Prevalence of transthyretin amyloidosis among heart failure patients with preserved ejection fraction in Japan

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Short title: Prevalence of ATTR in HFpEF

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Abstract

Aims: Heart failure with preserved ejection fraction (HFpEF), which is caused by wide various conditions, has become a major public health problem. Transthyretin amyloid cardiomyopathy (ATTR-CM), which is thought to be an underdiagnosed disease, can cause HFpEF. Noninvasive diagnosis using ^{99m}Technetium (Tc)- pyrophosphate (PYP) scintigraphy enables accurate diagnosis of ATTR-CM. The aim of this study was to clarify the prevalence and characteristics of ATTR-CM among Japanese patients with HFpEF.

Methods: This study was a multicenter, prospective, observational study conducted in Japan. We enrolled 373 patients with HFpEF (left ventricular (LV) ejection fraction ≥ 50%) aged ≥ 65 years old who were admitted to the department of cardiology from September 2018 to January 2022. A ^{99m}Tc- PYP scintigraphy scan was performed during admission in all eligible patients. Cardiac ^{99m}Tc-PYP retention was graded according to a previously reported visual scale ranging from 0 to 3 points. The scan was considered positive when it revealed moderate-to-severe ^{99m}Tc-PYP uptake (grade 2–3) in both ventricles. Patients were divided into ATTR-CM and non-ATTR-CM patients according to positive (grade 2-3) or negative (grade 0-1) 99mTc-PYP scintigraphy, respectively. Medical history, blood tests, electrocardiogram, echocardiography, and magnetic resonance imaging in the two groups of patients were compared.

Results: Among the 373 patients with HFpEF, 53 patients (14.2%; 95% confidence

interval: 10.7-17.7) showed positive uptake on ^{99m}Tc-PYP scintigraphy. An endomyocardial biopsy was performed in 32 patients and confirmed amyloidosis in all cases. There were no significant differences between the two groups in age, severity of heart failure as assessed by the NYHA functional classification, renal function values, left ventricular ejection fraction and tricuspid regurgitant pressure gradient (ATTR-CM, n=53 vs non-ATTR-CM, n=320). Patients in the ATTR-CM group had a higher N-terminal pro-brain natriuretic peptide level (2314 [1081-3398] vs 900 [415-1828] , $P<0.001$), higher sensitive troponin T level (0.074 ± 0.049 vs 0.035 ± 0.038, $P<0.001$) and higher mean LV maximal wall thickness (12.5 [11-14] vs 10.5 [9.5-11.5], $P<0.001$).

Conclusions: ATTR-CM is an underdiagnosed disease with a significant prevalence in Japanese patients with HFpEF. This study showed that results of examinations for ATTR-CM patients appear to be worse than those for non-ATTR-CM patients, but clinical severities of heart failure as assessed by the NYHA functional classification are similar in ATTR-CM and non-ATTR-CM patients and the clinical overlap between ATTR-CM and non-ATTR-CM is high.

Introduction

Heart failure with preserved ejection fraction (HFpEF), which has become a major public health problem, currently accounts for up to half of HF cases.[1,2] Because the wide heterogeneity in conditions leading to HFpEF, treatment for different conditions seems difficult. As epidemiological evolution is leading a predominance of HFpEF,[1,2] improvement in the phenotypic characterization of different subtypes is desirable to improve diagnosis and treatment.[2]

In recent years, transthyretin amyloidosis (ATTR) has been recognized to be the most common cause of cardiac amyloidosis (CA), which is a progressive and fatal disease.[3] The acquired form of ATTR amyloidosis is dominated by cardiac symptoms that cause myocardial infiltration and/or direct toxicity, leading to diastolic and systolic dysfunction, heart failure (HF), rhythm disturbances, and ischemia.[4 5] Although the contribution of ATTR to the burden of HFpEF is poorly defined,[6,7] transthyretin amyloid cardiomyopathy (ATTR-CM) has been reported to be a frequent cause of HFpEF in recent years.[6,8,9] ATTR-CM has traditionally been considered a rare disease and has been underdiagnosed and misdiagnosed as hypertrophic cardiomyopathy or as generic, undifferentiated HFpEF rather than as amyloidosis.[10 11] Although ATTR-CM previously was diagnosed only by endomyocardial biopsy (EMB), noninvasive techniques for diagnosing ATTR-CM have become available in the past decade.[12] Noninvasive diagnosis using bone-seeking radiopharmaceuticals such as ^{99m}Technetium

pyrophosphate (^{99m}Tc-PYP) scintigraphy allow for an accurate imaging diagnosis of ATTR-CM as long as diverse forms of cardiac amyloidosis and other cardiomyopathies have been ruled out.[12,13] There have been several reports on the prevalence of undiagnosed ATTRwt in elderly patients with HFpEF.[6,8,14] However, there has been no prospective large-scale study on the prevalence of the disease.

The aim of this study was to clarify the prevalence and the characteristics of ATTR-CM among Japanese patients with HFpEF by using a ^{99m}Tc-PYP scintigraphy-based protocol.

Methods

Study population and study design

We conducted a multi-center, prospective, observational study. A total of 373 consecutive newly diagnosed HFpEF patients who had been referred for evaluation to amyloidosis specialist centers in Japan during the period from September 2018 to January 2022 were enrolled in this study. The specialist centers included Department of Cardiovascular Medicine, Okayama University, Osaka City General Hospital, Tokyo Bay Urayasu Ichikawa Medical Center, National Hospital Organization Iwakuni Clinical Center, Kihara Cardiology Clinic, Okayama City Civic Hospital, Okayama, Fukuyama City Hospital, Mitoyo General Hospital, Japanese Red Cross Society Himeji Hospital and Department of Radiology in Asahikawa Medical University. Patients

fulfilling eligibility requirements and without exclusion criteria were offered participation in the study. All of the patients who accepted participation underwent diagnostic investigations including blood tests, an electrocardiogram (ECG), echocardiography, ^{99m}Tc-PYP scintigraphy, immunofixation electrophoresis of serum and urine, and serum free light chain assay. Contrast magnetic resonance imaging and biopsy were performed in patients according to local clinical practice. The final diagnosis was based on the results of those tests and histological findings. The protocol of this study was approved by the Faculty of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University Institutional Ethics Committee on Human Research (Approval no.1808-036) as well as the ethics committee of each participating center. Because of the anonymous nature of the data, the requirement for informed consent was waived. This study was conducted in compliance with the Declaration of Helsinki.

Patient selection

Patients aged ≥65 years with HFpEF were eligible for participation. HFpEF was defined as a left ventricular (LV) ejection fraction (EF) ≥50% by echocardiography within the prior 6 months, N-terminal pro B-type natriuretic peptide (NT-proBNP) >125 pg/mL during admission, and any symptoms including shortness of breath, orthopnea, and leg edema according to the European Society of Cardiology definition. [15] Patients previously diagnosed with infiltrative or restrictive cardiomyopathy and hypertrophic

cardiomyopathy were not excluded as long as they fulfilled all of the aforementioned criteria. Exclusion criteria included NT-proBNP < 125 pg/mL, LV ejection fraction < 50%, serious infection or severe trauma or perioperative patients, presence or history of stroke or significant coronary artery disease defined as myocardial infarction, stenosis of the left main artery or proximal left anterior descendent artery, or significant stenosis in two main coronary arteries within 6 months before participation, prognosis within two years, serious infection or severe trauma or perioperative patients, poorly controlled type 2 diabetes mellitus (HbA1c >9.0%), and uncontrolled hypertension (systolic blood pressure >160 mm Hg) at discharge, women who were pregnant or breastfeeding, and other medical reasons at the investigator's discretion including known congenital heart disease, constrictive pericarditis, isolated pulmonary arterial hypertension, or history of heart transplantation.

Data collection

Information on clinical characteristics and patients' outcomes was obtained from medical records for the patients during admission. Hypertension was defined on the basis of clinical history or use of antihypertensive medication at admission. Information on clinical histories such as prescribed medications and blood test parameters was obtained at discharge. ECG measures were based on standard definitions using the first ECG available during admission. Electrocardiographic LV hypertrophy was evaluated

according to the Sokolow-Lyon criteria.[16] Echocardiography was performed using a Philips iE33 system (PhilipsMedical Systems, Eindhoven, The Netherlands). Chamber and left ventricle ejection fraction quantification was based on standard recommendations.[17] End-diastolic LV wall thicknesses at the septum and the posterior wall were measured in the longitudinal parasternal view. Mean LV maximal wall thickness was defined as end-diastolic LV wall thickness at the septum plus the posterior wall divided by two.

Diagnosis of transthyretin amyloid cardiomyopathy

Patients were diagnosed with ATTR-CM based on consensus guidelines.[13 18] Briefly, patients were evaluated by either histology with positive cardiac biopsy for ATTR-CM or by imaging that included all of the following criteria: 1) echocardiography or cardiac magnetic resonance findings with increased LV wall thickness consistent with ATTR-CM, 2) grade 2 or 3 cardiac scintigraphy with ^{99m}Tc-PYP, and 3) negative monoclonal protein testing with both negative serum and urine protein electrophoresis with immunofixation and negative serum free light chains. Due to concerns that some positive scans may be due to blood pooling, we required that patients with grade 2 planar scans have myocardial uptake confirmed on SPECT to be classified as ATTR-CM.[13] Patients were considered to have no CA if they met the following criteria: negative EMB for amyloidosis, grade 0 or 1 ^{99m}Tc-PYP planar scan, and a grade 2

planar scan with SPECT showing neither blood pooling nor myocardial uptake. We divided patients into ATTR-CM patients (ATTR-CM) and patients with other HFpEF forms (non-ATTR-CM) as assessed by ^{99m}Tc-PYP scintigraphy.

Participating facilities were hospitals with board certified member of the Japanese Circulation Society with experience treating multiple patients with cardiac amyloidosis. Each examination including echocardiography, cardiac magnetic resonance imaging, ^{99m}Tc-PYP scintigraphy and pathological findings, was reviewed blindly by 3 physicians. In addition, ^{99m}Tc-PYP scintigraphy was reviewed blindly by 3 authors (T.N., K.N. and H.I.).

Scintigraphy protocol

Patients were scanned using two GE Medical Systems hybrid single photon emission computed tomography and computed tomography gamma cameras (Discovery NM/CT670) following administration of 740 MBq of intravenously injected ^{99m}Tc-PYP providing an expected radiation dose of ≈5 mSv per patient. Patients underwent a standardized imaging protocol with ^{99m}Tc-PYP imaged at 1 h after injection[19] In an estimated <10% of the scans, additional scans were conducted at 3 h to assess for blood pool washout.[13,20] Scans were graded by both experienced, board-certified nuclear cardiologists and cardiologists. The results of each examination were assessed to establish the existence and degree of uptake in the myocardium and to determine its

distribution. Cardiac ^{99m}Tc-PYP retention was graded using both anterior and lateral planar images according to a previously reported visual scale ranging from 0 to 3 points,[13,21] with 0 as no myocardial uptake, 1 as myocardial uptake less than ribs, 2 as myocardial uptake equal to ribs, and 3 as myocardial update greater than ribs with mild or absent rib uptake. The scan was considered positive when it revealed a moderate-to-severe ^{99m}Tc-PYP uptake (score 2–3) in both ventricles. Because only 1-h scanning protocols using planar imaging were increasing recognition of false positives,[13] SPECT scanning was also conducted.

Endomyocardial biopsy

EMB was performed in the supine position using the standard Seldinger technique with echocardiography. Biopsies were obtained from the right ventricle septum using a Jawz Endomyocardial Biotome (Argon Medical, Frisco, TX), and 3 specimens were sent for clinical histology.

Clinical histology, immunohistochemistry and proteomic analysis

Patients with severe ^{99m}Tc-PYP uptake underwent endomyocardial biopsies if considered necessary by the treating physician. Sections from formalin-fixed, paraffin-embedded biopsy specimens were stained with haematoxylin–eosin and Congo Red staining with at least moderate interstitial infiltration of amyloid fibrils.

Immunohistochemical staining of all amyloid deposits was performed with the use of monospecific antibodies reactive with serum amyloid A protein, κ and λ immunoglobulin light chains, and transthyretin, as previously described. [22]

Statistical analysis

Normally distributed variables were expressed as mean and standard deviation, and abnormally distributed variables were expressed as median and interquartile range (IQR). Univariate analysis for comparison of data in the two groups was performed using an unpaired Student's t-test for continuous variables if normally distributed or an x2 test for categorical variables. The Mann–Whitney U-test was used in cases of non-normally distributed variables. We conducted all statistical analyses by using IBM SPSS Statistics 24 (IBM, Armonk, NY). Statistical significance was defined as $P<0.05$.

Results

Patient characteristics

Among the 373 patients included in this study, 202 (54%) were men and the mean age of the patients was 79 ± 7 years (Table 1). A total of 253 patients (68%) were admitted for treatment and 120 patients (32%) were tested. Most of the patients had NYHA class ≥II. Many patients had a history of hypertension (263; 71%), 116 patients (31%) were diabetic, and 23 patients (6%) had a history of coronary artery disease and had already

undergone percutaneous coronary intervention.

The median level of high sensitivity troponin T was 0.040 ± 0.042 and the median level of N-terminal pro-brain natriuretic peptide (NT-proBNP) was 1003 pg/L (IQR: 485–21069). AF was present in 113 patients (30%), and 34 patients (9%) had a permanent pacemaker inserted. Mean left ventricular ejection fraction (LVEF) was 65.3 ± 6.6% and median maximal wall thickness at the septum was 11 mm (IQR: 10–12). Other clinical, electrocardiographic and echocardiographic characteristics are presented in Table 2.

Prevalence of ATTR-CM in patients with HFpEF

During the study period, 373 patients were admitted due to confirmed HFpEF and agreed to undergo a ^{99m}Tc-PYP scan. Figure 1 shows the study flowchart of results. A total of 53 patients (14.2%, 95% confidence interval (CI) [10.7–17.7]) showed positive uptake on the ^{99m}Tc-PYP scan. Eleven patients (2.9%) were grade 2 and 42 patients (11.3%) were grade 3 (Figure 1A and 1B). EMB was performed in 32 patients with grade 2 or 3 (5 patients with grade 2 and 27 patients with grade 3) and ATTR-CM was confirmed in all cases. EMB was performed in 46 patients with grade 0 or 1 and all of them were non-ATTR-CM patients. Three other patients with positive PYP scans underwent extracardiac biopsies performed (two skin biopsies and one abdominal fat biopsy) and were also positive. The prevalence of positive ^{99m}PYP scans graded by age

is shown in Figure 2. Many positive cases were found in the age range of 75-84 years.

ATTR-CM vs non-ATTR-CM

There were no significant differences between the two groups of patients in age and severity of heart failure as assessed by the NYHA functional classification (ATTR-CM, n=53 vs non ATTR-CM, n=320) (Table 1). There were several significant differences between the ATTR-CM and non-ATTR-CM groups. The percentages of male patients, patients with a past medical history of cardiomyopathy, patients with a past smoking habit, and patients receiving treatment with diuretics including tolvaptan were large in the ATTR-CM group, whereas the percentages of patients with arrhythmia, especially atrial fibrillation or atrial flutter, valvular disease, dyslipidemia, and current smoking habit and patients being treated with an angiotensin II receptor blocker were larger in the non-ATTR-CM group.

Table 2 shows the results of examinations. There were no significant differences between the two groups, in renal function values including blood urea nitrogen and creatinine, left ventricular ejection fraction and tricuspid regurgitant pressure gradient. ATTR-CM patients had a higher median NT-proBNP levels (2314 [1081-3398] vs 900 [415-1828], $P < 0.001$)] and a high median high -sensitivity troponin T level (0.074 vs. 0.035 mg/L, $P < 0.001$). **ECG features in ATTR-CM patients had high frequencies of atrial fibrillation and atrial flutter (51% vs. 29%,**

P < 0.001) and low frequencies of LVH with ST-T change (8% vs. 31%, P < 0.001). Echocardiographic analysis showed that although mean LVEF and left atrial dimensions were similar in the ATTR-CM and non-ATTR-CM groups (Table 2), median maximal wall thickness at the septum and that at the posterior wall were significantly increased in the ATTR-CM group [median 13 mm (IQR 11–14) vs. 10.7mm (IQR 9.6–12), P < 0.001 and 13 mm (IQR 11–14) vs. 10 mm (IQR 9–11], P < 0.001, respectively]. In contrast to LVEF, septal e' was lower in the ATTR-CM group (3.9 ± 1.6 vs. 5.0 ± 4.2 mmHg, P < 0.001) and E/e' was higher in the ATTR CM group (23.7 ± 13.8 vs. 19.5 ± 11.0 mmHg, P < 0.001). The percentages of patients with aortic stenosis and regurgitation were lower in the ATTR-CM group (P < 0.035 and P < 0.036, respectively). The percentages of patients with myocardial delayed enhancement on magnetic resonance imaging were different in the two groups (21% vs. 3%, P < 0.001).

These results showed that results of examinations for patients with ATTR-CM appear to be worse than those for non-ATTR-CM patients, but clinical severities of heart failure as assessed by the NYHA functional classification are similar in ATTR-CM and non-ATTR-CM patients.

Discussion

We conducted a multi-center prospective study of 373 consecutive patients who underwent a ^{99m}Tc-PYP scan for the diagnosis of ATTR-CM. This study is, to the

best of our knowledge, the first study in which the prevalence of ATTR-CM among Japanese patients with HFpEF was investigated. The main findings of this study are as follows: 1) ATTR-CM is an underdiagnosed disease with a significant prevalence (14.2%) in Japanese patients with HFpEF, 2) ^{99m}Tc-PYP scintigraphy is acceptable to those for detecting ATTR-CM due to HFpEF, and 3) severities of heart failure as assessed by the NYHA functional class are similar in ATTR-CM and non ATTR-CM patients and the clinical overlap between ATTR-CM and non-ATTR-CM is high.

Despite major efforts to improve mortality and morbidity in patients with HFpEF, none of the recommended treatments for heart failure with reduced ejection fraction have been effective treatments so far. [15,23] In the future, it is expected that new drugs such as SGLT2 inhibitors, which have ongoing trials, will show effectiveness for reducing mortality and cardiovascular hospitalization in patients with HFpEF.

Considering ATTR-CM, which has been reported as a frequent cause of HFpEF[6,8,9], on the other hand, novel stabilizing therapies[24] and results of investigations of alternative TTR stabilizers, TTR silencers, and amyloid fibril degraders have been in recent years. Contemporary clinical data suggest that there is a lag period of 2 or more years between the patient's first presentation to a physician and the diagnosis of CA or that multiple physicians observe the disease over a period of months to years before a correct diagnosis is made.[25] In the modern era of expensive therapeutics and lack of biopsy confirmation, a misdiagnosis has serious medical and financial ramifications.[13]

Furthermore, since ATTR stabilizing therapy is likely to have more benefits for patients with disease in an early stage before extensive end-organ damage has occurred,[24] it is important to recognize patients at risk and diagnose them earlier. To improve early and accurate identification and as a result of early treatments of ATTR-CM, noninvasive imaging plays a clinically important role.

In our study, we used a ^{99m}Tc-PYP scan, which was introduced as a tracer for imaging of subacute myocardial infarction and for which uptake has been shown to correlate with calcium deposits in the infarcted myocardium.[26] Of clinical relevance, some cases of AL showed densities of microcalcification comparable to those in ATTR cases, consistent with the clinical finding of ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD)/^{99m}Tc-PYP positivity in some patients with AL amyloidosis.[27] Thus, we routinely check negative monoclonal protein testing with negative serum and urine protein electrophoresis with immunofixation and negative serum free light chains to exclude amyloid light chain (AL) amyloidosis as described in previous reports.[13,18]

Furthermore, the addition of SPECT to planar imaging has been the key to the noninvasive testing pathway in the past several years. Whereas grade 2 planar scans were considered positive in early reports,[20] nearly two-thirds of grade 2 scans were either entirely negative or equivocal with blood pooling when evaluated by SPECT.[13] Grade 2 planar scans at 1 h were frequently being found to be false positive by SPECT[28]

with additional studies confirming the importance of SPECT in making an accurate diagnosis.[29,30] **Poterucha et al reported that with respect to SPECT as a reference standard, planar imaging showed 64% false positives in grade 2 scans.[13] In the present study, 31 patients diagnosed as grade 2 by planar scan were evaluated by SPECT, and 20 (64.5%) were false positive. The results were similar to those previously reported. In our study, immunofixation of serum and urine and serum free light chain assay ruled out AL amyloidosis in all cases; immunohistochemical staining of EMB also showed no patients with AL. Thus, the cause of the ^{99m}Tc-PYP false-positive cases is unknown. Further studies are needed to clarify this point.**

So far, ^{99m}Tc-PYP scintigraphy has shown excellent results in differentiating ATTR-CM from AL cardiac amyloidosis [21,31] and other cardiomyopathies.[13] Based on previous studies, since ^{99m}Tc-PYP scintigraphy has shown a high sensitivity and specificity, enabling amyloidosis to be differentiated from other types of cardiomyopathy,[31] ^{99m}Tc-PYP cardiac imaging has recently been incorporated into ESC guidelines as a recommended test for detecting ATTR-CM.[15] In our multiple-center study, for 373 unselected HFpEF patients, a ^{99m}Tc-PYP cardiac scan show a sensitivity and specificity of both 100% for ATTR-CM. Available evidence suggests that the ability of a ^{99m}Tc-PYP cardiac scan to predict survival needs more investigation.

Recent developments in noninvasive testing in preference to biopsy have

transformed our ability to diagnose ATTR-CM and unmasked the unrecognized prevalence of the disease.[7] The reported prevalence of ATTR-CM in HFpEF patients ranges from 5% to 14% [6,8,14,32,33]. In an autopsy analysis of patients with a history of HF and LVEF > 40%, amyloid fibrils were found in 19% of the patients, but only 4.6% of the patients had sufficient amyloid deposition to be clinically classified as CA.[8] In another study using ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid, it was shown that an underdiagnosed cause of HFpEF, with a prevalence of 13% in HFpEF patients ≥60 years old with a LV wall thickness of 12 mm or greater, is wild-type ATTR-CM.[6] Another study showed a myocardial biopsy-proven CA prevalence of 14% in HFpEF patients.[14] ATTR-CM was originally thought to be a rare disease. However, we showed that the prevalence of ATTR-CM in HFpEF patients was 14.2%, being consistent with prior reports, although the patient recruitment in this study differs from that in previous studies. We confirmed that ATTR-CM is far more common that was previously thought in Japanese patients with HFpEF and we recommend routine evaluation for CA.

As shown by previous studies, HFpEF with ATTRwt is related to the presence of hypertension, diabetes, obesity, CAD, and LV hypertrophy, which are also very common among the elderly.[6] In ATTR-CM, on the other hand, destabilization of the TTR protein promotes the release of monomers, which aggregate into amyloid fibrils that deposit in organs and tissues. Although the means by which TTR protein is deposited in the heart and how amyloid damages cardiomyocytes are not fully

understood,[5] extracellular amyloid deposition in the heart causes diastolic dysfunction that progresses to restrictive cardiomyopathy and congestive heart failure. [34] Our findings suggest that the following diagnostic clues should heighten suspicion for ATTR-CM in patients with HFpEF: advanced age, male, history or presence of atrial fibrillation or atrial flutter, low blood pressure, increased LV wall thickness, presence of a diastolic disorder, diffuse subendocardial or transmural late gadolinium enhancement on cardiac magnetic resonance imaging, elevated mean pulmonary artery pressure, and high NT-proBNP and troponin T levels, some of which have been shown by previous studies[6,28,11,35]. A previous study showed ATTR wild-type patients had a significantly higher rate of pacemaker implantation.[6] However, our study showed that ATTR-CM patients did not have a significantly higher rate of pacemaker implantation. **These different results might be due to ATTR-CM patients were diagnosed at early stage and there are few severe condition ATTR-CM patients who require PMI in this study.**

Although the severities of heart failure as assessed by the NYHA functional class and renal function values were similar in the two groups in our study, ATTR-CM patients had a higher median NT-proBNP value, higher median high -sensitivity troponin T level, and higher rate of diffuse subendocardial or transmural late gadolinium enhancement on cardiac magnetic resonance imaging. A previous study showed that a high extracellular volume fraction, which is associated with a poor

prognosis, is more frequent in amyloidosis than other encountered conditions in cardiac magnetic resonance imaging.[36] Furthermore, a high prevalence of pulmonary hypertension, especially combined post and pre-capillary pulmonary hypertension, in ATTR-CM patients has been reported.[37] However, tricuspid regurgitant pressure gradients were similar in ATTR-CM and non-ATTR-CM patients in our study. Our findings might be explained by the fact that clinical overlap between ATTR-CM and non-ATTR-CM is high.

Diagnosis of ATTR-CM might be useful for personalizing drug therapy. In our study, a larger percentage of ATTR-CM patients were receiving treatment with tolvaptan. Tolvaptan (TLV) is an oral selective vasopressin type 2 receptor antagonist that is used as an add-on therapy to loop diuretics for managing patients with HF in Japan. TLV inhibits the binding of vasopressin and increases electrolyte-water clearance without activating the renin-angiotensin–aldosterone system or reducing the glomerular filtration rate. [38] A recent study showed that the long-term use of TLV may be beneficial for patients with HFpEF.[39] However, treatment with TLV in patients with HFpEF is still controversial and additional investigation is needed to determine whether TLV is effective for reducing mortality and cardiovascular hospitalizations in ATTR-CM patients.

To facilitate early diagnosis of ATTR-CM, our findings indicate that evaluation by ^{99m}Tc-PYP scintigraphy with SPECT should be routinely considered for patients with

HFpEF, especially patients with an unexplained increase in LV wall thickness. Novel stabilizing therapies should be considered for patients who presently do not have any specific treatment.

In general, HFpEF patients are more likely to be female.[40] However, in the present study, there was no gender difference in overall HFpEF patients. The fact that the participating institutions were hospitals with cardiologists who could diagnose amyloidosis may have been a bias. Interestingly, ATTR-CM was predominantly male and non-ATTR-HFpEF was predominantly female. This finding was consistent with a previous study in which 99mTc-PYP scintigraphy was performed to diagnose ATTR-CM.[13] Further study is needed to clarify this point.

Limitations

Our study has several limitations. First, whether ATTR-CM affects mortality synergistically with HFpEF is not known. Second, because the patients in this study were referral patients with a high pre-test probability for HFpEF, the relevance of the results of this study for populations with a lower pre-test probability is uncertain and the prevalence of ATTR-CM might be higher compared with that with a referral bias. Third, information on clinical characteristics and echocardiography was obtained as close as possible to the time of the ^{99m}Tc-PYP scan but should ideally be obtained on the

same day. Furthermore, the left atrial volume index should have been examined rather than the left atrial dimension. Fourth, genetic diagnosis for ATTR-CM was not performed in any of the cases. Thus, rare hereditary disorders that cause amyloid deposits different from ATTR such as ApoA1 amyloidosis were not fully excluded in this study. Fifth, although there might be a burden of deposit from which PYP becomes positive, there are no available data on a correlation between the degree of amyloid deposition and PYP grade uptake. Finally, because this study was a local study, the results of the study may not be representative of the general population of patients with ATTR-CM. Further large-scale worldwide cohort studies are needed.

Conclusions

ATTR-CM is an underdiagnosed disease with a significant prevalence (14.2%) in Japanese patients with HFpEF. This study showed that results of examinations for ATTR-CM patients appear to be worse than those for non-ATTR-CM patients, but clinical severities of heart failure as assessed by the NYHA functional classification are similar in ATTR-CM and non-ATTR-CM patients and the clinical overlap between ATTR-CM and non-ATTR-CM is high.

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Conflict of interests

All authors have no financial disclosures that are relevant to this article.

Appendix

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Figure legends

Figure 1. Prevalence of positive ^{99m}Technetium - pyrophosphate scintigraphy in heart failure patients with preserved ejection fraction. A. Study flow chart of the results. The chart shows a summary of the 373 heart failure patients with preserved ejection fraction who underwent a ^{99m}Technetium (Tc)- pyrophosphate (PYP) scintigraphy. Grade 0 and 1 scans are considered negative tests and grade 2 and 3 scans are considered positive tests. HFpEF, heart failure with preserved ejection fraction; HF, heart failure; LVEF left ventricular ejection fraction. B. Percentage of ^{99m}Technetium - pyrophosphate scintigraphy by grade. In the 373 heart failure patients with preserved ejection fraction, 14.2% of the patients had transthyretin amyloid cardiomyopathy of Grade 2 or 3. CI, confidence interval.

Figure 2. Prevalence of positive ^{99m}Technetium - pyrophosphate scintigraphy graded by age over 65 years. The figure shows a higher prevalence in the age group of 75-84 years.