Non-biopsy diagnosis of cardiac transthyretin amyloidosis

Short Title: Gillmore, Diagnosis of cardiac ATTR amyloidosis

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Abstract

Background

Cardiac transthyretin (ATTR) amyloidosis is a progressive and fatal cardiomyopathy for which several promising therapies are in development. The diagnosis is frequently delayed or missed due to limited specificity of echocardiography and the traditional requirement for histologic confirmation. It has long been recognised that technetium labelled bone scintigraphy tracers can localise to myocardial amyloid deposits and use of this imaging modality for diagnosis of cardiac ATTR amyloidosis has lately been revisited. We conducted a multicentre study to ascertain the diagnostic value of bone scintigraphy in this disease.

Methods and Results

Results of bone scintigraphy and biochemical investigations were analysed from 1217 patients with suspected cardiac amyloidosis referred for evaluation in specialist centers. Among 857 patients with histologically proven amyloid (374 with endomyocardial biopsies), and 360 patients subsequently confirmed to have non-amyloid cardiomyopathies, myocardial radiotracer uptake on bone scintigraphy was >99% sensitive and 86% specific for cardiac ATTR amyloid, with 'false positives' almost exclusively from uptake in patients with cardiac AL amyloidosis. Importantly, the combined findings of grade 2 or 3 myocardial radiotracer uptake on bone scintigraphy and absence of a monoclonal protein in serum or urine had a specificity and positive predictive value for cardiac ATTR amyloidosis of 100% (PPV CI 98.0-100).

Conclusions

Bone scintigraphy enables the diagnosis of cardiac ATTR amyloidosis to be made reliably without need for histology in patients who do not have a monoclonal gammopathy. We

propose non-invasive diagnostic criteria for cardiac ATTR amyloidosis that are applicable to the majority of patients with this disease.

Key Words: amyloid, cardiomyopathy, genetics, hypertrophy, transthyretin, ^{99m} technetium, scintigraphy

Introduction

Cardiac amyloidosis is a rare form of restrictive cardiomyopathy which is often challenging to diagnose and is almost always associated with a poor prognosis. The causative amyloid fibril deposits are of monoclonal light chain (AL)[1] or transthyretin (ATTR)[2-4] types in the vast majority of cases. ATTR amyloidosis may be acquired, associated with wild-type transthyretin (previously known as senile systemic amyloidosis), or hereditary, associated with variants in the transthyretin gene. Clinical features are varied, and whilst heart failure symptoms predominate, suspicion of cardiac amyloidosis can also be prompted by syncope, arrhythmias or unexplained left ventricular (LV) wall thickening on echocardiography. Diagnosis of amyloidosis is usually obtained through biopsy of a clinically affected organ, with Congo red histology demonstrating pathognomonic green birefringence. However, when amyloidosis is suspected clinically, biopsy of subcutaneous fat, salivary gland or rectum yields the diagnosis in 50-80 percent of patients with AL amyloidosis.[5] A much lower yield in patients with ATTR amyloidosis frequently results in a requirement for endomyocardial biopsy (EMB) to confirm the diagnosis.[6] EMB is associated with a risk of complications, including myocardial perforation and tamponade which may be fatal, and requires expertise that can introduce diagnostic delay.[7]

Autopsy studies have shown the presence of cardiac ATTR amyloid deposits in up to 25% of individuals over 80 years of age, although in many of these hearts the amount of amyloid was small.[8] Nevertheless, among patients with heart failure and preserved ejection fraction (HFpEF), post-mortem examination indicates that cardiac amyloid deposition is commoner than in an age-matched autopsy group without heart failure. The majority of patients with cardiac amyloid on post-mortem in these studies had not had amyloidosis diagnosed during life.[9] Echocardiography, whilst a valuable and widely accessible tool for investigating heart failure, is neither sensitive nor specific for cardiac amyloidosis.[10] Typical

findings on echocardiography include thickening of ventricular walls, restrictive filling, abnormal left and right ventricular longitudinal strain and atrial septal thickening.[11] Cardiac magnetic resonance imaging (CMR) has much greater diagnostic value in cardiac amyloidosis but false positive and false negative CMRs are not infrequent.[12] Typical findings include restrictive morphology, abnormal gadolinium kinetics and extracellular volume expansion on T1 mapping.[11] Furthermore, CMR is costly and only available in specialist centers, is contraindicated in a substantial proportion of patients, and cannot reliably distinguish different types of amyloid.[13, 14] To date, definitive diagnosis of cardiac amyloid requires histologic confirmation and typing of amyloid, recognizing issues of sampling error and disease expertise needed for proper histologic interpretation.[15] All of these factors frequently contribute to delay in diagnosis, which is critical given the poor prognosis of cardiac ATTR and AL amyloidosis and the increasing availability of therapies for both diseases.[4, 16, 17] Thus, there is a major unmet need to diagnose and characterise cardiac amyloidosis at the earliest opportunity, noting that cardiac ATTR amyloidosis in particular is probably much underdiagnosed and fast becoming a treatable cause of heart failure.[18, 19]

Radionuclide 'bone' scintigraphy with technetium labelled bisphosphonates has long been anecdotally reported to localize to cardiac amyloid deposits, although the molecular basis for this remains unknown.[20] Recent systematic evaluation of bone scintigraphy suggests that ^{99m}Tc-labeled 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD), ^{99m}Tc-labeled pyrophosphate (PYP) and ^{99m}Tc-labeled-hydroxymethylene diphosphonate (HMDP) may be remarkably sensitive and specific for imaging cardiac ATTR amyloid and may reliably distinguish other causes of cardiomyopathy that mimic amyloid such as hypertrophic cardiomyopathy.[21-25] Indeed, radionuclide 'bone' scintigraphy may identify cardiac ATTR amyloid deposits early in the course of the disease, sometimes before development of abnormalities on echocardiography or CMR,[26, 27] and has been used to diagnose ATTR

amyloidosis among patients with HFpEF.[28] Cardiac localization of radiotracer does occur in a small proportion of patients with AL amyloidosis, and although usually low grade, it can confound distinguishing between cardiac ATTR and AL types of amyloid.[24]

In October 2014, we established a collaboration of clinicians and scientists from internationally renowned centers with expertise in clinical amyloidosis to determine whether radionuclide 'bone' scintigraphy in conjunction with other imaging methods and non-invasive laboratory investigations might enable diagnosis of cardiac ATTR amyloidosis without need for confirmatory biopsy. Our findings presented here form the basis for a proposed diagnostic algorithm for patients with suspected cardiac amyloidosis in which non-biopsy diagnosis of ATTR amyloidosis can be achieved in the majority of cases.

Methods

Patients

The subjects comprised patients with suspected or histologically proven amyloidosis who had been referred for evaluation to the following specialist amyloidosis centers in Europe and the US: UK National Amyloidosis Centre; Amyloidosis Research and Treatment Center, Pavia and University of Bologna in Italy; Amyloidosis Mondor Network in France; University Medical Center, Groningen in The Netherlands; Columbia University Medical Center, Brigham and Women's Hospital Cardiac Amyloidosis Program, Boston, MA, Mayo Clinic, Rochester, MN, and Amyloidosis Center, Boston University in the USA. All patients with suspected or proven cardiac ATTR amyloidosis underwent diagnostic investigations including bone scintigraphy, immunofixation electrophoresis (IFE) of serum and urine and serum free light chain assay. All patients underwent echocardiography in their respective specialist amyloidosis center; previous CMR images were reviewed, and new CMRs were performed in patients according to local clinical practice. Final diagnosis was based on the

results of these tests accompanied by histologic and genetic findings. A minority of the subjects had been included in small prior published series from the individual centers. The study received ethical approval.

Echocardiography and Cardiac Magnetic Resonance imaging at Specialist Center

Detailed echocardiography was performed at the specialist amyloid centers and included an extensive analysis of left and right ventricular wall thickness, LV function including global and regional longitudinal strain and atrial parameters. Diastolic function was analysed in detail by tissue doppler imaging. Features that were characteristic of amyloid were defined as the combined findings of thickened LV walls, impaired global strain with relative sparing of the apical region in comparison to the base of the heart, and abnormal diastolic physiology.

CMRs were performed at the specialist amyloid centers as previously described.[12, 13] Features that were characteristic of amyloid were defined as presence of diffuse subendocardial or transmural late gadolinium enhancement coupled with abnormal myocardial and blood-pool gadolinium kinetics.

Bisphosphonate (^{99m}Tc-DPD/^{99m}Tc-PYP/^{99m}Tc-HMDP) Scintigraphy

Patients were scanned after intravenous injection of ~700 MBq of ^{99m}Tc-DPD (n=877), ^{99m}Tc-PYP (n=199) or ^{99m}Tc-HMDP (n=141), providing an expected radiation dose of ~5 mSv per patient. Whole body planar images were acquired 3 h post-injection (with the exception of some ^{99m}Tc-PYP images in which thoracic planar images were acquired 1 h post-injection). Images were acquired using low energy, high-resolution collimators and a scan speed of 10 cm/min (^{99m}Tc-DPD and ^{99m}Tc-HMDP) or for a total of 750000 counts (^{99m}Tc-PYP).[24]

Cardiac retention of all ^{99m}Tc-DPD and ^{99m}Tc-HMDP scans was defined by a single reader at each center according to the grading devised by Perugini *et al*.[21] ^{99m}Tc-PYP was scored by a single reader at each center using the following grading system: Grade 0 – absent cardiac uptake; Grade 1 – mild uptake less than bone; Grade 2 - moderate uptake equal to bone; Grade 3 - high uptake greater than bone.

Histology, Immunohistochemistry and Proteomic analysis

All formalin fixed paraffin-embedded biopsies were stained with Congo red dye and viewed under crossed polarized light. Immunohistochemical staining of all amyloid deposits was performed using monospecific antibodies reactive with serum amyloid A protein (SAA), kappa and lambda immunoglobulin light chains and transthyretin, as previously described,[29] and where necessary, apolipoprotein A-I, and fibrinogen Aα-chain. Where required, Congo red positive deposits were stained and viewed by immunoelectron microscopy or microdissected for proteomic analysis, as previously described.[30]

All biopsy specimens in which amyloid deposits were not detected by Congo red histology were further examined by light microscopy following staining with hematoxylin and eosin (H&E) and Masson's trichrome, and wherever possible, additionally examined by electron microscopy.

Monoclonal protein studies

Presence of a monoclonal protein was sought by IFE of serum and urine and by serum free light chain (sFLC) assay (Freelite, The Binding Site)[31, 32] in all patients. Presence of a monoclonal protein was defined as an abnormal FLC ratio (< 0.26 or > 1.65) on serum Freelite assay, or presence of a band on IFE of serum or urine.

Genetic testing

All patients with cardiac ATTR amyloid underwent sequencing of the *TTR* gene. DNA was extracted from whole blood, amplified by polymerase-chain-reaction assay and the whole coding region of the transthyretin (*TTR)* gene was sequenced, as previously described.[33]

Statistical analyses

Sensitivity and specificity of radionuclide scan findings accompanied by positive and negative predictive values for the proposed diagnostic criteria (including 95% confidence intervals) were calculated using IBM SPSS Statistics 22 software.

Results

Patients

A total of 1498 patients were referred for evaluation of suspected cardiac amyloidosis to the relevant specialist amyloidosis centers. EMBs were performed in a total of 374 patients, 327 with amyloid and 47 without amyloid. Among 1124 remaining patients in whom EMBs were not performed, 391 cases had cardiac amyloidosis on the basis of a characteristic echocardiogram +/- CMR from the specialist amyloidosis center and histological proof of amyloid in extra-cardiac tissue. There were 139 patients with histologically proven extra-cardiac amyloid without evidence of cardiac involvement by echocardiography +/- CMR. The remainder (n=360) had no histologic or cardiac imaging evidence of amyloid. Patients with cardiac imaging (echocardiogram +/- CMR) from the specialist amyloid center that was characteristic of amyloid but in whom histological proof of amyloid was lacking (n=281) were excluded from all analyses, due to diagnostic uncertainty. The diagnostic algorithm detailing selection of patients for analysis is shown in Figure 1. The final diagnoses among 1217 analysable patients (857 with amyloid and 360 without amyloid), established on the

basis of these investigations, coupled with immunohistochemical and/or proteomic typing of amyloid and genetic analyses, are shown in Table 1.

Histology

Amyloid was identified histologically in 857 patients from the following tissues; heart (n=327), fat (n=209), bowel (n=92), bone marrow (n=56), lymph node, including salivary gland (n=43), bladder (n=29), kidney (n=27), lung (n=20), nerve (n=14) and from 12 other sites (n=40). Of the 360 patients without amyloid, 47 had EMBs which were negative for amyloid by Congo red staining.

Radionuclide 'bone' scintigraphy with ^{99m}Tc-DPD, ^{99m}Tc-PYP, or ^{99m}Tc-HMDP

Radionuclide scans were validated against Congo red histology of an EMB, which is the current gold standard for diagnosis of cardiac ATTR amyloid, in 374 patients (261 with cardiac ATTR amyloid, and 113 without cardiac ATTR amyloid). The results for each of the three radionuclide tracers are shown in Table 2. These results indicate that the sensitivity of a positive (grade 1, 2 or 3 cardiac uptake) scan alone for detecting cardiac ATTR amyloid deposits is >99% (positive radionuclide scan in 259/261 patients) and the specificity of a positive scan (grade 1, 2 or 3 cardiac uptake) for cardiac ATTR amyloid deposits is 68% (negative radionuclide scan in 77/113 patients without cardiac ATTR amyloid) (Table 3). The low specificity of a positive scan for cardiac ATTR amyloid deposits was almost entirely due to cardiac uptake of tracer among patients with cardiac AL or cardiac AApoAI amyloidosis. The sensitivity and specificity of grade 2 or 3 cardiac uptake on radionuclide scan for cardiac ATTR amyloid deposits among the 374 patients in this cohort who underwent EMB was 91% and 87% respectively (Table 3). A single patient with cardiac ATTR amyloid deposits detected on EMB had a negative ^{99m}Tc-DPD scan (Table 2). Review

of this individual's case revealed scanty amyloid deposits on EMB, an interventricular septal thickness on echocardiography of only 10 mm, modest elevation of cardiac biomarkers (high sensitivity troponin 14 ng/ml and NT-proBNP 51 pmol/L) and a 6-minute walk test distance of 607 metres (120% of expected for age). This patient therefore had limited sub-clinical cardiac ATTR amyloid deposits rather than clinically significant cardiac ATTR amyloidosis. One patient referred with histologically proven cardiac ATTR amyloid had a negative ^{99m}Tc-PYP scan, but review of a sub-aortic myomectomy biopsy obtained at aortic valve replacement demonstrated only a single, tiny focus of sub-clinical amyloid. Of the two patients with no amyloid on EMB but with radionuclide scans that were grade 2 or higher, one had a Perugini grade 3 ^{99m}Tc-DPD scan and the other a grade 2 ^{99m}Tc-PYP scan (Table 2). Both of these patients had a plasma cell dyscrasia and an echocardiogram that was characteristic of amyloidosis, leading us to question whether the negative EMBs in both cases were 'false' negatives.

Radionuclide scintigraphy findings in all 1217 patients (including 374 patients who underwent EMB) corroborated the findings among those who underwent EMB and indicated that the sensitivity of a positive (grade 1, 2 or 3 cardiac uptake) scan alone for detecting cardiac ATTR amyloid deposits was >99% (positive scans in 528/530 with cardiac ATTR amyloid) with a specificity of 86% (negative scans in 591/687 patients without cardiac ATTR amyloid). The sensitivity of grade 2 or 3 cardiac uptake on a radionuclide scan for cardiac ATTR amyloid deposits was 90% with a specificity of 97%. The radionuclide scan findings for all 1217 patients are shown in Supplementary Table 1, and for each of the individual 'bone' tracers are shown in Supplementary Tables 2, 3 and 4. Sensitivity and specificity analyses for the whole cohort of 1217 patients are shown by individual tracer in Supplementary Tables 5, 6 and 7.

Monoclonal Protein studies

Among 237 with systemic AL amyloidosis in this cohort, 235 (99%) had evidence of a monoclonal protein by one or more of serum IFE, urine IFE and sFLC assay. Interestingly, among 562 patients from the cohort with ATTR amyloid, whose median age was 75 years, 107 (19%) cases had a detectable monoclonal protein using these very sensitive techniques.

The monoclonal protein findings presented here were corroborated by analysis of two separate, larger cohorts of patients with histologically proven systemic AL amyloidosis. Among 714 patients with systemic AL amyloidosis from the UK National Amyloidosis Centre, 98.9% had evidence of a monoclonal protein, and among 1465 patients from a collaborative project between The Amyloidosis Research and Treatment Center, Pavia, Italy and the Mayo Clinic, USA, 99.8% had a detectable monoclonal protein (data not shown).

Combined Radionuclide Scintigraphy and Monoclonal Protein findings

The combined finding of grade 2 or 3 cardiac uptake on radionuclide scintigraphy and the absence of a monoclonal protein by IFE of serum and urine and by serum FLC measurement, was 100% specific for presence of cardiac ATTR amyloid both in the subgroup of 374 patients who underwent EMB and in the whole cohort of 1217 patients (positive predictive value 100% (CI 98.0-100%)). Detailed sensitivity and specificity analyses are shown in Table 4.

Genotyping

Among 562 patients with ATTR amyloid (530 with cardiac involvement), all had their *TTR* gene sequenced. This was wild-type sequence in 304 cases. Thirty-five different pathogenic variants were identified among the remaining 258 patients.

Radionuclide scan results by individual amyloidogenic TTR variant are shown in Supplementary Table 8. These results indicate that radionuclide scan findings were consistent with that expected according to the clinical and echocardiographic findings as well as the known likelihood of cardiac involvement associated with individual TTR variants. For example, younger patients with V30M-associated ATTR amyloidosis have a predominant neuropathic phenotype without cardiac amyloidosis while older patients with V30M-associated amyloidosis can present with cardiac amyloidosis. The radionuclide scintigraphy findings among 48 patients with V30M-associated ATTR amyloidosis showed no cardiac uptake in 23 cases (median age 37 years, median interventricular wall thickness on echocardiography 10 mm), and grade 1, 2 and 3 uptake in 6, 18, and 1 patient respectively (median age 68 years, median interventricular wall thickness on echocardiography 15 mm among those with positive radionuclide scans). There did not appear to be any particular mutation in which there was a discrepancy between the clinical picture and radionuclide scan findings.

Discussion

The diagnosis of cardiac amyloidosis is often delayed or missed due to the poor sensitivity and specificity of echocardiography,[10] coupled with the current requirement for histologic confirmation of amyloid in a tissue biopsy. The diagnosis of wild-type cardiac ATTR amyloidosis is particularly challenging due both to it presenting in older age[34] (when confounding co-morbidities such as coronary heart disease, hypertension and aortic stenosis frequently co-exist), and to the absence of any specific extra-cardiac features or supportive biomarkers in the blood. This contrasts with variant ATTR amyloidosis and AL amyloidosis which are supported by the presence of a TTR gene mutation[33] or monoclonal immunoglobulin respectively.[31, 32] Currently, wild-type ATTR amyloidosis is mostly

diagnosed in men over 70 years and in fewer than three per million per year,[35] which contrasts hugely with the high frequency of ATTR amyloid deposits found at autopsy and the high prevalence in common syndromes such as HFpEF.[28] Despite the lack of a blood marker and the fact that myocardial biopsy is required for a definitive diagnosis, recent data from specialist amyloid centres suggest that wild-type ATTR amyloidosis is now diagnosed more frequently than variant ATTR amyloidosis.[7]

Our analyses of 374 patients with EMBs, corroborated in the whole cohort of 1217 patients, indicate that cardiac uptake (grade 1, 2 or 3) on a radionuclide 'bone' scan is >99% sensitive but not completely specific for cardiac ATTR amyloid (68% specificity compared to EMB histology), the low specificity resulting largely from low grade uptake in patients with cardiac AL or cardiac AApoAI amyloidosis.[36] The specificity for cardiac ATTR amyloid of grade 2 or 3 cardiac uptake on radionuclide imaging increases to ~87%, whilst the sensitivity falls to 91%. Since it is absolutely essential to avoid misdiagnosis of cardiac ATTR amyloidosis in a patient who actually has cardiac AL amyloidosis requiring chemotherapy, the primary aim of the proposed diagnostic criteria was to achieve very high diagnostic specificity. The specificity and positive predictive value for cardiac ATTR amyloid of the combination of grade 2 or 3 cardiac uptake on a radionuclide scan, and absence of a detectable monoclonal protein despite serum IFE, urine IFE and sFLC assay was 100% (PPV CI 99.0-100%) in this cohort of 1217 patients and was also 100% among each of the 3 different radiotracer cohorts. Although the authors acknowledge that further validation of ^{99m}Tc-HMDP scintigraphy against EMB histology is required, the presented preliminary data for ^{99m}Tc-HMDP, indicate that it behaves identically to ^{99m}Tc-DPD and ^{99m}Tc-PYP. The data clearly indicate that the combination of grade 2 or 3 cardiac uptake on a radionuclide 'bone' scan, and absence of a detectable monoclonal protein by serum IFE, urine IFE and sFLC (Freelite) assay is diagnostic of cardiac ATTR amyloid.

In a patient with symptoms of heart failure and an echocardiogram or CMR suggesting the possibility of amyloidosis, the combined findings listed above establish the diagnosis of cardiac ATTR amyloidosis without a requirement for positive Congo red histology. Patients with cardiac uptake on a radionuclide scan who have a monoclonal protein require additional diagnostic testing, including histologic or proteomic typing of amyloid. A diagnostic algorithm for cardiac amyloidosis based on the findings presented here is shown in Figure 2. A diagnosis of cardiac ATTR amyloidosis should be followed by *TTR* genotyping in all cases in order to differentiate between wild-type and variant ATTR cardiac amyloidosis. In patients with a family history of cardiac amyloidosis and wild-type *TTR* gene sequence, the authors would suggest additional sequencing of the apoAI gene to exclude the remote possibility of cardiac AApoAI amyloidosis.

It is noteworthy that 11 patients in the whole cohort of 1217 had low grade (1 or 2) cardiac uptake on radionuclide scintigraphy despite 'so called' absence of cardiac amyloidosis (i.e., 'false positive' scans). The majority did not undergo EMB and all but one with a known *TTR* mutation had undefined cardiomyopathies; it is thus likely that they did indeed have minor cardiac ATTR amyloid deposits without echocardiographic or CMR evidence of cardiac ATTR amyloidosis.

Importantly, the findings and recommendations documented here do not eliminate the need for histological demonstration and typing of amyloid among patients with cardiac amyloidosis generally. On the contrary, a monoclonal protein was detected in a surprisingly high proportion (19%) of patients with ATTR amyloidosis in this cohort (likely, at least in part, due to referral bias), and the sensitivity and specificity of grade 2 or 3 cardiac uptake on radionuclide scan for diagnosing cardiac ATTR amyloidosis as opposed to cardiac AL amyloidosis in patients who had a monoclonal protein were 92% and 91% respectively, highlighting the need for histological typing of amyloid by immunohistochemical staining

and/or proteomic analysis, often from an EMB, in any patient who does not fulfil the diagnostic criteria for cardiac ATTR amyloidosis proposed above.

It should be noted that the patients presented here were not unselected, but rather referred to specialist amyloid centers for evaluation of suspected amyloidosis. Further study is required to validate these findings within the general 'unselected' cardiology population.

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-PYP 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.

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Figure Legends

Figure 1. Selection of patients for analysis. Among 1217 'evaluable' patients, 374 underwent endomyocardial biopsy and 843 were diagnosed with presence and type or absence of amyloid on the basis of extra-cardiac histology coupled with echocardiography +/- cardiac magnetic resonance imaging (CMR). Patients in whom there was diagnostic uncertainty were excluded from all analyses (n=281).

Figure 2. Diagnostic algorithm for patients with suspected amyloid cardiomyopathy. Echocardiographic features suggesting/indicating cardiac amyloid include (but are not limited to) increased LV wall thickness, restrictive filling pattern, abnormal left and right ventricular longitudinal strain and atrial septal thickening. Features suggesting/indicating cardiac amyloid on CMR include (but are not limited to) restrictive morphology, abnormal gadolinium kinetics and extracellular volume expansion based on T1 mapping.

Table 1. Final diagnoses in patients with and without endomyocardial biopsy (EMB)

Patients	**EMB N=374 (31%)**	**No EMB† N=843 (69%)**	**All N=1217 (100%)**
Cardiac transthyretin (ATTR) amyloid	261	269	530
Cardiac AL amyloidosis	62	119	181
Cardiac Apolipoprotein A-I amyloidosis	2	3	5
Cardiac amyloidosis of unknown type	2	0	2
No cardiac amyloid	47	452	499
Amyloidosis without cardiac amyloid infiltration	*0*	139	*139*
Systemic AL amyloidosis, no cardiac involvement	*0*	*56*	*56*
Transthyretin amyloidosis, no cardiac involvement	*0*	*32*	*32*
AA amyloidosis, no cardiac involvement	*0*	*3*	*3*
ApoAI amyloidosis, no cardiac involvement	*0*	*2*	*2*
Fibrinogen A α-chain amyloidosis, no cardiac involvement	*0*	*3*	*3*
Gelsolin amyloidosis, no cardiac involvement	*0*	*1*	*1*
Lysozyme amyloidosis, no cardiac involvement	*0*	*1*	*1*
Amyloidosis of unknown fibril type, no cardiac involvement	*0*	*2*	*2*
Localised AL amyloidosis, no cardiac involvement	*0*	*39*	*39*
No amyloidosis	*47*	*313*	*360*
Hypertrophic CM (HCM)	*1*	*41*	*42*
Heart failure with preserved ejection fraction (HFPEF)	*8*	*52*	*60*
Hypertensive heart disease	*0*	*8*	*8*
Anderson-Fabry disease	*0*	*2*	*2*
Undetermined cardiomyopathy	*32*	*182*	*214*
TTR mutation carriers	*5*	*28*	*33*
Light chain deposition disease	*1*	*0*	*1*

†Presence/absence of cardiac amyloidosis determined on the basis of echocardiography within specialist centers +/- CMR

Table 2. Radionuclide 'bone' scintigraphy findings among 374 patients with endomyocardial biopsies

Endomyocardial biopsy findings		**^{99m}Tc-DPD scan findings**				
		Perugini 0	**Perugini 1**	**Perugini 2**	**Perugini 3**	**N**
	No cardiac amyloid	31	3	0	1	35
	Cardiac transthyretin amyloid deposits	1	8	130	23	162
	Cardiac AL amyloid deposits	21	13	7	2	43
	Cardiac apoAI amyloid deposits	0	2	0	0	2
	Cardiac amyloid deposits of unknown type	1	1	0	0	2
	Total N	**54**	**27**	**137**	**26**	**244**
		^{99m}Tc-PYP scan findings				
		Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	
	No cardiac amyloid	7	1	1	0	9
	Cardiac transthyretin amyloid	1	10	7	67	85
	Cardiac AL amyloid deposits	10	1	3	1	15
	Cardiac apoAI amyloid deposits	0	0	0	0	0
	Cardiac amyloid deposits of unknown type	0	0	0	0	0
	Total N	**18**	**12**	**11**	**68**	**109**
		^{99m}Tc-HMDP scan findings				
		Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	
	No cardiac amyloid	3	0	0	0	3
	Cardiac transthyretin amyloid deposits	0	3	4	7	14
	Cardiac AL amyloid deposits	4	0	0	0	4
	Cardiac apoAI amyloid deposits	0	0	0	0	0
	Cardiac amyloid deposits of unknown type	0	0	0	0	0
	Total N	**7**	**3**	**4**	**7**	**21**

Table 3. Sensitivity and specificity of radionuclide 'bone' scintigraphy vs EMB histology

Positive radionuclide scan vs cardiac amyloid deposits (N=374)			
	Positive scan (Grade 1, 2, or 3)	Negative scan (Grade 0)	Sensitivity and specificity (CI)
Cardiac amyloid deposits	289	38	88% (84-92%) sensitive†
No cardiac amyloid deposits	6	41	87% (73-95%) specific
Positive radionuclide scan vs cardiac ATTR amyloid deposits (N=374)			
	Positive scan (Grade 1, 2, or 3)	Negative scan (Grade 0)	
Cardiac ATTR amyloid deposits	259	2	>99% (97-100%) sensitive
No cardiac ATTR amyloid deposits	36	77	68% (59-77%) specific
Grade 2 or 3 radionuclide scan vs cardiac ATTR amyloid deposits (N=374)			
	Grade 2/3 scan	Grade 0/1 scan	
Cardiac ATTR amyloid deposits	238	23	91% (87-94%) sensitive
No cardiac ATTR amyloid deposits	15	98	87% (79-92%) specific

†The sensitivity of a positive radionuclide scan for detecting cardiac amyloid deposits of any type is likely to be falsely high due to the high proportion of patients with ATTR amyloid in the sample.

Table 4. Combined radionuclide 'bone' scintigraphy and monoclonal protein studies

Grade 2 or 3 radionuclide scan + absence of clone vs ATTR amyloid deposits on EMB (N=374)				
	Grade 2/3 scan + no clone	**Grade 0/1 scan OR clone**	**Sensitivity and specificity (CI)**	**Positive (PPV) and Negative predictive (NPV)values (CI)**
Cardiac ATTR amyloid deposits	182	79	70% (64-75%) sensitive	NPV 59% (52-66%)
No cardiac ATTR amyloid deposits	0	113	100% (96-100%) specific	PPV 100% (98-100%)
Grade 2 or 3 radionuclide scan + absence of clone vs ATTR amyloid deposits on histology from any organ (N=1217)				
	Grade 2/3 scan + no clone	**Grade 0/1 scan OR clone**		
Cardiac ATTR amyloid	391	139	74% (70-77%) sensitive†	NPV 83% (80-86%)
No cardiac ATTR amyloid	0	687	100% (99-100%) specific	PPV 100% (99-100%)

†The low sensitivity was due to the exclusion of patients with cardiac ATTR amyloid exhibiting a monoclonal protein (88 of 530 patients with cardiac ATTR amyloid), and grade 0 or 1 radionuclide scans among a further 51 patients with cardiac ATTR amyloid.