, when there is a clinical suspicion of systemic amyloidosis, a ‘screening’ biopsy from subcutaneous fat, salivary gland, or rectum yields the diagnosis in 50 to 80% of patients with systemic AL amyloidosis [9]. Unfortunately in patients with ATTR amyloidosis, screening biopsies demonstrate a lower yield thus frequently resulting in the need for an endomyocardial biopsy (EMB) to confirm the diagnosis [10]. EMB are not routinely performed outside specialist centres in the UK due to the need for technical expertise which can often lead to a delay in diagnosis [11]. Risks include myocardial perforation and cardiac tamponade, the latter of which can be fatal.

CA has been recently recognised as an underdiagnosed cause of heart failure and in combination with advances in imaging modalities including repurposed bone scintigraphy and cardiac magnetic resonance imaging (CMR) over the last ten years, has enabled earlier diagnosis, improved understanding of pathogenesis, and the capacity to track disease in response to novel amyloid-specific therapies.

2. Background

2.1. Methodology

This article explores the available literature on ATTR amyloidosis focusing on the noninvasive/non-invasive diagnosis of

Article highlights

- (1) ATTR-CM is a progressive and fatal infiltrative cardiomyopathy typically manifesting with LV thickening and diastolic HF.
- (2) wtATTR-CM classically presents in elderly Caucasian men in their sixth, seventh or eighth decade with a preceding history of carpal tunnel syndrome and/or spinal stenosis.
- (3) Diagnosis is often missed or delayed due to the poor specificity of echocardiography and the absence of cardiac histology.
- (4) A combination of CMR, repurposed radionuclide 'bone' scintigraphy, and biochemical analyses permit non-invasive diagnosis of ATTR-CM with high specificity.
- (5) Recent advances in disease modifying therapies make the accurate and rapid diagnosis of ATTR-CM imperative. This box summarizes key points contained in the article.

ATTR-CM. A focused and detailed literature search was performed concentrating on peer reviewed peer-reviewed articles, of sufficient power and relevance, on scientific databases (Pubmed, Cochrane Library Library, and Google Scholar) from 1995 onwards alongside; book chapters, international diagnostic guidelines as well as expert opinion in the field of ATTR-CM. This article will focus on the key elements pertaining to the current noninvasive non-invasive diagnostic criteria for ATTR-CM including the prevalence and phenotype of the disease, role of transthoracic echocardiography, cardiac MRI,

bone scintigraphy, and assessment for exclusion of a clonal dyscrasia.

2.2. Prevalence

Cardiac ATTR amyloid deposits have been reported in autopsy series, in up to a quarter of individuals over the age of 80 years [12], although often the quantity of amyloid was small [13] and the presence of ATTR cardiomyopathy was not demonstrated. Nonetheless, postmortem post-mortem studies have shown that cardiac amyloid deposition is commoner in patients with heart failure and preserved ejection fraction (HFpEF), than in an age-matched autopsy group without heart failure, with a more balanced gender split than is reported in clinical practice.

Diagnoses of wtATTR-CM have risen several fold in recent decades, with prevalence estimates of 10–16% in elderly patients presenting with HFpEF [14,15]. wtATTR-CM has a male preponderance and presents with a predominant cardiac phenotype although can often be preceded by lumbar canal stenosis, carpal tunnel syndrome, and/or tendinopathy [8,16]. This is in stark contrast to the phenotype of vATTR amyloidosis which classically presents at a younger age with a varied and often mixed presentation of peripheral neuropathy, autonomic neuropathy, and/or cardiomyopathy [17]. There are 132 known causative TTR mutations [18], the commonest encoding V122I (p. V142I) which is present in 3.43% of US African Americans, resulting in a clinical phenotype which closely mimics wtATTR-CM [19]. It is estimated that

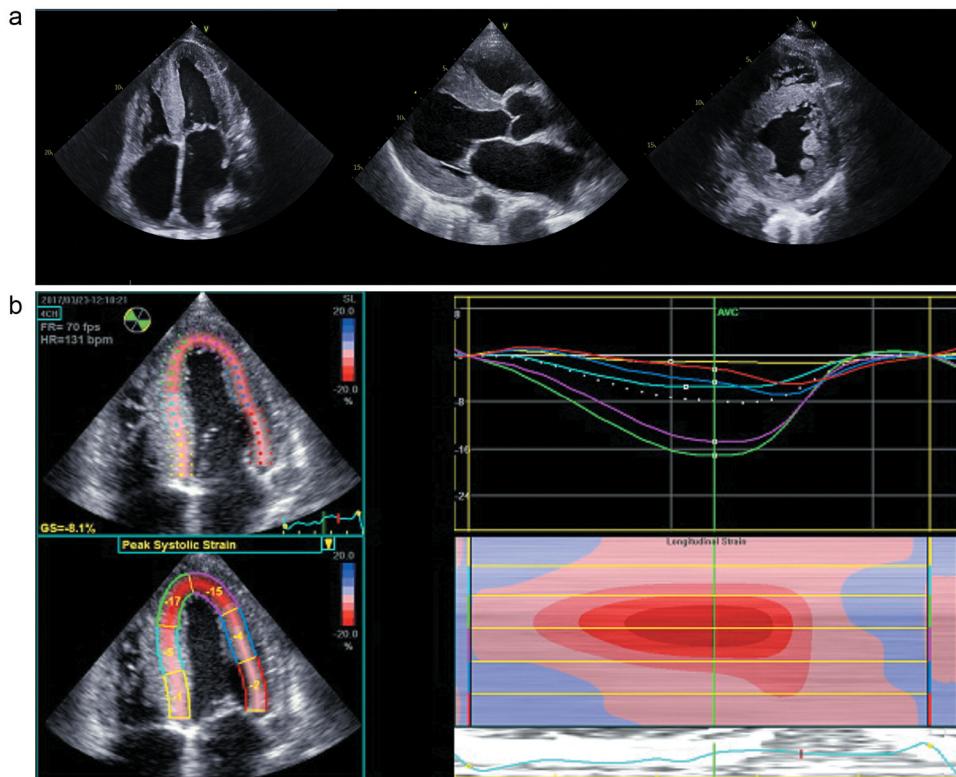


Figure 1. Transthoracic echocardiogram (TTE) of a patient with cardiac ATTR CM. TTE demonstrating concentric left ventricular hypertrophy (left to right) 4 Chamber view, parasternal and parasternal short axis view. 4 Chamber global longitudinal strain demonstrating a 'bull's eye pattern', characteristic of cardiac amyloidosis.

approximately 2 million people in the US are carriers of this variant, and thus at risk of developing ATTR-CM. Whilst patients with vATTR due to certain mutations may experience disabling autonomic neuropathy, cardiac involvement remains the key prognostic determinant in ATTR amyloidosis, with a median survival of 3–6 years from diagnosis [20].

3. Imaging

3.1. Echocardiography

Echocardiography, whilst the most accessible and commonly used tool for investigating heart failure, is neither specific nor sensitive for the diagnosis of CA [21]. Amyloidosis is characterized by thickened biventricular walls including left ventricular (LV) wall thickness greater than 12 mm and small, non-dilated ventricles (figure 1A). There is a propensity towards a symmetrical increase in LV wall thickness in AL CA, while ATTR-CM has been reported to manifest with a more asymmetrical pattern [22,23], with a sigmoid septal morphology in 70% and septal curvature inversion in up to 30% of cases [22]. Echocardiographic features of CA include thickened and at times sparkling appearance of the interatrial septum and valves as well as the classical ‘speckled’ myocardium. Extracellular amyloid infiltration leads to stiffening, impaired relaxation, and dysfunction of the ventricles, which in combination with atrial infiltration by amyloid, can lead to atrial dilatation, reduced blood flow, and a higher risk of thrombus formation [24–27]. Despite CA being categorised as a cause of HFpEF, there is typically both systolic and diastolic dysfunction [28]. Ejection fraction is a poor marker of systolic function in CA as it reflects radial contraction which is often not affected until the later stages of disease. Longitudinal function is classically impaired prior to radial contraction in CA and indices of longitudinal function such as longitudinal strain (LS) measurement by tissue Doppler and echocardiographic speckle tracking are important tools for diagnosing CA, demonstrating the distinctive appearance of a ‘bulls eye pattern’ due to apical sparing (figure 1B) as well as helping to distinguish CA from other hypertrophic cardiomyopathies [29]. Diastolic function is almost always impaired in CA and can span from impaired relaxation to restrictive filling defects [30]. Other markers of diastolic dysfunction can additionally be used to increase suspicion of CA, with TDI of the mitral annulus often being less than 6 cm/s [31].

3.2. Cardiac magnetic resonance imaging (CMR)

A now well-established imaging modality, particularly for investigation of cardiomyopathy, CMR provides unparalleled accuracy on cardiac morphology as well as myocardial tissue characterisation (figure 2). The deposition of amyloid fibrils leads to expansion of extracellular volume, which is well visualised by delayed or ‘late gadolinium enhancement’ (LGE) following administration of gadolinium-based contrast agents. Gadolinium passively accumulates extracellularly, giving rise to diffuse subendocardial or transmural LGE in CA and abnormal myocardial and blood pool gadolinium kinetics, described over 15 years ago [32]. There are three main patterns of LGE recognised; none (figure 3A), sub-endocardial (figure 3B) and transmural LGE (figure 3C), with transmurality showing good correlation with the extent of myocardial infiltration [33].

An important consideration when using gadolinium-based contrast agents (GBCA) is the association with nephrogenic systemic fibrosis (NSF), a grave and potentially fatal condition. Whilst the risk of developing NSF is strongly related to baseline renal excretory function (the highest risk associated with an eGFR < 30 mL/min), the chemical structure of the contrast agent contributes to the risk of NSF. American College of Radiology guidelines recommend the preferential use of Group II agents in patients with advanced CKD, emphasising the importance of a calculated assessment of both the benefits and risks of administering GBCA against the diagnostic challenges of performing a non-contrast scan. Whilst initially, the belief was that the gadolinium ion remained in a chelated state after intravenous administration, multiple studies have shown the presence of gadolinium deposits, in patients with normal renal function [34], in a range of tissues including neural matter (dentate nucleus, thalamus, pons, and globus pallidus) [35–37] and bone tissue [38]. To date, the clinical significance of this remains unclear; furthermore an additional challenge of LGE is that in light of its non-quantitative nature, its use to track disease over time remains limited.

T1 mapping which acts by direct quantification of an intrinsic myocardial signal, the longitudinal relaxation time has been used to overcome the limitations of GBCA. Pre-contrast or native myocardial T1 informs clinicians of disease severity by assessing both the degree of amyloid infiltration alongside both diastolic and systolic impairment (figure 4)[39]. Key benefits of native myocardial T1 are the accuracy with which it can diagnose ATTR-CM as well as its role as an early disease

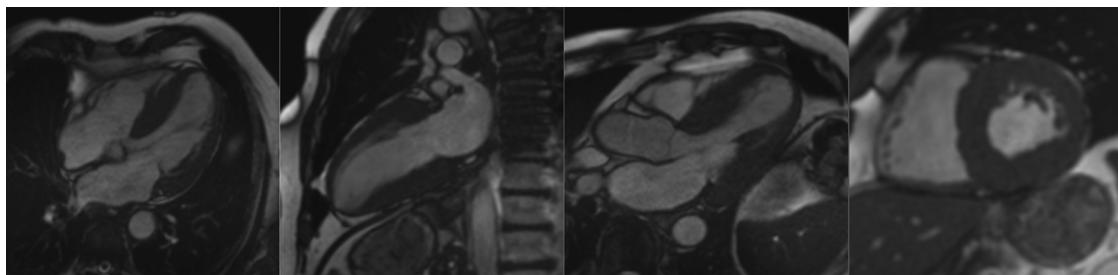


Figure 2. Cardiac MRI of a patient with ATTR CM (left to right) steady-state free precession cine; four chamber, two chamber, three chamber and short axis view demonstrating concentric LV hypertrophy.

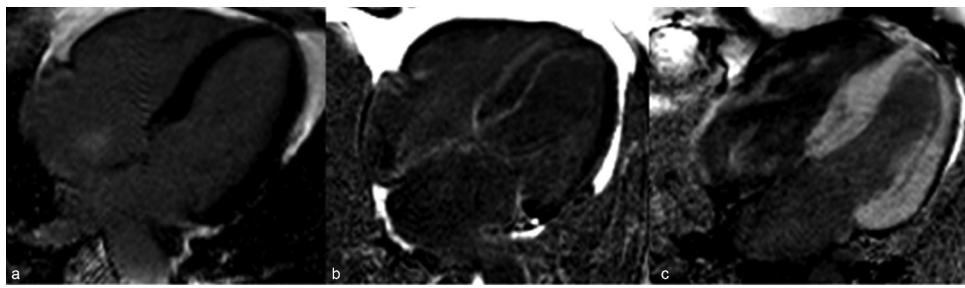


Figure 3. Cardiac MRI with phase sensitive inversion recovery reconstruction late gadolinium enhancement images demonstrating A) No LGE B) Sub-endocardial LGE and C) transmural LGE.

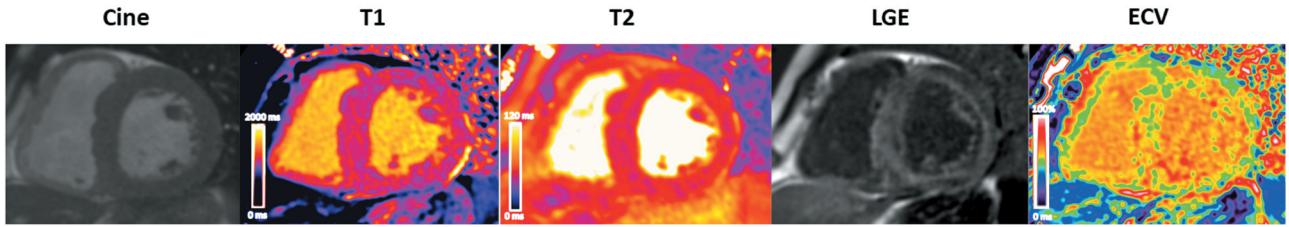


Figure 4. Cardiac MRI short axis steady-state free precession cine (left to right) with corresponding native myocardial T1 map, corresponding myocardial T2 map, corresponding phase sensitive inversion recovery late gadolinium enhancement image and corresponding ECV map.

marker, which often precedes left ventricular hypertrophy or LGE [39,40].

Extracellular volume (ECV) allows quantification of amyloid deposition in the myocardium and has been shown to correlate well with other markers of disease severity in both ATTR-CM and AL-CM [22,41]. The ECV is globally increased, with values frequently >40% in ATTR-CM (figure 4). Novel features of ECV measurement in CA include its distinctive ability to diagnose early disease, quantify the burden of amyloid deposition, and thus track disease change over time [42], a phenomenon recently reported with novel small interfering RNA therapies in ATTR-CM [43].

In conclusion, characterisation of myocardial tissue by CMR in CA provides clinicians with a detailed understanding of the multiple and varied disease processes that exist in CA, challenging the notion that CA's main pathology is that of tissue deposition. Exemplary of this, T2 relaxation time is a time constant representing the decay of transverse magnetization and detects oedema in a range of pathologies including myocardial infarction, myocarditis, and Takotsubo cardiomyopathy [44]. This has helped our understanding of CA as a heterogeneous condition with a range of contributing pathologies and was demonstrated by the discovery that T2 levels were found to be higher in patients with untreated AL CA compared with treated AL and ATTR-CM (figure 4); thereby demonstrating the potentially important pathophysiological and prognostic role of oedema [45].

3.3. Radionuclide 'bone' scintigraphy

It is well recognised that radionuclide 'bone' scintigraphy with technetium labelled bisphosphonates localizes to cardiac

amyloid deposits, although the reason for this remains unclear [46]. ^{99m}Tc -labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD), ^{99m}Tc -labelled pyrophosphate (PYP/PYP), and ^{99m}Tc -labelled-hydroxymethylene diphosphonate (HMDP) are both extremely sensitive (99%) and specific (86%) at detecting cardiac ATTR amyloid [47] and may have a role in differentiating it from other cardiomyopathies with similar echocardiographic features, such as hypertrophic cardiomyopathy [48–52]. Indeed, radionuclide 'bone' scintigraphy may even identify cardiac ATTR amyloid deposits early in the course of the disease, sometimes preceding abnormalities on echocardiography or CMR [40,53]. Whilst its use has been reported for the detection of ATTR amyloidosis among patients with known HFpEF [54], cardiac localization is reported in a proportion of patients with cardiac AL amyloidosis, and whilst usually low grade, it may be grade 2 or 3 such that bone scintigraphy alone is unable to establish a diagnosis of ATTR CA and exclude AL CA [51].

The role of bone scintigraphy as one component of an algorithm-based method for non-biopsy diagnosis of ATTR CM was highlighted in a seminal multicentre study. This diagnostic algorithm has been widely adopted in clinical practice, but needs to be followed to the letter in order for a secure diagnosis of ATTR-CM to be established [47]. In a patient with heart failure and an echocardiogram or CMR suggesting amyloid cardiomyopathy, if the ^{99m}Tc -PYP/ ^{99m}Tc -DPD/ ^{99m}Tc -HMDP scan is reported to be grade 2 or 3 (figure 5A) and there is no evidence of a monoclonal protein by serum free light chain assay (i.e. the kappa/lambda ratio is within the normal polyclonal range) or by both serum and urine immunofixation, ATTR CM can be diagnosed without a biopsy (specificity and positive predictive value >98%) [55]. There are a number of challenges with the non-biopsy diagnostic



Figure 5. A) ^{99m}Tc labeled labelled DPD scintigraphy demonstrating Perugini Grade 2 cardiac uptake in a patient with ATTR-CM.

criteria for ATTR-CM including the 'normal' polyclonal serum free light chain ratio in the context of CKD which may be up to 3.1 [56]. Similarly, there have been very occasional reports of both false negative bone scans (usually in association with particular and extremely rare pathogenic TTR variants) and false positive bone scans (possibly due to prior myocardial infarction or cardiac contusions). Overall however, the non-biopsy diagnostic algorithm for ATTR-CM appears to be extremely sensitive and specific for cardiac ATTR amyloidosis. Bone scintigraphy, if used in conjunction with the above tests for a monoclonal protein, also has a role in excluding cardiac amyloidosis although cannot do so entirely. However, in patients in whom there is no evidence of a monoclonal protein by serum free light chain assay together with negative serum and negative urine immunofixation, the absence of cardiac uptake on ^{99m}Tc -PYP (^{99m}Tc -Technetium labeled labelled pyrophosphate, ^{99m}Tc -DPDDPD, or ^{99m}Tc -HMDP (^{99m}Tc -Technetium labeled labelled hydroxymethylene diphosphonate) makes CA unlikely, although cannot exclude rare forms of cardiac amyloidosis such as apolipoprotein A-IV or apolipoprotein A-I amyloidosis. Similarly, if a patient has evidence of a plasma cell dyscrasia in the context of grade 2 or 3 cardiac uptake on bone scintigraphy, the differential diagnosis includes both cardiac AL and cardiac ATTR amyloidosis, such that definitive histological identification of the amyloid fibril protein by immunohistochemistry or proteomic analysis is required to establish the final diagnosis (figure 5B). Likewise, the presence of low grade (grade 1) uptake on a ^{99m}Tc -PYP/ ^{99m}Tc -DPD/ ^{99m}Tc -HMDP

scan requires further elucidation of the amyloid fibril protein and may be associated with cardiac AL, cardiac AApoAI AApoAI, or cardiac AA amyloidosis [47,57,58].

The use of radionuclide 'bone' scintigraphy to assess extra-cardiac involvement is a recently discovered and evolving phenomenon in patients with systemic amyloidosis. A typical pattern of muscle and soft-tissue uptake of ^{99m}Tc -DPD has been reported [58] and has been further characterised by the evidence of amyloid deposition (by soft tissue biopsy) in a series of positive patients [59]. Lung uptake may be present on ^{99m}Tc -HMDP scintigraphy [60], although the clinical implications of such findings remain poorly understood.

4. Investigations for Clonal Disease

In systemic AL amyloidosis, deposits are comprised of monoclonal immunoglobulin light chains, driven by an often subtle underlying clonal dyscrasia. Thus in any patient suspected of systemic amyloidosis, a complete clonal assessment should be undertaken. Whilst monoclonal proteins can be detected in the serum and/or urine by electrophoresis and immunofixation (IFE) it is imperative that serum free light chain assay is also tested in all suspected patients since in up to 20% of patients with systemic AL amyloidosis; serum or urine IFE alone may not detect a monoclonal protein. Nonetheless it has been demonstrated that fully quantitative high sensitivity serum free light chain immunoassay (Freelite) has improved the sensitivity of detection of an underlying clone [61]. The presence of a

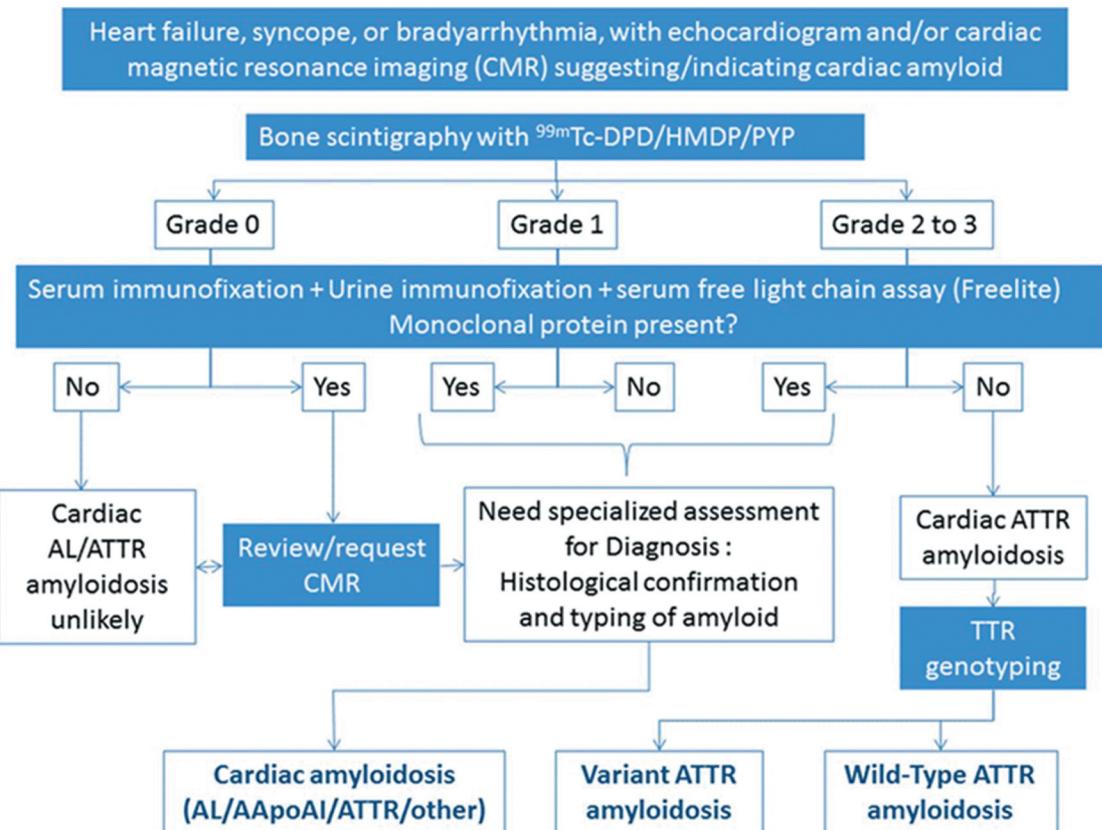


Figure 5b. B)Diagnostic algorithm for the noninvasive/non-invasive diagnosis of ATTR-CM.

monoclonal protein is defined as an abnormal FLC ratio (<0.26 or > 1.65 in the context of a normal eGFR, and >3.1 in the context of advanced CKD) on serum freelite assay, or the presence of a monoclonal band on IFE of serum or urine. Recent data indicates that <1% of patients with systemic AL amyloidosis have no identifiable clonal marker on the basis of these three assays [47]. Furthermore, the incidence of monoclonal gammopathy of unknown significance (MGUS) has been estimated to be ~5% in patients aged greater than 70 years and 7.5% in those 85 years or older [62], which is particularly relevant in patients with wtATTR-CM who are often in their seventh or eighth decade and in whom the clonal disease may be incidental to the ATTR amyloid rather than underlie AL amyloid. For this reason, all patients with high grade cardiac uptake on bone scintigraphy who have a clonal disease should have the amyloid type confirmed histologically, often via endomyocardial biopsy, in order to prevent inappropriate delivery of potentially toxic chemotherapy for presumed cardiac AL amyloidosis to patients who in fact have ATTR-CM [63].

5. Conclusion

In summary, cardiac ATTR amyloidosis can be reliably diagnosed in the absence of histology provided that all of the following criteria are met;

- Heart failure with an echocardiogram or CMR that is consistent with or suggestive of amyloidosis
- Grade 2 or 3 cardiac uptake on a radionuclide scan, using either ^{99m}Tc-DPD, ^{99m}Tc-PYPPYP, or ^{99m}Tc-HMDP
- Absence of a detectable monoclonal protein despite serum and urine IFE, and sFLC (Freelite) assay

Histological confirmation and typing of amyloid should be sought in all cases of suspected cardiac amyloidosis in which these criteria are not met, ideally with an endomyocardial biopsy.

6. Expert Opinion

Whilst improved diagnostic techniques including work up for a clonal disorder, cardiac imaging, and repurposed scintigraphic assessments have made the non-invasive diagnosis of ATTR-CM easier, it remains a rare and challenging disease to diagnose. Misdiagnosis most commonly takes the form of incorrect typing of amyloid such that chemotherapy is inappropriately administered for mistakenly identified cardiac AL amyloidosis or alternatively, not administered for mistakenly identified ATTR-CM. In patients with a clonal disorder and high-grade cardiac uptake on radionuclide imaging,

histological typing of amyloid, usually from an endomyocardial biopsy, remains essential.

Typically, wtATTR-CM (the commonest presentation of ATTR-CM) is a disease of elderly Caucasian men, who often give a history of sporting excellence or activity, with a preceding history of carpal tunnel syndrome or less commonly, spinal stenosis. Whilst vATTR secondary to V122I presents with a similar clinical phenotype to wtATTR, the key differentiator is that of African or Afro-Caribbean heritage and less of a male preponderance. Presence of the V122I allele has been shown to be a substantial risk factor for the development of ATTR-CM [64]. This is in contrast to cardiac AL amyloidosis which is present in up to 60% of patients diagnosed with systemic AL amyloidosis but is often accompanied by extra-cardiac manifestations including soft tissue amyloid (manifesting as macroglossia) as well as proteinuric renal insufficiency and tends to manifest with a more severe and symptomatic amyloid cardiomyopathy. Despite this, differentiating between ATTR-CM and AL-CM in the absence of a histological diagnosis can be made more challenging by a number of factors. One such factor which frequently accompanies ATTR-CM is CKD. Progressive renal impairment is typically associated with disproportionate retention of kappa as opposed to lambda LC such that the normal polyclonal FLC ratio may be up to 3.1 in advanced CKD (eGFR <30 ml/min). Correcting the normal range for FLC ratio in CKD can help avoid the need for unnecessary endomyocardial biopsies [56].

ATTR-CM is an emerging disease which may very well be a common cause of HFpEF in the elderly. Whilst not explored in this article, there have been a number of recent advances in treatment options for patients with ATTR-CM (both wild-type and variant) highlighting further the importance of accurate and rapid diagnosis of the disease.

In the coming years, with the increased use of both CMR and radionuclide 'bone' scintigraphy to diagnose and characterise cardiomyopathies and increasing awareness of cardiac amyloidosis within cardiology and geriatric communities alike, our prediction is that many more patients will attain an accurate and non-invasive diagnosis of ATTR-CM with only the more complex and challenging cases such as those who require an endomyocardial biopsy being referred to expert centres.

Funding

This paper was not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

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