Diagnosis of renal amyloidosis by liquid chromatography-tandem mass spectrometry: experience from a single-center cohort study in China

Ying Sun[1], Jian Sun[2], Jianling Tao[1], Wei Sun[3], Xuemei Li[1], Mingxi Li[1]

[1]Department of Nephrology, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100730, China;
[2]Department of Pathology, State Key Laboratory of Complex Severe and Rare Disease, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100730, China;
[3]Department of Core Instrument Facility, School of Basic Medicine, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100730, China.

To the Editor: Amyloidosis is caused by the extracellular deposition of insoluble beta-pleated protein fibrils in tissues and is classified according to the constituent proteins. The cause of amyloidosis can be traced to more than 30 kinds of malfolded proteins, and accurate amyloid typing is crucial for appropriate therapy.[1] The pathological diagnosis is confirmed with positive Congo red (CR) staining under a light microscope and 7 to 12 nm branching fibrils under an electron microscope. Further identification of some amyloid precursor proteins can resort to immunofluorescence (IF) and immunochemistry (IHC); however, the accuracy depends on the selected antibodies to a large extent. Laser microdissection coupled with liquid chromatography-tandem mass spectrometry (LMD/MS) can identify these proteins without the restriction of IF and IHC, although exquisite equipment and skilled specialists are required.[2] This study aimed to establish LMD/MS analysis of renal biopsy tissue and compare it with IHC on diagnostic sensitivity.

We retrospectively analyzed the clinical and pathological data of patients with renal amyloidosis diagnosed by kidney biopsy at the Peking Union Medical College Hospital during 2010 to 2015. First, 59 patients were divided into light chain amyloidosis (AL) and no light chain amyloidosis (non-AL) groups based on the presence of monoclonal protein (M protein) detected by serum immunofixation electrophoresis (IFE). The characteristics of the enrolled patients are presented in [Supplementary Table 1, http://links.lww.com/CM9/B59]. Within the AL group, 20 cases with inconsistent renal IF and IFE were further stained with IHC for kappa (κ) and lambda (λ) and then validated with LMD/MS. In the non-AL group, the tissue was subjected directly to LMD/MS analysis. This study was performed in accordance with the *Declaration of Helsinki* and approved by the Ethics Committee of PUMH (approval No. JS-1233-1). All the patients provided written informed consent for participation in the study.

Figure 1 and Supplementary Table 2, http://links.lww.com/CM9/B60 show patient selection and typing results. Overall, 59 cases of biopsy-proven renal amyloidosis were collected, and 51 cases had evidence of M protein; among them, 20 cases with no evidence of renal light chain deposition were chosen for IHC and LMD/MS. Meanwhile, eight cases without evidence of M protein in IFE were diagnosed directly by LMD/MS.

Formalin-fixed paraffin-embedded renal biopsy samples were analyzed using LMD/MS (LC-MS/MS; Linear Trap Quadrupole Orbitrap Velos Pro, Thermo Fisher Instruments, San Jose, USA). The Mascot database search engine (Matrix Science, London; version 2.3.02) was used for protein identification against the UniProt human database (www.uniprot.com, 84,910 entries). Searches were performed using a peptide and product ion tolerance of 0.05 Da. Scaffolds (v 4.3.2, Proteome Software Inc., Portland, OR, USA) were used to further filter the search results using the decoy database method. Protein identification was accepted when at least two unique peptides were detected, and the false discovery rate at the protein level was <1% based on decoy database searching.

The diagnosis of amyloidosis at the proteomic level is based on the presence of serum amyloid protein, apolipoprotein E, and amyloid precursor proteins. Quantitative information on each protein was based on the number of identified peptides, and the type of amyloidosis was based on the

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Correspondence to: Mingxi Li, Department of Nephrology, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100730, China
E-Mail: mingxili@hotmail.com

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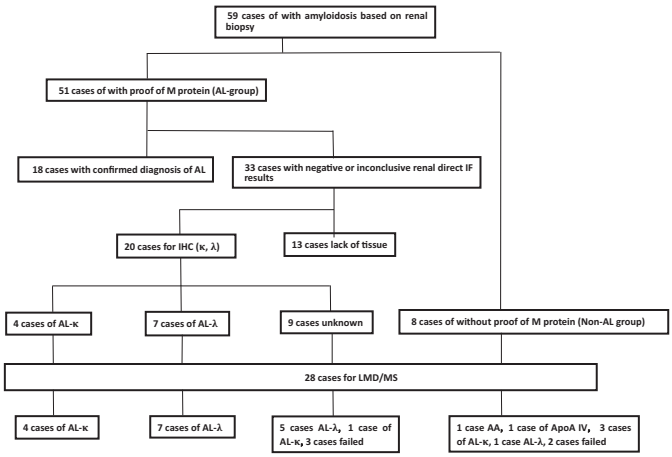

Figure 1: Flow chart of patient selection and LMD/MS results. IF: Immunofluorescence; ApoA IV: Apolipoprotein A-IV; IHC: Immunochemistry; LMD/MS: Liquid chromatography-tandem mass spectrometry; AL: Light chain amyloidosis; non-AL: No light chain amyloidosis.

highest abundance of proteins corresponding to the specific type of amyloid [Supplementary File, http://links.lww.com/CM9/B58]. A minimum of four mass spectra must be obtained to demonstrate significant amyloid deposition.[3]

Among the AL group patients, 11 cases had clear IHC results, as presented in [Figure 1]. Additionally, IHC diagnostic sensitivity was 55% (11 out of 20); and in the case of rest nine patients, it was not possible to distinguish between AL-λ and AL-κ. In terms of LMD/MS, for 11 cases with clear IHC results, LMD/MS yielded consistent results; among nine cases with unclear IHC staining, six were given precise results; 17 cases in the AL group were diagnosed using LMD/MS with a sensitivity of 85% (17 out of 20, 3 cases failed), showing that LMD/MD is superior to IHC in amyloid typing. In the eight patients with negative serum IFE but positive CR staining of renal tissues, LMD/MS could identify one case of AA amyloidosis, one case of apolipoprotein A-IV (ApoA IV) amyloidosis, three cases of AL-κ amyloidosis, and one case of AL-λ amyloidosis; however, two cases remained unclassified.

It is well accepted that the diagnosis of amyloidosis should include the nature of amyloid fibers and further classification. IF is the first step in the type of renal AL-amyloidosis; nonetheless, establishing the monotypic nature of κ or λ deposits by IF can be challenging. IHC is an easy, popular, and affordable procedure for type AL amyloidosis, but it is limited by the dependence on specific antibodies. However, proteomics is not bound by any such limitations. In this study, LMD/MS was shown to be much more sensitive and accurate for typing AL amyloidosis than IHC; LMD/MS is also efficient for diagnosing non-AL amyloidosis.

An analysis revealed the prevalence of certain systemic amyloidosis[4] in the kidney, with AL, amyloid A amyloidosis (AA), and leukocyte chemotactic factor 2 amyloidosis (ALECT2) being the most common. The number of non-AL amyloidosis cases in our cohort was small, and only AA and ApoA IV amyloids were identified using LMD/MS. We found no cases of ALECT2 amyloid, which is common in Hispanic patients.

LMD/MS is expensive and requires high-level expertise; at present, it is considered a supplement to amyloidosis diagnosis; however, the need for amyloidosis typing is brought out by the fact that up to 10% of all cases and 6.6% of renal amyloidosis cases are rare amyloid types.[4] Amyloid proteomics is currently carried out in relatively few centers worldwide,[2] but it is promising for diagnostically difficult amyloidosis to embrace correct treatment and improved prognosis in China.

In conclusion, this study demonstrated that LMD/MS is superior to IHC in amyloid typing, and is an important additional IHC method in the diagnosis of renal amyloidosis.

Declaration of patient consent

The authors certify that they obtained all appropriate patient consent forms. In these forms, the patient(s) gave consent for their images and other clinical information to be reported in the journal. Patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, although anonymity cannot be guaranteed.

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Conflicts of interest

None.

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