Inter-observer reproducibility and intra-observer repeatability in ^{99m}Tc-pyrophosphate scan interpretation for diagnosis of transthyretin cardiac amyloidosis

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Aim. **The purpose of this study was to determine the inter- and intra-observer variability in ^{99m}technetium-pyrophosphate (^{99m}Tc-PYP) scan interpretation for diagnosis of transthyretin cardiac amyloidosis (ATTR).**

Methods and Results. **Our study cohort comprised 100 consecutive subjects referred for ^{99m}Tc-PYP imaging based on clinical suspicion of ATTR cardiac amyloidosis. Myocardial ^{99m}Tc-PYP uptake was assessed by both visual (comparison of myocardial to rib uptake) and semi-quantitative (heart-to-contralateral lung uptake ratio, H:CL) methods. Twenty scans were analyzed twice, at least 48 hours apart, by each of two independent observers. Patients with visual scores of ≥ 2 on planar imaging as well as myocardial uptake on SPECT/CT were classified as ATTR positive. Diagnosis of ATTR by visual ^{99m}Tc-PYP grade was perfectly reproducible [concordance: positive and negative scans 100% (53/53 and 47/47, respectively). Both inter- and intra-observer correlations for H:CL ratio (r^2 = 0.90, 0.99 (Observer 1) and 0.98 (Observer 2), respectively) and repeatability values on Bland–Altman plots were excellent. The coefficient of variation (%) for Observers 1 and 2 was 3.21 (2.14 to 4.29) and 7.49 (4.95 to 10.09), respectively. In addition, there was 100% concordance in positive and negative scan interpretation by visual grading between novice CV imagers (< 3 years' experience) and an experienced CV imager (10 years' experience).**

Conclusions. **This study showed excellent inter-observer reproducibility and intra-observer repeatability of ^{99m}Tc-PYP visual scan interpretation and H:CL ratio for diagnosis of cardiac ATTR amyloidosis. Cardiac ATTR amyloidosis can be diagnosed reliably using ^{99m}Tc-PYP SPECT/CT by novice and experienced CV imagers. (J Nucl Cardiol 2022;29:440–6.)**

Key Words: Amyloid heart disease • SPECT • molecular imaging

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Abbreviations

AL	Light chain amyloidosis
ATTR	Cardiac transthyretin amyloidosis
ASNC	American Society of Nuclear Cardiology
CV	Cardiovascular
DPD	3,3-Diphosphono-1,2-propanedicarboxylic acid
GFR	Glomerular filtration rate
HMDP	Hydroxymethylene diphosphonate
H:CL	Heart-to-contralateral lung uptake ratio
Hs c-TnI	High sensitivity cardiac troponin I
NYHA	New York Heart Association
NT-ProBNP	N-terminal pro B-type natriuretic peptide
ROI	Region of interest
SPECT	Single-photon emission computed tomography
^{99m}Tc-PYP	^{99m}Tc-pyrophosphate

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INTRODUCTION

Cardiac amyloidosis is a disorder in which misfolded proteins deposit as insoluble amyloid fibrils in the myocardial extracellular space. Transthyretin protein (ATTR), derived from liver, or immunoglobulin light chains (AL), derived from a plasma cell dyscrasia, are the types of amyloidosis that most commonly involve the heart.[1,2] Studies have shown the prevalence of wild-type ATTR (ATTRwt) to be up to 16% in patients with severe aortic stenosis undergoing transcatheter aortic valve replacement,[3] 13% among patients with heart failure with preserved ejection fraction [4] and 5% in those presumed to have hypertrophic cardiomyopathy.[5] Despite substantial numbers of patients, therapy historically was limited to supportive care without specific disease-modifying treatment. Consequently, the reported median survival was 2.5 years in patients with mutant ATTR (ATTRm) with Val122Ile mutation, and 3.6 years in those with ATTRwt.[6] The most common cause of death is cardiovascular, particularly heart failure and sudden death.[6] However, a recent multicenter, double-blind, placebo-controlled, randomized clinical trial (RCT)[7] showed a reduction in cardiovascular hospitalizations and all-cause mortality due to treatment with tafamidis, which stabilizes TTR protein. In addition, the treatment group experienced lower rates of deterioration of quality of life and functional capacity compared with placebo. With the approval of this promising disease-modifying, but presently very expensive therapy, the focus has now shifted to non-invasive techniques for disease diagnosis, and likely following response to therapy in the near future. It is therefore crucial for such an important test that would direct initiation of now available high-priced treatment for ATTR cardiac amyloidosis, to have minimal variability in scan interpretation. It would also be important to know if there is a significant difference in scan interpretation between novice and experienced readers.

Radionuclide imaging plays a critical role in the diagnosis of ATTR cardiac amyloidosis.[8–10] ^{99m}Tc-pyrophosphate (PYP) imaging is a highly specific non-invasive imaging technique[11–13] to diagnose ATTR cardiac amyloidosis in patients with typical echocardiography or cardiac magnetic resonance imaging findings, if a plasma cell dyscrasia is excluded. Recent multisocietal expert consensus recommendations for multimodality imaging in cardiac amyloidosis suggest reporting of H:CL ratio in patients with positive scans.[10] Despite the central role of ^{99m}Tc-PYP imaging in the non-invasive diagnosis of ATTR cardiac amyloidosis, to the best of our knowledge, there have been no studies to evaluate the inter- and intra-observer variability[14] in visual scan interpretation and in estimation of H:CL ratio. Minimal variability in scan interpretation is even more critical as ^{99m}Tc-PYP imaging is now used to non-invasively diagnose ATTR cardiac amyloidosis and make treatment decisions. The primary aim of this study was to determine the inter-observer reproducibility and intra-observer repeatability of ^{99m}Tc-PYP scan interpretation using (1) visual grading and (2) H:CL ratio.

METHODS

Patient Selection

The study cohort comprised 100 consecutive subjects who were referred for ^{99m}Tc-PYP imaging based on the clinical suspicion of ATTR cardiac amyloidosis in a large tertiary care center from February 2015 through April 2018. This study was approved by Partners Human Research Committee.

^{99m}Tc-Pyrophosphate Scan Protocol

All subjects underwent a cardiac SPECT/CT and planar image acquisition at 1 to 3.5 hours (52% at 1 hour and 48% at 2 to 3.5 hours) after injection of a mean dose of 22.7 ± 2 mCi ^{99m}Tc-PYP. Our lab protocol initially was cardiac SPECT/CT and planar image acquisition at 1 hour after injection of ^{99m}Tc-PYP. However, due to excess blood pool activity in some patients, the time for image acquisition after injection of ^{99m}Tc-PYP was increased to 3 hours. All images were acquired on a Siemens Symbia T-6 SPECT/CT scanner (Knoxville, TN) using a low-dose CT for attenuation correction and anatomical co-localization. Myocardial ^{99m}Tc-PYP uptake was assessed by both visual (comparison of myocardial

to rib uptake) and semi-quantitative (ratio of heart to contralateral lung uptake, H:CL) approaches used in clinical practice and recommended by ASNC. Images with a visual score of 2 or 3 on planar imaging were classified as positive when diffuse myocardial uptake was also documented on SPECT/CT, whereas a visual grade of 0 or 1 on planar imaging when diffuse myocardial uptake was absent on SPECT/CT was classified as negative.

In addition, for each scan, both observers sequentially interpreted the planar images alone, followed by SPECT/CT images. Circular target regions of interest (ROI) were drawn over the heart on the planar images and were then mirrored over the contralateral lung to account for background and rib uptake. Mean counts were recorded from each ROI, and the H:CL ratio was calculated as the ratio of heart ROI mean counts to the contralateral lung ROI mean counts. Twenty out of the hundred scans were analyzed twice, at least 48 hours apart, by two independent observers who were cardiology imaging trainees with < 3 years' experience in ^{99m}Tc-PYP imaging. Both the visual grade assessment and H:CL ratio calculations were done by the observers twice (for each read) to arrive at the final interpretation of the scan. In addition to the two novice CV imagers, a senior faculty CV imager with 10 years' experience in performance, interpretation, and reporting of ^{99m}Tc-PYP performed visual grading of planar and SPECT/CT images for the 100 PYP scans. This was followed by an interval re-assessment of visual grading for 20 out of 100 scans. The 100 cases for initial read and 20 cases for re-reads were exactly the same for each of the novice CV imagers and the experienced CV imager. All the observers were blinded to the clinical characteristics, and other lab and imaging findings of the patients.

Statistical Analysis

Inter-observer reproducibility and intra-observer repeatability were evaluated using Pearson's correlation coefficient (r) and percent differences between measurements. Bland–Altman plots and scatter plots with fitted linear regression curves were created for inter-observer and intra-observer measurements. Coefficient of variation was reported for the repeat measurements.

RESULTS

Baseline characteristics of the 100 subjects are as shown in Table 1. The mean age of the study cohort was 75 ± 9 years, 84% were male, and 65% had New York Heart Association class $\geq$ 2 symptoms (median NT-ProBNP levels 5301 pg/mL). In this cohort, 27% (n = 27) had endomyocardial biopsies (2 = mutant-type ATTR, 11 = wild-type ATTR, 7 = AL, 7 = negative for cardiac amyloid). When retrospectively reviewed, among the 27 patients with biopsies, the ^{99m}Tc-PYP scan was interpreted as negative in all patients with a negative biopsy (n = 7) and in all AL CA patients (n = 7). The PYP scan was interpreted as positive in all patients with wild-type ATTR (n = 11). Of the 2 mutant-type ATTR subjects, ^{99m}Tc-PYP scan was interpreted as positive in the patient with Val122I or Ile122 mutation, but negative in the other m-ATTR patient with Ala60 mutation.

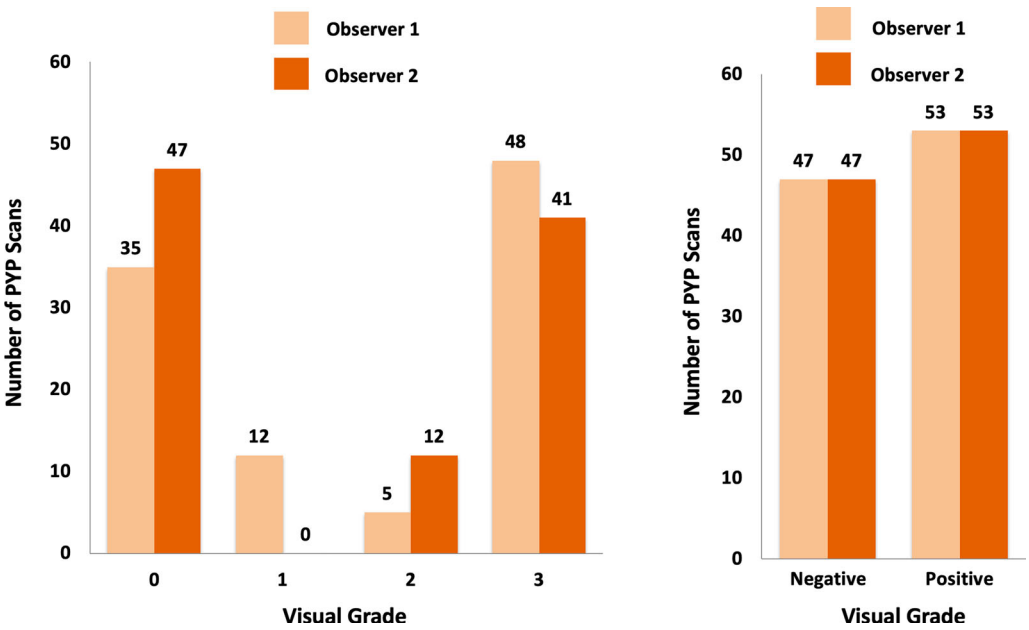
Inter-observer Reproducibility and Intra-observer Repeatability: Visual interpretation

Diagnosis of ATTR by visual ^{99m}Tc-PYP grade for the 100 studies was completely reproducible [concordance: positive and negative scans 100% (53/53 and 47/47, respectively) between the two observers, Figure 1. In addition, there was complete concordance of intra-observer visual grade assessments for the 20 studies that were re-interpreted by both observers independently, at least 48 hours after initial interpretation, Figure 2. The negative and positive patient groups were exactly the same for each reader. The distribution of ^{99m}Tc-PYP scans was equitable between positive and negative scans as shown in Figure 1. Furthermore, there was 100% concordance in final (positive vs negative) initial visual grade interpretation of 100 scans, and re-interpretation

Table 1. Baseline characteristics (N = 100)

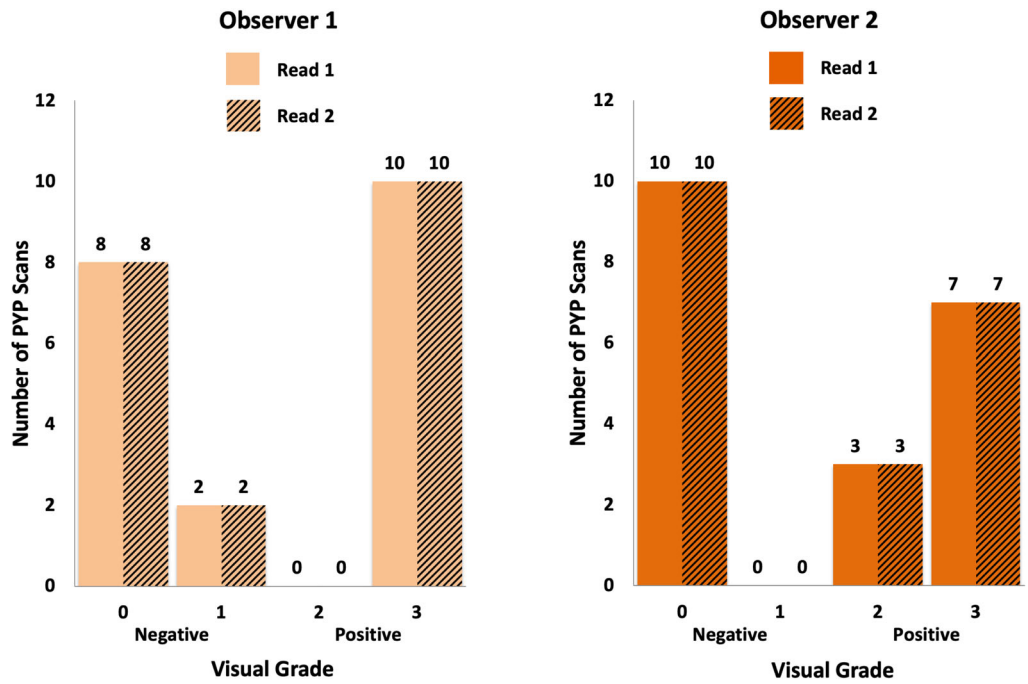
Age, mean, years	75 ± 9
Males, %	84
Weight, lbs	184 ± 40
Whites, %	73
Blacks, %	20
Carpal tunnel syndrome, %	30
Rotator cuff injury, %	3
Biceps tendon rupture, %	8
Neuropathy, %	30
NYHA class >2, %	65
Peripheral edema, %	43
NT-proBNP, pg/mL, median	5301
Hs c-TnI, ng/mL, mean	0.044 ± 0.04
Estimated GFR, mL/min/1.73 m^2, mean	50 ± 16
Hypertension, %	72
Hyperlipidemia, %	54
Atrial fibrillation, %	56
Coronary artery disease, %	37
Diabetes mellitus type2, %	25
Stroke, %	10
Smokers, %	43

NYHA: New York Heart Association; *NT-ProBNP*: N-terminal pro B-type natriuretic peptide; *Hs c-TnI*: high sensitivity cardiac troponin I; *GFR*: glomerular filtration rate



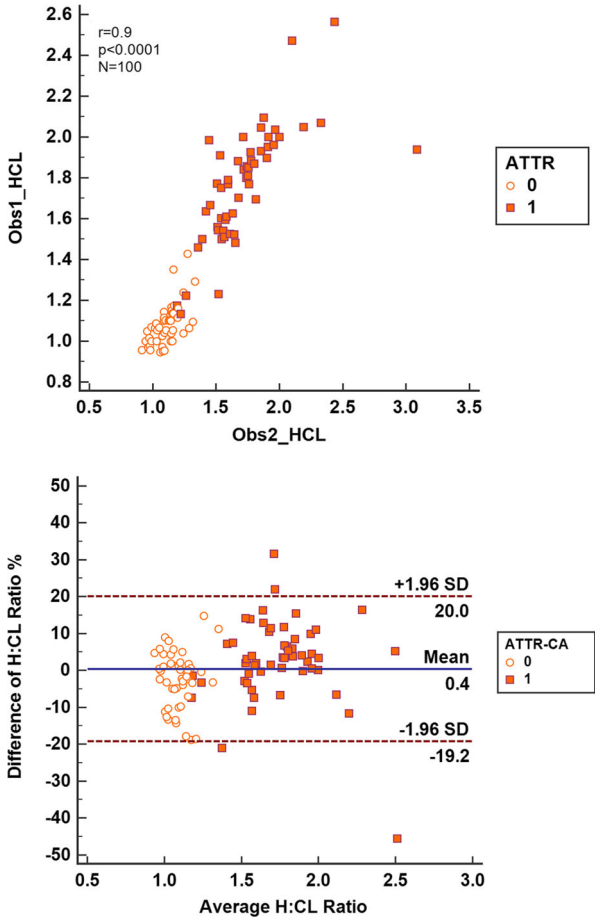
^{99m}Tc-PYP: pyrophosphate; * Scan interpretation based on planar and SPECT/CT images.

Figure 1. Inter-Observer Visual Grade Assessment of ^{99m}Tc-PYP Scans* (n = 100). *^{99m}Tc-PYP*: pyrophosphate; *Scan interpretation based on planar and SPECT/CT images.



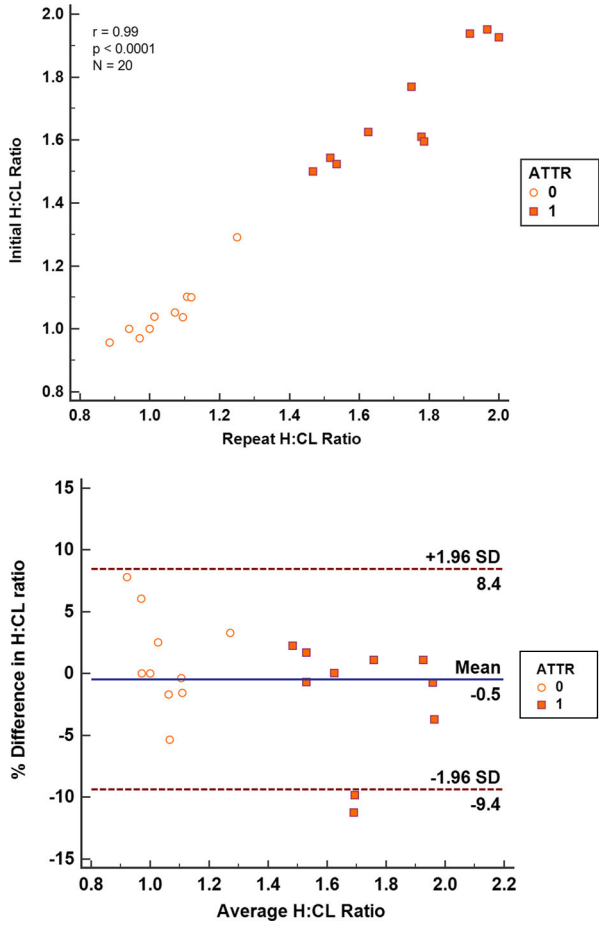
^{99m}Tc-PYP: pyrophosphate; * Scan interpretation based on planar and SPECT/CT images.

Figure 2. Intra-observer visual grade assessment of ^{99m}Tc-PYP Scans* (n = 20). *^{99m}Tc-PYP*: pyrophosphate; *Scan interpretation based on planar and SPECT/CT images.



Obs: Observer; H:CL: Heart to contralateral lung tracer uptake ratio; ^{99m}Tc-PYP: pyrophosphate; SD: Standard Deviation

Figure 3. Inter-observer correlations for H:CL ratio on ^{99m}Tc-PYP scans. *Obs*: observer; *H:CL*: heart-to-contralateral lung tracer uptake ratio; *^{99m}Tc-PYP*: pyrophosphate; *SD*: standard deviation.



H:CL: Heart to contralateral lung; ^{99m}Tc-PYP: pyrophosphate; SD: Standard Deviation

Figure 4. Intra-observer correlations for H:CL ratio on ^{99m}Tc-PYP scans: Observer 1. *H:CL*: heart to contralateral lung; *^{99m}Tc-PYP*: pyrophosphate; *SD*: standard deviation.

of 20 scans by the two novice CV imagers and the experienced CV imager.

For each scan, both novice CV imager sequentially interpreted the planar images alone, followed by SPECT/CT images. For Observer 1, there were 4 scans, and for Observer 2, there were 15 scans for which visual grades were changed from sequential planar only to planar + SPECT/CT image interpretation. However, for each of these 19 studies, the change in visual grade was within the same category of overall negative (grade 0 or 1) or positive (grade 1 or 2) scan read. Therefore, in this small cohort, 100% of the overall scan interpretations (positive or negative) were concordant between the planar alone read vs. planar followed by SPECT/CT read.

Inter-observer Reproducibility and Intra-observer Repeatability: H:CL Ratio

As shown in Figures 3, 4, and 5, inter- and intra-observer correlations for H:CL ratio were very strong (r = 0.90, 0.99 (Observer 1) and 0.98 (Observer 2), respectively); repeatability and reproducibility on Bland–Altman plots were excellent. The inter-observer coefficient of variation was 7.35 % (6.28 to 8.43), and 3.21 (2.14 to 4.29) and 7.49 (4.95 to 10.09) for Observers 1 and 2, respectively.

DISCUSSION

In this study of 100 patients, we showed excellent concordance between two observers for visual scan interpretation and excellent inter-observer reproducibility for H:CL ratio of ^{99m}Tc-PYP scans.

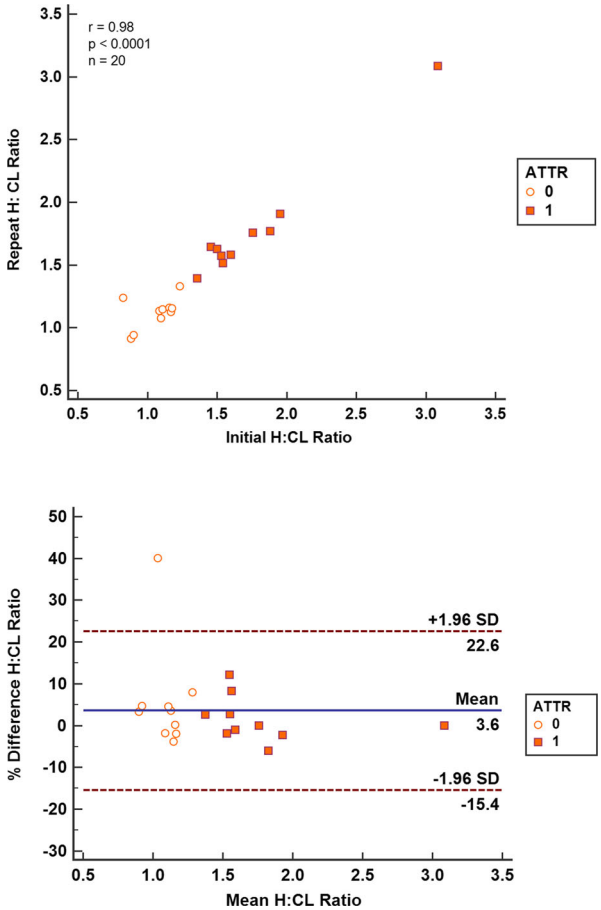


Figure 5. Intra-observer correlations for H:CL ratio on ^{99m}Tc-PYP scans: Observer 2. *H:CL*: heart to contralateral lung; *^{99m}Tc-PYP*: pyrophosphate; *SD*: standard deviation.

Once considered a inexorably progressive disease, the outlook of ATTR cardiac amyloidosis has now improved due to advances in non-invasive imaging,[8,15] and the recent approval of targeted breakthrough therapies that improve symptoms, reduce heart failure hospitalization, and prolong survival.[7,16,17] Non-invasive diagnosis is now feasible with ^{99m}Tc-PYP imaging; patients diagnosed with ATTR cardiac amyloidosis by ^{99m}Tc-PYP without biopsy become candidates for novel highly expensive therapies. Therefore, it is crucial that inter- and intra-observer variability in scan interpretation and semi-quantitative scan metrics be minimal, in order for these measures to be used to identify disease accurately. Currently, a diagnosis of ATTR cardiac amyloidosis is based on subjective ^{99m}Tc-PYP scan interpretation, using a visual comparison of rib and myocardial tracer uptake. Grade 2 or 3 uptake implies treatment with novel drugs [7,16,17], while Grade 0 or 1 uptake (negative) means no treatment; reporting of semi-quantitative evaluation of H:CL ratio on planar images is recommended for positive scans. Our results suggest that visual and quantitative measures of ^{99m}Tc-PYP imaging are highly reproducible and repeatable. One H:CL ratio was an outlier on Bland–Altman Analysis (Observer 2), and that was from a negative scan and reporting of H:CL ratio is only recommended for positive scans.[10] Notably, imaging trainee observers with < 3 years of experience in ^{99m}Tc-PYP interpretation achieved high reproducibility and repeatability in visual scan interpretation comparable to that of a cardiologist with 10 years of experience.

SPECT imaging is important to distinguish blood pool activity from myocardial uptake, assess the distribution of myocardial ^{99m}Tc-PYP uptake, to avoid overlap of bone uptake, and to quantify the degree of myocardial uptake in comparison to rib uptake. To study the added value of SPECT over planar imaging, both observers sequentially interpreted the planar images alone, followed by SPECT/CT. We found no significant differences in overall scan interpretation for SPECT and planar imaging. However, we believe that SPECT is clinically essential, and this difference was not apparent in this study because 48 of the 100 scans were performed 3 hours after injection of ^{99m}Tc-PYP when blood pool activity is expected to be minimal.

CONCLUSIONS

This study showed excellent inter-observer reproducibility and intra-observer repeatability of visual grade and H:CL ratio measurements for interpretation of ^{99m}Tc-PYP SPECT/CT scans for the diagnosis of ATTR cardiac amyloidosis. Cardiac ATTR amyloidosis can be diagnosed reliably using ^{99m}Tc-PYP SPECT/CT by novice and experienced CV imagers.

NEW KNOWLEDGE GAINED

The study demonstrates that cardiac ATTR amyloidosis can be diagnosed reliably using ^{99m}Tc-PYP SPECT/CT with excellent inter-observer reproducibility and intra-observer repeatability by novice CV imagers comparable to experienced CV imagers.

Disclosures

Dr. Singh has research grant from American Society of Nuclear Cardiology (ASNC). Dr. Cuddy has research grant from Pfizer. Dr. Skali received stock options from OptimizeRx for consulting/advisory roles. Dr. Blankstein has research grants from Amgen Inc and Astellas Inc. Dr. Falk has research grants from Ionnis, Alnylam, Glaxo Smith Kline and Pfizer. He serves as a consultant for Proclara. Dr. Di Carli has research

grant from Spectrum Dynamics and Gilead, he serves as a consultant for Janssen and Bayer. Dr. Dorbala has research grant from Pfizer and GE. Ms. Taylor, Dr. Kijewski, Dr. Park and Dr. Taqueti have no disclosures.

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