The value of screening biopsies in light chain (AL) and transthyretin (ATTR) amyloidosis

Running Title: Screening biopsies in amyloidosis

Oliver C. Cohen, Faye Sharpley, Janet A. Gilbertson, Ashutosh D. Wechalekar, Sajitha Sachchithanantham, Shameem Mahmood, Carol J. Whelan, Ana Martinez-Naharro, Marianna Fontana, Helen J. Lachmann, Phillip N. Hawkins, Julian D. Gillmore

National Amyloidosis Centre, Centre for Amyloidosis & Acute Phase Proteins, Division of Medicine, University College London

There are no conflicts of interest to declare

Correspondence:
Prof Julian D Gillmore
Centre for Amyloidosis and Acute Phase Proteins, Division of Medicine, University College London
Email: j.gillmore@ucl.ac.uk
Telephone: +44 (0)20 74332816, Fax: +44 (0)20 74332844

Abstract Word Count: 231
Manuscript Word count: 1767
Number of References: 14
Number of Figures: 1
Number of Tables: 3

Summary Statement

What is the new aspect of your work?

We present data examining the single and combined diagnostic sensitivity of three screening biopsies modalities in systemic amyloidosis. Organ involvement and disease burden both correlate with diagnostic sensitivity in AL amyloidosis. Whilst we confirm that diagnostic sensitivity of abdominal fat aspiration in ATTR amyloidosis is low, we find that ATTR amyloid is demonstrated in approximately one-third of bone marrow and gastrointestinal biopsies when abdominal fat aspiration failed to detect amyloid.

What is the central finding of your work?

We demonstrate that the combination of abdominal fat and bone marrow trephine biopsies avoid the need for critical organ biopsy in >80% patients with systemic AL amyloidosis. In patients with cardiac, renal or hepatic AL amyloid, screening biopsy sensitivity is higher leaving just 13.7% requiring target organ biopsy. Furthermore, we find that total body amyloid burden, based on ^{123}I-SAP scintigraphy, correlates with diagnostic sensitivity of screening biopsies.

What is the specific clinical relevance of your work?

We would advocate routine abdominal fat aspiration in patients undergoing a bone marrow assessment to investigate suspected AL amyloidosis in order minimize the requirement for higher-risk and more invasive target-organ biopsies.

Abstract

Introduction

Systemic amyloidosis is a histological diagnosis, often achieved via critical organ biopsy. Screening biopsies represent a low–risk approach to diagnosis.

Objectives and Methods

Patients with systemic AL and ATTR amyloidosis who underwent abdominal fat aspiration (AFA) and either a bone marrow (BM) or gastrointestinal (GI) biopsy at the UK National Amyloidosis Centre (2006-2019) were identified. We sought to determine diagnostic sensitivity in relation to whole body amyloid burden, amyloid type, and organ involvement

Results

Diagnostic sensitivity, established in 471 patients with AL (n=321) and ATTR (n=150) amyloidosis respectively was 73.2% and 27.3% for AFA ($p< 0.001$), 59.7% and 42.2% for BM ($p< 0.001$), 74.6% and 44.6% for GI biopsy ($p< 0.001$). ATTR amyloid deposits were detected in 35.4% BMs and 33.3% of GI biopsies when AFA did not demonstrate amyloid. In AL amyloidosis, sensitivity of combined AFA and BM biopsy in AL amyloidosis was 82.9%. There was a strong association between whole body amyloid burden and sensitivity of each screening biopsy method (80.0-90.5% if large load vs. 53.9-79.0% if no visceral deposits).

Conclusion

Performing both AFA and BMB should be considered in suspected AL amyloidosis to substantially reduce the clinical risk associated with critical organ biopsy. The sensitivity of screening biopsies in ATTR amyloidosis is poor.

Keywords: amyloidosis, histology, biopsy, diagnosis, fat aspirate

Introduction

Systemic AL amyloidosis is a histological diagnosis usually established via biopsy of a clinically involved organ[1], which is costly, has clinical risk[2,3], and requires expertise[4]. Despite the high diagnostic specificity of non-biopsy diagnosis of cardiac transthyretin (ATTR) amyloidosis, up to 30% patients have an identifiable monoclonal protein, such that histology, usually via an endomyocardial biopsy, is required to differentiate it from cardiac AL amyloidosis[5].

Screening biopsies represent a low–risk approach to histological diagnosis. The use of abdominal fat aspiration as a low-risk screening biopsy was first identified by Westermark and colleagues almost 50 years ago[6]. Reported sensitivities of screening biopsies in AL amyloidosis vary between 75-96% for abdominal fat aspiration (AFA)[2,7-12], 65% for bone marrow trephine (BMT)[13] and 50-70% for rectal biopsies[1]. The sensitivity of AFA also differs widely between amyloid types[8].

Few studies have examined the diagnostic sensitivity and concordance of combining different screening biopsy methods[11]. Patients with suspected AL amyloidosis routinely undergo diagnostic BMT biopsy[3] and the high sensitivity of AFA in AL amyloidosis, coupled with its procedural ease and safety[14], warrant its consideration as a routine adjunct to diagnosis. In addition, there are limited data on the relationship between organ involvement and whole body amyloid burden in AL amyloidosis[8,11].

We report here, the diagnostic sensitivity of screening biopsies and their concordance in patients with AL and ATTR amyloidosis.

Methods

All confirmed cases of ATTR and AL amyloidosis who underwent AFA and either BMT or GIB at the UK National Amyloidosis Centre (NAC), 2006-2019, were included. Confirmation of amyloid type was by immunohistochemistry or mass spectrometry except in cases of ATTR amyloidosis in whom criteria for non-histological diagnosis were met[5].

All patients underwent a detailed baseline clinical evaluation at NAC including echocardiography and ^{123}I-SAP scintigraphy. Involvement of the heart, liver and kidneys by amyloid

was defined according to International Consensus Criteria[15] and amyloid load was determined by ^{123}I-SAP scintigraphy.

AFA was performed as previously described [14]. Fat smears underwent Congo red staining and then formalin-fixed and double-embedded in agar before production of a paraffin block. All other biopsies were received as formalin-fixed paraffin blocks, which were sectioned for Congo red staining and immunohistochemistry. All slides were interpreted by two experienced personnel (one senior clinician and one senior laboratory scientist) blind to the clinical details. In the rare event of any disagreement regarding final diagnosis, the case was discussed in a multi-disciplinary team setting to reach a consensus. Gastrointestinal biopsies were performed prospectively, prior to review at NAC, either to screen for amyloidosis or for another indication. The level of CR staining was graded from 1-5 (1-single or very scanty deposits; 2-scanty deposits such as along 1-2 vessels; 3-throughout within selected areas; 4-throughout the sample; 5-complete replacement of normal tissue structure by sheets of amyloid).

Statistical analysis was performed using SPSS version 25. All statistical tests were 2-sided with *p* values of <0.05 considered significant.

Approval for analysis and publication was obtained from the Royal Free Hospital institutional review board and written consent was obtained from all patients in accordance with the Declaration of Helsinki.

Results

471 patients with a confirmed diagnosis of AL (n=321) or ATTR amyloidosis (n=150) were identified. Median age was 75 (41-95) years; 338 (71.6%) were male. Amyloidotic involvement of the heart, kidneys and liver was present in 68.8%, 43.9% and 19.0% of the cohort respectively. AL amyloidosis was lambda subtype in 231/321 (72.0%) and kappa subtype in the remainder.

Amyloid was detected on AFA in 235/321 (73.2%) patients with systemic AL amyloidosis. There was no association between presence of amyloid on AFA and light chain isotype (p=0.158). AL

amyloid was detected in 166/278 (59.7%) BMT and 53/71 (74.6%) GI biopsies (GIB). For GIB, the biopsy sites were: 36 (50.7%) rectal, 18 (25.4%) colon, 9 (12.7%) stomach, 6 (8.5%) duodenal and 2 (2.8%) oesophageal. Among patients with AL amyloid detected on AFA who also underwent a BMT (n=195) or GIB (n=50), amyloid was detected in 131 (67.2%) BMT and in 39 (78.0%) GIB. Among 86 patients with systemic AL amyloidosis in whom the AFA did not show amyloid, an alternative screening biopsy did identify amyloid in 49 (47.1%) cases (35 BMT and 14 GIB) (Figure 1).

The diagnostic sensitivity of screening biopsies was substantially lower among 150 patients with ATTR amyloidosis than in AL amyloidosis (AFA: 41/150 (27.3%); p=0.0001, BM: 35/83 (42.2%); p=0.004, GI: 33/74 (44.6%), p=0.0001). In ATTR amyloidosis, GIB sites were: 29 (39.2%) rectal, 24 (32.4%) colon, 10 (13.5%) duodenal, 6 (8.1%) oesophageal and 5 (6.8%) stomach. The ATTR amyloidosis cohort consisted of 117 patients with wild-type and 38 patients with hereditary ATTR amyloidosis; sensitivity of AFA was significantly lower in wild-type (16%) than hereditary (61%) ATTR amyloidosis (p=0.0001) and there appeared to be a similar, albeit non-significant, trend with both BMT (57% v 38%) and GI biopsies (50% v 42%). ATTR amyloid deposits were detected in 35.4% BMs and 34.6% of GI biopsies when AFA did not demonstrate amyloid; conversely, when ATTR amyloid was present in the AFA, it was also identified in 66.6% BMs and 68.2% GI biopsies.

The grade of amyloidosis on CR staining by biopsy site and amyloid subtype is documented in Table 1. The grading was higher in AFA than BM samples in both AL (p<0.0001) and ATTR amyloidosis (p=0.05). In AL amyloidosis, grading of CR staining was higher in AFA than GI biopsies (p<0.0001), which was not the case in ATTR amyloidosis (p=0.21). There was no significant difference in sensitivity of GIB between AL and ATTR amyloidosis (p=0.07). Furthermore, there was no difference in diagnostic sensitivity of GIB between asymptomatic patients and those with a clinical indication for GIB (p=0.26).

In systemic AL amyloidosis, the diagnostic sensitivity of screening biopsies was associated with organ involvement (Table 2). Patients with hepatic involvement were significantly more likely than those without to have amyloid detected on AFA (91.8% vs 69.0%; p=0.0002) and GI biopsy (100% vs 71.4%; p=0.04) although the sensitivity of BM biopsies between these two groups of patients was

similar (71.4% vs 69.5% respectively; p=0.10). AFA had a similar diagnostic sensitivity in patients with cardiac and renal AL amyloidosis (76.9% vs 78.0%; p=0.898), as did other screening biopsies. The combination of AFA and BM trephine biopsy, was associated with an overall diagnostic sensitivity in systemic AL amyloidosis of 82.9% and a diagnostic sensitivity of 86.3% in patients with clinically important organ involvement (85%, 96% and 87% in patients with cardiac, hepatic and renal amyloidosis respectively). Among patients with AL amyloidosis who did not have cardiac, hepatic or renal involvement (n=48), diagnostic sensitivity for AFA, BM and GI biopsies were 64.6%, 40.5% and 77.8% respectively; the combination of AFA and BM trephine had a diagnostic sensitivity of 71.4%.

Total body amyloid load, defined by ^{123}I-SAP scintigraphy, was also strongly associated with diagnostic sensitivity of screening biopsies in systemic AL amyloidosis, although even among patients without visceral amyloid deposits by SAP scintigraphy, the diagnostic sensitivity of both AFA (69.0%) and GI biopsy (68.0%) was more than two thirds (Table 3).

Discussion

This study underlines the value of screening biopsies, particularly in AL amyloidosis, for establishing the diagnosis without recourse to higher-risk, higher-cost target organ biopsies. We found the diagnostic sensitivity of AFA to be 73.2%, increasing to 82.9% when combined with BMT, in patients with systemic AL amyloidosis. These figures are analogous to the 89% sensitivity of combining these procedures previously reported[11]. Notably, biopsies reported in these two studies were processed and reported within specialist amyloidosis centres thereby minimising the risks of previously reported high inter-observer variability and false negatives[16]. Consequently, the authors favour central review of screening biopsies to maximise diagnostic sensitivity. The combined use of AFA and BM biopsy left just 17.1% requiring an alternative biopsy to establish a histological diagnosis.

Perhaps unsurprisingly, screening biopsies are more likely to be positive if overall disease burden is higher and if there is extensive or critical organ (cardiac, hepatic, renal) involvement by amyloid. The diagnostic sensitivity in patients with hepatic amyloidosis was highest, likely reflecting

the fact that presence of amyloid in the liver is invariably associated with presence of amyloid in other organs[17], and typically indicates a high overall disease burden. The combined diagnostic sensitivity of AFA and BM in patients with cardiac, renal or hepatic AL amyloidosis, those that are most likely to be referred for target organ biopsy, was 86.3% (compared to 59.7% with BM alone), leaving just 13.7% patients requiring a so called 'high risk' biopsy. Most of the remaining patients with AL amyloidosis had predominant soft tissue amyloid (e.g. macroglossia), easily amenable to tissue sampling by an oral surgeon. BM biopsy is routinely undertaken in patients with suspected AL amyloidosis to ascertain plasma cell percentage [3] and we would suggest that AFA should be performed at the same time in such cases.

In ATTR amyloidosis, the need to establish a definitive histological diagnosis in patients' who do not meet non-biopsy diagnostic criteria[5] has increased with the availability of new gene silencing medications, available for hereditary ATTR amyloidosis, and clinical trials of novel therapeutics such as ATTRibute-CM (NCT03860935), access to which require a firm diagnosis. Consequently, histological proof of diagnosis is paramount to allow access to disease-modifying therapies for eligible patients. Whilst diagnostic sensitivity of AFAs is considerably lower in ATTR amyloidosis, frequent involvement of the heart in this type of amyloidosis and the significant, albeit low, mortality and serious complication risk associated with endomyocardial biopsy, quoted as ~6% in most studies[16], nonetheless encourages their initial use. We would not advocate performing BM biopsies as a means of screening for ATTR deposits. These tests were conducted for other indications (e.g. the finding of a paraprotein) prior to referral to our centre and we retrospectively requested the tissue to assess for the presence of amyloidosis as a means of differentiating between AL and ATTR amyloidosis.

In conclusion, AFA is a simple, low risk procedure that can be performed at the bedside at the same time as the BM trephine in patients with suspected AL amyloidosis which we believe ought to be introduced as 'standard' diagnostic practice in order to minimise the need for more invasive, higher risk target organ biopsies. Screening biopsy review in a specialist amyloidosis centre leaves < 20%

patients with AL amyloidosis requiring target organ biopsy although cannot exclude a diagnosis of systemic amyloidosis. The sensitivity of screening biopsies in ATTR amyloidosis remains poor.

Acknowledgements

OCC, FS and JDG conceived the study and analysed data. OCC and JDG wrote the manuscript. FS, JG, HL, SS, SM, MF, CW, AMN, ADW and PH contributed to the manuscript and provided critical input. All authors reviewed the final version of the manuscript.

References

1. Vaxman I, Gertz M. Recent Advances in the Diagnosis, Risk Stratification, and Management of Systemic Light-Chain Amyloidosis. *Acta Haematol.* 2019;141(2):93-106.
2. Li T, Huang X, Cheng S, et al. Utility of abdominal skin plus subcutaneous fat and rectal mucosal biopsy in the diagnosis of AL amyloidosis with renal involvement. *PLoS One.* 2017;12(9):e0185078.
3. Gillmore JD, Wechalekar A, Bird J, et al. Guidelines on the diagnosis and investigation of AL amyloidosis. *Br J Haematol.* 2015;168(2):207-218.
4. Maurer MS. Noninvasive Identification of ATTRwt Cardiac Amyloid: The Re-emergence of Nuclear Cardiology. *Am J Med.* 2015;128(12):1275-1280.
5. Gillmore JD, Maurer MS, Falk RH, et al. Nonbiopsy Diagnosis of Cardiac Transthyretin Amyloidosis. *Circulation.* 2016;133(24):2404-2412.
6. Westermark P, Stenkvist B. A new method for the diagnosis of systemic amyloidosis. *Arch Intern Med.* 1973;132(4):522-523.
7. Kimmich C, Schonland S, Kraker S, et al. Amyloid in bone marrow smears in systemic light-chain amyloidosis. *Amyloid.* 2017;24(1):52-59.
8. Quarta CC, Gonzalez-Lopez E, Gilbertson JA, et al. Diagnostic sensitivity of abdominal fat aspiration in cardiac amyloidosis. *Eur Heart J.* 2017;38(24):1905-1908.
9. Vrana JA, Theis JD, Dasari S, et al. Clinical diagnosis and typing of systemic amyloidosis in subcutaneous fat aspirates by mass spectrometry-based proteomics. *Haematologica.* 2014;99(7):1239-1247.
10. Fernandez de Larrea C, Verga L, Morbini P, et al. A practical approach to the diagnosis of systemic amyloidoses. *Blood.* 2015;125(14):2239-2244.
11. Muchtar E, Dispenzieri A, Lacy MQ, et al. Overuse of organ biopsies in immunoglobulin light chain amyloidosis (AL): the consequence of failure of early recognition. *Ann Med.* 2017;49(7):545-551.
12. Staron A, Connors LH, Ruberg FL, Mendelson LM, Sanchorawala V. A new era of amyloidosis: the trends at a major US referral centre. *Amyloid.* 2019;26(4):192-196.
13. Cowan AJ, Seldin DC, Skinner M, et al. Amyloid deposits in the bone marrow of patients with immunoglobulin light chain amyloidosis do not impact stem cell mobilization or engraftment. *Biol Blood Marrow Transplant.* 2012;18(12):1935-1938.
14. Shidham VB, Hunt B, Jardeh SS, Barboi AC, Devata S, Hari P. Performing and processing FNA of anterior fat pad for amyloid. *J Vis Exp.* 2010(44).
15. Gertz MA, Comenzo R, Falk RH, et al. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18-22 April 2004. *Am J Hematol.* 2005;79(4):319-328.
16. Devata S, Hari P, Markelova N, Li R, Komorowski R, Shidham VB. Detection of amyloid in abdominal fat pad aspirates in early amyloidosis: Role of electron microscopy and Congo red stained cell block sections. *Cytojournal.* 2011;8:11.
17. Lovat LB, Persey MR, Madhoo S, Pepys MB, Hawkins PN. The liver in systemic amyloidosis: insights from 123I serum amyloid P component scintigraphy in 484 patients. *Gut.* 1998;42(5):727-734.

Tables and Figure Legends

Figure 1: Diagnostic sensitivity and concordance of screening biopsies in amyloidosis
A) Number of biopsies included in light-chain (AL) amyloidosis
B) Number of biopsies included in transthyretin (ATTR) amyloidosis
C) Concordance of abdominal fat aspiration with bone marrow and gastrointestinal biopsies in light chain (AL) amyloidosis
D) Concordance of abdominal fat aspiration with bone marrow and gastrointestinal biopsies in transthyretin (ATTR) amyloidosis

Table 1: Histological grading of amyloid based on Congo red staining by biopsy site and amyloid subtype

Table 2: Diagnostic sensitivity of abdominal fat aspirate, bone marrow and gastrointestinal biopsies by organ involvement in AL amyloidosis

Table 3: Diagnostic sensitivity of abdominal fat aspirate, bone marrow and gastrointestinal biopsy in AL amyloidosis according to whole body amyloid load on ^{123}I-SAP scintigraphy