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ORIGINAL ARTICLE

Bone scintigraphy with ^{99m}technetium-hydroxymethylene diphosphonate allows early diagnosis of cardiac involvement in patients with transthyretin-derived systemic amyloidosis

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Abstract

Objective: To assess the usefulness of bone scintigraphy with ^{99m}Technetium-hydroxymethylene diphosphonate (^{99m}Tc-HDP) for the detection of cardiac involvement in a group of patients with ATTR amyloidosis in different phases of disease, to relate the findings to echocardiography, ECG and cardiac biomarkers, and to evaluate different bone scintigraphic techniques and calculation methods for quantification of the cardiac uptake and for correlation with echocardiographic features and cardiac biomarkers.
Methods: Forty-one patients underwent clinical examinations, echocardiography, ECG, measurement of cardiac biomarkers and bone scintigraphy (planar imaging and SPECT-CT) and were subsequently subdivided into three groups: (1) carriers of an amyloidogenic TTR mutation, $n = 11$, (2) proven ATTR amyloidosis without echocardiographically-defined (mean wall thickness >12 mm) cardiac amyloidosis (AC), $n = 19$, and (3) ATTR amyloidosis with echocardiographically-defined cardiac amyloidosis, $n = 11$. Planar and SPECT-CT images were analyzed visually according to a routine scoring system (grade 0–3) and semi-quantitatively by heart-to-whole body (H/WB) and heart-to-skull (H/S) ratio on planar images and by a left ventricle-blood pool ratio on SPECT-CT images.
Results: All patients with ATTR and echocardiographically-defined AC and none of the carriers showed high cardiac uptake on bone scintigraphy. Furthermore, 8 out of 19 patients with ATTR without echocardiographically-defined AC showed high cardiac uptake. Highest correlations were found between H/S ratio on planar bone scintigraphy with troponin T ($r = 0.76$, $p < 0.0001$) and H/WB ratio with left ventricular mass index ($r = 0.73$, $p < 0.0001$).
Conclusions: Bone scintigraphy with ^{99m}Tc-HDP may detect cardiac involvement in patients with ATTR amyloidosis prior to echocardiographic evidence of cardiac involvement. Cardiac uptake on bone scintigraphy correlates with severity of cardiac involvement using echocardiography, ECG and cardiac biomarkers. Visual grading and calculation of H/S ratio on planar imaging are the preferred methods to assess cardiac uptake.

Abbreviations: ^{99m}Tc-DPD: ^{99m}Technetium-3,3-diphosphone-1,2-propanodicarboxylic acid; ^{99m}Tc-HDP: ^{99m}Technetium-hydroxymethylene diphosphonate; ^{123}I-MIBG: ^{123}Iodine-metaiodobenzylguanidine; AC: cardiac amyloidosis; ATTR: amyloid TTR; E/A ratio: ratio of peak flow velocity of left ventricle rapid diastolic filling (E) to peak flow velocity during atrial contraction (A); ECG: electrocardiography; E/e′ ratio: ratio between mitral inflow velocity (E) and average of the lateral and septal e′ values; eGFR: estimated glomerular filtration rate; e′lat: early diastolic velocity of the lateral mitral annulus; E′sep: early diastolic velocity of the septal mitral annulus; H/WB: heart-to-whole body; H/S: heart-to-skull; LV: left ventricle; MBq: megabequerel; MRI: magnetic resonance imaging; NT-proBNP: N-terminal prohormone of brain natriuretic peptide; ROI: region of interest; SPECT/CT: single photon emission computed tomography/computed tomography; TnT: cardiac troponin T; TTR: transthyretin; VOI: volume of interest.

Keywords

Amyloidosis, ATTR, bone scintigraphy, cardiac amyloidosis, cardiac biomarkers, echocardiography

History

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Introduction

Systemic amyloidoses are diseases characterized by deposition throughout the body of insoluble fibrils (amyloid) derived from soluble serum proteins. One of the major systemic types is ATTR amyloidosis that may be hereditary or

age-related (senile) [1]. The hereditary type is caused by autosomal dominantly inherited point mutations (about 100 mutations have been described) of the precursor protein transthyretin (TTR). In the age-related type it is the normal (''wild-type'') TTR that behaves as precursor protein. In both ATTR-types myocardial involvement is frequently seen, characterized by a restrictive cardiomyopathy due to amyloid deposition in both myocardial tissue and atrioventricular conduction system, leading to intractable heart failure, conduction disturbances or fatal arrhythmias.

Diagnosing ATTR amyloidosis and typing with confidence is challenging since patients often present with a wide variety of symptoms. In the hereditary type, as TTR is primarily produced by the liver, liver transplantation is the only effective way to get rid of the mutated protein precursor as treatment for the disease. However, this only applies for patients in which the disease is minimally interfering with organ function, particularly cardiac function. Treatment of the age-related form is restricted to symptom relief with conventional heart failure therapy [2].

Echocardiography, 12-lead electrocardiography (ECG), and magnetic resonance imaging (MRI) are currently considered as the standard for non-invasive diagnosis of cardiac amyloidosis [3–6]. But these techniques are neither suitable for differentiating between amyloid cardiomyopathy and other forms of cardiomyopathy, nor for determining the type of amyloidosis. Furthermore, these techniques have only limited diagnostic utility in the early phases of the disease [1,2,5,7–9].

Bone scintigraphy with radiolabeled diphosphonate was found to be a new diagnostic tool for defining the type of amyloid cardiomyopathy, since high uptake was found in ATTR amyloid cardiomyopathy whereas absent or weak uptake was found in AL (immunoglobulin light chain-derived) amyloid cardiomyopathy [10]. In ATTR amyloidosis, bone scintigraphy was able to reflect the extent of cardiac amyloid deposition [11] and to identify myocardial infiltration by amyloid across a wide spectrum of morphological and functional cardiac involvement, allowing early diagnosis of the disease [12]. Furthermore, myocardial uptake was found to be a prognostic determinant of cardiac outcome in ATTR, either alone or in combination with left ventricular (LV) wall thickness [12].

The aims of this study were (1) to further assess the usefulness of bone scintigraphy with ^{99m}Technetium labeled hydroxymethylene diphosphonate (^{99m}Tc-HDP) for cardiac involvement in a group of patients with ATTR amyloidosis in three different phases of their disease (carriers of the mutation without the disease, ATTR amyloidosis patients without echocardiographic signs of cardiomyopathy, and ATTR amyloidosis patients with echocardiographically-defined cardiomyopathy) and (2) to correlate the cardiac uptake on bone scintigraphy with echocardiographic findings, ECG characteristics, and cardiac biomarkers (N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and cardiac troponin T (TnT)). Furthermore, we performed not only planar bone scintigraphy but also single photon emission computed tomography/computed tomography (SPECT/CT) in a large subset of patients, enabling other methods to quantify the uptake of the diphosphonate tracer in the heart. As third aim of this study, different bone scintigraphic imaging techniques and calculation methods were evaluated for quantification of the cardiac uptake and for correlation with echocardiographic features and cardiac biomarkers.

Methods

Patients

In our referral center for the diagnosis and treatment of amyloidosis, all patients with suspected or known ATTR amyloidosis, either TTR mutation carriers without amyloid or ATTR patients with histologically proven disease activity (hereditary and age-related form, after or without liver transplantation), underwent clinical examination, echocardiography, ECG and quantification of cardiac biomarkers (NT-proBNP and TnT) as part of the usual diagnostic workup to exclude or confirm cardiac amyloidosis. Furthermore, neurological tests (peripheral neuropathy), autonomic function tests (autonomic neuropathy), eye examinations (vitreous involvement), and examination of the kidney function (estimated glomerular filtration rate (eGFR) and evaluation of serum proteins in the urine (proteinuria)) were performed. Between March 2012 and March 2013, these patients were also asked to provide standard informed consent for acquiring bone scintigraphy with ^{99m}Tc-HDP. This research study was approved by the local medical ethics committee of our hospital.

Histology

Systemic amyloidosis was diagnosed by the presence of typical apple-green birefringence in polarized light in a tissue specimen stained with Congo red dye in a biopsy of subcutaneous fat or in at least one involved organ. Typing of ATTR amyloidosis was performed by immunohistology of a biopsy or by a transthyretin ELISA of abdominal fat tissue [13]. Hereditary ATTR was defined by a documented TTR gene mutation at DNA analysis. When there was no TTR mutation found with this analysis, wild-type (age-related form) ATTR was diagnosed.

Echocardiography

The following echocardiographic variables were measured according to standard recommendations and using standard techniques: left ventricle (LV) ejection fraction, septum thickness, LV posterior wall thickness, LV mass index and right ventricle free wall thickness. Using Doppler recording of transmitral flow velocity, the ratio of peak flow velocity of LV rapid diastolic filling (peak E) to peak flow velocity during atrial contraction (peak A) was measured (E/A ratio). Furthermore, the early diastolic velocity of the lateral (e'lat) and septal (e'sep) mitral annulus was measured and the ratio between mitral inflow velocity (E) and average of the lateral and septal e' values (E/e' ratio) was calculated.

Cardiac amyloidosis was defined as a mean left ventricular wall thickness (septum and posterior wall) greater than 12 mm in the absence of hypertension or other potential causes of left ventricular hypertrophy [14]. Other echocardiographic signs suggesting cardiac amyloidosis were: right ventricular free wall thickening in the presence of left ventricular thickening

and in the absence of pulmonary or systemic hypertension, granular sparkling appearance of ventricular myocardium, and pericardial effusion [2,9]. Left ventricular mass index was classified as increased when $>130\,g/m^2$ in men and $>110\,g/m^2$ in women [15], E/A ratio >2 indicated restrictive filling, and E/e' ratio was judged normal when <8, judged as possible increased LV filling pressure between 8 and 15, and judged as increased filling pressure when >15 [16,17].

Other diagnostic definitions

The presence of low voltage on 12-lead electrocardiography, intraventricular conduction delay and anteroseptal pseudo-infarction were considered suggestive for cardiac amyloidosis. Elevation of NT-pro BNP (<75 years: >125 ng/l; >75 years: >450 ng/l) and TnT (>14) may be seen in cardiac amyloidosis. Although elevation of these biomarkers are seen in a wide variety of cardiac disorders, normal values of NT-pro BNP and TnT exclude significant cardiac amyloidosis.

Peripheral neuropathy was defined clinically by a typical symmetric ascending sensorimotor peripheral neuropathy. The presence of carpal tunnel syndrome alone did not constitute peripheral nerve involvement. Autonomic neuropathy was defined by presence of orthostatic hypotension, gastric-emptying disorder, intestinal pseudo-obstruction or bladder dysfunction not related to direct organ infiltration [14]. Eye involvement was defined by infiltration of amyloid deposits in the vitreous, producing characteristic glass-wool-like sheets and globular opacities [18]. Impaired kidney function was defined as an eGFR $<60\,ml/min/1.73\,m^2$ (generic sign of impaired renal function) and/or a 24-h urine protein excretion ≥ 0.5 g/day (a marker of amyloidotic kidney involvement), with other causes of proteinuria excluded.

Bone scintigraphy protocol, image analysis and uptake quantifications

All patients received 700 MBq ^{99m}Tc-HDP intravenously. Planar anterior and posterior whole body scans were obtained 3 h after injection. In a large subset of patients ($n = 36$), a SPECT-CT of the thorax including the heart was also performed. The SPECT images were acquired using a 128×128 matrix, 180° degrees of rotation, 64 views, 15 s per view. All scans were acquired on a SPECT/CT dual head gamma camera system (Siemens Symbia T, Siemens Medical Systems, Knoxville, TN). Visual image analysis was performed in consensus by two experienced nuclear medicine physicians (RS and AG) blinded to all other patient data. Visual scoring of cardiac uptake was scored on planar images using a routine scoring system [12,19,20], see Figure 1: (0) absent cardiac uptake and normal bone uptake, (1) mild cardiac uptake, inferior to bone uptake, (2) moderate cardiac uptake associated with attenuated bone uptake, and (3) high cardiac uptake with decreased or absent bone uptake. Visual scoring of cardiac uptake on SPECT was scored as follows: (0) absent cardiac uptake, (1) mild cardiac uptake, lower than mediastinal background, (2) moderate cardiac uptake higher than mediastinal background but lower than bone uptake, and (3) high cardiac uptake higher than bone uptake.

Semi-quantitative analysis on planar images was performed by calculating the heart-to-whole-body (H/WB) and heart-to-skull (H/S) ratio. For this purpose a geometric mean image was calculated on a pixel by pixel basis using the anterior and posterior image data. Regions of interest (ROIs) were placed over the heart, kidneys, bladder and within the skull on this geometric mean image. If residual activity was detected at the injection site, a ROI was defined on that location as well. This way, the geometric means for each ROI were calculated. The H/WB method was described earlier [12,19,20] with the total image counts corrected for kidney and bladder uptake and residual activity at the injection site considered as whole-body uptake. The H/S ratio was also calculated for each patient as the ratio between heart uptake and uptake in the skull.

Semi-quantitative analysis on SPECT-CT images was performed by drawing volumes of interest (VOIs) over the left ventricle and on the ascending aorta (valve area till aortic arch, defined as blood pool background). The total counts in the VOIs were divided by the volume of the VOIs, leading to a VOI concentration. By dividing the VOI concentration in the left ventricle by the VOI concentration in the bloodpool, the LV/blood pool ratio was calculated.

Statistical analysis

Continuous data are expressed as median (range). Categorical variables are expressed as absolute numbers and percentages. For wide ranging right-skewed quantities (NT-proBNP) values were normalized using logarithmic scales. This log transformation of NT-proBNP was already described to improve the prognostic value in patients with pulmonary arterial hypertension [21]. Analyses between bone scintigraphic results and echocardiography, ECG and cardiac biomarkers were performed with unpaired *t* test (two subgroups). Analyses between different subgroups of patients were performed with one-way ANOVA (three subgroups) and Bonferroni post-test to compare all pairs of subgroups. Analyses of categorical variables were performed with Fisher's exact tests. Correlation analyses were performed by linear regression and calculation of Pearson's correlation coefficient (r). $p < 0.05$ was considered statistically significant. All tests were performed using GraphPad Prism version 5.0 for Windows, GraphPad Software, San Diego, CA.

Results

Patient population

Forty-one patients fulfilled the study criteria and entered the analysis. Eleven of them (27%) were carriers of a TTR gene mutation, 19 patients (46%) were diagnosed histologically with ATTR amyloidosis but had no echocardiographic signs of cardiac involvement, and 11 patients (27%) were diagnosed echocardiographically as ATTR amyloidosis with amyloid cardiomyopathy (mean LV wall thickness >12 mm), six of them proven by endomyocardial biopsies. Table 1 summarizes the main clinical findings in the patient population, divided into these three subgroups. In the whole patient group, half of the patients ($n = 20$, 49%) were diagnosed with a Val30Met

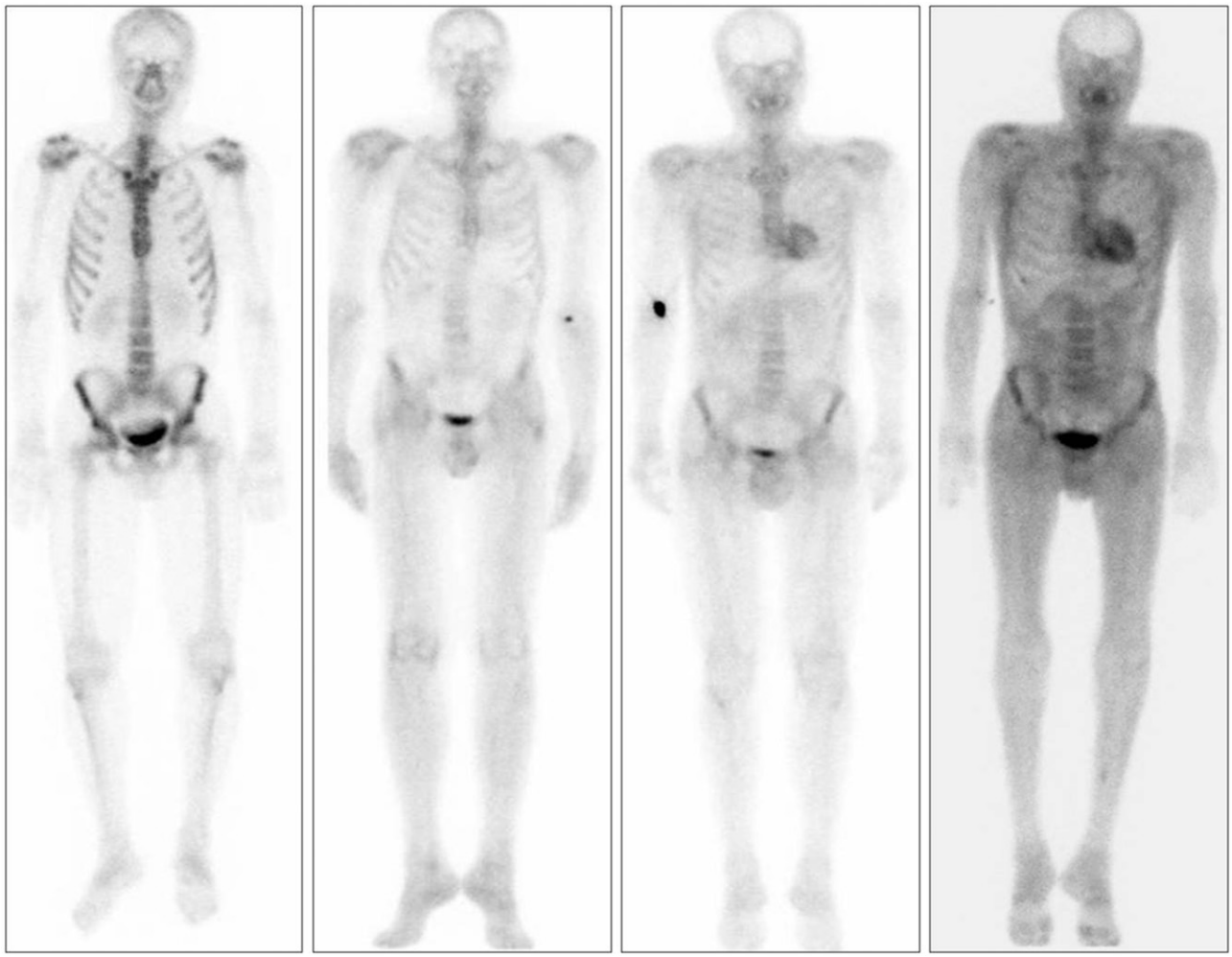

Figure 1. Visual grading scale for cardiac uptake on planar imaging (from left to right: grade 0, 1, 2 and 3).

mutation. Other TTR mutations (non-Val30Met) found were Val71Ala (10 patients), Tyr114Cys (2), Gly47Glu (1), Glu89Lys (1), Val122Ile (1), whereas no mutation was found in six patients (wild-type ATTR). In total, 17 patients underwent liver transplantation, 13 because of diagnosed ATTR amyloidosis, and four for other reasons. Of interest, these last four patients received a donor liver of a patient with ATTR amyloidosis; three patients are considered as carriers without clinical signs of amyloidosis, but one patient with clinical signs has been histologically diagnosed to have systemic ATTR amyloidosis.

Laboratory findings, echocardiography and ECG

Characteristics of cardiac biomarkers, eGFR, echocardiography and ECG are shown in Table 1 for the three groups. In summary, TnT, log NT-proBNP, many echocardiographic features (LV ejection fraction, mean LV wall thickness, septum thickness, LV posterior wall thickness, LV mass index, E/A ratio, E'-lat, E'-sep and E/e' ratio) and ECG features (PR duration, QRS duration and QTc duration) differed significantly between the ATTR group with echocardiographically-defined amyloidotic cardiomyopathy (AC) and each of the two other groups, respectively. Furthermore, NT-proBNP also differed significantly between carriers and ATTR group without echocardiographically-defined AC.

Bone scintigraphy

Visual and semi-quantitative scintigraphic findings, both on planar imaging and with SPECT-CT, are summarized in Table 1. None of the 11 carriers tested positive at visual score analysis on planar images, and only one of 11 carriers showed slightly elevated (grade 1) uptake on SPECT-CT. Among the 19 patients with ATTR without echocardiographically-defined AC, eight patients on planar imaging showed uptake grade 2 or 3. In these eight patients with cardiac uptake, NT-proBNP, TnT, echocardiographic and ECG findings did not significantly differ from the other patients ($n = 11$) in this group without cardiac uptake on the bone scan. All 11 patients with ATTR and echocardiographically-defined AC showed high uptake (grade 2 or 3), both on planar images and on SPECT-CT.

At semi-quantitative analysis, H/WB ratio, H/S ratio and SPECT LV/bloodpool ratio, were significantly higher in patients with ATTR and echocardiographically-defined AC, compared to both carriers and ATTR patients without echocardiographically-defined AC. Concerning the H/S ratio, there was also a significant difference between

Table 1. Patient characteristics, laboratory, echocardiographic, ECG and bone scintigraphy findings.

	Carriers $N=11$	ATTR without AC $N=19$	ATTR with AC $N=11$	p Value
Clinical characteristics				
Age (yrs)	55 (25–76)	53 (32–72)	68 (55–83)	0.006*
Male	5 (45)	11 (58)	9 (82)	0.23
NYHA functional class ≥ 2	1 (9)	5 (26)	10 (91)	0.0001*
Peripheral neuropathy	0	18 (95)	4 (36)	<0.0001**
Autonomic neuropathy	0	6 (32)	2 (18)	0.12
Eye involvement	0	7 (37)	3 (27)	0.06
Disease duration after histological proof (mo)	n.a.	40 (19–208)	19 (1–119)	0.38
TTR mutations				0.0003*
Val30Met	8 (73)	10 (53)	2 (18)	
non-Val30Met	3 (27)	9 (47)	3 (27)	
wild type	0	0	6 (55)	
Liver transplantation	3 (27)	12 (63)	2 (18)	0.06
Laboratory markers				
NT-proBNP plasma level (ng/l)	75 (10–557)	247 (86–797)	2660 (368–9227)	<0.0001***,†
Troponin T plasma level (ng/l)	5 (4–11)	10.5 (4–117)	56 (25–178)	<0.0001*
eGFR (ml/min*1,73^2)	72 (33–111)	76 (46–134)	60 (27–92)	0.08
Echocardiographic findings				
LV ejection fraction (%)	60 (55–60)	60 (52–60)	44 (28–60)	<0.0001*
LV mean wall thickness (mm)	8.8 (7.0–10.5)	9.5 (8.0–12.0)	18.9 (13.9–22.5)	<0.0001*
Septum thickness (mm)	9.0 (8.0–12.0)	10.0 (7.0–14.0)	18.0 (16.0–24.0)	<0.0001*
LV posterior wall thickness (mm)	8.3 (6.0–9.0)	9.6 (7.8–13.0)	16.3 (9.8–26.0)	<0.0001*
Right ventricular wall thickness (mm)	4.0 (3–4.5)	4.0 (3.0–7.0)	9.0 (5.0–12.0)	<0.0001*
LV mass index (g/m^2)	63.5 (62–69)	76.0 (51.0–103.0)	210.5 (167.0–270.0)	<0.0001*
E/A ratio	1.04 (0.70–2.20)	1.06 (0.70–2.50)	3.25 (0.80–14.00)	0.002*
e'-lat (cm/s)	9.6 (5.7–15.5)	10.7 (5.0–17.1)	5.9 (2.5–7.5)	0.0004*
e'-sept (cm/s)	8.6 (5.1–15.5)	9.3 (3.8–12.1)	3.3 (1.9–5.4)	<0.0001*
E/e'	7.2 (4.3–10.7)	7.3 (4.3–15.7)	15.3 (7.5–38.7)	0.0005*
ECG findings				
PR duration (ms)	150 (132–202)	169 (132–215)	202 (138–192)	0.004*
QRS duration (ms)	84 (70–102)	89 (78–152)	118 (78–156)	<0.0001*
QT duration (ms)	392 (348–502)	396 (330–465)	433 (336–514)	0.10
QTc duration (ms)	402 (381–446)	432 (375–509)	467 (437–558)	<0.0001*
Bone scintigraphic findings				
Visual grading score, planar				<0.0001***
0	11 (100)	6 (32)	0 (0)	
1	0 (0)	5 (26)	0 (0)	
2	0 (0)	6 (32)	5 (45)	
3	0 (0)	2 (10)	6 (55)	
Visual grading score, SPECT				<0.0001***
0	10 (91)	7 (41)	0 (0)	
1	1 (9)	4 (23)	0 (0)	
2	0 (0)	3 (18)	4 (50)	
3	0 (0)	3 (18)	4 (50)	
Heart-to-whole-body-ratio, planar	1.48 (1.30–2.07)	1.88 (1.28–2.93)	2.88 (2.04–3.91)	<0.0001*
Heart-to-skull-ratio, planar	1.20 (0.40–2.70)	2.16 (1.17–9.06)	5.43 (2.74–8.18)	0.0002***
LV-to-bloodpool ratio, SPECT	0.70 (0.60–1.00)	1.50 (0.80–5.30)	4.60 (2.60–5.20)	0.0002*

Values are in median (range) or in absolute numbers (percentage).
p Values between the three subgroups calculated by one-way ANOVA with the Bonferroni post-test. p Values of categorical variables calculated with Fisher's exact test. p Values <0.05 were considered significant.
*Statistically significant between ATTR with AC and the other two groups (ATTR without AC and carriers).
** Statistically significant between ATTR without AC and the other two groups (ATTR with AC and carriers).
***Statistically significant between each group and the other groups.
†Log-transformed NT-proBNP.
AC = amyloid cardiomyopathy (defined by echocardiography), NYHA = New York Heart Association, TTR = transthyretin, yrs = years, mo = months, n.a. = not applicable.

carriers and ATTR patients without echocardiographically-defined AC. However, there is overlap between the H/S ratio of patients with and without echocardiographically-defined AC, so for the individual patient it remains difficult to provide conclusions based on the H/S ratio alone. The semi-quantitative scintigraphic findings (H/S ratio) of the carriers, ATTR amyloidosis patients with echocardiographically-defined AC, and ATTR patients without echocardiographically-defined AC are shown in Figure 2.

Scintigraphic correlations with cardiac biomarkers and echocardiographic findings

In the overall population, the highest correlation was found between H/S ratio and TnT ($r=0.76$, $p<0.0001$), followed by the correlation between H/WB ratio and LV mass index ($r=0.73$, $p<0.0001$, SPECT ratio with LV mass index ($r=0.72$, $p=0.0005$), and SPECT ratio with TnT ($r=0.71$, $p<0.0001$) (Figure 3). Interestingly, the correlation between

all semi-quantitative scintigraphic findings was better for LV mass index compared to LV mean wall thickness, till now the standard echocardiographic measurement for AC. Taken all scintigraphic quantifications together, H/S ratio was on average found to correlate best with cardiac biomarkers and echocardiographic findings, with significant correlations between H/S ratio and TnT, log NT-proBNP ($r = 0.63$, $p = 0.0005$), mean wall thickness ($r = 0.68$, $p < 0.0001$) and LV mass index ($r = 0.71$, $p < 0.0001$), see Table 2.

If restricted to only the patients with ATTR amyloidosis, the same correlations were found, with the best correlation between H/S ratio and TnT ($r = 0.71$, $p < 0.0001$). In the subgroup of ATTR amyloidosis patients without echocardiographically-defined AC, a correlation was found between NT-proBNP and cardiac uptake on bone scintigraphy (visual grade on planar imaging, $r = 0.49$, $p = 0.04$).

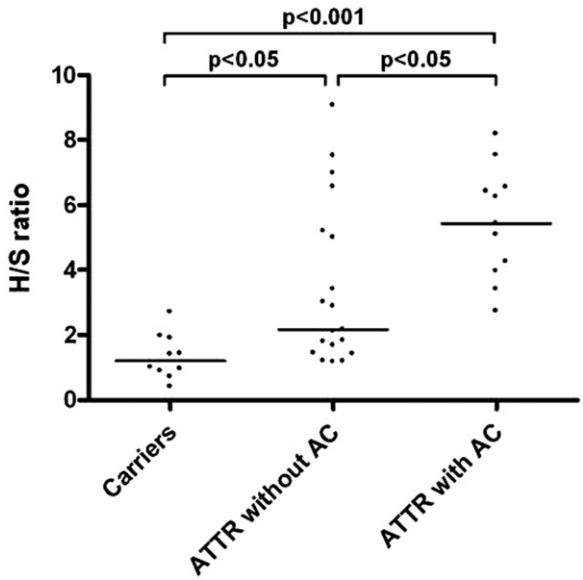

Figure 2. Semi-quantitative scintigraphic measurements (H/S ratio) for different patient subgroups.

Subgroups of ATTR patients

We divided the patients with proven ATTR amyloidosis into three subgroups to search for differences between ATTR patients with Val30Met gene mutation ($n = 12$), ATTR patients with all other gene mutations (non-Val30Met, $n = 12$) and wild-type TTR ($n = 6$). As expected, significantly higher TnT values, thicker LV mean wall thickness and higher LV/bloodpool SPECT were found in patients with wild type TTR. With visual grading score, both on planar images and on SPECT images, most patients with Val30Met mutation had lower scores, whereas most patients with non-Val30Met mutation had scores grade 2 or 3. The H/S ratio seemed to be most appropriate for differentiating Val30Met from non-Val30Met mutation patients. In a scatter plot (Figure 4), it is shown that half of the patients with non-Val30Met mutation (black bullets) had a high H/S ratio (>3) but a normal LV

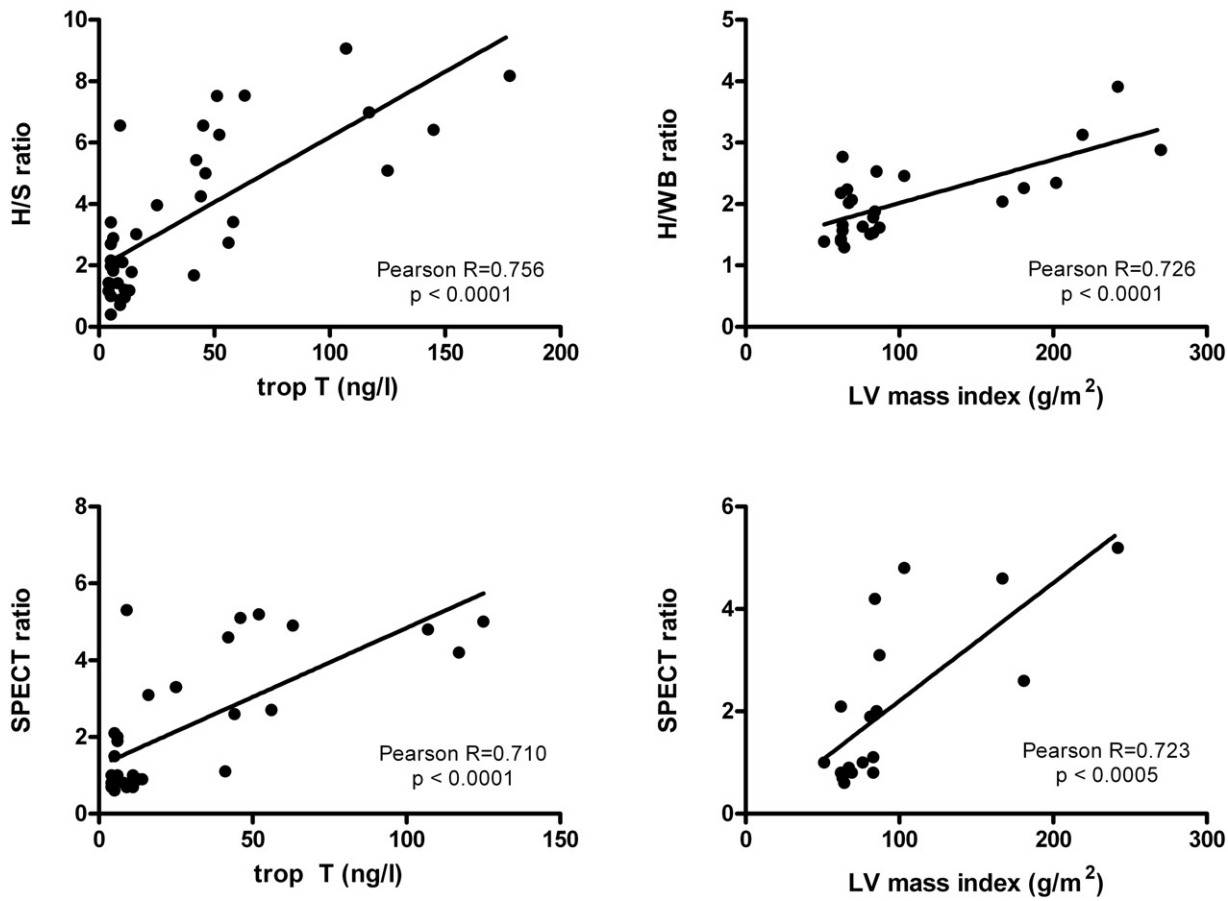

Figure 3. Highest correlations found between semi-quantitative scintigraphic results and laboratory and echocardiographic findings. In all patients planar images were acquired and H/S ratio calculated. SPECT/CT images and SPECT calculations were performed in 32 patients.

mean wall thickness (<12 mm, no echocardiographically-defined AC), whereas only one patient with Val30Met (open bullet) had a high H/S ratio but a normal LV mean wall thickness. This may be an important observation since these patients, defined as having no AC based on echocardiographic findings, do show high uptake on bone scintigraphy and therefore may already have cardiac involvement.

Thirteen patients underwent liver transplantation because of ATTR amyloidosis, the median time between liver transplantation and bone scan was 36 months (range 6–181). Before liver transplantation, the median LV mean wall thickness was 9 mm (range 8–10). At the time of bone scintigraphy, 11 patients were still in the ATTR group without echocardiographically-defined AC with a median LV mean wall thickness of 9.3 mm (range 8–11.5), not different from before. However, five out of these 11 patients showed cardiac uptake on bone scintigraphy (two patients with planar grade 1, two patients with grade 2 and one patient with grade 3) and four of the five had a TTR-non-Met30 mutation. No differences were found in NT-proBNP and TnT in the patients with and without cardiac uptake. Two patients, both with ATTR-non-Met30, developed echocardiographically-defined cardiac involvement in the period between liver transplantation and bone scintigraphy (group ATTR with AC): one patient already one year after transplantation, and the other patient three years after transplantation. At the moment of bone scintigraphy, the LV mean wall thickness of these patients was 20.2 and 18.9 mm, respectively. Both patients had grade 3 uptake on planar bone scintigraphy.

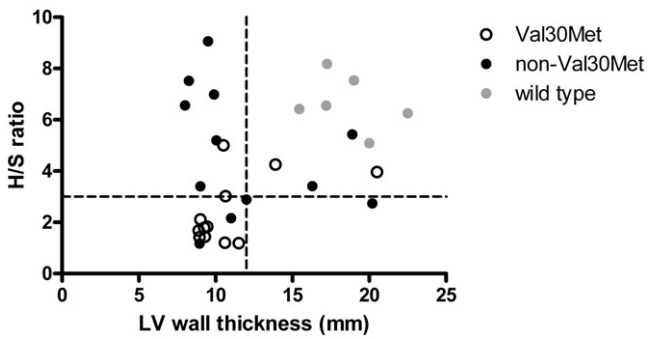

Figure 4. Scatterplot of H/S ratio versus LV mean wall thickness in patients with ATTR amyloidosis subdivided into Val30Met (open bullets), non-Val30Met (black bullets) and wild type TTR (gray bullets) groups.

Discussion

Diagnosing cardiac amyloidosis is a challenge in clinical practice. Histological confirmation obtained by endomyocardial biopsy is the gold standard; however, this is limited to centers with cardiopathology facilities, is an invasive procedure, and harbors a non-negligible risk of perforation and bleeding. Furthermore, typing of amyloid using immunohistochemistry is fraught with errors and more definitive techniques such as mass spectroscopy are not widely available. In patients with known systemic amyloidosis, typical findings on echocardiography and/or ECG might help for this diagnosis. However, these techniques are neither able to differentiate between cardiac amyloidosis and other causes of restrictive cardiomyopathy, nor to differentiate between the etiologic types of amyloidosis.

Cardiac uptake on bone scintigraphy has been found useful to distinguish ATTR type (high uptake) from AL type (no or weak cardiac uptake) and to allow early diagnosis of the disease, even before abnormalities can be found with echocardiography [10–12]. Cardiac uptake on bone scintigraphy (planar visual grade >0) reflects the extent of amyloid infiltration histologically confirmed by endomyocardial biopsy [12].

Our results strengthen earlier findings [11,12]. Correlations were found between cardiac uptake on bone scintigraphy and findings on echocardiography and laboratory parameters. Highest correlations were found between cardiac uptake and TnT and between cardiac uptake and LV mass index. LV mass index correlated better with cardiac uptake than LV mean wall thickness. This is good to notice, since LV mean wall thickness >12 mm is the current criterion to define cardiac involvement in amyloidosis [14]. If confirmed in other studies, this finding indicates that LV mass index may be a better criterion for cardiac involvement in amyloidosis than LV mean wall thickness.

As expected, this study showed various parameters to differentiate ATTR patients with echocardiographically-defined AC from carriers and from ATTR patients without echocardiographically-defined AC (see Table 1). An interesting finding, however, is that NT-proBNP also differentiated carriers from ATTR patients without echocardiographically-defined AC. This is really worthwhile to be explored by additional analyses. For example, one could follow the patients longitudinally in time from being carrier to full blown AC and evaluate which of the three (i.e. cardiac biomarkers, echocardiography or bone

Table 2. Correlations between different bone scintigraphic calculations techniques and most important echocardiographic features and cardiac biomarkers.

	Mean wall thickness (mm)		LV mass index (g/m^2)		NT-proBNP (ng/l)*		Troponin T (ng/l)	
	Pearson *r*	*p* Value	Pearson *r*	*p* Value	Pearson *r*	*p* Value	Pearson *r*	*p* Value
Planar ratios								
Heart-to-skull	0.68	<0.0001	0.71	<0.0001	0.63	0.0005	0.76	<0.0001
Heart-to-whole-body	0.57	0.003	0.73	<0.0001	0.60	0.01	0.56	0.0002
SPECT ratio								
LV-blood pool	0.59	0.0006	0.72	<0.0005	0.57	0.0006	0.71	<0.0001

*Log-transformed NT-proBNP.
Correlation analyses expressed as Pearson's correlation coefficient (*r*).
$p<0.05$ was considered statistically significant.

scintigraphy) will be the most sensitive to reflect the onset of cardiac involvement.

In the already mentioned studies, only planar bone scintigraphy was performed but with dual time point imaging (5 min and 3 h after injection). Heart tracer retention, heart/whole body retention and visual cardiac score were found to correlate with echocardiographically-defined AC. In these studies, also H/WB ratio was determined and found higher in patients with cardiomyopathy. In our opinion, H/WB ratio is not the best calculation method on planar images, since whole body counts, although corrected for renal and bladder uptake, may potentially be confounded by high uptake of the isotope in bones due to other osteoblastic bone diseases (arthritis, osteoarthritis, recent fractures, etc). Calculating the H/S ratio may be a better method, since uptake within the skull is rather stable among patients. Since SPECT/CT was also performed in most of our patients, SPECT was visually graded and the uptake on SPECT was calculated by determining the ratio between LV uptake and uptake in the ascending aorta (LV-blood pool ratio). Both planar and SPECT visual grade and H/S ratio on planar imaging were found to differ between carriers and ATTR patients without echocardiographically-defined AC, between carriers and ATTR patients with echocardiographically-defined AC and between ATTR patients without and with echocardiographically-defined AC. H/S ratio was found to correlate better than H/WB ratio and LV/blood pool ratio on SPECT imaging. As a consequence of these results and the correlation of cardiac uptake on the bone scan with echocardiographic features and cardiac biomarkers, visual analysis of and calculation of H/S ratio at planar images is in our opinion preferred in these patient groups. SPECT/CT is not necessary to perform since it showed no advantages in visual and semiquantitative analysis over planar imaging, whereas it has some disadvantages compared to planar imaging (extension of the image acquisition time and radiation dose of the CT). Furthermore, there is nearly always homogenous increased uptake in the whole left ventricle, as can already be seen on planar imaging. It is not necessary to perform SPECT/CT to define the extent of amyloid infiltration in various segments of the myocardium, since AC is nearly always a diffuse disease of the myocardium.

In the patient group with ATTR amyloidosis without echocardiographically-defined AC (as defined by LV mean wall thickness), 13 of the 19 patients (68%) showed cardiac uptake on bone scintigraphy (five patients with planar visual grade scale 1, six patients with grade 2, and two patients with grade 3). In this group, a linear correlation was found between cardiac uptake and NT-proBNP. Bone scintigraphy could therefore be an early marker of cardiac involvement in this patient group. This is supported by the finding that all patients with echocardiographically-defined AC showed high uptake (grade 2 and 3 on visual grading scale) on bone scintigraphy, whereas none of the carriers did.

Patients with ATTR and echocardiographically-defined AC caused by non-Val30Met mutations tend to have a worse prognosis compared to patients with a Val30Met mutation [22,23]. An important finding was that half of the patients with non-Val30Met mutations had high cardiac uptake on the bone scan (H/S ratio >3), whereas other findings were not clearly indicating AC. A similar finding was found in ATTR patients after liver transplantation, with five out of 11 patients showing cardiac uptake without other signs of AC. We agree with other authors that it is reasonable to hypothesize that the severity of cardiac uptake on bone scintigraphy could be a prognostic determinant of cardiac events [12]. Patients with non-Val30Met mutation have a worse prognosis, and significant organ involvement precludes approval of liver transplantation (for the heart based on echocardiography). One may argue that if patients with non-Val30Met mutation show cardiac uptake on bone scintigraphy, liver transplantation should be seriously reconsidered or even combined liver and heart transplantation should be considered since cardiac infiltration is already present and may progress in spite of liver transplantation.

This study has some limitations. Although the number of patients in this study is high compared to the low incidence of this rare disease, it is still a relatively small population, restricting the statistical power of the analyses. Analysis was performed transversally. To strengthen the results, prospective and longitudinal studies are necessary. We performed many statistical tests on the data which may increase the risk of some false positive correlations due to the small number of patients and the high number of statistical tests. The data presented in Table 1 could be influenced by the six patients with wild-type ATTR amyloidosis. These patients have by definition cardiomyopathy with enlarged hearts and impaired heart function and this may have impact on the comparison with the other patient subgroups. Endomyocardial biopsies were not performed in all patients in order to prove AC in patients with high uptake on bone scintigraphy. This had already been proven in another study [12]; furthermore, endomyocardial biopsy may lead to complications as stated above.

Other studies in this field were performed with ^{99m}Tc-3,3-diphosphone-1,2-propanodicarboxylic acid (^{99m}Tc-DPD). Earlier studies did not show any significant differences in diagnostic accuracy among the different bone seeking agents used in bone scintigraphy [24,25]. However, the imaging agent that was used in this study (^{99m}Tc-hydroxymethylene diphosphonate) was not described earlier in this patient population. Since the results of this study may be confounded by subgrouping of patients that may bias results in favor of the technique, further studies in larger populations are necessary to confirm its utility in these patient groups. Several other nuclear tracers have been investigated for imaging of AC, however with little results. ^{99m}Tc-pyrophospate uptake was found higher in subjects with ATTR cardiac amyloid compared to a cohort of patients with AL amyloidosis [26]. Some clinical evidence was found for the use of ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) in which myocardial defects in MIBG activity correlates with impaired cardiac sympathetic nerve endings due to amyloid deposits [1]. MIBG heart-to-mediastinum ratio was found lower and wash-out higher in patients with echocardiographic signs of amyloidosis and may even detect cardiac denervation in ATTR patients before signs of AC were evident on echocardiography [1,27]. A recently published study in two patients with ATTR amyloidosis showed that both bone scintigraphy and MIBG scintigraphy seem to be able to detect cardiac involvement before echocardiographic parameters are present [28].

The exact mechanism of diphosphonate binding to amyloid is still not completely elucidated. It may be explained by the high calcium concentration in amyloid tissue [29,30]. The presence of calcium in amyloid may be due to the non-fibrillar glycoprotein amyloid P component [29]. The finding that uptake is almost exclusively seen in ATTR patients might be explained by a different composition of amyloid fibrils, different affinities of diphosphonate for amyloid proteins and differences in tissue involvement [11]. *In vitro* analysis provided evidence that TTR binds to calcium [31].

Conclusions

The results of this study confirm that bone scintigraphy identifies cardiac involvement in patients with ATTR amyloidosis. Bone scintigraphy with ^{99m}Tc-HDP may detect cardiac involvement in patients with ATTR amyloidosis prior to echocardiographic evidence of cardiac involvement. Cardiac uptake on bone scintigraphy correlates better with LV mass index than with LV mean wall thickness.

Visual analysis of and calculation of H/S ratio at planar images is the preferred method for interpretation of the scintigraphic results in these patient groups.

Declaration of interest

The authors declare that they have no conflict of interest.

This manuscript has not been submitted or is not simultaneously being submitted elsewhere; no portion of the data has been or will be published in proceedings or transactions of meetings or symposium volumes. The authors declare that they have no financial support or other benefits from commercial sources for the work reported in this manuscript, or other financial interests which could create a potential conflict of interest or the appearance of a conflict of interest with regard to the work.

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