

Nonbiopsy Diagnosis of Cardiac Transthyretin Amyloidosis

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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 because of the limited specificity of echocardiography and the traditional requirement for histological confirmation. It has long been recognized that technetium-labeled bone scintigraphy tracers can localize to myocardial amyloid deposits, and use of this imaging modality for the diagnosis of cardiac ATTR amyloidosis has lately been revisited. We conducted a multicenter study to ascertain the diagnostic value of bone scintigraphy in this disease.

Methods and Results—Results of bone scintigraphy and biochemical investigations were analyzed from 1217 patients with suspected cardiac amyloidosis referred for evaluation in specialist centers. Of 857 patients with histologically proven amyloid (374 with endomyocardial biopsies) and 360 patients subsequently confirmed to have nonamyloid 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 the absence of a monoclonal protein in serum or urine had a specificity and positive predictive value for cardiac ATTR amyloidosis of 100% (positive predictive value confidence interval, 98.0–100).

Conclusions—Bone scintigraphy enables the diagnosis of cardiac ATTR amyloidosis to be made reliably without the need for histology in patients who do not have a monoclonal gammopathy. We propose noninvasive diagnostic criteria for cardiac ATTR amyloidosis that are applicable to the majority of patients with this disease. (*Circulation*. 2016;133:2404–2412. DOI: 10.1161/CIRCULATIONAHA.116.021612.)

Key Words: amyloid ■ cardiomyopathies ■ genetics ■ hypertrophy ■ radionuclide imaging

Cardiac amyloidosis is a rare form of restrictive cardiomyopathy that 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

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transthyretin (previously known as senile systemic amyloidosis), or hereditary, associated with variants in the transthyretin gene. Clinical features are varied, and although heart failure symptoms predominate, suspicion of cardiac amyloidosis can also be prompted by syncope, arrhythmias, or unexplained left ventricular wall thickening on echocardiography. The 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% to 80% 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, that may be fatal and requires expertise that can introduce diagnostic delay.⁷

Clinical Perspective on p 2412

Autopsy studies have shown the presence of cardiac ATTR amyloid deposits in up to 25% of individuals >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, postmortem 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 postmortem in these studies had not had amyloidosis diagnosed during their lifetime.⁹ Echocardiography, although 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, is available only 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 histological confirmation and typing of amyloid, recognizing issues of sampling error and the disease expertise needed for proper histological 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 characterize cardiac amyloidosis at the earliest opportunity, noting that cardiac ATTR amyloidosis in particular is probably greatly underdiagnosed and fast becoming a treatable cause of heart failure.^{18,19}

Radionuclide bone scintigraphy with technetium-labeled 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 the development of abnormalities on echocardiography or CMR,^{26,27} and has been used to diagnose ATTR amyloidosis among patients with heart failure and preserved ejection fraction.²⁸ Cardiac localization of radiotracer occurs 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 noninvasive laboratory investigations, might enable the diagnosis of cardiac ATTR amyloidosis without the need for confirmatory biopsy. Our findings presented here form the basis for a proposed diagnostic algorithm for patients with suspected cardiac amyloidosis in whom non-biopsy diagnosis of ATTR amyloidosis can be achieved in the majority of cases.

Methods

Patients

The subjects were patients with suspected or histologically proven amyloidosis who had been referred for evaluation to the following specialist amyloidosis centers in Europe and the United States: UK National Amyloidosis Center; 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, New York, NY; Brigham and Women's Hospital Cardiac Amyloidosis Program, Boston, MA; Mayo Clinic, Rochester, MN; and Amyloidosis Center, Boston University, Boston, MA. 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 (sFLC) assay. All patients underwent echocardiography in their respective specialist amyloidosis center; previous CMRs 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 histological and genetic findings. A minority of the subjects had been included in small previously published series from the individual centers. The study received ethics approval.

Echocardiography and CMR at Specialist Centers

Detailed echocardiography was performed at the specialist amyloid centers and included an extensive analysis of left and right ventricular wall thickness, left ventricular function, including global and regional longitudinal strain, and atrial parameters. Diastolic function was analyzed in detail by tissue Doppler imaging. Features that were characteristic of amyloid were defined as the combined findings of thickened left ventricular walls, impaired global strain with relative sparing of the apical region compared with 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 the 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 ^{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 hours after injection (except for some ^{99m}Tc -PYP images in which thoracic planar images were acquired 1 hour after injection). Images were acquired with 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 750 000 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; and 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 with the use of monospecific antibodies reactive with serum amyloid A protein, κ and λ immunoglobulin light chains, and transthyretin, as previously described²⁹ and, when necessary, apolipoprotein AI, and fibrinogen A α -chain. When 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 after staining with hematoxylin and eosin and Masson trichrome and, whenever possible, additionally examined by electron microscopy.

Monoclonal Protein Studies

The presence of a monoclonal protein was sought by IFE of serum and urine and by sFLC assay (Freelite, The Binding Site)^{31,32} in all patients. The 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 transthyretin (*TTR*) gene. DNA was extracted from whole blood and amplified by polymerase chain reaction assay, and the whole coding region of the *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 with 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. Of the remaining 1124 patients in whom EMBs were not performed, 391 had cardiac amyloidosis on the basis of a characteristic echocardiogram with or without CMR from the specialist amyloidosis center and histological proof of amyloid in extracardiac tissue. There were 139 patients with histologically proven extracardiac amyloid without evidence of cardiac involvement by echocardiography with or without CMR. The remainder (n=360) had no histological or cardiac imaging evidence of amyloid. Patients with cardiac imaging (echocardiogram with or without 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 because of diagnostic uncertainty. The diagnostic algorithm detailing the selection of patients for analysis is shown in Figure 1. The final diagnoses of 1217 analyzable patients (857 with amyloid and 360 without amyloid), established on the basis of these investigations, coupled with immunohistochemical or proteomic typing of amyloid and genetic analyses, are shown in Table 1.

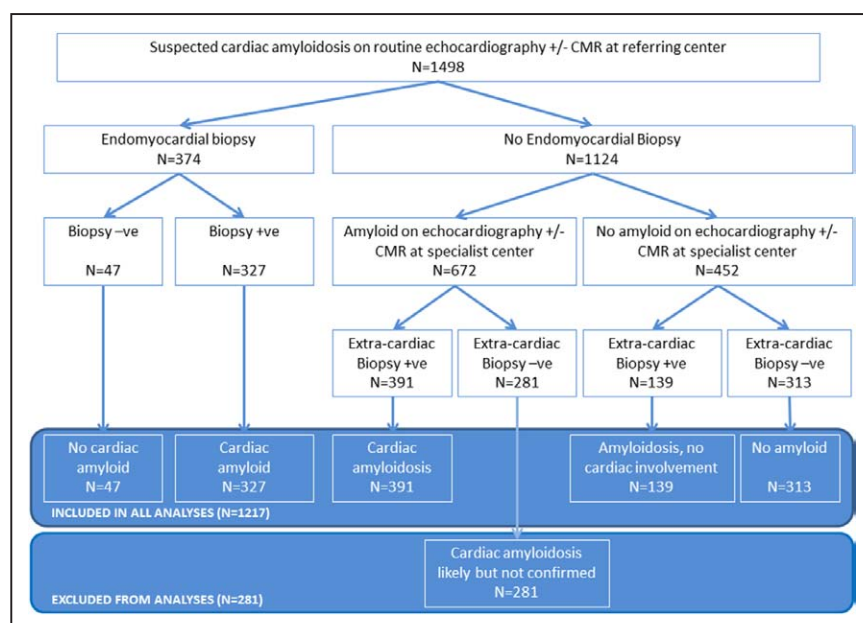


Figure 1. Selection of patients for analysis. Of 1217 evaluable patients, 374 underwent endomyocardial biopsy, and 843 were diagnosed with presence and type or absence of amyloid on the basis of extracardiac histology coupled with echocardiography with or without cardiac magnetic resonance imaging (CMR). Patients in whom there was diagnostic uncertainty were excluded from all analyses (n=281).

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 12 other sites (n=40). Of the 360 patients without amyloid, 47 had EMBs that 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 the diagnosis of cardiac ATTR amyloid, in 374 patients (261 with cardiac ATTR amyloid, 113 without cardiac ATTR amyloid). The results for each of the 3 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 of 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 of 113 patients without cardiac ATTR amyloid; Table 3). The low specificity of a positive scan for cardiac ATTR amyloid deposits was due almost entirely to cardiac uptake of tracer among patients with cardiac AL or cardiac apolipoprotein A-I 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 were 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; NT-pro-brain natriuretic peptide, 51 pmol/L), and a 6-minute walk test distance of 607 m (120% of expected for age). This patient therefore had limited subclinical 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 subaortic myomectomy biopsy obtained at aortic valve replacement demonstrated only a single, tiny focus of subclinical amyloid. Of the 2 patients with no amyloid on EMB but with radionuclide scans that were grade 2 or higher, 1 patient had a Perugini grade 3 ^{99m}Tc -DPD scan, and the other had 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 of 530 with cardiac ATTR amyloid) with a specificity of 86% (negative

scans in 591 of 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

Table 1. Final Diagnoses in Patients With and Without EMB

Patients	EMB (n=374, 31%), n	No EMB* (n=843, 69%), n	All (n=1217, 100%), n
ATTR amyloid	261	269	530
Cardiac AL amyloidosis	62	119	181
Cardiac ApoA1 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
ATTR amyloidosis, no cardiac involvement	0	32	32
AA amyloidosis, no cardiac involvement	0	3	3
ApoA1 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
Localized AL amyloidosis, no cardiac involvement	0	39	39
No amyloidosis	47	313	360
Hypertrophic cardiomyopathy	1	41	42
Heart failure with preserved ejection fraction	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

ApoA1 indicates apolipoprotein A-I; and EMB, endomyocardial biopsy.

*Presence/absence of cardiac amyloidosis was determined on the basis of echocardiography within specialist centers with or without cardiac magnetic resonance imaging.

Table 2. Radionuclide ‘Bone’ Scintigraphy Findings Among 374 Patients With EMBs

EMB Findings	^{99m} Tc-DPD Scan Findings, n				n
	Perugini 0	Perugini 1	Perugini 2	Perugini 3	
No cardiac amyloid	31	3	0	1	35
Cardiac ATTR 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	54	27	137	26	244
^{99m} Tc-PYP Scan Findings					
	Grade 0	Grade 1	Grade 2	Grade 3	n
No cardiac amyloid	7	1	1	0	9
Cardiac ATTR 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	18	12	11	68	109
^{99m} Tc-HMDP Scan Findings					
	Grade 0	Grade 1	Grade 2	Grade 3	n
No cardiac amyloid	3	0	0	0	3
Cardiac ATTR 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	7	3	4	7	21

ApoAI indicates apolipoprotein A-I; DPD, 3,3-diphosphono-1,2-propanodicarboxylic acid; EMB, endomyocardial biopsy; HMDP, hydroxymethylene diphosphonate; and PYP, pyrophosphate.

findings for all 1217 patients are shown in Table I in the [online-only Data Supplement](#), and findings for each of the individual bone tracers are shown in Tables II through IV in the [online-only Data Supplement](#). Sensitivity and specificity analyses for the whole cohort of 1217 patients are shown by individual tracer in Tables V through VII in the [online-only Data Supplement](#).

Monoclonal Protein Studies

Of 237 patients with systemic AL amyloidosis in this cohort, 235 (99%) had evidence of a monoclonal protein by ≥1 of serum IFE, urine IFE, and sFLC assay. Interestingly, of 562 patients from the cohort with ATTR amyloid whose median age was 75 years, 107 (19%) had a detectable monoclonal protein with these very sensitive techniques.

The monoclonal protein findings presented here were corroborated by analysis of 2 separate, larger cohorts of patients with histologically proven systemic AL amyloidosis. Of 714 patients with systemic AL amyloidosis from the UK National Amyloidosis Center, 98.9% had evidence of a monoclonal protein, and of 1465 patients from a collaborative project between the Amyloidosis Research and Treatment Center, Pavia and the Mayo Clinic, 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 sFLC 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%; confidence interval, 98.0–100). Detailed sensitivity and specificity analyses are shown in Tables 4 and 5.

Genotyping

All 562 patients with ATTR amyloid (530 with cardiac involvement) 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 Table VIII in the [online-only Data Supplement](#). These results indicate that radionuclide scan findings were consistent with that expected according to the clinical and echocardiographic findings and the known likelihood of cardiac involvement associated with individual *TTR* variants. For example, younger patients with V30M-associated ATTR

Table 3. Sensitivity and Specificity of Radionuclide ‘Bone’ Scintigraphy Compared With EMB Histology

Positive Radionuclide Scan vs Cardiac Amyloid Deposits (n=374)			
	Positive Scan (Grade 1, 2, or 3), n	Negative Scan (Grade 0), n	Sensitivity and Specificity (CI), %
Cardiac amyloid deposits	289	38	88 (84–92) sensitive*
No cardiac amyloid deposits	6	41	87 (73–95) specific
	Positive Scan (Grade 1, 2, or 3), n	Negative Scan (Grade 0), n	
Cardiac ATTR amyloid deposits	259	2	>99 (97–100) sensitive
No cardiac ATTR amyloid deposits	36	77	68 (59–77) specific
	Grade 2/3 Scan, n	Grade 0/1 Scan, n	
Cardiac ATTR amyloid deposits	238	23	91 (87–94) sensitive
No cardiac ATTR amyloid deposits	15	98	87 (79–92) specific

CI indicates confidence interval; DPD, 3,3-diphosphono-1,2-propanodicarboxylic acid; EMB, endomyocardial biopsy; HDMP, hydroxymethylene diphosphonate; and PYP, pyrophosphate.

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

amyloidosis have a predominant neuropathic phenotype without cardiac amyloidosis, whereas 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 patients (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 as a result of the poor sensitivity and specificity of echocardiography,¹⁰ coupled with the current requirement for histological confirmation of amyloid in a tissue biopsy. The diagnosis of wild-type cardiac ATTR amyloidosis is particularly challenging because it presents in older age³⁴ (when confounding comorbidities such as coronary heart disease, hypertension, and aortic stenosis frequently coexist) and because of the absence of any specific extracardiac 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 mutation³³ or monoclonal immunoglobulin, respectively.^{31,32} Currently, wild-type ATTR amyloidosis is diagnosed mostly in men >70 years of age and in <3 per million per year,³⁵ which contrasts greatly with the

high frequency of ATTR amyloid deposits found at autopsy and the high prevalence in common syndromes such as heart failure and preserved ejection fraction.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 centers 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 with EMB histology); the low specificity results largely from low-grade uptake in patients with cardiac AL or cardiac apolipoprotein A-I amyloidosis.³⁶ The specificity for cardiac ATTR amyloid of grade 2 or 3 cardiac uptake on radionuclide imaging increases to ≈87%, but the sensitivity falls to 91%. Because 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 the absence of a detectable monoclonal protein despite serum IFE, urine IFE, and sFLC assay were 100% (positive predictive value confidence interval, 99.0–100%) in this cohort of 1217 patients and were also 100% among each of the 3 different radiotracer cohorts. Although we acknowledge that further validation of ^{99m}Tc-HMDP scintigraphy against EMB histology is

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, n	Grade 0/1 Scan or Clone, n	Sensitivity and Specificity (CI), %	PPV and NPV (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)

CI indicates confidence interval; EMB, endomyocardial biopsy; NPV, negative predictive value; and PPV, positive predictive value.

Table 5. Combined Radionuclide ‘Bone’ Scintigraphy and Monoclonal Protein Studies Compared With Amyloid Histology

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, n	Grade 0/1 Scan or Clone, n	Sensitivity and Specificity (CI), %	PPV and NPV (CI), %
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)

CI indicates confidence interval; NPV, negative predictive value; and PPV, positive predictive value.

*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.

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 the 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 histological 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 patients 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, we suggest additional sequencing of the *APOA1* gene to exclude the remote possibility of cardiac apolipoprotein AI 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 the so-called absence of cardiac amyloidosis (ie, false-positive scans). The majority did not undergo EMB, and all but 1 patient 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 (probably due, at least in part, 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 or proteomic analysis, often from an EMB, in any patient who does not fulfill the diagnostic criteria for cardiac ATTR amyloidosis proposed above.

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

Conclusions

Cardiac ATTR amyloidosis can be reliably diagnosed in the absence of histology provided that all of the following criteria

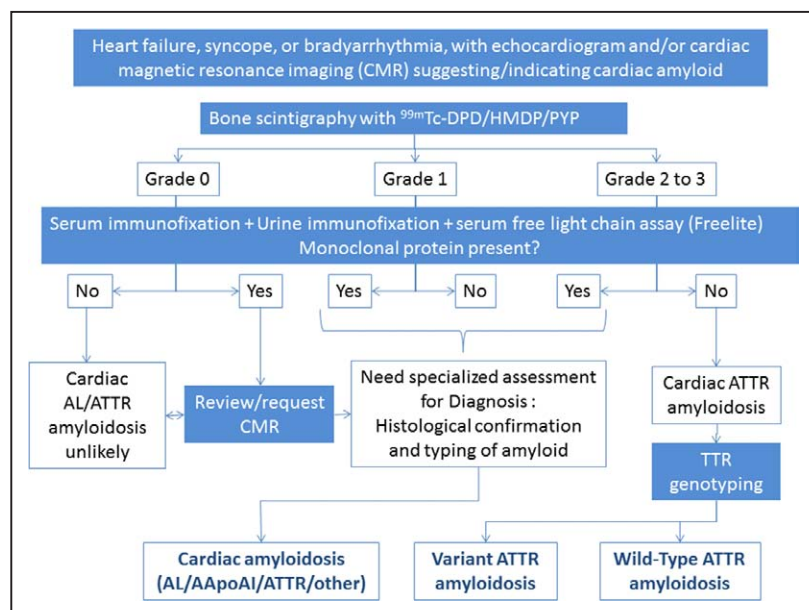


Figure 2. Diagnostic algorithm for patients with suspected amyloid cardiomyopathy. Echocardiographic features suggesting/indicating cardiac amyloid include (but are not limited to) increased left ventricular wall thickness, restrictive filling pattern, abnormal left and right ventricular longitudinal strain, and atrial septal thickening. Features suggesting/indicating cardiac amyloid on cardiac magnetic resonance imaging (CMR) include (but are not limited to) restrictive morphology, abnormal gadolinium kinetics, and extracellular volume expansion based on T1 mapping. AApoA1 indicates apolipoprotein A-I; DPD, 3,3-diphosphono-1,2-propanodicarboxylic acid; HMDP, hydroxymethylene diphosphonate; and PYP, pyrophosphate.

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 with ^{99m}Tc -DPD, ^{99m}Tc -PYP, or ^{99m}Tc -HMDP, and absence of a detectable monoclonal protein despite serum IFE, 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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References

- Kyle RA, Gertz MA. Primary systemic amyloidosis: clinical and laboratory features in 474 cases. *Semin Hematol*. 1995;32:45–59.
- Benson MD. The hereditary amyloidoses. *Best Pract Res Clin Rheumatol*. 2003;17:909–927. doi: 10.1016/j.berh.2003.09.001.
- Ruberg FL, Berk JL. Transthyretin (TTR) cardiac amyloidosis. *Circulation*. 2012;126:1286–1300. doi: 10.1161/CIRCULATIONAHA.111.078915.
- Pinney JH, Whelan CJ, Petrie A, Dunge J, Banypersad SM, Sattianayagam P, Wechalekar A, Gibbs SD, Venner CP, Wassef N, McCarthy CA, Gilbertson JA, Rowczenio D, Hawkins PN, Gillmore JD, Lachmann HJ. Senile systemic amyloidosis: clinical features at presentation and outcome. *J Am Heart Assoc*. 2013;2:e000098. doi: 10.1161/JAHA.113.000098.
- Ansari-Lari MA, Ali SZ. Fine-needle aspiration of abdominal fat pad for amyloid detection: a clinically useful test? *Diagn Cytopathol*. 2004;30:178–181. doi: 10.1002/dc.10370.
- Fine NM, Arruda-Olson AM, Dispenzieri A, Zeldenrust SR, Gertz MA, Kyle RA, Swiecicki PL, Scott CG, Grogan M. Yield of noncardiac biopsy for the diagnosis of transthyretin cardiac amyloidosis. *Am J Cardiol*. 2014;113:1723–1727. doi: 10.1016/j.amjcard.2014.02.030.
- Maurer MS. Noninvasive identification of ATTRwt cardiac amyloid: the re-emergence of nuclear cardiology. *Am J Med*. 2015;128:1275–1280. doi: 10.1016/j.amjmed.2015.05.039.
- Tanskanen M, Peuralinna T, Polvikoski T, Notkola IL, Sulkava R, Hardy J, Singleton A, Kiuru-Enari S, Paetau A, Tienari PJ, Myllykangas L. Senile systemic amyloidosis affects 25% of the very aged and associates with genetic variation in alpha2-macroglobulin and tau: a population-based autopsy study. *Ann Med*. 2008;40:232–239. doi: 10.1080/07853890701842988.
- Mirzoyev SA, Edwards WD, Mohammed SF, Donovan JL, Roger VL, Grogan DR, Redfield MM. Cardiac amyloid deposition is common in elderly patients with heart failure and preserved ejection fraction. *Circulation*. 2010;122:A17926.
- Falk RH. Diagnosis and management of the cardiac amyloidoses. *Circulation*. 2005;112:2047–2060. doi: 10.1161/CIRCULATIONAHA.104.489187.
- Falk RH, Quarta CC, Dorbala S. How to image cardiac amyloidosis. *Circ Cardiovasc Imaging*. 2014;7:552–562. doi: 10.1161/CIRCIMAGING.113.001396.
- Maceira AM, Joshi J, Prasad SK, Moon JC, Perugini E, Harding I, Sheppard MN, Poole-Wilson PA, Hawkins PN, Pennell DJ. Cardiovascular magnetic resonance in cardiac amyloidosis. *Circulation*. 2005;111:186–193. doi: 10.1161/01.CIR.0000152819.97857.9D.
- Fontana M, Pica S, Reant P, Abdel-Gadir A, Treibel TA, Banypersad SM, Maestrini V, Barcella W, Rosmini S, Bulluck H, Sayed RH, Patel K, Mamhood S, Bucciarelli-Ducci C, Whelan CJ, Herrey AS, Lachmann HJ, Wechalekar AD, Manisty CH, Schelbert EB, Kellman P, Gillmore JD, Hawkins PN, Moon JC. Prognostic value of late gadolinium enhancement cardiovascular magnetic resonance in cardiac amyloidosis. *Circulation*. 2015;132:1570–1579. doi: 10.1161/CIRCULATIONAHA.115.016567.
- Dunge J, Valencia O, Pinney JH, Gibbs SD, Rowczenio D, Gilbertson JA, Lachmann HJ, Wechalekar A, Gillmore JD, Whelan CJ, Hawkins PN, Anderson LJ. CMR-based differentiation of AL and ATTR cardiac amyloidosis. *JACC Cardiovasc Imaging*. 2014;7:133–142. doi: 10.1016/j.jcmg.2013.08.015.
- Pepys MB. Amyloidosis. *Annu Rev Med*. 2006;57:223–241. doi: 10.1146/annurev.med.57.121304.131243.
- Sekijima Y, Kelly JW, Ikeda S. Pathogenesis of and therapeutic strategies to ameliorate the transthyretin amyloidosis. *Curr Pharm Des*. 2008;14:3219–3230.
- Wechalekar AD, Schonland SO, Kastritis E, Gillmore JD, Dimopoulos MA, Lane T, Foli A, Foard D, Milani P, Rannigan L, Heegenbart U, Hawkins PN, Merlini G, Palladini G. A European collaborative study of treatment outcomes in 346 patients with cardiac stage III AL amyloidosis. *Blood*. 2013;121:3420–3427. doi: 10.1182/blood-2012-12-473066.
- Dubrey S, Ackermann E, Gillmore J. The transthyretin amyloidosis: advances in therapy. *Postgrad Med J*. 2015;91:439–448. doi: 10.1136/postgradmedj-2014-133224.
- Coelho T, Adams D, Silva A, Lozeron P, Hawkins PN, Mant T, Perez J, Chiesa J, Warrington S, Tranter E, Munisamy M, Falzone R, Harrop J, Cehelsky J, Bettencourt BR, Geissler M, Butler JS, Sehgal A, Meyers RE, Chen Q, Borland T, Hutabarat RM, Clausen VA, Alvarez R, Fitzgerald K, Gamba-Vitalo C, Nochur SV, Vaishnav AK, Sah DW, Gollob JA, Suhr OB. Safety and efficacy of RNAi therapy for transthyretin amyloidosis. *N Engl J Med*. 2013;369:819–829. doi: 10.1056/NEJMoa1208760.
- Ali A, Turner DA, Rosenbush SW, Fordham EW. Bone scintigram in cardiac amyloidosis: a case report. *Clin Nucl Med*. 1981;6:105–108.
- Perugini E, Guidalotti PL, Salvi F, Cooke RM, Pettinato C, Riva L, Leone O, Farsad M, Ciliberti P, Bacchi-Reggiani L, Fallani F, Branzi A, Rapezzi C. Noninvasive etiologic diagnosis of cardiac amyloidosis using ^{99m}Tc -3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy. *J Am Coll Cardiol*. 2005;46:1076–1084. doi: 10.1016/j.jacc.2005.05.073.
- Rapezzi C, Guidalotti P, Salvi F, Riva L, Perugini E. Usefulness of ^{99m}Tc -DPD scintigraphy in cardiac amyloidosis. *J Am Coll Cardiol*. 2008;51:1509–1510. doi: 10.1016/j.jacc.2007.12.038.
- Rapezzi C, Quarta CC, Guidalotti PL, Longhi S, Pettinato C, Leone O, Ferlini A, Salvi F, Gallo P, Gagliardi C, Branzi A. Usefulness and limitations of ^{99m}Tc -3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy in the aetiological diagnosis of amyloidotic cardiomyopathy. *Eur J Nucl Med Mol Imaging*. 2011;38:470–478. doi: 10.1007/s00259-010-1642-7.
- Bokhari S, Castaño A, Pozniakoff T, Deslisle S, Latif F, Maurer MS. (99m)Tc-pyrophosphate scintigraphy for differentiating light-chain cardiac amyloidosis from the transthyretin-related familial and senile cardiac amyloidosis. *Circ Cardiovasc Imaging*. 2013;6:195–201. doi: 10.1161/CIRCIMAGING.112.000132.
- Quarta CC, Guidalotti PL, Longhi S, Pettinato C, Leone O, Ferlini A, Biagini E, Grigioni F, Bacchi-Reggiani ML, Lorenzini M, Milandri A, Branzi A, Rapezzi C. Defining the diagnosis in echocardiographically suspected senile systemic amyloidosis. *JACC Cardiovasc Imaging*. 2012;5:755–758. doi: 10.1016/j.jcmg.2012.02.015.
- Glaudemans AW, van Rheeën RW, van den Berg MP, Noordzij W, Koole M, Blokzijl H, Dierckx RA, Slart RH, Hazenberg BP. Bone scintigraphy with (99m)technetium-hydroxymethylene diphosphate allows early diagnosis of cardiac involvement in patients with

- transthyretin-derived systemic amyloidosis. *Amyloid*. 2014;21:35–44. doi: 10.3109/13506129.2013.871250.
27. Fontana M, Banyersad SM, Treibel TA, Maestrini V, Sado DM, White SK, Pica S, Castelletti S, Piechnik SK, Robson MD, Gilbertson JA, Rowczenio D, Hutt DF, Lachmann HJ, Wechalekar AD, Whelan CJ, Gillmore JD, Hawkins PN, Moon JC. Native T1 mapping in transthyretin amyloidosis. *JACC Cardiovasc Imaging*. 2014;7:157–165. doi: 10.1016/j.jcmg.2013.10.008.
 28. Castaño A, Bokhari S, Maurer MS. Unveiling wild-type transthyretin cardiac amyloidosis as a significant and potentially modifiable cause of heart failure with preserved ejection fraction. *Eur Heart J*. 2015;36:2595–2597. doi: 10.1093/eurheartj/ehv328.
 29. Tennent GA, Cafferty KD, Pepys MB, Hawkins PN. Congo red overlay immunohistochemistry aids classification of amyloid deposits. In: Kyle RA, Gertz MA, eds. *Amyloid and Amyloidosis 1998*. Pearl River, NY: Parthenon Publishing; 1999:160–162.
 30. Vrana JA, Gamez JD, Madden BJ, Theis JD, Bergen HR 3rd, Dogan A. Classification of amyloidosis by laser microdissection and mass spectrometry-based proteomic analysis in clinical biopsy specimens. *Blood*. 2009;114:4957–4959. doi: 10.1182/blood-2009-07-230722.
 31. Bradwell AR, Carr-Smith HD, Mead GP, Tang LX, Showell PJ, Drayson MT, Drew R. Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. *Clin Chem*. 2001;47:673–680.
 32. Drayson M, Tang LX, Drew R, Mead GP, Carr-Smith H, Bradwell AR. Serum free light-chain measurements for identifying and monitoring patients with nonsecretory multiple myeloma. *Blood*. 2001;97:2900–2902.
 33. Rowczenio DM, Noor I, Gillmore JD, Lachmann HJ, Whelan C, Hawkins PN, Obici L, Westermark P, Grateau G, Wechalekar AD. Online registry for mutations in hereditary amyloidosis including nomenclature recommendations. *Hum Mutat*. 2014;35:E2403–E2412. doi: 10.1002/humu.22619.
 34. Pinney JH, Smith CJ, Taube JB, Lachmann HJ, Venner CP, Gibbs SD, Dzung J, Banyersad SM, Wechalekar AD, Whelan CJ, Hawkins PN, Gillmore JD. Systemic amyloidosis in England: an epidemiological study. *Br J Haematol*. 2013;161:525–532. doi: 10.1111/bjh.12286.
 35. Gillmore JD, Hawkins PN. V122I transthyretin variant in elderly black Americans. *N Engl J Med*. 2015;372:1769. doi: 10.1056/NEJMc1503222#SA2.
 36. Hutt DF, Quigley AM, Page J, Hall ML, Burniston M, Gopaul D, Lane T, Whelan CJ, Lachmann HJ, Gillmore JD, Hawkins PN, Wechalekar AD. Utility and limitations of 3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy in systemic amyloidosis. *Eur Heart J Cardiovasc Imaging*. 2014;15:1289–1298. doi: 10.1093/ehjci/jeu107.

CLINICAL PERSPECTIVE

Cardiac transthyretin (ATTR) amyloidosis is a progressive and fatal cardiomyopathy that is greatly underdiagnosed, largely because of the limited specificity of echocardiography and the traditional requirement for histological confirmation. This multicenter study establishes the exquisite (>99%) sensitivity of technetium-labeled bone scintigraphy for diagnosing cardiac ATTR amyloid and shows that when scintigraphy is combined with biochemical testing for a monoclonal protein in serum and urine, cardiac ATTR amyloidosis can be diagnosed in the absence of histology with >98% certainty. This represents a paradigm shift in the diagnosis of cardiac ATTR amyloidosis and removes the need for endomyocardial biopsy, with its attendant risks and costs, in the majority of patients experiencing this disease.