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ORIGINAL RESEARCH

Biomarkers to Predict Abnormal Technetium-99m Pyrophosphate Scans in Patients With Suspected Transthyretin Amyloidosis

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ABSTRACT

BACKGROUND Technetium Tc 99m pyrophosphate scintigraphy (^{99m}Tc PYP imaging) is a diagnostic tool for transthyretin amyloid cardiomyopathy (ATTR-CM). Cardiac biomarkers, particularly high-sensitivity cardiac troponin (hs-cTn) and N-terminal pro–B-type natriuretic peptide (NT-proBNP), may help identify patients at low or high risk for ATTR-CM.

OBJECTIVES The authors sought to evaluate the predictive value of hs-cTnT and NT-proBNP in patients undergoing ^{99m}Tc PYP imaging for suspected ATTR-CM in a large U.S. cohort.

METHODS This was a retrospective study of patients who underwent ^{99m}Tc PYP imaging between May 2013 and September 2022, including those with at least 1 hs-cTnT measurement within 6 months of the scan.

RESULTS ATTR-CM was diagnosed in 427 of 1,442 patients (29.6%). A hs-cTnT level <6 ng/L (n = 50, 3.5%) showed a negative predictive value of 100% (95% CI: 93%-100%) and sensitivity of 100% (95% CI: 99%-100%) for ruling out ATTR-CM. As the hs-cTnT threshold increased, the number of patients who could be ruled out also increased, but false negatives emerged. The positive predictive value for ruling in ATTR-CM remained low. NT-proBNP showed similar results (n = 1,378). The combination of hs-cTnT <14 ng/L and NT-proBNP <60 ng/L identified 45 patients (3.3%) without ATTR-CM.

CONCLUSIONS In patients undergoing ^{99m}Tc PYP imaging for suspected ATTR-CM, very low hs-cTnT levels can effectively rule out the diagnosis, although in a small subset of patients. Higher thresholds increase the risk of false negatives. NT-proBNP and combined biomarker strategies showed similar trends, the utility of hs-cTnT and NT-proBNP for ruling in the disease is limited. (JACC CardioOncol. 2025;7:70–78) © 2025 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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Transthyretin (TTR) amyloid cardiomyopathy (ATTR-CM) is an infiltrative disease characterized by the progressive deposition of misfolded TTR in the extracellular matrix.[1-3] The condition is now recognized in 6%[4] to 13%[5] of patients with heart failure with preserved ejection fraction (HFpEF) and increased left ventricular wall thickness.

Diagnostic scores[6-8] and clinical "red flags"[9,10] have been proposed to identify patients who need definitive diagnostic testing. After excluding monoclonal proteins, technetium pyrophosphate single-photon emission computed tomography (^{99m}Tc PYP) enables accurate, noninvasive diagnosis of ATTR-CM.[9]

The role of cardiac biomarkers—particularly high-sensitivity cardiac troponin (hs-cTn) and N-terminal pro-B-type natriuretic peptide (NT-proBNP)—in identifying patients for referral to ^{99m}Tc PYP imaging for suspected ATTR-CM has not been evaluated in a U.S. cohort. This study aimed to assess the predictive value of high-sensitivity cardiac troponin T (hs-cTnT) and NT-proBNP in patients undergoing ^{99m}Tc PYP for suspected ATTR-CM, focusing on whether specific levels of these biomarkers could provide sufficient negative predictive value (NPV) to clinically rule out ATTR-CM.

METHODS

STUDY DESIGN. The study was approved by the Mayo Clinic Institutional Review Board and funded by the Mayo Clinic Department of Cardiovascular Medicine. Only patients who provided written informed consent for the use of their medical records in research were included.

All patients who underwent ^{99m}Tc PYP imaging at the Mayo Clinic (Rochester, Minnesota) from May 2013 to September 2022, with at least 1 hs-cTnT measurement available within 6 months of the imaging, were included in the study. Among these, 607 patients had been included in a previous study for the derivation and validation of an ATTR-CM score in patients with HFpEF.[6] Additionally, patients from a separate community HFpEF cohort (n = 261), whose characteristics were previously published, were included in the present analysis.[4]

NT-proBNP values within 6 months of ^{99m}Tc PYP imaging were extracted from electronic health records. When multiple hs-cTnT or NT-proBNP values were available within this time frame, the value closest to the ^{99m}Tc PYP imaging date was extracted and used for this analysis. Other laboratory and echocardiographic data, performed within 1 year of the ^{99m}Tc PYP imaging, were also collected.

Hs-cTnT was measured using the Elecsys Troponin T Gen 5 STAT assay (Roche Diagnostics). Concentrations are reported in whole units (ng/L) down to the limit of quantitation of <6 ng/L, which is the lowest reportable value per the U.S. Food and Drug Administration. Sex-specific 99th percentile upper reference limits (URLs) of 10 ng/L for women and 15 ng/L for men[11] are used at the Mayo Clinic to define myocardial injury.[12] A value of 14 ng/L for both sexes, which has been explored in other studies, was also evaluated.[13] NT-proBNP levels were measured using the Elecsys analyzer, with age- and sex-specific criteria applied to determine normal values at the Mayo Clinic.[14]

For ^{99m}Tc PYP imaging, 3-hour planar imaging followed by single-photon emission computed tomography with computed tomography (SPECT/CT) imaging was performed for each patient. To classify a "positive" ^{99m}Tc PYP study, which is highly suggestive of ATTR-CM, 2 criteria had to be met: 1) visual interpretation of the SPECT/CT image showing characteristic "diffuse" tracer update greater than the background blood pool; and 2) a heart-to-contralateral lung ratio of 1.3 or higher on the 3-hour planar image.

In cases where the heart-to-contralateral lung ratio was considered inaccurate—such as due to patient anatomy such as rib fractures—visual interpretation of the SPECT/CT image was used alone. Each study was performed and cointerpreted by experienced nuclear cardiologists and radiologists/nuclear medicine physicians.

All patients undergoing ^{99m}Tc PYP were evaluated for serum free light chain abnormalities and the presence of monoclonal proteins in serum and urine, to allow for non-tissue biopsy diagnosis of ATTR-CM. A final diagnosis of ATTR-CM was confirmed by a cardiologist (L.D.M.) and 2 cardiology fellows (M.A..A, D.R.D.) using all available information. Challenging cases were further reviewed by an expert amyloid specialist (O.F.A.) to ascertain a final diagnosis. Additional details on the diagnostic and adjudication process are provided in the Supplemental Appendix. The primary endpoint of the study was the final diagnosis of ATTR-CM, not the frequency of positive ^{99m}Tc PYP scans.

ABBREVIATIONS AND ACRONYMS

^{99m}Tc PYP imaging = technetium Tc 99m pyrophosphate single-photon emission-computed tomography

AL = light chain amyloidosis

ATTR-CM = transthyretin amyloid cardiomyopathy

AUC = area under the curve

CA = cardiac amyloidosis

CKD = chronic kidney disease

eGFR = estimated glomerular filtration rate

HFpEF = heart failure with preserved ejection fraction

hs-cTnT = high-sensitivity cardiac troponin T

NPV = negative predictive value

NT-proBNP = N-terminal pro-B-type natriuretic peptide

PPV = positive predictive value

SPECT/CT = single-photon emission computed tomography with computed tomography

TTR = transthyretin

URL = upper reference limit

STATISTICAL ANALYSIS. Continuous variables are reported as medians with 25th and 75th percentiles (Q1-Q3), whereas categorical variables are presented as absolute numbers and percentages. Group comparisons were performed with the Kruskal-Wallis test for continuous variables and the chi-square test for categorical variables.

Logistic regression was used to calculate the area under the curve (AUC) with associated 95% confidence intervals (CIs). Receiver-operating characteristic analysis evaluated the performance of hs-TnT, NT-proBNP, and their combination in identifying ATTR-CM. The diagnostic accuracy of hs-cTnT and NT-proBNP for ruling out or ruling in ATTR-CM is described using NPV, positive predictive value (PPV), sensitivity, and specificity, each with corresponding 95% CIs.

To assess how renal dysfunction affects the performance of these biomarkers, a separate analysis was performed in patients with chronic kidney disease (CKD), defined as an estimated glomerular filtration rate (eGFR) below 60 mL/min/m^2. The 95% CIs for these values were derived using the exact binomial distribution. All analyses were performed using SAS version 9.4 software (SAS Institute), with 2-sided P values <0.05 considered statistically significant.

RESULTS

Of 2,291 unique patients evaluated with ^{99m}Tc PYP imaging, 52 were excluded due to lack of research authorization, and 797 were excluded due to the absence of hs-cTnT values within 6 months of imaging, leaving 1,442 patients for analysis. Clinical characteristics of excluded patients who authorized the use of their records for research are reported in Supplemental Table 1.

Among those included in the study, 1,378 also had NT-proBNP levels assessed. Hs-cTnT values were measured at a median of 1 day (Q1-Q3: 0-5 days) from the date of ^{99m}Tc PYP imaging. Similarly, NT-proBNP levels were measured at a median of 1 day (Q1-Q3: 0-4 days) from imaging.

A total of 427 patients (29.6%) had a final diagnosis of ATTR-CM. The majority (93%) were men, and the median age was 77 years (Q1-Q3: 72-83 years). Patients with ATTR-CM were older, predominantly male, and had fewer cardiovascular comorbidities compared with those without ATTR-CM (**Table 1**). Median hs-cTnT levels were significantly higher in patients with ATTR-CM (48 [Q1-Q3: 31-66] ng/L) than in those without ATTR-CM (26 [Q1-Q3: 13-49] ng/L; $P < 0.001$). Myocardial injury, defined as hs-cTnT levels above the sex-specific 99th percentile URLs, was observed in 407 patients (95.3%) with ATTR-CM, compared with 770 patients (75.9%) without ATTR-CM ($P < 0.001$). Similarly, NT-proBNP levels were higher in the ATTR-CM group (2,119 [Q1-Q3: 996-3,683] ng/L) compared with those without ATTR-CM (1,134 [Q1-Q3: 331-3,012] ng/L; $P < 0.001$). Kidney function (creatinine) did not differ significantly between groups.

A total of 18 patients had a positive ^{99m}Tc PYP scan but were not diagnosed with ATTR-CM. Among these, 15 had cardiac (n = 8) or extracardiac (n = 7) histologic diagnosis of light chain amyloidosis (AL). For the remaining 3 patients, very subtle myocardial radiotracer (PYP) uptake was observed, and they were offered confirmatory endomyocardial biopsy. One patient underwent the biopsy, which revealed mild myocyte hypertrophy and moderate focal interstitial fibrosis without amyloidosis (assessed using sulfated Alcian blue and Congo red stains). The other 2 patients declined the biopsy.

PREDICTIVE VALUE OF HS-cTnT. The AUC of hs-cTnT for predicting ATTR-CM was 0.69 (95% CI: 0.66-0.71). Among patients with very low hs-cTnT levels (<6 ng/L; n = 50, 3.5% of the overall cohort), none had ATTR-CM, yielding an NPV of 100% (95% CI: 93%-100%) and a sensitivity of 100% (95% CI: 99%-100%) for ruling out ATTR-CM (**Figure 1**, **Central Illustration**, **Table 2**).

Using sex-specific 99th percentile URLs of 10 ng/L for women and 15 ng/L for men, 265 patients (18.4%) had hs-cTnT levels below or equal to these thresholds, with 20 (7.6%) diagnosed with ATTR-CM, yielding an NPV of 92% (95% CI: 89-95%) and a sensitivity of 95% (95% CI: 93-97). When using a threshold of 14 ng/L, as suggested by others,[15] 277 patients (19.2%) had hs-cTnT levels below this threshold, with 16 (5.9%) having ATTR-CM (NPV 94%; 95% CI: 91%-97%; sensitivity 96%; 95% CI: 94%-98%).

The diagnostic performance of these and other hs-cTnT thresholds is shown in **Figure 1** and **Table 2**. Evaluating progressively higher hs-cTnT levels for ruling in ATTR-CM (**Figure 1**, **Table 3**) revealed increased specificity, but the PPV remained low (<45%).

The diagnostic performance of hs-cTnT in patients with CKD (n = 710) is reported in Supplemental Table 2. Compared with those with an eGFR above 60 mL/min/m^2, patients with eGFR below 60 mL/min/m^2 were older (median age 76 vs 72 years) and had higher median hs-cTnT levels (45 [Q1-Q3: 26-75] ng/L vs 23 [Q1-Q3: 12-42] ng/L). Consequently, the percentage of patients correctly

TABLE 1 Baseline Characteristics of the Overall Cohort and by Final Diagnosis (ATTR-CM vs No ATTR-CM)

	Available, n	Total (N = 1,442)	No ATTR-CM (n = 1,015)	ATTR-CM (n = 427)	*P* Value
Age, y	1,442	74 (67-81)	73 (65-80)	77 (72-83)	<0.001
Male	1,442	1,024 (71.0)	623 (61.4)	401 (93.9)	<0.001
Caucasian[a]	1,423	1,316 (92.5)	926 (92.2)	390 (93.1)	0.58
Black[a]	1,423	76 (5.3)	52 (5.2)	24 (5.7)	0.67
Asian[a]	1,423	13 (0.9)	10 (1.0)	3 (0.7)	0.61
Coronary artery disease	1,442	352 (24.4)	274 (27.0)	78 (18.3)	<0.001
COPD	1,442	257 (17.8)	209 (20.6)	48 (11.2)	<0.001
Ischemic heart disease	1,442	102 (7.1)	84 (8.3)	18 (4.2)	0.006
Chronic kidney disease	1,442	460 (31.9)	368 (36.3)	92 (21.5)	<0.001
Hypertension	1,442	752 (52.1)	603 (59.4)	149 (34.9)	<0.001
Diabetes	1,442	465 (32.2)	385 (37.9)	80 (18.7)	<0.001
History of stroke	1,442	206 (14.3)	162 (16.0)	44 (10.3)	0.005
Peripheral vascular disease	1,442	512 (35.5)	413 (40.7)	99 (23.2)	<0.001
Atrial fibrillation	1,442	669 (46.4)	454 (44.7)	215 (50.4)	0.051
Carpal tunnel syndrome	1,442	159 (11.0)	99 (9.8)	60 (14.1)	0.017
Spinal stenosis	1,442	204 (14.1)	144 (14.2)	60 (14.1)	0.95
Systolic blood pressure, mm Hg	1,310	129 (114-143)	130 (115-146)	124 (113-138)	<0.001
Heart rate, beats/min	1,344	70 (62-80)	71 (62-81)	69 (61-78)	0.008
Echocardiography					
Ejection fraction, %	1,370	58 (49-64)	60 (52-64)	52 (43-60)	<0.001
Left ventricular end-diastolic diameter, mm	1,238	49 (45-53)	50 (45-54)	47 (44-52)	<0.001
Septal wall thickness, mm	1,156	13 (11-16)	12 (10-14)	16 (14-18)	<0.001
Posterior wall thickness, mm	1,153	12 (10-14)	11 (10-13)	15 (12-16)	<0.001
Relative wall thickness	1,141	0.49 (0.40-0,61)	0.45 (0.38-0.54)	0.60 (0.50-0.73)	<0.001
Laboratory					
Creatinine, mg/dL	1,427	1.2 (1.0-1.6)	1.2 (1.0-1.6)	1.2 (1.1-1.5)	0.39
N-terminal pro-B-type natriuretic peptide, ng/L	1,378	1,463 (462-3,276)	1,134 (331-3,012)	2,119 (996-3,683)	<0.001
High-sensitivity cardiac troponin T, ng/L	1,442	33 (17-58)	26 (13-49)	48 (31-66)	<0.001

Values are median (Q1-Q3) or n (%). [a]Race was self-reported.
ATTR-CM = transthyretin amyloid cardiomyopathy; COPD = chronic obstructive pulmonary disease.

ruled out using the evaluated thresholds was lower in this subset than in the overall cohort.

PREDICTIVE VALUE OF NT-proBNP. The AUC of NT-proBNP for predicting ATTR-CM was 0.62 (95% CI: 0.59-0.65). An NT-proBNP level below 60 ng/L (n = 53, 3.8%) had a sensitivity and NPV of 100% for ruling out ATTR-CM (**Figure 1**, **Central Illustration**, **Table 2**). At a threshold of 180 ng/L, as suggested by others,[15] 180 patients (13.1%) had NT-proBNP levels below this value, with 12 (6.7%) diagnosed with ATTR-CM (NPV 93%; 95% CI: 89-97; sensitivity 97%; 95% CI: 95%-99%).

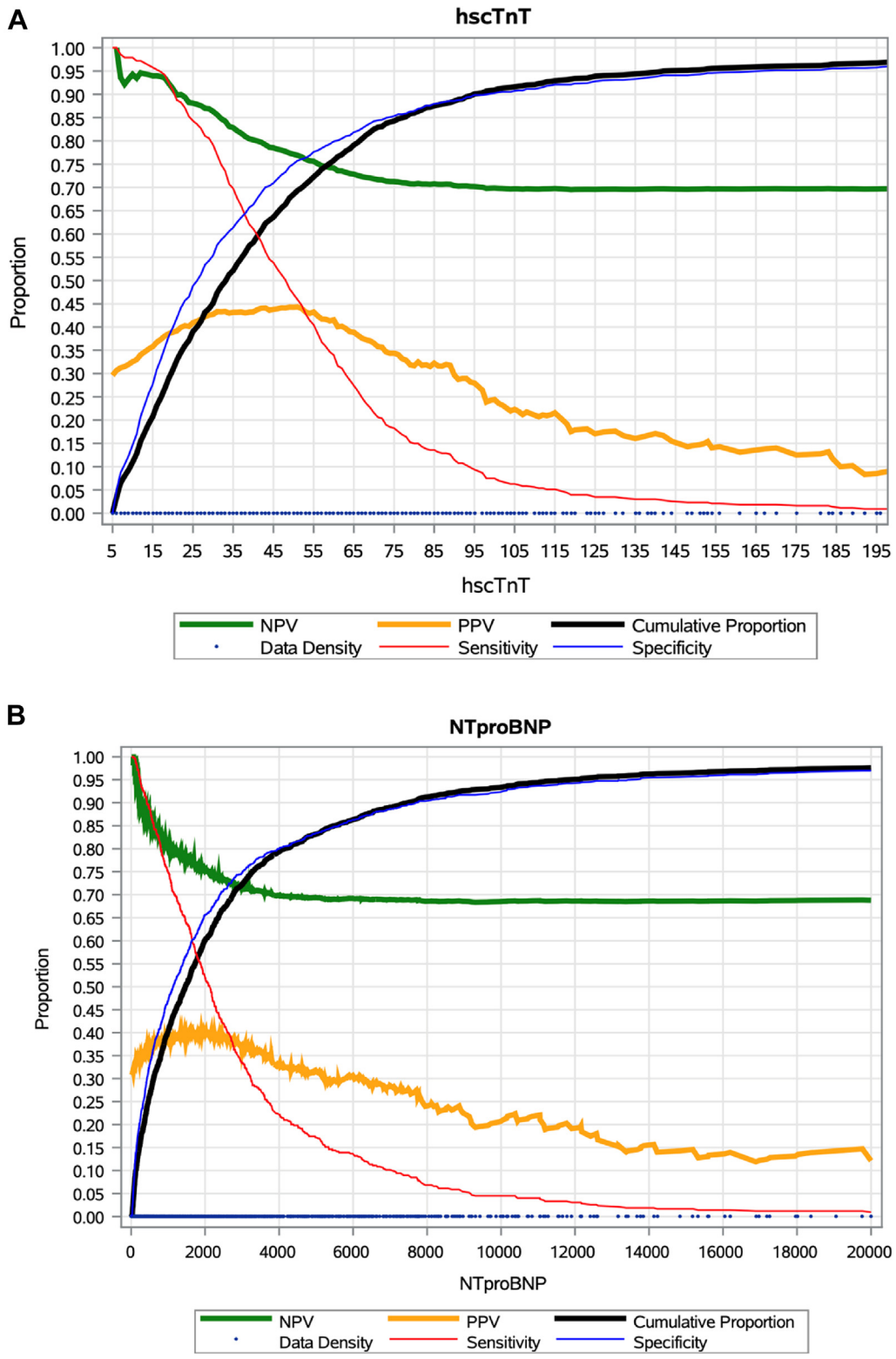
When considering progressively increasing NT-proBNP levels as potential thresholds to rule in ATTR-CM, specificity increased, but the PPV remained low (<45%) (**Figure 1**, **Table 3**). Patients with CKD had higher median NT-proBNP values (2,363 [Q1-Q3: 882-5,412] ng/L vs 901 [Q1-Q3: 260-1,989] ng/L). The diagnostic performance of NT-proBNP in this subset is detailed in Supplemental Table 2.

COMBINED NT-proBNP AND HS-cTnT. Using a combination of hs-cTnT and NT-proBNP, the AUC for predicting ATTR-CM was 0.68 (95% CI: 0.65-0.71). To identify the most optimal threshold for diagnostic performance, a combination of hs-cTnT <14 ng/L and NT-proBNP <60 ng/L was observed in 45 patients (3.3%) and showed a sensitivity and NPV of 100% for ruling out ATTR-CM. Other combinations of hs-cTnT and NT-proBNP thresholds for ruling out ATTR-CM are presented in **Table 2**. The diagnostic performance of these combined biomarkers in patients with CKD is detailed in Supplemental Table 2.

DISCUSSION

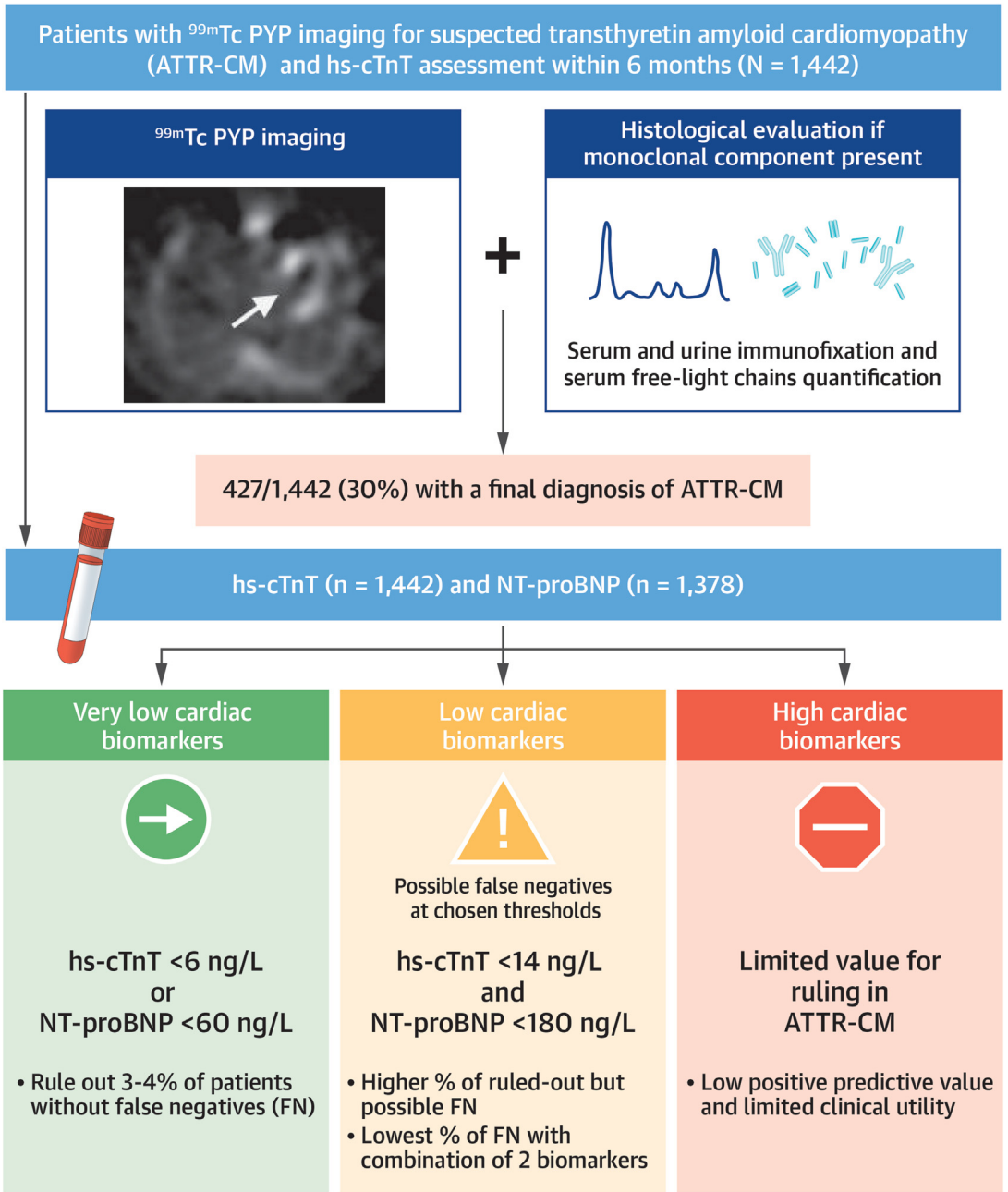
To our knowledge, this is the first study to investigate the predictive value of cardiac biomarkers in a large U.S. cohort undergoing ^{99m}Tc PYP imaging for suspected ATTR-CM. We report several important observations. Very low hs-cTnT levels (<6 ng/L)

FIGURE 1 Diagnostic Performance of hs-cTnT and NT-proBNP in Patients Undergoing Imaging for Suspected ATTR-CM



Diagnostic performance of high-sensitivity cardiac troponin T (hs-cTnT) (A) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) (B) is shown in patients undergoing imaging for suspected transthyretin amyloid cardiomyopathy (ATTR-CM). ^{99m}Tc PYP = technetium pyrophosphate single-photon emission-computed tomography; NPV = negative predictive value; PPV = positive predictive value.

CENTRAL ILLUSTRATION **Cardiac Biomarkers in Patients Undergoing ^{99m}Tc PYP Imaging for Suspected Transthyretin Amyloid Cardiomyopathy**



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In this cohort, 30% were diagnosed with transthyretin amyloid cardiomyopathy (ATTR-CM). Very low cardiac biomarker levels effectively ruled out a small subset of patients, whereas higher thresholds led to some false negatives (FN). The utility of cardiac biomarkers for confirming the disease was limited. ^{99m}Tc PYP = technetium pyrophosphate single-photon emission computed tomography; hs-cTnT = high-sensitivity cardiac troponin T; NT-proBNP = N-terminal pro-B-type natriuretic peptide.

TABLE 2 Performance of hs-cTnT and NT-proBNP Thresholds for Ruling out ATTR-CM in Patients Undergoing ^{99m}Tc PYP Imaging

Biomarker	Threshold	Sensitivity	Specificity	NPV	PPV	TN	FN
Hs-cTnT	<6 ng/L	100 (99-100)	5 (4-6)	100 (93-100)	31 (28-33)	50	0
	<12 ng/L	97 (95-99)	21 (18-23)	95 (91-97)	34 (31-37)	211	12
	<14 ng/L	96 (94-98)	26 (23-29)	94 (91-97)	35 (33-38)	261	16
	<20 ng/L	91 (88-93)	40 (37-43)	91 (88-94)	39 (36-42)	405	39
	≤10 ng/L F ≤15 ng/L M	95 (93-97)	24 (22-27)	92 (89-95)	35 (32-37)	245	20
NT-proBNP	<60 ng/L	100 (99-100)	6 (4-7)	100 (93-100)	32 (29-35)	53	0
	<180 ng/L	97 (95-99)	18 (15-20)	93 (89-97)	34 (32-37)	168	12
Hs-cTnT/ NT-proBNP	<6 ng/L <60 ng/L	100 (99-100)	2 (1-3)	100 (79-100)	31 (29-34)	16	0
	<6 ng/L <180 ng/L	100 (99-100)	4 (3-5)	100 (90-100)	32 (29-34)	36	0
	<12 ng/L <60 ng/L	100 (99-100)	4 (3-6)	100 (91-100)	32 (29-34)	41	0
	≤10 ng/L F or ≤15 ng/L M <60 ng/L	100 (99-100)	5 (3-6)	100 (92-100)	32 (29-34)	45	2
	<14 ng/L <60 ng/L	100 (99-100)	5 (3-6)	100 (92-100)	32 (29-34)	45	0
	<12 ng/L <180 ng/L	99 (98-100)	10 (8-12)	96 (90-99)	33 (30-36)	97	4
	<14 ng/L <180 ng/L	99 (97-100)	11 (9-13)	96 (90-99)	33 (31-36)	107	5
	≤10 ng/L F or ≤15 ng/L M <180 ng/L	98 (97-100)	11 (9-13)	95 (90-99)	33 (31-36)	107	5

Values are estimate % (95% CI) or n, except as noted.

^{99m}Tc PYP imaging = technetium Tc 99m pyrophosphate single-photon emission-computed tomography; F = female; FN = false negative; hs-cTnT = high-sensitivity cardiac troponin T; M = male; NPV = negative predictive value; NT-proBNP = N-terminal pro-B-type natriuretic peptide; PPV = positive predictive value; TN = true negative.

demonstrated good performance with a sensitivity and NPV of 100% for ruling out ATTR-CM, but these levels were observed in only 3.5% of the cohort. Higher thresholds led to an increasing percentage of false-negative results. NT-proBNP showed similar trends; levels below 60 ng/L had a sensitivity and NPV of 100%, but these were found in only 3.8% of the cohort, with higher thresholds also increasing false negatives. The percentage of patients accurately ruled out was even lower among those with CKD. Neither high hs-cTnT nor high NT-proBNP levels was effective for ruling in ATTR-CM due to their low PPV. Combining hs-cTnT <14 ng/L and NT-proBNP <60 ng/L achieved a 100% sensitivity and NPV for ruling out ATTR-CM, but this applied to only 3.3% of our cohort, offering no advantage over using each biomarker alone.

TABLE 3 Performance of hs-cTnT and NT-proBNP Thresholds for Ruling in ATTR-CM in Patients Undergoing ^{99m}Tc PYP Imaging

Biomarker	Threshold	Sensitivity	Specificity	NPV	PPV	TP	FP
hs-cTnT	>153 ng/L	2 (1-4)	95 (93-96)	70 (67-72)	15 (8-26)	10	55
NT-proBNP	>12,421 ng/L	3 (1-5)	95 (93-96)	69 (66-71)	17 (9-29)	11	52

Values are estimate % (95% CI) or n, except as noted. The thresholds reported in the table are those that performed best in our cohort for ruling in ATTR-CM.

Abbreviations as in Tables 1 and 2.

Given the low discriminatory value of hs-cTnT and NT-proBNP alone or in combination, our findings suggest that a biomarker-based approach to identify which patients should undergo ^{99m}Tc PYP imaging for suspected ATTR-CM is less than ideal. The ability to make a noninvasive diagnosis of ATTR-CM in selected cases, combined with new therapeutic advances,[9] has increased the need to identify patients at higher risk for ATTR-CM. For these reasons, various scores and "red flags" have been proposed to identify ATTR-CM in patients with HFpEF,[6] aortic stenosis,[7] or carpal tunnel syndrome.[10] Additionally, it is important to identify patients who may not require further testing. Although cardiac biomarkers have been explored for prognostic purposes in ATTR-CM,[16] data on their diagnostic significance remain limited.

Vergaro et al[15] reported that hs-cTnT and NT-proBNP have diagnostic value in patients with suspected cardiac amyloidosis (CA). In their study, a final diagnosis of CA (either AL or ATTR) was made in over 60% of cases. Low hs-cTnT levels, defined as <14 ng/L, and low NT-proBNP levels (<180 ng/L) were useful in identifying patients at low risk for CA, whereas hs-cTnT levels >86 ng/L helped identify those with a high probability of disease. In the validation cohort, an hs-cTnT level of 14 ng/L demonstrated a sensitivity of

98% (95% CI: 96%-99%) and an NPV of 91% (95% CI: 86%-96%) for excluding disease. Similar results were reported for an NT-proBNP level of 180 ng/L. The combination of both biomarkers below these thresholds was observed in 78 patients of the validation cohort, with 4 false negatives. For ruling in CA, an hs-cTnT level of 86 ng/L had a specificity of 95% (95% CI: 91%-97%) and a PPV of 89% (95% CI: 84%-94%). These thresholds were further validated in cohorts with varying disease prevalence using bootstrapping methods.

Compared with the study by Vergaro et al,[15] our analysis focused on lower threshold biomarker values in a U.S. cohort with a lower pretest likelihood of ATTR-CM (30% final diagnosis rate). The lower percentage of patients in our cohort referred for ^{99m}Tc PYP imaging for suspected ATTR-CM who ultimately had a confirmed diagnosis is more likely reflective of most physicians' practices. Very low hs-cTnT and low NT-proBNP values identified patients at low risk for ATTR-CM. For hs-cTnT, the lowest reportable U.S. value of <6 ng/L (limit of quantitation of the assay) avoided false-negative results. A similar trend was observed for very low NT-proBNP values (<60 ng/L). However, these findings applied to only a small proportion (3.5%-3.8%) of our cohort.

The combination of hs-cTnT <14 ng/L and NT-proBNP <60 ng/L offered a sensitivity and NPV of 100% but applied to just 3.3% of patients in our data set, providing no advantage over using each biomarker alone. At progressively higher thresholds, more patients could be ruled out, but this came with an increased rate of false negatives, consistent with findings by Vergaro et al.[15] The overall number of false negatives remained low but was not insignificant among those with biomarker levels below these specific thresholds.

For instance, among patients with hs-cTnT levels below 14 ng/L, approximately 6% had ATTR-CM, indicating a sensitivity that may be inadequate given the critical importance of accurate diagnosis. Similarly, 7% of patients with NT-proBNP levels below 180 ng/L had the disease. The combination of hs-cTnT <14 ng/L and NT-proBNP <180 ng/L, as used in the prior European study, was present in 112 patients but resulted in 5 false negatives (4%). The different composition of patients in Vergaro et al's cohort (both AL and ATTR-CM) compared with ours (ATTR-CM only) should also be considered. However, their results were consistent in the subgroup referred for suspected ATTR-CM.

Our data suggest that very low biomarker thresholds can safely rule out ATTR-CM, whereas higher thresholds, even those below the 99th percentile URL, carry a risk of false negatives, requiring closer clinical assessment. Nonetheless, integrating cardiac biomarkers into the diagnostic algorithm for ATTR-CM is reasonable and may help in selecting patients who require further, more costly imaging tests.

In contrast to the findings of Vergaro et al[15] for ruling in ATTR-CM, high hs-cTnT and NT-proBNP values in our study achieved a specificity of 95%, but the PPV remained low. Consequently, we were unable to identify specific, clinically useful thresholds for hs-cTnT and NT-proBNP to confirm ATTR-CM. This difference may be attributed to the clinical characteristics of patients referred for ^{99m}Tc PYP imaging in our study, who were generally older and had comorbidities beyond ATTR-CM that could elevate hs-cTnT levels. Additionally, the lower prevalence of ATTR-CM in our cohort (30% vs 60% in the European cohort) contributed to the lower PPV observed. Similarly, Vergaro et al[15] could not establish a reliable NT-proBNP cutoff for ruling in CA due to the high percentage of patients with elevated NT-proBNP in their overall cohort.

STUDY LIMITATIONS. Our study has several strengths, including a large sample size, robust case and control ascertainment at a well-established amyloidosis center of excellence, consistent cardiac biomarker assays across patients, and rigorous statistical methods.

However, several limitations are to be acknowledged due to the retrospective, single-center design. First, the retrospective nature of the study and the referral pattern of the Mayo Clinic practice introduce selection and referral bias. Moreover, the probed thresholds were not derived and then validated against a separate data set, highlighting the need for prospective validation in external cohorts, particularly those with lower disease prevalence.

Second, cardiac biomarkers are an established component of the ATTR-CM diagnostic pathway, which may have influenced how cases were adjudicated. Third, we included only patients with hs-cTnT values available within 6 months, and NT-proBNP values were not available for all patients, which may have impacted our findings. Fourth, given the known predisposition of elderly males to ATTR-CM, most patients in our cohort were male (71%). Therefore, applying these data to predominantly female cohorts requires caution. Finally, the small number of patients with hereditary ATTR-CM prevented a dedicated analysis of biomarker performance in this subgroup.

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CONCLUSIONS

In patients undergoing ^{99m}Tc PYP imaging for suspected ATTR-CM, very low hs-cTnT values can effectively rule out the diagnosis. However, as thresholds increase, the risk of false-negative results rises, which should be considered in clinical practice. High hs-cTnT levels have limited predictive value for ruling-in the disease. Similar findings apply to NT-proBNP. Although these biomarkers can help identify patients at low risk for ATTR-CM, the potential for false negatives must be acknowledged.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Very low hs-cTnT and NT-proBNP levels can identify patients at low risk for ATTR-CM, though they apply to only a small subset. As thresholds increase, a multiparametric approach is necessary to avoid false negatives and misdiagnosis. By contrast, the utility of hs-cTnT and NT-proBNP for ruling in ATTR-CM is limited.

TRANSLATIONAL OUTLOOK: Future prospective studies are needed to validate these findings in broader and less-selected patient populations with suspected ATTR-CM.

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APPENDIX For an expanded Methods section and supplemental tables, please see the online version of this paper.