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...
Fat pad fine needle aspiration

By definition, all patients included in the study underwent FPFNA, which was performed as previously described.\(^1\) 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 monoclonal antibodies against the most common amyloidogenic proteins, including kappa and lambda light chains, and transthyretin, as previously described.\(^1\) Interpretation of all stained slides was carried out independently by two experienced examiners.

\(^{123}\text{I}-\text{serum amyloid P component scintigraphy}\)

Whole body anterior and posterior scintigraphic imaging was undertaken 6 or 24 h after administration of \(^{123}\text{I}-\text{serum amyloid P component scintigraphy}\), as previously described.\(^1\) Whole body amyloid load burden was categorized into small, moderate, or large in each patient, as previously described.\(^2\) Labelled SAP studies were interpreted by a panel of physicians with experience of over 10 000 SAP scans who were blinded to the FNFPA 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 hadATTRm amyloidosis (age 68 ± 8 years), and 271 hadATTRwt 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.\(^2\)

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).
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 ATTRm amyloidosis compared with 14 of 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).

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%).

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 FPFNA | Diagnostic sensitivity |
|--------------|---------------------------------------------|---------------------------|------------------------|
| Systemic AL  | Large                                       | 28/28                     | 100%                   |
| amyloidosis  | Moderate                                    | 33/34                     | 97%                    |
|              | Small                                       | 120/154                   | 78%                    |
| ATTRm        | Small<sup>a</sup>                           | 51/113                    | 45%                    |
| ATTRwt       | Small<sup>a</sup>                           | 42/271                    | 15%                    |

Amyloid deposits in the gastrointestinal tract<sup>a</sup> and nerves<sup>b</sup> 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).

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).

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.<sup>72</sup>

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 Thr60 Ala 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 PFPNA 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 FPFNA 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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