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

Bokhari et al: 99mTc-PYP Scintigraphy for Differentiating AL from ATTR and Senile cardiac Amyloidoses

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Abstract

Background—Differentiating immunoglobulin 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 $^{99m}$Tc-pyrophosphate scintigraphy ($^{99m}$Tc-PYP) in AL vs. TTR-related cardiac amyloidoses.

Methods and Results—45 subjects (12 AL, 16 ATTR wild-type, and 17 ATTR mutants) underwent $^{99m}$Tc-PYP planar and single-photon positive emission computed tomography (SPECT) cardiac imaging. Scans were performed by experienced nuclear cardiologists blinded to the subjects’ cohort assignment. Cardiac retention was assessed with both a semi-quantitative visual score (range 0, no uptake to 3, diffuse uptake) and by quantitative analysis by drawing a region of interest (ROI) over the heart corrected for contralateral counts and calculating a heart-to-contralateral ratio (H/CL). Subjects with ATTR cardiac amyloid had a significantly higher semi-quantitative cardiac visual score than the AL cohort (2.9±0.06 vs. 0.8±0.27, p<0.0001) as well as a higher quantitative score (1.80±0.04 vs.1.21±0.04, p<0.0001). Using a H/CL 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—$^{99m}$Tc-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.

Key Words: AL amyloid, ATTR transthyretin cardiomyopathy, technetium, 99m-TcPYP scintigraphy

Abbreviations: AL=amyloid light-chain; ATTR=transthyretin amyloidosis; EF=ejection fraction; HFpEF=heart failure preserved ejection fraction; ROI=region of interest; SPECT=single-photon positive emission computed tomography; $^{99m}$Tc-PYP=technetium pyrophosphate
Cardiac amyloidosis is an underappreciated and under-diagnosed cause of heart failure\textsuperscript{1}. While often considered as a single entity attributable to extracellular deposition of fibrillary proteins, there are at least three different pathophysiologic substrates for cardiac amyloidosis that have differing clinical courses and require distinctly different treatment\textsuperscript{2}. In AL cardiac amyloid, 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 (ATTR) related cardiac amyloidosis, 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, SSA) 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 while ATTRwt is predominately described in older adult males.

The most common ATTRm allele in the United States, the valine to isoleucine substitution at position 122 (V122I)\textsuperscript{3}, is found in approximately 3.5% of African Americans\textsuperscript{4,5}. ATTRwt cardiomyopathy has been found at autopsy in over 30% of patients with heart failure with a preserved ejection fraction (HFpEF) $\geq$75 years\textsuperscript{6}. These latter forms of cardiac amyloidosis are becoming increasingly recognized in part due the aging of the population, enhancements in the understanding of the disease’s pathobiology, and the potential benefit from emerging therapies\textsuperscript{7}.

The diagnosis of cardiac amyloidosis, however, and subsequent differentiation of AL from ATTR, remains challenging and misdiagnosis is associated with potential for significant harm.\textsuperscript{8} 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 either immunohistochemistry\textsuperscript{9,10} or in cases in which this is inconclusive, mass spectroscopy\textsuperscript{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 non-invasive diagnosis and has potential as a tool for ongoing follow-up of disease progression. Recent reports from European investigators have demonstrated that \textsuperscript{99m}Tc-DPD scintigraphy is useful in distinguishing AL from ATTR amyloid\textsuperscript{12} and may have prognostic significance\textsuperscript{13}. However, this isotope is not available for use in the United States. Previous reports regarding the utility of \textsuperscript{99m}Tc-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 \textsuperscript{99m}Tc-PYP in subjects with AL vs. ATTR-related cardiac amyloidosis secondary to wild-type and several different mutant variants, including the most common in the United States, the V122I mutation.

\textbf{Methods}

\textbf{1. Patient population}

Patients with biopsy proven AL or ATTR-related amyloidosis undergoing routine follow-up at the Columbia College of Physicians & Surgeons Center for Advanced Cardiac Care participated
in this study. 45 patients (12 AL, 16 ATTRwt, and 17 ATTRm) were enrolled. Inclusion criteria for the diagnosis of cardiac amyloidosis were one of the following: (a) biopsy proven cardiac amyloidosis (n=37); (b) in the absence of an endomyocardial biopsy, histologic documentation of Congo red staining in at least one involved organ with echocardiographically defined evidence of amyloid cardiomyopathy (thickness of the left ventricular septum or posterior wall of >12 mm without another cause of LVH) (n=5); or (c) 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.

2. Study design

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

3. $^{99m}$Tc-PYP SPECT scintigraphy

Planar imaging with $^{99m}$Tc-PYP was performed with a dual head Philips Precedence SPECT/CT camera (Philips Healthcare, Guildford, United Kingdom) equipped with low energy, high resolution (LEHR) collimators. Patients received 15-25 mCi of $^{99m}$Tc-PYP intravenously and anterior, lateral, and left anterior oblique planar views were obtained at one 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 256 x 256 matrix with 1.46 zoom factor. The SPECT imaging was performed if there was myocardial uptake of $^{99m}$Tc-PYP on planar images. Acquisition parameters for the SPECT imaging were LEHR collimators, matrix 64 x 64 with 1.46 zoom. The Butterworth filter was used with a cutoff of 0.50 and order of 5.00.

For the primary analysis, which was based on myocardial tracer uptake, two methods were used: (1) semi-quantitative 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 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 spillover 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 H/CL ratio.

4. Statistical analyses

Demographic, laboratory, and imaging data were collected and analyzed with descriptive statistics using mean±standard error for continuous variables and as relative percentages for categorical variables. Statistical analyses were performed using Statistical Analysis Software (SAS; Cary, North Carolina).

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

**Results**

1. **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 three groups are shown in Table 1. Subjects were, on average predominately male (84%) older adults with a mean of 70±2 years-old. Those with ATTRwt were older than those in the AL group ($p=0.0008$), while those with ATTRm were predominantly African American 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 (NYHA) Class III/IV heart failure with an average EF of 45%±2 that did not differ between groups.

Assessment of serum biomarkers, troponin I, brain natriuretic peptide (BNP), and modified BMI (mBMI), 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, while 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 reported², 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 and/or an infarct pattern¹⁶, and 20% had a pacemaker defibrillator.

⁹⁹mTc-PYP SPECT imaging

Representative examples of ⁹⁹mTc-PYP uptake among subjects and controls are shown in Figure 1. Semi-quantitative visual cardiac scores were significantly higher in patients with ATTR cardiac amyloidosis than in the AL cohort (2.9±0.06 vs. 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 usual TTR mutation (Thr59Lys) but who did not 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 vs. 22±3, p=0.04) overall but the trend across the three 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 heart/contralateral ratio (H/CL). This ratio was significantly higher among ATTR patients as compared with AL patients (1.80±0.04 vs.1.21±0.04) as well and was significant by ANOVA (p<0.0001). ROC 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=0.001). Heart maximum counts per pixel were also significantly higher in the ATTRm group than in the AL group (p=0.01).

\(^{99m}\)Tc-PYP myocardial uptake as measured by the H/CL ratio correlated with LV septal wall thickness (0.3172, p=0.04) and with LV mass index (r=0.42828, p=0.007) but not with ejection fraction (r=0.00122 p=0.9938). Furthermore, in multivariate logistic regression analysis adjusted for potentially confounding variables, neither calcium nor measures of renal function were significantly associated with H/CL ratio. Rather, the only independent variable associated with the H/CL ratio was ATTR vs. AL amyloid (OR 9.8, 95% CI 2.6-37.5).

**Discussion**

The result of this study confirms that \(^{99m}\)Tc-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 non-invasive 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 technetium-3,3-diphosphono-1,2-propanodicarboxylic acid ($^{99}$Tc-DPD), 99m-technetium-methylene diphosphonate ($^{99}$Tc-MDP), and $^{99}$Tc-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\textsuperscript{17, 18}. Moreover, the mechanism by which $^{99}$Tc-PYP distinguishes ATTR from AL amyloidosis remains to be elucidated. One hypothesis is that $^{99}$Tc-PYP may bind TTR amyloid fibrils more intensely than AL fibrils as a result of higher calcium containing compounds in ATTR hearts. Pepys and colleagues 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\textsuperscript{19, 20}. Additionally, since SAP self-aggregation is enhanced by the presence of calcium\textsuperscript{21} and is resistant to proteases in the presence of calcium,\textsuperscript{22} perhaps varying degrees of calcium in different amyloid subtypes may account for different levels of tissue enhancement. In this population, while calcium levels did differ between cohorts and were higher in ATTR subjects than AL, when calcium was 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, while calcium levels were significantly correlated with the H/CL ratio ($r=0.36$, $p=0.02$), there was no correlation for corrected calcium levels ($r=0.14$, $p=0.36$).

Furthermore, in multivariate analysis neither calcium nor measures of renal function were significantly associated with H/CL ratio. The only independent variable associated with the H/CL ratio was ATTR vs. AL amyloid (OR 9.8, 95% CI 2.6-37.5).
Another theory that may explain the mechanism of myocardial enhancement in ATTR subjects proposes that the intensity of $^{99m}$Tc-PYP binding relates to the duration over which amyloid deposition has occurred in the affected tissue. In AL patients, fibrils tend to accumulate over shorter time periods than in ATTR patients, whose disease course is typically more indolent. Accordingly, the characteristics of amyloidogenic fibrils in patients with ATTR cardiac amyloid may differ from those of AL amyloid thereby resulting in higher levels of $^{99m}$Tc-PYP uptake. Finally, it has not escaped our attention that the degree of amyloid infiltration in the myocardium may influence the H/CL ratio. However, multivariate analysis in our population did not show that wall thickness was independently associated with $^{99m}$Tc-PYP uptake, rather this was related to amyloid subtype.

Results from early technetium isotope studies may differ from ours due to inconsistent differentiation between AL and ATTR, lack of a quantitative measure of tracer retention (assessment have usually been semi-quantitative), and improvements in imaging techniques and quality since the 1980s. Of the bone isotopes employed for non-invasive identification of cardiac amyloid, $^{99m}$Tc-DPD has been the most studied to date. Perugini et al. demonstrated that in 25 patients with cardiac amyloidosis (15 ATTR and 10 AL), all 15 ATTR patients had strong myocardial uptake of $^{99m}$Tc-DPD while no uptake was observed in AL patients suggesting that $^{99m}$Tc-DPD myocardial uptake was 100% sensitive and 100% specific for diagnosing ATTR cardiac amyloidosis $^{12}$. However, in a larger cohort of 79 patients (45 ATTR and 34 AL) where tracer retention was calculated by a heart-to-whole body ratio (H/WB), the diagnostic accuracy of $^{99m}$Tc-DPD scintigraphy was found to be lower than previously reported due to unexpected tracer uptake in about one third of AL patients. Further studies demonstrated that in ATTR subjects, $^{99m}$Tc-DPD myocardial uptake is of prognostic value for predicting major adverse
cardiac events (MACE), either alone or in combination with LV wall thickness\textsuperscript{13}. Therefore, it appears that $^{99}$m-Tc-DPD scanning can assist in the differential diagnosis of ATTR and AL cardiac amyloidosis when tracer retention is either intense or absent, respectively (intermediate $^{99}$m-Tc-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, $^{99}$m-Tc-MDP has been employed in several case reports and small studies for the diagnosis of cardiac amyloidosis, but has demonstrated lower sensitivity than with $^{99}$m-Tc-PYP\textsuperscript{23, 24}.

Several case reports and larger studies dating back to the 1980s have described the utility of $^{99}$m-Tc-PYP, as used in this study, in identifying cardiac amyloidosis\textsuperscript{2, 25-32}. However, $^{99}$m-Tc-PYP scintigraphy has not yet been established for the non-invasive 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 ATTRwt patients only, missing the most common ATTRm allele in the United States, V122I\textsuperscript{3} and finally, most studies have analyzed scans using a semi-quantitative visual scoring system, not a quantitative method. In a 1982 report, intensely diffuse cardiac uptake of $^{99}$m-Tc-PYP was reported in all 10 subjects with biopsy proven cardiac amyloidosis, suggesting that $^{99}$m-Tc-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\textsuperscript{31}. 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 $^{99}$m-Tc-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, $^{99m}$Tc-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 semi-quantitatively).

Most recently, Yamamoto et al. described a quantitative method, the “PYP score”, to assess the utility of $^{99m}$Tc-PYP to evaluate for cardiac amyloidosis in 13 subjects with heart failure due to amyloid (1 AL, 3 ATTRm, 8 ATTRwt) and 37 subjects with heart failure due to non-amyloid 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 non-amyloid causes of heart failure. However, to the best of our knowledge, no study has compared AL subjects against ATTRwt and ATTRm groups using $^{99m}$Tc-PYP and the quantitative methodology we describe here. Our study sheds light on the fact that while 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, while sensitive and specific is not perfect as demonstrated by a single AL subject who had increased focal tracer retention that was due to a previous myocardial infarction and a single ATTR subject with a false negative scan result with minimal increase in wall thickness due 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 semi-quantitative approaches. Understanding that unrecognized myocardial infarction is a known reason for technetium uptake in the myocardium\textsuperscript{34} is essential so that potential contributors to false elevated visual scores are identified. Additionally, the association of uptake with wall thickness and LV mass in studies of \textsuperscript{99}Tc-DPD\textsuperscript{35} suggests that non-invasive identification of ATTR cardiac amyloidosis is dependent on the magnitude of myocardial amyloid infiltration and that intense \textsuperscript{99}Tc-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 vs. 1.5 cm, respectively), yet H/CL ratio was significantly higher in ATTRm patients, suggesting that \textsuperscript{99}Tc-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 \textsuperscript{99}Tc-PYP has diagnostic utility in genotype positive phenotype negative individuals with TTR mutations and if this technique is useful in monitoring disease progression and even response to therapy.

While reliable confirmation of amyloid is important, diagnosis of the specific etiologic subtype early in the disease course is essential for improving outcomes since 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 ATTRwt subjects in whom serologic testing for early diagnosis is not available\textsuperscript{36}. \textsuperscript{99}Tc-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 etiologic subtypes that specifically focused on the utility of $^{99m}$Tc-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, though 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 left ventricular walls, which while similar to other cohorts of patients with cardiac amyloidosis, may be characterized by enhanced uptake of technetium pyrophosphate. Future studies will need to evaluate the utility of this approach to prospectively identify patients 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 $^{99m}$Tc-PYP binds to ATTR more than AL fibrillar deposits remains to be elucidated.

In conclusion, $^{99m}$Tc-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.

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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

## Table 1. Baseline mean clinical, laboratory, and echocardiographic characteristics

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<th>Category</th>
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<th>AL n=12</th>
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<td>0.1±0.03</td>
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<td>1.7±0.06</td>
<td>1.6±0.1</td>
<td>1.9±0.1(a)</td>
<td>1.5±0.1</td>
<td>0.0078</td>
</tr>
<tr>
<td>LV posterior wall thickness (cm)</td>
<td>1.6±0.05</td>
<td>1.6±0.1</td>
<td>1.8±0.1(b)</td>
<td>1.4±0.1</td>
<td>0.0131</td>
</tr>
<tr>
<td>LV mass (gm/m²)</td>
<td>29±14</td>
<td>255±22</td>
<td>358±24(b)</td>
<td>253±14</td>
<td>0.0014</td>
</tr>
</tbody>
</table>

Continuous data are expressed as mean± SE, and categorical data are expressed as percentages.
\(a\)P<0.05 by ANOVA with Bonferroni correction in comparison to AL.
\(b\)P<0.05 by ANOVA with Bonferroni correction in comparison to ATTRm.
Table 2. $^{99m}$Tc-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>Semi-quantitative Visual Cardiac Score (%)</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>Quantitative Cardiac Score (cts)</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>&lt;0.0001</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>38,966±4,528</td>
<td>60,709±4,423*</td>
<td>58,346±4,764*</td>
<td>0.008</td>
</tr>
<tr>
<td>CL mean total cts/ROI</td>
<td>32,142±3,591</td>
<td>33,904±1,724</td>
<td>35,285±3,140</td>
<td>0.72</td>
</tr>
<tr>
<td>Heart mean area ROI (mm²)</td>
<td>4,672±217</td>
<td>5,356±302</td>
<td>5,303±436</td>
<td>0.36</td>
</tr>
<tr>
<td>CL mean area ROI (mm²)</td>
<td>4,619±412</td>
<td>5,295±298</td>
<td>5,212±342</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Continuous data are expressed as mean ± SE, and categorical data are expressed as percentages. Semi-quantitative and quantitative data were obtained 1 hour post $^{99m}$Tc-PYP infusion. Cts indicates counts; CL, contralateral; SD, standard deviation; Pix, pixels; ROI, region of interest. *P<0.05 by ANOVA with Bonferroni correction in comparison to AL.
Figure Legends

Figure 1 (A-B). Semi-quantitative method of calculating the distribution of $^{99m}$Tc-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. ROI = region of interest; C/L = contralateral; Cts = counts; Std Dev = standard deviation.

Figure 2. Mean heart to contralateral ratio according to amyloid subtype. Comparison of $^{99m}$Tc-PYP mean H/CL ratio between patients with AL, ATTRwt, and ATTRm cardiac amyloidosis. AL and transthyretin-related amyloidoses are differentiated by mean H/CL ratio of 1.5. The outlier with H/CL 1.3 is an ATTRm patient with the unusual Thr59Lys mutation. AL = amyloid light-chain; ATTRwt = wild-type transthyretin amyloidosis; ATTRm = mutant transthyretin amyloidosis.
**Negative Uptake**

- **Mean cts/pixel**: 15
- **Max cts**: 34
- **Std Dev**: 4.3
- **Total cts**: 41,794
- **Area of ROI**: 6,717 mm²

**Positive Uptake**

- **Mean cts/pixel**: 15
- **Max cts**: 29
- **Std Dev**: 4.3
- **Total cts**: 40,934
- **Area of ROI**: 6,795 mm²

**Patient CL**

**ROI**

**Raw Image**
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

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