Non-cardiac uptake of technetium-99m pyrophosphate in transthyretin cardiac amyloidosis

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Background. **Technetium-based bone scintigraphy is rapidly becoming the most common non-invasive imaging tool in the diagnosis of Transthyretin cardiac amyloidosis (ATTR). Skeletal muscle uptake has been described with technetium-99m-3,3-diphosphono-1,2-propanodicarboxylic acid (TcDPD), and may account for masking of bony uptake. We sought to investigate skeletal muscle uptake of technetium-99m-pyrophosphate (TcPYP) in patients with ATTR.**

Methods and Results. **This was a retrospective analysis of 57 patients diagnosed with ATTR who underwent TcPYP scintigraphy. Cardiac uptake was assessed on whole-body planar imaging using a semiquantitative scale (grades 0 to 3) and on single-photon emission computed tomography (SPECT) with CT attenuation correction using total myocardial counts per voxel after a 3-hour incubation. Skeletal muscle (psoas and biceps), vertebral body, LV myocardium, and blood pool mean counts were calculated. In the cohort (age 78 ± 9 years, 77% male, and 30% hereditary ATTR), there was no visualized tracer uptake in skeletal muscle or soft tissue on qualitative SPECT assessment. Total and blood pool-corrected uptake in the muscle groups were significantly less than myocardium and bone ($P < 0.001$). Blood pool-corrected muscle uptake was not associated with semiquantitative grade 3 vs 2 uptake (psoas $P = 0.66$, biceps $P = 0.13$) or presence of hereditary ATTR (psoas $P = 0.43$, biceps $P = 0.69$). As bony uptake decreased, there was no corresponding increase in skeletal muscle uptake.**

Conclusions. **In patients with ATTR cardiac amyloidosis, skeletal muscle uptake of TcPYP is minimal when assessed by qualitative and quantitative metrics, and is not significantly different in patients with grade 2 vs 3 semiquantitative uptake. The properties of this tracer may be different than TcDPD with respect to non-cardiac uptake. (J Nucl Cardiol 2019;26:1630–7.)**

Key Words: Infiltrative cardiomyopathies • heart failure with preserved ejection fraction • nuclear cardiac imaging • advanced cardiac imaging

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Abbreviations	
ATTR	Transthyretin cardiac amyloidosis
H/CL	Heart-to-contralateral lung
SPECT	Single-photon emission computed tomography
TcPYP	Technetium 99m-pyrophosphate
TcDPD	Technetium-99m 3,3-diphosphono-1,2-propanodicarboxylic acid
TcHMDP	Technetium-99m hydroxymethylene diphosphonate

See related editorial, pp. 1638–1641

INTRODUCTION

Transthyretin cardiac amyloidosis (ATTR) is increasingly being recognized as a potential cause of heart failure with preserved ejection fraction, and stems from pathologic TTR protein dissociation and subsequent amyloid fibril deposition into the myocardium. There has been increased clinical appreciation of this disease entity due to non-invasive nuclear imaging with off-label bone scintigraphic agents. This diagnostic imaging clinical practice was recently formalized via a Practice Points document from the American Society of Nuclear Cardiology.[1] While technetium-99m pyrophosphate (TcPYP) is available in the United States, other agents such as technetium-99m 3,3-diphosphono-1,2-propanodicarboxylic acid (TcDPD) and technetium-99m hydroxymethylene diphosphonate (TcHMDP) are available in Europe and other parts of the world. Uptake of these bone tracers is classically evaluated on planar images using a semiquantitative scale (grades 0 to 3), where grade 3 indicates significant uptake in the myocardium greater than bone.[2] Skeletal muscle uptake has been described with TcDPD, and may account for masking of bony uptake seen in grade 3 patients.[3] However, the presence of significant skeletal muscle uptake with other bone tracers is unknown. We sought to investigate skeletal muscle uptake of TcPYP in patients with transthyretin cardiac amyloidosis.

METHODS

Study Population

Patients were included in the analysis if they underwent TcPYP nuclear scintigraphy at our institution from December 2011 to November 2016 for the evaluation of cardiac amyloidosis. Only patients with confirmed ATTR by endomyocardial biopsy or by nuclear scintigraphy with visual semiquantitative grades 2 and 3 were included[4] Patients with light chain (AL) amyloidosis were excluded from the analysis. Serum-free light chains and immunofixation were used to rule out AL. In patients with visual TcPYP uptake and monoclonal gammopathy, ATTR was confirmed after endomyocardial or extracardiac biopsy utilizing tissue typing with immunohistochemistry or mass spectrometry as needed.

Measurement Techniques

Electronic medical records were retrospectively reviewed for demographic and clinical data. Twelve-lead electrocardiograms were performed using standard equipment and retrospectively reviewed for voltage as previously described.[5] Echocardiography was performed using Vivid 7 or Vivid 9 (GE Medical, Milwaukee) or EPIQ (Philips Medical Systems, Bothell, WA) ultrasound systems and ejection fraction was calculated using Simpson's biplane method.

TcPYP scintigraphy using SPECT-CT on Siemens Symbia T6 cameras was performed. Patients were dosed with 20 mCi ± 10% of TcPYP intravenously and imaged 3 h later. Whole-body planar images were acquired with the heart centered on a 256 × 1024 matrix. Cardiac SPECT images used a low-energy high-resolution collimator, a 15% energy window, and CT attenuation correction on a 128 × 128 matrix with no zoom. Planar and SPECT images were analyzed offline using 4DM software (INVIA, Ann Arbor, MI). Planar images were analyzed using visual semiquantitative scoring (score 0 to 3).[6] and the heart-to-contralateral lung ratio[2,7] as previously described. SPECT images were analyzed to obtain quantitative measurements of total left ventricular myocardial uptake in counts per voxel as previously described.

Skeletal muscle uptake was visually assessed on fusion imaging and assessed quantitatively by drawing a region of interest over bilateral psoas muscles and averaging the mean counts per voxel. This was repeated with the proximal portion of the biceps muscle. Care was taken to avoid spillover of counts from adjacent bone. These two muscle groups were chosen as they were the largest muscles captured in our SPECT field of view with enough distance from bone to avoid count spillover. Intensity of bone uptake was obtained by drawing a region of interest over two representative vertebral bodies and taking their average. Mean counts in the blood pool were calculated by averaging counts obtained from 3 regions of interest in the proximal, mid, and distal ascending aorta.

Statistical Analysis

Categorical variables are presented as percentages and compared with Fisher's Exact test. Continuous variables are expressed as mean ± standard deviation or median with interquartile range and compared with the paired *t* test or Wilcoxon rank-sum test where appropriate. Variable correlations were assessed using Pearson's correlation coefficients, and scatter and box plots were used to visualize data. All statistical tests were 2-sided and P values $< .05$ were considered statistically significant. Statistical analysis was performed using Stata (version 13, College Station, Texas). The study was approved by the Institutional Review Board.

Table 1. Baseline demographics

	Total cohort (n = 57)	Semiquantitative grade 2 (n = 43)	Semiquantitative grade 3 (n = 14)	P value
Age (years)	78.3 ± 9.1	79.3 ± 7.9	75.4 ± 12.1	0.17
Male	44 (77%)	33 (77%)	11 (79%)	1.00
Caucasian	37 (65%)	29 (67%)	8 (57%)	0.53
NYHA class 3-4	37 (69%)	30 (70%)	7 (50%)	0.21
Hereditary ATTR	17 (30%)	12 (28%)	5 (36%)	0.74
Obesity	11 (19%)	9 (21%)	2 (14%)	0.71
Hypertension	41 (72%)	31 (72%)	10 (71%)	1.00
Hyperlipidemia	43 (75%)	32 (74%)	11 (79%)	1.00
Diabetes	18 (32%)	14 (33%)	4 (29%)	1.00
Atrial fibrillation	30 (53%)	25 (58%)	5 (36%)	0.22
GFR (mL/min per 1.73 m^{2+})	54.7 ± 19.7	53.6 ± 18.3	58.5 ± 24.2	0.44
Troponin T (ng/mL)	0.053 (0.031, 0.134)	0.043 (0.029, 0.129)	0.1 (0.088, 0.16)	0.055
NTproBNP (pg/dL)	4252 (2641, 8542)	5264 (2990, 9545)	3108 (2546, 4049)	0.13
Sokolow voltage (mm)	16.3 ± 8.4	15.9 ± 8.2	18.0 ± 9.6	0.49
Limb lead voltage (mm)	19.6 ± 8.4	19.6 ± 8.4	19.5 ± 8.8	0.95
Ejection fraction (%)	46.1 ± 12.9	48.1 ± 12.3	39.9 ± 13.1	0.038

NYHA, New York Heart Association; *ATTR*, transthyretin amyloidosis; *GFR*, glomerular filtration rate; *NTproBNP*, N-terminal of pro-brain natriuretic peptide

RESULTS

A total of 57 cases were analyzed for non-cardiac uptake on TcPYP nuclear scintigraphy. Baseline characteristics are seen in Table 1. Mean age was 78.3 years, 77% were male, and 30% had hereditary ATTR. The most common variant was V122I (n = 15), followed by Ser84Ile (n = 1) and V30Met (n = 1). Patients with semiquantitative grade 3 vs 2 uptake had a lower ejection fraction.

Qualitatively, there was no visualized tracer uptake in the psoas, biceps, thoracic, and other upper extremity skeletal muscles in all patients on SPECT imaging (Figure 1). Figure 2 shows an example of planar images with decreased bony and increased myocardial uptake in grade 3 vs 2 disease. There were no patients with absent or grade 1 disease on planar images who subsequently had significant counts on SPECT that were masked by soft tissue uptake. Mean counts in the blood pool, myocardium, psoas and biceps muscles, and bone are seen in Table 2. Patients with semiquantitative grade 3 vs 2 had greater LV myocardial uptake, but no difference in vertebral or muscle uptake. Total and blood pool-corrected uptake in both muscle groups were significantly less than myocardium and bone ($P < .001$ for both). As total absolute myocardial counts increased, so did skeletal muscle, vertebral, and blood pool counts (Figure 3, all $P < .001$). However, there was no relationship between blood pool-corrected psoas ($r = 0.173$, $P = .202$), biceps ($r = -0.156$, $P = .260$), or vertebral body ($r = 0.144$, $P = .292$) counts and corrected myocardial counts (Figure 4). There was no decrease in SPECT measured vertebral counts as psoas muscle counts increased; in fact, these two measures were positively correlated ($r = 0.290$, $p = .029$, Figure 4). Neither psoas nor biceps muscle uptake (corrected and uncorrected) was associated with semiquantitative grade (Table 2) or presence of hereditary ATTR ($P = .434$ and 0.357 for psoas; $P = .691$ and .648 for biceps, respectively). Vertebral body uptake was numerically lower in grade 3 vs 2 patients, but this was not significant (corrected $P = .301$, uncorrected $P = .909$). Vertebral uptake was not associated with hereditary ATTR (corrected $P = .513$, uncorrected 0.914). Box plots of blood pool-corrected psoas and vertebral

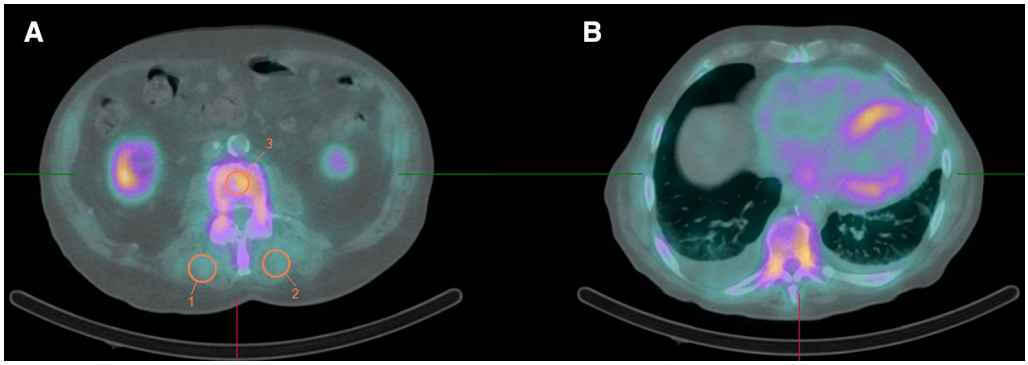

Figure 1. SPECT example. Example of intense vertebral and lack of psoas or soft tissue uptake in a patient with wild-type ATTR (**A**). There is myocardial uptake with an apical sparing pattern (**B**).

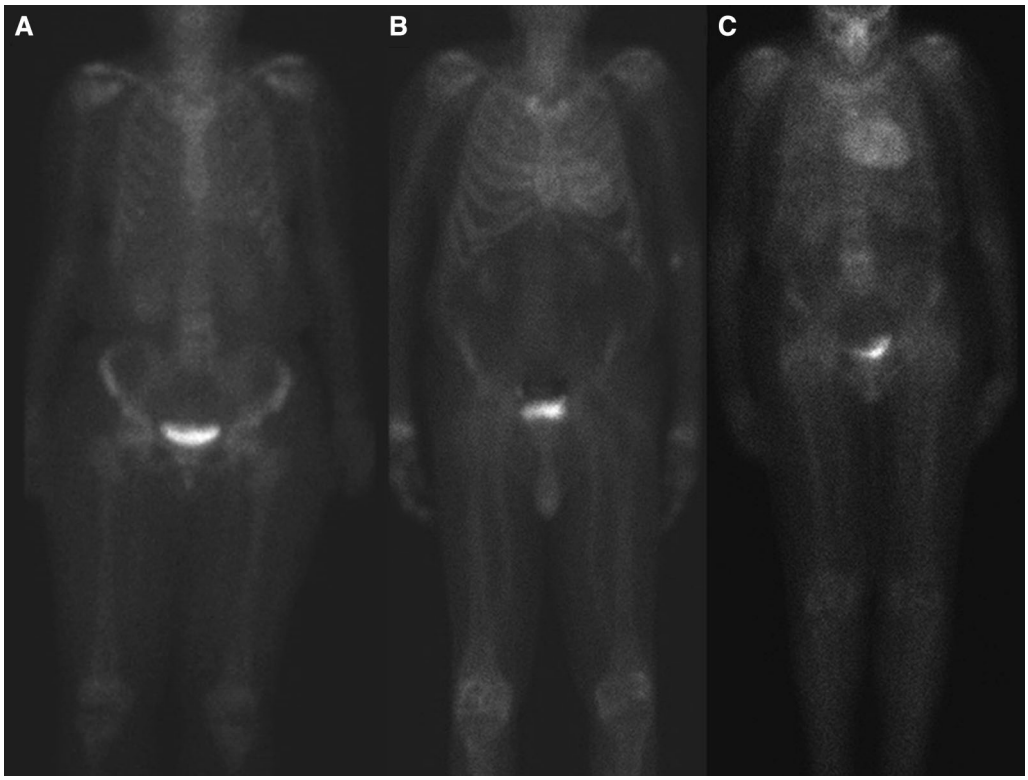

Figure 2. Planar examples. Example of planar images with grade 0 (**A**), grade 2 (**B**), and grade 3 (**C**) uptake. There is a relative increase in myocardial and decrease in bone uptake seen in the patient with grade 3 disease (**C**).

uptake with respect to semiquantitative grade and hereditary ATTR are seen in Figures 5 and 6.

DISCUSSION

Cardiac utility of bone scintigraphy agents using technetium-based radionuclides has found a second life as a non-invasive diagnostic tool for transthyretin cardiac amyloidosis. Various radionuclides are in use around the world to make this diagnosis, despite displaying different properties with respect to bone and soft tissue uptake. In this study, we assessed skeletal muscle and bony uptake in patients with ATTR cardiac amyloidosis. Patients generally had advanced disease

Table 2. Imaging findings

	Total cohort (n = 57)	Semiquantitative grade 2 (n = 43)	Semiquantitative grade 3 (n = 14)	p value
H/CL ratio	1.65 ± 0.26	1.56 ± 0.16	1.90 ± 0.33	< 0.001
Blood pool counts	243 ± 75	237 ± 76	259 ± 73	0.33
Total LV counts	9193 ± 3912	8296 ± 2985	11885 ± 5120	0.002
Total LV counts, blood pool corrected	38.15 ± 9.47	35.85 ± 8.22	45.05 ± 9.89	0.001
Psoas muscle counts	105 ± 42	102 ± 44	115 ± 36	0.34
Psoas muscle counts, blood pool corrected	0.44 ± 0.15	0.44 ± 0.14	0.46 ± 0.17	0.66
Biceps muscle counts	49 ± 38	53 ± 42	37 ± 21	0.19
Biceps muscle counts, blood pool corrected	0.21 ± 0.16	0.22 ± 0.17	0.16 ± 0.10	0.13
Vertebral body counts	925 ± 264	927 ± 272	918 ± 247	0.91
Vertebral body counts, blood pool corrected	4.04 ± 1.41	4.15 ± 1.45	3.70 ± 1.28	0.30

LV, left ventricular; *H/CL*, heart-to-contralateral lung

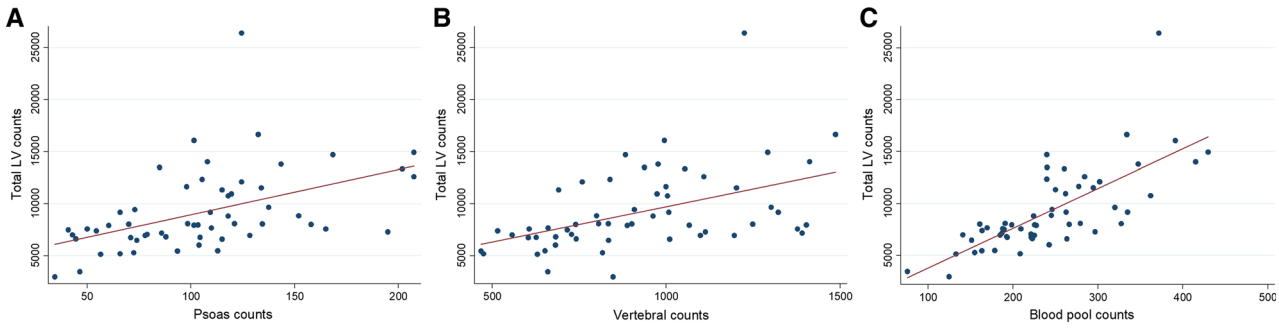

Figure 3. Total counts. Total left ventricular counts are positively correlated with psoas counts (**A**, $r = 0.473$, $P < .001$), vertebral counts (**B**, $r = 0.464$, $P < .001$), and blood pool counts (**C**, $r = 0.734$, $P < .001$) in patients with ATTR.

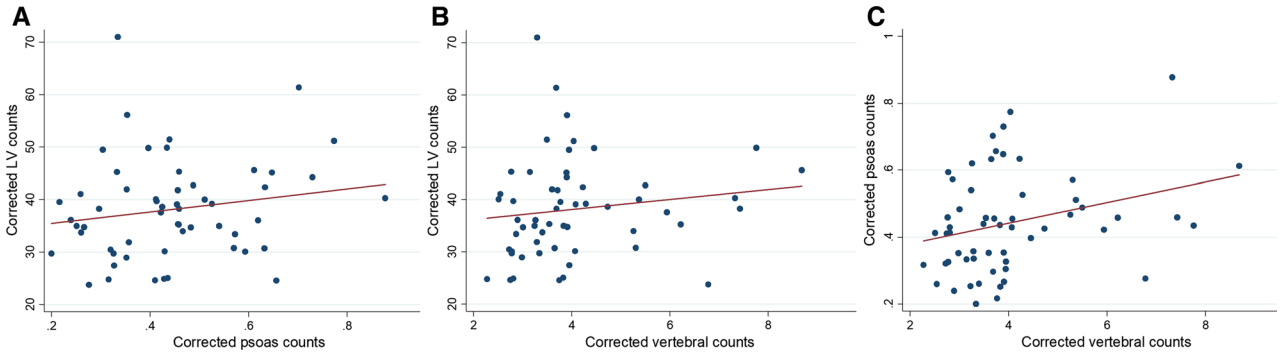

Figure 4. Blood pool-corrected counts. There is no association between blood pool-corrected left ventricular counts and blood pool-corrected psoas (**A** $r = 0.173$, $P = .202$) or vertebral body (**B** r = 0.144, $P = .292$) counts. SPECT measured vertebral counts and psoas muscle counts were positively correlated (**C** $r = 0.290$, $P = .029$).

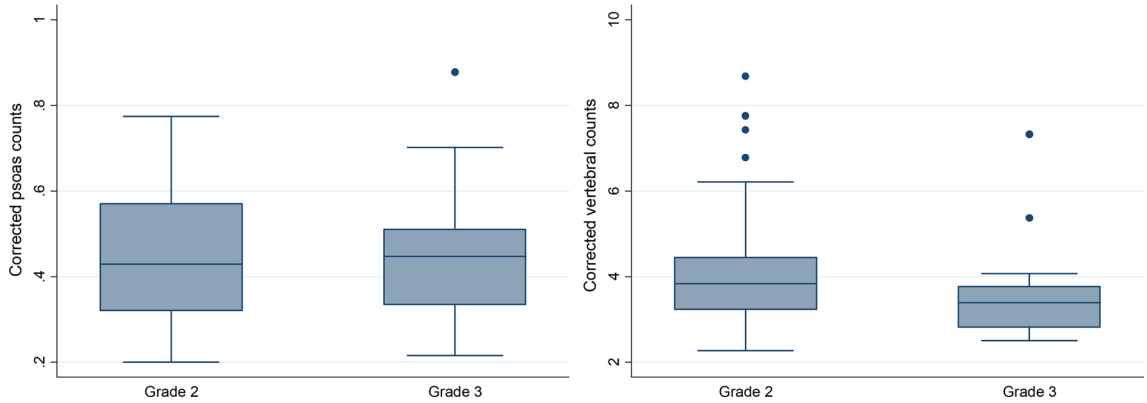

Figure 5. Skeletal muscle and bone counts based on semiquantitative grade. There is no association between blood pool-corrected psoas counts and semiquantitative grade 3 vs 2 ($P = .664$). There are numerically lower vertebral counts in patients with a semiquantitative grade of 3 vs 2 which was not statistically significant ($P = .301$).

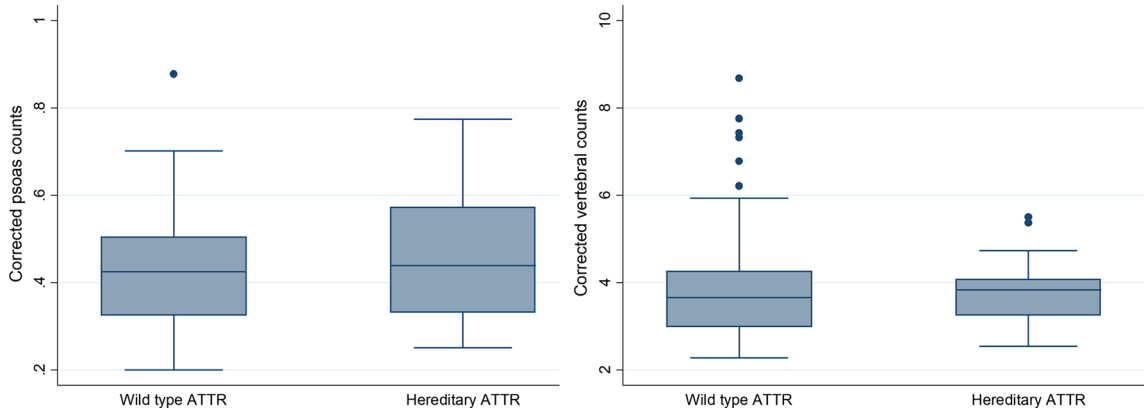

Figure 6. Skeletal muscle and bone counts based upon presence of hereditary ATTR. There is no association between blood pool-corrected psoas ($P = .434$) or vertebral body counts ($P = .513$) based upon presence of TTR gene mutation.

with over two-thirds having NYHA class 3–4 symptoms, mean EF 46%, and an elevated troponin and N-terminal of pro-brain natriuretic peptide at the time of imaging. Using technetium pyrophosphate, minimal uptake was found in muscle via qualitative and quantitative measures, and significantly less than blood pool. These results have clinical implications and may reflect differences among various technetium-based bone tracers used in ATTR.

A previous study has demonstrated soft tissue uptake in ATTR. Hutt et al. described a large cohort of suspected ATTR using TcDPD.[3] They employed a similar imaging protocol with late phase image acquisition (whole-body planar images acquired 3 h after tracer injection followed by cardiac SPECT-CT). The investigators found visual diffuse skeletal muscle uptake in all patients with ATTR which was more pronounced in those with ATTRwt and V122I mutation. One patient with V122I actually had complete obscurement of cardiac uptake on planar images due to extensive soft tissue uptake; cardiac uptake could only be seen on SPECT-CT. The authors postulated that decreased bone activity in patients with grade 3 planar uptake was likely due to extensive soft tissue uptake which masks bony uptake. Even in Perugini's original work with this tracer, soft tissue uptake appears to increase as semiquantitative grade increases and bony uptake decreases (Figure 1 of Ref 6). The only prior report of significant soft tissue uptake with TcPYP was after direct current transthoracic countershock in a canine model.[8]

The lack of soft tissue uptake seen in our study with TcPYP is likely due to differences in properties between the PYP vs DPD tracer. Perhaps DPD has a higher affinity for soft tissue, and thus bony uptake is less pronounced in patients with semiquantitative grade 3 uptake. Anecdotally, there is sometimes significant muscle uptake in the psoas and other large muscle groups using F18-florbetapir which specifically binds to amyloid fibrils. Histologically, we do know that amyloid deposits are found in soft tissue structures such as muscle,[3] ligaments and tendons,[9] and subcutaneous fat.[10] It is important to note that the degree of abdominal fat involvement may be patchy or non-existent as the diagnostic sensitivity of abdominal fat pad biopsy is only 15% in ATTRwt and 33% in V122I mutation.[10]

The prior study using TcDPD[3] noted skeletal muscle uptake predominantly with wild-type and V122I mutation, and less so with other mutations. The vast majority of our cohort was wild type or V122I, as it commonly encountered in the United States. Thus, skeletal muscle uptake with TcPYP in other mutations cannot be adequately quantified in this cohort. Even in our cohort with almost exclusively wild-type and V122I patients, skeletal muscle uptake was not significant.

This investigation has clinical implications for patients undergoing TcPYP scintigraphy. Differentiating between grade 2 and 3 uptake on phosphate-based nuclear scintigraphy may not be clinically meaningful with the TcPYP tracer. In fact, outcomes are similar between patients with grade 2 and 3 uptake in prior studies.[2,11] Amyloid myopathy is a known entity, particularly in AL amyloidosis, and is becoming more recognized in ATTR. This likely contributes to symptoms of fatigue in these patients in excess of what is expected based upon the degree of heart failure. However, the TcPYP tracer in contrast with TcDPD is not likely to aid in diagnosing this extracardiac large muscle involvement for the clinician. Significant muscle and soft tissue uptake with DPD or amyloid-specific agents like F18-florbetapir may point to amyloid myopathy as a contributor for symptoms of muscle fatigue and weakness. Unfortunately, TcDPD is not available in the United States and the diagnostic criteria for extracardiac involvement from European and Asian studies may not be applicable to images generated by TcPYP. Conversely, it is unlikely given our findings, that muscle uptake would mask cardiac uptake and therefore lead to false-negative results as is possible with TcDPD when significant muscle uptake is present.

Limitations

This is a single-center study and limited by local protocols and image analysis expertise. This study does not directly compare PYP with DPD or HMDP. This study also only assesses uptake after a 3-h imaging delay. Characteristics of muscle and bony uptake could be different at different time points. We know that soft tissue uptake generally decreases and bony uptake increases from DPD injection to 3 h.[3] Only psoas, biceps, and vertebral body uptake were quantitatively assessed. This may not have reflected the extent of skeletal muscle or bony uptake in a given patient. However, there was no visualization of significant counts in other skeletal muscle.

NEW KNOWLEDGE GAINED

As compared to what is reported with TcDPD, skeletal muscle uptake of TcPYP is minimal when assessed by qualitative and quantitative metrics in patients with cardiac ATTR. Skeletal muscle uptake is not associated with grade 2 vs 3 semiquantitative uptake or presence of TTR gene mutation. The properties of the TcPYP tracer may be different than TcDPD with respect to non-cardiac uptake.

CONCLUSION

In patients with ATTR cardiac amyloidosis, skeletal muscle uptake of TcPYP is minimal when assessed by qualitative and quantitative metrics, and is not significantly different in patients with grade 2 vs 3 semiquantitative uptake. The properties of this tracer may be different than TcDPD where skeletal muscle uptake may mask cardiac uptake and potentially lead to false-negative results on planar images.

Disclosures

Dr. Sperry, Dr. Hanna, Brunken, Dr. Jaber and Gonzalez have no disclosures to report. Dr. Cerqueira is a consultant for Astellas Pharmaceuticals.

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