

# Noninvasive Etiologic Diagnosis of Cardiac Amyloidosis Using $^{99m}\text{Tc}$ -3,3-Diphosphono-1,2-Propanodicarboxylic Acid Scintigraphy

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**OBJECTIVES** We investigated the diagnostic accuracy of  $^{99m}\text{Tc}$ -3,3-diphosphono-1,2-propanodicarboxylic acid ( $^{99m}\text{Tc}$ -DPD) scintigraphy for differentiation of monoclonal immunoglobulin light-chain (AL) and transthyretin (TTR)-related cardiac amyloidosis.

**BACKGROUND** Differential diagnosis between TTR-related and AL amyloidosis is often complex and time-consuming.

**METHODS** Patients under routine observation with TTR-related/AL systemic amyloidosis and echocardiographic evidence of cardiac involvement were studied with  $^{99m}\text{Tc}$ -DPD scintigraphy.

**RESULTS** Patients with cardiac involvement of TTR-related (group A; n = 15) and AL (group B; n = 10) etiology were comparable for left ventricular mass and renal function. Heart and heart/whole-body tracer retention were significantly higher ( $p < 0.05$ ) in group A as compared with group B and with 10 unaffected controls. At visual scoring, cardiac  $^{99m}\text{Tc}$ -DPD uptake was present in all group A patients and absent in all group B patients; thus, using genotyping/immunohistochemistry as the reference technique, the accuracy of  $^{99m}\text{Tc}$ -DPD scintigraphy for distinction of TTR-related and AL etiology was 100%. Cardiac  $^{99m}\text{Tc}$ -DPD uptake was also absent among unaffected controls. Using echocardiography as the reference standard for recognition of cardiac involvement, sensitivity and specificity of scintigraphy were both 100% for group A patients; in group B, sensitivity was 0% and specificity was 100% (accuracy, 50%). Eleven patients with myocardial  $^{99m}\text{Tc}$ -DPD uptake underwent  $^{99m}\text{Tc}$ -methylene diphosphonate ( $^{99m}\text{Tc}$ -MDP) scintigraphy; all patients showed a  $^{99m}\text{Tc}$ -MDP myocardial visual score of 0.

**CONCLUSIONS** Etiology is a third major cause—in addition to type of organ-involved (soft-tissue/heart) and tracer type—of scintigraphic variability in cardiac amyloidosis. This is a highly relevant consideration for future studies. We conclude that  $^{99m}\text{Tc}$ -DPD scintigraphy is a useful step in the workup of the differential diagnosis of TTR versus AL etiology in patients with documented cardiac amyloidosis. (J Am Coll Cardiol 2005;46:1076–84) © 2005 by the American College of Cardiology Foundation

Cardiac involvement has major clinical and prognostic implications in amyloidosis. Acquired monoclonal immunoglobulin light-chain (AL) and transthyretin (TTR)-related (familial and wild-type/senile) disease are the most frequent causes of cardiac amyloidosis (1). In hereditary amyloidosis, cardiac involvement can limit short-term and long-term results of orthotopic liver transplantation (OLT) (2,3) and influence decisions to perform combined heart-liver transplantation (4). Severe cardiac involvement in AL amyloidosis can limit optimal hematologic treatments, including bone marrow transplantation (5).

Differential diagnosis between TTR-related and AL amyloidosis is often complex and time-consuming. Echocardiography is considered the gold standard for noninva-

sive diagnosis of amyloidotic cardiomyopathy (1,6), but it is not etiologically informative. A recent study showed a 10% misdiagnosis rate between familial and AL amyloidosis (7). Although genotyping and/or immunohistochemical analysis are mandatory for a definitive diagnosis, they are not routine examinations and thus require accurate patient selection (7,8).

Various tracers (mainly phosphonates) have been used in the past for scintigraphic assessment of cardiac amyloidosis, and they have produced heterogeneous results (9–22). These discrepancies might partially be explained by the variable composition of the amyloid substance (14,17). We hypothesized that this apparent limit might translate into a noninvasive tool for diagnostic evaluation of etiology. In this pilot study, we investigated the uptake of  $^{99m}\text{Tc}$ -3,3-diphosphono-1,2-propanodicarboxylic acid ( $^{99m}\text{Tc}$ -DPD) in a population of patients with TTR-related or AL cardiac amyloidosis. The main goal of the study was to assess the

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Manuscript received March 4, 2005; revised manuscript received May 18, 2005, accepted May 24, 2005.

#### Abbreviations and Acronyms

AL	= monoclonal immunoglobulin light-chain
CA	= cardiac amyloidosis
LV	= left ventricle/ventricular
OLT	= orthotopic liver transplantation
ROI	= region of interest
SPECT	= single-photon emission computed tomography
<sup>99m</sup> Tc-DPD	= <sup>99m</sup> Tc-3,3-diphosphono-1,2-propano-dicarboxylic acid
<sup>99m</sup> Tc-MDP	= <sup>99m</sup> Tc-methylene diphosphonate
TTR	= transthyretin

diagnostic accuracy and predictive value of <sup>99m</sup>Tc-DPD scintigraphy for differentiation of TTR-related and AL cardiac amyloidosis using genotyping/immunohistochemistry as a reference standard. Subsidiary goals were to explore patterns of myocardial and soft-tissue DPD uptake and relationships between scintigraphic and echocardiographic/histologic findings.

## METHODS

**Study design and selection criteria.** All patients under routine observation in our cardiology and neurology units between January 2004 and January 2005 with a diagnosis of TTR-related or AL amyloidosis with echocardiographically demonstrated cardiac involvement were enrolled in this study (no exclusion criteria were applied). External controls were 10 unaffected patients (6 men, 4 women; median age, 68 years; age range, 55 to 74 years) undergoing scintigraphy for oncologic reasons. All participants provided informed consent for participation. Ethical approval was not required for this noninvasive study.

**Diagnostic definitions.** Diagnosis of amyloidosis was defined by histologic documentation of Congo-red staining and apple-green birefringence under cross-polarized light in at least one involved organ (1). Cardiac involvement was echocardiographically defined as end-diastolic thickness of the interventricular septum >1.2 cm (in the absence of any other cause of ventricular hypertrophy) plus two or more of the following features: 1) homogeneous atrioventricular valve thickening; 2) atrial septal thickening; and 3) sparkling appearance of the ventricular septum (6). A clear-cut distinction between TTR-related and AL amyloidosis was based on genotyping and/or immunohistochemistry (7). Diagnosis of familial TTR-related amyloidosis was defined by a documented TTR mutation at DNA analysis; TTR-related wild-type (senile) amyloidosis by positive immunohistochemistry for TTR in the absence of any TTR mutation at DNA analysis (23). Monoclonal immunoglobulin light-chain amyloidosis was diagnosed by the presence of monoclonal plasma cells in the bone marrow, plus: 1) negative immunohistochemistry for TTR, and 2) absence of any TTR mutation at DNA analysis (7).

**Genotyping/immunohistochemistry and histology.** For genotyping, DNA was isolated from peripheral blood and exons 2, 3, and 4 of the *TTR* gene (accession number m11844) were amplified by polymerase chain reaction (Takara ExTaq polymerase, TaKaRa Shuzo, Otsu, Japan) by using primers previously described (24). Amplified DNA fragments were directly sequenced by using an ABI Prism 3100 automated sequencer (Applied Biosystems, Foster City, California).

Endomyocardial biopsies were microwave fixed/processed, and multiple 2- $\mu$ m sections were tested for the presence of amyloid by Congo-red staining and apple-green birefringence under cross-polarized light microscopy. Amounts of amyloid deposits were semiquantitatively classified as mild (<30% involvement of the tissue fragments), moderate (30% to 60% involvement), or severe (>60% involvement) (25). Immunohistochemical analysis was performed by the Labeled StreptAvidin Biotin (LSAB) method using an antibody against TTR (R. P. Linke, Max Plank Institute of Biochemistry, Martinsried, Germany).

**Scintigraphy protocol and image analysis.** Scintigraphy was always performed  $\leq 2$  months after echocardiography and genotyping/immunohistochemistry. All patients received 740 MBq of <sup>99m</sup>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 five minutes (early) and three hours (late) after injection. In patients showing cardiac uptake, a myocardial single-photon emission computed tomography (SPECT) study was acquired after the late whole-body scan. Image analysis was independently performed by three experienced analysts (P.G., C.P., and M.F.) blinded to all patient data (any disagreement was settled by group discussion). Visual scoring of cardiac retention was routine (i.e., score 0, absent cardiac uptake and normal bone uptake; score 1, mild cardiac uptake, inferior to bone uptake; score 2, moderate cardiac uptake accompanied by attenuated bone uptake; score 3, strong cardiac uptake with mild/absent bone uptake). Semiquantitative analysis of heart retention, whole-body retention, and heart/whole-body ratio were evaluated from region of interest (ROI) drawings in the standard manner. In brief, on anterior images rectangular ROIs were drawn over the heart, and irregular ROIs over the kidneys and bladder. These ROIs were copied and mirrored on posterior images, and for each ROI geometric means of the two projections were calculated. 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. Heart/

**Table 1.** Clinical, Morphologic and Functional Parameters in the Overall Patient Population

	Group A TTR-Related Cardiac Amyloidosis (15 Patients)	Group B AL Cardiac Amyloidosis (10 Patients)	p Value
Age (yrs)			
Median	56	60	0.91
Interquartile range	48–76	58–65	
NYHA functional class >II (% patients)	13	50	0.075
Neurologic involvement (% patients)	53	42	0.41
Glomerular filtration rate (ml/min)			
Median	64	66	0.95
Interquartile range	47–85	59–80	
Abnormal electrocardiogram (% patients)	93	100	1.00
LV mass (g/m <sup>2</sup> )			
Median	184	170	0.64
Interquartile range	160–291.5	136–215	
Interventricular septal thickness (mm)			
Median	16	15.5	0.69
Interquartile range	13.5–20	13–17	
LV ejection fraction (%)			
Median	53	54	0.77
Interquartile range	41–66	45–57	
Restrictive LV filling pattern at echo-Doppler (% patients)	26	66	0.049

AL= monoclonal immunoglobulin light-chain; LV = left ventricular; NYHA = New York Heart Association; TTR = transthyretin.

whole-body ratios were calculated by dividing counts in the heart by counts in late whole-body images. Soft tissue uptake (particularly skeletal muscle and splanchnic organs) was also visually assessed using an analogous scoring system to that used for the heart.

Patients showing cardiac uptake of <sup>99m</sup>Tc-DPD were invited to receive <sup>99m</sup>Tc-methylene diphosphonate (<sup>99m</sup>Tc-MDP) scintigraphy using the same protocol and heart and soft-tissue visual scoring systems.

**Statistical analysis.** Statistical analysis was performed using SPSS 11.0 statistical software (SPSS Inc., Chicago, Illinois). Continuous variables are expressed as median (interquartile range), categorical variables as percentages. When comparing subgroups, the Fisher exact test or contingency tables were used (the former when the expected frequency of counts in at least one cell of 2 × 2 tables was <5), alongside the Mann-Whitney U or the Kruskal-Wallis test. For multiple comparisons, the Dunn test was also applied. Any p values <0.05 were considered statistically significant.

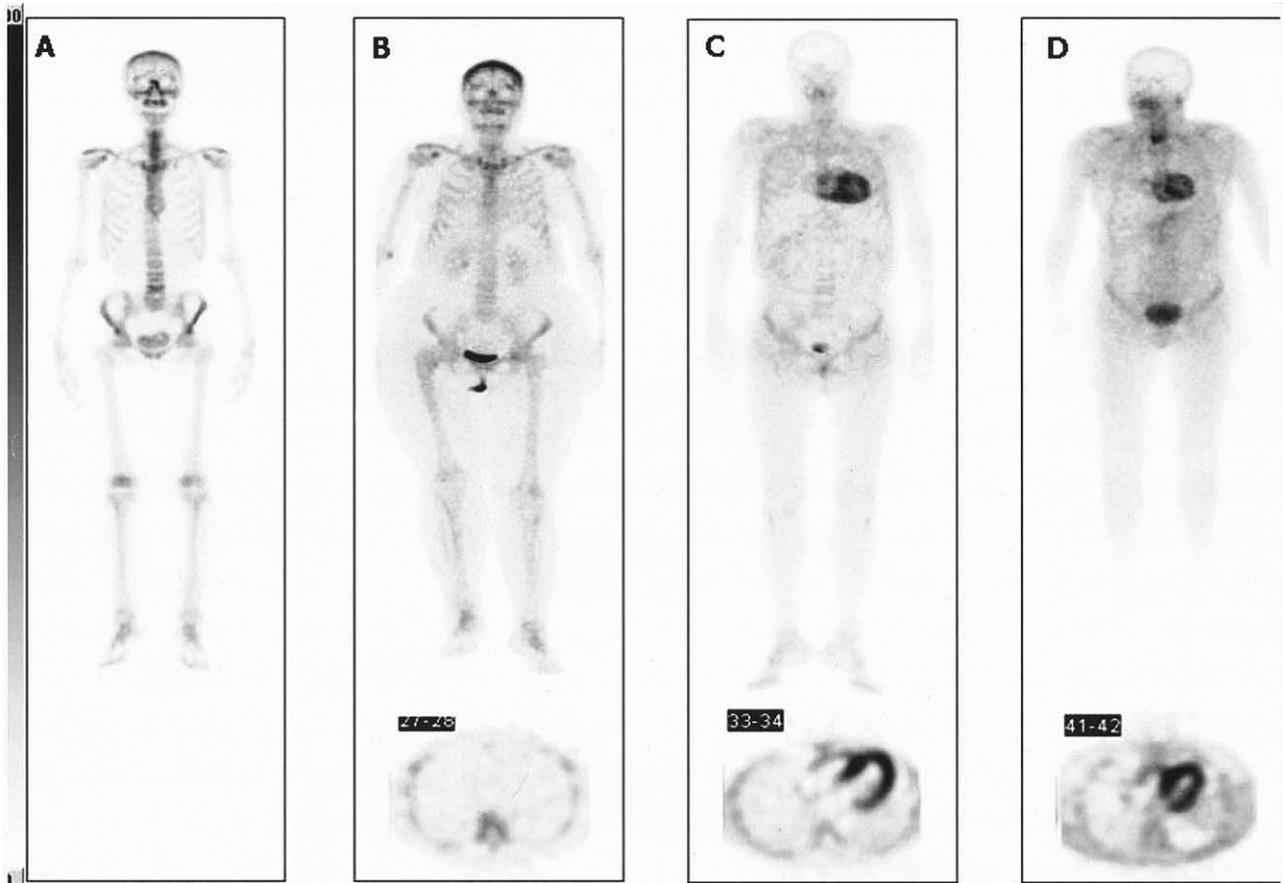
## RESULTS

**Patient population.** Twenty-five cardiac amyloidosis patients were enrolled, all of whom completed the study protocol. Table 1 summarizes the principal clinical, electrocardiographic, and echocardiographic findings in the patient population, subdivided into two groups according to etiology. Of the 15 patients with TTR-related amyloidosis with cardiac involvement (group A), 10 had familial TTR amyloidosis (carrying Glu89Gln [n = 1], Val30Met [n = 2], Ala36Pro [n = 2], Thr49Ala [n = 2], Phe33Val [n = 1], or

Ile68Leu [n = 2] TTR mutations), and 5 had senile TTR. No difference was apparent regarding age, renal function, and left ventricular (LV) mass (all p > 0.40). None of the patients had any associated cardiovascular disease, including systemic hypertension and coronary artery disease (the latter was excluded on clinical grounds, as well as by coronary angiography in three patients with suspected angina).

**Scintigraphy findings.** Figure 1 shows representative examples of the spectrum of <sup>99m</sup>Tc-DPD uptake among patients and control patients. Semiquantitative and visual scintigraphic findings are summarized in Figure 2 and Table 2, according to the two subgroups of the patient population and the unaffected controls. At semiquantitative analysis, heart tracer retention and heart/whole-body retention ratio were both about two-fold higher in group A as compared with group B and also with controls (Fig. 2). Smaller differences were also observed between group B and controls, plausibly because of intense mediastinal/sternal uptake in a single patient in group B (see asterisk in Fig. 2A). Regarding the visual score, myocardial uptake (score >0) was detected in 15 of 15 (100%) group A patients, as compared with 0 of 10 (0%) in group B and 0 of 10 (0%) in the control group.

We were able to compare the macroscopic, histologic, and <sup>99m</sup>Tc-DPD scintigraphic appearance of myocardium from one group A patient who underwent combined heart–liver transplantation (one month after scintigraphy) and one group B patient who died (after three months). Myocardial uptake of <sup>99m</sup>Tc-DPD occurred only in the patient with TTR-related amyloidosis, despite similar LV mass and myocardial infiltration (Fig. 3).



**Figure 1.** Representative examples illustrating the spectrum of  $^{99m}\text{Tc}$ -3,3-diphosphono-1,2-propanodicarboxylic acid ( $^{99m}\text{Tc}$ -DPD) uptake among patients with transthyretin (TTR)-related or monoclonal immunoglobulin light-chain (AL) cardiac amyloidosis and unaffected controls (top row = whole-body scans, anterior view; bottom row = cross sectional views of cardiac single-photon emission computed tomography in the same patients). (A) Unaffected control subject without visually detectable uptake. (B) Patient with AL amyloidosis and echocardiographic documentation of cardiac involvement without any visually detectable sign of myocardial  $^{99m}\text{Tc}$ -DPD uptake; mild uptake of the tracer is visible only at the soft tissue level. (C and D) Two patients with TTR-related amyloidosis and echocardiographic documentation of cardiac involvement, both showing strong myocardial  $^{99m}\text{Tc}$ -DPD uptake (with absent bone uptake); in one of the patients (D), splanchnic uptake is also visible.

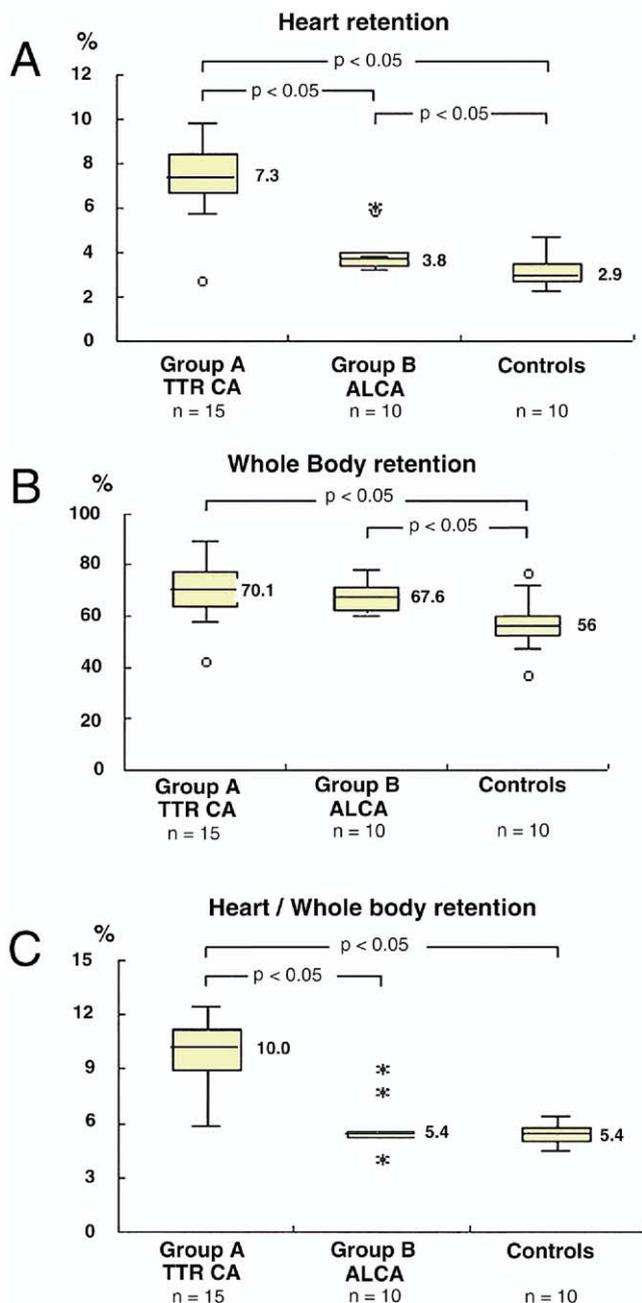
Eleven patients with myocardial uptake of  $^{99m}\text{Tc}$ -DPD agreed to undergo  $^{99m}\text{Tc}$ -MDP scintigraphy. The visual score for  $^{99m}\text{Tc}$ -MDP myocardial uptake was 0 in all of these patients. A representative example of differences in myocardial uptake of the two tracers can be seen in Figure 3. The visual score for  $^{99m}\text{Tc}$ -MDP soft tissue uptake was 0 in 8 patients and 1 in the remaining 3.

**Diagnostic accuracy of  $^{99m}\text{Tc}$ -DPD scintigraphy for differentiation of TTR-related and AL cardiac amyloidosis.** We assessed the diagnostic accuracy of  $^{99m}\text{Tc}$ -DPD scintigraphy (visual score  $>0$ ) for differentiation of TTR-related and AL cardiac amyloidosis using genotyping/immunohistochemistry as the reference technique. Within our subpopulation of amyloidosis patients with echocardiographic evidence of cardiac involvement (groups A and B), sensitivity and specificity of  $^{99m}\text{Tc}$ -DPD scintigraphy were both 100%.  
**Diagnostic accuracy of  $^{99m}\text{Tc}$ -DPD scintigraphy for detection of amyloidotic cardiomyopathy within different etiologic settings.** We also considered the diagnostic accuracy of a scintigraphy visual score  $>0$  between each of the two groups of patients and the control patients for detection

of cardiac amyloidosis using echocardiography as the reference standard. Sensitivity and specificity of scintigraphy were both 100% for group A (TTR-related patients). By contrast, for group B (AL patients), sensitivity was 0% and specificity was 100%. Thus, the overall accuracy for detection of amyloid of both types was only 50%. These findings further illustrate the concept that etiology can be a major cause of variability in the diagnostic performance of  $^{99m}\text{Tc}$ -DPD scintigraphy.

**Patterns of myocardial uptake of  $^{99m}\text{Tc}$ -DPD.** All but one of the group A patients had widespread involvement of all myocardial segments (Fig. 4A), whereas the remaining patient showed localized septal accumulation (Fig. 4B). These observations paralleled the patterns of increased wall thickness visible at echocardiography (Fig. 4).

**$^{99m}\text{Tc}$ -DPD scintigraphy findings in soft tissue.** Regarding extracardiac uptake of  $^{99m}\text{Tc}$ -DPD, at semiquantitative analysis whole-body retention was significantly higher in both groups A and B with respect to controls (Table 2, Fig. 2C). The increased uptake of  $^{99m}\text{Tc}$ -DPD was visually detectable at the muscular and/or splanchnic level in 13 of



**Figure 2.** Comparison of  $^{99m}\text{Tc}$ -3,3-diphosphono-1,2-propanodicarboxylic acid heart retention (A), whole-body retention (B), and heart/whole-body retention ratio (C) between group A patients (transthyretin [TTR]-related cardiac amyloidosis [CA]), group B patients (monoclonal immunoglobulin light-chain [AL] cardiac amyloidosis), and controls. The lower/upper limits of the boxes indicate 25th/75th percentiles; horizontal lines (and values) within boxes indicate medians. Values lying more than 1.5 and 3 box-lengths from the 75th percentile are indicated by circles and asterisks, respectively.

15 group A patients and 5 of 5 group B patients. No difference was apparent between groups A and B. The visual score for  $^{99m}\text{Tc}$ -MDP soft tissue uptake was 0 in eight patients and 1 in the remaining three.

**Relationships between scintigraphic and echocardiographic/histologic findings.** We investigated whether the degree of myocardial  $^{99m}\text{Tc}$ -DPD uptake as assessed by visual score could be related to LV mass and/or interven-

tricular septal thickness. No evidence of relationships with LV mass or interventricular septal thickness emerged ( $p = 0.54$  and  $p = 0.43$ , respectively, using Kruskal-Wallis test). As can be seen from Figure 5, the vast majority of patients had a similar spectrum of LV masses irrespective of visual score at scintigraphy.

In myocardial biopsies (available from 8 of 15 group A patients and all 10 group B patients), the degree of amyloidotic infiltration was similar in patients with or without myocardial  $^{99m}\text{Tc}$ -DPD uptake (severe infiltration in 5 of 8 vs. 7 of 10 patients, respectively;  $p = 0.87$ ).

## DISCUSSION

To our knowledge, this is the first assessment of  $^{99m}\text{Tc}$ -DPD scintigraphy as a potential diagnostic tool for assessment of etiology in cardiac amyloidosis. Our findings could have potential clinical implications (especially in the light of recent therapeutic advances), and they also could help clarify the conflicting data in published reports on the use of bone tracers in the scintigraphic evaluation of amyloidosis.

Noninvasive detection of amyloid has been investigated using three main groups of radiolabeled tracers:  $^{125}\text{I}$ -labeled serum amyloid P protein (26,27); aprotinin (28,29); and bone tracers, mainly  $^{99m}\text{Tc}$ -pyrophosphate (9-15) and  $^{99m}\text{Tc}$ -HDP (20,21). Labeled serum amyloid P protein localizes rapidly and specifically to amyloid deposits (in proportion to the amount of amyloid) and persists there, allowing quantitative monitoring of amyloid deposition and regression during therapy (low quality of heart visualization and limited availability are current limitations of this tracer [12,13]).  $^{99m}\text{Tc}$ -aprotinin (a protease inhibitor) has been mainly studied in AL patients, in whom it accumulates in various organs, including myocardium: available experience is limited and quantitative assessment is not feasible (14,15). Data on bone tracers are numerous but heterogeneous.  $^{99m}\text{Tc}$ -pyrophosphate is the most widely studied: because it binds avidly to many types of amyloid, it was a popular imaging agent for cardiac amyloidosis during the 1980s. However, amyloidotic uptake of this tracer has not been quantitated, and it has lower diagnostic sensitivity than echocardiography (2-8,10).

Relatively few studies are available on the diagnostic performance of  $^{99m}\text{Tc}$ -DPD (16,17),  $^{99m}\text{Tc}$ -methylene diphosphonate, or  $^{99m}\text{Tc}$ -hydroxymethylene-diphosphonate in amyloidosis (18-20). The numbers of patients included in these studies were often low, and the type of amyloidosis was frequently unspecified. None of these studies used immunohistochemistry/genotyping to exclude misdiagnosis between TTR and AL forms. Both positive and negative myocardial uptake of bone tracers has been reported in patients with definite cardiac amyloidosis, generating a confusing scenario.

In the present study, abnormal cardiac uptake of  $^{99m}\text{Tc}$ -DPD (whether at visual scoring or semiquantitative analysis) occurred exclusively in patients with familial or senile TTR-related cardiac amyloidosis, despite quite similar signs

**Table 2.** Scintigraphic Findings in the Patient Population and Control Group

	Group A TTR-Related CA (15 Patients)	Group B AL CA (10 Patients)	Unaffected Control Patients (10 Patients)	p Value (Kruskal-Wallis Test/ Contingency Tables)
Heart tracer retention (%)				
Median	7.3*†	3.8‡	2.9	0.0001
Interquartile range	6.7–8.4	3.4–4.05	2.7–3.5	
Whole-body tracer retention (%)				
Median	70.1†	67.6‡	56	0.010
Interquartile range	63.6–77.3	61.8–71.3	52–60	
Heart/whole-body ratio				
Median	10.0*†	5.4	5.4	0.0001
Interquartile range	8.9–11.2	5.2–5.5	5.0–5.7	
Visual cardiac score				
0	0 (0%)	10 (100%)	10 (100%)	0.0001
1	0 (0%)	0 (0%)	0 (0%)	
2	3 (20%)	0 (0%)	0 (0%)	
3	12 (80%)	0 (0%)	0 (0%)	

\*p < 0.05 group A vs. B. †p < 0.05 group A vs. control group. ‡p < 0.05 group B vs. control group.  
 CA = cardiac amyloidosis; TTR = transthyretin.

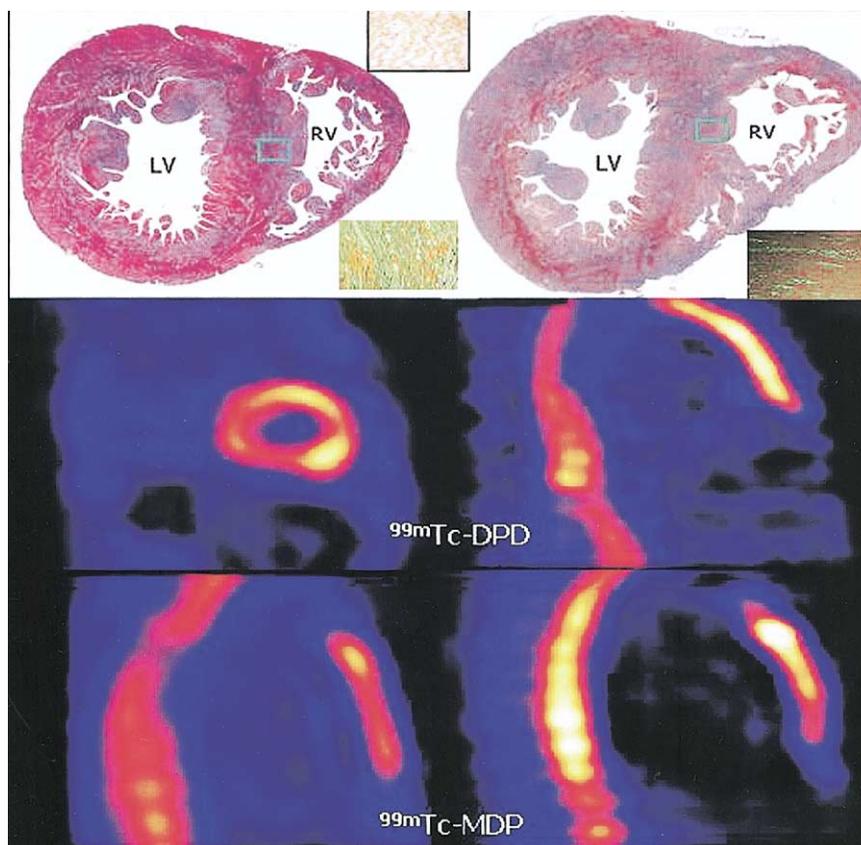
of amyloidotic myocardial infiltration in the two groups, as expressed by comparable values of interventricular septal thickness and LV mass. The strength of these findings was reinforced by the use of immunohistochemistry/genotyping for differentiation between AL and TTR-related amyloidosis. The data are broadly in line with the single published study of <sup>99m</sup>Tc-DPD scintigraphy in a small series of patients in familial TTR-related cardiac amyloidosis (17). In the only patient with AL amyloidosis reported in the literature to have myocardial uptake of <sup>99m</sup>Tc-DPD, the etiologic diagnosis was based only on the presence of abnormal protein at serum and urine electrophoresis (a potentially misleading finding) (16).

In our study sample, selective myocardial uptake of <sup>99m</sup>Tc-DPD provided 100% accuracy for differentiation of TTR-related and AL etiology of cardiac amyloidosis. This potential diagnostic application is confined to amyloidotic patients with myocardial involvement and cannot be extended to patients with involvement limited to soft tissues and/or splanchnic organs. Differential diagnosis of TTR-related and AL cardiac amyloidosis is often problematic, especially from the clinical standpoint. Electrocardiographic and echocardiographic findings are superimposable, irrespective of the composition of the deposits (30,31). Clinically, AL cardiac amyloidosis more often affects older patients and is associated with a more rapid course with prominent symptoms of heart failure. However, exceptions are frequent. For example, patients carrying the TTR Glu89Gln mutation (4) can experience an accelerated course strongly influenced by cardiologic problems. Furthermore, the first clinical manifestations of amyloidosis can be cardiologic with a variety of other mutations. A relatively frequent diagnostic pitfall occurs in elderly patients with monoclonal gammopathy and echocardiographic signs of cardiac amyloidosis. Because of the high prevalence of subtle monoclonal gammopathies in the general population, it is not possible automatically to assume a diagnosis of AL

cardiac amyloidosis in such cases (7) (as in one of our patients with TTR-related cardiac amyloidosis casually associated with a monoclonal gammopathy). Despite these obstacles, a correct differential diagnosis of TTR-related and AL cardiac amyloidosis is now mandatory in the light of the availability of highly specific therapeutic options: OLT or combined heart–liver transplantation for familial TTR (2–4); high-dose chemotherapy and bone marrow transplantation for AL amyloidosis (5). Moreover, definite diagnosis of a hereditary TTR form enables appropriate genetic counseling.

Regarding our wider understanding of the conflicting scintigraphic data available on amyloidosis, the present study offers a new interpretative key. Our analysis reveals that the etiology of the amyloidosis is a third major variable, in addition to the type of organ involved (soft tissues versus heart) and bone tracer type. Etiologic variability must be taken into account in future studies of scintigraphy in cardiac amyloidosis.

What possible explanations are there for selective myocardial uptake of <sup>99m</sup>Tc-DPD in TTR-related cardiac amyloidosis? Analysis of the echocardiographic and histologic characteristics of the two groups largely excludes the possibility of different levels of myocardial infiltration (Figs. 3 and 5). The affinity of all bone tracers for calcium determines their uptake by bone (and especially by bone metastases and post-traumatic lesions), tumors, infected/inflammatory tissues, and some neoplastic lesions (18). It has been hypothesized that uptake of phosphonates in amyloidotic tissues might be explained by high calcium concentration in amyloid deposits (10,32,33). A model capable of fully explaining our findings would have to account for all three forms of variability (etiology, type of organ involved, tracer type), and in particular why <sup>99m</sup>Tc-DPD uptake is massive in myocardium of patients with TTR-related amyloidosis, absent in myocardium of AL patients, and present in soft tissues of both groups. No such



**Figure 3.** Macroscopic/histologic findings (top) and scintigraphic images using  $^{99m}\text{Tc}$ -3,3-diphosphono-1,2-propanodicarboxylic acid ( $^{99m}\text{Tc}$ -DPD) (middle) or  $^{99m}\text{Tc}$ -methylene diphosphonate ( $^{99m}\text{Tc}$ -MDP) (bottom) as the tracer in a group A patient with transthyretin amyloidosis who underwent combined heart-liver transplantation (left) and a group B patient with monoclonal immunoglobulin light-chain (AL) amyloidosis (right). Two paradoxes are apparent: 1) despite similar left ventricular (LV) thickness and amount of amyloidotic infiltration in the two patients, myocardial  $^{99m}\text{Tc}$ -DPD uptake is characteristically strong (without bone uptake) in the patient with TTR-related amyloidosis and absent in the patient with AL amyloidosis (who had only bone uptake); 2) conversely, use of a different tracer ( $^{99m}\text{Tc}$ -MDP) results in absence of visually detectable myocardial uptake (accompanied by normal bone uptake) in both patients. The macrohistologic sections show pale gray-blue amyloid infiltrations at Azan Mallory trichrome staining, whereas Congo-red staining (lower insets) shows typical green birefringence under cross-polarized light microscopy. In the patient with TTR, immunohistochemical staining against TTR is positive (upper inset). RV = right ventricle.

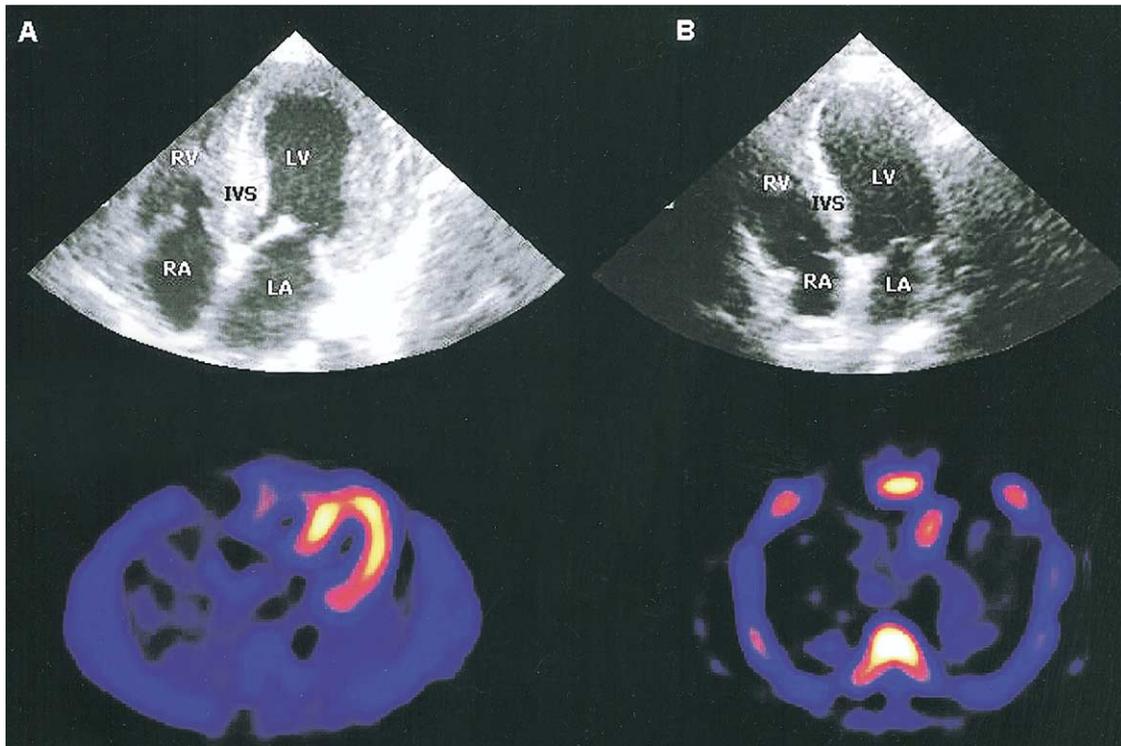
model currently exists. Onset of amyloidosis is a very complex process, eventually leading to tissue deposition of amyloid fibrils (34). Although amyloid fibrils share a common chemical structure (i.e., serum amyloid protein) irrespective of etiology, some characteristics vary between specific etiologies (allowing differential immunostaining). We can only speculate that  $^{99m}\text{Tc}$ -DPD might bind to specific fragments of the mutated TTR protein or of the wild-type protein (present in the senile form), and that this phenomenon occurs predominantly in the myocardium either because of the large amount of amyloid or some unknown features of its spatial arrangement at this level.

It should be noted that in our study sample,  $^{99m}\text{Tc}$ -MDP, unlike  $^{99m}\text{Tc}$ -DPD, did not show selective myocardial uptake in 11 patients with TTR-related cardiac amyloidosis. This finding agrees with one reported case (9), but contrasts with a further two (19,20) with an apparently definite diagnosis of TTR-related cardiac amyloidosis.

Our findings have potential clinical implications in a clinical setting, where misdiagnosis rates between hereditary TTR and AL amyloidosis can be as high as 7% (7) and

where the repercussions can be disastrous (chemotherapy with/without bone marrow transplantation instead of OLT). In patients with diagnosis of cardiac amyloidosis associated with monoclonal gammopathy, negative  $^{99m}\text{Tc}$ -DPD scintigraphy would reinforce a diagnostic hypothesis of AL etiology, whereas a positive test result would direct the diagnostic workup toward DNA analysis and immunohistochemistry. Also,  $^{99m}\text{Tc}$ -DPD scintigraphy could be used as an orientative diagnostic step in cases of suspected senile (wild-type TTR) cardiac amyloidosis.

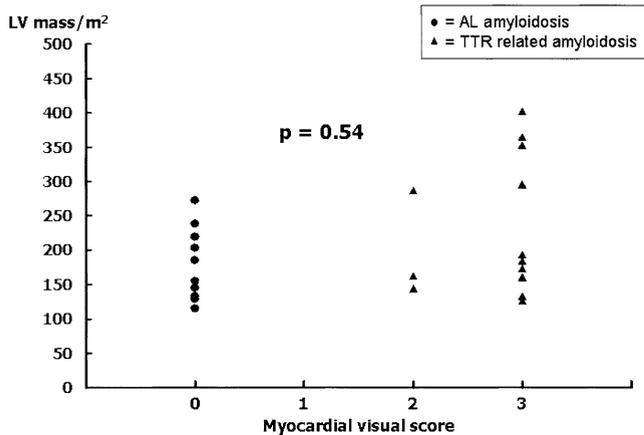
**Study limitations.** Considering the restricted number of patients (especially in the AL cardiac amyloidosis subgroup), independent confirmation is required on a larger study sample. Our tertiary center included cardiology/neurology units particularly active in the field of amyloidosis, and especially familial amyloidotic polyneuropathy. Therefore, the results cannot automatically be extended to populations with a lower prevalence of the disease. It should be noted that our study does not provide information on the rarer hereditary forms of amyloidosis related to mutations of apolipoprotein A, fibrinogen, lysozyme, or the acquired AA type.



**Figure 4.** Apical four-chamber-view echocardiograms (top row) and cross-sectional views of cardiac single-photon emission computed tomography (SPECT) (bottom row) in two patients with transthyretin (TTR)-related cardiac amyloidosis. Topographic correspondences between increased left ventricular parietal thickness and <sup>99m</sup>Tc-3,3-diphosphono-1,2-propanodicarboxylic acid (<sup>99m</sup>Tc-DPD) uptake is evident: (A) increased thickness of both the interventricular septum and the lateral wall of the left ventricle at echocardiography in correspondence with diffuse myocardial <sup>99m</sup>Tc-DPD uptake at SPECT; (B) increased thickness of the medial portion of the interventricular septum (with normal lateral wall) at echocardiography in correspondence with localized myocardial <sup>99m</sup>Tc-DPD uptake at the same level. IVS = intraventricular septum; LA = left atrium; LV = left ventricle; RA = right atrium; RV = right ventricle.

**Conclusions.** Etiology is an important determinant of <sup>99m</sup>Tc-DPD scintigraphic variability in cardiac amyloidosis, a highly relevant consideration for future studies. Our analysis indicates that <sup>99m</sup>Tc-DPD scintigraphy could offer a useful first step in the workup of differential diagnosis of TTR versus AL etiology in patients with documented cardiac amyloidosis.

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**Figure 5.** Distribution of left ventricular (LV) mass values according to visual scores at myocardial <sup>99m</sup>Tc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy. No relationship between the two variables is discernible ( $p = 0.54$  by Kruskal-Wallis test). AL = monoclonal immunoglobulin light-chain; TTR = transthyretin.

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