

Diagnostic sensitivity of abdominal fat aspiration in cardiac amyloidosis

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Aims

Congo red staining of an endomyocardial biopsy is the diagnostic gold-standard in suspected cardiac amyloidosis (CA), but the procedure is associated with the risk, albeit small, of serious complications, and delay in diagnosis due to the requirement for technical expertise. In contrast, abdominal fat pad fine needle aspiration (FPFNA) is a simple, safe and well-established procedure in systemic amyloidosis, but its diagnostic sensitivity in patients with suspected CA remains unclear.

Methods and results

We assessed the diagnostic sensitivity of FPFNA in 600 consecutive patients diagnosed with CA [216 AL amyloidosis, 113 hereditary transthyretin (ATTRm), and 271 wild-type transthyretin (ATTRwt) amyloidosis] at our Centre. Amyloid was detected on Congo red staining of FPFNAs in 181/216 (84%) patients with cardiac AL amyloidosis, including 100, 97, and 78% of those with a large, moderate, and small whole-body amyloid burden, respectively, as assessed by serum amyloid P (SAP) component scintigraphy ($P < 0.001$); the deposits were successfully typed as AL by immunohistochemistry in 102/216 (47%) cases. Amyloid was detected in FPFNAs of 51/113 (45%) patients with ATTRm CA, and only 42/271 (15%) cases with ATTRwt CA.

Conclusions

FPFNA has reasonable diagnostic sensitivity in cardiac AL amyloidosis, particularly in patients with a large whole-body amyloid burden. Although the diagnostic sensitivity of FPFNA is substantially lower in transthyretin CA, particularly ATTRwt, it may nevertheless sometimes obviate the need for endomyocardial biopsy.

Keywords

Amyloid • Amyloidosis • Cardiomyopathy • Diagnosis • Fat aspiration • Scintigraphy

Introduction

Systemic amyloidosis is usually diagnosed via Congo red staining of a biopsy from a clinically affected organ but, when suspected, may be diagnosed through a so-called 'screening biopsy' of rectum,^{1,2} salivary

gland,³ or fat,⁴ avoiding the risks, costs and delay associated with biopsies of organs such as the heart and liver.⁵

Cardiac amyloidosis (CA) is a common manifestation and the major determinant of prognosis in systemic immunoglobulin light chain (AL) amyloidosis.^{6,7} CA is also the commonest presenting

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feature of transthyretin (ATTR) amyloidosis,^{8,9} and is the dominant phenotypic manifestation in both wild-type ATTR amyloidosis (ATTRwt), which is increasingly diagnosed in the elderly male population,¹⁰ and the most common form of mutant cardiac ATTR amyloidosis (ATTRm), that associated with the V122I variant.⁸

Endomyocardial biopsy, the diagnostic gold standard for detection of CA, is associated with a small risk of serious complications, high cost, and a requirement for specialist technical expertise and hospital equipment which are not widely available and may introduce delay.⁵ An alternative diagnostic test with high sensitivity which can be performed at the bedside, is thus particularly desirable in patients with suspected CA. Congo red staining of an abdominal fat pad fine needle aspiration (FPFNA) is a simple, cheap, low risk procedure that is currently the most widely used screening biopsy technique in patients with suspected systemic amyloidosis.¹¹ Unlike other screening biopsy techniques such as rectal, salivary gland or formal fat biopsy, it does not require a surgical incision, blunt dissection, surgical re-approximation, or suture. Although the specificity of FPFNA for amyloid is unequivocally high,⁴ its diagnostic sensitivity has been reported to be as low as 14% in certain series and >90% in others.^{12,13}

The diagnostic performance of FPFNA in certain types of ATTR amyloidosis has previously been reported,¹² but there has never been a systematic study comparing its diagnostic sensitivity in a large cohort of patients with different types of CA, including subtypes of ATTRm, and the basis for the reported discrepancy in sensitivity is not known.

We sought to determine the diagnostic sensitivity of FPFNA in identifying and typing amyloid deposits in patients with the three main types of CA, namely cardiac AL, cardiac ATTRm, and cardiac ATTRwt amyloidosis. Further, we studied the relationship between the diagnostic sensitivity of FPFNA and total body amyloid burden hypothesizing that the presence and degree of amyloid infiltration in fat may correlate with the extent of amyloid throughout the body in general.

Methods

Patients

Patients with suspected CA attending the UK National Amyloidosis Centre between 2010 and 2015 underwent a comprehensive protocol of evaluation comprising ECG, echocardiogram, cardiac magnetic resonance (CMR) imaging, biochemical tests including serum and urine immunofixation electrophoresis and serum free light chain assay, ¹²³I-serum amyloid P component (SAP) scintigraphy,¹⁴ ^{99m}Tc-labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) scintigraphy,¹⁵ TTR gene sequencing,¹⁶ and where necessary, sequencing of other hereditary amyloidosis genes.

Six hundred consecutive patients with an unequivocal diagnosis and type of CA were included in the analysis. The diagnosis of presence and type of cardiac amyloid was established on the basis of a characteristic echocardiogram and/or CMR for amyloid, in conjunction with histological proof of presence and type of amyloid in 380 patients (including all those with cardiac AL amyloidosis) and on the basis of published non-biopsy diagnostic criteria in 220 patients with ATTR amyloidosis.¹⁷

All patients were managed in accordance with the Declaration of Helsinki and provided informed consent for anonymous publication of scientific data.

Fat pad fine needle aspiration

By definition, all patients included in the study underwent FPFNA, which was performed as previously described.¹¹ Median (range) weight of aspirated material was 0.050 g (0.004–0.131). Smears from each FPFNA were prepared on five glass slides for Congo red staining. The remainder of the aspirated fat tissue in each case was briefly fixed in formalin, double embedded in agar, and a paraffin block was produced for further Congo red staining and routine immunohistochemistry (IHC). IHC was performed using a panel of monospecific antibodies against the most common amyloidogenic proteins, including kappa and lambda light chains, and transthyretin, as previously described.¹⁸ Interpretation of all stained slides was carried out independently by two experienced examiners.

¹²³I-serum amyloid P component scintigraphy

Whole body anterior and posterior scintigraphic imaging was undertaken 6 or 24 h after administration of ¹²³I-labelled SAP, as previously described.¹⁹ Whole body amyloid load burden was categorized into small, moderate, or large in each patient, as previously described.²⁰ Labelled SAP studies were interpreted by a panel of physicians with experience of over 10 000 SAP scans who were blinded to the FNFA results.

Statistical analysis

Summary statistics were expressed as mean (SD) or median (interquartile range) for numerical variables and frequencies (percentages) for categorical variables. Independence of the two categorical variables defining a contingency table was tested using Fisher's exact test or Pearson's chi-square test (according to Cochran's rule) using IBM SPSS Statistics 23 software. *P*-values < 0.05 were considered significant.

Results

Among 600 patients with an unequivocal diagnosis of CA, 216 had systemic AL amyloidosis (age 65 ± 10 years), 113 had ATTRm amyloidosis (age 68 ± 8 years), and 271 had ATTRwt amyloidosis (age 71 ± 6 years). Details of the diagnostic pathway are provided in a Supplementary material online, *Figure S1*. Cardiac AL amyloidosis was lambda light chain isotype in 188/216 (86%) cases. Among patients with ATTRm, TTR variants were distributed as follows: Val122Ile (*n* = 69), Thr60Ala (*n* = 21), Val30Met (*n* = 7), Ser77Tyr (*n* = 5), Glu89Gln (*n* = 3), Phe44Leu (*n* = 2), Ile68Leu (*n* = 2), Ile107Phe (*n* = 2), Cys10Gly (*n* = 1), Glu54Leu (*n* = 1). Of note, the most prevalent TTR variant in our population, Val122Ile, was typically associated with an exclusive cardiac phenotype, similar to ATTRwt,⁹ whereas the next most prevalent, Thr60Ala, was associated with a variably mixed cardiomyopathy and neuropathy phenotype.²¹

Among 216 patients with systemic AL amyloidosis, 181 (84%) had amyloid detected on Congo red staining of their FPFNA (*Table 1*). The amyloid was definitively typed as AL by IHC of the FPFNA in 102/181 (56%) patients with amyloid present and in 102/216 (47%) of total AL patients. Interestingly, amyloid was present in the FPFNA of 28/28 (100%) AL amyloidosis patients with a large whole body amyloid burden by SAP scintigraphy, 33/34 (97%) AL amyloidosis patients with a moderate whole body amyloid burden, and 120/154 (78%) of those with systemic AL amyloidosis and a small whole body amyloid burden (*P* < 0.001; large/moderate vs. small load, Fisher's exact test).

Table 1 Diagnostic sensitivity of fat pad fine needle aspiration in different cardiac amyloidoses

Amyloid type	n	Number positive by Congo red staining	Diagnostic sensitivity (CI)
Systemic AL amyloidosis	216	181	84% (78–88%)
ATTRm	113	51	45% (36–54%)
Val122Ile	69	23	33%
Thr60Ala	21	14	67%
ATTRwt	271	42	15% (11–20%)

Systemic AL amyloidosis vs. ATTR amyloidosis, $P < 0.001$ (Chi square test). The combination of absence of amyloid on FPFNA and absence of a *TTR* mutation on gene sequencing, had a positive predictive value for ATTRwt in this series of 87% (CI 82–91%) and a negative predictive value of 81% (CI 75–86%).

Among 113 patients with ATTRm CA, 51 (45%) had amyloid detected on Congo red staining of their FPFNA (Table 1). The amyloid was definitively typed as TTR by IHC of the FPFNA in 37/51 (73%) patients with amyloid present and in 37/113 (33%) total ATTRm patients. More specifically, amyloid was identified in the fat samples of 23/69 (33%) patients with Val122Ile-associated ATTRm amyloidosis compared with 14/21 (67%) patients with Thr60Ala-associated ATTRm amyloidosis.

Among 271 patients with ATTRwt CA, amyloid was identified in the FPFNA of only 42 (15%) cases (Table 1), 27 (65%) of whom had diagnostic IHC for TTR (10% of total with ATTRwt).

Discussion and conclusions

Our study, which comprises the largest cohort to date of consecutive patients with CA to undergo FPFNA as a diagnostic tool, supports its use in systemic AL amyloidosis but highlights its limitations for diagnosis of ATTR amyloidosis, particularly ATTRwt amyloidosis. Amyloid was identified in the FPFNA specimens of only 15% of patients with ATTRwt and the fibril protein was definitively typed as TTR by IHC in only 10% of cases. Similarly, amyloid was only detected in one third of FPFNAs from patients with Val122Ile-associated CA, the most common type of ATTRm CA worldwide, who typically present with a cardiomyopathic phenotype that is indistinguishable from ATTRwt. It is noteworthy however, that the finding of grade 2 or 3 cardiac uptake on bone scintigraphy in a patient with a suggestive echocardiogram or CMR for CA who does not have evidence of a monoclonal protein by serum or urine immunofixation electrophoresis or by serum free light chain assay, is sufficient to diagnose cardiac ATTR amyloidosis without need for histological identification of amyloid (i.e. in the context of a negative FPFNA or without an FPFNA); further differentiation between ATTRwt and ATTRm CA can then be established by genetic testing.¹⁷ When cardiac AL amyloidosis is suspected however, typically in the context of a monoclonal protein or grade 0 or 1 cardiac uptake on bone scintigraphy, FPFNA remains clinically valuable, since it is known to have high diagnostic specificity and may obviate the need for invasive EMB. Furthermore, in this cohort of patients, all of whom had CA, a

Table 2 Relationship between diagnostic sensitivity of fat pad fine needle aspiration and total body amyloid burden

Amyloid type	Total body amyloid load by SAP scintigraphy	Amyloid detected on FNFA	Diagnostic sensitivity
Systemic AL amyloidosis	Large	28/28	100%
	Moderate	33/34	97%
	Small	120/154	78%
ATTRm	Small ^{a,b}	51/113	45%
ATTRwt	Small ^a	42/271	15%

Amyloid deposits in the gastrointestinal tract^a and nerves^b are not visualized by SAP scintigraphy. Large/moderate load vs. Small load in AL, $P < 0.001$ (Fisher's exact test), Large/moderate load vs. Small load (all patients), $P < 0.001$ (Chi Square test).

negative result (i.e. absence of amyloid on FPFNA) in the absence of a *TTR* mutation on gene sequencing, had a positive predictive value for wild-type ATTR amyloidosis of 87% (CI 82–91%).

In a general cardiological setting, imaging modalities with high diagnostic specificity for cardiac amyloid, such as bone scintigraphy and/or CMR imaging are likely to have higher sensitivity for CA, particularly ATTR CA, than FPFNA and should probably therefore, be employed earlier in the course of the investigative pathway. Although there are no formal guidelines on the diagnostic pathway for patients with suspected CA, existing guidelines on diagnosis and management of hypertrophic cardiomyopathy suggest consideration of EMB in suspected infiltrative cardiomyopathy. Due to the simplicity and rapidity of the procedure, we would suggest that FPFNA should be performed before recourse to EMB in such cases.²²

The strong association between total body amyloid burden as estimated by SAP scintigraphy and the likelihood of identifying amyloid deposits on FPFNA is noteworthy, although perhaps not surprising. Quite simply, the more extensive the amyloid, the higher the chance of finding deposits within a FPFNA sample. Since all FPFNAs were performed and analysed at a single centre which has widely recognized experience in the histological assessment of amyloid deposits from a variety of different tissues, the differences in diagnostic yield identified here between phenotypically different patients, are likely to reflect 'true' amyloid deposition rather than differing experience or practice, or varying diagnostic technique. SAP scintigraphy is most sensitive for identifying amyloid in large solid organs such as liver, spleen, kidney, and bone, which are commonly involved in AL amyloidosis, but is unable to identify amyloid deposits in non-solid or very diffuse organs such as the heart, nerves or gastrointestinal tract which are those involved in ATTR amyloidosis. Nonetheless, an extensively amyloidotic liver and spleen may contain ~8 kg of amyloid compared with an extensively amyloidotic heart which contains ~500 mg such that the estimate of total amyloid burden by SAP scintigraphy remains valid even among those with extensive CA. The higher diagnostic sensitivity of FPFNA in Thr60Ala-associated ATTRm amyloidosis, which typically involves the peripheral and autonomic nerves as well as the heart, compared with Val122Ile-associated ATTRm and ATTRwt amyloidosis, which typically cause a late onset restrictive

cardiomyopathy in the absence of significant extra-cardiac involvement, is consistent with greater systemic involvement in Thr60Ala patients. It is not known whether the diagnostic yield of screening biopsies from other sites has an association with whole body amyloid load. The possibility that proteomic analysis of PPFNA specimens might result in higher diagnostic sensitivity was not addressed here, and also merits further evaluation.²³

In conclusion, diagnosing systemic amyloidosis may be challenging, particularly in centres with no access to newer non-invasive diagnostic tools such as DPD or SAP scintigraphy. Although PPFNA is a simple, safe, and inexpensive technique with a recognized role in diagnosis of amyloidosis generally, it has important limitations, including low sensitivity in patients with a small total body amyloid burden and those with ATTR amyloidosis and predominant cardiac disease, and cannot be used to exclude amyloidosis. Obtaining a biopsy from an organ that is thought to be clinically affected by amyloid continues to be a diagnostic requirement in a substantial proportion of patients who are suspected of having systemic amyloidosis.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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