Cardiac amyloidosis is an underappreciated and underdiagnosed cause of heart failure. Although often considered as a single entity attributable to extracellular deposition of fibrillary proteins, there are at least 3 different pathophysiologic substrates for cardiac amyloidosis that have differing clinical courses and require distinctly different treatment. In cardiac amyloid light-chain (AL), the fibrils are composed of immunoglobulin light chains that are produced by a clonal population of plasma cells in the bone marrow. Treatment involves chemotherapeutic agents targeted at the plasma cell. In transthyretin-related cardiac amyloidosis (ATTR), misfolded monomers or dimers of the normally tetrameric transthyretin protein (TTR) from either mutant TTR (ATTRm, also referred to as familial amyloid cardiomyopathy) or wild-type TTR (ATTRwt, also known as senile systemic amyloidosis) deposit in the myocardium. ATTRm is caused by >100 mutations in the TTR protein that are inherited in an autosomal dominant fashion and can affect individuals of all ages whereas ATTRwt is predominately described in older adult males.

The diagnosis of cardiac amyloidosis, however, and subsequent differentiation of amyloid light-chain from ATTR, remains challenging, and misdiagnosis is associated with potential for significant harm. Clinically, signs and symptoms of cardiac amyloidosis often overlap with other causes of heart failure, and electrocardiographic and echocardiographic features can be nonspecific. Currently, the gold standard for definitive diagnosis is endomyocardial biopsy coupled with emerging therapies.

99mTc-Pyrophosphate Scintigraphy for Differentiating Light-Chain Cardiac Amyloidosis From the Transthyretin-Related Familial and Senile Cardiac Amyloidoses

Sabahat Bokhari, MD; Adam Castaño, MD; Ted Pozniakoff, BS, RT(N)(R); Susan Deslisle, MS; Farhana Latif, MD; Mathew S. Maurer, MD

Background—Differentiating amyloid light-chain (AL) from transthyretin-related cardiac amyloidoses (ATTR) is imperative given implications for prognosis, therapy, and genetic counseling. We validated the discriminatory ability of 99mTc-pyrophosphate (99mTc-PYP) scintigraphy in AL versus ATTR.

Methods and Results—Forty-five subjects (12 AL, 16 ATTR wild type, and 17 ATTR mutants) underwent 99mTc-PYP planar and single-photon positive emission computed tomography cardiac imaging. Scans were performed by experienced nuclear cardiologists blinded to the subjects’ cohort assignment. Cardiac retention was assessed with both a semiquantitative visual score (range, 0; no uptake to 3, diffuse uptake) and by quantitative analysis by drawing a region of interest over the heart corrected for contralateral counts and calculating a heart-to-contralateral ratio. Subjects with ATTR cardiac amyloid had a significantly higher semiquantitative cardiac visual score than the AL cohort (2.9±0.06 versus 0.8±0.27; P<0.0001) as well as a higher quantitative score (1.80±0.04 versus 1.21±0.04; P<0.0001). Using a heart-to-contralateral ratio >1.5 consistent with intensely diffuse myocardial tracer retention had a 97% sensitivity and 100% specificity with area under the curve 0.992, P<0.0001 for identifying ATTR cardiac amyloidosis.

Conclusions—99mTc-PYP cardiac imaging distinguishes AL from ATTR cardiac amyloidosis and may be a simple, widely available method for identifying subjects with ATTR cardiac amyloidosis, which should be studied in a larger prospective manner. (Circ Cardiovasc Imaging. 2013;6:195-201.)

Key Words: 99mTc-PYP scintigraphy ■ AL amyloid ■ ATTR transthyretin cardiomyopathy ■ technetium

The most common ATTRm allele in the United States, the valine to isoleucine substitution at position 122 (V122I), is found in ≈3.5% of blacks.ATTRwt cardiomyopathy has been found at autopsy in >30% of patients with heart failure with a preserved ejection fraction >75 years. These latter forms of cardiac amyloidosis are becoming increasingly recognized, in part, because of the aging of the population, enhancements in the understanding of the disease’s pathobiology, and the potential benefit from emerging therapies.

The diagnosis of cardiac amyloidosis, however, and subsequent differentiation of amyloid light-chain from ATTR, remains challenging, and misdiagnosis is associated with potential for significant harm. Clinically, signs and symptoms of cardiac amyloidosis often overlap with other causes of heart failure, and electrocardiographic and echocardiographic features can be nonspecific. Currently, the gold standard for definitive diagnosis is endomyocardial biopsy coupled with emerging therapies.

Received September 5, 2012; accepted January 8, 2013.

From the Nuclear Cardiology Laboratory (S.B., T.P.) and Clinical Cardiovascular Research Laboratory for the Elderly, Center for Advanced Cardiac Care, Division of Cardiology (A.C., S.D., F.L., M.S.M.), Columbia College of Physicians & Surgeons, New York, NY.

Correspondence to Mathew S. Maurer, MD, Clinical Cardiovascular Research Laboratory for the Elderly, Center for Advanced Cardiac Care, Division of Cardiology, Columbia College of Physicians & Surgeons, 622 W 168th St, P.H. 12–1291, New York, NY. E-mail msm10@columbia.edu

© 2013 American Heart Association, Inc.

Circ Cardiovasc Imaging is available at http://circimaging.ahajournals.org

DOI: 10.1161/CIRCIMAGING.112.000132
either immunohistochemistry\(^9,10\) or in cases in which this is inconclusive, mass spectroscopy.\(^11\) Unfortunately, these diagnostic requirements are typically performed only in specialized centers with particular expertise, do not provide sufficient information about the extent or distribution of cardiac amyloidosis, disease progression, or response to treatment, and in practice can lead to delayed care. Additionally, many older adults are reluctant to undergo invasive procedures.

Therefore, a clinical unmet need in this arena is the development of a noninvasive imaging modality that can diagnose cardiac amyloid, differentiate AL from ATTR subtypes, quantify the extent of myocardial amyloid infiltration, and monitor disease progression and response to treatment. Nuclear scintigraphy holds promise for noninvasive diagnosis and has potential as a tool for ongoing follow-up of disease progression. Recent reports from European investigators have demonstrated that \(^99m\text{Tc}-3,3\)-diphenyl-1,2-propanodicarboxylic acid (\(^99m\text{Tc}-\text{DPD}\)) scintigraphy is useful in distinguishing AL from ATTR amyloid\(^12\) and may have prognostic significance.\(^13\) However, this isotope is not available for use in the United States. Previous reports regarding the utility of \(^99m\text{Tc}\)-pyrophosphate (\(^99m\text{Tc}-\text{PYP}\)) were confounded by the grouping of patients with AL amyloidosis together with ATTR amyloid patients and by the lack of modern quantitative imaging techniques to measure isotope uptake. Accordingly, we aimed to validate the discriminatory ability of \(^99m\text{Tc}-\text{PYP}\) in subjects with AL versus ATTR-related cardiac amyloidosis secondary to wild-type and several different mutant variants, including the most common in the United States, the V122I mutation.

**Methods**

**Patient Population**

Patients with biopsy-proven AL or ATTR undergoing routine follow-up at the Columbia College of Physicians & Surgeons Center for Advanced Cardiac Care participated in this study. Forty-five patients (12 AL, 16 ATTR\(\text{wt}\), and 17 ATTR\(\text{m}\)) were enrolled. Inclusion criteria for the diagnosis of cardiac amyloidosis were one of the following: (1) biopsy-proven cardiac amyloidosis (\(n=37\)); (2) in the absence of an endomyocardial biopsy, histological documentation of Congo red staining in at least 1 involved organ with echocardiographically defined evidence of amyloid cardiomyopathy (thickness of the left ventricular [LV] septum or posterior wall of \(\geq12\) mm without another cause of LV hypertrophy; \(n=5\)); or (3) documented amyloidogenic TTR mutation by DNA analysis and echocardiographically defined evidence of amyloid cardiomyopathy without evidence of a plasma cell dyscrasia (\(n=3\)). Exclusion criteria included women of childbearing potential, minors, inability to provide informed consent, and inability to lie still for 15 minutes under the camera. All patients provided written informed consent. The study protocol was approved by the Columbia Joint Radiation Safety Committee and Institutional Review Board.

**Study Design**

This was a single-center, blinded, prospective cohort study aimed at evaluating whether \(^99m\text{Tc}-\text{PYP}\) could differentiate AL from ATTR cardiac amyloidosis in 45 subjects. All subjects underwent a single \(^99m\text{Tc}-\text{PYP}\) cardiac imaging scan as described below. Scans were performed and interpreted by experienced nuclear cardiologists blinded to the subjects’ cohort assignment.

\(^99m\text{Tc}-\text{PYP}\) Single-photon Positive Emission Computed Tomography Scintigraphy

Planar imaging with \(^99m\text{Tc}-\text{PYP}\) was performed with a dual head Philips Precedence SPECT/CT camera (Philips Healthcare, Guildford, United Kingdom) equipped with low-energy, high-resolution collimators. Patients received 15 to 25 mCi of \(^99m\text{Tc}-\text{PYP}\) intravenously and anterior, lateral, and left anterior oblique planar views were obtained at 1 hour over 8 minutes duration. The planar images were acquired for a total of 750,000 counts, with the heart centered in the field of view. The acquisition parameters used for planar imaging were 256x256 matrix with 1.46 zoom factor. The single-photon positive emission computed tomography (SPECT) imaging was performed if there was myocardial uptake of \(^99m\text{Tc}-\text{PYP}\) on planar images. Acquisition parameters for the SPECT imaging were low-energy, high-resolution collimators, matrix 64x64x64 with 1.46 zoom. The Butterworth filter was used with a cut off of 0.50 and order of 5.00.

For the primary analysis, which was based on myocardial tracer uptake, 2 methods were used: (1) semiquantitative visual scoring of cardiac retention (0=absent cardiac uptake, 1=mild uptake less than bone, 2=moderate uptake equal to bone, 3=high uptake greater than bone), and (2) quantitative analysis of heart retention was calculated by drawing a region of interest (ROI) over the heart in the standard manner (Figure 1). A circular ROI was drawn over the heart, copied, and mirrored over the contralateral chest to normalize for the spill-over from the ribs. Mean total and absolute counts were measured correcting for background counts, and the fraction of mean counts in the heart ROI-to-contralateral chest ROI was calculated as the heart-to-contralateral (H/CL) ratio.

**Statistical Analyses**

Demographic, laboratory, and imaging data were collected and analyzed with descriptive statistics using mean±SE for continuous variables and as relative percentages for categorical variables. Statistical analyses were performed using Statistical Analysis Software (SAS Institute, Inc., Cary, NC).

For comparisons between study subgroups, differences in continuous variables were analyzed using a 1-way ANOVA with post hoc Bonferroni correction, and differences in categorical variables were analyzed using the \(\chi^2\) test or when appropriate, Fisher exact test. Multivariate logistic regression analysis using a forward selection model was performed to evaluate for factors independently associated with the H/CL ratio including group (ATTR versus AL), age, LV wall thickness, estimate glomerular filtration rate, and calcium levels. All \(P\) values used were 2 sided, with \(P<0.05\) considered significant.

**Figure 1.** A and B. Semiquantitative method of calculating the distribution of \(^99m\text{Tc}\)-pyrophosphate (\(^99m\text{Tc}-\text{PYP}\)) uptake. Raw images of a representative negative (A) and positive subject (B) are shown 1 hour after radiotracer infusion. ROI circles are depicted in red, and the contralateral comparison circle is depicted in blue. C/L indicates contralateral; cts, counts; ROI, region of interest; and Std Dev, standard deviation.
Results

Demographics of Study Population

Forty-five patients with cardiac amyloidosis (12 AL, 16 ATTRwt, and 17 ATTRm) were enrolled and completed the study protocol. Of the patients with ATTRm cardiac amyloidosis, the following TTR mutations were included: Val122Ile (n=12), Thr60Ala (n=2), Ser23Asn (n=1), Thr59Lys (n=1), and Ala120Ser (n=1). The demographic, clinical, and echocardiographic features of the 3 groups are shown in Table 1. Subjects were, on average predominately male (84%) older adults with a mean of 70±2 years of age. Those with ATTRwt were older than those in the AL group (P=0.0008), whereas those with ATTRm were predominantly black given the known demographics of the condition and the strong association of the V122I mutation with black race. At baseline, individuals presented with a phenotype consistent with cardiac amyloidosis as described previously.14 Functionally, these symptoms translated to 31% with New York Heart Association Class III/IV heart failure with an average ejection fraction of 45±2% that did not differ between groups.

Assessment of serum biomarkers, troponin I, brain natriuretic peptide, and modified body mass index, a reflection of cardiac cachexia,15 did not differ between cohorts, suggesting similar degrees of disease severity. Calcium levels were higher in ATTR than AL subjects but when corrected for decrements in albumin (as some subjects with AL amyloid had concomitant nephrotic syndrome with a low serum albumin) differences were no longer observed. Thus, although calcium levels were significantly correlated with the H/CL ratio (r=0.36; P=0.02), there was no correlation for corrected calcium levels (r=0.14; P=0.36).

As previously reported,2 subjects with ATTRwt cardiac amyloidosis had significantly increased LV wall thickness and hence greater LV mass compared with AL or ATTRm groups, respectively. A vast majority of subjects across all groups had an abnormal ECG characteristic of amyloidosis evidenced by baseline low-QRS voltage, an infarct pattern,16 or both; 20% had a pacemaker defibrillator.

99mTc-PYP SPECT Imaging

Representative examples of 99mTc-PYP uptake among subjects and controls are shown in Figure 1. Semiquantitative visual cardiac scores were significantly higher in patients with ATTR cardiac amyloidosis than in the AL cohort (2.9±0.6 versus 0.8±0.27; P<0.0001). Two AL patients had more intense uptake than other AL subjects. The first, who was assigned a visual score of 3, had a history of myocardial infarction and was the only subject across groups whose distribution of myocardial uptake was focal. The second, who received a visual score of 2, had no history of myocardial infarction and had diffuse myocardial tracer uptake. One ATTRm patient with an unusual TTR mutation (Thr59Lys) but who did not

| Table 1. Baseline Mean Clinical, Laboratory, and Echocardiographic Characteristics |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Category                        | Total (n=45)    | AL (n=12)       | ATTRwt (n=16)   | ATTRm (n=17)    | P Value         |
| Clinical                        |                |                 |                 |                 |                 |
| Age, y                          | 70±2           | 63±3            | 77±2*           | 70±3            | 0.0017          |
| Male sex, %                     | 84             | 75              | 100             | 82              | 0.1483          |
| White race, %                   | 60             | 67              | 88              | 29              | <0.0001         |
| Black race, %                   | 24             | 0               | 65              | 65              | <0.0001         |
| NYHA functional class           | 2.3±0.1        | 2.4±0.2         | 2.2±0.2         | 2.2±0.1         | 0.65            |
| Laboratory                      |                |                 |                 |                 |                 |
| Troponin I, ng/mL               | 0.3±0.2        | 0.7±0.6         | 0.1±0.03        | 0.1±0.02        | 0.34            |
| BNP, pg/mL                      | 808±103        | 692±246         | 720±115         | 826±182         | 0.86            |
| mBMI, kg/m2                     | 108±4          | 105±12          | 108±5           | 110±5           | 0.90            |
| Calcium, mg/dL                  | 9.3±0.1        | 9.0±0.7         | 9.5±0.6         | 9.5±0.5         | 0.0391          |
| Correct calcium, mg/dL          | 9.4±0.1        | 9.2±0.7         | 9.3±0.7         | 9.5±0.4         | 0.4530          |
| Creatinine, mg/dL               | 1.5±0.1        | 1.6±0.4         | 1.7±0.5         | 1.2±0.4         | 0.0052          |
| eGFR, mL/(min·m²)               | 70±4.7         | 71±30           | 54±19           | 86±36           | 0.0124          |
| Echocardiographic               |                |                 |                 |                 |                 |
| Abnormal ECG, %                 | 89             | 83              | 94              | 88              | 0.85            |
| LV ejection fraction, %         | 45±2           | 52±4            | 48±4            | 39±4            | 0.08            |
| LV end-diastolic diameter, cm   | 4.2±0.1        | 4±0.1           | 4.3±0.2         | 4.4±0.1         | 0.45            |
| Interventricular septal thickness, cm | 1.7±0.06       | 1.6±0.1         | 1.9±0.1†        | 1.5±0.1         | 0.0078          |
| LV posterior wall thickness, cm | 1.6±0.05       | 1.6±0.1         | 1.8±0.1†        | 1.4±0.1         | 0.0131          |
| LV mass, g/m²                   | 291±14         | 255±22          | 358±24†         | 253±14          | 0.0014          |

Continuous data are expressed as mean±±SE, and categorical data are expressed as percentages. AL indicates amyloid light-chain; ATTRm, mutant transthyretin amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; LV, left ventricular; mBMI, modified body mass index; and NYHA, New York Heart Association.

*P<0.05 by ANOVA with Bonferroni correction in comparison to AL.
†P<0.05 by ANOVA with Bonferroni correction in comparison to ATTRm.
have a thickened myocardium relative to other ATTR patients received a lower than expected visual score of 1.

For quantitative scoring of cardiac tracer uptake (Table 2), subjects with ATTR cardiac amyloidosis had higher absolute counts within the heart ROI than those with AL amyloid (29±2 versus 22±3; P=0.04) overall, but the trend across the 3 groups was not statistically significant (P=0.11). Accordingly, we indexed the absolute heart ROI counts according to the absolute background counts over the contralateral chest as the H/CL ratio. This ratio was significantly higher among ATTR patients as compared with AL patients (1.80±0.04 versus 1.21±0.04) as well and was significant by ANOVA (P<0.0001). Receiver operating characteristic curves demonstrated an area of 0.992, P<0.001 for distinguishing ATTR and AL cardiac amyloidosis with a ratio of H/CL >1.5 consistent with intensely diffuse myocardial tracer retention having a 97% sensitivity and 100% specificity for identifying ATTR cardiac amyloidosis (Figure 2). When analyzing heart total counts per ROI, measurements were also significantly greater in ATTR subjects as compared with AL subjects (P=0.0001). Heart maximum counts per pixel were also significantly higher in the ATTRm group than in the AL group (P=0.01).

99mTc-PYP myocardial uptake as measured by the H/CL ratio correlated with LV septal wall thickness (0.3172; confidence interval, 2.6–37.5). CL ratio was ATTR versus AL amyloid (odds ratio, 9.8; 95% CI, 2.6–37.5) was significantly greater in ATTR subjects as compared with AL patients (P=0.001). Heart maximum counts per pixel were also significantly higher in the ATTRm group than in the AL group (P=0.01).

Quantitative and semiquantitative data were obtained 1 hour post 99mTc-PYP infusion. The result of this study confirms that 99mTc-PYP cardiac imaging can differentiate ATTR from AL cardiac amyloidosis. Our findings are relevant to the noninvasive differential diagnosis of cardiac amyloidosis, clarify conflicting data in prior reports of bone-seeking radiotracers, and may have clinical implications for noninvasive identification of affected individuals and in the follow-up of disease progression and response to therapy.

Observations during the 1970s and 1980s of myocardial uptake during whole-body planar imaging with bone-seeking radiotracers roused suspicion for cardiac amyloidosis and were subsequently confirmed by tissue biopsy. Various groups went on to investigate the utility of imaging cardiac amyloidosis with different bone-seeking radiotracers, including 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid, 99mTc-methylene diphosphonate, and 99mTc-PYP. The precise mechanism by which these bone-seeking radiotracers accumulate in the myocardium of patients with cardiac amyloidosis remains unclear but may be related to high calcium levels in amyloidosis.17–18 Moreover, the mechanism by which 99mTc-PYP distinguishes ATTR from AL amyloidosis remains to be elucidated. One hypothesis is that 99mTc-PYP may bind TTR amyloid fibrils more intensely than AL fibrils as a result of higher calcium containing compounds in ATTR hearts. Pepys et al19 observed that the normal human serum amyloid protein P (SAP) binds many different types of amyloid fibrils with a high degree of affinity and in a highly specific calcium-dependent manner.20 Additionally, since SAP self-aggregation is enhanced by the presence of calcium11 and is resistant to proteases in the presence of calcium,22 perhaps varying degrees of calcium in different amyloid subtypes may account for different levels of affinity and in a highly specific calcium-dependent manner.

### Table 2. 99mTc-PYP SPECT Data According to Amyloid Subtype

<table>
<thead>
<tr>
<th>Category</th>
<th>AL (n=12)</th>
<th>ATTRwt (n=16)</th>
<th>ATTRm (n=17)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Semiquantitative visual cardiac score, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Score &lt;2</td>
<td>83</td>
<td>0</td>
<td>6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Score ≥2</td>
<td>17</td>
<td>100</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td><strong>Quantitative Cardiac Score, cts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart absolute cts</td>
<td>21.8±2.7</td>
<td>27.7±0.9</td>
<td>30.4±3.6</td>
<td>0.10</td>
</tr>
<tr>
<td>CL absolute cts</td>
<td>18.1±2.2</td>
<td>15.3±0.6</td>
<td>17.5±2.4</td>
<td>0.48</td>
</tr>
<tr>
<td>H/CL ratio</td>
<td>1.21±0.04</td>
<td>1.84±0.06</td>
<td>1.77±0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Heart SD mean cts</td>
<td>5.4±0.3</td>
<td>6.5±0.2</td>
<td>6.7±0.5</td>
<td>0.033</td>
</tr>
<tr>
<td>CL SD mean cts</td>
<td>4.4±0.3</td>
<td>4.6±0.1</td>
<td>4.7±0.4</td>
<td>0.73</td>
</tr>
<tr>
<td>Heart mean max cts/pix</td>
<td>40.2±3.7</td>
<td>52.0±1.8</td>
<td>54.2±4.8</td>
<td>0.041</td>
</tr>
<tr>
<td>CL mean max cts/pix</td>
<td>35.3±3.1</td>
<td>34.6±0.8</td>
<td>35.5±3.2</td>
<td>0.88</td>
</tr>
<tr>
<td>Heart mean total cts/ROI</td>
<td>38966±4528</td>
<td>60709±4423</td>
<td>58346±4764</td>
<td>0.008</td>
</tr>
<tr>
<td>CL mean total cts/ROI</td>
<td>32132±3591</td>
<td>33904±1724</td>
<td>35285±3140</td>
<td>0.72</td>
</tr>
<tr>
<td>Heart mean area ROI, mm²</td>
<td>4672±417</td>
<td>5356±302</td>
<td>5303±336</td>
<td>0.36</td>
</tr>
<tr>
<td>CL mean area ROI, mm²</td>
<td>4619±412</td>
<td>5295±298</td>
<td>5262±342</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Continuous data are expressed as means± SE, and categorical data are expressed as percentages.

Semiquantitative and quantitative data were obtained 1 hour post 99mTc-PYP infusion.

99mTc-PYP indicates technetium pyrophosphate; AL, amyloid light-chain; ATTRm, mutant transthyretin amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; CL, contralateral; Cts, counts; CL, contralateral; H/CL ratio, heart-to-contralateral ratio; pix, pixels; ROI, region of interest; and SPECT, single-photon positive emission computed tomography.

*P=0.05 by ANOVA with Bonferroni correction in comparison to AL.
The only independent variable associated with myocardial uptake of 99mTc-PYP relates to the duration over which amyloid deposition has occurred in the affected tissue. In AL patients, 99mTc-DPD myocardial uptake is of prognostic value for predicting major adverse cardiac events, either alone or in combination with LV wall thickness. Therefore, it seems that 99mTc-DPD scanning can assist in the differential diagnosis of ATTR and AL cardiac amyloidosis when tracer retention is either intense or absent, respectively (intermediate 99mTc-DPD myocardial uptake was concluded to be of indeterminate significance), and has prognostic significance, leading to its widespread use among amyloid centers in Europe. However, this isotope is not approved by the Food and Drug Administration and thus is not available for clinical use in the United States. Regarding other radiotracers, 99mTc-methylene diphosphonate is not approved by the United States Food and Drug Administration, and thus is not available for clinical use in the United States. However, 99mTc-PYP scintigraphy has not yet been established for the noninvasive evaluation of cardiac amyloidosis for several reasons: results to date have been in large part conflicting and with variable sensitivity; amyloid subtype was not defined in many of the early studies and those that defined it were limited to ATTRw patients only, missing the most common ATTRm allele in the United States, V122I; and finally, most studies have analyzed scans using a semiquantitative visual scoring system, not a quantitative method. In a 1982 report, intensely diffuse cardiac uptake of 99mTc-PYP was reported in all 10 subjects with biopsy-proven cardiac amyloidosis, suggesting that 99mTc-PYP scintigraphy might function as a useful adjunct to biopsy and echocardiographic imaging in the diagnosis of amyloid heart disease. However, amyloid subtype was not defined in this study. In a larger study in 34 patients all of whom had biopsy-proven amyloidosis but where subtype was not defined and not all subjects had cardiac involvement, only 3 of 14 retrospectively reviewed cases had intense 99mTc-PYP myocardial uptake and 17 of 20 prospectively reviewed cases had abnormal scans. Of these 17, 14 had only mild uptake, which was similar to 15 of 20 control subjects. Based on these results, 99mTc-PYP was judged not to be sufficiently sensitive to warrant routine screening in patients with cardiac amyloidosis. However, in addition to the above mentioned limitations, this study did not measure myocardial tracer uptake in a quantitative fashion (only semiquantitatively).

Most recently, Yamamoto et al described a quantitative method, the PYP score, to assess the utility of 99mTc-PYP to evaluate for cardiac amyloidosis in 13 subjects with heart failure due to amyloid (1 AL, 3 ATTRm, 8 ATTRw) and 37 subjects with heart failure attributable to nonamyloid causes. PYP score, defined as the ratio of myocardial mean counts to ventricular cavity mean counts, was found to have a sensitivity of
84.6% and specificity of 94.5% for distinguishing cardiac amyloidosis from nonamyloid causes of heart failure. However, to the best of our knowledge, no study has compared AL subjects against ATTRwt and ATTRm groups using 99mTc-PYP and the quantitative methodology we describe here. Our study sheds light on the fact that although AL subjects may indeed have mild uptake with varying degrees of sensitivity compared with normal controls, quantification of counts using standard ROI technique adjusted for background counts over the contralateral chest as a H/CL ratio provides a sensitive and specific numerical index for the diagnosis of and differentiation between AL (H/CL<1.5) and ATTR (H/CL>1.5) cardiac amyloidosis.

This technique, although sensitive and specific, is not perfect as demonstrated by a single AL subject who had increased focal tracer retention that was attributable to a previous myocardial infarction and a single ATTR subject with a false-negative scan result with minimal increase in wall thickness attributable to the unusual TTR mutation, Thr59Lys. Accordingly, qualitative analyses to identify diffuse versus focal uptake, the latter of which is characteristic of a myocardial infarction, has added clinical value to quantitative and semiquantitative approaches. Understanding that unrecognized myocardial infarction is a known reason for technetium uptake in the myocardium is characteristic of a myocardial infarction, has added clinical value to quantitative and semiquantitative approaches. The utility of this approach to identify patients prospectively with ATTR cardiac amyloidosis is dependent on the magnitude of myocardial amyloid infiltration and that intense 99mTc-PYP uptake reflects a thick-walled heart seen in advanced stages of cardiac amyloidosis. Notably, wall thickness was not that dissimilar in our AL cohort compared with ATTRm (IVS 1.6 cm versus 1.5 cm, respectively), yet H/CL ratio was significantly higher in ATTRm patients, suggesting that 99mTc-PYP possesses a unique affinity for the TTR fibril. Regardless, this approach may not be useful for early identification of cardiac amyloidosis in affected individuals with less severe phenotypes. Further work is needed to examine whether 99mTc-PYP has diagnostic utility in genotype-positive, phenotype-negative individuals with TTR mutations and whether this technique is useful in monitoring disease progression and even response to therapy.

Although reliable confirmation of amyloid is important, diagnosis of the specific pathogenic subtype early in the disease course is essential for improving outcomes because all current therapies for ATTR amyloid are targeted at preventing further deposition of amyloid fibrils but do not remove fibrils from the myocardium. In a previous study that examined clinical features and outcomes in 58 ATTR patients, we found that despite the ability to test for the V122I allele, these patients typically present later and at a more advanced stage of cardiac disease than ATTRw subjects in whom serological testing for early diagnosis is not available. Stated another way, 99mTc-PYP scanning may facilitate earlier differentiation of AL and ATTR cardiac amyloidosis while arrangements for confirmatory tissue biopsy are underway. Further prospective studies using the imaging technique and H/CL ratio >1.5 determined to be sensitive and specific for ATTR cardiac amyloidosis are warranted.

Several limitations to our investigation are worth noting. This was a small single-center study. However, to the best of our knowledge, this is the largest study to date with AL, ATTRwt, and ATTRm pathogenic subtypes that specifically focused on the utility of 99mTc-PYP cardiac imaging. A large percentage of the ATTRm subjects had the V122I mutation, the most common ATTRm allele in the United States. Future studies in larger cohorts that include the spectrum of TTR mutations will determine the utility of this technique in identifying cardiac involvement in other TTR mutations, although our preliminary data suggests excellent performance irrespective of mutation. The generalizability of these results to other populations is unknown. Indeed, many of these subjects enrolled had severe phenotypes with markedly thickened LV walls, which although similar to other cohorts of patients with cardiac amyloidosis, may be characterized by enhanced uptake of 99mTc-PYP. Future studies will need to evaluate the utility of this approach to identify patients prospectively with ATTR cardiac amyloidosis with less severe phenotypes. The cross sectional nature of this study and the absence of serial scanning provides no information on the ability of this technique to monitor progression of disease over time, but this is a focus on ongoing investigation. Finally, the mechanism by which 99mTc-PYP binds to ATTR more than AL fibrillar deposits remains to be elucidated.

In conclusion, 99mTc-PYP SPECT is able to distinguish AL from ATTR cardiac amyloidosis and may be a simple, widely available method for identifying subjects with ATTR cardiac amyloidosis which should be studied in a larger prospective manner.

Acknowledgments
We would like to acknowledge the patients with cardiac amyloidosis who participated in this study and who continue to hope for methods to improve outcomes including earlier and more efficient diagnosis and better therapeutics.

Sources of Funding
Pfizer, Inc. provided a restricted grant to support the imaging performed as part of this study.

Disclosures
Dr Maurer serves on the Executive Board of THAOS (Transthyretin Amyloid Outcomes Survey), an international registry of patients with ATTR amyloidosis that is funded by FoldRx Pharmaceuticals, Inc, a wholly owned subsidiary of Pfizer, Inc.

References
99mTc-pyrophosphate imaging may serve as a noninvasive adjunct in the differential diagnosis of cardiac amyloidosis. The implications for prognosis, therapy, and genetic counseling. We validated the discriminatory ability of 99mTc-pyrophosphate scintigraphy in AL versus TTR cardiac amyloidoses in a population of 45 subjects with biopsy-proven cardiac amyloidosis. 99mTc-pyrophosphate imaging distinguishes AL from TTR cardiac amyloidosis. Subjects with TTR cardiac amyloidosis had a significantly higher semiquantitative cardiac visual score than the AL cohort (2.9±0.06 versus 0.8±0.27; P<0.0001 for identifying TTR cardiac amyloidosis. This has important diagnostic implications. 99mTc-pyrophosphate myocardial uptake and peripheral neuropathy in a rare variant of familial transthyretin (TTR) amyloidosis (Ser23Asn): a case report and literature review. Amyloid. 2012;19:41–46.


99mTc-Pyrophosphate Scintigraphy for Differentiating Light-Chain Cardiac Amyloidosis From the Transthyretin-Related Familial and Senile Cardiac Amyloidoses

Sabahat Bokhari, Adam Castaño, Ted Pozniakoff, Susan Deslisle, Farhana Latif and Mathew S. Maurer

_Circ Cardiovasc Imaging_. 2013;6:195-201; originally published online February 11, 2013;
doi: 10.1161/CIRCIMAGING.112.000132

_Circulation: Cardiovascular Imaging_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-9651. Online ISSN: 1942-0080

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circimaging.ahajournals.org/content/6/2/195

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation: Cardiovascular Imaging_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation: Cardiovascular Imaging_ is online at:
http://circimaging.ahajournals.org/subscriptions/