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SHORT COMMUNICATION

Non-cardiac biopsy sites with high frequency of transthyretin amyloidosis

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Abstract

Aims Cardiac scintigraphy, a non-invasive technique for diagnosing ATTR cardiac amyloidosis, lacks specificity in patients with concomitant monoclonal gammopathy (up to 40% of cases). For these patients, amyloid type is often established by endomyocardial biopsy (EMB), which has clinical risk. This study aimed to investigate the frequency of ATTR in amyloid-positive tendon/synovium, urinary bladder, and prostate biopsies, sites for which prior biopsy specimens might exist for patients suspected of having cardiac amyloidosis, and, when available, determine the amyloid type concordance rate with other anatomic sites and provide clinical data regarding subsequent development of cardiac amyloidosis.

Methods and results We queried our reference laboratory database of 19,298 amyloid specimens from myriad anatomic sites typed by mass spectrometry-based proteomics (LC-MS/MS) to investigate the frequency of ATTR amyloid in tendon/synovium, urinary bladder, and prostate. The amyloid type was ATTR in 104/138 (75.4%) tendon/synovium, 173/453 (38.0%) urinary bladder, and 27/81 (33.3%) prostate samples. Of 62 patients with available clinical data, 12 (19%) had bona fide ATTR cardiac amyloidosis prior to/concomitant with the non-cardiac site biopsy. Of the remaining 14 with follow-up, 8 developed bona fide and 2 probable cardiac amyloidosis; at last follow-up 4 had no evidence of cardiac amyloidosis. Fourteen of 16 patients (87.5%) for whom we typed both non-cardiac and cardiac sites had concordant amyloid types. There were 2 discordant cases (prostate = ASem1/heart = AL and urinary bladder = AL/heart = ATTR); only the latter is potentially clinically consequential.

Conclusions In patients suspected of having cardiac amyloidosis based on cardiac scintigraphy, LC-MS/MS typing of Congophilic deposits in pre-existing biopsy specimens from non-cardiac sites may help establish the cardiac amyloid type, obviating the need for EMB. However, if the amyloid type identified in the non-cardiac site is not in keeping with other clinical features, then EMB for typing the cardiac amyloid might be indicated.

Keywords Amyloid typing; ATTR; Tendon; Synovium; Prostate; Urinary bladder

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Background

The clinical characteristics of amyloidoses, a group of clinical syndromes characterized by abnormal deposition of misfolded proteins in organs,[1] are determined by the identity of the deposited protein. Nearly all cardiac amyloidoses result from deposition of monoclonal light chains (AL) or transthyretin (ATTR).[1,2] However, AL and ATTR cardiac amyloidosis are separate diseases with distinct therapies and clinical courses, so accurate typing of the amyloid deposits is critical for patient care. Recently, technetium-labelled cardiac scintigraphy (99mTc-PYP and 99mTc-DPD) has been shown to be an effective method for detecting ATTR cardiac amyloidosis.[3,4] However, nuclear scintigraphy lacks specificity for ATTR in the setting of a monoclonal gammopathy,[3] which is present in up to 40% of ATTR patients.[3,5–7] In these patients, the standard of care is to obtain an endomyocardial biopsy (EMB)[3] or

subcutaneous fat aspirate[3] to establish the amyloid type by mass spectrometry-based proteomics. However, EMB has clinical risk. Moreover, only about 15% of ATTR cardiomyopathy patients have a positive subcutaneous fat aspirate.[8] Hence, we investigated the utility of using biopsies from alternative sites, which might be available due to concomitant clinical care, to establish an ATTR diagnosis.

We chose to study tendon/synovium, urinary bladder, and prostate biopsies for the following reasons. Cardiac ATTR patients often have an antecedent history of bilateral carpal tunnel syndrome or lumbar spinal stenosis and thus may have a pre-existing tendon/synovium specimen for analysis. ATTR amyloid has been identified in 34% of patients with idiopathic carpal tunnel syndrome[9,10] and 45% of patients with lumbar spinal stenosis.[11] Most ATTR amyloidosis patients are elderly men, and both urinary bladder and prostate are frequently biopsied sites in older men. Up to 83% of ATTR patients have urinary tract dysfunction,[12] and ATTR may be a common amyloid type in urinary bladder.[13] Also, approximately 10% of prostate biopsies from random populations and 25% from adenocarcinoma patients contain Congo red (CR)-positive amyloid deposits.[14]

Aims

This study aimed to study the frequency of ATTR in CR-positive amyloid deposits obtained from biopsies of tendon/synovium, urinary bladder, and prostate and, when available, determine the amyloid-type concordance rate with other anatomic sites and provide clinical data regarding subsequent development of cardiac amyloidosis.

Methods

Clinical cohort

Mayo Clinic Institutional Review Board approved this retrospective study, and it was conducted in accordance with the Declaration of Helsinki. A database of all CR-positive, paraffin-embedded, surgical specimens obtained from 19 298 amyloidosis patients typed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) in a reference laboratory at the Mayo Clinic between 1 January 2008 and 19 January 2020 was searched for three anatomic sites: urinary bladder (n = 453), tendon/synovium (n = 138), and prostate (n = 81). From this cohort of 672 specimens, we identified 30 patients who also had an amyloid specimen from a separate anatomic site typed by LC–MS/MS, as well as a separate cohort of 62 patients for whom clinical data were available.

Tissue amyloid typing

The CR-positive amyloid deposits were typed by LC–MS/MS using previously published methods.[15,16] A pathologist scrutinized the morphologic features and amyloid proteome of each specimen and determined an amyloid type based on the most abundant amyloidogenic protein consistently detected across all replicate analyses.

Clinical data analysis

Bona fide ATTR cardiac amyloidosis was defined as presence of positive PYP scan with Grade 2 or 3 myocardial uptake of radiotracer AND negative monoclonal gammopathy workup (serum protein electrophoresis, urine protein electrophoresis, and K/L ratio) AND suggestive cardiac imaging (either transthoracic echocardiogram with left ventricular wall thickness $>$12 mm, low global longitudinal strain with relative apical sparing pattern and Grade II diastolic dysfunction or more, OR cardiac magnetic resonance with left ventricular wall thickness $>$ upper limit of normal for sex and age, and diffuse late gadolinium enhancement), OR absence of PYP scan AND positive biomarkers (human cardiac troponin T or N-terminal pro-brain natriuretic peptide) AND suggestive cardiac imaging AND unexplained heart failure. A probable diagnosis of cardiac amyloidosis was made if full criteria were not met but the clinical impression after cardiologist review of the data was suggestive of cardiac amyloidosis. Cardiac amyloidosis was considered to be absent when the aforementioned criteria of bona fide and probable were not met.

Results

Table 1 shows the patient demographics of the first cohort and the frequency of ATTR, AL, and non-AL/non-ATTR types detected in each anatomic site. Together, AL and ATTR accounted for 94%, 93%, and 47% of the types observed in tendon/synovium, urinary bladder, and prostate biopsies, respectively. Tendon/synovium biopsies had the highest frequency of ATTR (75.4%), and urinary bladder and prostate biopsies had similar frequencies of ATTR (38.0% and 33.3%). Urinary bladder had the highest frequency of AL (54.5%), and tendon/synovium and prostate had similar frequencies of AL (18.1% and 13.6%). The non-AL/non-ATTR types were Aβ2M (N = 8) and AH (N = 1) in tendon/synovium; AH (N = 33) in urinary bladder; and ASem1 (N = 38), ALect2 (N = 4), and AH (N = 1) in prostate. Except for ASem1 in prostate, none of the non-AL/non-ATTR types reached more than 5% in frequency in any organ.

Mutant peptides indicative of pathogenic mutations were detected in 17 ATTR cases, including 11 urinary bladder

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Table 1 Frequency of amyloid types in organs of interest

Tissue	Age[a]	Gender (F/M/U)[b]	# of cases	TTR	AL	Non-AL/non-ATTR[c]
Tendon/synovium	77.4 ± 11.6	29/107/2	138	75.4%	18.1%	6.5%
Urinary bladder	74.8 ± 15.2	119/329/5	453	38.2%	54.5%	7.3%
Prostate	73.7 ± 8.5	0/81/0	81	33.3%	13.6%	53.1%

Demographics and frequency of amyloid types observed in tendon/synovium, urinary bladder, and prostate.
[a]Average and standard deviations are shown.
[b]M, male; F, female; and U, unknown.
[c]Non-AL/non-ATTR amyloid types are other canonical types listed in Benson *et al.*[1]

(*N* = 7 p.Val142Ile/Leu, *N* = 3 p.Thr80Ala, and *N* = 1 p.Val50Met), 5 tendon/synovium (*N* = 4 p.Val142Ile/Leu and *N* = 1 p.Thr80Ala), and 1 prostate (*N* = 1 p.Val50Met).

Table 2 shows organ and amyloid type detected in each patient in the second cohort, representing 30 patients with LC–MS/MS performed at two anatomic sites. The amyloid types in the two anatomic sites were concordant in all patients but two (93% concordance). There was 100% concordance if one of the two sites of involvement was tendon/synovium (*N* = 11). The concordance rate was 87.5% for the 16 patients in whom the second site was heart. The two cases with discordant amyloid types were prostate (ASem1)/heart (AL) and urinary bladder (AL)/heart (ATTR).

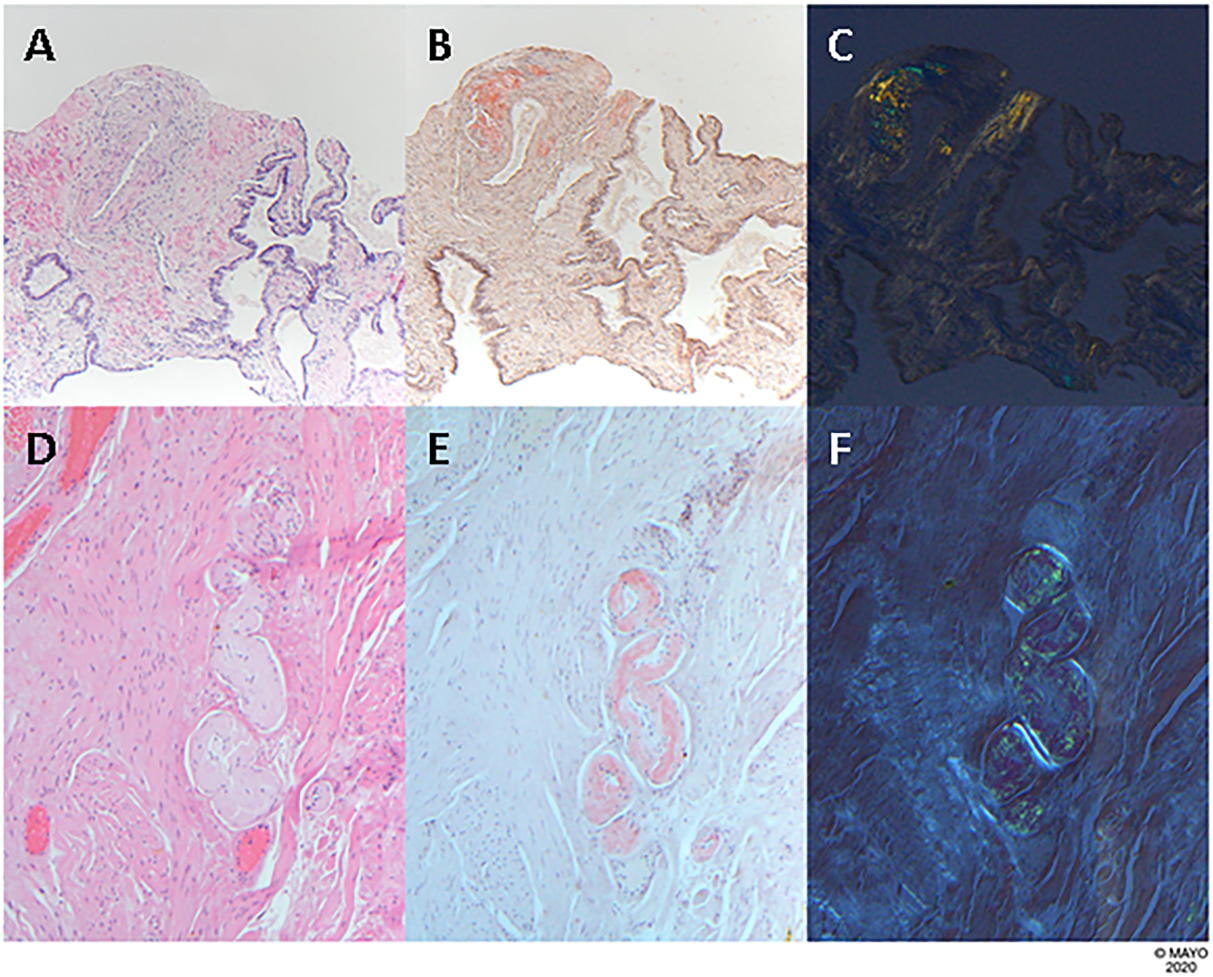
Clinical data regarding the development of cardiac amyloidosis were available for 62 patients. Cardiac involvement by ATTR amyloidosis prior to/concomitant with the non-cardiac site biopsy was present in 12 patients (19%) and probably present in three patients (5%), two of whom went on to develop bona fide disease (the third was lost to follow-up). Of the 50 patients without clinical evidence of bona fide cardiac disease at the time of the non-cardiac site biopsy, eight (16%) developed cardiac disease (including the two who previously had probable disease), two (4%) developed probable cardiac disease, four (8%) did not develop cardiac disease, and the rest were lost to follow-up. The median time to progression to cardiac disease was 3 years (range 1–17 years). For the

Table 2 Concordance of amyloid types in different organs

Patient ID	Organ of interest[a]	Other organ[b]
1	Urinary bladder = ATTR	GI tract = ATTR
2	**Urinary bladder = ATTR**	**Heart = ATTR, fat aspirate = ATTR**
3	**Urinary bladder = ATTR**	**Heart = ATTR**
4	**Urinary bladder = ATTR**	**Heart = ATTR**
5	**Urinary bladder = ATTR**	**Heart = ATTR**
6	Urinary bladder = ATTR	Lung = ATTR
7	Urinary bladder = AL	Bone marrow = AL
8	Urinary bladder = AL	Bone marrow = AL
9	**Urinary bladder = AL**	**Heart = AL**
10*	***Urinary bladder = AL***	***Heart = ATTR***
11	Urinary bladder = AL	Urethra = AL
12	Urinary bladder = AA	Bone marrow = AA, fat aspirate = AA
13	Urinary bladder = AA	GI tract = AA
14	**Tendon/synovium = ATTR**	**Heart = ATTR**
15	**Tendon/synovium = ATTR**	**Heart = ATTR**
16	**Tendon/synovium = ATTR**	**Heart = ATTR**
17	**Tendon/synovium = ATTR**	**Heart = ATTR**
18	**Tendon/synovium = ATTR**	**Heart = ATTR**
19	Tendon/synovium = ATTR	Nerve = ATTR
20	Tendon/synovium = ATTR	Right arm soft tissue = ATTR
21	Tendon/synovium = AL	Bone marrow = AL
22	Tendon/synovium = AL	Fat aspirate = AL
23	Tendon/synovium = AL	Fat aspirate = AL
24	Tendon/synovium = AL	Skin = AL
25	**Prostate = AL**	**Heart = AL**
26	**Prostate = ATTR**	**Heart = ATTR**
27	**Prostate = ATTR**	**Heart = ATTR**
28	**Prostate = ATTR**	**Heart = ATTR**
29*	***Prostate = ASem1***	***Heart = AL***
30	Prostate = AL	Bone marrow = AL

GI, gastrointestinal.
Patients in whom the second organ is heart are highlighted with bold font. Patients with discordant amyloid types between organs are highlighted with italicized font and an asterisk.
[a]Organ of interest and amyloid type.
[b]Other organ(s) from same patient and amyloid type(s).

Figure 1 Prototypical histopathology of ATTR in prostate and tenosynovial biopsies. Prostate (A–C) biopsy with subtle vascular ATTR amyloid deposits. (A) Haematoxylin and eosin, (B) Congo red, and (C) Congo red with cross-polarized light demonstrating apple green birefringent amyloid. Tenosynovial (D–F) tissue with vascular ATTR amyloid deposits. (D) Haematoxylin and eosin, (E) Congo red, and (F) Congo red with cross-polarized light demonstrating apple green birefringent amyloid.



20 patients (18 men and 2 women) with bona fide ATTR cardiomyopathy, the median age at diagnosis of cardiac involvement was 78.5 years (range 65–95 years). Of the 16 patients with ATTR amyloidosis in an alternate site with available serum or urine protein immunoelectrophoresis data, 5 (31.2%) had an M-protein, including 2 of 10 patients with tendon/synovium ATTR and 3 of 6 patients with urinary bladder ATTR.

Conclusions

There is heightened interest in identifying patients with cardiac ATTR amyloid as treatment options have recently become available, including a Food and Drug Administration approved drug that has been shown to prolong life in patients with ATTR cardiomyopathy.[17] Herein, we demonstrate that utilizing pre-existing biopsy specimens from sites such as tendon/synovium, urinary bladder, or prostate for CR staining, with subsequent typing by mass spectrometry-based proteomics if positive, may help confirm ATTR cardiomyopathy. Furthermore, as the morphologic features of amyloidosis are also often subtle (*Figure 1*), it is not unreasonable to perform a CR stain on a pre-existing specimen even if amyloidosis had not been diagnosed initially. However, it is imperative not to rely solely on the amyloid type identified in the alternate site to determine the identity of the cardiac amyloid, as rarely two different amyloid types can exist in the same patient.[18,19] Tendon/synovium may have the greatest utility for confirming ATTR cardiomyopathy, both because of its high frequency of ATTR (75%) and because of the recognized association between an

antecedent history of bilateral carpal tunnel syndrome and/or lumbar spinal stenosis and ATTR cardiomyopathy. Although our overall amyloid-type concordance rate was 87.5% among the 16 patients for whom both cardiac and non-cardiac specimens were typed, it was 100% when the non-cardiac specimen was tendon/synovium.

We identified two patients with discordant amyloid types. The first, prostate (ASem1)/heart (AL), would not be clinically problematic as ASem1 is typically localized exclusively to seminal vesicles and is clinically inconsequential. Also, it is easy to distinguish amyloid involving seminal vesicles from amyloid involving prostatic parenchyma on pathologic review. If seminal vesicle ASem1 cases are excluded from analysis in our study, then the frequency of ATTR amyloid in prostate is 69%, which is similar to that of tendon/synovium. Furthermore, in prostate, the frequency of AL amyloid is low (14%).

The second case, urinary bladder (AL)/heart (ATTR), illustrates that relying solely on the alterate site result could potentially be misleading. It is also not surprising that this case involved urinary bladder. AL amyloid is the most common amyloid type in urinary bladder (55%), unlike tendon/synovium (18%) and prostate (14%). Furthermore, localized AL amyloidosis of the urinary bladder is a recognized phenomenon.[20] Thus, it is imperative to assess all clinical features for each amyloid cardiomyopathy patient. If the amyloid type identified in an alternate site is not in keeping with other clinical features, then EMB for verification of amyloid type in the heart might be indicated.

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Conflict of interest

None declared.

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