

[CASE REPORT]

AL-Kappa Primary Amyloidosis with Apolipoprotein A-IV Deposition

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Abstract:

A 70-year-old woman with complaints of edema, general malaise, and hypotension was diagnosed with renal amyloidosis, and laser microdissection mass spectrometry revealed her amyloidosis to predominantly comprise the apolipoprotein A-IV type. The M-protein turned from negative to positive during the course, and a bone marrow biopsy showed smoldering myeloma. Treatment with bortezomib and dexamethasone failed to save her from heart failure six months after the onset. Western blotting of urine samples at the time of the renal biopsy showed that amyloid light-chain κ amyloidosis had been present since the onset. Unlike the myeloma, Congo red staining was positive in the plasma cells of the bone marrow.

Key words: AL amyloidosis, Apolipoprotein A-IV amyloidosis, myeloma, Congo red staining, plasma cell

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Introduction

Amyloid light-chain (AL) amyloidosis is classified into primary amyloidosis and amyloidosis secondary to multiple myeloma. Primary AL amyloidosis has a poor prognosis, and patients often die of heart failure within one to two years of the diagnosis (1). In contrast, for cases of multiple myeloma, the 5-year survival rate has improved to around 60% due to advances in autologous peripheral blood stem cell transplantation, proteasome inhibitors, high-dose dexamethasone therapy, and thalidomide (2, 3). Patients with primary AL amyloidosis are now often being treated with drugs used for myeloma, but the prognosis has not markedly improved (1, 4).

Primary AL amyloidosis and secondary AL amyloidosis associated with myeloma are considered to be similar entities associated with plasma cell dyscrasia, but their clinical prognosis appears to be different. Immunoelectrophoresis and immunofixation electrophoresis methods used for the di-

agnosis of myeloma are insensitive compared to free light chain and Western blotting (5), and M protein and Bence Jones protein (BJP) cannot be detected at an early stage. However, immunostaining methods failed to diagnose the typing of amyloidosis in 7.3% of AL amyloidosis and 21% of AA amyloidosis in an analysis of 474 cases of renal amyloidosis (6), and laser microdissection mass spectrometry (LMD-MS) has been shown to be a powerful tool for typing amyloid such as transthyretin, leukocyte cell-derived chemotaxin-2, fibrinogen- α chain, apolipoprotein (Apo)A-I/A-II/A-IV, and gelsolin (6-8).

We herein report a case of renal amyloidosis that was negative for light chain by immunofluorescence and suspected of ApoA-IV amyloidosis by LMD-MS, but developed smoldering myeloma. Chemotherapy was ineffective in this patient, and she ultimately died of heart failure six months later. The rapid progression of heart failure seems to indicate AL amyloidosis rather than myeloma, and a Western blotting analysis of urine revealed that AL κ amyloidosis had existed from the onset, and plasma cells in the bone marrow

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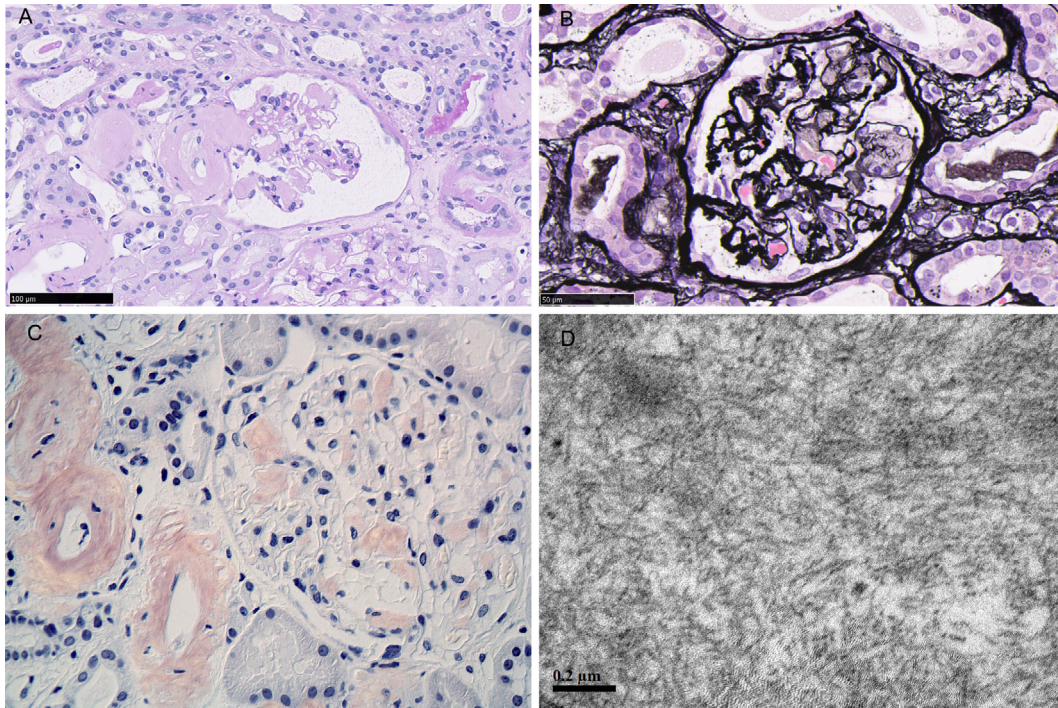


Figure 1. Renal biopsy findings. **A:** PAS staining showing weakly PAS-positive deposits in afferent arterioles and mesangium. **B:** PAM staining showing PAM-negative deposits in mesangium. **C:** Congo red staining showing polarized amyloid deposits in the interlobular arteries, afferent arterioles, and mesangium. **D:** Electron microscopy revealed amyloid fibrils with an average diameter of 11.7 nm in mesangial deposits. The bar indicates 100 μ m (A), 50 μ m (B, C) and 0.2 μ m (D).

were positive for Cong red staining, suggesting amyloid production.

We also discuss the effectiveness and limitations of LMD-MS in the typing of amyloidosis.

Case Report

A 70-year-old woman visited our hospital in May with a 2-week history of edema, diarrhea, general malaise, cough, and shortness of breath. A review of her family history revealed no renal diseases.

Her physical findings showed hypotensive blood pressure 96/64 mmHg, pulse rate 89/min, no heart murmur, and no abnormalities other than edema of her lower legs. Due to diarrhea and general malaise, she weighed 55.6 kg, which was less than the usual 57 kg. She had nephrotic syndrome with serum total protein (TP) 5.3 g/dL, serum albumin (Alb) 2.7 g/dL and urinary protein 3.73 g/g creatinine (Cr) with a selectivity index (SI) of 0.06, and her renal function was normal, with a serum Cr of 0.50 mg/dL and an estimated glomerular filtration rate (eGFR) of 90 mL/min/1.73 m². She had no obvious immunoglobulin abnormalities (IgG 748 mg/dL, IgA 134 mg/dL, IgM 28 mg/dL, IgE 4.4 mg/dL), hypercalcemia, anemia, serum M protein, urinary Bence Jones protein, or urinary casts found in myeloma. Her chest X-ray showed a slight pleural effusion with a cardiothoracic ratio (CTR) of 50.5%. She had a high brain natriuretic peptide (BNP) level of 421.5 pg/mL and a low voltage on an elec-

trocardiogram; however, an ultrasound cardiogram showed a normal ejection fraction of 63% and a normal wall motion of the left ventricle, with an inter-ventricular septum thickness of 10 mm, posterior wall thickness of 9 mm, and no sparkling glittering.

A renal biopsy was performed in June, and periodic acid-schiff (PAS)-weakly-positive and periodic acid-methenamin silver (PAM)-negative substances were found to have been deposited in the mesangium and vascular wall of the interlobular arteries and afferent arterioles (Fig. 1A, B). Congo red and Direct Fast Scarlet staining were positive in the vascular wall and mesangium, showing polarization (Fig. 1C), and electron microscopy revealed fibrils with an average diameter of 11.7 nm in the deposit (Fig. 1D); these findings resulted in a diagnosis of renal amyloidosis. The type of amyloid was not identified by immunofluorescence for immunoglobulin, κ , and λ light chain nor by immunostaining for amyloid A or amyloid β . No transthyretin gene mutation was observed. Therefore, LMD-MS was performed to diagnose the amyloidosis typing.

LMD-MS revealed that Apo A-IV increased most in both the vascular wall and glomerulus compared to the control, and ApoA-IV renal amyloidosis was diagnosed (Fig. 2). A reduced amount of κ light chain was also detected at the same time. Following this diagnosis, her edema worsened with an increased urinary protein level of 8.3 g/gCr and an SI <0.1, so treatment with 40 mg prednisolone was started. The urinary protein level decreased to 3.2 g/gCr, but it in-

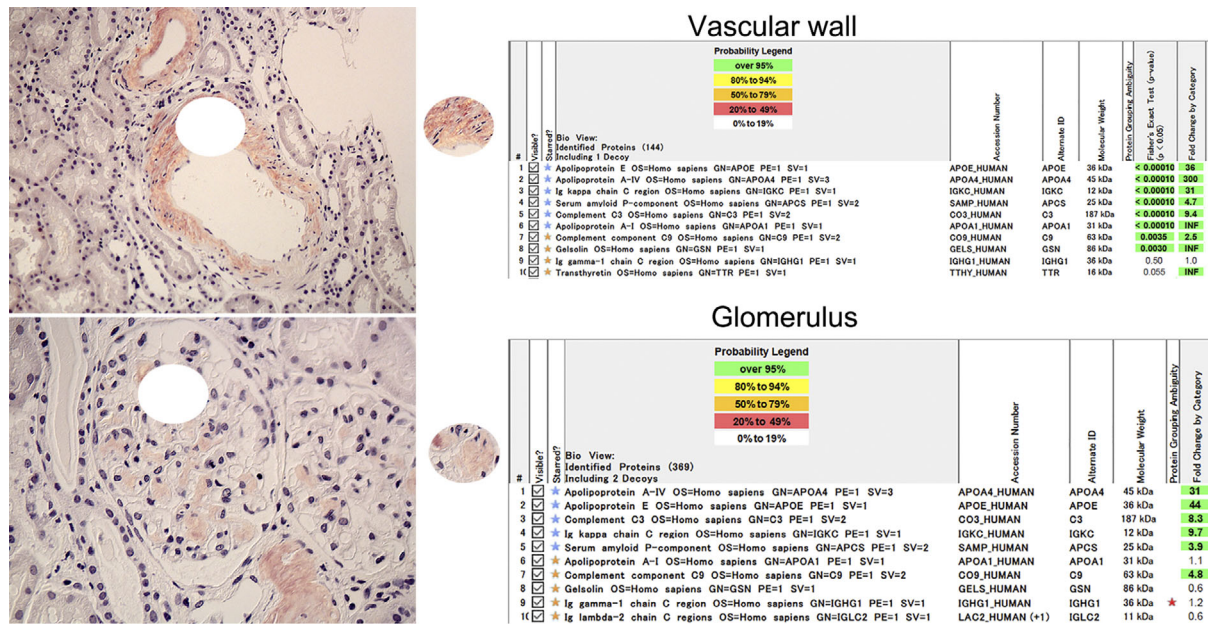


Figure 2. Laser microdissection mass spectrometry. The top 10 proteins analyzed by mass spectrometry of the tissue excised by laser microdissection from the vascular wall and glomerular mesangial region.

creased again after tapering the steroid dose.

As M protein became positive in urine for the first time with an increased free light chain (FLC)-kappa/lambda level (524/9.4 mg/L) and difference in FLC (dFLC) of 515 mg/L, a bone marrow biopsy was performed in September, revealing smoldering myeloma with a plasma cell rate of 15% (Fig. 3). However, there were no CRAB symptoms including hyperkalemia, renal dysfunction, anemia, or bone lesions associated with myeloma. In addition, there were no chromosomal translocations such as t(4;14), t(11;14) and t(14;16), or NRAS and KRAS activated gene abnormalities, or TP53 gene mutations as seen in myeloma. A gastroesophageal biopsy also found amyloids, and cardiac amyloidosis was diagnosed based on the increased BNP level of 4,082 pg/mL and the granular sparkling echocardiograph findings with a decreased cardiac ejection fraction (EF 53%) and wall thickening at 13 mm. M protein damaged multiple organs as monoclonal gammopathy of renal significance (MGRS), so bortezomib and dexamethasone (BD) therapy was applied with a slight reduction in the kappa/lambda light chain level (363/10.6 mg/L) and dFLC of 352 mg/L. After 1 month of BD therapy, the patient was unable to get out of bed due to general malaise and orthostatic hypotension, and her systolic blood pressure was about 80 mmHg with midodrine hydrochloride. However, her blood pressure suddenly dropped, and unfortunately, she died with heart failure with high BNP levels (2,453 pg/mL) in December. The progression was very rapid, with death occurring approximately six months after the discovery of the disease, as is typical of primary AL amyloidosis (Fig. 4).

The detection sensitivity of M protein is >100 mg/dL by immunoelectrophoresis, and >5 mg/dL by immunofixation electrophoresis. Therefore, urine that had been preserved at

the time of the renal biopsy was re-examined by Western blotting, which is the most sensitive approach, and the κ light chain was found to have been predominantly excreted in the urine at the time of the renal biopsy in June (Fig. 5A). Furthermore, when the epoxy resin section of the renal biopsy was stained by the enzyme immunostaining method with 0.25% trypsin treatment, the κ chain was predominantly stained (Fig. 5B, C). Thus, a re-evaluation of the renal biopsy findings resulted in a diagnosis of AL κ amyloidosis at the onset.

Interestingly, Congo red staining was strongly positive in the cytoplasm of the plasma cells in the bone marrow of this patient (Fig. 3E), and an electron micrograph of these cells showed fibrils (Fig. 3E insert). In contrast, Congo red staining of a sample from a 68-year-old patient with myeloma without amyloid was negative (Fig. 3F). Our findings suggested that amyloid fibrils were produced in the plasma cytoplasm of primary AL amyloidosis, but the plasma cells of myeloma did not produce any amyloid fibril in the cytoplasm.

Discussion

In the present case of renal amyloidosis, the type of amyloid could not be diagnosed by immunofluorescence, and LMD-MS suggested the patient to have ApoA-IV renal amyloidosis. However, M protein and BJP turned from negative to positive during the clinical course, and a bone marrow biopsy resulted in a diagnosis of smoldering myeloma. BD therapy was ineffective and the patient ultimately died of heart failure after a rapid course of six months.

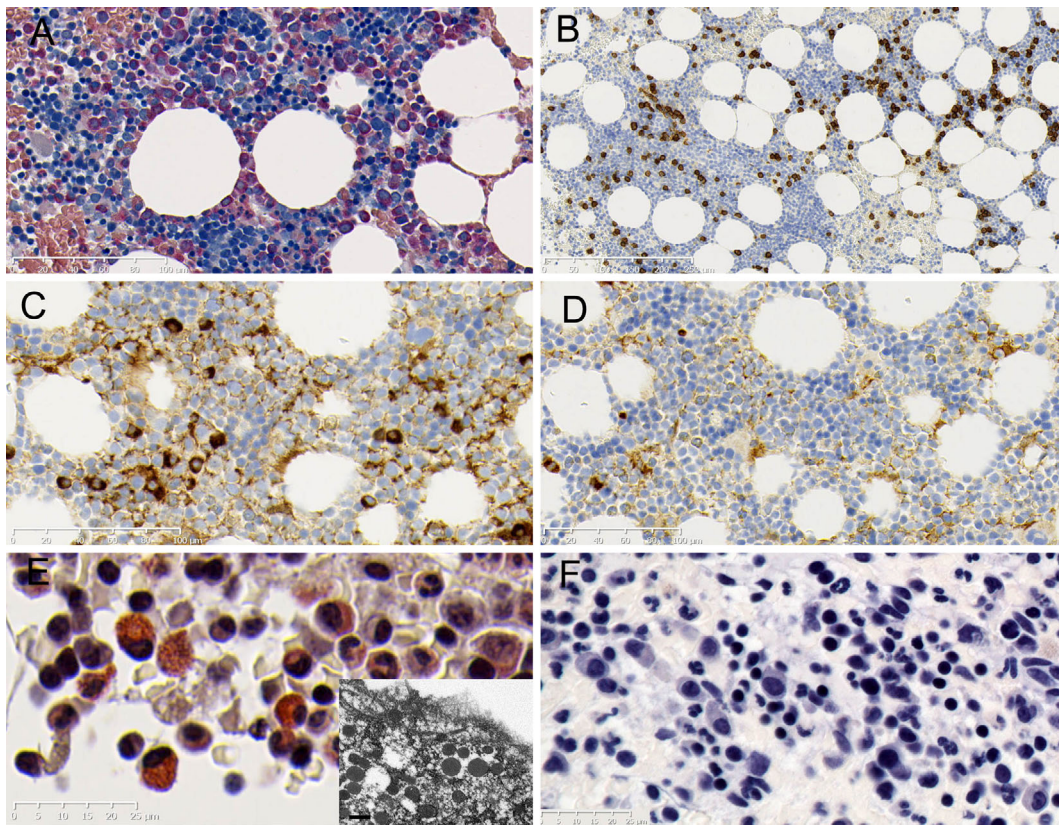


Figure 3. Findings of a bone marrow biopsy. A: ASD-Giemsa staining, B: CD138 immunostaining, C: κ -light chain, D: λ -light chain, E: Congo red staining in the present case and electron micrograph of plasma cell showing fibrils (insert) F: Congo red staining in a case of BJP- λ myeloma without amyloidosis. The bars indicate 100 μ m (A-D), 25 μ m (E, F) and 500 nm (insert).

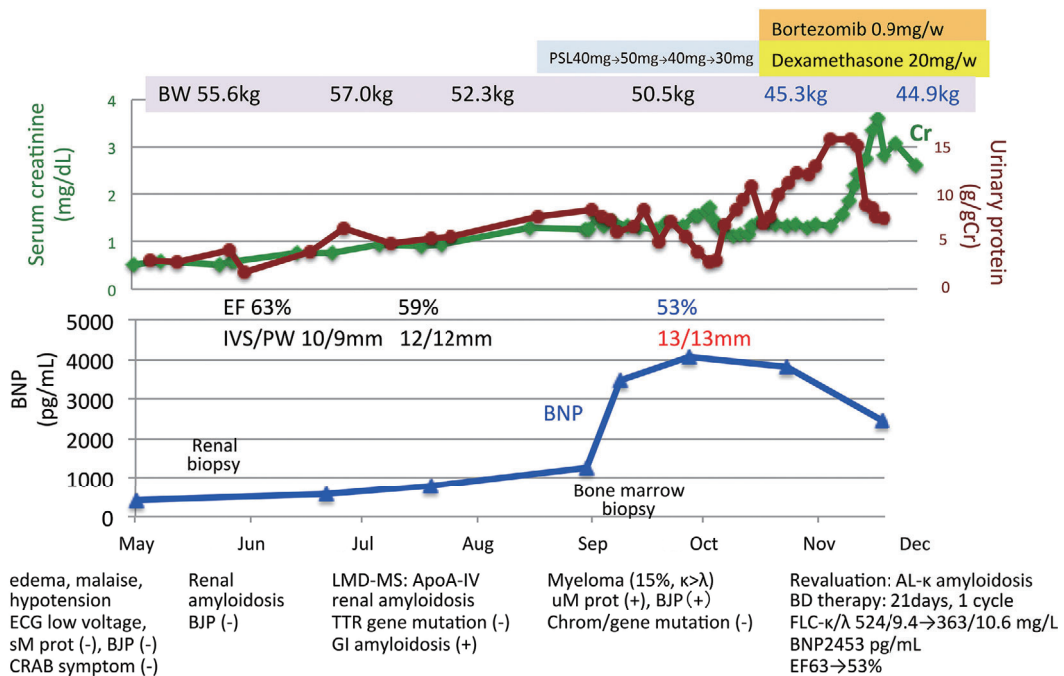


Figure 4. Clinical course and changes in the values for BNP, serum creatinine, urinary protein, ejection fraction (EF), and inter-ventricular septum (IVS) and posterior wall (PW) thickness by an ultrasound cardiogram.

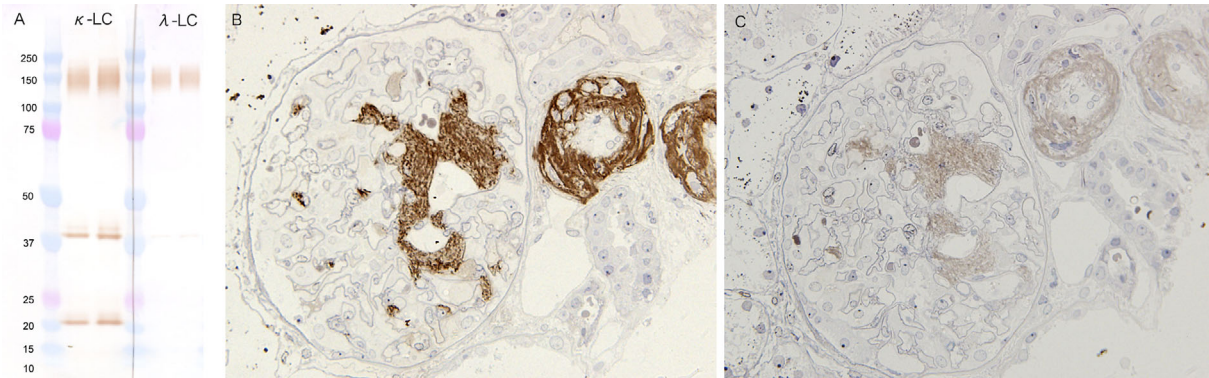


Figure 5. Results of a Western blotting analysis of κ and λ light chain in urine at a renal biopsy (A) and immunohistochemistry of κ (B) and λ (C) light chain using epoxy resin-embedded renal biopsy samples.

Issue with amyloidosis typing by mass spectrometry of a renal biopsy sample

For amyloid typing, immunostaining has a sensitivity of 84.6% and a specificity of 92.4%, and there is a 15% chance that AL amyloidosis cannot be diagnosed (9). In contrast, mass spectrometry has almost 100% sensitivity and specificity provided the existing proteins can be ionized (7, 10). However, the limitation of the LMD-MS method is how laser microdissection is limited to specific tissue regions, as it is impossible to remove proteins from surrounding cells and matrices other than amyloid fibrils. In the present case, 146 proteins were identified in the blood vessel wall and 369 proteins were identified in the glomerulus. The top five most abundantly expressed proteins were ApoA-IV, Apo-E, kappa light chain, complement C3, and amyloid P, so it is difficult to determine which was the main component of amyloid fibrils. The amyloid obtained from the formalin-fixed paraffin-embedded section is sticky (11). In the present case, complement C3 was a serum-derived deposit that attached to amyloid fibrils. In addition, extracellular matrix tenascin and the adhesion molecule vitronectin, which easily attaches itself to amyloid, were detected by LMD-MS. In contrast, ApoA-IV and Apo-E are considered to be amyloidogenic proteins as well as the kappa light chain.

ApoA-IV renal amyloidosis was first reported by Sethi et al (12), and 11 cases have been summarized since then. Amyloid deposits are predominantly found in the renal medulla and have few glomerular lesions, resulting in low urinary protein levels, and in most cases, a slow reduction in the renal function (13). The present case showed nephrotic syndrome, a rapid decline in the renal function and a short prognosis of six months, findings that are inconsistent with ApoA-IV renal amyloidosis. In ApoA-IV renal amyloidosis, ApoE, serum amyloid P, and leukocyte cell-derived chemotaxin-2 (LECT2) have been identified along with ApoA-IV (13). In cerebral amyloidosis, the apoproteins ApoC-III, ApoA-I, ApoE4, and ApoB bind to A β in blood, preserve and strengthen non-polar spiral structures, and are

involved in A β fibril formation (14). Serum amyloid A (SAA) of reactive amyloidosis is also a lipid surface-binding protein containing amphipathic α -helices such as apolipoprotein, where the N-terminal fragment of SAA increased by inflammation replaces apoA-I in high-density lipoprotein (HDL) to deposit type A amyloid (15). Therefore, APO abnormalities promote amyloid fibril formation in brain amyloid and reactive amyloid, but whether or not APO α -helices are converted to amyloid cross β -sheet is not yet well understood (16).

There may be two different types of amyloid fibers in the same organ. Indeed, 2 of the 11 cases of ApoA-IV renal amyloidosis also had AL amyloidosis (13). In addition, there have been reports of cases in which ApoA-IV and transthyretin amyloid had two types of amyloid, although the organs and localization did not match (17).

Myeloma-like lesions of AL amyloidosis

Conventionally, AL amyloidosis has been considered to be classified as either primary amyloidosis or amyloidosis associated with myeloma. Amyloidogenic mutations are found in the monoclonal immunoglobulin light chain proteins produced by approximately 15% of plasma cells in myeloma, where amyloid fibrils are composed of the extracellular β -sheet structure of light chain proteins (18). Recently, the 5-year survival rate for myeloma has improved (2, 3), but the prognosis of primary AL amyloidosis remains poor, even with the use of treatments for myeloma (1, 4). The present case was unlikely to have had myeloma, as there were no CRAB symptoms, chromosomal abnormalities, or genetic mutation seen in myeloma, and also because the changes in the left ventricle wall thickening and BNP elevation were too rapid for the transition from smoldering myeloma to amyloidosis.

A Western blot analysis and immunohistochemistry using epoxy resin specimens revealed that AL κ amyloidosis had occurred from the time of the onset. ApoA-IV may block the antigenicity of AL amyloid fibrils. Immunostaining of the epoxy resin section activated the antigenicity after treating with potassium hydroxide-saturated ethanol to remove

the resin and then adding 3% trypsin in order to digest lipoproteins that cover the antigen. Furthermore, in the present case, AL amyloidosis plasma cells were found to be Congo red-positive, thus suggesting the production of amyloid fibril in AL amyloidosis plasma cells. In contrast, myeloma plasma cells were negative for Congo red staining. Given these findings, the characteristics of plasma cells in bone marrow may differ between primary AL amyloidosis and myeloma. Further research is needed to clarify the difference between AL amyloidosis plasma cells and myeloma plasma cells.

Conclusion

We experienced a patient with AL amyloidosis that was first diagnosed with ApoA-IV renal amyloidosis by LMD-MS but who developed smoldering myeloma and ultimately died of heart failure. Western blotting of urinary protein showed AL amyloidosis predominantly in the κ light chain from the time of the renal biopsy, and it was speculated that the Congo red-positive bone marrow plasma cells had produced amyloid fibers.

The authors state that they have no Conflict of Interest (COI).

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References

- Gertz MA. Immunoglobulin light chain amyloidosis: 2018 update on diagnosis, prognosis, and treatment. *Am J Hematol* **93**: 1169-1180, 2018.
- Shah V, Sherborne AL, Walker BA, et al. Prediction of outcome in newly diagnosed myeloma: a meta-analysis of the molecular profiles of 1905 trial patients. *Leukemia* **32**: 102-110, 2018.
- Rajkumar SV. Multiple myeloma: every year a new standard? *Hematol Oncol* **37**: 62-65, 2019.
- Dittrich T, Kimmich C, Hegenbart U, Schonland SO. Prognosis and staging of AL amyloidosis. *Acta Haematol* **143**: 388-399, 2020.
- Leung N, Barnidge DR, Hutchison CA. Laboratory testing in monoclonal gammopathy of renal significance (MGRS). *Clin Chem Lab Med* **54**: 929-937, 2016.
- Said SM, Sethi S, Valeri AM, et al. Renal amyloidosis: origin and clinicopathologic correlations of 474 recent cases. *Clin J Am Soc Nephro* **8**: 1515-1523, 2013.
- Sethi S, Vrana JA, Theis JD, et al. Laser microdissection and mass spectrometry-based proteomics aids the diagnosis and typing of renal amyloidosis. *Kidney Int* **82**: 226-234, 2012.
- Aoki M, Kang DD, Katayama A, et al. Optimal conditions and the advantages of using laser microdissection and liquid chromatography tandem mass spectrometry for diagnosing renal amyloidosis. *Clin Exp Nephrol* **22**: 871-880, 2018.
- Suarez MLG, Zhang PC, Nasr SH, et al. The sensitivity and specificity of the routine kidney biopsy immunofluorescence panel are inferior to diagnosing renal immunoglobulin-derived amyloidosis by mass spectrometry. *Kidney Int* **96**: 1005-1009, 2019.
- Sethi S, Vrana JA, Theis JD, Dogan A. Mass spectrometry based proteomics in the diagnosis of kidney disease. *Curr Opin Nephrol Hypertens* **22**: 273-280, 2013.
- Picken MM, Herrera GA. The burden of "sticky" amyloid - typing challenges. *Arch Pathol Lab Med* **131**: 850-851, 2007.
- Sethi S, Theis JD, Shiller SM, et al. Medullary amyloidosis associated with apolipoprotein A-IV deposition. *Kidney Int* **81**: 201-206, 2012.
- Dasari S, Amin MS, Kurtin PJ, et al. Clinical, biopsy, and mass spectrometry characteristics of renal apolipoprotein A-IV amyloidosis. *Kidney Int* **90**: 658-664, 2016.
- Gursky O, Atkinson D. Thermodynamic analysis of human plasma apolipoprotein C-1: High-temperature unfolding and low-temperature oligomer dissociation. *Biochemistry* **37**: 1283-1291, 1998.
- van der Westhuyzen DR, de Beer FC, Webb NR. HDL cholesterol transport during inflammation. *Curr Opin Lipidol* **18**: 147-151, 2007.
- Das M, Gursky O. Amyloid-forming properties of human apolipoproteins: sequence analyses and structural insights. *Adv Exp Med Biol* **855**: 175-211, 2015.
- Bergstrom J, Murphy CL, Weiss DT, et al. Two different types of amyloid deposits - apolipoprotein A-IV and transthyretin - in a patient with systemic amyloidosis. *Lab Invest* **84**: 981-988, 2004.
- Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. *N Engl J Med* **349**: 583-596, 2003.

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