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CLINICAL RESEARCH ARTICLE

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^{99m}Technetium-pyrophosphate bone scan: A potential biomarker for the burden of transthyretin amyloidosis in skeletal muscle: A preliminary study

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Abstract

Introduction/Aims: Transthyretin amyloidosis (ATTR) proteins can infiltrate skeletal muscle and infrequently cause a myopathy. ^{99m}Technetium-pyrophosphate (^{99m}Tc-PYP) is a validated biomarker for cardiac involvement in variant and wild-type ATTR (ATTRv and ATTRwt, respectively). The aim of this study was to test the hypothesis that ^{99m}Tc-PYP is a biomarker for muscle burden of ATTR.

Methods: Radioisotope uptake in the deltoid muscles of patients with ATTR was compared to uptake in control subjects without amyloidosis in a retrospective study. ^{99m}Tc-PYP scans were evaluated in 11 patients with ATTR (7 ATTRv, 4 ATTRwt) and 14 control subjects. Mean count (MC) values were measured in circular regions of interest (ROIs) 2.5–3.8 cm^2 in area. Tracer uptake was quantified in the heart, contralateral chest (CC), and deltoid muscles.

Results: Tracer uptake was significantly higher over the deltoids and heart but not the CC, in patients with ATTR than in control subjects. MC values were 120.1 ± 43.7 (mean ± SD) in ATTR patients and 78.9 ± 20.4 in control subjects over the heart ($p = 0.005$), 73.3± 21.0 and 63.5 ± 14.4 over CC ($p = 0.09$), and 37.0 ± 11.7 and 26.0 ± 7.1 averaged over both deltoid muscles ($p = 0.014$).

Discussion: ^{99m}Tc-PYP is a potential biomarker for ATTR amyloid burden in skeletal muscle.

KEYWORDS
^{99m}Tc-PYP, amyloidosis, ATTR, myopathy, transthyretin

1 | INTRODUCTION

Transthyretin amyloidosis (ATTR) is a multi-system disease caused by the deposition of a transthyretin (TTR) variant (ATTRv) or wild-type TTR (ATTRwt), the latter being a common entity associated with

LIST OF ABBREVIATIONS: ^{99m}Tc-DPD, technetium-99m-labeled 3,3-diphosphono-1,2-propanodicarboxylic acid; ^{99m}Tc-PYP, ^{99m}Technetium-pyrophosphate; ASNC, American Society of Nuclear Cardiology; ATTR, transthyretin amyloidosis; ATTRv, variant transthyretin amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; AUC, area under the curve; CC, contralateral chest; CTS, carpal tunnel syndrome; EMG, electromyography; MC, mean count; MRC, Medical Research Council; ROC, receiver operating characteristic; ROI, region of interest; TTR, transthyretin.

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aging.[1] ATTR may present with a variety of musculoskeletal manifestations, such as carpal tunnel syndrome (CTS), lumbar spinal stenosis, and myopathy, several years before the onset of cardiomyopathy or polyneuropathy, which are the cardinal manifestation of ATTRwt and ATTRv.[2–5] Disease modifying treatment of ATTR is rapidly evolving, with two TTR gene silencers and a TTR stabilizer already approved by Food and Drug Administration (FDA) and European Medicines Agency (EMA) for ATTR neuropathy and cardiomyopathy.[6–8] ATTR myopathy is likely underdiagnosed partly because of weakness being attributed to the systemic disease. As the disease-modifying treatment of ATTR neuropathy is more effective when started early in the disease course,[9,10] and musculoskeletal manifestations often precede the diagnosis of cardiomyopathy and neuropathy by years,[2,4,5] biomarkers to assess ATTR burden in musculoskeletal tissue are needed. The diagnosis of ATTR is based on demonstration of tissue deposition of amyloid and then confirmation of ATTR through amyloid subtyping. However, ATTR cardiomyopathy can be diagnosed based on nuclear scintigraphy without a tissue biopsy.[11]

Nuclear scintigraphy with ^{99m}Technetium-pyrophosphate (^{99m}Tc-PYP scan) is a validated disease biomarker for cardiac involvement in ATTR,[12] with a high sensitivity and specificity in differentiating ATTR and non-ATTR cardiomyopathy.[11,13] A previous study demonstrated extensive uptake of technetium-99m-labeled 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) in the skeletal muscle of patients with ATTRwt and ATTRv (specially due to V122I mutation).[14] Furthermore, increased musculoskeletal uptake of ^{99m}Tc-DPD nuclear scintigraphy was reported in a patient with ATTR myoneuropathy.[15] ^{99m}Tc-DPD and ^{99m}Tc-PYP are both bone seeking radiotracers that have a high uptake in the myocardium of patients with ATTR[16] . ^{99m}Tc-DPD is not approved by FDA and is not available in the United States.

We hypothesized that ^{99m}Tc-PYP scanning may be a biomarker to assess muscle burden of ATTR. In this proof-of-concept study, we investigated whether there is an increased uptake of ^{99m}Tc-PYP in the deltoid muscles of patients with confirmed ATTR compared to the patients who did not have ATTR.

2 | METHODS

2.1 | Subjects

The study was approved by the University of Chicago Biological Science Division Institutional Review Board before any data collection. This was a retrospective study using a database of 176 patients who underwent ^{99m}Tc-PYP cardiac imaging at the University of Chicago Hospitals between March 1, 2015, and March 1, 2020. Only patients whose arms were at their sides during the scanning were included, 151 patients were excluded because their scan was done with the arm stretched above the head, as the deltoid muscle tissue could not be clearly and reliably delineated. We identified 11 patients who carried a diagnosis of ATTR cardiac amyloidosis and 14 who had non-amyloid cardiac disease (control subjects). ATTR was diagnosed based on a cardiac visual score ≥ 2 on ^{99m}Tc-PYP, and exclusion of amyloid light chain (AL) amyloidosis with serum protein immunofixation and light chain panel[11]; ATTRwt and ATTRv were then differentiated with TTR gene sequencing. Only one of the 11 patients underwent a muscle biopsy to confirm the diagnosis of ATTR. Control patients were those with cardiomyopathy and heart failure with negative ^{99m}Tc-PYP cardiac scans, defined by American Society of Nuclear Cardiology (ASNC) guidelines as grade 0.[17]

2.2 | Methods

^{99m}Tc-PYP planar cardiac imaging of the chest was done using two-headed gamma cameras with low-energy, high-resolution collimators. The dose of ^{99m}Tc-PYP ranged from 10 to 25 mCi which was then allowed to incubate for 1 h with the option of extending to 3 h if additional information was needed.[18] The cardiac retention was determined using a semiquantitative visual score ranging from 0 (no uptake) to 3 (uptake greater than rib)[17,19] and a quantitative heart to contralateral (H/CL) ratio of total counts in the region of interest (ROI) over the heart divided by background counts in an identical size ROI of the contralateral chest (CC).[17,18] Results were considered positive for ATTR cardiac amyloidosis if there was a visual score ≥ 2 or an H/CL ratio ≥ 1.5.[18]

Mean counts (MCs) over the deltoid muscles, heart, and CC were measured using IntelliSpace PACS 4.4 Enterprise software (Intelerad, Montreal, Canada), in circular ROIs of 2.5–3.8 cm^2 in circumference. Only anterior views of the thorax at 1-h incubations were used to assess the MCs. To avoid contamination with bony structure uptake, ROIs for the deltoids were located lateral and inferior to the shoulder joint.

2.3 | Statistical analysis

Data analysis was performed using Stata 17 (College Station, TX). Continuous variables are presented as mean (±SD) and categorical variables are summarized with counts (percentages). The 2 tailed *t*-test was used with continuous variables to compare between ATTR patients, their subgroups, and control subjects. The Fisher exact test was used to compare categorical variables. A p-value <0.05 was considered statistically significant.

3 | RESULTS

Seven of the 11 ATTR patients had ATTRv due to the V122I mutation and the rest had ATTRwt (Table 1). Neurological signs or symptoms were present in 8 of 11 ATTR patients, including distal sensory symptoms in 4, asymmetrical upper limb predominant neuropathy in 1, and proximal more than distal limb weakness in 3 (Table 2 and Supplemental Table S1). Autonomic symptoms were not documented, and autonomic testing was not done in any patient. Deltoid weakness as assessed with Medical Research Council (MRC) testing (ranging from 0 to 5)[20] was noted in four of the ATTR patients, of whom, needle electromyography (EMG) showed spontaneous activity (fibrillation

TABLE 1 Characteristics of patients with ATTR

Patient	A (yr)/G	Grade	Mutation	Deltoid MC	Heart MC
1	66M	0[a]	V122I	32.5	44.4
2	80F	3	V122I	27.20	93.9
3	41F	3	V122I	26	126.7
4	71F	3	V122I	51.2	192.1
5	76M	3	V122I	41.1	174.4
6	83M	2	V122I	41.4	135.2
7	90F	3	V122I	47.3	144.1
8	76M	3	None	22.625	115.4
9	81M	1	None	58.4	73
10	81M	3	None	32.0	135.8
11	66F	2	None	27.6	85.7

Abbreviations: A (yr)/G, age in years at the time of nuclear scintigraphy, and gender; F, female; Grade, semi-quantitative visual grading of myocardial ^{99m}Tc-PYP uptake by comparison to bone (rib) uptake[17]; M, male.
[a]History of heart transplantation.

TABLE 2 Summary of clinical and electrodiagnostic findings

Patient	Neurological history	Neurological examination	Nerve conduction study	EMG
1	Progressive weakness, more severe in the R.UE, numbness in hands > feet	Distal > proximal weakness, more severe in the R.UE, DTRs absent, multimodal distal sensory loss in the UEs and distal LEs	Severe, multifocal axonal neuropathy, B. CTS and L. ulnar neuropathy at the elbow	Polyradiculopathy
2	Progressive proximal weakness of LEs > UEs and numbness in the feet	Proximal > distal weakness, more severe in LEs, distal foot sensory loss and ankle areflexia	Length dependent axonal polyneuropathy	Myopathy
3	Foot paresthesia	Diminished pinprick and temperature in the forefeet	Normal	Normal
4	None	ND	ND	ND
5	Numbness in the hands and feet	Normal strength, distal LE multimodal sensory loss, ankle areflexia	Length dependent axonal polyneuropathy and B.CTS	Normal
6	Progressive shortness of breath, dysphagia, dysarthria, and limb weakness	Severe weakness UE > LEs, DTRs absent in UEs and ankles, brisk at the knees. Multimodality sensory loss in the distal UEs and LEs	Length dependent axonal polyneuropathy	Polyradiculopathy versus motor neuron disease
7	None	ND	ND	ND
8	Progressive numbness in the feet	Normal strength, distal LE sensory loss, normal DTRs	Length dependent sensory axonal polyneuropathy	Normal
9	Progressive proximal weakness	Proximal > distal weakness of UEs and LEs, distal LE sensory loss, DTRs normal in the UEs, absent at the knees and ankles	Length dependent axonal polyneuropathy	Polyradiculopathy
10	None	ND	ND	ND
11	Numbness and neuropathic pain in the feet	Atrophy of distal foot muscles, distal LE sensory loss, ankle areflexia	Distal axonal polyneuropathy	Distal axonal polyneuropathy

Note: More detailed data are presented in Table SS1.
Abbreviations: B, bilateral; DTR, deep tendon reflex; L, left; ND, not done; R, right; UE and LE, upper extremity and lower extremity, respectively.

potentials, positive waves), with large motor unit action potentials and reduced recruitment in three, and short duration motor unit action potentials of low amplitudes in one (Table 2). Three patients in ATTR group did not have any neurological symptoms and an examination with MRC grading was not documented in those patients. There was no significant difference in the age and gender distributions between patients with ATTR versus controls ($p = 0.06$ and 0.90), ATTRv versus ATTRwt ($p = 0.64$ and 0.54), ATTR with versus without neurological

Group	N	Age	Gender (M/F)	Deltoid MC	Heart MC
Control	14	63.1 (16.6)	8/6	26.0 (7.1)	78.9 (20.4)
ATTR	11	73.6 (13.0)	6/5	37.0 (11.7)	120.1 (43.7)
ATTRwt	4	76 (6.1)	3/1	35.2 (15.9)	102.4 (28.4)
ATTRv	7	72.3 (14.6)	3/4	38.1 (9.8)	130.1 (49.5)
Neuro+	7	75.4 (6.4)	5/2	35.8 (11.3)	103.1 (39.7)
Neuro−	4	70.8 (18.4)	1/3	39.2 (12.2)	149.7 (25.3)
Deltoid+	4	81 (9.5)	2/4	39.9 (13.7)	86.6 (38.2)
Deltoid−	4	52.1 (25.4)	1/3	35.5 (10.4)	125.6 (36.9)

TABLE 3 Characteristics of control and patient groups

Abbreviations: Deltoid+ and Deltoid−, with and without deltoid weakness; M/F, male/female; Neuro+ and Neuro−, with and without neurological symptoms.

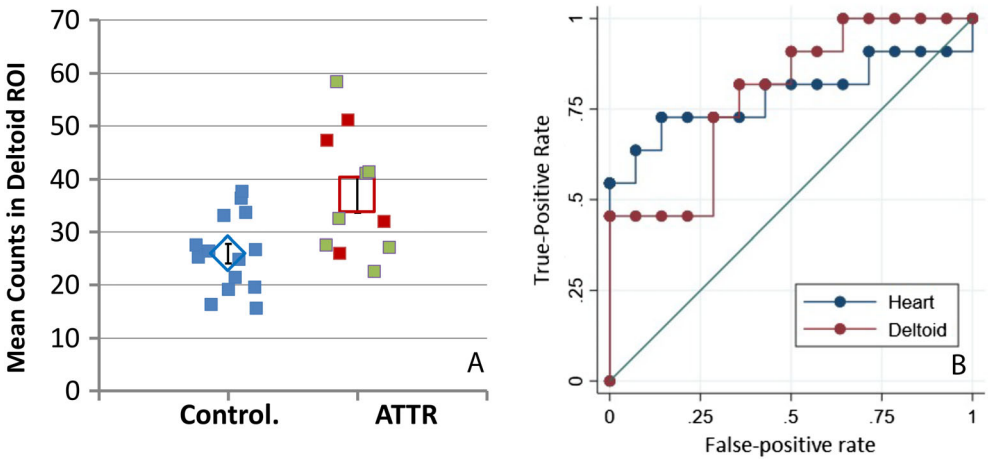

FIGURE 1 A, MC of deltoids in patients with ATTR and controls. The green and red cubes represent patients with and without neurological symptoms respectively. B, ROC curves for distinguishing ATTR patients from control subjects using ^{99m}Tc-PYP measured over the heart (blue) or over the deltoids (red). The AUCs were the same (AUC = 0.786)

symptoms ($p = 0.55$ and 0.24) and ATTR with versus without deltoid weakness ($p = 0.07$ and 0.77) (Table 3).

Nine of 11 (81.8%) patients in the ATTR group and none in the control group had a cardiac semi-quantitative visual score ≥ 2 on the ^{99m}Tc-PYP scan. The MC of the heart and average deltoids were significantly increased in the ATTR patients compared to the controls, $p = 0.005$ and $p = 0.014$, respectively (Table 3, Figure 1(A), Figure SS1). The MC of the CC was not significantly different between the ATTR patients and control subjects ($p = 0.17$). The average MC of the deltoids were not significantly different between ATTRv versus ATTRwt ($p = 0.71$), ATTR with versus without neurological symptoms ($p = 0.87$), and ATTR with versus without deltoid weakness ($p = 0.62$).(Table 1). Compared to measurements over the heart, the MC from the deltoids were equally useful in differentiating control subjects from ATTR patients: the area under the receiver operating characteristic (ROC) curve (AUC) was the same for measurements from the heart and deltoids (AUC = 0.786; Figure 1(B)).

4 | DISCUSSION

In this small proof-of-concept study, we demonstrated that the uptake of ^{99m}Tc-PYP is increased in the deltoid muscles of patients with ATTR compared to control subjects.

There was no significant difference in the ^{99m}Tc-PYP uptake in patients with or without deltoid weakness or EMG abnormality in that muscle. Possible explanations include: (1) small sample size of this study; and (2) asymptomatic nature of TTR deposition in some of the patients.

Two of the patients in our ATTR cohort had increased deltoid with normal heart uptake (Table 1): patient 1, who had heart transplantation before the scintigraphy, and patient 9 who had limb weakness without heart disease.

Patient 6 presented with rapidly progressive respiratory, bulbar, and limb weakness, and a distal axonal polyneuropathy. He was diagnosed with amyotrophic lateral sclerosis (ALS) superimposed on an underlying ATTRv related cardiomyopathy and polyneuropathy. ATTRv cases with a presentation mimicking ALS have been previously reported,[21,22] although with a slower rate of disease progression and a purely lower motor neuron phenotype. The patient did not have a nerve and muscle biopsy as he went on comfort care, and a post-mortem examination was not conducted.

The limitations of this study are its retrospective nature, the small sample size, lack of data on ^{99m}Tc-PYP uptake in the lower limbs, demonstration of amyloid deposition in the muscle biopsy in only one patient, and lack of variants other than V122I, which was the sole mutation in our ATTRv cohort due to its high prevalence in the metropolitan United States.[23]

There was significant overlap between the uptake of ^{99m}Tc-PYP in the deltoids of patients with and without ATTR raising the question of whether skeletal muscle ^{99m}Tc-PYP uptake will ultimately prove useful as a clinical diagnostic test. The small sample size of the study prevents a definitive answer to this question, but even with the overlap the sensitivity and specificity of deltoid uptake of ^{99m}Tc-PYP for ATTR diagnosis was comparable to that of the currently accepted measure of heart ^{99m}Tc-PYP, with the same AUC for both measures. While these findings suggest that ^{99m}Tc-PYP is a potentially useful biomarker for ATTR amyloid burden in the skeletal muscle, a larger, prospective study that includes the lower limbs will be needed to determine the applicability of this test in clinical practice.

5 | CONCLUSION

This preliminary, proof of concept study suggests that ^{99m}Tc-PYP may be a viable biomarker to assess the muscle burden of ATTR.

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CONFLICT OF INTEREST

Dr. Rezania has received honoraria from Alnylam and Akcea for serving in the advisory boards and as a speaker. The remaining authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ETHICS STATEMENT

A preliminary version of this work was presented as a poster in American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM) virtual meeting 2020.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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