Abdominal Fat Pad Fine-Needle Aspiration for Diagnosis of Cardiac Amyloidosis in Patients with Non-Ischemic Cardiomyopathy
Evidence from a Cohort of 77 Cardiac Biopsy Cases

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Summary
The diagnosis of cardiac amyloidosis is frequently delayed because histological confirmation is often challenging. Few studies have attempted to clarify the utility and safety of abdominal fat pad fine-needle aspiration (FPFNA) for an initial screening test in patients with suspected cardiac amyloidosis.

This study included 77 consecutive patients with suspected non-ischemic cardiomyopathy who had left ventricular dysfunction and/or hypertrophy. All patients underwent abdominal FPFNA and an endomyocardial biopsy. In all patients, the abdominal FPFNA could be performed within less than 5 minutes with no complications; however, in 1 patient (1.3%), the obtained specimen was too small to evaluate. Among the remaining 76 patients, 5 (6.6%) were positive for amyloid (FPFNA+[+]) and 7 (9.2%), including the 5 FPFNA+[+], were diagnosed with cardiac amyloidosis (AL = 1, ATTR = 6) by endomyocardial biopsy. Positive abdominal FPFNA indicated cardiac amyloidosis with high accuracy (sensitivity, 71.4%; specificity, 100%).

Positive abdominal FPFNA is directly linked to diagnoses of cardiac amyloidosis. Abdominal FPFNA is simple and useful for the initial screening test for cardiac amyloidosis in patients with non-ischemic cardiomyopathy.

Key words: Heart failure

Cardiac involvement of systemic amyloidosis (cardiac amyloidosis) may be divided into the following three types of amyloidosis: immunoglobulin light chain-related [AL] amyloidosis, wild-type transthyretin (TTR)-related (ATTR) amyloidosis (ATTRwt), and variant ATTR amyloidosis (ATTRv). The clinical spectrum of cardiac amyloidosis is heterogeneous, and its incidence is higher than previously thought in the aging society. The effective treatment options are increasing, and the benefits of an earlier diagnosis and treatment are manifold. Many patients who had not previously been assumed to have cardiac amyloidosis have now to be tested for it.

Histological confirmation by endomyocardial biopsy is the diagnostic gold standard for detecting cardiac amyloidosis. However, it might not be suitable for the first screening biopsy to diagnose systemic or cardiac amyloidosis. Several studies have reported abdominal fat pad fine-needle aspiration (FPFNA) is a useful test for detecting systemic amyloidosis. However, in those studies, the number of patients who had definitely been diagnosed with cardiac amyloidosis by endomyocardial biopsy was none or very small. The utility and safety of abdominal FPFNA for an initial screening test for cardiac amyloidosis have not been examined thus far. The purpose of this study was to clarify this point in heterogeneous patients with non-ischemic cardiomyopathy.

Methods

Study population: The data that support the findings of this study are available from the corresponding author upon reasonable request. This prospective study included 77 consecutive patients with non-ischemic cardiomyopathy who were admitted to the Department of Cardiology of the University of Fukui Hospital from January 2016 to March 2020. For the purpose of this study, abdominal FPFNA and endomyocardial biopsy from the right ventri...
In the heart, the ventricular fibrillation and ventricular tachycardia, the patients were divided into the following two groups: patients with and without cardiac amyloidosis. The clinical characteristics and electrocardiographic, echocardiographic, and blood test parameters at the time of hospitalization were collected and compared between the two groups. The safety and usefulness of abdominal FPFNA for a diagnostic first screening test of cardiac amyloidosis was examined. This study was approved by the Research Ethics Committee of the University of Fukui. All patients provided informed consent. The study complied with the Declaration of Helsinki.

**Diagnosis and classification of cardiac amyloidosis:** The endomyocardial biopsy samples from the right ventricle were fixed with formalin and embedded in paraffin. The sections were stained with H&E and phenol Congo red. A definitive diagnosis of cardiac amyloidosis was made on the positive Congo-red staining with apple-green birefringence under a polarized light microscope. Immunohistochemical staining was carried out using a panel of type-specific antibodies13-15) at the Department of Medicine (Neurology and Rheumatology), Shinshu University, or at the Department of Molecular Pathology, University of Fukui, both of which belong to the government-funded group for surveys and research of amyloidosis in Japan. These specific antibodies included anti-κ light chain118-13 (rabbit polyclonal),14) anti-λ light chain118-13 (rabbit polyclonal),14) anti-TTR115-124 (rabbit polyclonal),14) anti-AA (mouse monoclonal; DAKO),16) and anti-β2-m (rabbit polyclonal; DAKO) antibodies. The dilutions used were 1:5,000, 1:800, 1:8,000, 1:500, and 1:5,000, respectively. In all cardiac ATTR amyloidosis patients, AL amyloidosis was also excluded by serum and urine immunofixation electrophoresis and serum free light chain assay. Genotyping was performed using the genomic DNA obtained, polymerase chain reaction amplification assays, and sequencing of TTR exons 1 to 4, if available. 99mTc-labeled pyrophosphate (PYP) scintigraphy was performed, if available. Abdominal FPFNA was performed using an 18-gage, 38-mm needle, and the tissue was fixed in formalin. The abdominal FPFNA procedures were based on previous reports.16

**Definitions:** Low voltage in the limb leads was defined as a QRS amplitude of ≤ 0.5 mV in all limb leads. A poor R wave progression was defined as a loss or absence of R waves in leads V1-V3. The echocardiographic parameters included the chamber size, wall thickness, left ventricular (LV) ejection fraction (LVEF), and pericardial effusions using standard procedures. LV hypertrophy was defined as an interventricular septal thickness of ≥ 12 mm and/or LV posterior wall thickness of ≥ 12 mm. Asymmetric septal hypertrophy was defined as an interventricular septal thickness to LV posterior wall thickness ratio of ≥ 1.3. Left ventricular dysfunction was defined as an LVEF of < 50%. Fatal ventricular arrhythmias included ventricular fibrillation and ventricular tachycardia. The hospitalization for heart failure was defined incorporating both worsening symptoms and signs of heart failure and the need to hospital and increase specific heart failure-related intravenous and/or mechanical therapy (ventilator or circulatory).

**Statistical analysis:** Data were expressed as the mean ± SD or number (percentage). Differences in the parameters were analyzed using a paired t-test. Continuous data and categorical variables were compared using the Welch’s t- and Fisher’s exact tests, respectively. The diagnostic performance of various criteria for cardiac amyloidosis was assessed by the analysis of their accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). All statistical analyses were performed with SPSS (Statistical Package for the Social Sciences) version 20 software (IBM Inc., Armonk, NY, USA). A two-sided P < 0.05 was considered statistically significant.

**Results**

**Characteristics of the cohort (Table I):** Seventy-seven consecutive patients underwent endomyocardial biopsy and abdominal FPFNA. Abdominal FPFNA was performed by a single physician. All abdominal FPFNA procedures took less than 5 minutes in all patients. However, the FPFNA sample could not be evaluated in 1 (1.3%) patient because of inadequate volume of material. There were no complications associated with endomyocardial biopsy or abdominal FPFNA.

In 76 patients with available data on both the endomyocardial biopsy and abdominal FPFNA, the mean age was 62 ± 13 (range, 26-90) years. Forty-five (59.2%) patients had LV hypertrophy, 2 (2.6%) had asymmetric LV hypertrophy, and 24 (31.6%) had increased wall thickness of ≥ 14 mm. The mean LVEF was 42.9% ± 16.0% (range, 14.7-78.2), and 61 patients (80.3%) had LV dysfunction. Thirty-two patients (42.1%) had both LV dysfunction and hypertrophy. Twenty-four patients (31.6%) had fatal ventricular arrhythmias. Fifty-five patients (72.4%) had been hospitalized for heart failure at least once. Amyloid was detected in the abdominal FPFNA samples of 5 (6.6%) patients.

**Characteristics of the patients with cardiac amyloidosis (Tables I, II and Figure):** In 76 patients (mean age, 62 ± 13 [range, 26-90]) who underwent both endomyocardial biopsy and abdominal FPFNA, the data obtained were assessed (Table I). Among those patients, 7 (9.2%) were definitely diagnosed with cardiac amyloidosis by endomyocardial biopsy (Tables I, II); one patient was diagnosed with cardiac AL amyloidosis (Patient 1), and the remaining 6 were immunohistochemically diagnosed with cardiac ATTR amyloidosis (Patients 2-7) at the Department of Medicine (Neurology and Rheumatology), Shinshu University (Patients 1, 2, 3, and 5), or at the Department of Molecular Pathology, University of Fukui (Patients 4, 6, and 7). Representative histopathological images of amyloid deposits (Patient 4) are shown in the Figure. Five (83.3%) of 6 ATTR amyloidosis patients (Patients 2, 3, and 5-7) were diagnosed with ATTRwt by genetic testing (Table II). The remaining patient (Patient 4) refused genetic testing.

All 7 patients with cardiac amyloidosis were more
than 65 years old, older than those without \((P = 0.001,\) Table I). The prevalence of a low QRS voltage in the limb leads \((P = 0.015)\) and that of a poor R progression in the precordial leads \((P = 0.004)\) were higher in the patients with cardiac amyloidosis than in those without. All 7 patients with cardiac amyloidosis had LV hypertrophy, and 2 \((28.6\%)\) had asymmetric LV hypertrophy. The LV end-diastolic \((P < 0.001)\) and end-systolic \((P = 0.003)\) diameters were smaller in the patients with cardiac amyloidosis than in those without. The LVEF was greater \((P = 0.018)\) and the LV dysfunction was less frequently observed \((P = 0.003)\) in patients with cardiac amyloidosis than in those without. One patient with cardiac ATTRwt amyloidosis (Patient 6) was negative for FPFFNA. \(^{99m}\)Tc-PYP accumulation was as high as those of positive FPFFNA. In this study, among the patients aged \(\geq 65\) years, the likelihood of cardiac amyloidosis was high \((19.4\%)\), and the rate of positive FPFFNAs was high \((13.9\%)\). Moreover, among the patients of \(\geq 65\) years and LV hypertrophy, the likelihood of cardiac amyloidosis was very high \((35.0\%)\) and the rate of positive FPFFNA was high \((25.0\%)\) as well.

### Table I. Characteristics of the Cohort

| Age, years | Total \(n = 76\) | Cardiac amyloidosis \(n = 7\) | No cardiac amyloidosis \(n = 69\) | \(P\)-value |
|------------|----------------|-----------------|----------------|-----------|
| Male, \(\%\) | 62 ± 13 | 77 ± 8 | 61 ± 12 | 0.001 |
| Body mass index, kg/m\(^2\) | 55 \((72.4)\) | 6 \((85.7)\) | 49 \(71.0\) | 0.371 |

NYHA functional class, \(\%\)

- I: \(27 \quad (35.5)\) \(1 \quad (14.3)\) \(26 \quad (37.7)\) \(P = 0.018\)
- II: \(31 \quad (40.8)\) \(5 \quad (71.4)\) \(25 \quad (36.2)\) \(P < 0.001\)
- III: \(17 \quad (22.4)\) \(1 \quad (14.3)\) \(16 \quad (23.1)\) \(P < 0.001\)
- IV: \(1 \quad (1.3)\) \(0 \quad 0\) \(1 \quad (1.4)\) \(P = 0.379\)

Hospitalization for heart failure, \(\%\)

- \(55 \quad (72.4)\) \(7 \quad (100)\) \(48 \quad (68.6)\) \(P = 0.180\)

Hypertension, \(\%\)

- \(53 \quad (69.7)\) \(4 \quad (57.1)\) \(49 \quad (71.0)\) \(P = 0.356\)

Atrial fibrillation, \(\%\)

- \(23 \quad (30.3)\) \(1 \quad (14.2)\) \(22 \quad (31.9)\) \(P = 0.620\)

Fatal arrhythmias, \(\%\)

- \(24 \quad (31.6)\) \(1 \quad (14.2)\) \(23 \quad (33.3)\) \(P = 0.421\)

Electrocardiographic parameters

- Low QRS voltage in limb leads, \(\%\)
  - \(7 \quad (9.2)\) \(3 \quad (42.8)\) \(4 \quad (5.8)\) \(P = 0.015\)
- Poor R progression, \(\%\)
  - \(25 \quad (32.9)\) \(6 \quad (85.7)\) \(19 \quad (27.5)\) \(P = 0.004\)

Echocardiographic findings

- LA diameter, mm
  - \(41.1 \pm 5.9\)
  - \(42.1 \pm 4.8\)
  - \(41.0 \pm 6.0\)
  - \(P = 0.624\)
- LV septal wall thickness, mm \((\text{range})\)
  - \(12.5 \pm 3.0 \quad (6.4–22.4)\)
  - \(16.2 \pm 1.8 \quad (14.0–18.1)\)
  - \(12.1 \pm 2.8 \quad (6.4–22.4)\)
  - \(P < 0.001\)
- LV posterior wall thickness, mm \((\text{range})\)
  - \(12.0 \pm 2.6 \quad (8.6–22.5)\)
  - \(14.8 \pm 2.1 \quad (12.4–17.2)\)
  - \(11.6 \pm 2.5 \quad (6.4–22.5)\)
  - \(P = 0.003\)
- LV hypertrophy, \(\%\)
  - \(61 \quad (80.3)\) \(2 \quad (28.6)\) \(59 \quad (85.5)\)
  - \(P = 0.003\)
- Asymmetric LV hypertrophy, \(\%\)
  - \(2 \quad (2.6)\) \(0 \quad 0\)
  - \(P = 0.007\)
- LV end-diastolic diameter, mm
  - \(53.0 \pm 8.5\)
  - \(40.5 \pm 5.5\)
  - \(54.1 \pm 8.0\)
  - \(P < 0.001\)
- LV end-systolic diameter, mm
  - \(41.5 \pm 10.5\)
  - \(30.4 \pm 7.0\)
  - \(42.6 \pm 10.2\)
  - \(P = 0.003\)
- LV ejection fraction, \(\%\) \((\text{range})\)
  - \(42.9 \pm 16.0 \quad (14.7–78.2)\)
  - \(56.4 \pm 13.4 \quad (38.0–76.8)\)
  - \(41.6 \pm 15.7 \quad (14.7–78.2)\)
  - \(P = 0.018\)
- LV dysfunction, \(\%\)
  - \(61 \quad (5.8)\) \(2 \quad (28.6)\) \(59 \quad (85.5)\)
  - \(P = 0.003\)
- Plasma BNP level, pg/dL
  - \(501.6 \pm 570.7\)
  - \(575.1 \pm 463.1\)
  - \(493.4 \pm 584.0\)
  - \(P = 0.722\)
- Positive abdominal FPFFNA, \(\%\)
  - \(5 \quad (6.6)\) \(5 \quad (71.4)\) \(0 \quad (0)\)
  - \(P < 0.001\)

Values are reported as the mean ± standard deviation or number of patients \((\%\), unless otherwise noted. LV, left ventricular; LA, left atrial; NYHA, New York Heart Association.

In this study, among the patients aged \(\geq 65\) years, the likelihood of cardiac amyloidosis was high \((19.4\%)\), and the rate of positive FPFFNAs was high \((13.9\%)\). Moreover, among the patients of \(\geq 65\) years and LV hypertrophy, the likelihood of cardiac amyloidosis was very high \((35.0\%)\) and the rate of positive FPFFNA was high \((25.0\%)\) as well.

### Sensitivity, specificity, and predictive accuracy for the diagnosis of cardiac amyloidosis

Table IV shows the diagnostic values of the various criteria for cardiac amyloidosis. An age of \(\geq 65\) years and the echocardiographic findings of LV hypertrophy, interventricular septal thickness of \(\geq 12\) mm, and LV end-diastolic dimension of \(\leq 55\) mm all had 100% sensitivity and 100% NPV. However, their specificities were moderate and the PPVs were low. In contrast, positive FPFFNAs had 100% specificity and 100% PPV with high NPV \((97.2\%)\) and moderate sensitivity \((71.4\%)\). Although the number of patients was small and limited \((33\) patients \([43.4\%]\)), the sensitivity, specificity, PPV, NPV, and accuracy of \(^{99m}\)Tc-PYP accumulation in the myocardium were as high as those of positive FPFFNAs. The sensitivity, specificity, PPV, NPV, and accuracy of positive FPFFNAs and \(^{99m}\)Tc-PYP accumulation in the myocardium were lower than those of each test alone.

Table V shows the diagnostic values of the various criteria for cardiac amyloidosis among patients with LV hypertrophy. The accuracy of positive FPFFNAs and \(^{99m}\)Tc-PYP accumulation were higher than the others.

### Abdominal FPFFNA for first screening test for cardiac amyloidosis

In Tables II, III, IV: In this study, amyloid was histopathologically detected by abdominal FPFFNA (positive FPFFNAs) in 5 \((6.6\%)\) patients (Tables I, III), and all of 5 had cardiac involvement of the amyloidosis \((71\%)\) of the patients with cardiac amyloidosis. In the patients with no cardiac amyloidosis, no amyloid was detected by abdominal FPFFNA. No remarkable differences were observed in the characteristics and results of the measurement variables between the patients with positive FPFFNAs (Patients 1-5) and those with negative FPFFNAs (Patients 6 and 7; Table II).
Figure. Representative histopathological images of amyloid deposits (Patient 4). A: Congo-red staining (heart). B: Amyloid deposits exhibit apple-green birefringence under a polarized light microscope after Congo-red staining (heart). C: The amyloid deposits are positive for anti-TTR115-124 (rabbit polyclonal) (heart). D: Congo-red staining (abdominal fatty tissue). E: The amyloid deposits exhibit apple-green birefringence under a polarized light microscope after Congo-red staining (abdominal fatty tissue). The bar indicates 200 μm.

Table II. Patient Characteristics and Results of the Measurement Variables in Patients with Cardiac Amyloidosis

| Pt. No. | Age, years | Gender (M/F) | NYHA class | HT | ECG rhythm | IVS, mm | PW, mm | LVDD, mm | LVDs, mm | LVEF, % | LAd, mm | Plasma BNP, pg/mL | FPFNA amyloid uptake | Cardiac MRI Type |
|---------|------------|--------------|------------|----|------------|---------|--------|----------|----------|---------|---------|------------------|-------------------|-----------------|
| 1       | 74         | M            | 3          | –  | AF         | 17.0    | 17.2   | 37.7     | 26.7     | 57.0    | 42.0    | 639              | 335               | +               |
| 2       | 81         | M            | 2          | +  | SR         | 17.3    | 16.9   | 40.6     | 28.8     | 55.3    | 44.3    | 374              | 191               | +               |
| 3       | 82         | M            | 2          | +  | JER        | 17.6    | 12.6   | 42.0     | 25.7     | 69.7    | 31.7    | 487              | 184               | +               |
| 4       | 90         | F            | 2          | +  | SR         | 14.0    | 15.6   | 40.8     | 22.4     | 76.8    | 44.6    | 677              | 317               | +               |
| 5       | 69         | M            | 1          | +  | SR         | 15.5    | 16.0   | 37.1     | 31.9     | 44.3    | 42.1    | 95               | 95                | +               |
| 6       | 76         | M            | 2          | –  | SR         | 13.6    | 12.7   | 52.9     | 43.9     | 38.0    | 44.5    | 1,513            | 110               | –               |
| 7       | 69         | M            | 2          | –  | SR         | 18.1    | 12.4   | 46.6     | 33.6     | 54.0    | 45.6    | 240              | 203               | –               |

AF indicates atrial fibrillation; AL, immunoglobulin light chain-related amyloid; ATTR, transthyretin-related amyloid; BNP, brain natriuretic peptide; BNPh, brain natriuretic peptide at the time of hospitalization; BNPe, brain natriuretic peptide after compensated heart failure; ECG, electrocardiogram; FPFNA, fat pad fine-needle aspiration; HT, hypertension; IVS, intraventricular septum; JER, junctional escape rhythm; Lad, left atrial diameter; LGE, late gadolinium enhancement; LVDD (s), left ventricular end-diastolic (end-systolic) diameter; LVEF, left ventricular ejection fraction; NA, not available; NYHA, New York Heart Association; M, male; Pt., patient; PW, posterior wall; SR, sinus rhythm; and wt, wild-type.

Table III. Likelihood of Cardiac Amyloidosis and Positive Rate of FPFNA

| Population          | n   | Likelihood of cardiac amyloidosis, % | Positive rate of abdominal FPFNA, % |
|---------------------|-----|-------------------------------------|------------------------------------|
| All patients        | 76  | 9.2                                 | 6.6                                |
| A: Age ≥ 65 years old | 36  | 19.4                                | 13.9                               |
| B: LV hypertrophy   | 45  | 15.6                                | 11.6                               |
| A or/and B          | 61  | 11.4                                | 8.2                                |
| A and B             | 20  | 35.0                                | 25.0                               |

FPFNA indicates fat pad fine-needle aspiration; and LV, left ventricular.

Discussion

Major findings: The results of this study that included 77 patients with non-ischemic cardiomyopathy demonstrated the following: 1) 9.2% of the patients were definitely diagnosed with cardiac amyloidosis by endomyocardial biopsy; 2) abdominal FPFNA was a simple and safe procedure that could be performed by one person, 3) all 5 patients (6.6%) with positive FPFNA were positive for cardiac amyloidosis; 4) positive FPFNA had 100% specificity and PPV with relatively high sensitivity and NPV for diagnosing cardiac amyloidosis; 5) the factor of elderly patients ≥ 65 years old with LV hypertrophy indicated a high likelihood of having cardiac amyloidosis (35.0%) and high rate of positive FPFNA (25.0%); and 6) the factors of elderly patients ≥ 65 years old or with characteris-
tients with AL amyloidosis.\(^{22}\) ATTRwt amyloid deposits:
Cardiac involvement occurs in as many as 60% of people,\(^{1}\) and ATTRwt amyloidosis is responsible for approximately 15%-30% of heart failure with preserved ejection fraction in elderly patients.\(^{1,23}\) Classically, cardiac amyloidosis is characterized by low QRS voltage and poor R progression, elevation of the BNP and NT-pro-BNP levels, concentric LV hypertrophy, and a granular sparkling appearance.\(^{1}\) However, the clinical spectrum of ATTRwt amyloidosis is highly dependent on the type of amyloid precursor proteins, early recognition and precise typing of the amyloid are indispensable.\(^{13}\)

As the detection rate of amyloid deposits by myocardial biopsy is almost 100%, an endomyocardial biopsy is the gold standard technique to establish the definite diagnosis of cardiac amyloidosis and amyloid typing. However, because histopathological confirmation by endomyocardial biopsy is accompanied with a high cost, risk of serious complications, and requirement for specialist technical expertise and hospital equipment, a delayed diagnosis is still common. Thus, endomyocardial biopsy might not be suitable for the first screening for cardiac amyloidosis, and it might be better to perform a biopsy for detecting amyloid deposits from locations other than the myocardium.

Abdominal FPFNA for diagnosing cardiac amyloidosis: Cardiac involvement occurs in as many as 60% of patients with AL amyloidosis.\(^{20}\) ATTRwt amyloid deposits are often found in the autopsied myocardium of elderly people,\(^{1}\) and ATTRwt amyloidosis is responsible for approximately 15%-30% of heart failure with preserved ejection fraction in elderly patients.\(^{1,23}\) Classically, cardiac amyloidosis is characterized by low QRS voltage and poor R progression, elevation of the BNP and NT-pro-BNP levels, concentric LV hypertrophy, and a granular sparkling appearance.\(^{1}\) However, the clinical spectrum of ATTRwt is heterogeneous and differs from the classic phenotype,\(^{13}\) and cardiac amyloidosis is more prevalent than previously thought in the aging society.\(^{4,5}\) Cardiac involvement is the most significant predictor of poor prognosis in patients with systemic amyloidosis.\(^{16,24}\) To avoid deterioration of the cardiac function and progression of heart failure, it is crucial to delay or prevent the accumulation of amyloid in the heart at an earlier stage of the disease. Recent progress in therapeutic interventions, such as chemotherapy, liver transplantation, and transthyretin (TTR)-modifying therapeutics, could improve the patients’ prognosis.\(^{5}\) As those treatment strategies are highly dependent on the type of amyloid precursor proteins, early recognition and precise typing of the amyloid are indispensable.\(^{13}\)

Table IV. Sensitivity, Specificity, and Predictive Accuracy of the Criteria for Cardiac Amyloidosis

| Criteria                                         | n   | Sensitivity, % | Specificity, % | Positive predictive value, % | Negative predictive value, % | Accuracy, % |
|-------------------------------------------------|-----|----------------|----------------|-----------------------------|-----------------------------|-------------|
| Age ≥ 65 years old                               | 76  | 100            | 58.0           | 19.4                        | 100                         | 61.8        |
| Poor R progression                               | 76  | 85.7           | 72.5           | 24.0                        | 98.0                        | 73.7        |
| LV hypertrophy                                   | 76  | 100            | 42.0           | 14.9                        | 100                         | 47.4        |
| Interventricular septal thickness ≥ 12 mm        | 76  | 100            | 58.0           | 19.4                        | 100                         | 61.8        |
| LV end-diastolic dimension < 55 mm               | 76  | 100            | 43.5           | 15.2                        | 100                         | 48.7        |
| Abdominal FPFNA (+)                              | 76  | 71.4           | 100            | 100                         | 97.2                        | 97.4        |
| PYP scan (uptake+)                               | 33  | 83.3           | 100            | 100                         | 96.7                        | 97.5        |
| Abdominal FPFNA (+) + PYP scan (uptake+)         | 33  | 66.7           | 100            | 100                         | 93.1                        | 93.9        |
| Cardiac MRI (LGE+)                               | 44  | 66.7           | 73.2           | 15.4                        | 96.8                        | 72.7        |

FPFNA indicates fat pad fine-needle aspiration; PYP, pyrophosphate; LV, left ventricular; LGE, late gadolinium enhancement; and MRI, magnetic resonance imaging.

Table V. Sensitivity, Specificity, and Predictive Accuracy of the Criteria for Cardiac Amyloidosis Among Patients with LV Hypertrophy

| Criteria                                         | n   | Sensitivity, % | Specificity, % | Positive predictive value, % | Negative predictive value, % | Accuracy, % |
|-------------------------------------------------|-----|----------------|----------------|-----------------------------|-----------------------------|-------------|
| Age ≥ 65 years old                               | 45  | 100            | 65.8           | 35.0                        | 100                         | 71.1        |
| Poor R progression                               | 45  | 85.7           | 76.3           | 40.0                        | 96.7                        | 77.8        |
| LV end-diastolic dimension < 55 mm               | 45  | 100            | 42.1           | 24.1                        | 100                         | 51.1        |
| Abdominal FPFNA (+)                              | 45  | 71.4           | 100            | 100                         | 95.0                        | 95.6        |
| PYP scan (uptake+)                               | 25  | 83.3           | 100            | 100                         | 95.0                        | 96.0        |
| Abdominal FPFNA (+) + PYP scan (uptake+)         | 25  | 66.7           | 100            | 100                         | 100                         | 92.0        |
| Cardiac MRI (LGE+)                               | 22  | 66.7           | 73.7           | 28.6                        | 93.3                        | 72.7        |

FPFNA indicates fat pad fine-needle aspiration; PYP, pyrophosphate; LV, left ventricular; LGE, late gadolinium enhancement; and MRI, magnetic resonance imaging.
tients with ATTR cardiac amyloidosis from 1990 to 2013. Many patients (131 patients, 46%) underwent endomyocardial biopsy. However, in the study, the clinical spectrum included the classic phenotype: 75 ± 6-year-old, male dominant (93%), LVH, and a low LVEF (mean ejection fraction, 47%). Those three studies included patients with classic cardiac amyloidosis. Our study differed from previous studies in that the rate of endomyocardial biopsies was lower and the study subjects consisted of patients with classic amyloidosis not non-ischemic cardiomyopathy. Recently, a large-scale study demonstrated that abdominal FPFNA had a valid diagnostic sensitivity for cardiac amyloidosis (AL, 84%; ATTR, 45%; ATTRwt, 15%). However, that study included many patients who were diagnosed with cardiac amyloidosis only by bone scintigraphy without performing endomyocardial biopsies. In the present study, we performed both abdominal FPFNA and endomyocardial biopsy in all patients and found that positive FPFNA had 100% specificity and PPV with relatively high sensitivity and NPV for a definitive diagnosis of cardiac amyloidosis. No complications occurred and the FPFNA could be performed in less than 5 minutes by a single person in all patients. Therefore, we believe that FPFNA is the most suitable option for an initial screening test of cardiac amyloidosis to differentiate it from other cardiomyopathies among patients with heterogeneous non-ischemic cardiomyopathies. In recent years, the usefulness of 99mTc-PYP accumulation in the myocardium has been particularly shown. In this study, although the number of patients was limited, the accuracy of 99mTc-PYP accumulation in the myocardium was as high as that of positive FPFNAS.

**Clinical implications:** Several findings obtained from non-invasive diagnostic modalities, such as the electrocardiogram (ECG), echocardiography, nuclear scintigraphy, and MRI, have been reported as characteristic in patients with cardiac amyloidosis. However, nothing is more definitively diagnostic for cardiac amyloidosis by itself than endomyocardial biopsy. In this study, we strictly defined cardiac amyloidosis as the presence of amyloid in the myocardium, and the diagnostic accuracy of the findings was assessed in patients with non-ischemic cardiomyopathy. In this study, all patients with positive FPFNA had cardiac amyloidosis, and no amyloid was detected by abdominal FPFNA in patients without cardiac amyloidosis. Therefore, if the patient have positive for FPFNA, the patients with non-ischemic cardiomyopathy are surely diagnosed with cardiac amyloidosis. As the result, the patients have the advantage of omitting the endocardial biopsy, and the possibility of a prompt treatment. Conversely, if the patient is negative for FPFNA, nuclear imaging, for example 99mTc-PYP, plays an important role in cardiac amyloidosis diagnosis as the next step. If there is PYP uptake in the myocardium, an endomyocardial biopsy is recommended for a definitive diagnosis.

In this study, FPFNA was negative in 2 (28.6%) patients with histologically proven cardiac amyloidosis. In those cases, it might be difficult to definitely differentiate hypertrophic cardiomyopathies from cardiac amyloidosis only with echocardiographic parameters. Nuclear imaging and cardiac MRI may be helpful for diagnosing cardiac amyloidosis. However, patient 6 was negative for FPFNA, 99mTc-PYP accumulation in the myocardium, and late gadolinium enhancement on MRI (Table II). A similar case with cardiac amyloidosis in whom the bone scintigraphy and/or MRI were negative, was reported. Thus, the absence of characteristic findings of non-invasive modalities could not deny the presence of cardiac amyloidosis. Moreover, not all patients with suspicious diagnoses of cardiac amyloidosis have access to facilities where the scintigram can be done. As previously mentioned, the detection rate of amyloid deposits by myocardial biopsy is almost 100%. In those cases like patient 6, endomyocardial biopsy should be performed for a definite diagnosis without hesitation, and the identification of the specific protein causing amyloidosis and starting amyloid type-dependent therapeutic interventions should also be performed.

As previously reported, the patients characteristics, ECG, and echocardiographic findings were useful for diagnosing cardiac amyloidosis. From the results of our study (Tables III, IV, V), if the patient is < 65 years, or if the patient does not have LV hypertrophy, interventricular septal thickness ≥ 12 mm, or LV end-diastolic dimension < 55 mm, there is a high probability that the patient does not have cardiac amyloidosis. Conversely, the presence of echocardiographic findings of LV hypertrophy, interventricular septal thickness ≥ 12 mm, or LV end-diastolic dimension < 55 mm indicates cardiac amyloidosis with a moderate possibility, and FPFNA and/or endomyocardial biopsy are recommended. In particular, elderly patients with LV hypertrophy had a high likelihood of cardiac amyloidosis and high rate of positive FPFNAS. For those patients, there is no reason not to do the FPFNA for the first screening test for cardiac amyloidosis.

We highly recommend FPFNA, and as the first screening test for cardiac amyloidosis. FPFNA could be performed at the bedside during an office visit or the hospital stay for patients with non-ischemic cardiomyopathy. Especially, in patients ≥ 65 years old with ECG and echocardiographic findings suspected for cardiac amyloidosis, patients could benefit from a FPFNA providing a rapid diagnosis, while waiting for PYP scan and evaluation of monoclonal components, allowing for the prompt initiation of disease modifying therapies.

**Study limitations:** This study had several limitations. First, it was a single center study and the sample size was relatively small. Second, we could not demonstrate or assess the LV longitudinal strain assessed by color tissue Doppler and speckle tracking echocardiography because it was measured in a small number of patients. Third, we could not accurately evaluate the utility of 99mTc-PYP scintigraphy because the number of patients was small and limited.

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Disclosure

Conflicts of interest: None.

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