The complementary role of histology and proteomics for diagnosis and typing of systemic amyloidosis

Tamer Rezk1,2 †, Janet A Gilbertson1 †, P Patrizia Mangione3,4, Dorota Rowczenio1, Nigel B Rendell3, Diana Canetti3, Helen J Lachmann1, Ashutosh D Wechalekar1, Paul Bass2, Philip N Hawkins1, Vittorio Bellotti3,4, Graham W Taylor3 and Julian D Gillmore1*

1National Amyloidosis Centre, University College London, London, UK
2Department of Renal Medicine, University College London, London, UK
3Wolfson Drug Discovery Unit, Centre for Amyloidosis and Acute Phase Proteins, Division of Medicine, Royal Free Campus, University College London, London, UK
4Department of Molecular Medicine, Institute of Biochemistry, University of Pavia, Pavia, Italy

*Correspondence: Julian D Gillmore, National Amyloidosis Centre, Division of Medicine, Royal Free Campus, University College London, Rowland Hill Street, London NW3 2PF, UK. E-mail: j.gillmore@ucl.ac.uk
†Joint first authors.

Abstract

The tissue diagnosis of amyloidosis and confirmation of fibril protein type, which are crucial for clinical management, have traditionally relied on Congo red (CR) staining followed by immunohistochemistry (IHC) using fibril protein specific antibodies. However, amyloid IHC is qualitative, non-standardised, requires operator expertise, and not infrequently fails to produce definitive results. More recently, laser dissection mass spectrometry (LDMS) has been developed as an alternative method to characterise amyloid in tissue sections. We sought to compare these techniques in a real world setting. During 2017, we performed LDMS on 640 formalin-fixed biopsies containing amyloid (CR+ve) comprising all 320 cases that could not be typed by IHC (IHC−ve) and 320 randomly selected CR+ve samples that had been typed (IHC+ve). In addition, we studied 60 biopsies from patients in whom there was a strong suspicion of amyloidosis, but in whom histology was non-diagnostic (CR−ve). Comprehensive clinical assessments were conducted in 532 (76%) of cases. Among the 640 CR+ve samples, 602 (94%) contained ≥2 of 3 amyloid signature proteins (ASPs) on LDMS (ASP+ve) supporting the presence of amyloid. A total of 49 of the 60 CR−ve samples were ASP−ve; 7 of 11 that were ASP+ve were glomerular. The amyloid fibril protein was identified by LDMS in 255 of 320 (80%) of the IHC−ve samples and in a total of 545 of 640 (85%) cases overall. The LDMS and IHC techniques yielded discordant results in only 7 of 320 (2%) cases. CR histology and LDMS are corroborative for diagnosis of amyloid, but LDMS is superior to IHC for confirming amyloid type.

Keywords: amyloid; mass spectrometry; proteomics; immunohistochemistry

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Introduction

The amyloidoses are a group of rare diseases caused by extracellular accumulation of amyloid, a fibrillar material derived from a variety of precursor proteins that can aggregate in a highly abnormal cross B sheet conformation [1,2]. Amyloid deposits progressively disrupt tissue structure and function [3], the various clinical syndromes being classified according to the respective fibril precursor protein, of which more than 30 are known [4]. Amyloid is identified by the pathognomonic finding of apple-green dichroism when affected tissue sections are stained with Congo red (CR) dye and visualised under polarised light microscopy. Under electron microscopy, amyloid fibrils have a characteristic appearance of rigid non-branching fibrils with a diameter of ~10 nm [5].

Systemic amyloidosis is highly heterogeneous not only with respect to amyloid fibril protein type but also to the range of organ involvement and broader clinical phenotype. Therapy is aimed at reducing the production of the respective amyloid fibril precursor...
protein, and there is therefore a critical need to definitively determine the amyloid fibril protein type in every patient [6]. For example, chemotherapy, which can be very toxic, is potentially beneficial only in immunoglobulin light chain (AL) amyloidosis, which can be particularly challenging to confirm [7].

CR histology followed by immunohistochemical (IHC) staining of biopsy samples is the classical and widely available method for identifying amyloid and determining the fibril type. Whilst this is the historical gold standard for diagnosis and typing of amyloid, many different staining methods and antisera are used in different labs, and substantial operator experience is required for best results. The highly idiosyncratic nature of monoclonal AL amyloid, largely due the fact that the fibrils comprise the hypervariable region of the immunoglobulin light chains, presents particular challenges for IHC staining of AL deposits. Consequently, the sensitivity and specificity of IHC varies with type of amyloid, local methods and experience [8]. IHC studies of thousands of samples in our own centre have failed to confirm amyloid type beyond doubt in up to 30% of cases, mostly when AL amyloidosis is probable on clinical grounds. Immunofluorescence (IF) is a more reliable technique for thetyping of AL amyloid deposits than IHC [9], although it requires frozen amyloidotic tissue. Immunoelectron microscopy (IEM) is a technique that combines IHC and electron microscopy and allows for the correct characterisation of the amyloid protein in virtually all cases but IEM is not widely available and is currently performed in only a select number of specialist centres [10]. Fibril extraction and direct protein sequencing is the gold standard method for typing of amyloid; however it is time consuming, expensive and requires substantial quantities of frozen tissue which are rarely available in clinical practice.

Laser dissection and tandem mass spectrometry (LDMS) is an alternative, reportedly accurate tool for identification and typing of amyloid deposits which has gained popularity. Importantly, it can be performed using tiny quantities of formalin fixed amyloidotic tissue. LDMS was first validated by the Mayo group in 2009, in 102 predominantly endomyocardial biopsy specimens; LDMS was 98–100% sensitive and specific in comparison to clinicopathological criteria for identification and typing of amyloid [11]. LDMS has also been reported in non-amyloid fibrillary monoclonal gammopathies of renal significance (MGRS), namely fibrillary glomerulonephritis (fibrillary GN) and light chain deposition disease (LCDD); hitherto, the diagnosis has been established by integrating morphological findings on light microscopy along with IF and EM [12]. DNAJB9 is a recently reported novel and specific IHC marker [13], and its presence on LDMS of a glomerular sample, is pathognomonic of fibrillary GN [14].

We sought to compare and evaluate the roles of CR/IHC and LDMS for identifying and typing amyloid in a diverse population of suspected amyloidosis patients referred to our single national centre.

Methods

Samples

During the calendar year 2017, 1864 formalin-fixed paraffin wax-embedded (FFPE) biopsies from various tissues of patients suspected to have amyloid were studied in the UK National Amyloidosis Centre (NAC). They were received from many local hospitals across the UK and overseas. Tissue fixation and processing into paraffin blocks had been performed according to the referring hospitals’ protocols and was therefore not standardised. Details on the duration of fixation and processing were unavailable.

Sections from all FFPE biopsy samples were cut and stained with CR and a panel of anti-fibril protein antibodies as described below. Amyloid, identified by pathognomonic green birefringence of CR stained tissue sections viewed under crossed polarised light, was present in 1109 of 1864 (59%) biopsy samples (CR+ve); 755 (41%) were negative for presence of amyloid (CR−ve). The amyloid fibril protein was determined by IHC in 789 of 1109 (71%) CR+ve samples, but could not be characterised definitively in the remaining 320 CR+ve samples.

For the purposes of this study, we processed a total of 700 samples for LDMS comprising all 320 CR+ve samples in which IHC was non-diagnostic of the amyloid fibril protein, 320 randomly selected CR+ve biopsy samples in which the amyloid type was definitively determined by IHC, and 60 randomly selected CR−ve biopsy samples.

All patients were managed in accordance with the declaration of Helsinki and institutional review board approval from the Royal Free Hospital Ethics committee was obtained for this study (REC/06/Q0501/42).

CR and IHC staining at NAC

Twenty-two serial sections were cut from each FFPE block for CR and IHC staining. Sections of 2 μm thick were used for IHC and sections of 6 μm thick were used for CR staining and IHC/CR overlay [15]. CR
staining was by the method of Puchler et al and amyloid was identified by the presence of apple green birefringence when viewed under crossed polarised light [16]. Immunohistochemical staining was with a panel of 11 antibodies against known amyloid fibril proteins using the Shandon Sequenza™ (Thermo Shandon Ltd, Runcorn, England), namely, kappa and lambda immunoglobulin light chains, transthyretin (ATTR), amyloid A protein, fibrinogen, LECT2, apolipoprotein A-I, and lysozyme, as previously described [17]. Antigen retrieval was not performed with the exception of ATTR staining, which uses oxidation steps 1% aqueous sodium periodate (10 min) and also blocked with normal serum. Sections were incubated overnight at 4°C and then digested with trypsin (25 ng/sample) overnight (~18 h) at 37°C. Each digested sample was reduced with dithiothreitol (50 μg) at 99°C for 5 min, freeze dried, reconstituted in 0.1% (v/v) trifluoroacetic acid in HPLC grade water (20 μl) and analysed by HPLC–MSMS, using a Thermo Scientific Q-Exactive Plus mass spectrometer (Thermo Scientific, Bremen, Germany) coupled to a Dionex Ultimate 3000 RSLC nanoLC (Dionex Softron GmbH, Bavaria, Germany) using a Thermo Easy-spray Acclaim Pepmap (Fisher Scientific UK Ltd, Loughborough) column (75 μm × 15 cm, 3 μm/100 Å packing), as previously described [18,19].

MS raw data files were queried using Matrix Science’s Mascot search engine (http://www.matrixscience.com/) and the SwissProt database to assign peptide and protein probability scores. Amyloid was established by LDMS on the basis of the presence of the ‘amyloid signature proteins’ (ASP) defined by the presence (≥2 unique specific peptide) of two or more of the following proteins; apolipoprotein E (APOE), apolipoprotein A-IV (AApoAIV) and serum amyloid P (SAP) component, as previously described [11]. The amyloid fibril protein was determined by a Mascot score of >80 coupled with at least two unique specific peptides of a known amyloid fibril protein together with absence (Mascot score < 80 or fewer than two unique specific peptides) of other known amyloid fibril proteins.

Analyses
The results of CR and IHC were compared with results of LDMS in a total of 700 samples (Figure 1). Specifically, they were compared with respect to identification of amyloid and identification of the amyloid fibril protein (amyloid type).

Results
The 700 samples (640 CR+ve and 60 CR–ve) were from a total of 50 different tissue types with renal (n = 188), cardiac (n = 126) and bone marrow trephine (n = 44) comprising over 50% of all samples processed.

Diagnosis of amyloid
A total of 602 of 640 (94%) CR+ve samples were ASP+ve by MS and 38 (6%) were ASP–ve. A total of 15 of 38 (39%) CR+ve/ASP–ve samples were classified by MS as ‘insufficient for analysis’ due to scanty tissue (n = 14) or scanty amyloid (n = 1) with a total
of <5 proteins identified within the sample. In the remaining 23/38 (61%) CR+ve/ASP−ve samples there was adequate tissue for analysis. The details of these 23 tissues samples (obtained from 20 patients) are listed in Table 1 which also includes the findings of a comprehensive clinical, biochemical and imaging review that was conducted in 15 of 20 individuals. In each of the 15 cases, an unequivocal diagnosis of amyloidosis was established on the basis of combined clinical, biochemical, echocardiographic, cardiac magnetic resonance (CMR) imaging, and scintigraphic analyses [20]. The final diagnosis was localised AL amyloidosis in four cases, ATTR amyloidosis in four cases, systemic AL amyloidosis in three cases, leukocyte chemotactic factor 2 (ALECT2) amyloidosis in two cases, beta 2 microglobulin (β2M) amyloidosis in one case, and the final patient was a 59 year old male with carpal tunnel amyloid deposits in the absence of any critical amyloidotic organ involvement or dysfunction. The remaining 5 of 20 patients were not seen at our centre, and their biopsy sample had been sent to NAC solely for histological analysis.

A total of 49 of the 60 (82%) CR−ve samples were ASP−ve by MS and 11 of 60 (18%) were ASP+ve (Table 2). There were no CR−ve/ASP+ve cardiac, gastrointestinal, fat or bone marrow trephine biopsy samples (Table 3). However, there were seven renal biopsy specimens, all glomerular, from a total of 188 (4%) renal biopsies which were CR−ve/ASP+ve. In four of seven such cases, a diagnosis of fibrillar

Figure 1. Flowchart of the results of 700 samples processed for CR with IHC and LDMS.
GN was established on the basis of the clinical and electron microscopy findings coupled with the presence of DNAJB9 protein by MS [14]. One of the remaining three patients with a CR−ve/ASP+ve glomerular sample was eventually diagnosed with fibronectin glomerulopathy, and the diagnosis in the remaining two cases was not established at the time of writing (Table 2).

Typing of amyloid

Of the 320 samples in which the amyloid fibril protein was determined by IHC, the amyloid type by MS concurred in 290 of 320 (91%), whereas in 30 samples there was a discrepancy between the amyloid type identified by IHC and the MS results. This discrepancy was due to the absence of ASP (i.e. CR+ve/ASP−ve) by MS in 13 of 30 (43%) cases and, in the remaining 17 samples, the MS was either non-diagnostic of amyloid type (n = 10) or discordant (n = 7) (Table 4). Of those seven cases in which the amyloid fibril protein by IHC and MS were discordant, four were AL lambda sub-type by IHC but AL kappa sub-type by MS. Two of the four relevant patients had a circulating monoclonal protein, in both cases of lambda isotype. The amyloid fibril protein was discordant in a further three of seven samples; two were from the same patient (renal and testicular biopsy) in which IHC showed AA amyloid and MS showed AApoAI amyloid and in whom a novel APOA1 mutation was discovered, and the remaining sample was a bladder biopsy in which AL (lambda sub-type) amyloid was identified by IHC and MS revealed ATTR amyloid. This last patient did not undergo clinical evaluation at our centre.

Table 1. Samples diagnostic of amyloid by CR staining but with no amyloid by MS (CR+ve/ASP−ve)

| Sample/patient no | Age and sex | Tissue | Clinical/histology review | Final diagnosis* | Organ involvement | SAP scintigraphy | DPD scan (Perugini grade) |
|------------------|-------------|--------|---------------------------|-----------------|------------------|-----------------|--------------------------|
| 1/1              | 62 F        | Lymph node | Clinical                  | Localised AL amyloidosis | No vital organ involvement | No visceral amyloid | Not done                  |
| 2/2              | 87 M        | Fat aspirate | Clinical                  | ATTR amyloidosis | Cardiac          | No visceral amyloid | Grade 2                  |
| 3/3              | 65 M        | Prostate | Clinical                  | Localised AL amyloidosis | No vital organ involvement | No visceral amyloid | Not done                  |
| 4/4              | 67 M        | Renal | Clinical                  | Systemic AL amyloidosis | Renal          | Isolated renal amyloid | Not done                  |
| 5/5              | 84 M        | Carpal tunnel | Clinical                  | Systemic AL amyloidosis | No vital organ involvement | No visceral amyloid | Not done                  |
| 6/6              | 68 F        | Renal | Clinical                  | ALECT2 amyloidosis | Renal           | Amyloid in spleen and kidneys | Not done                  |
| 7/7              | 67 F        | BMT | Histology                  | Uncertain | Not done | Not done | Not done                  |
| 8/8              | 55 M        | Muscle | Histology                  | Uncertain | Not done | Not done | Not done                  |
| 9/9              | 84 M        | Cardiac | Clinical                  | ATTR amyloidosis | Cardiac          | No visceral amyloid | Grade 2                  |
| 10/10            | 68 M        | Tonsil | Clinical                  | Localised AL amyloidosis | No vital organ involvement | No visceral amyloid | Not done                  |
| 11/11            | 82 M        | Tongue | Clinical                  | ATTR amyloidosis | Cardiac          | No visceral amyloid | Grade 2                  |
| 12/12            | 54 M        | Brain | Clinical                  | Uncertain | Not done | Not done | Not done                  |
| 13/13            | 72 M        | BMT | Clinical                  | Systemic AL amyloidosis | Cardiac and soft tissue | No visceral amyloid | Not done                  |
| 14/14            | 74 M        | Renal (glomeruli) | Clinical                  | Renal amyloid – uncertain type | Renal            | Isolated renal amyloid | Not done                  |
| 15/14            | 82 M        | Renal (medulla) | As above                  | As above | Not done | As above | As above                  |
| 16/15            | 34 F        | Mucosa | History                    | Uncertain | Not done | As above | As above                  |
| 17/16            | 63 M        | Skin | Clinical                  | Localised AL amyloidosis | No vital organ involvement | No visceral amyloid | Not done                  |
| 18/17            | 68 M        | Renal (interstitium) | Histology | ALECT2 amyloidosis | Not done | Not done | Not done                  |
| 19/18            | 59 M        | Carpal tunnel | Clinical                  | Amyloid – uncertain type | No vital organ involvement | No visceral amyloid | Grade 0                    |
| 20/19            | 92 M        | Prostate | Clinical                  | ATTR amyloidosis | Cardiac          | No visceral amyloid | Grade 2                  |
| 21/19            | 92 M        | Fat aspirate #2 | As above                  | As above | As above | As above | As above                  |
| 22/19            | 84 M        | Fat aspirate | As above                  | Systemic AL amyloidosis | Cardiac and soft tissue | Amyloid in liver and spleen | Not done                  |
| 23/20            | 61 F        | BMT | Clinical                  | Systemic AL amyloidosis | Cardiac and soft tissue | Amyloid in liver and spleen | Not done                  |

DPD, 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid.

*The final diagnosis in patients who underwent clinical review was established on the basis of a comprehensive clinical assessment, biochemical investigations including serum and urine immunofixation, serum free light chains and cardiac biomarkers and specialist imaging including SAP scintigraphy, DPD scintigraphy, echocardiography and CMR imaging.
Of the 320 CR+ve samples in which the amyloid type was not diagnosed by IHC, amyloid was identified and typed by MS in 255 (80%) cases but was not definitively typed in the remaining 65 cases. In the 255 samples which were inconclusive by IHC but definitely typed by MS, the latter correlated with extensive clinical assessment in 252 of 255 (99%) cases. In the remaining three cases, tissue was received for a histology review alone such that limited clinical details were available. MS established a diagnosis of: AL lambda in 135 cases, AL lambda in 35 cases, ATTR in 55 cases, ATTR in 26 cases, fibrinogen alpha chain (AFib) in eight cases, localised semenogelin amyloid deposits in six cases, immunoglobulin heavy chain in six cases, hereditary apolipoprotein AI (AApoAI) in six cases, apolipoprotein C2 (AApoC2) in four cases, AApoAIV in four cases, galectin 7 (cutaneous) amyloid deposits in three cases, insulin derived (Alns) amyloid deposits in one case and isolated atrial amyloidosis in the final case.

Of the 65 cases in which the amyloid type could not be determined by MS, 12 samples were ‘insufficient for analysis’ by MS, 25 samples were ASP–ve, and 7 contained no known amyloid fibril protein. Twenty-one samples contained more than one known amyloid fibril protein each with a similar Mascot and specific peptide score including seven cases with both TTR and a light chain (either kappa or lambda), and five cases with both kappa and lambda light chains.

**Discussion**

CR staining and IHC remains the most commonly used method for the diagnosis and typing of amyloid worldwide, although some specialist centres have recently moved towards the use of LDMS alone [21]. Here we report for the first time a single centre direct comparison of the methods, supported where necessary by correlation with specialist clinical and imaging assessments, which highlights the advantages and limitations of each method and argues strongly in favour of their use as complementary techniques for diagnosis and typing of amyloid.

Our study showed a high concordance between CR staining and LDMS for the identification of amyloid with 94% of CR+ve samples showing amyloid by LDMS and 82% CR–ve samples not showing amyloid by LDMS. Nonetheless, LDMS in our hands failed to identify amyloid in a small proportion of cases, even when an adequate quantity of amyloidotic tissue was captured, and the reasons for this remain unclear. It is
also worth highlighting that 7 of 11 CR−ve/ASP+ve cases were glomerular and that the final diagnosis in 5 of these 7 glomerular samples was either fibrillary GN or fibronectin glomerulopathy. In 2015 the Mayo group reported LDMS in a small cohort of MGRS cases which included seven cases of renal amyloidosis and eight cases of fibrillary GN. LDMS revealed the presence of two or more ASP (SAP and APOE) in all amyloidotic samples and the presence of only APOE in the absence of CR staining in these tissues should therefore be determined by MS. Furthermore, a small number of cases were not typed by MS due to the

With respect to typing of amyloid, MS was superior to IHC. The amyloid type was established by MS in 255 (80%) of the 320 cases that were not typed by IHC and in a total of 545 of 640 (85%) CR+ve samples. A sub-analysis according to tissue type showed that the amyloid type was definitively determined by MS in 95% of gastrointestinal, 88% of endomyocardial, 82% of renal, 78% of fat, and 75% of bone marrow trephine CR+ve samples (Table 3). However, despite a very high concordance between the amyloid type established by MS and that established by IHC, there were occasional discordant results (n = 7; 2%) of which a proportion appeared to be more consistent with the IHC finding than that by MS, based on clinical and biochemical parameters. In addition, a number of CR+ve samples that were definitively typed by IHC did not contain ASP and the amyloid type could not therefore be determined by MS. Furthermore, a small number of cases were not typed by MS due to the

**Table 4.** Samples in which MS was either non-diagnostic of amyloid type (n = 10) or discordant (n = 7) with the results of IHC

| Sample/patient no | Age/sex | Clinical/histology review | Tissue | Final diagnosis* | Organ involvement | Systemic clone (n/k/λ) | IHC | MS | SAP scintigraphy |
|------------------|---------|---------------------------|--------|------------------|------------------|----------------------|-----|----|-----------------|
| 1/1              | 78 M    | Clinical                  | Laryngeal | Localised AL amyloidosis | No vital organ involvement | No | AL (k) | AL (k) | No visceral amyloid |
| 2/2              | 65 F    | Clinical                  | Lymph node | Localised AL amyloidosis | No vital organ involvement | Lambda | AL (λ) | AL (k) | No visceral amyloid |
| 3/3              | 84 F    | Clinical                  | Renal    | Systemic AL amyloidosis | Renal | Lambda | AL (λ) | Uncertain | Isolated renal amyloid |
| 4/4              | 83 M    | Histology                 | Cardiac  | ATTR amyloidosis | Cardiac | Not known | ATTR | Uncertain | Not done |
| 5/5              | 25 F    | Clinical                  | Laryngeal | Localised AL amyloidosis | No vital organ involvement | No | AL (k) | AL (k) | No visceral amyloid |
| 6/6              | 58 M    | Histology                 | Renal    | Amyloid – uncertain type | Renal | Not known | AL (λ) | Uncertain | Not done |
| 7/7              | 64 F    | Clinical                  | Breast   | Localised AL amyloidosis | No vital organ involvement | No | AL (k) | AL (k) | No visceral amyloid |
| 8/8              | 79 M    | Clinical                  | Renal    | Systemic AL amyloidosis | Renal and Liver | Lambda | AL (λ) | Uncertain | Amyloid in liver and kidneys |
| 9/9              | 78 M    | Clinical                  | Cardiac  | ATTR amyloidosis | Cardiac | Kappa | ATTR | Uncertain | No visceral amyloid |
| 10/10            | 66 M    | Clinical                  | Cardiac  | Systemic AL amyloidosis | Renal and autonomic nerve | Lambda | AL (λ) | Uncertain | Amyloid in liver, spleen and kidneys |
| 11/11            | 77 M    | Histology                 | Bladder  | Amyloid – uncertain type | Bladder | Not known | AL (λ) | TTR | Not done |
| 12/12            | 67 F    | Clinical                  | Lymph node | Insulin amyloid | No vital organ involvement | No | Alns | Uncertain | No visceral amyloid |
| 13/13            | 46 M    | Histology                 | Testes   | Amyloid – uncertain type | Renal and testicular | Not known | AA | AApoAl | Not done |
| 14/13            | As above | Clinical                  | Renal    | As above | As above | AA | Al (k) | Al (k) | Amyloid in spleen and kidneys |
| 15/14            | 74 M    | Clinical                  | Renal    | Systemic AL amyloidosis | Renal | Lambda | AL (λ) | Uncertain | Not done |
| 16/15            | 82 F    | Histology                 | Soft tissue | Insulin amyloid | Soft tissue | Not known | Alns | Uncertain | Not done |
| 17/16            | 47 F    | Clinical                  | Laryngeal | Localised AL amyloidosis | No vital organ involvement | No | AL (λ) | Uncertain | No visceral amyloid |

*The final diagnosis in patients who underwent clinical review was established on the basis of a comprehensive clinical assessment, biochemical investigations including serum and urine immunofixation, serum free light chains and cardiac biomarkers and specialist imaging including SAP scintigraphy, DPD scintigraphy, echocardiography and CMR imaging.
presence of more than one amyloid fibril protein, each with a similar Mascot and specific peptide score [23]. It is noteworthy that these cases frequently contained a combination of immunoglobulins and ATTR, both of which are abundant plasma proteins and may therefore be related to fixation and processing factors over which specialist referral centres, such as ours, have no control.

Whilst LDMS may successfully determine the amyloid fibril protein from tiny quantities of amyloidotic tissue, there are important factors to consider as to whether a sample is sufficient for analysis, including the distribution of amyloid deposits within the specimen. The challenges of dissecting ‘chicken-wire’ type amyloid deposits has been noted previously [24] and may result in abundance of other proteins within the microdissected sample. In addition, the limitations of amyloid typing by MS on fat samples containing ‘scanty’ amyloid deposits have previously been highlighted [25]. Further work is needed to avoid the small proportion of samples that cannot be typed by MS. To this end, recent work from our centre has looked at the role of tissue decellularisation in samples in which more than one potentially amyloidogenic protein is identified. In a small case series, pre-treatment of tissue with decellularisation enhanced the specificity of the identification of the correct amyloid fibril protein in samples in which more than one protein was detected [23].

In 2015, we undertook a smaller collaborative study of a similar nature in which 142 biopsy samples were stained with CR and IHC at the NAC and processed for LDMS at the Mayo Clinic, USA. The results of this earlier study were remarkably similar to our findings here; there was a very high degree of concordance between the two techniques and LDMS enabled typing of amyloid in 74% of 34 cases that could not be typed by IHC compared to 80% here. Furthermore, the reasons for the failure of MS to determine the amyloid type in the earlier study were listed as insufficient amyloid, insufficient tissue and technical failure [26], almost identical to our findings here.

Our study’s main limitation is that it is a retrospective analysis of biopsies from a single centre. There is, by definition, a selection bias based on the fact that all patients have been suspected of having amyloidosis or MGRS by the referring histopathologist or clinician in order to reach us in the first place. Second, due to the fact that fixation and processing of tissue occurred in the local referring centre, this was not controlled for and may have contributed to some of the failures of both IHC and LDMS [19]; as a consequence, however, our study does reflect ‘real-world’ clinical practice.

In conclusion we show here that the presence of ASP on MS is a sensitive and specific method for the identification of amyloid deposits but highlight the fact that, particularly in glomerular samples from patients with other deposition diseases such as fibrillary GN, the presence of ASP may not be due to amyloid. The addition of LDMS to CR histology and IHC markedly increased the proportion of amyloidotic samples in which the amyloid type was definitively determined to between 74 and 95%, depending on the biopsy tissue. Nonetheless, challenges remain; namely the occasional detection of more than one potential amyloid fibril protein by LDMS and the laser capture and identification of scanty material. Whilst LDMS remains a powerful tool for the diagnosis and typing of amyloid, our experience suggests that it should be used in conjunction with comprehensive clinical and histological assessment to ensure optimal patient care.

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Author contributions statement

JDG, JAG, and TR were responsible for conceiving the study, interpreting the results and drafting the manuscript. DR, NR, PPM, TR, DC, HJL, ADW, PB, PNH, VB, and GWT were responsible for data collection and interpretation of results.

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