Original Article

Non-invasive detection and differentiation of cardiac amyloidosis using ^{99m}Tc -pyrophosphate scintigraphy and ^{11}C -Pittsburgh compound B PET imaging

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Running head: Non-invasive diagnosis of cardiac amyloidosis

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Abstract

Purpose: To investigate the utility of the combined use of ^{11}C -Pittsburgh compound B (^{11}C -PiB) positron emission tomography (PET) imaging and $^{99\text{m}}\text{Tc}$ -pyrophosphate ($^{99\text{m}}\text{Tc}$ -PYP) scintigraphy for detection and differentiation of three major types of cardiac amyloidosis, i.e., immunoglobulin light chain (AL), hereditary transthyretin (ATTR ν), and wild-type transthyretin (ATTRwt) amyloidosis.

Methods: Whole-body ^{11}C -PiB PET and $^{99\text{m}}\text{Tc}$ -PYP scintigraphy were performed in 17 patients with AL amyloidosis, 22 patients with ATTR ν , and eight patients with ATTRwt amyloidosis. The correlations between organ involvement and the uptake of ^{11}C -PiB and $^{99\text{m}}\text{Tc}$ -PYP were analyzed in each patient.

Results: Cardiac amyloidosis was detectable by $^{99\text{m}}\text{Tc}$ -PYP scintigraphy or ^{11}C -PiB PET in all systemic amyloidosis patients with cardiac involvement. $^{99\text{m}}\text{Tc}$ -PYP scintigraphy and ^{11}C -PiB PET showed an interesting complementary relation. Strict combination of positive ^{11}C -PiB and negative $^{99\text{m}}\text{Tc}$ -PYP uptake (PiB pattern) was observed in all AL amyloidosis patients with cardiac involvement. In contrast, strict combination of positive $^{99\text{m}}\text{Tc}$ -PYP and negative ^{11}C -PiB uptake (PYP pattern) was observed in all ATTRwt amyloidosis patients with cardiac involvement. ATTR ν amyloidosis patients with cardiac involvement were divided into two groups: PiB pattern or PYP pattern. All of the early-onset V30M (p.V50M) ATTR ν patients showed the PiB pattern, whereas all of the late-onset V30M and non-V30M ATTR ν patients showed the PYP pattern.

Conclusions: All three major types of cardiac amyloidosis can be detected and differentiated non-invasively by combined use of the two amyloid imaging methods and *TTR* gene testing.

Key words: pyrophosphate scintigraphy, Pittsburgh compound B PET, light chain amyloidosis, hereditary transthyretin amyloidosis, wild-type transthyretin amyloidosis

Introduction

Systemic amyloidosis is a multisystem disorder caused by extracellular deposition of insoluble amyloid fibrils. Cardiac amyloidosis commonly occurs in systemic amyloidosis patients and is the most serious clinical complication. Cardiac amyloidosis is characterized by extracellular infiltration of amyloid fibrils into the myocardium, which causes ventricular wall thickening and diastolic kinetic dysfunction, resulting in restrictive cardiomyopathy.

Immunoglobulin light chain (AL), hereditary transthyretin (ATTRv), and wild-type transthyretin (ATTRwt) amyloidosis are major systemic amyloidoses that involve the heart, each of which has a different pathological background, prognosis, and therapeutic approach.

Systemic AL amyloidosis is caused by abnormal immunoglobulin light chains, which form amyloid fibrils and undergo deposition in the whole body, mainly involving the heart, kidney, gastrointestinal tract, liver, peripheral nervous system, and soft tissues [1] [2]. Amyloidogenic light chains are produced by a clonal population of plasma cells in the bone marrow, and treatment therefore involves chemotherapy targeting abnormal plasma cells [3]. Among the three types of amyloidosis (i.e., AL, ATTRv, and ATTRwt), patients with cardiac AL amyloidosis have the poorest prognosis. The median survival of patients with congestive heart failure is less than 6 months if untreated [4]. ATTRv amyloidosis is caused by variants of the *transthyretin* (*TTR*) gene, which show an autosomal dominant inheritance pattern. Patients present with sensorimotor neuropathy, autonomic neuropathy, gastrointestinal symptoms, glaucoma, and central nervous system disorders as well as cardiomyopathy [5]. The prognosis of cardiac ATTRv amyloidosis is better than that of AL amyloidosis [6]. Effective disease-modifying therapies, including liver transplantation, TTR tetramer stabilizers (i.e.,

diflunisal [7] and tafamidis [8]), and gene silencing therapies (i.e., patisiran [9] and inotersen [10]) are now available. ATTRwt amyloidosis is caused by accumulation of wild-type TTR. ATTRwt amyloidosis is characterized by heart failure and carpal tunnel syndrome in elderly patients. The reported median survival of cardiac ATTRwt amyloidosis patients varies from 43 to 75 months [11]. Recently, the clinical effects of tafamidis on ATTRwt amyloidosis have been demonstrated [12]. Thus, AL and ATTR amyloidosis have become treatable diseases, and therefore early detection of amyloid deposition and early determination of its subtype are critically important to improve outcomes.

Although endomyocardial biopsy is needed for histopathological diagnosis of cardiac amyloidosis [13], this technique is not applicable in all patients due to its relatively high invasiveness and requirement for specific operator skills. Therefore, non-invasive methods to detect cardiac amyloidosis and determine its amyloid subtype are required. Recently, the value of radionuclide molecular imaging for the diagnosis of cardiac amyloidosis has been reported. Technetium-99m (^{99m}Tc)-3,3-diphosphono-1,2-propanedicarboxylic acid (DPD), ^{99m}Tc -hydroxymethylene diphosphonate (HMDP), and ^{99m}Tc -pyrophosphate (PYP) were originally developed as bone-seeking tracers, but are now also utilized in the evaluation of cardiac ATTR amyloidosis [14]. On the other hand, ^{11}C -labeled Pittsburgh compound B (^{11}C -PiB; 2-[4'-methylaminophenyl]-6-hydroxybenzothiazole) positron emission tomography (PET) was developed to evaluate localized A β amyloid deposition in the brains of patients with Alzheimer's disease. Recently, our group showed that ^{11}C -PiB PET can also detect ATTRv and AL amyloid deposition [15] [16].

Here, we investigated the efficacy and limitations of a combination of ^{99m}Tc -PYP

scintigraphy and ^{11}C -PiB PET for evaluating AL, ATTRv, and ATTRwt cardiac amyloidosis. The results indicated that we can detect and differentiate between these three types of amyloidosis non-invasively using $^{99\text{m}}\text{Tc}$ -PYP scintigraphy, ^{11}C -PiB PET, and *TTR* gene testing.

Material and methods

Patients

The clinical information of participants is summarized in Table 1. Consecutive patients with histologically proven systemic AL amyloidosis ($n = 17$; mean age $\pm$ SD, 68 ± 9 years; range 52 – 82 years) and patients with histologically and genetically proven ATTRv amyloidosis ($n = 22$; mean age $\pm$ SD, 50 ± 18 years; range 26 – 77 years) and ATTRwt amyloidosis ($n = 8$; mean age $\pm$ SD, 76 ± 4 years; range 71 – 82 years) were prospectively enrolled in this study. All patients were diagnosed and examined at Shinshu University Hospital. All patients were confirmed to have Congo red staining-positive amyloid deposition in biopsy specimens taken from at least one organ site. Amyloid subtype was confirmed by immunohistochemical staining and supplemental mass spectrometric analysis. Underlying plasma cell dyscrasia was confirmed by M-proteinemia on serum and urine immunofixation tests and abnormally elevated serum free light chain level in all patients with systemic AL amyloidosis. In the patients with ATTR amyloidosis, normal *TTR* genotype was confirmed in all ATTRwt amyloidosis patients and variant *TTR* genotype was confirmed in all ATTRv amyloidosis patients. *TTR* genotypes of the ATTRv amyloidosis patients were as follows: V30M (p.V50M) heterozygous ($n = 16$), D38A (p.D58A) heterozygous ($n = 1$), G47R (p.G67R) heterozygous

($n = 1$), S50I (p.S70I) heterozygous ($n = 1$), T60A (p.T80A) heterozygous ($n = 1$), Y69H (p.Y89H) heterozygous ($n = 1$), and V122I (p.V142I) heterozygous ($n = 1$). ATTRv amyloidosis patients were divided into three clinical subtypes according to the *TTR* genotype and age at disease onset, i.e., early-onset V30M (patient No. 18 – 28, age at disease onset < 50 years), late-onset V30M (patient No. 29 – 33, age at disease onset $\geq$ 50 years), and non-V30M (patient No. 34 – 39) (Table 1). The duration between ^{99m}Tc -PYP scintigraphy and ^{11}C -PiB PET were no longer than one year for any patient.

Clinical cardiac evaluation

Clinical cardiac involvement was determined by increased mean left ventricular wall thickness > 12 mm on echocardiography [13], abnormal electrocardiographic findings (combination of low voltage in limb lead and QS pattern in anterior precordial lead, sick sinus syndrome, AV block and/or atrial fibrillation), and/or elevation of plasma brain natriuretic peptide (BNP) > 100 pg/mL, in the absence of other cardiac causes.

^{99m}Tc -PYP myocardial scintigraphy imaging and evaluation

Planar cardiac imaging was performed using a triple-head gamma camera (PRISM IRIX; Shimadzu, Kyoto, Japan) equipped with low-energy, high-resolution collimators. ^{99m}Tc -PYP (555 – 740 MBq) was administered intravenously and planar images (anterior, lateral and left anterior oblique views) with the heart centered in the field of view were obtained approximately 3.5 hours after injection. The acquisition parameters were as follows: matrix = 512×512 , zoom factor = 1.422, acquisition time = 240 s. Single photon emission computed

tomography (SPECT) images were obtained with the following acquisition parameters: matrix = 64×64 , zoom factor = 1.488. The Butterworth filter was used with a cut-off of 0.24 cycle/pixel and order of 8.00. All scans were performed at Shinshu University. Imaging results of ^{99m}Tc -PYP myocardial accumulation were scored visually by a single reader (K.T.) using the following grading system: grade 0 = absent cardiac uptake; grade 1 = mild uptake less than bone; grade 2 = moderate uptake equal to bone; and grade 3 = high uptake greater than bone [14]. In this study, visual score higher than grade 1 was regarded as significant positive cardiac ^{99m}Tc -PYP uptake.

^{11}C -PiB PET imaging and evaluation

All imaging studies were performed using a PET/CT scanner with an axial spatial resolution of 5.1 mm (Discovery PET/CT 600; GE Healthcare, Milwaukee, WI, USA). Patients received an intravenous injection of 500 – 636 MBq of ^{11}C -PiB. After whole-body low-dose CT from the vertex to the pelvis, three-dimensional emission scans with seven bed positions were followed by a scan at 2 minutes per bed position 30 minutes after tracer injection. PET images were reconstructed on 128×128 matrices using an ordered subset expectation maximization method (2 iterations, 16 subsets) with a 3.5 mm Gaussian filter (VUE Point plus®). All scans were performed at Aizawa Hospital (Matsumoto, Japan). For evaluation of cardiac tracer accumulation, the maximal standardized uptake value (SUV_{max}) was used. The SUV_{max} was defined as the maximal SUV in all of the volume of interest (VOI) analyzed. SUV_{max} of the myocardium was measured with a spherical VOI 2 – 3 cm in diameter placed over the region of highest uptake in the left myocardium. SUV_{max} of the background was measured by VOI

placed over the blood pool in the left atrium. Because SUV_{max} of myocardium (1.4 ± 0.3) and background (1.1 ± 0.2) were similar in healthy individuals, an SUV_{max} cut-off value of 2.11 was determined by the mean + 2SD value of the background SUV_{max} .

Statistical analysis

To compare the presence of cardiac ^{99m}Tc -PYP uptake among clinical groups, Fisher's exact test with Bonferroni correction was applied. To compare SUV_{max} values among clinical groups, the Kruskal–Wallis test and Mann–Whitney U test with Bonferroni correction were applied. These tests were performed using Excel Statistics 2012 for Windows (Social Survey Research Information Co., Ltd, Tokyo, Japan) and IBM SPSS Statistics - Essentials for Python 25.0 (IBM, Armonk, NY, USA).

Histopathological analysis

To confirm that positive cardiac tracer uptake truly indicates histopathological amyloid deposition in the myocardium, the correlation between cardiac tracer uptake and histopathological amyloid infiltration in myocardial biopsy specimens was investigated retrospectively in patients with clinically detectable cardiac involvement (patient No. 6, AL amyloidosis; patient No. 35, ATTRv amyloidosis; patient No. 41, 42, 45, 46, ATTRwt amyloidosis). Histopathological analysis, including Congo red staining and immunohistochemical staining, was performed as described previously [17].

Results

Correlation between clinical organ involvement and amyloid imaging

Radiological findings of ^{99m}Tc -PYP scintigraphy and ^{11}C -PiB PET of the enrolled patients and their clinical backgrounds are summarized in Table 1. Thirty-four of the 47 enrolled patients were diagnosed with clinically detectable cardiac amyloidosis by echocardiographic, electrocardiographic, and/or BNP criteria. Representative ^{99m}Tc -PYP scintigraphy and ^{11}C -PiB PET images of the patients with clinical cardiac involvement in each clinical subtype group are shown in Figure 1. Correlations between clinical cardiac involvement and cardiac tracer uptake intensities, i.e., visual score in ^{99m}Tc -PYP scintigraphy and SUV_{max} value in ^{11}C -PiB PET, are shown in Figure 2a and 3a, respectively.

As shown in Table 1 and Figure 3a, the presence of clinical cardiac involvement in AL patients was consistent with positive results regarding ^{11}C -PiB uptake. All of the AL patients with clinical cardiac involvement (patient No. 6 – 17) showed positive ^{11}C -PiB uptake, whereas all of those without cardiac involvement (patient No. 1 – 5) showed negative uptake (Table 1). Statistical analysis showed that SUV_{max} value of AL patients with clinical cardiac involvement (median SUV_{max} 6.16; range 2.43 – 19.2) was significantly increased in comparison to those without clinical cardiac involvement (median SUV_{max} 1.23; range 0.4 – 1.31; $p = 0.0016$). On the other hand, ^{99m}Tc -PYP scintigraphy was not useful to detect cardiac AL amyloidosis (Figure 1, Figure 2a). In contrast to AL amyloidosis, all of the ATTRwt patients with clinical cardiac involvement (patient No. 40 – 47) showed positive ^{99m}Tc -PYP uptake (Figure 1, Figure 2a), whereas ^{11}C -PiB PET could not detect cardiac ATTRwt amyloidosis (Figure 1, Figure 3a). Amyloid imaging was also sensitive in detecting cardiac ATTRv amyloidosis. All of the ATTRv patients with clinical cardiac involvement (patient No.

23 – 28, 31 – 33, 35 – 39) were positive for either ^{99m}Tc -PYP or ^{11}C -PiB, with the exception of three patients (patient No. 32, 35 and 39) who showed positive ^{99m}Tc -PYP uptake with modestly increased ^{11}C -PiB uptake (Table 1).

Amyloid imaging patterns in AL, ATTRv, and ATTRwt amyloidosis

^{99m}Tc -PYP scintigraphy and ^{11}C -PiB PET showed an interesting complementary relation. Strict combination of positive ^{11}C -PiB and negative ^{99m}Tc -PYP uptake (defined as PiB pattern) was observed in all of the AL amyloidosis patients with cardiac involvement (Table 1, Figure 1). In contrast, strict combination of positive ^{99m}Tc -PYP and negative ^{11}C -PiB uptake (defined as PYP pattern) was observed in all of the ATTRwt amyloidosis patients with cardiac involvement (Table 1, Figure 1).

ATTRv amyloidosis patients with cardiac involvement were divided into two groups: negative ^{99m}Tc -PYP and positive ^{11}C -PiB pattern (PiB pattern) or positive ^{99m}Tc -PYP and negative ^{11}C -PiB pattern (PYP pattern) (Table 1). Although three ATTRv amyloidosis patients (No. 32, 35 and 39) showed positive ^{99m}Tc -PYP and increased ^{11}C -PiB uptake, these patients were regarded as PYP pattern in this study because the degrees of elevation of SUV_{max} values in these patients were very small (SUV_{max} values in patient No. 32, 35 and 39 were 2.12, 2.19, and 2.19, respectively, whereas the cut-off value was 2.11) (Table 1). Interestingly, among the patients with clinical cardiac involvement, all with the early-onset V30M subtype showed PiB pattern, whereas all of the late-onset V30M and non-V30M patients showed PYP pattern (Table 1, Figure 1, Figure 2b and 3b). It was noteworthy that one late-onset V30M patient without cardiac involvement (No. 30) showed obvious PiB pattern (SUV_{max} value of

4).

Statistical analysis showed that the ^{99m}Tc -PYP positive rates in the AL (0%) and early-onset V30M ATTRv groups (0%) were lower than those in the late-onset V30M ATTRv (100%, $p = 0.0022$ [vs. AL], $p = 0.0119$ [vs. early-onset V30M ATTRv]), non-V30M ATTRv (100%, $p = 0.0002$ [vs. AL], $p = 0.0022$ [vs. early-onset V30M ATTRv]), and ATTRwt groups (100%, $p < 0.0001$ [vs. AL], $p = 0.0003$ [vs. early-onset V30M ATTRv]) among the patients with clinical cardiac involvement (Figure 2b). On the other hand, SUV_{max} values in AL (median 6.16; range 2.43 – 19.2) and early-onset V30M ATTRv groups (median 5.64; range 3.47 – 8.18) were higher than those in the late-onset V30M ATTRv (median 1.65; range 1.21 – 2.12; $p = 0.0094$ [vs. AL], $p = 0.0201$ [vs. early-onset V30M ATTRv]), non-V30M ATTRv (median 2.01; range 1.5 – 2.19; $p = 0.0016$ [vs. AL], $p = 0.0061$ [vs. early-onset V30M ATTRv]), and ATTRwt groups (median 1.42; range 0.38 – 1.92, $p = 0.0002$ [vs. AL], $p = 0.0019$ [vs. early-onset V30M ATTRv]) among the patients with clinical cardiac involvement (Figure 3b).

A clear complementary relation was demonstrated among the patients with clinical cardiac involvement regardless of amyloidosis subtype, i.e., those with PYP uptake showed negative PiB uptake and those without PYP uptake showed positive PiB uptake (Figure 4). In addition, ^{11}C -PiB PET may be more sensitive than clinical criteria for detecting early-onset V30M ATTRv cardiac amyloid, as all of the early-onset V30M ATTRv patients without clinical cardiac involvement (patient No. 18 – 22, Table 1) showed increased ^{11}C -PiB uptake in the heart.

Correlation between amyloid imaging tracer uptake and histopathological amyloid

deposition in the heart

Figure 5 shows representative images of cardiac tracer uptake and corresponding histopathological findings. The positive correlation between tracer uptake and histological amyloid deposition was confirmed in all three types of amyloidosis, i.e., AL (patient No. 6, Figure 5 a – d), ATTRv (patient No. 35, Figure 5 e – h), and ATTRwt (patient No. 41, Figure 5 i – l; patient No. 42, 45, 46, images not shown) amyloidosis.

Discussion

Recently, disease-modifying therapies for cardiac amyloidosis have been developed [3] [7] [8] [9] [10] [18], and therefore early precise diagnosis is becoming increasingly important. However, biopsy-based subtyping of cardiac amyloidosis is occasionally difficult. Especially, differential diagnosis between cardiac AL and ATTRwt amyloidosis is often problematic due to low sensitivity of non-cardiac biopsy [19] [20], and the high frequency of monoclonal gammopathy in ATTRwt amyloidosis [14] [21] [22] frequently results in a requirement for endomyocardial biopsy to confirm the diagnosis [19]. Recently, an algorithm for non-biopsy diagnosis of cardiac ATTR amyloidosis using bone scintigraphy and monoclonal gammopathy was proposed [14]. The algorithm indicated that the combined findings of grade 2 or 3 myocardial radiotracer uptake on bone scintigraphy and the absence of monoclonal protein in serum or urine had specificity and positive predictive value for cardiac ATTR amyloidosis of 100% [14]. On the other hand, sensitivity and negative predictive value for cardiac ATTR amyloidosis were 74% and 83%, respectively, [14]. One reason for the relatively low sensitivity and negative predictive value was that no cardiac tracer uptake was observed in 23

of 48 V30M patients [14]. Our study indicated that all ATTRv amyloidosis patients with clinical cardiac involvement were positive on either ^{99m}Tc -PYP scintigraphy or ^{11}C -PiB PET. Among ATTRv amyloidosis patients, all of the early-onset V30M ATTRv patients with or without cardiac involvement were positive for ^{11}C -PiB PET, but not for ^{99m}Tc -PYP scintigraphy. Positivity for ^{11}C -PiB PET without clinical cardiac involvement suggested that ^{11}C -PiB PET may be more sensitive than clinical criteria for detecting early-onset V30M ATTRv amyloid and could be useful for early diagnosis. Taken together, it is likely that we will be able to increase the sensitivity of cardiac ATTRv amyloid detection by additional ^{11}C -PiB PET analysis in cases where ^{99m}Tc -PYP scintigraphy is negative. Furthermore, our observations indicated that positive ^{11}C -PiB PET and negative ^{99m}Tc -PYP scintigraphy without *TTR* gene mutation are strongly suggestive of AL-type cardiac amyloidosis. Similarly, positive ^{99m}Tc -PYP scintigraphy and negative ^{11}C -PiB PET without *TTR* gene mutation are strongly suggestive of ATTRwt cardiac amyloidosis. Patients with negative ^{99m}Tc -PYP scintigraphy and ^{11}C -PiB PET are less likely to have cardiac amyloidosis. Previous reports in AL amyloidosis showed that cardiac ^{99m}Tc -PYP uptake was absent in most patients [14, 23], but ^{11}C -PiB uptake was increased [16, 24]. Our findings regarding AL amyloidosis were consistent with these reports. ^{99m}Tc -PYP scintigraphy is known to be effective to visualize cardiac involvement in ATTRwt amyloidosis [12] [23] [25]. However, cardiac ^{11}C -PiB uptake in ATTRwt amyloidosis patients has not been reported previously except in only one case with decreased cardiac ^{11}C -PiB uptake [26]. Therefore, this is the first report regarding investigation of cardiac ^{11}C -PiB uptake in ATTRwt amyloidosis in a relatively large patient population.

Another intriguing aspect of this study was the strict complementary relationship between ^{11}C -PiB PET and $^{99\text{m}}\text{Tc}$ -PYP scintigraphy. (i.e., only ^{11}C -PiB PET was positive in AL and early-onset V30M ATTRv amyloidosis with cardiac involvement, whereas only $^{99\text{m}}\text{Tc}$ -PYP scintigraphy was positive in ATTRwt, non-V30M ATTRv and most cases of late-onset V30M ATTRv amyloidosis with cardiac involvement) (Table 1, Figure 4). Recently, Pilebro et al. [27] analyzed a small number of V30M ATTRv patients and reported that the PiB-retention index of type B fibrils (composed of full-length TTR) was significantly higher than that of type A fibrils (mixture of full-length TTR and TTR fragments), suggesting that ^{11}C -PiB bound much more strongly to type B than to type A. They also showed that $^{99\text{m}}\text{Tc}$ -3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) uptake was elevated in patients with type A fibrils, but absent in patients with type B fibrils [27] [28]. In addition, previous studies in patients with V30M ATTRv amyloidosis revealed that the presence of long amyloid fibrils composed of full-length TTR was a characteristic feature in early-onset cases, while short fibrils composed of TTR fragments were characteristic of late-onset cases [29] [30] [31]. Short fibrils composed of TTR fragments have also been reported in cardiac amyloid deposits in ATTRwt amyloidosis patients [32]. Pathologically, the presence of long amyloid fibrils composed of full-length TTR is strongly congophilic and birefringent, whereas short fibrils composed of TTR fragments showed weak Congo red staining [30]. These previous studies and the similarity between Congo red and PiB, i.e., both compounds recognize β sheet structures, suggest that ^{11}C -PiB binds strongly to long amyloid fibrils composed of full-length TTR (type B fibrils), but weakly to short fibrils composed of TTR fragments (type A fibrils). As $^{99\text{m}}\text{Tc}$ -PYP is a bone tracer, $^{99\text{m}}\text{Tc}$ -PYP uptake may be influenced by microcalcification.

However, it is unlikely that microcalcification can completely explain the complementary relationship between ^{11}C -PiB PET and $^{99\text{m}}\text{Tc}$ -PYP scintigraphy. $^{99\text{m}}\text{Tc}$ -PYP may have high affinity to short ATTR fibrils composed of TTR fragments [28]. by a pathomechanism other than microcalcification.

In this study, we divided the V30M ATTRv patients according to early-onset (< 50 years) or late-onset ($\geq$ 50 years). Ihse et al reported that patients with type A fibrils are correlated to a general higher age of onset than patients with type B fibrils, and almost all early-onset patients have fibrils of type B [29]. However, they also showed that patients with type B fibrils do not have to have an age of onset before 50. Intriguingly, one late-onset V30M patient (No. 30; onset age, 63 years) had a PiB SUV_{max} value of 4, suggesting that this patient had type B fibrils compatible with the previous report [29]. It is likely that the radioactive substances used in this study have different affinity to the two different fibril conformation types, as the fibril types differ in structure. Taken together, grouping of ATTR amyloidosis according to fibril composition type is considered to be more reasonable rather than grouping by onset age.

We can detect all three types of cardiac amyloidosis with increased uptake of either tracer, and can distinguish clearly between AL amyloidosis and ATTRwt amyloidosis by these two imaging methods. However, we cannot distinguish ATTRv amyloidosis from the other two types using only these imaging techniques, and therefore supplementary *TTR* gene testing is required for non-invasive diagnosis. In addition, appropriate testing for AL amyloidosis (i.e, monoclonal protein in serum and urine) should be necessary in order to increase the reliability of diagnosis, as some AL patients showed bone scintigraphic uptake grade 2 or 3 [14].

Limitations

This study was performed in a relatively small number of patients with AL, ATTRv, and ATTRwt amyloidosis. In addition, we did not analyze rare cardiac amyloidoses, such as AA and AApoAI amyloidosis, and localized AANF amyloidosis. With regard to bone tracers, no significant correlation was observed between cardiac amyloidosis and cardiac uptake in AA amyloidosis [33], whereas high ^{99m}Tc-DPD myocardial uptake was observed in a patient with AApoAI amyloidosis with cardiac involvement [34]. Concerning PiB PET, no clinical study was reported in AA, AApoAI, or AANF amyloidosis. Studies involving rare cardiac amyloidoses are needed to gain further insight into the cardiac amyloid imaging.

Another limitation of this study is that endomyocardial biopsy was performed in only six patients. All the six patients showed clinical cardiac involvement, amyloid deposition on endomyocardial biopsy, and positive amyloid imaging, however, absence of amyloid deposits in the myocardium of patients with negative examinations were not settled in this study. Therefore, biopsy-based diagnosis, supported by genetic testing and appropriate AL diagnostic tests are still the first step to secure the diagnosis.

Compliance with Ethical Standards:

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Conflict of Interest: The authors declare that they have no conflicts of interest.

Ethical approval: All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Ethics Committee of Shinshu University School of Medicine (No. 4446).

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Figure legends

Fig. 1 Representative ^{99m}Tc -PYP scintigraphy and ^{11}C -PiB PET images in amyloidosis patients with clinical cardiac involvement

a – e ^{99m}Tc -PYP scintigraphy planar images; **f – j** ^{99m}Tc -PYP scintigraphy axial SPECT images; **k – o** ^{11}C -PiB PET maximum intensity projection images; **p – t** Fused ^{11}C -PiB PET/CT axial images; **a, f, k, p** AL amyloidosis patient with cardiac involvement (No. 8); **b, g, l, q** early-onset V30M ATTRv amyloidosis patient with cardiac involvement (No. 26); **c, h, m, r** late-onset V30M ATTRv amyloidosis patient with cardiac involvement (No. 31); **d, i, n, s** non-V30M ATTRv amyloidosis patient with cardiac involvement (No. 35); **e, j, o, t** ATTRwt amyloidosis patient with cardiac involvement (No. 45).

Positive cardiac ^{99m}Tc -PYP uptake was demonstrated in **c, d, e, h, i, and j**. Positive cardiac ^{11}C -PiB uptake was demonstrated in **k, l, p, and q**. Negative ^{99m}Tc -PYP uptake and positive ^{11}C -PiB uptake pattern (PiB pattern) was observed in AL and early-onset V30M ATTRv amyloidosis patients. In contrast, positive ^{99m}Tc -PYP uptake and negative ^{11}C -PiB uptake pattern (PYP pattern) was observed in late-onset V30M ATTRv, non-V30M ATTRv, and ATTRwt amyloidosis patients.

Fig. 2 Comparison of intensity of cardiac ^{99m}Tc -PYP uptake (visual score) between clinical subgroups

The y-axis represents the visual score and the dotted line indicates the cut-off value.

a Comparison of visual scores between AL, ATTRv, and ATTRwt amyloidosis patients with

(CI(+)) and without (CI(-)) cardiac involvement. All ATTRwt patients were CI(+).

b Visual scores in patients with clinical cardiac involvement in comparison between clinical subgroups.

Fig. 3 Comparison of intensity of cardiac ^{11}C -PiB uptake (SUV_{max} value) between clinical subgroups

The y-axis represents SUV_{max} value and the dotted line indicates the cut-off value of $\text{SUV}_{\text{max}} = 2.11$.

a Comparison of SUV_{max} value between AL, ATTRv, and ATTRwt amyloidosis patients with (CI(+)) and without (CI(-)) cardiac involvement. All ATTRwt patients were CI(+).

b SUV_{max} values in patients with clinical cardiac involvement in comparison between clinical subgroups.

Fig. 4 Complementary relation between $^{99\text{m}}\text{Tc}$ -PYP scintigraphy and ^{11}C -PiB PET

The y-axis represents cardiac SUV_{max} value in patients with clinical cardiac involvement, with (PYP(+)) or without (PYP(-)) cardiac $^{99\text{m}}\text{Tc}$ -PYP uptake regardless of amyloidosis subtype. The patients with positive $^{99\text{m}}\text{Tc}$ -PYP uptake (visual score 2 or 3) showed significantly decreased SUV_{max} value (negative ^{11}C -PiB uptake), whereas the patients without $^{99\text{m}}\text{Tc}$ -PYP uptake (visual score 0 or 1) showed significantly increased SUV_{max} value (positive ^{11}C -PiB uptake) ($p < 0.0001$).

Fig. 5 Correlation between amyloid imaging tracer uptake and histological amyloid

infiltration in the heart

a Fused ^{11}C -PiB PET/CT axial image of the heart; **e** and **i** $^{99\text{m}}\text{Tc}$ -PYP scintigraphy axial SPECT images of the heart; **b – d, f – h, j – l** Histopathological images of biopsied myocardium showing Congo red-positive amyloid deposition with immunoreactivity with specific antibodies (all scale bars 200 μm); **b, f, j** Congo red staining; **c, g, k** Congo red staining (polarized view); **d** Immunostaining with anti- λ light chain antibody; **h** and **l** Immunostaining with anti-TTR antibody; **a – d** AL amyloidosis patient (No. 6); **e – h** ATTRv patient (No. 35); **i – l** ATTRwt patient (No. 41).

Fused ^{11}C -PiB PET/CT axial image (**a**) shows mild ^{11}C -PiB cardiac uptake, consisting of mildly elevated SUV_{max} value in this patient (No. 6, Table 1). Positive ^{11}C -PiB uptake was confirmed to represent histological AL amyloid infiltration (**b – d**). $^{99\text{m}}\text{Tc}$ -PYP scintigraphy images in ATTRv (**e**) and ATTRwt (**i**) amyloidosis patients (No. 35 and No. 41, respectively, Table 1) showed significant $^{99\text{m}}\text{Tc}$ -PYP cardiac uptake. Positive $^{99\text{m}}\text{Tc}$ -PYP uptake was confirmed to represent histological ATTR amyloid infiltration (**f – h, j – l**).

^{99m}Tc-PYP scintigraphy images

¹¹C-PiB PET images

AL

ATTRv
early-onset V30M

ATTRv
late-onset V30M

ATTRv
nonV30M

ATTRwt

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Figure 5

Table 1. Clinical background, organ involvement and ^{99m}Tc-PYP and ¹¹C-PiB tracer uptake

Patient No.	Diagnosis	Age at amyloid imaging analyses	Age at disease onset	Sex	Cardiac biopsy	BNP (pg/ml)	Mean LV wall thickness (mm)	Cardiac involvement determined by				^{99m} Tc-PYP scintigraphy		¹¹ C-PiB PET	
								Echo	ECG	BNP	Total	Visual score	Uptake	SUV _{max}	Uptake
1	Systemic AL amyloidosis	61	60	F	–	42.9	11.05	–	–	–	–	0	–	1.23	–
2	Systemic AL amyloidosis	80	79	F	–	40.6	10.15	–	–	–	–	0	–	0.4	–
3	Systemic AL amyloidosis	57	56	F	–	24	7.9	–	–	–	–	0	–	1.11	–
4	Systemic AL amyloidosis	75	74	M	–	19.9	9.7	–	–	–	–	0	–	1.26	–
5	Systemic AL amyloidosis	69	68	F	–	20.5	11.15	–	–	–	–	0	–	1.31	–
6	Systemic AL amyloidosis	60	60	M	+	458.5	19.95	+	+	+	+	0	–	2.43	+
7	Systemic AL amyloidosis	55	52	M	–	119.3	12.25	+	–	+	+	0	–	10.99	+
8	Systemic AL amyloidosis	73	72	M	–	363.3	18.45	+	+	+	+	0	–	19.2	+
9	Systemic AL amyloidosis	69	68	M	–	556.2	19.75	+	–	+	+	1	–	8.04	+
10	Systemic AL amyloidosis	59	57	M	–	292.3	18	+	+	+	+	0	–	10.66	+
11	Systemic AL amyloidosis	82	80	F	–	679.9	23.3	+	–	+	+	1	–	3.04	+
12	Systemic AL amyloidosis	52	50	F	–	548.8	19.1	+	+	+	+	0	–	2.91	+
13	Systemic AL amyloidosis	71	70	M	–	163.9	15.4	+	–	+	+	0	–	5.56	+
14	Systemic AL amyloidosis	69	68	F	–	813.4	15.95	+	–	+	+	0	–	3.47	+
15	Systemic AL amyloidosis	80	79	M	–	111	14.05	+	–	+	+	1	–	6.76	+
16	Systemic AL amyloidosis	77	76	M	–	171.9	18.65	+	–	+	+	1	–	10.35	+
17	Systemic AL amyloidosis	72	72	M	–	148.2	18.35	+	+	+	+	0	–	3.42	+
18	Hereditary ATTR amyloidosis (V30M)	32	30	F	–	11.6	8.6	–	–	–	–	0	–	6.65	+
19	Hereditary ATTR amyloidosis (V30M)	38	29	M	–	<5	10.45	–	–	–	–	1	–	4.9	+
20	Hereditary ATTR amyloidosis (V30M)	37	29	M	–	20.5	10.85	–	–	–	–	1	–	8.24	+
21	Hereditary ATTR amyloidosis (V30M)	26	23	M	–	7.2	7.8	–	–	–	–	0	–	8.85	+
22	Hereditary ATTR amyloidosis (V30M)	27	23	M	–	<5	8.8	–	–	–	–	0	–	4.22	+
23	Hereditary ATTR amyloidosis (V30M)	55	24	M	–	86	13.85	+	+	–	+	0	–	8.18	+
24	Hereditary ATTR amyloidosis (V30M)	45	28	M	–	65.4	11	–	+	–	+	1	–	6.23	+
25	Hereditary ATTR amyloidosis (V30M)	42	22	F	–	39.7	8.95	–	+	–	+	0	–	3.88	+
26	Hereditary ATTR amyloidosis (V30M)	55	35	F	–	150.5	13.15	+	+	+	+	1	–	6.68	+
27	Hereditary ATTR amyloidosis (V30M)	36	27	M	–	9	12.4	+	–	–	+	1	–	3.47	+
28	Hereditary ATTR amyloidosis (V30M)	40	32	M	–	8.6	13.1	+	–	–	+	0	–	5.05	+
29	Hereditary ATTR amyloidosis (V30M)	62	54	M	–	12	10.1	–	–	–	–	1	–	1.34	–
30	Hereditary ATTR amyloidosis (V30M)	76	63	F	–	44.5	9.15	–	–	–	–	1	–	4	+
31	Hereditary ATTR amyloidosis (V30M)	77	67	M	–	216.7	20.5	+	+	+	+	3	+	1.21	–
32	Hereditary ATTR amyloidosis (V30M)	67	57	M	–	145.5	17.55	+	+	+	+	2	+	2.12	+(–)
33	Hereditary ATTR amyloidosis (V30M)	53	52	M	–	28	12.65	+	+	–	+	3	+	1.65	–
34	Hereditary ATTR amyloidosis (Y69H)	44	42	M	–	<5	9.7	–	–	–	–	0	–	1.56	–
35	Hereditary ATTR amyloidosis (T60A)	58	51	M	+	110.9	18.4	+	+	+	+	3	+	2.19	+(–)
36	Hereditary ATTR amyloidosis (G47R)	23	18	F	–	29.9	14.45	+	–	–	+	3	+	1.5	–
37	Hereditary ATTR amyloidosis (S50I)	77	58	F	–	336.2	21.35	+	+	+	+	3	+	2.01	–
38	Hereditary ATTR amyloidosis (D38A)	75	67	M	–	416.6	23.4	+	+	+	+	3	+	1.85	–
39	Hereditary ATTR amyloidosis (V122I)	75	71	F	–	582.3	21.7	+	–	+	+	3	+	2.19	+(–)
40	wild-type ATTR amyloidosis	72	62	M	–	108.7	9.2	–	–	+	+	3	+	1.48	–
41	wild-type ATTR amyloidosis	75	70	M	+	412	19.3	+	+	+	+	3	+	1.74	–
42	wild-type ATTR amyloidosis	82	82	M	+	279.7	20.7	+	+	+	+	3	+	1.35	–
43	wild-type ATTR amyloidosis	76	75	F	–	323.6	19.15	+	+	+	+	3	+	0.38	–
44	wild-type ATTR amyloidosis	82	81	F	–	285	17.8	+	–	+	+	3	+	1.36	–
45	wild-type ATTR amyloidosis	73	72	M	+	81	20.6	+	–	–	+	3	+	1.73	–
46	wild-type ATTR amyloidosis	71	65	M	+	733	18.85	+	–	+	+	3	+	1.92	–
47	wild-type ATTR amyloidosis	79	74	F	–	190.2	17.5	+	–	+	+	3	+	1.05	–

BNP: brain natriuretic peptide, LV: left ventricle, PYP: pyrophosphate, PiB: Pittsburgh compound B, SUV: standardized uptake value, AL amyloidosis: light chain amyloidosis, ATTR amyloidosis: transthyretin amyloidosis

Supplemental table. Affected organs and dominating symptoms and/or signs

Patient No.	Diagnosis	Age at amyloid analyses	Age at disease onset	Sex	Affected organs	Dominating symptoms and/or signs
1	Systemic AL amyloidosis	61	60	F	K	Proteinuria
2	Systemic AL amyloidosis	80	79	F	L, K	Hepatic and renal dysfunction, Proteinuria
3	Systemic AL amyloidosis	57	56	F	K	Proteinuria
4	Systemic AL amyloidosis	75	74	M	K	Proteinuria
5	Systemic AL amyloidosis	69	68	F	K	Proteinuria
6	Systemic AL amyloidosis	60	60	M	H	Heart failure
7	Systemic AL amyloidosis	55	52	M	H, K, PN, GI, AN	Heart failure, proteinuria, numbness in the limbs
8	Systemic AL amyloidosis	73	72	M	K, H	Proteinuria, leg edema
9	Systemic AL amyloidosis	69	68	M	H	Heart failure
10	Systemic AL amyloidosis	59	57	M	K, H	Proteinuria, pleural effusion
11	Systemic AL amyloidosis	82	80	F	Muscle, H	Dropped head
12	Systemic AL amyloidosis	52	50	F	H, K	Leg edema
13	Systemic AL amyloidosis	71	70	M	H, K	Leg edema, proteinuria
14	Systemic AL amyloidosis	69	68	F	H, K	Leg numbness
15	Systemic AL amyloidosis	80	79	M	GI, H	Bloody stool
16	Systemic AL amyloidosis	77	76	M	K, H	Proteinuria, leg edema
17	Systemic AL amyloidosis	72	72	M	H	Heart failure
18	Hereditary ATTR amyloidosis (V30M)	32	30	F	GI, AN	Constipation/diarrhea
19	Hereditary ATTR amyloidosis (V30M)	38	29	M	GI, PN, AN	Constipation, leg numbness
20	Hereditary ATTR amyloidosis (V30M)	37	29	M	PN, AN	Leg numbness, impotence
21	Hereditary ATTR amyloidosis (V30M)	26	23	M	GI, PN, AN	Constipation/diarrhea, leg numbness
22	Hereditary ATTR amyloidosis (V30M)	27	23	M	PN	Leg numbness
23	Hereditary ATTR amyloidosis (V30M)	55	24	M	PN, H, E	Leg numbness, Af, glaucoma
24	Hereditary ATTR amyloidosis (V30M)	45	28	M	PN, AN, GI, H	Leg numbness, constipation/diarrhea
25	Hereditary ATTR amyloidosis (V30M)	42	22	F	AN, H, GI	Constipation/diarrhea, SSS
26	Hereditary ATTR amyloidosis (V30M)	55	35	F	AN, H, PN	Diarrhea, Cardiac conduction disorder
27	Hereditary ATTR amyloidosis (V30M)	36	27	M	PN, H	Leg pain
28	Hereditary ATTR amyloidosis (V30M)	40	32	M	PN, AN, H	Leg pain, vomiting attack
29	Hereditary ATTR amyloidosis (V30M)	62	54	M	V, PN	Vitreous opacity , leg numbness
30	Hereditary ATTR amyloidosis (V30M)	76	63	F	PN	Leg numbness
31	Hereditary ATTR amyloidosis (V30M)	77	67	M	PN, AN, GI, H	Leg numbness, vomiting, constipation
32	Hereditary ATTR amyloidosis (V30M)	67	57	M	PN, AN, H, GI	Leg numbness, constipation/diarrhea, heart failure
33	Hereditary ATTR amyloidosis (V30M)	53	52	M	PN, H	Leg numbness
34	Hereditary ATTR amyloidosis (Y69H)	44	42	M	LM, CT, V	TFNE
35	Hereditary ATTR amyloidosis (T60A)	58	51	M	H, CT	Heart failure, CTS
36	Hereditary ATTR amyloidosis (G47R)	23	18	F	V, PN, AN, H	Vitreous opacity , leg numbness
37	Hereditary ATTR amyloidosis (S50I)	77	58	F	H, CT, PN	AV block
38	Hereditary ATTR amyloidosis (D38A)	75	67	M	H, CT	Heart failure, Af
39	Hereditary ATTR amyloidosis (V122I)	75	71	F	H, CT	Heart failure, arrhythmia
40	wild-type ATTR amyloidosis	72	62	M	CT, H	CTS
41	wild-type ATTR amyloidosis	75	70	M	H, SC	Af, cardiac conduction disorder
42	wild-type ATTR amyloidosis	82	82	M	H	Heart failure
43	wild-type ATTR amyloidosis	76	75	F	H, CT	AV block
44	wild-type ATTR amyloidosis	82	81	F	H, SC, CT	Heart failure
45	wild-type ATTR amyloidosis	73	72	M	H, SC, CT	IVS thickening
46	wild-type ATTR amyloidosis	71	65	M	H, CT, SC	Heart failure, Af
47	wild-type ATTR amyloidosis	79	74	F	H, CT	Heart failure

BNP: brain natriuretic peptide, LV: left ventricle, PYP: pyrophosphate, PiB: Pittsburgh compound B, SUV: standardized uptake value, AL amyloidosis: light chain amyloidosis, ATTR amyloidosis: transthyretin amyloidosis, K: kidney, L: liver, H: heart, PN: peripheral nerve, GI: gastrointestinal tract, AN: autonomic nerve, E: eye, Af: atrial fibrillation, SSS: sick sinus syndrome, V: vitreous, LM: leptomeninges, CT: carpal tunnel, CTS: carpal tunnel syndrome, TFNE: transient focal neurological episodes, SC: Spinal canal, AV: atrioventricular, IVS: interventricular septum