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Role of ^{99m}Tc-DPD Scintigraphy in Diagnosis and Prognosis of Hereditary Transthyretin-Related Cardiac Amyloidosis

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OBJECTIVES In a cohort of patients with hereditary transthyretin-related amyloidosis (ATTR), we aimed to assess the role of ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) in detecting myocardial amyloid infiltration across a wide spectrum of cardiac involvement and in predicting major adverse cardiac events (MACE).

BACKGROUND Hereditary transthyretin-related amyloidosis is a challenging and underdiagnosed condition where both early diagnosis and prognosis remain problematic.

METHODS We evaluated 63 patients with ATTR: 40 with and 23 without echocardiographically diagnosed amyloidotic cardiomyopathy (AC). Myocardial uptake of ^{99m}Tc-DPD scintigraphy was semiquantitatively and visually assessed at 5 min and 3 h.

RESULTS All patients with AC showed moderate-to-severe myocardial tracer uptake (i.e., visual score $\geq$2). Within the subgroup without AC, only 4 patients (with Ala36Pro, Gly47Ala, Thr49Ala, and Glu89Gln transthyretin mutations) showed myocardial tracer uptake and abnormal heart/whole body retention (H/WB) values: in all these cases endomyocardial biopsies showed amyloidotic infiltration. The H/WB was positively correlated with left ventricular (LV) mean wall thickness (Pearson's $r = 0.695$, $p < 0.001$) and negatively with LV ejection fraction ($r = -0.368$, $p = 0.004$). The H/WB was an unfavorable predictor of MACE-free survival at Cox univariate analysis and contributed to the multivariate model. Notably, LV wall thickness >12 mm in combination with H/WB >7.5 was associated with the highest event rate.

CONCLUSIONS In ATTR, ^{99m}Tc-DPD scintigraphy can identify myocardial infiltration across a wide spectrum of morphologic/functional cardiac involvement, allowing an early diagnosis of the disease (even before the appearance of echocardiographic abnormalities). The ^{99m}Tc-DPD myocardial uptake is a prognostic determinant of "cardiac" outcome in ATTR, either alone or in combination with LV wall thickness. (J Am Coll Cardiol Img 2011;4:659–70) © 2011 by the American College of Cardiology Foundation

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Hereditary transthyretin-related amyloidosis (ATTR) is the most frequent form of familial systemic amyloidoses, a group of severe diseases with neurological and polyvisceral involvement (1–3). Hereditary transthyretin-related amyloidosis is challenging and widely underdiagnosed, because of an extreme phenotypic variability that ranges from almost exclusive neurologic involvement to cases with a strictly cardiologic presentation (1–3). Such heterogeneity is mainly linked to specific transthyretin (TTR) mutations, geographic distribution, endemic/nonendemic type of aggregation (1–5). Echocardiography and electrocardiography (ECG) have an established role in the diagnosis of overt forms of amyloidotic cardiomyopathy (AC) (4–7). Unfortunately, none of them gives information with regard to the etiologic type of amyloidosis; furthermore, their diagnostic role in the early phases or in the mild forms of the disease is limited by a low specificity (4,5,7,8).

ABBREVIATIONS AND ACRONYMS

^{99m}Tc-DPD = ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid

AC = amyloidotic cardiomyopathy

AL = immunoglobulin light-chain

ATTR = hereditary transthyretin-related amyloidosis

ECG = electrocardiogram/electrocardiographic

EMB = endomyocardial biopsy

H/WB = heart/whole body retention

LV = left ventricle/ventricular

MACE = major adverse cardiac events

ROI = region of interest

TTR = transthyretin

^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) scintigraphy has proved to be useful in differential diagnosis between TTR (both mutant and wild-type) and immunoglobulin light-chain (AL) etiologies in patients with echocardiographically defined AC (9–11): ^{99m}Tc-DPD uptake is absent in unaffected control subjects, strong in TTR-related AC, and absent or weak in AL-derived AC (10,11). However, apart from the contribution in tissue diagnosis, the diagnostic possibilities of ^{99m}Tc-DPD in ATTR have not yet been fully assessed. It also remains to be determined whether ^{99m}Tc-DPD myocardial uptake has major prognostic implications on cardiac events.

The present study was designed to evaluate the diagnostic and prognostic contribution of ^{99m}Tc-DPD in ATTR across a wide spectrum of morphologic and functional cardiac involvement, including early stages of the disease.

METHODS

Setting and study design. We enrolled all patients diagnosed with ATTR who presented to our center from March 2004 to March 2010. In our referral center for the diagnosis and treatment of cardiomyopathies, clinical examination, echocardiogram, ECG, and ^{99m}Tc-DPD scintigraphy are part of the usual diagnostic workup of patients with suspected or confirmed cardiac amyloidosis. Each patient was required to provide standard informed consent for the execution of ^{99m}Tc-DPD scintigraphy. Cardiac catheterization and endomyocardial biopsy (EMB) were performed in selected cases and in all potential candidates for liver or combined liver/heart transplantation.

All patients provided informed consent for anonymous publication of scientific data. Formal ethical approval was not applicable for this retrospective study involving only routinely performed procedures.

Our study included a cross-sectional analysis of baseline clinical and instrumental profiles and a longitudinal analysis of ATTR patients to assess the role of myocardial ^{99m}Tc-DPD uptake in predicting major adverse cardiovascular events (MACE).

Diagnostic definitions. Diagnosis of systemic amyloidosis was based on histological documentation of Congo-red staining and apple-green birefringence under cross-polarized light in at least 1 involved organ (12,13). The AC was echocardiographically defined as end-diastolic thickness of the interventricular septum >1.2 cm (in the absence of any other plausible cause of ventricular hypertrophy) (4,14). Other echocardiographic signs suggesting cardiac amyloidosis were: granular sparkling appearance of ventricular myocardium, increased thickness of right ventricular free wall, atrioventricular valves or interatrial septum, and pericardial effusion (4–6). Diagnosis of ATTR was defined by a documented TTR mutation at deoxyribonucleic acid analysis (15). Renal insufficiency was defined as glomerular filtration rate <60 ml/min (14). The assessment of peripheral nervous system involvement was based on characteristic neurologic signs and symptoms (typical symmetric ascending sensorimotor peripheral neuropathy) (14). Autonomic impairment was defined by presence of orthostatic hypotension, gastric-emptying disorder, pseudoobstruction, and voiding dysfunction not related to direct organ infiltration (14).

Instrumental definitions. The ECG and echocardiographic measures were based on standard definitions (16,17). Left ventricular (LV) mass was calculated according to Devereux and was classified as increased when >130 g/m^2 in men and >110 g/m^2 in women (18). The LV restrictive filling pattern was defined in terms of E-wave deceleration time <150 ms accompanied by E/A-wave ratio >2.5 at pulsed Doppler echocardiography (19).

Histology and immunohistochemistry. Histological documentation of amyloid deposition was obtained either in subcutaneous adipose tissue from abdominal fat or from EMB. All myocardial samples (5 per patient) were microwave fixed/processed, and multiple 2-μm sections were tested for presence of amyloid by Congo-red staining and apple-green birefringence under cross-polarized light microscopy. The amount of amyloid infiltration at EMB was semiquantitatively classified as: mild (<30% of the analyzed tissue specimen), moderate (30% to 60% of the analyzed tissue specimen), or severe (more than 60% of the analyzed tissue specimen). Immunohistochemical analysis was performed by the labeled Streptavidin-Biotin LSAB method with an antibody against TTR (R.P. Linke, Max Planck Institute of Biochemistry, Martinsried, Germany), or by immunoelectron microscopy with specific antibodies proteins (DAKO, Ely, United Kingdom).

Scintigraphy protocol and image analysis. Patients received 740 MBq of ^{99m}Tc-DPD intravenously; a dual-head camera (ECAM, Siemens Medical Systems, Hoffman Estates, Illinois) equipped with low-energy, high-resolution collimators was used. Whole body scans were obtained 5 min (early) and 3 h (late) after injection. In patients showing cardiac uptake, a myocardial single-photon emission computed tomography study was acquired after the late whole-body scan. Image analysis was independently performed by experienced analysts unaware of all patient data. Visual scoring of cardiac retention was routine: score 0, absent cardiac uptake and normal bone uptake; score 1, mild cardiac uptake, inferior to bone uptake; score 2, moderate cardiac uptake associated with attenuated bone uptake; or score 3, strong cardiac uptake with mild/absent bone uptake. Semiquantitative analysis of heart retention, whole-body retention, and heart/whole body ratio (H/WB) were evaluated from region-of-interest (ROI) drawings in the standard manner. In brief, rectangular ROIs were drawn over the heart, and irregular ROIs were drawn over the kidneys and bladder on anterior images. These ROIs were copied and mirrored on posterior images, and geometric means of the 2 projections were calculated for each ROI. All ROIs were corrected for background counts. Total counts in the images were considered as whole-body counts. Early whole-body counts were used to represent the injected activity. Whole-body retention was evaluated by comparing counts in the late images (corrected for decay and scan speed, and subtracting the activity in the urinary tract and bladder) with the counts in the early whole-body images. Heart retention was evaluated by comparing decay-corrected counts of the heart in late images with counts in early whole-body images. The H/WB ratios were calculated by dividing counts in the heart by counts in late whole-body images.

Follow-up. Planning of follow-up visits was based on clinical needs (or 6 monthly). Data collection was closed in September 2010; for patients who had not attended a visit in the previous 6 months, vital status was ascertained by telephone and/or by contacting referring physicians.

We defined MACE as the occurrence of death from cardiovascular causes (due to either fatal arrhythmias or severe worsening heart failure), hospital stay due to congestive heart failure, complete atrioventricular block, atrial fibrillation/flutter, myocardial infarction, or stroke. Each prognostic outcome was assessed by contacting the patients, the family members, or the referring physician.

Statistical analysis. Summary statistics were expressed as median [interquartile range] or frequencies (percentages). Independence of categorical variables was tested using Fisher exact test or Pearson chi-square test. Continuous variables were analyzed using Mann-Whitney *U* test. Pearson correlation coefficient (r) was used to study the possible correlation of H/WB with mean LV wall thickness and LV ejection fraction. MACE-free survival was analyzed with Kaplan-Meier curves. Patients were censored at time of solid organ transplantation. To explore possible associations with MACE-free survival, univariate Cox regression analysis was initially performed with clinical, echocardiographic, ECG, and scintigraphic variables. Multivariate analysis was then performed by entering into the model the variables that were significant ($p < 0.05$) on univariate analysis but unrelated to each other. A formal search for colinearity (with the variance inflation factor test) and interactions (with the test of proportional hazard assumption) was performed to guide the construction of the final statistical model. Analyses were conducted with STATA (version 11.1 SE, Stata Corporation, College Station, Texas). Values of $p < 0.05$ were considered significant.

RESULTS

Study population. A total of 63 patients with ATTR fulfilled the study protocol and entered the analysis. Twenty-eight of these patients were in-

Table 1. Clinical and Genetic Characteristics of Patients With ATTR According to the Presence of Echocardiographically Defined AC

	Overall Population (n = 63)	Patients With AC (n = 40)	Patients Without AC (n = 23)	p Value
Age, yrs	53 (41–66)	57 (48–67)	39 (33–57)	<0.001
Male	39 (62)	31 (78)	8 (35)	0.002
NYHA functional class >II	10 (16)	10 (25)	0 (0)	0.024
Neurologic/autonomic involvement	53 (84)	30 (75)	23 (100)	0.024
Carpal tunnel syndrome	17 (27)	14 (35)	3 (14)	0.111
TTR mutations				0.234
Val30Met	11 (17)	4 (10)	7 (30)	
Ile68Leu	18 (29)	14 (35)	4 (17)	
Thr49Ala	9 (14)	5 (12)	4 (17)	
Glu89Gln	10 (16)	7 (18)	3 (13)	
Other	15 (24)	10 (25)	5 (23)	
Referral route				<0.001
Cardiology	21 (33)	21 (53)	0 (0)	
Neurology	27 (43)	18 (45)	9 (39)	
Family screening	15 (24)	1 (2)	14 (61)	
Disease duration, months	21 (3–53)	30 (7–63)	7 (0–38)	0.050
Glomerular filtration rate, ml/min	77 (64–94)	74 (58–92)	82 (73–99)	0.125

Values are n (%) or median (interquartile range).
AC = amyloidotic cardiomyopathy; ATTR = hereditary transthyretin-related amyloidosis; NYHA = New York Heart Association; TTR = transthyretin.

cluded in a previous study (11) analyzing the role of ^{99m}Tc-DPD scintigraphy in the differential diagnosis between AL and TTR-related AC. Fourteen patients with ATTR evaluated during the same period were excluded from this study due to lack of informed consent or to a severe global clinical impairment (that was judged incompatible with the execution of scintigraphy).

Distribution of TTR mutations was as follows: Ile68Leu (n = 18), Val30Met (n = 11), Glu89Gln (n = 10), Thr49Ala (n = 9), Phe64Leu (n = 3), Ser23Asn (n = 2), Ala36Pro (n = 2), Arg34Thr

Table 2. ECG and Echocardiographic Characteristics of Patients With ATTR According to the Presence of Echocardiographically Defined AC

	Overall Population (n = 63)	Patients With AC (n = 40)	Patients Without AC (n = 23)	p Value
Abnormal ECG	49 (78)	40 (100)	5 (22)	<0.001
Low QRS voltages	13/60 (22)	11/38 (29)	0 (0)	0.140
Atrial fibrillation	4/62 (7)	4 (10)	0 (0)	0.293
Pacemaker	3/62 (5)	3 (7)	0 (0)	0.545
Prolonged P-R interval	12/60 (20%)	10/39 (26)	2/21 (10)	0.185
Left bundle branch block	1/60 (2)	1/38 (3)	0/22 (0)	>0.999
Right bundle branch block	7/60 (12)	6/38 (16)	1/22 (5)	0.408
Left anterior hemiblock	22/60 (37)	19/38 (50)	2/22 (9)	0.003
Repolarization abnormalities	20/60 (33)	19/38 (50)	1/22 (5)	<0.001
Indexed LV mass, g/m^2	161 (118–231)	194 (159–256)	106 (89–119)	<0.001
Indexed LV mass/volume ratio	2.8 (2.1–4.4)	3.9 (2.7–4.9)	1.8 (1.5–2.3)	<0.001
Interventricular septal thickness, mm	14 (11–18)	16 (14–20)	10 (9–11)	<0.001
LV mean wall thickness, mm	14 (11–18)	16 (13–19)	10 (9–11)	<0.001
LVEF, %	61 (50–68)	53 (48–65)	66 (61–73)	<0.001
LVEF <50%	16 (25)	15 (38)	0/23 (0)	0.002
E-wave deceleration time, ms	170 (142–201)	160 (135–215)	190 (160–200)	0.296
Restrictive LV filling pattern	19 (30)	17 (43)	0 (0)	<0.001
Mild pericardial effusion	30/61 (49)	28 (70)	0 (0)	<0.001

Values are n (%) or median (interquartile range).
ECG = electrocardiographic; LV = left ventricular; LVEF = left ventricular ejection fraction; other abbreviations as in Table 1.

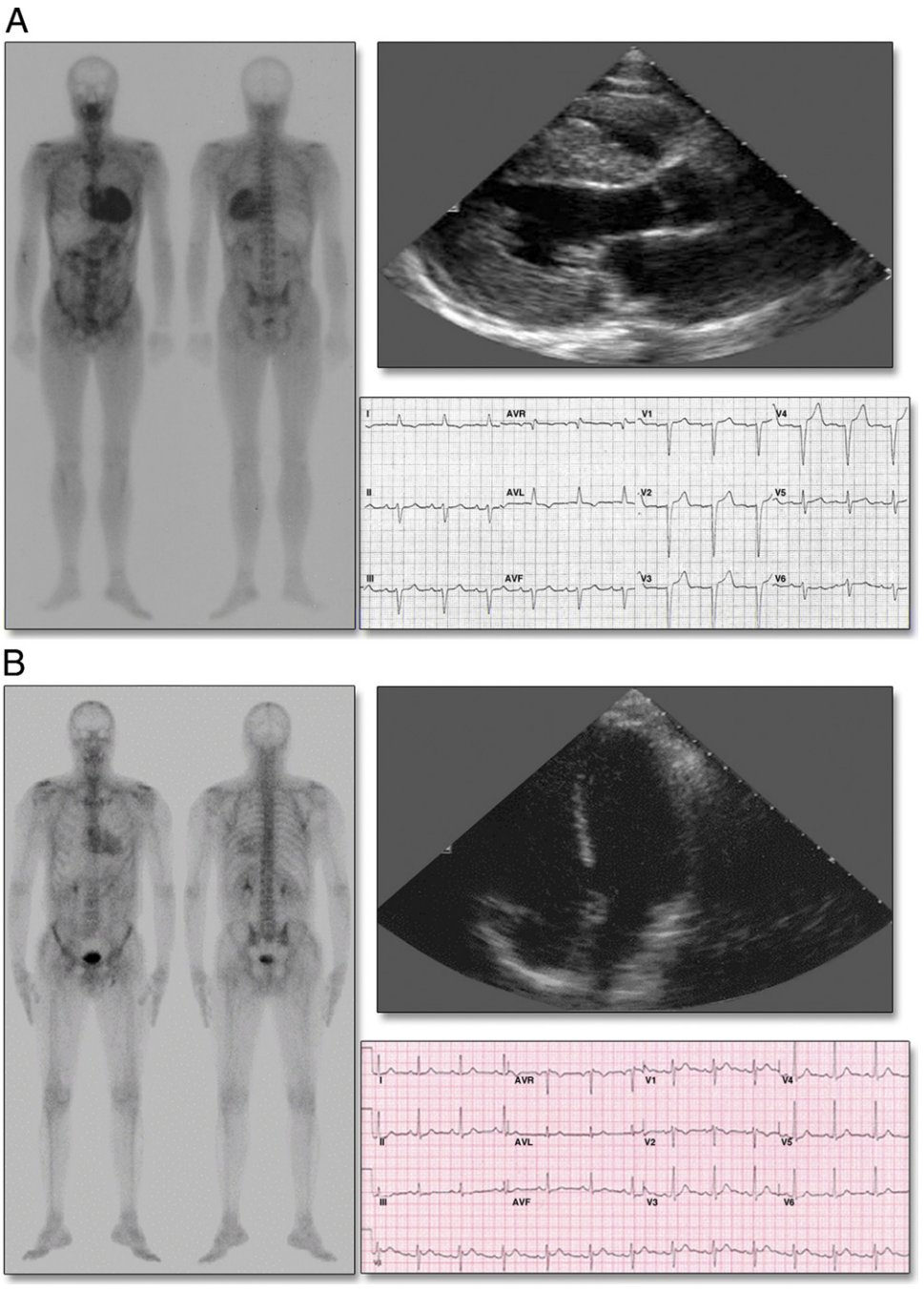

Figure 1. Scintigraphic, Echocardiographic, and ECG Findings in ATTR With and Without Cardiomyopathy

The patient with echocardiographically defined cardiomyopathy **(A)** shows strong myocardial ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid uptake and very attenuated bone uptake. The patient with normal echocardiographic and electrocardiographic assessment **(B)**, carrying a non-Val30Met TTR mutation, shows a mild myocardial tracer uptake. ATTR = hereditary transthyretin-related amyloidosis; ECG = electrocardiographic; TTR = transthyretin.

(n = 2), Phe33Val (n = 1), His88Arg (n = 1), Thr59Lys (n = 1), Ser50Arg (n = 1), Gly47Ala (n = 1), and Val14Leu (n = 1), a novel TTR mutation not previously described.

At baseline, 40 patients (63%) fulfilled the echocardiographic diagnostic criteria of AC (14), whereas 23 (37%) patients did not. In particular, among patients without echocardiographically de-

Table 3. Scintigraphic Findings in the Overall Population and According to the Presence of an Echocardiographically Diagnosed AC

	Overall Population (n = 63)	Patients With AC (n = 40)	Patients Without AC (n = 23)	p Value
Whole-body tracer retention (%)				<0.0001
Median	62.4	65.6	41.5	
IQR	47.7–68.5	57.9–74.0	37.8–57.8	
Heart tracer retention (%)				<0.0001
Median	5.3	7.0	1.5	
IQR	2.3–7.8	5.2–7.9	0.9–3.2	
Heart/whole body retention ratio				<0.0001
Median	7.5	10.0	3.1	
IQR	4.1–10.6	7.4–11.3	2.0–5.0	
Visual cardiac score				<0.0001
0		0 (0)	19 (81)	
1		0 (0)	1 (5)	
2		14 (35)	2 (9)	
3		26 (65)	1 (5)	

IQR = interquartile range; other abbreviations as in Table 1.

fined cardiomyopathy, echocardiogram was normal in 16 (69%) patients, whereas in 7 cases (31%) some echocardiographic abnormalities were detectable: interventricular septal thickness = 12 mm in 3 cases (13%), and increased interatrial septum thickness (>5 mm) in 2 cases (9%). Tables 1 and 2 summarize the main clinical and instrumental (ECG/echocardiographic) profiles of the patients. Patients with AC were older than those without, with a higher prevalence of male sex and a higher prevalence of TTR Ile68Leu mutation. The most represented mutation in patients without AC was the Val30Met. Only 43% of patients received the (final) diagnosis of ATTR due to neurologic manifestations; the diagnostic route was cardiologic in 33% and related to family screening in 24%. Of note, approximately 40% of cases without AC showed ECG abnormalities (vs. 100% in the other group, $p < 0.001$), most frequently consisting in nonspecific repolarization abnormalities, first degree atrioventricular block or QRS axis deviation.

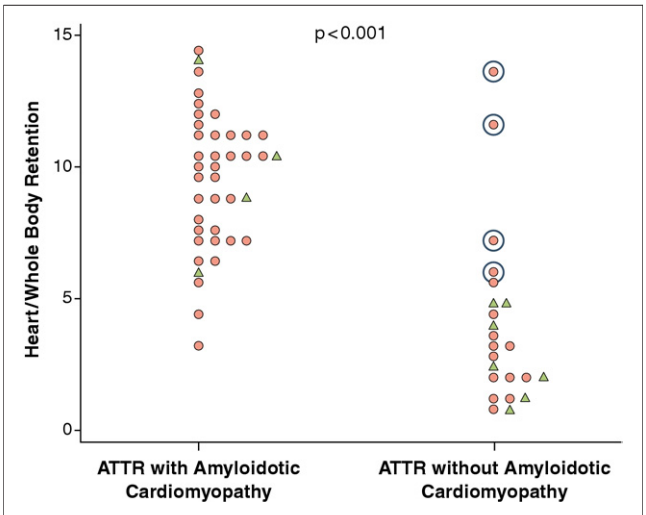

Figure 2. Comparison of ^{99m}Tc-DPD Heart/Whole Body Retention Between ATTR Patients With and Without AC

Patients with **(on the left)** and without **(on the right)** amyloidotic cardiomyopathy (AC). The **triangles** represent patients with Val30Met transthyretin (TTR) mutation; the **dots** represent patients with other than Val30Met TTR mutations. The **circles** surrounding the **dots** in the **upper right** indicate the 4 patients with other than Val30Met TTR mutation without cardiomyopathy but with scintigraphic myocardial tracer uptake. ATTR = hereditary transthyretin-related amyloidosis; ^{99m}Tc-DPD = ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid.

Scintigraphic findings and scintigraphic-echocardiographic correlations. Figure 1 shows examples illustrating the spectrum of ^{99m}Tc-DPD uptake among the groups under investigation, with the corresponding echocardiographic and ECG images. Semiquantitative and visual scintigraphic findings are summarized in Table 3 and Figure 2, in the overall population and according to the presence of cardiomyopathy. At semiquantitative analysis, heart retention and H/WB were both higher in patients with cardiomyopathy. A visual myocardial uptake of the scintigraphic tracer (i.e., visual score ≥1) was present in all patients with echocardiographically defined cardiomyopathy. Within the subgroup without cardiomyopathy, 19 patients (81%) did not show any visible myocardial tracer uptake, whereas 4 patients (18%) showed myocardial tracer uptake (mild in 1 case, moderate in 2 cases, strong in 1 case) and higher H/WB values (Fig. 2, Table 3). In all these 4 cases, LV wall thickness, left ventricular ejection fraction (LVEF), and trans-mitral Doppler

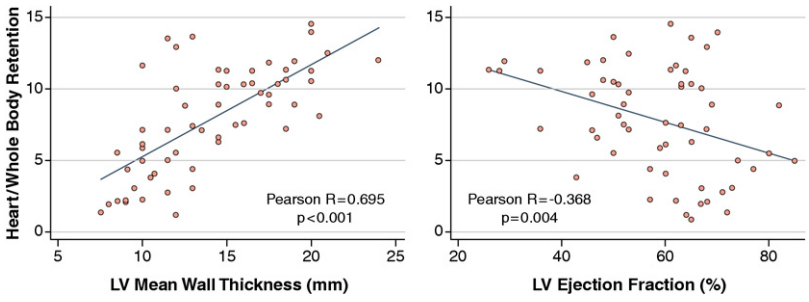

Figure 3. Correlation Between H/WB and Mean LV Wall Thickness and LV Ejection at Pearson's Correlation Test

Mean left ventricular (LV) wall thickness **(left)**, and LV ejection **(right)**. The heart/whole body retention (H/WB) was positively correlated with LV mean wall thickness and inversely correlated with LV ejection fraction.

profile were normal, whereas E/E′ was abnormal in 1 case. The ECG showed nonspecific T-wave abnormalities in 1 patient. All 4 cases carried non-Val30Met TTR mutations (Thr49Ala, n = 1; Ala36Pro, n = 1; Glu89Gln, n = 1; Gly47Ala, n = 1). As can be seen in Figure 3, in the overall population, H/WB was positively correlated with LV mean wall thickness (Pearson r = 0.695, p < 0.001) and inversely correlated with LVEF (Pearson r = −0.368, p = 0.004).

Scintigraphic-histologic correlations. An EMB was performed in 28 (70%) patients with echocardiographically defines AC. Among patients without AC, EMB was performed only in the 4 cases with myocardial ^{99m}Tc-DPD tracer uptake (all candidates for orthotopic liver transplantation) and showed a mild-to-moderate amyloid infiltration in all cases. We did not find any correlation between the extent of the histological infiltration at EMB and the degree of myocardial tracer uptake (p = 0.86 at Kruskal-Wallis test) (Fig. 4).

Outcome and prognosis. The median duration of follow-up was 14 (6.2 to 32) months. No patients were lost to follow-up. Sixteen patients (25%) were submitted to solid organ transplantation (liver transplantation, n = 11; heart-liver transplantation, n = 4; heart-liver-kidney transplantation, n = 1). With regard to MACE, we recorded 15 total events: deaths from cardiovascular causes were recorded in 2 (3%) patients; hospital stay due to heart failure was recorded in 9 (14%) patients; complete atrioventricular block was recorded in 2 (3%) patients; new-onset atrial fibrillation/flutter was recorded in 1 (2%) patient; and sustained ventricular tachyarrhythmia was recorded in 1 (2%) case. Annualized rate of MACE was 0.121/year.

Figure 5 shows MACE-free survival in ATTR patients according to (Fig. 5A) mean LV wall thickness (≤ or >12 mm), (Fig. 5B) H/WB (≤ or >7.5, which represented the median value), and (Fig. 5C) the combination of the previous variables. Notably, the combination of LV wall thickness >12 and H/WB >7.5 was associated with the highest event rate (Fig. 5C).

Table 4 reports univariate and multivariate Cox models on MACE-free survival. At univariate analysis, increasing age, left atrial diameter, mean LV wall thickness, and LV mass/volume ratio as well as increasing New York Heart Association functional class at first evaluation, presence of low QRS voltage, presence of restrictive filling pattern, and increasing scintigraphic heart retention and H/WB were unfavorable predictors of MACE-free survival, whereas increasing LVEF was a favorable

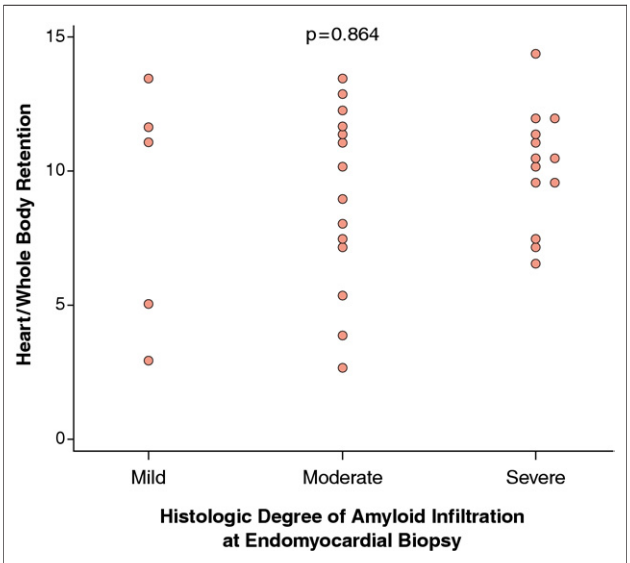

Figure 4. Relation Between ^{99m}Tc-DPD H/WB Retention and EMB Findings

No correlation was evident between the extent of the histologic infiltration at endomyocardial biopsy (EMB) and the degree of myocardial tracer uptake (heart/whole body [H/WB] retention), p = 0.864 at Kruskal-Wallis test.

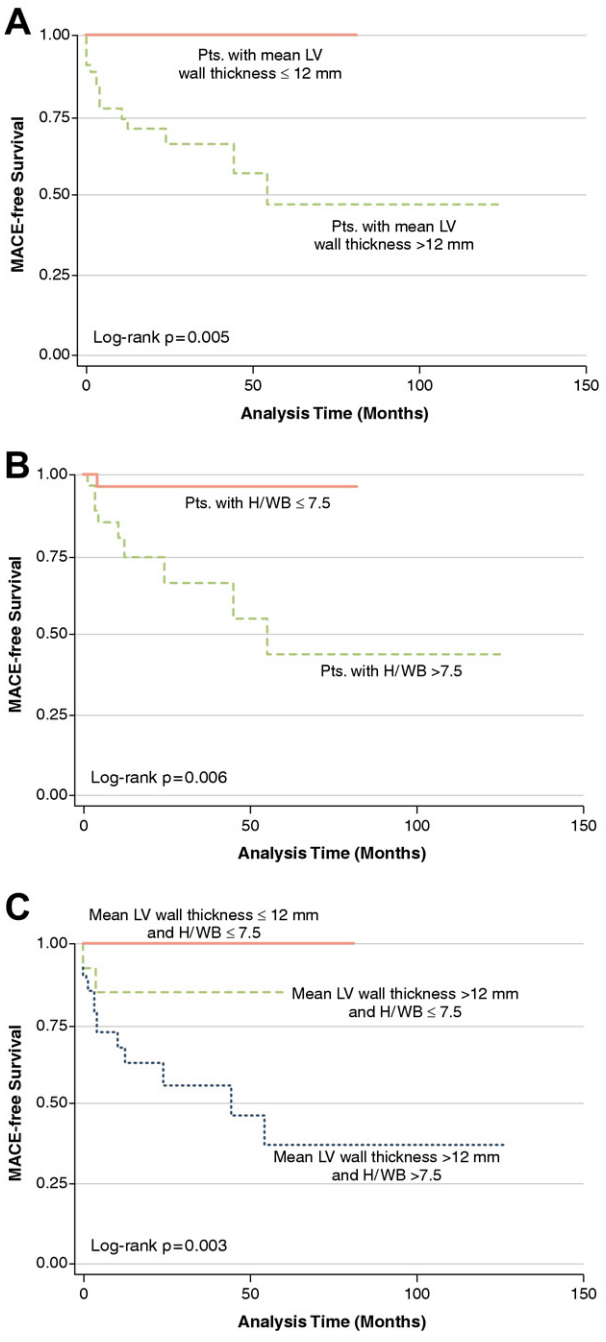

Figure 5. MACE-Free Survival in ATTR Patients

Freedom from major adverse cardiac events (MACE) in patients with hereditary transthyretin-related amyloidosis (ATTR) according to **(A)** the presence of increased thickness of left ventricular (LV) parietal wall (i.e., a mean LV wall thickness >12 mm), **(B)** the median values of heart/whole body retention (H/WB) (= 7.5), and **(C)** the combination of both variables (in all cases, MACE-free survival was censored at time of solid organ transplantation).

predictor (a trend was also recorded for increasing glomerular filtration rate). Therefore, we constructed 2 multivariate analysis models: the first one included only significant and unrelated variables on univariate analysis (i.e., age and filling pattern). The second was obtained by forcing H/WB into the previous one. Likelihood ratio chi-square was 24.13 in the first model and 20.10 in the second (likelihood ratio test, p = 0.044) (Table 4). No interactions emerged between the variables included in the models.

DISCUSSION

Our study highlights the clinical relevance of ^{99m}Tc-DPD scintigraphy in the evaluation of ATTR patients with assessed or suspected AC. Indeed, our data provide 2 main findings: 1) in patients with ATTR, ^{99m}Tc-DPD scintigraphy can identify myocardial infiltration across a wide spectrum of morphologic and functional cardiac involvement, increasing the accuracy of echocardiographic diagnosis of AC and allowing early diagnosis of the disease; and 2) ^{99m}Tc-DPD myocardial uptake is a prognostic determinant of "cardiac" outcome in ATTR.

^{99m}Tc-DPD detection of myocardial involvement in ATTR. In clinical practice, scintigraphy has provided conflicting results in the diagnosis of cardiac amyloidosis. ^{123}I-labeled serum amyloid P (the so-called "SAP") protein binds in a calcium-dependent way to all amyloid deposits, irrespective of the protein of origin (20–22). Unfortunately, the myocardium does not apparently uptake the tracer, probably due to high background (blood) activity and decreased permeability of the cardiac tissue to the tracer (9,20). ^{99m}Tc-aprotinin has been mainly studied in AL patients, in whom it accumulates in various organs, including the myocardium (23,24). However, available experience is limited, and quantitative assessment is not feasible (20,23–25). ^{123}I-metaiodobenzylguanidine does not bind directly to amyloid deposits and has proved useful only in the functional evaluation of amyloid cardiomyopathy, because it indirectly reveals the impairment of cardiac sympathetic nerve endings due to amyloid deposition (20,26,27). Studies on bone tracers in cardiac amyloidosis have produced conflicting results, with significant variations in diagnostic performance (20,28–33). Our previous studies showed that this variability was mainly related to the etiology of amyloid deposits and that this characteristic could be used for noninvasive differential diagnosis

Table 4. Univariate and Multivariate Analyses of Risk of MACE in the Overall Population With ATTR

	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	p Value	HR	95% CI	p Value
Male	1.7	0.5–5.2	0.86			
ATTR Val30Met vs. other mutations	0.3	0.04–2.3	0.25			
Age (per yr)	1.1	1.0–1.2	0.001	1.1	1.0–1.1	0.015
Autonomic imbalance	1.9	0.7–5.4	0.213			
Advanced neurosensorial stage	1.1	0.4–3.5	0.87			
Disease duration (per month)	1.0	1.0–1.0	0.33			
NYHA functional class at observation	8.8	2.0–39.2	0.004			
Glomerular filtration rate	0.9	0.9–1.0	0.073			
Body mass index	0.9	0.8–1.1	0.468			
Low QRS voltage on ECG	6.2	2.2–17.3	0.001			
Left atrial diameter	1.1	1.0–1.2	0.002			
Mean LV wall thickness (per mm)	1.3	1.1–1.5	0.001			
LVEF	0.9	0.9–1.0	0.001			
Indexed LV mass/volume ratio	1.5	1.2–2.0	0.003			
Pericardial effusion	5.7	1.6–20.5	0.007			
LV restrictive filling pattern	3.6	1.3–9.9	0.014	3.2	1.0–9.6	0.043
Heart retention	7.2	1.6–31.9	0.01			
Heart/whole body retention ratio	7.2	1.6–32.1	0.009	3.9	0.8–18.4	0.081

CI = confidence interval; HR = hazard ratio; MACE = major adverse cardiac events; other abbreviations as in Tables 1 and 2.

between AL and TTR-related AC at least in cases with intense or null tracer uptake (10,11).

In the present study, ^{99m}Tc-DPD was able to disclose cardiac amyloidosis across a wide spectrum of morphologic involvement, ranging from patients with overt cardiomyopathy to cases with normal echocardiograms and normal or near-normal ECGs. Our data suggest that the myocardial tracer uptake is an expression of the extent of amyloidotic infiltration: amyloidosis was detected at myocardial histology in all cases with "positive" myocardial scintigraphy (visual score >0), and mean LV wall thickness was linearly and positively correlated to the cardiac tracer retention (Fig. 3). The absence of a significant correlation between the extent of amyloid infiltration at EMB and the degree of myocardial tracer uptake (Fig. 4) does not necessarily contrast with this interpretation, considering the intrinsic "spotty" nature of the EMB and the lack of a quantitative morphometric evaluation of amyloidotic infiltration in our protocol.

Interestingly, ^{99m}Tc-DPD scintigraphy was positive in 4 cases with normal LV wall thickness and circumferential function (diastolic longitudinal function was slightly depressed in only 1 case). All these patients were affected by TTR mutations other than Val30Met. This finding is consistent with the current knowledge that some non-Val30Met TTR mutations are more aggressive in the development and progression of myocardial involvement, even in the absence of overt neurologic manifestations or clear familial transmission.

The possibility of identifying myocardial involvement before overt morphological abnormalities are echocardiographically visible is potentially relevant from a clinical perspective, particularly when considering that drugs that stabilize the tetramer and possibly slow disease progression are starting to be available (4,5,8). Diagnostic performance of ^{99m}Tc-DPD scintigraphy favorably compares with the other noninvasive techniques. With regard to 2-dimensional echocardiography, data and diagnostic criterion reported so far all refer to cases with overt cardiomyopathy (14,34). Similar considerations can be made with regard to standard ECG (4,35) and magnetic resonance (36). Tissue-Doppler and deformation imaging techniques have proved to identify early impairment of LV mechanics (37). However, these functional (all nonspecific) abnormalities have been described mainly in patients with AL-amyloidosis (38) and are shared by other pathological conditions (such as hypertensive heart disease). The possibilities offered by scintigraphy could usefully integrate with those of Doppler echocardiography, increasing its specificity and therefore its clinical usefulness. Furthermore, the integration between echocardiography and scintigraphy could also be decisive in cases with echocardiographically overt cardiomyopathy. Indeed, it must be considered that in many cases (one-third

in our series) patients present with an exclusively cardiologic phenotype, typically with an LV hypertrophy compatible with different interpretations ranging from hypertensive heart disease to hypertrophic cardiomyopathy. This is the case of some TTR mutations including Val122Ile (probably the most frequent TTR mutation, because its prevalence is more than 4% in African Americans), Leu111Met (the so called "FAC," familial AC, of Danish origin), Thr60Ala, and Ile68Leu. The documentation of myocardial ^{99m}Tc-DPD tracer uptake would be decisive in the diagnostic workup of such cases.

Prognostic implication of ^{99m}Tc-DPD myocardial uptake. This is the first study that investigates the prognostic role of ^{99m}Tc-DPD in ATTR. The natural history of ATTR is very difficult to assess due to the high genotypic heterogeneity and the rarity of the disease. These 2 factors explain the paucity of large systematic studies of ATTR as a whole. The vast majority of data with regard to outcome and prognostic stratification of cardiac amyloidosis derive from studies of patients with AL amyloidosis (4,39,40). In this setting, the main known (negative) prognostic factors are advanced congestive heart failure, increased N-terminal pro–B-type natriuretic peptide, increased parietal thickness, and trans-mitral restrictive filling pattern at Doppler evaluation (41). In general, groups of patients with ATTR AC caused by non-Val30Met mutations tend to have a worse prognosis (compared with Val30Met), even after liver transplantation (3,5,42). Because amyloidotic infiltration is responsible for the myocardial tracer uptake, it is reasonable to hypothesize that the amount of tracer uptake could be a prognostic determinant of cardiac events. Our data seem to confirm this hypothesis: H/WB is not only a significant predictor of MACE-free survival at Cox univariate analysis, but it also interacts with the mean LV wall thickness in predicting cardiovascular events: when mean LV wall thickness is >12 mm, a high myocardial tracer uptake is associated with a particularly poor MACE-free survival.

However, it should be noted that, in this relatively small case series with a relatively low number of events, only increasing age and the presence of LV restrictive filling pattern were independently associated with MACE-free survival. To correctly interpret these prognostic data, it is necessary to consider that our series was mostly formed by patients with TTR mutations other than Val30Met, with a high prevalence of cardiologic abnormalities even at first evaluation. The prognostic contribution could be quite different in patients with Val30Met TTR mutation where myocardial involvement is infrequent (and age-dependent) and neurologic progression is generally the main prognostic determinant.

Study limitations. Although this study represents the largest report of patients with ATTR ever studied with myocardial scintigraphy, the relatively small population (typical of a rare disease) might represent an unavoidable limitation. In particular, only 4 cases in our study showed myocardial tracer uptake, despite normal echocardiography. So, the statement that ^{99m}Tc-DPD scintigraphy can be useful in the early diagnosis of ATTR-related myocardial disease needs to be confirmed by further studies. In this retrospective study, where all the examinations were performed on the basis of a clinical indication, EMB was not performed when echocardiogram and ^{99m}Tc-DPD scintigraphy were both normal. Therefore, the study does not provide information on the sensitivity of ^{99m}Tc-DPD scintigraphy in this context.

B-type natriuretic peptide or N-terminal pro–B-type natriuretic peptide collection (which was not systematically performed in this study) could have been usefully integrated with DPD uptake data. Finally, it should be emphasized that the clinical relevance of ^{99m}Tc-DPD might only apply to ATTR patients and not to cases with AL amyloidosis.

CONCLUSIONS

In patients with ATTR, ^{99m}Tc-DPD scintigraphy can identify myocardial infiltration across a wide spectrum of morphologic and functional cardiac involvement, increasing the accuracy of the echocardiographic diagnosis of AC and allowing an early diagnosis of the disease (even before the appearance of echocardiographic abnormalities), at least in cases with non-Val30Met TTR mutations. ^{99m}Tc-DPD myocardial uptake is a prognostic determinant of "cardiac" outcome in ATTR, either alone or in combination with LV wall thickness.

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REFERENCES

1. Ando Y, Nakamura M, Araki S. Transthyretin-related familial amyloidotic polyneuropathy. Arch Neurol 2005;62:1057–62.
2. Benson MD, Kincaid JC. The molecular biology and clinical features of amyloid neuropathy. Muscle Nerve 2007;36:411–23.
3. Rapezzi C, Perugini E, Salvi F, et al. Phenotypic and genotypic heterogeneity in transthyretin-related cardiac amyloidosis: towards tailoring of therapeutic strategies? Amyloid 2006;13:143–53.
4. Falk RH, Dubrey SW. Amyloid heart disease. Prog Cardiovasc Dis 2010;52:347–61.
5. Rapezzi C, Quarta CC, Riva L, et al. Transthyretin-related amyloidoses and the heart: a clinical overview. Nat Rev Cardiol 2010;7:398–408.
6. Selvanayagam JB, Hawkins PN, Paul B, et al. Evaluation and management of the cardiac amyloidosis. J Am Coll Cardiol 2007;50:2101–10.
7. Rapezzi C, Merlini G, Quarta CC, et al. Systemic cardiac amyloidoses: disease profiles and clinical courses of the 3 main types. Circulation 2009;120:1203–12.
8. Sekijima Y, Kelly JW, Ikeda S. Pathogenesis of and therapeutic strategies to ameliorate the transthyretin amyloidoses. Curr Pharm Des 2008;14:3219–30.
9. Glaudemans AW, Slart RH, Zeebregts CJ, et al. Nuclear imaging in cardiac amyloidosis. Eur J Nucl Med Mol Imaging 2009;36:702–14.
10. Perugini E, Guidalotti PL, Salvi F, et al. Noninvasive etiologic diagnosis of cardiac amyloidosis using 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy. J Am Coll Cardiol 200520;46:1076–84.
11. Rapezzi C, Quarta CC, Guidalotti PL, et al. Usefulness and limitations of 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy in the aetiological diagnosis of amyloidotic cardiomyopathy. Eur J Nucl Med Mol Imaging 2011;38:4708.
12. Arbustini E, Verga L, Concardi M, et al. Electron and immunoelectron microscopy of abdominal fat identifies and characterizes amyloid fibrils in suspected cardiac amyloidosis. Amyloid 2002;9:108–14.
13. Benson MD, Breall J, Cummings OW, Liepnieks JJ. Biochemical characterisation of amyloid by endomyocardial biopsy. Amyloid 2009;16:9–14.
14. Gertz MA, Comenzo R, Falk RH, et al. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th international symposium on amyloid and amyloidosis. Am J Hematol 2005;79:319–28.
15. Ferlini A, Fini S, Salvi F, et al. Molecular strategies in genetic diagnosis of transthyretin-related hereditary amyloidosis. FASEB J 1992;6:2864–6.
16. Surawicz B, Knilans TK. Chou's Electrocardiography in Clinical Practice. 5th edition. Philadelphia, PA: WB Saunders, 2001.
17. Cheitlin MD, Armstrong WF, Aurigemma GP, et al. ACC/AHA/ASE 2003 guideline update for the clinical application of echocardiography—summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (ACC/AHA/ASE Committee to Update the 1997 Guidelines for the Clinical Application of Echocardiography). J Am Coll Cardiol. 2003;42:954–70.
18. Devereux RB, Alonso DR, Lutas EM, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 1986;57:450–8.
19. Garcia MJ, Thomas JD, Klein AL. New Doppler echocardiographic applications for the study of diastolic function. J Am Coll Cardiol 1998;32:865–75.
20. Hazenberg BP, van Rijswijk MH, Lub-de Hooge MN, et al. Diagnostic performance and prognostic value of extravascular retention of 123I-labeled serum amyloid P component in systemic amyloidosis. J Nucl Med 2007;48:865–72.
21. Hawkins PN, Lavender JP, Pepys MB. Evaluation of systemic amyloidosis by scintigraphy with 123I-labeled serum amyloid P component. N Engl J Med 1990;323:508–13.
22. Hawkins P. Serum amyloid P component scintigraphy for diagnosis and monitoring amyloidosis. Curr Opin Nephrol Hypertens 2002;11:649–55.
23. Aprile C, Marinone G, Saponaro R, et al. Cardiac and pleuropulmonary. AL amyloid imaging with technetium-99m labelled aprotinin. Eur J Nucl Med 1995;22:1393–401.
24. Han S, Chong V, Murray T, et al. Preliminary experience of 99mTc-aprotinin scintigraphy in amyloidosis. Eur J Haematol 2007;79:494–500.
25. Schaadt BK, Hendel HW, Gimsing P, et al. 99mTc-aprotinin scintigraphy in amyloidosis. J Nucl Med 2003;44:177–83.
26. Hongo M, Urushibata K, Kai R, et al. Iodine-123 metaiodobenzylguanidine scintigraphic analysis of myocardial sympathetic innervation in patients with AL (primary) amyloidosis. Am Heart J 2002;144:122–9.
27. Tanaka M, Hongo M, Kinoshita O, et al. Iodine-123 metaiodobenzylguanidine scintigraphic assessment of myocardial sympathetic innervation in patients with familial amyloid polyneuropathy. J Am Coll Cardiol 1997;29:168–74.
28. Leinonen H, Tötterman KJ, Korppi-Tommola T, Korhola O. Negative myocardial technetium-99m pyrophosphate scintigraphy in amyloid heart disease associated with type AA systemic amyloidosis. Am J Cardiol 1984;53:380–1.
29. Eriksson P, Backman C, Bjerle P, et al. Non-invasive assessment of the presence and severity of cardiac amyloidosis. A study in familial amyloidosis with polyneuropathy by cross sectional echocardiography and technetium-99m pyrophosphate scintigraphy. Br Heart J 1984;52:321–6.
30. Worsley DF, Lentle BC. Uptake of technetium-99m MDP in primary amyloidosis with a review of the mechanisms of soft tissue localization of bone seeking radiopharmaceuticals. J Nucl Med 1993;34:1612–5.
31. Ak I, Vardareli E, Erdinc O, et al. Myocardial Tc-99m MDP uptake on a bone scan in senile systemic amyloidosis with cardiac involvement. Clin Nucl Med 2000;25:826–7.
32. Puille M, Altland K, Linke RP, et al. 99mTc-DPD scintigraphy in transthyretin-related familial amyloidotic polyneuropathy. Eur J Nucl Med Mol 2002;29:376–9.
33. Kulhanek J, Movahed A. Uptake of technetium 99m HDP in cardiac amyloidosis. Int J Cardiovasc Imaging 2003;19:225–7.
34. Tsang W, Lang RM. Echocardiographic evaluation of cardiac amyloid. Curr Cardiol Rep 2010;12:272–6.
35. Rahman JE, Helou EF, Gelzer-Bell R, et al. Noninvasive diagnosis of biopsy-proven cardiac amyloidosis. J Am Coll Cardiol 2004;43:410–5.
36. Maceira AM, Joshi J, Prasad SK, et al. Cardiovascular magnetic resonance in cardiac amyloidosis. Circulation 2005;111:186–93.
37. Bellavia D, Pellikka PA, Abraham TP, et al. Evidence of impaired left ventricular systolic function by Doppler myocardial imaging in patients with systemic amyloidosis and no evidence of cardiac involvement by standard two-dimensional and

Doppler echocardiography. Am J Cardiol 2008;101:1039–45.

38. Koyama J, Ray-Sequin PA, Falk RH. Longitudinal myocardial function assessed by tissue velocity, strain, and strain rate tissue Doppler echocardiography in patients with AL (primary) cardiac amyloidosis. Circulation 2003;107:2446–52.
39. Kyle RA, Gertz MA. Primary systemic amyloidosis: clinical and laboratory features in 474 cases. Semin Hematol 1995;32:45–59.
40. Merlini G, Palladini G. Amyloidosis: is a cure possible? Ann Oncol 2008; 19:63–6.
41. Klein AL, Hatle LK, Burstow DJ, et al. Doppler characterization of left ventricular diastolic function in cardiac amyloidosis. J Am Coll Cardiol 1989;13:1017–26.
42. Herlenius G, Wilczek HE, Larsson M, et al. Ten years of international experience with liver transplantation for familial amyloidotic polyneuropathy: results from the Familial Amyloidotic Polyneuropathy World Transplant Registry. Transplantation 2004; 77:64–71.

Key Words: ^{99m}Tc-DPD scintigraphy ■ cardiac amyloidosis ■ diagnosis ■ hereditary transthyretin-related amyloidosis ■ prognosis.