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ORIGINAL ARTICLE
Heart Failure

Utility of ^{99m}Tc-Pyrophosphate Scintigraphy in Diagnosing Transthyretin Cardiac Amyloidosis in Real-World Practice

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Background: Amyloid transthyretin (ATTR) cardiac amyloidosis has now been recognized as one of the major causes of heart failure, especially in elderly patients. The purpose of the present study was to validate the usefulness of technetium-99 m (^{99m}Tc)-pyrophosphate (^{99m}Tc-PYP) scintigraphy in the screening diagnosis for ATTR amyloidosis in daily clinical practice.

Methods and Results: Ninety-eight patients underwent ^{99m}Tc-PYP scintigraphy in the previous 3 years (PYP positive/negative, 18/80), of whom 29 underwent concomitant endomyocardial biopsy (ATTR positive/negative, 9/20). The sensitivity and specificity of ^{99m}Tc-PYP scintigraphy for the diagnosis of biopsy-proven ATTR amyloidosis were 0.889 and 0.950, respectively. Age, gender, N-terminal prohormone of brain natriuretic peptide (NT-proBNP) level, or electrocardiogram findings did not differ significantly between PYP-positive and PYP-negative patients. Left ventricular (LV) wall thickness was significantly greater in PYP-positive than in PYP-negative patients, but LV ejection fraction or prevalence of atrial fibrillation was similar between groups. In the PYP-positive patients, higher uptake of PYP correlated with younger age and lower NT-proBNP.

Conclusions: ^{99m}Tc-PYP scintigraphy was useful, with high sensitivity and specificity in the screening diagnosis for ATTR cardiac amyloidosis, which is difficult to diagnose on clinical characteristics alone. ^{99m}Tc-PYP scintigraphy should be considered to elucidate the underlying causes of heart failure, especially in elderly patients based on the higher prevalence of ATTR cardiac amyloidosis in this population.

Key Words: ^{99m}Tc-pyrophosphate scintigraphy; Endomyocardial biopsy; Transthyretin cardiac amyloidosis

Amyloidosis is a localized or systemic deposition disease in which proteins with unstable tertiary structures misfold, aggregate, and form amyloid fibrils that are deposited, with a range of chaperone proteins, in the heart, kidneys, liver, gastrointestinal tract, lungs, and soft tissues.[1] More than 30 proteins can form amyloid fibrils, 5 of which frequently infiltrate the heart and cause cardiac amyloidosis: immunoglobulin light chain (AL), immunoglobulin heavy chain (AH), amyloid transthyretin (ATTR), serum amyloid A (AA), and apolipoprotein A I (AApoA I). Although cardiac amyloidosis has been considered a rare condition, recent studies suggest that ATTR amyloidosis may be a major cause of heart failure (HF), especially in elderly patients.[2–5] Indeed, Ueda et al reported that 12% of autopsy cases in patients >80 years of age involved deposition of ATTR in the heart, with the percentage increasing with age.[2] ATTR cardiac amyloidosis, however, appears underdiagnosed because of a relatively high threshold for performing endomyocardial biopsy in daily clinical practice.[6]

Technetium (Tc)-labeled bone scintigraphy, including ^{99m}Tc-pyrophosphate (^{99m}Tc-PYP), has recently been revisited as a screening tool for ATTR cardiac amyloidosis.[7–10] The myocardial uptake of bone scintigraphy is reportedly >99% sensitive and 86% specific for ATTR cardiac amyloidosis, with false positives almost exclusively due to the uptake in patients with AL amyloidosis.[9] Furthermore, although ATTR amyloidosis has been considered a disease predominantly associated with elderly men, characterized by concentric left ventricular hypertrophy (LVH), a preserved left ventricular ejection fraction (LVEF), and low QRS voltages, a recent study with a large cohort of 108 patients showed that the clinical spectrum of ATTR amyloidosis is heterogeneous and differs from the classic phenotype.[11]

Therefore, the purpose of the present study was to validate the accuracy of ^{99m}Tc-PYP scintigraphy in the screening for biopsy-proven ATTR cardiac amyloidosis in

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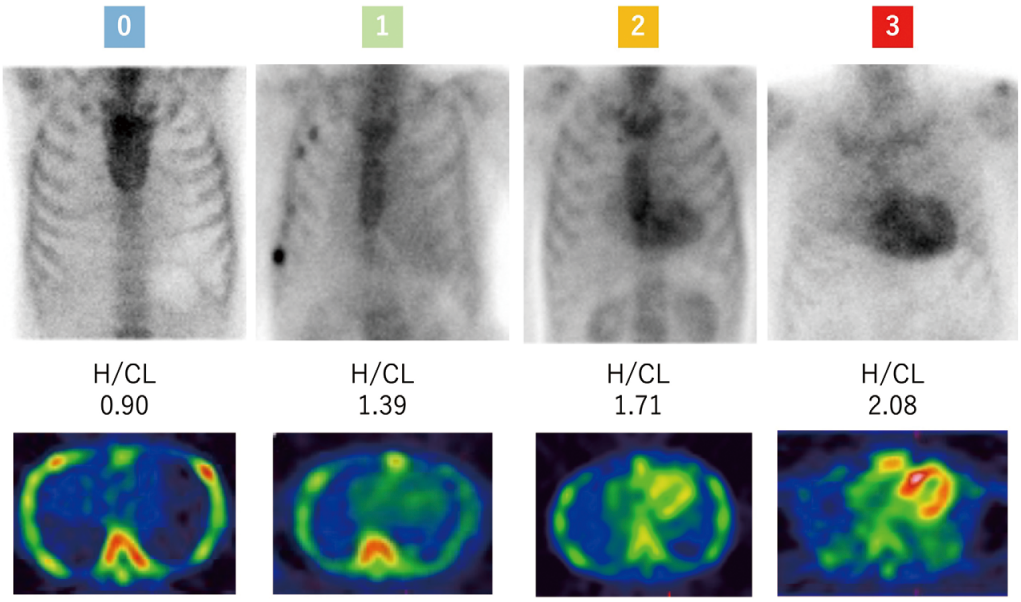

Figure 1. Representative technetium-99m pyrophosphate scintigraphy of visual scores 0–3 with concomitant H/CL ratios. (**Upper panels**) Planar images by which the H/CL ratios were evaluated; (**Lower panels**) single-photon emission computed tomography, with which the visual scores were graded. H/CL, mean counts in the heart region of interest (ROI) relative to the contralateral chest ROI.

daily clinical practice and to elucidate the patient characteristics of positive ^{99m}Tc-PYP uptake.

Methods

Patients

All patients who received ^{99m}Tc-PYP scintigraphy for screening of ATTR cardiac amyloidosis in Saiseikai Fukuoka General Hospital between 2015 and 2017 were retrospectively analyzed based on clinical records. Although the physicians were encouraged to perform ^{99m}Tc-PYP scintigraphy in elderly patients with HF without evident coronary or valvular heart disease, patients with coronary artery disease or valvular heart disease were not excluded in the present study. The study protocol was approved by the Ethics Committee of Saiseikai Fukuoka General Hospital. The investigation conformed to the principles outlined in the Declaration of Helsinki.

^{99m}Tc-PYP Single-Photon Emission Computed Tomography Scintigraphy

Planar imaging with ^{99m}Tc-PYP was performed with a dual-head Siemens Synvia E single-photon emission computed tomography (SPECT) camera (Siemens, Germany) equipped with low-energy, high-resolution collimators. Patients received 740 MBq of ^{99m}Tc-PYP i.v., and anterior and posterior planar views were obtained at 2.5 h. The planar images were acquired for 5 min, with the heart centered in the field of view. The acquisition parameters used for planar imaging were a 128×128 matrix with a 1.45 zoom factor. The acquisition parameters for SPECT imaging were low-energy, high-resolution collimators and a 64×64 matrix with a 1.45 zoom. The Butterworth filter was used with a cut-off of 0.25 cycle/pixel and order of 8.

Based on Bokhari et al,[8] 2 methods were used to evaluate the myocardial tracer uptake: (1) semiquantitative visual scoring of cardiac retention (0, no cardiac uptake; 1, mild uptake less than bone; 2, moderate uptake equal to bone; 3, high uptake greater than bone) based on SPECT; and (2) quantitative analysis of the heart retention calculated by drawing a region of interest (ROI) over the heart in the standard anterior planar view. A circular ROI was drawn over the heart, copied, and mirrored over the contralateral chest to normalize for the spillover from the ribs. The proportion of mean counts in the heart ROI relative to the contralateral chest ROI was calculated as the heart-to-contralateral (H/CL) ratio.

Endomyocardial Biopsy

Endomyocardial biopsies were obtained from the right ventricular septum. Tissue samples were fixed in 10% buffered formalin overnight. For conventional histochemistry, paraffin-embedded tissues were cut into 4-μm-thick slices and stained with hematoxylin-eosin and Masson trichrome. Amyloid deposition in the cardiac interstitium and vascular wall was defined as Congo-red stain followed by confirmation of apple green birefringence under polarized light. For the amyloid typing, immunohistochemical staining was performed using an automated slide stainer (BenchMark ULTRA, Ventana Medical Systems, Tucson, AZ, USA), and the sections were incubated with mouse monoclonal antibody (mAb) for human amyloid A (clone mc1; Dako, Glostrup, Denmark), mouse mAb for human kappa light chain (clone H16-E; Abnova, Taipei, Taiwan), mouse mAb

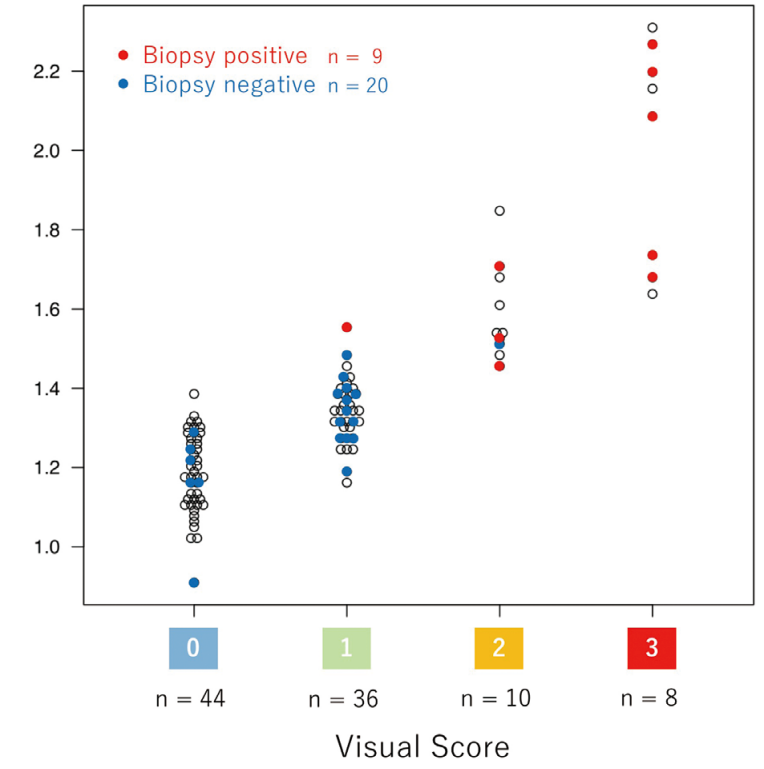

Figure 2. H/CL ratio vs. visual score and endomyocardial biopsy results in the diagnosis of amyloid transthyretin cardiac amyloidosis. Open circles, patients without endomyocardial biopsy. Visual score was significantly correlated with H/CL ratio (r=0.868, P<0.0001). The cut-off by which H/CL ratio discriminates technetium-99m pyrophosphate scintigraphy-positive from -negative patients was 1.46. H/CL, mean counts in the heart region of interest (ROI) relative to the contralateral chest ROI.

for human lambda light chain (clone HP6052; Genemed Biotechnologies, South San Francisco, CA, USA) or rabbit mAb anti-prealbumin (transthyretin; clone EPR3219; Abcam, Cambridge, UK) following manufacturer instructions. Alternatively, samples were fixed in 2.5% glutaraldehyde, and the deposition of amyloid microfibrils was detected on transmission electron microscopy. Representative histological pictures of ATTR cardiac amyloidosis are shown in **Supplementary Figures 1,2**.

Statistical Analysis

All statistical analyses were performed using EZR 1.32 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (R Foundation for Statistical Computing, Vienna, Austria).[12] Continuous variables are presented as mean±SD unless mentioned otherwise. Comparison of continuous variables between 2 independent groups was performed using unpaired Student's t-test (if normally distributed) or Mann–Whitney U-test (non-normally distributed variables) and in cases where more than 2 groups were compared, 1-way analysis of variance (ANOVA) or Kruskal–Wallis test was used. Categorical data are reported as frequencies and percentages and were compared using chi-squared or Fisher's exact test. Receiver operating characteristic (ROC) analysis was used to obtain cut-off points to convert continuous parameters to categorical variables. The diagnostic accuracy was calculated using estimated 95% CI. All tests were 2-tailed, and P<0.05 was considered to be statistically significant.

Results

Ninety-eight patients underwent ^{99m}Tc-PYP scintigraphy (mean age, 78.9±11.0 years; male/female, 56/42). **Figure 1** shows the representative images of ^{99m}Tc-PYP scintigraphy of visual scores 0–3 with concomitant H/CL ratios. Eighteen patients (18.4%) were diagnosed as PYP positive based on visual score 2–3, of whom 9 underwent endomyocardial biopsy. Eighty patients (81.6%) were diagnosed as PYP negative based on visual score 0–1, of whom 20 underwent endomyocardial biopsy. As shown in **Figure 2**, visual score significantly correlated with H/CL ratio (r=0.868, P<0.0001). The H/CL ratio cut-off to discriminate PYP-positive from PYP-negative patients was 1.46 (sensitivity, 1; specificity, 0.975; area under the curve [AUC], 0.995).

^{99m}Tc-PYP Scintigraphy and Endomyocardial Biopsy

The clinical characteristics of 29 patients who underwent both ^{99m}Tc-PYP scintigraphy and endomyocardial biopsy, of whom 9 patients were histologically ATTR positive and 20 were ATTR negative, are summarized in **Table 1**. None of 6 patients who had undergone genetic analysis had a mutation in the transthyretin gene. There were no significant differences in age, gender, or N-terminal prohormone of brain natriuretic peptide (NT-proBNP) level between histologically ATTR-positive and ATTR-negative patients, although 8 of 9 ATTR-positive patients (88.9%) were male. The prevalence of atrial fibrillation (AF; paroxysmal or persistent) and poor R progression (including pseudoinfarction pattern) were relatively high, but there were no significant differences on electrocardiography (ECG). In

Table 1. EMB: Clinical Patient Characteristics

	EMB		P-value
	Negative	Positive	
n	20	9	
Patient characteristics			
Age (years)	74.2±11.4	74.6±7.2	0.932
Gender (M/F)	13/7	8/1	0.371
NT-proBNP (pg/dL)	2,811 (37–93,312)	3,692 (671–34,988)	0.863
Electrocardiography			
Atrial fibrillation (+/–)	6/14	6/3	0.106
Atrioventricular block (+/–)	0/20	1/8	0.31
LBBB/RBBB/BBB (–)	4/1/15	3/1/5	0.539
Voltage (low/normal/high)	0/19/1	0/9/0	1
Poor R progression (+/–)	6/14	6/3	0.106
Echocardiography			
LVEDD (mm)	51.9±8.0	47.6±7.8	0.182
LVESD (mm)	40.0±10.7	32.3±8.3	0.068
LVEF (%)	46.2±16.5	54.4±12.6	0.191
HFrEF/HFmrEF/HFpEF	7/6/7	1/2/6	0.279
IVST (mm)	10.5±2.0	14.8±4.4	0.001
PWT (mm)	10.7±1.5	13.6±3.1	0.002
LAD (mm)	44.0±10.7	48.4±6.7	0.18
MRI			
LGE (+/–)	3/6	5/0	0.031
^{99m}Tc-PyP scintigraphy			
Visual score 0/1/2/3	6/13/1/0	0/1/3/5	<0.001
H/CL ratio	1.30±0.13	1.80±0.30	<0.001

Data given as mean±SD, n, or median (range). ^{99m}Tc-PyP, technetium-99m pyrophosphate; EMB, endomyocardial biopsy; H/CL, mean counts in the heart region of interest (ROI) relative to the contralateral chest ROI; HFr(mr,p)EF, heart failure with reduced (mid-range, preserved) ejection fraction; IVST, interventricular septum thickness; LAD, left atrial dimension; LGE, late gadolinium enhancement; L(R)BBB, left (right) bundle branch block; LVED(S)D, left ventricular end-diastolic (systolic) dimension; LVEF, left ventricular ejection fraction; MRI, magnetic resonance imaging; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PWT, posterior wall thickness.

Table 2. Diagnostic Accuracy of Clinical Parameters for Screening ATTR Cardiac Amyloidosis

	^{99m}Tc-PYP scintigraphy		Echocardiography		MRI
	Visual score	H/CL ratio	IVST	PWT	LGE
Cut-off point	2	1.46	11 mm	12 mm	
AUC (95% CI)	0.950 (0.866–1)	0.989 (0.962–1)	0.853 (0.709–0.996)	0.794 (0.592–0.997)	
Sensitivity (95% CI)	0.889 (0.518–0.997)	1.000 (0.555–1.000)	0.889 (0.518–0.997)	0.778 (0.400–0.972)	1.000 (0.359–1.000)
Specificity (95% CI)	0.950 (0.751–0.999)	0.900 (0.683–0.988)	0.650 (0.408–0.846)	0.700 (0.457–0.881)	0.667 (0.299–0.925)
PPV (95% CI)	0.889 (0.518–0.997)	0.818 (0.482–0.977)	0.553 (0.266–0.787)	0.538 (0.251–0.808)	0.625 (0.245–0.915)
NPV (95% CI)	0.950 (0.751–0.999)	1.000 (0.740–1.000)	0.929 (0.661–0.998)	0.875 (0.617–0.984)	1.000 (0.421–1.000)
Accuracy (95% CI)	0.931 (0.772–0.992)	0.931 (0.772–0.992)	0.724 (0.528–0.873)	0.724 (0.528–0.873)	0.786 (0.492–0.953)
PLR (95% CI)	17.778 (2.595–121.778)	10.000 (2.685–37.239)	2.540 (1.339–4.818)	2.593 (1.218–5.516)	3.000 (1.191–7.588)
NLR (95% CI)	0.117 (0.018–0.744)	0.000 (0.000–NaN)	0.171 (0.026–1.115)	0.317 (0.090–1.114)	0.000 (0.000–NaN)

ATTR, amyloid transthyretin; AUC, area under the curve; NaN, not a number; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value. Other abbreviations as in Table 1.

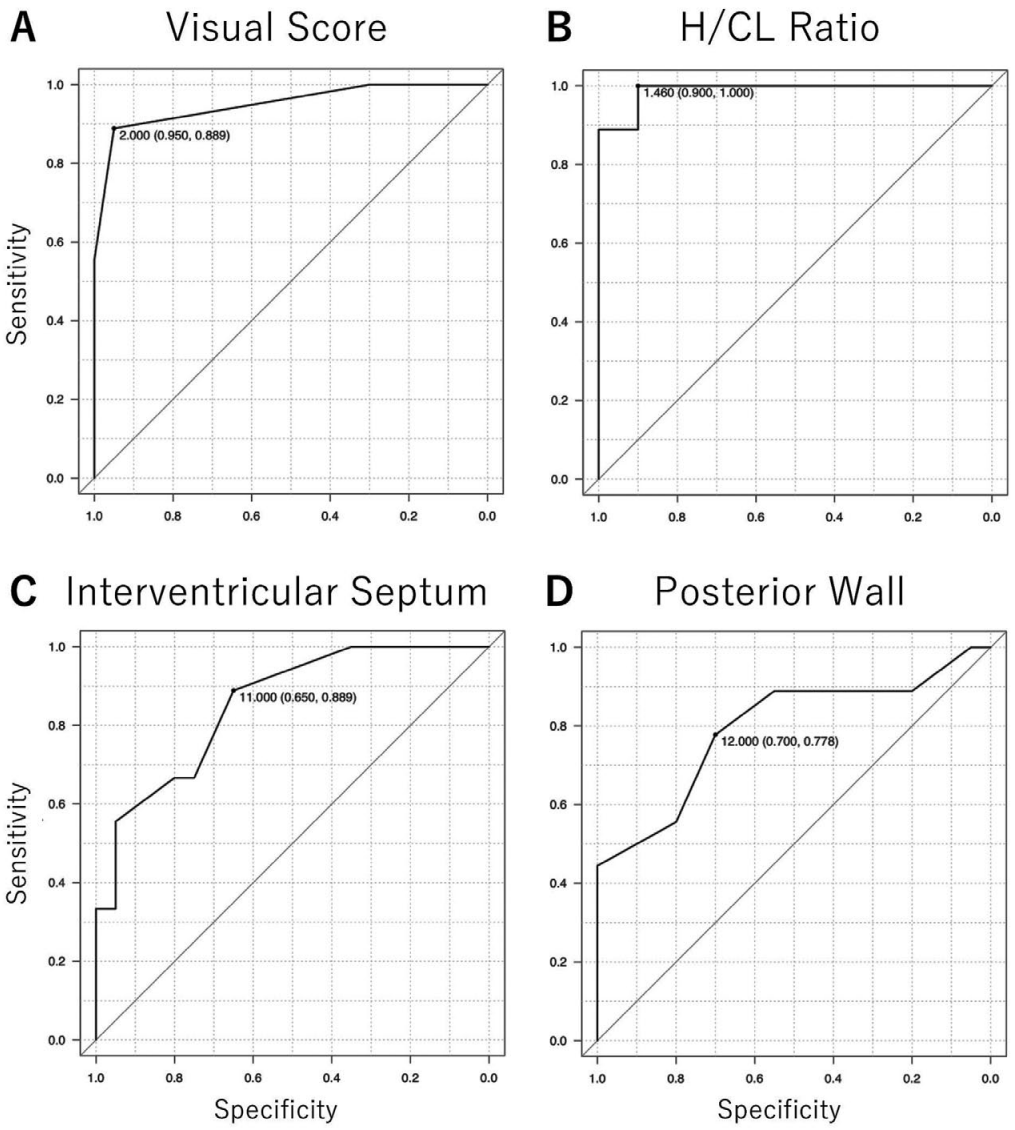

Figure 3. Receiver operating characteristic analysis of (**A**) visual score, (**B**) H/CL ratio, and (**C**) interventricular septum and (**D**) posterior wall thickness for the diagnosis of biopsy-proven amyloid transthyretin cardiac amyloidosis. The cut-off points in (**A**), (**B**), (**C**), and (**D**) were 2, 1.46, 11 mm and 12 mm, with areas under the curve of 0.950, 0.989, 0.853 and 0.894, respectively. H/CL, mean counts in the heart region of interest (ROI) relative to the contralateral chest ROI.

contrast, on echocardiography the interventricular septum and posterior wall were significantly thicker in ATTR-positive than in ATTR-negative patients (14.8±4.4 mm vs. 10.5±2.0 mm, P=0.001; 13.6±4.4 mm vs. 10.7±1.5 mm, P=0.002; respectively). The LV dimensions and EF, however, were not significantly different. Furthermore, of the 14 patients evaluated on cardiac magnetic resonance imaging (MRI) concomitantly, all 5 ATTR-positive patients but only 3 of 9 ATTR-negative patients had late gadolinium enhancement (LGE) in the myocardium, indicating a high prevalence of LGE in patients positive for ATTR (P=0.031). The number of patients with ^{99m}Tc-PYP scintigraphy visual score 0, 1, 2, or 3 was 0, 1, 3, and 5 of 9 patients with an ATTR-positive biopsy, and 6, 13, 1, and 0 patients of the 20 with an ATTR-negative biopsy, indicating a significantly greater prevalence of higher score in the ATTR-positive patients than in the ATTR-negative ones (P<0.001). Consistent with these visual scores, quantitative H/CL ratio was significantly higher in the ATTR-positive than in the ATTR-negative patients (1.80±0.30 vs. 1.30±0.13, P<0.001).

The diagnostic accuracy of visual score, H/CL ratio, interventricular septum and posterior wall thickness, and LGE on MRI is summarized in **Table 2**. On ROC analysis the cut-off points of visual score, H/CL ratio, and interventricular septum and posterior wall thickness were 2, 1.46, and 11 mm and 12 mm, respectively (**Figure 3**). Both the visual score and H/CL ratio had high sensitivity and specificity (0.889 and 0.950; 1.000 and 0.900, respectively) with high accuracy (0.931 and 0.931, respectively), resulting

Table 3. ^{99m}Tc-Pyrophosphate Scintigraphy: Clinical Patient Characteristics

	^{99m}Tc-PYP scintigraphy		P-value
	Negative	Positive	
n	80	18	
Patient characteristics			
Age (years)	78.1±10.7	82.3±11.7	0.143
Gender (M/F)	44/36	12/6	0.436
NT-proBNP (pg/dL)	3,085 (20–167,647)	4,658 (126–31,096)	0.506
Electrocardiography			
Atrial fibrillation (+/–)	32/48	9/9	0.443
Atrioventricular block (+/–)	3/77	1/17	0.562
LBBB/RBBB/BBB (–)	11/4/65	4/1/13	0.591
Voltage (low/normal/high)	6/57/17	1/15/2	0.738
Poor R progression (+/–)	19/61	9/9	0.041
Echocardiography			
LVEDD (mm)	48.2±8.2	45.7±8.8	0.247
LVESD (mm)	34.5±10.2	33.2± 9.1	0.602
LVEF (%)	54.0±16.6	53.8±12.7	0.962
HFrEF/HFmrEF/HFpEF	19/14/47	2/5/11	0.387
IVST (mm)	11.2±2.3	13.4±3.6	0.001
PWT (mm)	11.1±1.9	12.8±2.5	0.001
LAD (mm)	42.9±7.5	44.9±5.0	0.265
MRI			
LGE (+/–)	9/13	5/1	0.165
^{99m}Tc-PYP scintigraphy			
Visual score 0/1/2/3	44/36/0/0	0/0/10/8	<0.001
H/CL ratio	1.26±0.12	1.78±0.29	<0.001

Data given as mean±SD, n, or median (range). Abbreviations as in Table 1.

in statistically significant positive and negative likelihood ratios (17.778 and 0.117; 10.000 and 0.000). In contrast, the interventricular septum and posterior wall thickness and LGE had relatively low specificity with moderate–high sensitivity (0.650 and 0.889; 0.700 and 0.778; 0.667 and 1.000; respectively), resulting in moderate accuracy (0.724, 0.724 and 0.786, respectively). Although the positive likelihood ratios of both the interventricular septum and posterior wall were significantly different from 1 (2.540 and 2.593, respectively), their negative likelihood ratios were not different from 1 (0.171 and 0.317, respectively). This indicates that if patients were screened based on the thickness of the LV wall alone, a substantial number of cases of ATTR cardiac amyloidosis might have been overlooked. Based on these results, ^{99m}Tc-PYP scintigraphy is an extremely useful and accurate screening tool for ATTR cardiac amyloidosis in daily clinical practice.

Positive ^{99m}Tc-PYP Scintigraphy: Clinical Characteristics

The clinical characteristics of all patients who underwent ^{99m}Tc-PYP are summarized in **Table 3**, **Figure 4**. Based on visual score, 18 patients (18.4%) were PYP positive, and 80 were PYP negative. As in the biopsy cases, there were no significant differences in age, gender, NT-proBNP, or ECG findings, except for poor R progression between PYP-positive and PYP-negative patients. The interventricular septum and posterior wall of PYP-positive patients were significantly thicker than those of PYP-negative patients (13.4±3.6 mm vs. 11.2±2.3 mm, P=0.001; 12.8±2.5 mm vs. 11.1±1.9 mm, P=0.001; respectively), but the LV dimensions and EF were not significantly different. The prevalence of LGE in the myocardium was high in PYP-positive patients (5/6, 83%) compared with PYP-negative patients (9/22, 41%), although this was not statistically significant (P=0.165).

The diagnostic accuracy of interventricular septum and posterior wall thickness and LGE for predicting positive ^{99m}Tc-PYP uptake is summarized in **Supplementary Table**. The cut-off points of the interventricular septum and posterior wall thickness for PYP positivity were both 12 mm based on ROC analysis. Interventricular septum and posterior wall thickness and LGE all had relatively low–moderate sensitivity with low specificity (0.661 and 0.612; 0.778 and 0.638; 0.833 and 0.619; respectively), resulting in low–moderate accuracy (0.612, 0.663 and 0.667, respectively). Given that none of these clinical parameters satisfied the high positive or low negative likelihood ratios, there appears to be no clinical parameters that can reliably predict positive uptake of ^{99m}Tc-PYP.

^{99m}Tc-PYP Uptake Intensity and ATTR Cardiac Amyloidosis Severity

To examine the correlation between radiographic ^{99m}Tc-PYP uptake intensity and clinical severity of ATTR cardiac amyloidosis, 1 patient with positive ^{99m}Tc-PYP uptake but negative histology on endomyocardial biopsy was excluded in the analysis, while 1 patient with negative ^{99m}Tc-PYP uptake but positive histology on endomyocardial biopsy was included. As summarized in **Table 4**, the ATTR patients with mild–moderate ^{99m}Tc-PYP uptake (≤2+) were significantly older than those with high uptake (3+; 87.6±11.7 years vs. 76.0±7.6 years, P=0.028). Although the mild–moderate ^{99m}Tc-PYP uptake group tended to be have a

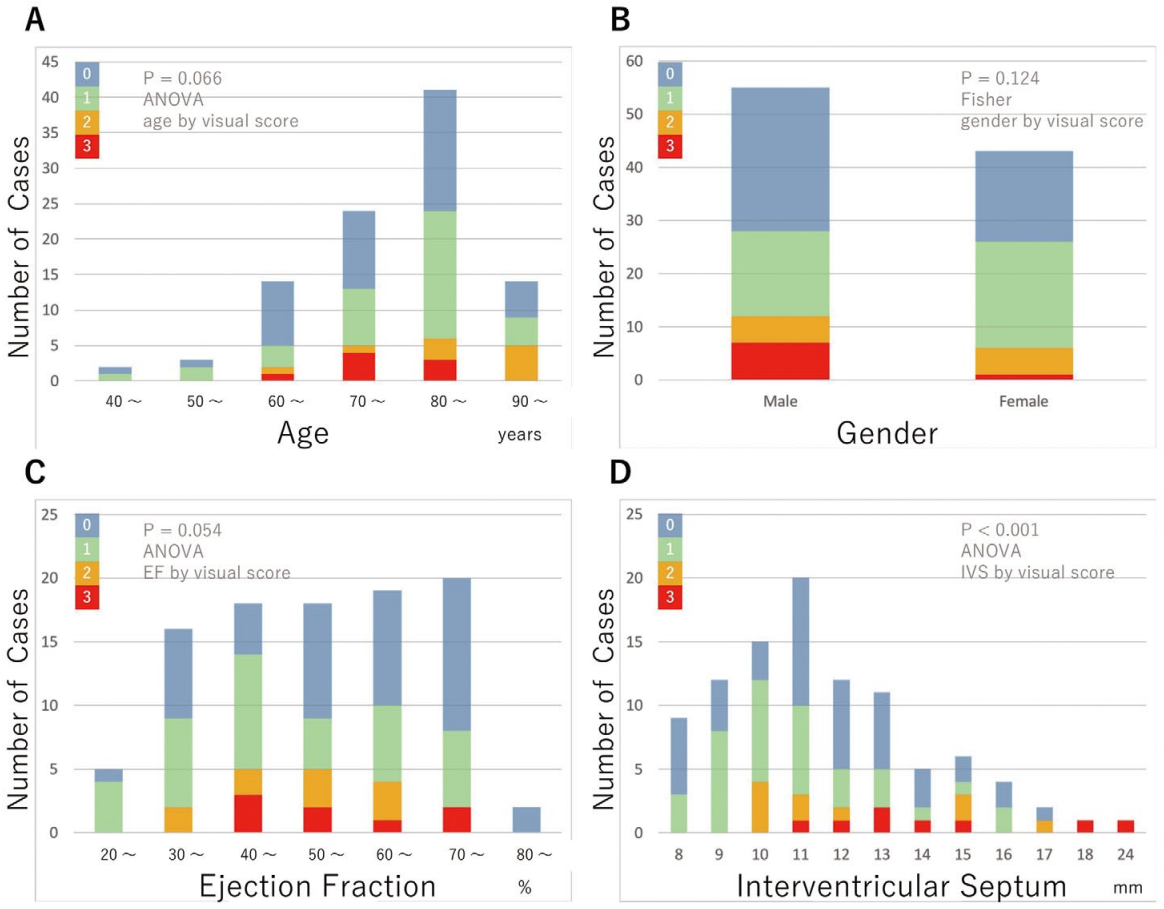

Figure 4. Distribution of visual score according to (**A**) age, (**B**) gender, (**C**) left ventricular ejection fraction and (**D**) interventricular septum (IVS) thickness.

higher proportion of female patients and a lower prevalence of LVH (P=NS), NT-proBNP at the time of diagnosis was significantly higher than in the high ^{99m}Tc-PYP group. On multivariate analysis with age, gender, LV diastolic dimension, LVEF, interventricular septum thickness, presence of AF, visual score and H/CL ratio thickness, higher LV diastolic dimension, presence of AF and lower ^{99m}Tc-PYP uptake were significantly correlated with higher NT-proBNP in patients with ATTR amyloidosis.

Discussion

In the present study, we showed that ^{99m}Tc-PYP scintigraphy was both sensitive and specific in diagnosing ATTR cardiac amyloidosis by comparing the scintigraphy findings with those of endomyocardial biopsy. Although approximately 20% of patients who underwent ^{99m}Tc-PYP scintigraphy had suspected ATTR cardiac amyloidosis, the clinical characteristics of the PYP-positive patients were slightly different from the classic phenotype of ATTR cardiac amyloidosis, suggesting that we might have overlooked some ATTR patients if ^{99m}Tc-PYP scintigraphy had not been performed. Considering the non-invasiveness of ^{99m}Tc-PYP scintigraphy, we advocate that ^{99m}Tc-PYP scintigraphy be considered for screening for ATTR cardiac amyloidosis in patients with HF without evident coronary or valvular heart disease, especially when endomyocardial biopsy cannot be performed due to extremely advanced age or other clinical conditions. Needless to say, patients with LVH are good candidates for ^{99m}Tc-PYP scintigraphy especially given the relatively low voltage on ECG.

Technetium-labeled bone scintigraphy has recently been reconsidered as a screening tool for ATTR cardiac amyloidosis.[7–10] Although the precise mechanism by which ^{99m}Tc-PYP accumulates in the myocardium in patients with ATTR cardiac amyloidosis remains unclear, it is considered to be related to high calcium in ATTR amyloid.[8] Gillmore et al reported that the myocardial uptake of bone scintigraphy was >99% sensitive and 86% specific for ATTR cardiac amyloidosis, with false positives almost exclusively due to the uptake in patients with cardiac AL amyloidosis.[9] In the present study, by investigating 29 patients who underwent both ^{99m}Tc-PYP scintigraphy and endomyocardial biopsy, we showed that the sensitivity and specificity of ^{99m}Tc-PYP scintigraphy for diagnosing histologically proven ATTR cardiac amyloidosis were 0.889 (95% CI: 0.518–0.997) and 0.950 (95% CI: 0.751–0.999) for visual score (≥2), and 1 (95% CI: 0.555–1) and 0.9 (95% CI: 0.683–0.988) for H/CL ratio (≥1.46). Given that the positive and negative likelihood ratios were 17.118 (95% CI: 2.595–

Table 4. ATTR Cardiac Amyloidosis Diagnosed on ^{99m}Tc-PYP Scintigraphy and/or EMB: Clinical Patient Characteristics

	^{99m}Tc-PYP scintigraphy		P-value
	≤2+	3+	
n	10	8	
Patient characteristics			
Age (years)	87.6±11.7	76.0±7.6	0.028
Gender (M/F)	5/5	7/1	0.152
NT-proBNP (pg/dL)	9,151.5 (671–34,900)	3,024.5 (126–4,938)	0.003
Electrocardiography			
Atrial fibrillation (+/–)	5/5	4/4	1
Atrioventricular block (+/–)	0/10	1/7	0.444
LBBB/RBBB/BBB (–)	1/1/8	3/1/4	0.487
Voltage (low/normal/high)	1/8/1	0/7/1	1
Poor R progression (+/–)	3/7	3/5	1
Echocardiography			
LVEDD (mm)	47.5±11.3	44.5±6.4	0.513
LVESD (mm)	33.2±10.1	31.4±8.0	0.683
LVEF (%)	52.1±11.1	57.1±13.9	0.405
HFrEF/HFmrEF/HFpEF	2/1/7	0/3/5	0.397
IVST (mm)	12.2±2.5	15.0±4.2	0.099
PWT (mm)	12.0±2.1	13.9±2.6	0.109
LAD (mm)	45.5±6.8	46.6±4.6	0.695
MRI			
LGE (+/–)	1/1	4/0	0.333
^{99m}Tc-PYP scintigraphy			
Visual score 0/1/2/3	0/1/9/0	0/0/0/8	<0.001
H/CL ratio	1.60±0.12	2.01±0.28	0.001

Data given as mean±SD, n, or median (range). Abbreviations as in Table 1.

121.77) and 0.117 (95% CI: 0.018–0.744) for visual score and 10 (95% CI: 2.685–32.329) and 0 (0–not a number) for H/CL ratio, respectively, ^{99m}Tc-PYP scintigraphy is a useful, non-invasive screening tool for diagnosing ATTR cardiac amyloidosis.

ATTR amyloidosis is one of the major causes of HF, especially in elderly patients.[1–6] González-López et al prospectively screened 120 consecutive patients ≥60 years of age admitted due to HF with preserved EF (HFpEF) and LVH on technetium-labeled bone scintigraphy, and 16 of those patients (13.3%) were diagnosed with ATTR cardiac amyloidosis.[4] In the present study, 18 of 98 patients (18.4%) had positive ^{99m}Tc-PYP uptake in the myocardium, supporting the relatively high prevalence of ATTR cardiac amyloidosis in elderly patients with HF without evident coronary or valvular heart diseases.

Wild-type ATTR amyloidosis has been considered a disease predominantly associated with elderly men, characterized by concentric LVH, preserved LVEF, and low QRS voltages. A recent large cohort study, however, showed that the clinical spectrum of wild-type ATTR amyloidosis is heterogeneous and differs from the classic phenotype.[11] Indeed, the present 18 PYP-positive patients consisted of 12 men and 6 women (33%), and the prevalence of PYP positivity was independent of LVEF: 3 of 25 HF with reduced EF patients (12%), 5 of 19 HF with mid-range EF patients (26%), and 10 of 54 HFpEF patients (18%; P=0.432). Although LVH was more common in PYP-positive than in PYP-negative patients, it was difficult to diagnose ATTR cardiac amyloidosis based on clinical characteristics alone. Therefore, we consider that ^{99m}Tc-PYP scintigraphy should be performed to confirm or exclude ATTR amyloidosis as a cause of HF.

Rapezzi et al reported that myocardial uptake of ^{99m}Tc-DPD was a prognostic determinant of adverse cardiovascular events in patients with cardiac amyloidosis due to mutated ATTR.[13] In addition, Kristen et al reported that myocardial uptake of ^{99m}Tc-DPD positively correlated with LV wall thickness and plasma NT-proBNP and troponin T in mutated as well as wild-type ATTR patients.[14] In the present study, ATTR patients with higher ^{99m}Tc-PYP uptake were significantly younger and were more likely to be female and less likely to have LVH, although the differences were not statistically significant. The younger age of ATTR patients with higher ^{99m}Tc-PYP uptake might reflect earlier accumulation of ATTR in the myocardium. It was of interest that NT-proBNP at the time of diagnosis was significantly higher in patients with lower ^{99m}Tc-PYP uptake than in those with higher uptake. On multivariate analysis, lower ^{99m}Tc-PYP uptake was significantly correlated with higher NT-proBNP regardless of age, gender, LV diastolic dimension, LVEF, interventricular septum thickness, and the presence of AF. Precise mechanisms by which higher ^{99m}Tc-PYP uptake correlates with lower NT-proBNP remain to be elucidated.

There has been no specific treatment approved yet for this disease. Recently, tafamidis, a transthyretin stabilizer, has been shown to reduce all-cause mortality and cardiovascular-related hospitalization in patients with ATTR cardiac amyloidosis as compared with placebo.[15] When it

is approved for the treatment of ATTR cardiac amyloidosis, early diagnosis will be needed in order to optimize drug therapy and improve prognosis. Furthermore, several compounds are now under development at different levels.[1,6]

Study Limitations

Several limitations warrant mention in the present study. First, we cannot exclude selection bias because ^{99m}Tc-PYP scintigraphy was performed at the discretion of the individual physician. The physicians, however, were encouraged to perform ^{99m}Tc-PYP scintigraphy in elderly patients with HF without evident coronary or valvular heart disease. Indeed, the number of patients undergoing ^{99m}Tc-PYP scintigraphy increased during the study period (4 in 2015, 30 in 2016, and 64 in 2017). As the absolute number of patients with positive ^{99m}Tc-PYP uptake increased annually (2 in 2015, 6 in 2016, to 10 in 2017), the prevalence of positive ^{99m}Tc-PYP uptake conversely decreased (50% in 2015, 20% in 2016, and 16% in 2017). Therefore, the prevalence of ATTR amyloidosis might vary from 10% to 20%, depending on patient selection. Second, only 6 of 9 biopsy-proven ATTR-positive patients underwent genetic analysis. Although none of them had a mutation in the transthyretin gene, those with mutated as well as wild-type ATTR might have been included in the present analysis. Third, AL amyloidosis was not systematically excluded. Although all of the cases of endomyocardial biopsy-proven amyloid were confirmed as ATTR on immunohistochemistry in the present study, some patients with mild ^{99m}Tc-PYP uptake (visual score 1) without endomyocardial biopsy might have had AL amyloidosis. Finally, quantification of ^{99m}Tc-PYP uptake using visual score and H/CL ratio was somewhat arbitrary. As shown in **Figure 2**, although 2 independent investigators analyzed the data carefully, visual score was sometime discordant with H/CL ratio. In the present study, we showed that H/CL ratio ≥1.46 had 100% sensitivity and 90% specificity, with AUC 0.989, while Bokhari et al reported that H/CL ratio >1.5 had 97% sensitivity and 100% specificity, with AUC 0.992.[8] After analyzing approximately 100 patients, however, we prefer to use visual score rather than H/CL ratio, because H/CL ratio varies substantially with the ROI used. Furthermore, the incubation time and other factors may cause variation in the H/CL cut-off. Castano et al reported that for the scans that used a 3-h incubation, the H/CL ratio threshold should be lowered to 1.3 or greater, adjusting for higher bone activity seen with longer exposures.[10] Additionally, bone tracer is known to stay in the cardiac blood pool, and can cause false-positive scintigrams.[16] Therefore, it might be better to use H/CL ratio as a supplement to visual score to confirm the accuracy.

Conclusions

^{99m}Tc-PYP scintigraphy was both sensitive and specific in the screening of ATTR cardiac amyloidosis in real-world practice. Given that ATTR cardiac amyloidosis appears to be difficult to diagnose based on the clinical characteristics alone, ^{99m}Tc-PYP scintigraphy should be performed for the early detection of ATTR cardiac amyloidosis.

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Disclosures

The authors declare no conflicts of interest.

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Supplementary Files

Please find supplementary file(s);
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