JAMA Cardiology | Original Investigation

Multicenter Study of Planar Technetium 99m Pyrophosphate Cardiac Imaging

Predicting Survival for Patients With ATTR Cardiac Amyloidosis

Adam Castano, MD, MS; Muhammad Haq, MD; David L. Narotsky, MD; Jeff Goldsmith, PhD; Richard L. Weinberg, MD, PhD; Rachelle Morgenstern, MPH; Ted Pozniakoff, ARRT; Frederick L. Ruberg, MD; Edward J. Miller, MD, PhD; John L. Berk, MD; Angela Dispenzieri, MD; Martha Grogan, MD; Geoffrey Johnson, MD, PhD; Sabahat Bokhari, MD; Mathew S. Maurer, MD

IMPORTANCE Transthyretin cardiac amyloidosis (also known as ATTR cardiac amyloidosis) is an increasingly recognized cause of heart failure with preserved ejection fraction. In single-center studies, technetium 99m pyrophosphate (Tc 99m PYP) cardiac imaging noninvasively detects ATTR cardiac amyloidosis, but the accuracy of this technique in a multicenter study and the association of Tc 99m PYP myocardial uptake with survival are unknown.

OBJECTIVE To assess Tc 99m PYP cardiac imaging as a diagnostic tool for ATTR cardiac amyloidosis and its association with survival in a multicenter study.

DESIGN, SETTING, AND PARTICIPANTS Retrospective cohort study performed at 3 academic specialty centers for cardiac amyloidosis in the United States in which 229 participants were evaluated for cardiac amyloidosis and also underwent Tc 99m PYP cardiac imaging. The date of analysis and final confirmation from the statistician was May 4, 2016.

EXPOSURE Tc 99m PYP cardiac imaging for detection of ATTR cardiac amyloidosis.

MAIN OUTCOMES AND MEASURES Retention of Tc 99m PYP in the heart was assessed using both a semiquantitative visual score (range, 0 [no uptake] to 3 [uptake greater than bone]) and a quantitative heart to contralateral (H/CL) ratio. The H/CL ratio was calculated as total counts in a region of interest over the heart divided by background counts in an identical size region of interest over the contralateral chest. The outcome measured was time to death after Tc 99m PYP imaging.

RESULTS Tc 99m PYP imaging of 171 participants (121 with ATTR cardiac amyloidosis and 50 with non-ATTR cardiac amyloidosis [34 with AL amyloidosis and 16 with nonamyloid heart failure with preserved ejection fraction]; 86% male; median [IQR] age, 73 years [65-79 years]) demonstrated 91% sensitivity and 92% specificity for detecting ATTR cardiac amyloidosis with an area under the curve of 0.960 (95% CI, 0.930-0.981). Univariable and multivariable Cox proportional hazards regression analyses among participants with ATTR cardiac amyloidosis showed that an H/CL ratio of 1.6 or greater predicted worse survival (hazard ratio, 3.911 [95% CI, 1.155-13.247]; $P = .03$ for univariable analysis and 7.913 [95% CI, 1.679-37.296]; $P = .01$ for multivariable analysis). In Kaplan-Meier analysis over a 5-year follow-up period, survival was significantly worse if the H/CL ratio was 1.6 or greater rather than less than 1.6 (log-rank $P = .02$).

CONCLUSIONS AND RELEVANCE In this multicenter study, Tc 99m PYP cardiac imaging conferred a high level of sensitivity and specificity for differentiation of patients with ATTR cardiac amyloidosis (irrespective of genotype) from patients with AL cardiac amyloidosis and patients with nonamyloid heart failure with preserved ejection fraction. An H/CL ratio of 1.6 or greater was associated with worse survival among patients with ATTR cardiac amyloidosis. Among patients for whom there is a high clinical suspicion of cardiac amyloidosis, Tc 99m PYP may be of diagnostic and prognostic importance.

JAMA Cardiol. 2016;1(8):880-889. doi:10.1001/jamacardio.2016.2839
Published online August 24, 2016.

Supplemental content

CME Quiz at jamanetworkcme.com and CME Questions page 968

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Adam Castano, MD, MS, Division of Cardiology, Department of Medicine, Center for Advanced Cardiac Care, Columbia University Medical Center, 622 W 168th St, PH 10-203, New York, NY 10032 (ac3220@cumc.columbia.edu).

Transthyretin cardiac amyloidosis (also known as ATTR cardiac amyloidosis) is increasingly recognized as an important cause of heart failure with preserved ejection fraction (HFpEF).[1-3] In ATTR cardiac amyloidosis, destabilization of the transthyretin (TTR) protein from either inherited mutations (ATTRm) or the aging process in wild-type disease (ATTRwt) promotes the release of monomers, which aggregate into amyloid fibrils that deposit in organs and tissues. In the heart, extracellular amyloid deposition causes diastolic dysfunction that progresses to restrictive cardiomyopathy and congestive heart failure.[4] More than 100 mutations in the TTR protein inherited in autosomal dominant fashion affect persons of all ages with ATTRm, with the most common allele in the United States the valine to isoleucine substitution at position 122 (Val122Ile),[5] found in approximately 4% of black persons.[6] ATTRwt cardiomyopathy predominantly affects older adult men and has been detected at autopsy in 20% of patients with HFpEF without antemortem suspicion of amyloid deposition and in 13.3% of hospitalized patients with HFpEF with a left ventricular wall thickness of 12 mm or greater.[2,3]

The detection of ATTR cardiac amyloidosis, subtyping into ATTRm or ATTRwt, and the exclusion of light chain–related amyloidosis (AL amyloidosis) are imperative not just for diagnostic accuracy but because these 3 pathophysiologic amyloid types have differing clinical courses and management. The identification of ATTR cardiac amyloidosis is clinically important because treatments for this form of heart failure differ from other cardiomyopathies (including the avoidance of digitalis, calcium channel blockers, and high-dose beta-blockade); targeted therapies for ATTR cardiac amyloidosis comprising at least 3 new drug classes (transthyretin stabilizers, silencers, and fibril degraders) are now in phase 3 clinical trials.[7] Unfortunately, standard tests to definitively diagnose and subtype cardiac amyloidosis (endomyocardial biopsy coupled with either immunohistochemistry[8,9] or mass spectroscopy[10]) have limitations. These specialized tests are only performed in centers with experienced staff, do not yield prognostically useful information, may be inadvisable for frail older adults, and often present logistical challenges that lead to delayed care. Given the limitations of current diagnostic testing for amyloidosis, a noninvasive imaging test that is able to detect cardiac amyloidosis, differentiate AL from ATTR subtypes, and provide prognostic information would significantly improve the identification and early treatment of patients. Recently, the preferential myocardial uptake in patients with cardiac amyloidosis has been described using technetium 99m pyrophosphate (Tc 99m PYP) planar cardiac imaging.[11] While Tc 99m PYP imaging was able to detect ATTR amyloidosis and differentiate it from AL amyloidosis with a sensitivity of 97% and a specificity of 100% in participants with advanced disease at a single center, the diagnostic accuracy of Tc 99m PYP imaging across multiple centers and its ability to predict survival remain unknown. Therefore, in a multicenter study, we sought to evaluate the utility of Tc 99m PYP cardiac imaging for detecting ATTR cardiac amyloidosis and to predict survival among patients who received a diagnosis of cardiac amyloidosis.

Key Points

Question Among patients evaluated at amyloid centers, does technetium 99m pyrophosphate (Tc 99m PYP) imaging accurately detect transthyretin cardiac amyloidosis (also known as ATTR cardiac amyloidosis) and predict survival?

Findings For 171 patients, Tc 99m PYP cardiac imaging demonstrated 91% sensitivity and 92% specificity for ATTR cardiac amyloidosis. In Cox proportional hazards analyses, a heart to contralateral (H/CL) ratio of 1.6 or greater predicted worse survival. Over 5 years, survival was significantly worse if the H/CL ratio was 1.6 or greater rather than less than 1.6.

Meaning Among patients with highly suspected amyloid deposition, Tc 99m PYP cardiac imaging may be of diagnostic and prognostic importance.

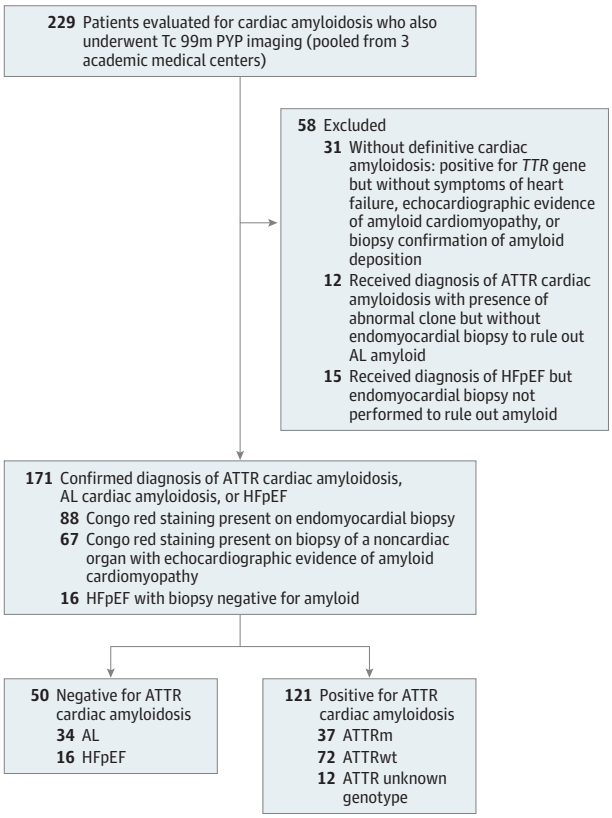
Methods

Study Population and Study Design

A total of 229 patients undergoing evaluation for cardiac amyloidosis, including Tc 99m PYP cardiac imaging between December 1, 2010, and November 1, 2015, were pooled from 3 amyloid centers in the United States (100 patients from the Columbia University Center for Advanced Cardiac Care, 72 patients from the Boston University Amyloidosis Center, and 57 patients from the Mayo Clinic). All patients provided written informed consent, and the study was approved by each center's institutional review board. Cardiac amyloidosis was defined by (1) endomyocardial biopsy-proven congophilic deposits or (2) histological documentation of congophilic deposits in at least 1 noncardiac organ and echocardiographic evidence of infiltrative cardiomyopathy defined as a left ventricular septum or a posterior wall of 12 mm or greater in thickness without another cause of left ventricular hypertrophy. Classification of amyloid type was established by immunohistochemical analysis or mass spectrometry on biopsied tissue specimens. The presence of an abnormal clone in AL cardiac amyloidosis was defined by the presence of a band during immunofixation electrophoresis of serum or urine or by an abnormal free light chain ratio (<0.26 or >1.65) after a serum Freelite assay. Nonamyloid HFpEF was determined if the patient had heart failure with a left ventricular ejection fraction (LVEF) of 50% or greater without echocardiographic evidence of amyloid deposition and a heart biopsy negative for Congo red staining.

Among the 229 patients pooled from 3 centers who underwent Tc 99m PYP cardiac imaging, we first excluded 58 patients who did not undergo standard testing and for whom the diagnosis of cardiac amyloidosis was uncertain (**Figure 1**). Specifically, these included 31 patients who tested positive for the *TTR* gene but who did not have phenotypic or biopsy-proven disease, 12 patients who received a diagnosis of ATTR but who also had an abnormal clone without an endomyocardial biopsy, and 15 patients with HFpEF without a biopsy demonstrating or excluding amyloid cardiomyopathy. The remaining 171 patients included those with ATTR cardiac amyloidosis

Figure 1. Study Population and Breakdown by Amyloid Diagnosis



Echocardiographic evidence of amyloid cardiomyopathy was defined as a left ventricular or posterior wall thickness of 12 mm or greater without another cause of hypertrophy. ATTRm indicates mutated transthyretin; ATTRwt, wild-type transthyretin; HFpEF, heart failure preserved ejection fraction; and Tc 99m PYP, technetium 99m pyrophosphate.

(n = 121), AL cardiac amyloidosis (n = 34), or nonamyloid HFpEF (n = 16).

All participants underwent a single Tc 99m PYP cardiac scan that was interpreted by nuclear cardiologists and radiologists as part of a routine amyloid workup. Biochemistry analysis included serum cardiac biomarkers, troponin I and brain natriuretic peptide (BNP) at Columbia University and Boston University and troponin T and amino-terminal pro-B-type natriuretic peptide at the Mayo Clinic. In addition, the estimated glomerular filtration rate (eGFR), the serum free light chain level, the serum and urine immunofixation electrophoresis results, and the modified body mass index (BMI) (ie, the serum albumin level multiplied by the BMI), as a reflection of gastrointestinal dysmotility, were recorded.[12] Doppler echocardiography was used, along with commercially available ultrasonographic systems, to assess the left ventricular dimensions from parasternal long-axis views by 2-dimensional-guided measurements at the end of diastole and at the end of systole. The myocardial contraction fraction (MCF), a novel volumetric index of myocardial shortening, was calculated from linear dimensions as described previously.[13] All-cause mortality was ascertained by the patients' medical records, by contacting the patients' primary physicians, and by cross-referencing the social security death index. Of note, among the 229 patients pooled from the 3 centers, the first 199 patients were also grouped into a separate multicenter international collaboration to ascertain the diagnostic value of the most promising bone scintigraphy tracers in ATTR cardiac amyloidosis.[14]

Tc 99m PYP Planar Cardiac Scintigraphy

Planar cardiac imaging of the chest was performed using 2-headed gamma cameras with low-energy, high-resolution collimators. First, 10 to 25 mCi of Tc 99m PYP was administered intravenously and incubated for either 1 hour (Columbia University and Boston University), based on prior published amyloid imaging data,[11] or 3 hours (Mayo Clinic), based on original ischemia literature.[15,16] Following incubation, anterior and lateral planar views of the heart were obtained simultaneously for a total 750 000 counts (approximately 3-8 minutes). Cardiac retention was assessed by both a semiquantitative visual score (range, 0 [no uptake] to 3 [uptake greater than bone)[17] and a quantitative heart to contralateral (H/CL) ratio of total counts in a region of interest over the heart divided by background counts in an identical size region of interest over the contralateral chest, including soft tissue, ribs, and blood pool[11] (**Figure 2**). Based on prior published data using a 1-hour incubation, we defined a visual score of 2 or higher, or an H/CL ratio of 1.5 or greater, to indicate ATTR cardiac amyloidosis.[11] For the scans that used a 3-hour incubation, the H/CL ratio threshold was lowered to 1.3 or greater, corresponding to the best calculated area under the curve (AUC) adjusting for higher bone activity seen with longer exposures. Single-photon emission computed tomography was performed after planar scans to evaluate the distribution of myocardial uptake minimizing the confounding factor of increased bone activity at centers using a 3-hour incubation.

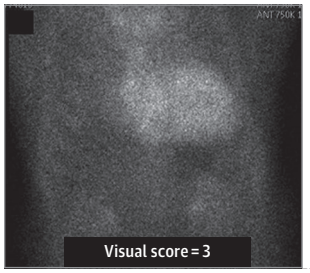
Statistical Analysis

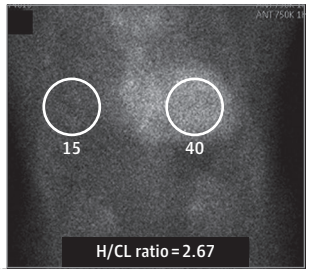
Analyses were performed using Statistical Analysis Software version 9.4 (SAS Institute, Inc) and R (R Core Team 2015). Continuous variables were presented as mean (SD) or median (interquartile range [IQR]) values and categorical variables were summarized as counts (percentages). The χ^2 test or the Fisher exact test (when cell counts were small) were used to compare categorical variables, and the Wilcoxon rank sum test was used to compare continuous variables, between patients with and patients without ATTR cardiac amyloidosis. The sensitivity and the specificity of Tc 99m PYP scans for detecting ATTR cardiac amyloidosis were calculated from standard 2 × 2 tables. Using either a visual score or an H/CL ratio as a diagnostic marker for ATTR cardiac amyloidosis, we estimated the area under the corresponding receiver operating characteristic curve (ie, the AUC, C statistic) with corresponding 95% CIs. Univariable regression and multivariable logistic regression were used to identify factors associated with the outcome of ATTR. The reproducibility of scans in terms of interobserver and intraobserver variability and interinstitutional variability was analyzed with the κ statistic for the visual score and H/CL ratio for a subset of scans (n = 20).

Univariable Cox proportional hazards models were created to identify factors associated with death. The following

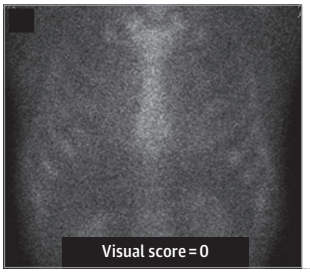
Figure 2. Technetium 99m Pyrophosphate (Tc 99m PYP) Semiquantitative and Quantitative Scoring

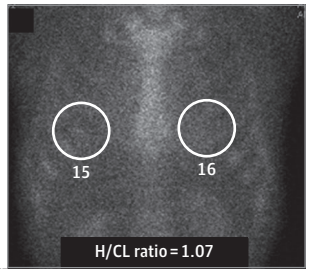
A Tc 99m scan of patient with ATTR cardiac amyloidosis





B Tc 99m scan of patient without ATTR cardiac amyloidosis





C Calculations

Visual score
0 = Myocardial uptake absent
1 = Myocardial uptake < rib
2 = Myocardial uptake = rib
3 = Myocardial uptake > rib

$$\text{H/CL ratio} = \frac{\text{(heart ROI mean counts/pixel)}}{\text{(contralateral ROI mean counts/pixel)}}$$

D Visual score

1-h Incubation

	No. of Patients		
	ATTR+	ATTR−	Total
Positive scan ≥2	92	6	98
Negative scan <2	5	23	28
Total	97	29	126

Sensitivity = 95%
Specificity = 79%

AUC, 0.938 (95% CI, 0.873-0.984)

3-h Incubation

	No. of Patients		
	ATTR+	ATTR−	Total
Positive scan ≥2	14	0	14
Negative scan <2	10	21	31
Total	24	21	45

Sensitivity = 58%
Specificity = 100%

AUC, 0.980 (95% CI, 0.932-1.000)

Multicenter Analysis

	No. of Patients		
	ATTR+	ATTR−	Total
Positive scan	106	6	112
Negative scan	15	44	59
Total	121	50	171

Sensitivity = 88%
Specificity = 88%

AUC, 0.945 (95% CI, 0.901-0.977)

E H/CL ratio

1-h Incubation

	No. of Patients		
	ATTR+	ATTR−	Total
Positive scan ≥1.5	89	1	90
Negative scan <1.5	8	28	36
Total	97	29	126

Sensitivity = 92%
Specificity = 97%

AUC, 0.971 (95% CI, 0.940-0.992)

3-h Incubation

	No. of Patients		
	ATTR+	ATTR−	Total
Positive scan ≥1.3	21	3	24
Negative scan <1.3	3	18	21
Total	24	21	45

Sensitivity = 88%
Specificity = 86%

AUC, 0.935 (95% CI, 0.848-0.988)

Multicenter Analysis

	No. of Patients		
	ATTR+	ATTR−	Total
Positive scan	110	4	114
Negative scan	11	46	57
Total	121	50	171

Sensitivity = 91%
Specificity = 92%

AUC, 0.960 (95% CI, 0.930-0.981)

Representative scans of a patient who tested positive for ATTR cardiac amyloidosis (A) and a patient who tested negative for ATTR cardiac amyloidosis (B) are shown with corresponding visual cardiac scores and heart to contralateral (H/CL) ratios (C). Sensitivity and specificity of Tc 99m PYP for detecting ATTR cardiac amyloidosis based on either visual score (D) or H/CL ratio (E). The centers that used a 1-hour radioisotope incubation were compared with the center that used a 3-hour radioisotope incubation, and then they were combined. A visual score of higher than 2 was considered indicative of ATTR cardiac amyloidosis. The cutoff H/CL ratio that indicated a scan positive for ATTR was greater than 1.5 for centers that scanned patients after 1-hour radioisotope incubation and was greater than 1.3 for the center that scanned patients after 3-hour radioisotope incubation. The multicenter analysis for the H/CL ratio combined cell counts from 1- and 3-hour scan positivity thresholds. The area under the receiver operating curve is shown as AUC (95% CI). ATTR+ indicates patient with ATTR cardiac amyloidosis; ATTR−, patient without ATTR cardiac amyloidosis.

variables were dichotomized around the median or clinically meaningful thresholds[18] (troponin I level of ≥0.10 ng/mL, troponin T level of ≥0.02 ng/mL, BNP level of ≥500 pg/mL, amino-terminal pro-B-type natriuretic peptide level of ≥2000 pg/mL, eGFR < 60 mL/min, LVEF < 45%, interventricular septal wall thickness of ≥15 mm, MCF < 20, and H/CL ratio of ≥1.6). Multivariable Cox proportional hazards modeling was subsequently performed to determine whether the H/CL ratio was significantly associated with mortality after controlling for demographics (age, sex, black race, and mutation status; model 1), demographics and clinical factors (New York Heart Association [NYHA] classification; model 2), and demographics, clinical factors, and biomarkers of disease severity (troponin levels, concentration of natriuretic peptides, and eGFR; model 3). In addition to models 1 to 3, we considered all available variables and used forward selection to develop a model by

sequentially including the individual factors most associated with death, until no remaining factors were significant (model 4). Finally, Kaplan-Meier curves were used to estimate survival in the high and low H/CL ratio groups using the log-rank test. All *P* values were 2-sided with $P < .05$ considered to be statistically significant.

Results

Study Population

The study cohort totaled 171 patients, with a median age of 73 years (IQR, 65-79 years), including 121 (70.8%) with ATTR cardiac amyloidosis and 50 (29.2%) without ATTR cardiac amyloidosis. Of 109 patients with ATTR cardiac amyloidosis (90.1%) who underwent genotyping, 72 (59.5%) had ATTRwt and 37 (30.6%) had ATTRm (15 with Val122Ile, 9 with Thr60Ala, 2 with Ser23Asn, 2 with Phe64Leu, 2 with Ser77Tyr, 2 with Thr59Lys, 1 with Val30Met, 1 with Ala120Ser, 1 with Phe33Leu, 1 with Gln89Glu, and 1 with Gln42Gly). Of the 50 patients without ATTR cardiac amyloidosis, 34 (68.0%) had AL amyloidosis and 16 (32.0%) had HFpEF. The cohort was predominantly male (147 of 171 patients [86.0%]) with NYHA class II or class III symptoms (108 of 134 patients [80.6%]) (**Table 1**). Patients with ATTR cardiac amyloidosis were typically older, more likely to be male, and had a lower NYHA class, a lower LVEF, thicker interventricular septal walls, a lower stroke volume index, and a lower MCF compared with patients without ATTR cardiac amyloidosis. In contrast, patients with AL amyloidosis had lower serum albumin levels and a lower modified BMI.[12] The presence of a monoclonal protein was detected in all patients with AL amyloidosis and in 11 of 121 patients (10.0%) with ATTR cardiac amyloidosis, all of whom had an endomyocardial biopsy confirming TTR as the precursor protein.

Accuracy of Tc 99m PYP Cardiac Imaging for Detecting ATTR Cardiac Amyloidosis

Representative Tc 99m PYP cardiac scans for patients with or without ATTR cardiac amyloidosis are shown with corresponding visual scores and H/CL ratios (Figure 2). Significantly more patients with ATTR cardiac amyloidosis than patients with AL amyloidosis and HFpEF had a semiquantitative visual score of 2 or higher (106 of 121 [87.6%] vs 6 of 50 [12.0%]; $P < .001$) and a greater H/CL ratio (median value, 1.73 [IQR, 1.53-1.98] vs 1.16 [IQR, 1.02-1.30]; $P < .001$), consistent with previously published single-center data.[11] When analyzing Tc 99m PYP uptake by clinical disease state, we found that 62 of 134 patients (46%) had NYHA class I or class II symptoms but showed no difference in H/CL ratio compared with patients who had NYHA class III or class IV symptoms (median H/CL ratio, 1.51 [IQR, 1.20-1.83] vs1.60 [IQR, 1.33-1.88]; $P = .82$). Using logistic regression, we found that age, male sex, albumin level, interventricular septal wall thickness, stroke volume index, and H/CL ratio were significant univariable predictors of ATTR, but in a multivariable model containing all significant univariable covariates, only H/CL ratio independently predicted ATTR (odds ratio, 3.34 [95% CI, 1.86-5.98]; $P < .001$). The Tc 99m PYP visual score and H/CL ratio were highly reproducible. Interobserver and intraobserver variability, in addition to interinstitutional variability, demonstrated 100% agreement ($\kappa = 1.0$, $P < .001$).

The overall sensitivity and the overall specificity for detecting ATTR cardiac amyloidosis by the use of a semiquantitative visual score of 2 or higher were 88% and 88%, respectively, with an AUC of 0.943 (95% CI, 0.902-0.977) (Figure 2). Increasing the cutoff for the visual score to 3 or higher lowered the sensitivity to 76% but increased the specificity to 96%. In contrast to the 3-hour incubation time, the 1-hour incubation time increased sensitivity (95% vs 58%) but lowered specificity (79% vs 100%). When analyzing myocardial Tc 99m PYP uptake quantitatively by combining an H/CL ratio of 1.5 or greater (for centers that used a 1-hour incubation) and an H/CL ratio of 1.3 or greater (for 3-hour incubation time), the overall sensitivity and the overall specificity were 91% and 92%, respectively, with an AUC of 0.960 (95% CI, 0.930-0.981). By quantitative analysis, 1-hour and 3-hour incubation times had similar sensitivity (92% and 88%, respectively) but improved specificity (97% and 86%, respectively). Sensitivity and specificity did not change when analyzing patients with ATTR cardiac amyloidosis whose condition was diagnosed exclusively by endomyocardial biopsy.

H/CL Ratio Predicting Survival

We observed a median follow-up of 365 days (IQR, 219-803 days) from the time of scan. In the course of the study, 33 patients (19.3%) died. Among subtypes, probability of death was 32.4% for patients with AL amyloidosis, 26.4% for patients with ATTR cardiac amyloidosis, and 6.3% for patients with HFpEF. Using Cox proportional hazards regression models among only patients with ATTR cardiac amyloidosis, we found that significant univariable predictors of death included older age ($P = .002$), a lower modified BMI ($P = .002$), a higher NYHA class ($P = .01$), a troponin I level of 0.10 ng/mL or higher ($P = .01$), a BNP level of 500 pg/mL or higher ($P = .03$), and an eGFR of less than 60 mL/min ($P = .01$) (**Table 2**; eTable 1 in the Supplement). Troponin T and amino-terminal pro-B-type natriuretic peptide levels were not included in the predictive modeling because only 1 center used these measures. We observed a trend toward worse survival among patients with an MCF of less than 20 ($P = .07$), as previously reported.[19] Male sex, self-reported black race, presence of an ATTR mutation, LVEF of less than 45%, or interventricular septal wall thickness of 15 mm or greater did not significantly predict death in this cohort. Notably, while a visual score of 3 vs 2 was not related to death ($P = .22$), the H/CL ratio cut point of 1.6 or greater was significantly associated with death (hazard ratio, 3.911 [95% CI, 1.155-13.247]; $P = .03$). In multivariable Cox analysis, the association between an H/CL ratio of 1.6 or greater and death persisted when adjusted by the successive addition of demographic variables (age, sex, black race, and presence of TTR mutation), NYHA class, and serum biomarkers (troponin I, BNP, and eGFR) (Table 2, models 1-3). In a forward selection model that considered all significant univariable predictors of death, an H/CL ratio of 1.6 or greater ($P = .01$), age ($P = .03$), and NYHA class ($P = .01$) remained significantly associated with death

Table 1. Baseline Characteristics of Patients With or Without ATTR Cardiac Amyloidosis[a]

Characteristic	Patients, No. (%)			P Value
	Overall (n = 171)	Without ATTR Cardiac Amyloidosis[a] (n = 50)	With ATTR Cardiac Amyloidosis[b] (n = 121)	
Demographic				
Age, median (IQR), y	73 (65-79)	66 (61-75)	75 (68-80)	<.001
Male sex	147 (86.0)	38 (76.0)	109 (90.1)	.03
Race				
White	139 (81.3)	43 (86.0)	96 (79.3)	.65
Black	21 (12.3)	5 (10.0)	16 (13.2)	
Other	11 (6.4)	2 (4.0)	9 (7.4)	
NYHA class				
I	20 (14.9)	1 (2.9)	19 (19.2)	.04
II	42 (31.3)	13 (37.1)	29 (29.3)	
III	66 (49.3)	18 (51.4)	48 (48.5)	
IV	6 (4.5)	3 (8.6)	3 (3.0)	
Biochemistry				
Troponin I,[c] median (IQR), ng/mL	0.12 (0.05-0.18)	0.13 (0.03-0.18)	0.11 (0.06-0.18)	.49
Troponin T, median (IQR), ng/mL	0.025 (0.01-0.05)	0.04 (0.02-0.07)	0.02 (0.01-0.04)	.04
BNP, median (IQR), pg/mL	460 (195-1212)	685 (171-1341)	434 (195-1190)	.62
NT pro-BNP, median (IQR), pg/mL	2059 (706-3106)	2541 (663-4175)	1922 (962-2571)	.32
Albumin, median (IQR), g/dL	4.0 (3.5-4.4)	3.5 (3.2-3.9)	4.1 (3.7-4.5)	<.001
eGFR, median (IQR), mL/min	74 (53-98)	67 (47-94)	75 (55-99)	.21
Modified BMI,[d] median (IQR)	109 (88-125)	102 (80-121)	112 (97-127)	.02
Presence of clone	50 (32)	39 (79.6)	11 (10.3)	<.001
Echocardiography				
LVEF, median (IQR), %	55 (42-60)	57 (50-63)	53 (39-60)	.02
LA size, median (IQR), cm	4.4 (3.9-5.0)	4.3 (3.8-4.9)	4.4 (4.0-5.0)	.56
IVSD, median (IQR), cm	1.5 (1.3-1.8)	1.4 (1.2-1.6)	1.6 (1.4-1.8)	.002
SVI, median (IQR), mL/m^2	25 (19-33)	28 (24-36)	23 (18-31)	.003
MCF	20 (14-26)	23 (19-32)	17 (13-25)	.001
Tc 99m PYP cardiac imaging				
Visual score				
0	38 (22.2)	36 (72.0)	2 (1.7)	<.001
1	21 (12.3)	8 (16.0)	13 (10.7)	
2	18 (10.5)	4 (8.0)	14 (11.6)	
3	94 (55.0)	2 (4.0)	92 (76.0)	
H/CL ratio, median (IQR)	1.60 (1.28-1.87)	1.16 (1.02-1.30)	1.73 (1.53-1.98)	<.001

Abbreviations: BMI, body mass index; BNP, brain natriuretic peptid; eGFR, estimated glomerular filtration rate; IQR, interquartile range; IVSD, interventricular septal thickness at diastole; LA, left atrium; LVEF, left ventricular ejection fraction; LVPW, left posterior wall thickness at diastole; MCF, myocardial contraction fraction; NYHA, New York Heart Association; NT pro-BNP, amino-terminal pro–B-type natriuretic peptide; SVI, stroke volume index; Tc 99m PYP, technetium 99m pyrophosphate.

[a] Thirty-four patients with AL amyloidosis and 16 patients with heart failure with preserved ejection fraction.

[b] Thirty-seven patients with ATTRm amyloidosis, 72 patients with ATTRwt amyloidosis, and 12 patients with ATTR amyloidosis.

[c] Columbia University and Boston University measured troponin I and BNP, whereas the Mayo Clinic measured troponin T and NT pro-BNP.

[d] Calculated as serum albumin multiplied by kilograms divided by height in meters squared.

(model 4). In Kaplan-Meier analysis among patients with ATTR cardiac amyloidosis over a 5-year follow-up (**Figure 3**), survival was significantly worse if the H/CL ratio was high (≥1.6) rather than low (<1.6) (log-rank P = .02). Comparing patients with high vs low H/CL ratios (eTable 2 in the Supplement), we found that there were no differences in age, sex, race, NYHA class, or LVEF but that those with a high H/CL ratio had a significantly thicker interventricular septal wall (P < .001), a lower stroke volume index (P = .001), and a lower MCF (P < .001).

Discussion

The results of this multicenter study verify previous results from other single-center studies that Tc 99m PYP planar cardiac imaging can accurately differentiate ATTR from AL cardiac amyloidosis. Furthermore, a greater H/CL ratio on Tc 99m PYP cardiac imaging among patients with ATTR cardiac amyloidosis is associated with lower survival.

Table 2. Cox Proportional Hazards Univariable and Multivariable Predictors of Death Among Patients With ATTR Cardiac Amyloidosis[a]

Predictor	Patients, No.	Hazard Ratio (95% CI)	*P* Value
Univariable			
Age, y	121	1.116 (1.042-1.194)	.002
Modified BMI[b]	118	0.959 (0.934-0.985)	.002
Male sex	121	2.643 (0.355-19.705)	.34
Black race	121	1.497 (0.582-3.848)	.40
ATTR mutation	121	0.779 (0.325-1.865)	.58
NYHA class	99	4.754 (1.555-14.529)	.01
Troponin I ≥ 0.10 ng/mL	93	17.156 (2.278-129.186)	.01
BNP ≥ 500 pg/mL	97	3.498 (1.166-10.498)	.03
eGFR < 60 mL/min	120	3.179 (1.353-7.469)	.01
LVEF < 45%	119	1.799 (0.778-4.159)	.17
LA size	104	1.255 (0.697-2.259)	.45
IVSD ≥ 15 mm	119	1.477 (0.498-4.382)	.48
MCF < 20	94	3.207 (0.901-11.410)	.07
Visual score 3 vs 2	121	3.543 (0.474-26.455)	.22
H/CL ratio ≥1.6	121	3.911 (1.155-13.247)	.03
Multivariable			
Model 1[c]	90	7.587 (1.304-44.129)	.02
Model 2[d]	90	6.442 (1.293-32.100)	.02
Model 3[e]	90	6.489 (1.214-34.695)	.03
Model 4[f]	90	7.913 (1.679-37.296)	.01

Abbreviations: BNP, brain natriuretic peptide; BMI, body mass index; eGFR, estimated glomerular filtration rate; H/CL, heart to contralateral; IVSD, interventricular septal thickness at diastole; LA, left atrium; LVEF, left ventricular ejection fraction; MCF, myocardial contraction fraction; NYHA, New York Heart Association.

[a] Cox proportional hazards modeling with time to death as outcome ($P < .05$ considered to be statistically significant).

[b] Calculated as serum albumin multiplied by kilograms divided by height in meters squared.

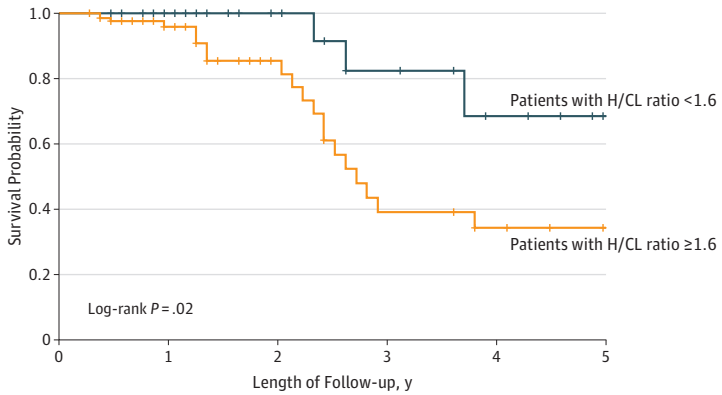
[c] Heart to contralateral ratio of 1.6 or greater adjusted by age, male sex, black race, and ATTR mutation.

[d] Heart to contralateral ratio of 1.6 or greater adjusted by age, male sex, black race, ATTR mutation, and NYHA class.

[e] Heart to contralateral ratio of 1.6 or greater adjusted by age, male sex, black race, ATTR mutation, NYHA class, troponin I ≥ 0.10 ng/mL, BNP ≥ 500 pg/mL, and eGFR < 60 mL/min.

[f] Forward selection: heart to contralateral ratio of 1.6 or greater adjusted by age and NYHA class.

Figure 3. Kaplan-Meier Survival Curves Among 121 Patients With ATTR Cardiac Amyloidosis Over the 5-Year Follow-up, Stratified by Heart to Contralateral (H/CL) Ratio



No. at risk

Patients with H/CL ratio <1.6	38	18	12	7	4	0
Patients with H/CL ratio ≥1.6	83	39	19	9	3	0

Recent nuclear imaging studies using Tc 99m 2,2-diphosphono-1,2 propanodicarboxylic acid, a radiotracer not available in the United States but similar to Tc 99m PYP and used routinely in Europe in the diagnostic workup of cardiac amyloidosis, suggest that ATTR cardiac amyloidosis may not be as rare as previously thought and is likely an underrecognized cause of HFpEF.[20] Heart failure with preserved ejection fraction is a heterogeneous syndrome for which clinical

trials have yet to demonstrate effective therapies leading to renewed focus on basic biologic mechanisms.[21] Notably, these findings coincide with the emergence of TTR-targeted therapies that are currently in phase 3 human clinical trials.[22-25] Therefore, detection of ATTR cardiac amyloidosis using Tc 99m PYP in vulnerable individuals may be important not only for diagnosis and prognostication, but also for early identification of the disease. The recognition of cardiac amyloidosis before overt HFpEF symptoms ensue may be a key therapeutic goal given that the majority of the emerging agents are designed to prevent further amyloid deposition with no direct effect on deposited amyloid. In the present study, the H/CL ratio was associated with the detection of ATTR cardiac amyloidosis across all NYHA classes, nearly half of which had NYHA class I or class II symptoms, which suggests that Tc 99m PYP may be used for patients with heart failure who are only mildly symptomatic. Furthermore, among patients with ATTR cardiac amyloidosis, those with an H/CL ratio of 1.6 or greater compared with those with an H/CL ratio of 1.6 or less not only had worse survival but also had echocardiographic features suggestive of more advanced disease, including a significantly thicker interventricular septal wall, a lower stroke volume index, and a lower MCF. Of note, a visual score of 3 vs 2 for patients with ATTR cardiac amyloidosis was not associated with death, whereas an H/CL ratio of 1.6 or greater vs less than 1.6 was associated with death, a difference that most likely highlights the higher sensitivity of the H/CL ratio for myocardial uptake because it normalizes for background, soft tissue, and blood pool, whereas the visual score does not.

Differences between 1-hour and 3-hour incubation times merit discussion. The H/CL ratio threshold of 1.5 or greater demonstrated high sensitivity and specificity for detecting ATTR cardiac amyloidosis at centers that used a 1-hour incubation, corroborating this previously published imaging method. The center that used a 3-hour incubation time did so based on protocols used in original myocardial ischemia Tc 99m PYP imaging studies.[15,16] With the longer 3-hour incubation, bone activity increases, leading to decreased H/CL ratios, which requires adjustment of the H/CL ratio threshold to 1.3 or greater, where the AUC was highest. In addition, a 3-hour incubation time requires concurrent single-photon emission computed tomography to eliminate the issue of increased bone activity. Despite this variation, the overall accuracy of the Tc 99m PYP methods across the 3 centers remained high. The visual score, however, appeared to be more sensitive at 1-hour vs 3-hour incubation time (95% vs 58%, respectively), although with lower specificity (79% vs 100%, respectively) because, after 3 hours of incubation, the Tc 99m PYP myocardial uptake was reduced and the bone uptake was increased. Overall, however, a 1-hour incubation time is likely superior to a 3-hour incubation time from the perspectives of the patient and systems efficiency, as well as from a diagnostic standpoint, where the AUC is higher, especially when using the H/CL ratio alone with a cutoff of 1.5 or greater.

Clinical Implications

Bone scintigraphy has long been established as safe and reproducible, and now the accuracy of the visual score and the H/CL ratio for detecting ATTR cardiac amyloidosis may galvanize a revival in nuclear cardiology. Refined scan protocols now require as little as 10 mCi of Tc 99m PYP, limit planar scanning to only the chest (vs whole-body scanning performed with other bone isotopes), and only acquire data for approximately 3- to 5-minute duration for a total of 750 000 counts after a radioisotope incubation period of 1 hour. Altogether, Tc 99m PYP planar imaging confers a total radiation exposure of approximately 3 mSv. Prior data indicate that measurements of the visual score and H/CL ratio are highly reproducible within and between readers,[26] and in the present study, these measures were 100% reproducible between institutions. Incorporation of Tc 99m PYP cardiac imaging into diagnostic algorithms may also lower costs relative to the costs of an endomyocardial biopsy, an invasive procedure, coupled with increased risk and specialized and sparsely available immunohistochemical and mass spectrometry expertise.[27] Magnetic resonance imaging of the heart often provides findings that strongly enhance the suspicion for cardiac amyloidosis. Although late gadolinium enhancement, especially with subendocardial distribution, is classically associated with cardiac amyloidosis, other patterns of late gadolinium enhancement are common.[28] In addition, the utility of a magnetic resonance imaging scoring system to differentiate ATTR and AL subtypes is limited by poor reproducibility.[29] When clinical findings and magnetic resonance imaging findings heighten suspicion for cardiac amyloidosis, Tc 99m PYP imaging can add certainty to the specific diagnosis of ATTR cardiac amyloidosis while excluding the diagnosis of AL cardiac amyloidosis. Therefore, our findings have direct clinical implications for the noninvasive detection of ATTR cardiac amyloidosis, the enhancement of patient safety, and the prognostication of disease severity at the time of diagnosis.

It should be noted that the molecular mechanism by which Tc 99m PYP selectively binds to ATTR amyloid fibrils in the myocardium is currently unknown. One theory is that Tc 99m PYP binds to amyloid deposits in a calcium-dependent manner. Calcium is known to enhance the affinity of the human serum amyloid P protein to bind different fibril types,[30,31] and perhaps different calcium concentrations in different fibril or tissue types can explain the varying degrees of subtype-specific Tc 99m PYP uptake. Another hypothesis is that Tc 99m PYP binding relates to the duration over which amyloid deposition has occurred in the affected tissue. In ATTR cardiac amyloidosis, the disease course is typically indolent, and therefore Tc 99m PYP myocardial enhancement is higher in patients with ATTR cardiac amyloidosis than in patients with AL cardiac amyloidosis, for which the fibrils are toxic and accumulate over a shorter period of time.[32,33] Further studies are needed to elucidate the molecular biology of Tc 99m PYP in different organs and tissues.

Limitations

Our study has several limitations. Referral bias and corresponding high pretest probability for cardiac amyloidosis influenced generalizability to a broader population. The distribution of ATTRm mutations was specific to North America, with the Val122Ile mutation known to carry an almost exclusive

cardiac phenotype, accounting for 41% of mutations in the cohort. Therefore, the accuracy of Tc 99m PYP to detect cardiac amyloidosis in individuals harboring mutations known to confer neuropathic or mixed neuropathic-cardiac phenotypes (eg, Val30Met) remains unknown. Furthermore, although nearly half the ATTRm population had NYHA class I or class II symptoms, suggesting utility for Tc 99m PYP cardiac imaging to detect ATTR cardiac amyloidosis early, its role among genotype-positive but phenotype-negative individuals needs to be determined. Another limitation is that echocardiography did not include strain-rate imaging, particularly in light of recent studies demonstrating the prognostic significance of the longitudinal strain in cardiac amyloidosis.[34,35] Magnetic resonance imaging was not performed either, and therefore our study lacks data on late gadolinium enhancement and extracellular volume. The results do not offer information about Tc 99m PYP tracking disease progression, although prior data on patients with advanced disease showed that myocardial uptake did not change over an average 1.5 years of follow-up, even though disease progressed markedly.[26] It is possible that follow-up over a period longer than 1.5 years in a population with less advanced disease is required to better test whether meaningful changes in the H/CL ratio occur temporally and whether Tc 99m PYP can identify patients with early disease.

Conclusions

In this multicenter study of patients evaluated for cardiac amyloidosis, Tc 99m PYP planar cardiac imaging was highly sensitive and specific for detecting ATTR cardiac amyloidosis and distinguishing it from AL cardiac amyloidosis. Among patients who received a diagnosis of ATTR cardiac amyloidosis, an H/CL ratio of 1.6 or greater was independently associated with lower survival. The visual score and the H/CL ratio in Tc 99m PYP planar cardiac imaging should be incorporated into diagnostic and prognostic algorithms for cardiac amyloidosis.

ARTICLE INFORMATION

Accepted for Publication: June 30, 2016.

Published Online: August 24, 2016. doi:10.1001/jamacardio.2016.2839.

Author Affiliations: Division of Cardiology, Department of Medicine, Center for Advanced Cardiac Care, Columbia University Medical Center, New York, New York (Castano, Narotsky, Maurer); Department of Medicine, Boston University School of Medicine, Boston, Massachusetts (Haq); Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, New York (Goldsmith); Nuclear Cardiology Laboratory, Columbia University, New York, New York (Weinberg, Morgenstern, Pozniakoff, Bokhari); Amyloidosis Center, Boston University School of Medicine, Boston, Massachusetts (Ruberg, Berk); Section of Cardiovascular Medicine, Yale University School of Medicine, New Haven, Connecticut (Miller); Department of Hematology, Mayo Clinic, Rochester, Minnesota (Dispenzieri); Department of Cardiovascular Diseases, Mayo Clinic, Rochester, Minnesota (Grogan); Department of Radiology and Immunology, Mayo Clinic, Rochester, Minnesota (Johnson).

Author Contributions: Drs Castano and Haq had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Castano and Haq are co–first authors.
Concept and design: Castano, Haq, Ruberg, Miller, Dispenzieri, Grogan, Johnson, Bokhari, Maurer.
Acquisition, analysis, or interpretation of data: Castano, Haq, Narotsky, Goldsmith, Weinberg, Morgenstern, Pozniakoff, Ruberg, Berk, Dispenzieri, Grogan, Johnson, Bokhari, Maurer.
Drafting of the manuscript: Castano, Haq, Narotsky, Goldsmith, Dispenzieri, Bokhari, Maurer.
Critical revision of the manuscript for important intellectual content: Castano, Narotsky, Goldsmith, Weinberg, Morgenstern, Pozniakoff, Ruberg, Miller, Berk, Dispenzieri, Grogan, Johnson, Bokhari, Maurer.
Statistical analysis: Castano, Haq, Goldsmith, Morgenstern, Miller, Dispenzieri, Johnson.
Obtaining funding: Castano, Maurer.
Administrative, technical, or material support: Castano, Narotsky, Weinberg, Morgenstern, Pozniakoff, Dispenzieri, Grogan, Bokhari.
Study supervision: Castano, Miller, Bokhari, Maurer.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Maurer's institution, Columbia University Medical Center, receives funding for research, and he reports serving on the advisory boards and data and safety monitoring boards of Pfizer Inc, Alnylam Pharmaceuticals, Ionis Pharmaceuticals, and Prothena Inc. Dr Dispenzieri's, Dr Gorgan's, and Dr Johnson's institution, the Mayo Clinic, receives funding for research from Alnylam Pharmaceuticals, Ionis Pharmaceuticals, and Prothena Inc. Dr Grogan reports receiving grants from Pfizer Inc and Alnylam Pharmaceuticals. Dr Berk's institution, Boston University Medical Center, receives funding for research from Alnylam Pharmaceuticals and Ionis Pharmaceuticals. Dr Miller reports receiving grants from Bracco Inc and fees for consulting from GE Inc. No other disclosures are reported.

Funding/Support: Dr Castano was funded by the American College of Cardiology–Merck Fellowship in Cardiovascular Disease and by the New York Academy of Medicine Glorney-Raisbeck Fellowship in Cardiovascular Disease. Dr Maurer received a National Institutes of Health K24 Midcareer Mentoring Award in Geriatric Cardiology.

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We acknowledge the patients with cardiac amyloidosis who continue to hope for methods to improve outcomes, including earlier and more efficient diagnoses and better therapeutics.

REFERENCES

1. González-Lopez E, Guzzo-Merello G, Gallego-Delgado M, et al. Prevalence of TTR senile cardiac amyloidosis among elderly patients with diastolic heart failure. In: ESC Congress 365 of the European Society of Cardiology; August 30–September 3, 2014; Barcelona, Spain. Abstract 5757. http://congress365.escardio.org/Search-Results?vgnextkeyword=C365PRESENTATION101203#.V5bUt4MrKHs. Accessed July 25, 2016.

2. González-López E, Gallego-Delgado M, Guzzo-Merello G, et al. Wild-type transthyretin amyloidosis as a cause of heart failure with preserved ejection fraction. *Eur Heart J*. 2015;36(38):2585-2594.

3. Mohammed SF, Mirzoyev SA, Edwards WD, et al. Left ventricular amyloid deposition in patients with heart failure and preserved ejection fraction. *JACC Heart Fail*. 2014;2(2):113-122.

4. Ton VK, Mukherjee M, Judge DP. Transthyretin cardiac amyloidosis: pathogenesis, treatments, and emerging role in heart failure with preserved ejection fraction. *Clin Med Insights Cardiol*. 2015;8(suppl 1):39-44.

5. Connors LH, Lim A, Prokaeva T, Roskens VA, Costello CE. Tabulation of human transthyretin (TTR) variants, 2003. *Amyloid*. 2003;10(3):160-184.

6. Jacobson DR, Pastore RD, Yaghoubian R, et al. Variant-sequence transthyretin (isoleucine 122) in late-onset cardiac amyloidosis in black Americans. *N Engl J Med*. 1997;336(7):466-473.

7. Castaño A, Drachman BM, Judge D, Maurer MS. Natural history and therapy of TTR-cardiac amyloidosis: emerging disease-modifying therapies from organ transplantation to stabilizer and silencer drugs. *Heart Fail Rev*. 2015;20(2):163-178.

8. Satoskar AA, Efebera Y, Hasan A, et al. Strong transthyretin immunostaining: potential pitfall in cardiac amyloid typing. *Am J Surg Pathol*. 2011;35(11):1685-1690.

9. Chee CE, Lacy MQ, Dogan A, Zeldenrust SR, Gertz MA. Pitfalls in the diagnosis of primary amyloidosis. *Clin Lymphoma Myeloma Leuk*. 2010;10(3):177-180.

10. Vrana JA, Gamez JD, Madden BJ, Theis JD, Bergen HR III, Dogan A. Classification of amyloidosis by laser microdissection and mass

spectrometry-based proteomic analysis in clinical biopsy specimens. *Blood*. 2009;114(24):4957-4959.

11. 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 amyloidoses. *Circ Cardiovasc Imaging*. 2013;6(2):195-201.

12. Suhr O, Danielsson A, Holmgren G, Steen L. Malnutrition and gastrointestinal dysfunction as prognostic factors for survival in familial amyloidotic polyneuropathy. *J Intern Med*. 1994;235(5):479-485.

13. King DL, El-Khoury Coffin L, Maurer MS. Myocardial contraction fraction: a volumetric index of myocardial shortening by freehand three-dimensional echocardiography. *J Am Coll Cardiol*. 2002;40(2):325-329.

14. Gillmore JD, Maurer MS, Falk RH, et al. Nonbiopsy diagnosis of cardiac transthyretin amyloidosis. *Circulation*. 2016;133(24):2404-2412.

15. Corbett JR, Lewis SE, Wolfe CL, et al. Measurement of myocardial infarct size by technetium pyrophosphate single-photon tomography. *Am J Cardiol*. 1984;54(10):1231-1236.

16. Rude RE, Parkey RW, Bonte FJ, et al. Clinical implications of the technetium-99m stannous pyrophosphate myocardial scintigraphic "doughnut" pattern in patients with acute myocardial infarcts. *Circulation*. 1979;59(4):721-730.

17. Perugini E, Guidalotti PL, Salvi F, et al. Noninvasive etiologic diagnosis of cardiac amyloidosis using ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy. *J Am Coll Cardiol*. 2005;46(6):1076-1084.

18. Kumar S, Dispenzieri A, Lacy MQ, et al. Revised prognostic staging system for light chain amyloidosis incorporating cardiac biomarkers and serum free light chain measurements. *J Clin Oncol*. 2012;30(9):989-995.

19. Tendler A, Helmke S, Teruya S, Alvarez J, Maurer MS. The myocardial contraction fraction is superior to ejection fraction in predicting survival in patients with AL cardiac amyloidosis. *Amyloid*. 2015;22(1):61-66.

20. 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(38):2595-2597.

21. Maurer MS, Mancini D. HFpEF: is splitting into distinct phenotypes by comorbidities the pathway forward? *J Am Coll Cardiol*. 2014;64(6):550-552.

22. Berk JL, Suhr OB, Obici L, et al; Diflunisal Trial Consortium. Repurposing diflunisal for familial amyloid polyneuropathy: a randomized clinical trial. *JAMA*. 2013;310(24):2658-2667.

23. Maurer MS, Grogan DR, Judge DP, et al. Tafamidis in transthyretin amyloid cardiomyopathy: effects on transthyretin stabilization and clinical outcomes. *Circ Heart Fail*. 2015;8(3):519-526.

24. Coelho T, Adams D, Silva A, et al. Safety and efficacy of RNAi therapy for transthyretin amyloidosis. *N Engl J Med*. 2013;369(9):819-829.

25. Lachmann HJ. A new era in the treatment of amyloidosis? *N Engl J Med*. 2013;369(9):866-868.

26. Castaño A, DeLuca A, Weinberg R, et al. Serial scanning with technetium pyrophosphate (^{99m}Tc-PYP) in advanced ATTR cardiac amyloidosis [published online October 9, 2015]. *J Nucl Cardiol*. doi:10.1007/s12350-015-0261-x.

27. Maurer MS. Noninvasive identification of ATTRwt cardiac amyloid: the re-emergence of nuclear cardiology. *Am J Med*. 2015;128(12):1275-1280.

28. Fontana M, Pica S, Reant P, et al. Prognostic value of late gadolinium enhancement cardiovascular magnetic resonance in cardiac amyloidosis. *Circulation*. 2015;132(16):1570-1579.

29. Dungu JN, Valencia O, Pinney JH, et al. CMR-based differentiation of AL and ATTR cardiac amyloidosis. *JACC Cardiovasc Imaging*. 2014;7(2):133-142.

30. Pepys MB, Dyck RF, de Beer FC, Skinner M, Cohen AS. Binding of serum amyloid P-component (SAP) by amyloid fibrils. *Clin Exp Immunol*. 1979;38(2):284-293.

31. Pepys MB, Butler PJ. Serum amyloid P component is the major calcium-dependent specific DNA binding protein of the serum. *Biochem Biophys Res Commun*. 1987;148(1):308-313.

32. Ng B, Connors LH, Davidoff R, Skinner M, Falk RH. Senile systemic amyloidosis presenting with heart failure: a comparison with light chain-associated amyloidosis. *Arch Intern Med*. 2005;165(12):1425-1429.

33. Quarta CC, Solomon SD, Uraizee I, et al. Left ventricular structure and function in transthyretin-related versus light-chain cardiac amyloidosis. *Circulation*. 2014;129(18):1840-1849.

34. Cacciapuoti F. The role of echocardiography in the non-invasive diagnosis of cardiac amyloidosis. *J Echocardiogr*. 2015;13(3):84-89.

35. Riffel JH, Mereles D, Emami M, et al. Prognostic significance of semiautomatic quantification of left ventricular long axis shortening in systemic light-chain amyloidosis. *Amyloid*. 2015;22(1):45-53.