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α1-Heavy Chain Deposition Disease With Negative Immunofluorescence Staining on Renal Biopsy

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INTRODUCTION

Monoclonal Ig deposition disease (MIDD) is a form of end-organ damage caused by extracellular deposition of pathogenic monoclonal Igs, or their fragments, secreted by B-cell or plasma-cell clones. Although rare, this disease has been most often described in the kidney, and is characterized by nonorganized deposits within the glomeruli, tubulointerstitium, and blood vessels. When the deposited protein is a heavy chain without an accompanying light chain, the entity is termed heavy chain deposition disease (HCDD). The most frequently deposited heavy chain is a truncated γ-chain (IgG), followed by α (IgA), and rarely δ (IgD) and μ-chains (IgM).[1–8] Clinical manifestations of the disease include renal insufficiency, proteinuria (often nephrotic range), hematuria, and hypertension in patients with monoclonal gammopathy of undetermined significance, B-cell lymphoma, smoldering multiple myeloma, symptomatic multiple myeloma, or rarely in patients with no detectable monoclonal Ig in serum or urine.[9] Diagnosis and early initiation of treatment is of utmost importance to prevent further renal damage, particularly in patients with otherwise no indication for therapy (such as low-grade B-cell neoplasms, monoclonal gammopathy of undetermined significance, or smoldering myeloma). In this setting, the diagnosis of HCDD would represent what has been recently recognized as monoclonal gammopathy of renal significance.[10] The diagnosis of HCDD is established by light, immunofluorescence, and electron microscopic examination of a renal biopsy. Regardless of the particular class of deposited heavy chain, the disease is morphologically characterized by nodular glomerulosclerosis by light microscopy, with linear staining of basement membranes by a single heavy chain on immunofluorescence, and frequent punctate-powdery electron-dense deposits by electron microscopy.[3] Here we present an unusual case of IgA HCDD with negative immunofluorescence staining that was eventually diagnosed by proteomic analysis using liquid chromatography and tandem mass spectrometry (LC-MS/MS).

CASE PRESENTATION

A 68-year-old Caucasian male with past medical history significant for hypertension, hyperlipidemia, valvular heart disease, and chronic anemia managed with iron supplementation, was found to have increased creatinine of 2.3 mg/dl during a routine annual visit (baseline creatinine of 1.5 mg/dl). No relevant findings were noted on physical examination. The patient was referred to nephrology and subsequent workup showed further increase in serum creatinine to 3.1 mg/dl. The urine protein/creatinine ratio was 0.53 g/g, serum albumin was 4.5 g/dl, and no monoclonal spike was evident on serum protein electrophoresis. Serum immunofixation electrophoresis revealed an IgA kappa monoclonal band, and urine protein electrophoresis with immunofixation electrophoresis showed a free kappa light chain monoclonal band. Free serum kappa/lambda ratio was 36.9 (free serum kappa light chain was 58.03 mg/l and free serum lambda light chain was 1.57 mg/l) and serum IgA levels were slightly increased at 432 mg/dl. No evidence of lytic lesions was present on skeletal survey or positron emission tomography/computed tomography. A bone marrow biopsy showed a hypocellular marrow with approximately 8% kappa-restricted plasma cells. The monoclonal plasma cells

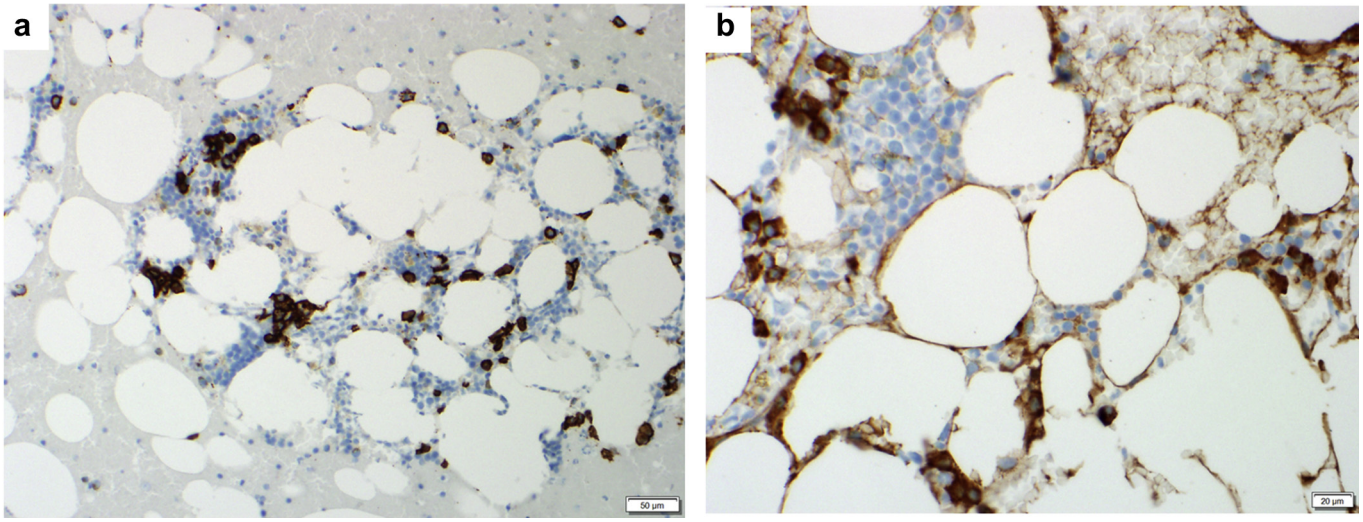

Figure 1. Bone marrow biopsy. Immunohistochemistry showing plasma cells staining for CD138 (a) and IgA (b) (original magnification ×200 for [a] and ×400 for [b]). The monoclonal plasma cells also showed kappa light chain restriction by in situ hybridization (not shown).

stained positive for IgA by immunohistochemistry (Figure 1). Congo red stain was negative for amyloid. There was no flow cytometric evidence of increased blasts, B- or T-cell lymphoma, and conventional cytogenetics was normal. At this point, the patient met diagnostic criteria for monoclonal gammopathy of undetermined significance, and a renal biopsy was performed to rule out monoclonal gammopathy of renal significance.

Pathologic Findings

Light Microscopy

One core of renal cortex was available for light microscopic examination, which contained a total of 15 glomeruli; 3 of them were globally sclerotic. The glomeruli showed moderate mesangial matrix expansion with frequent nodule formation, along with focal areas of segmental glomerulosclerosis (Figure 2a and b). The mesangial nodules stained strongly positive on periodic acid-Schiff stain and negative on Jones methenamine silver stain. There was moderate to severe tubular atrophy and interstitial fibrosis. Mild polymorphous lymphoplasmacytic inflammation was limited to areas of interstitial fibrosis. No atypical casts were present within the tubular lumens, and Congo red stain was negative for amyloid. The arteries showed severe arteriosclerosis. Immunoperoxidase staining, using the same IgA antibody that stained the monoclonal plasma cells in the bone marrow, showed absence of glomerular or tubular basement membrane staining.

Immunofluorescence

Twenty-three glomeruli were available for immunofluorescence, 3 of which were globally sclerotic. All stains, including IgA, IgG, IgM, C3, C1q, fibrinogen, and kappa and lambda light chains, were negative within the glomeruli and throughout the tubulointerstitium (IgA, IgG, IgM, C3, C1q [Kent Laboratories, Bellingham, WA]; fibrinogen, kappa, and lambda [Agilent, Carpinteria, CA]) (Figure 2c–e). There was no light chain restriction of glomerular basement membranes, tubular basement membranes, intratubular casts, or proximal tubule protein reabsorption droplets. Repeat staining with a rabbit polyclonal anti-human IgA primary antibody (Agilent) rendered similar negative results. The tissue was also stained and was negative with anti-IgD (Kent Laboratories).[6] To exclude a "masked" monoclonal Ig, staining for IgA, IgG, IgM, kappa, and lambda was subsequently performed on the formalin-fixed paraffin-embedded tissue after protease digestion.[11] All stains were negative within the glomeruli and throughout the tubulointerstitium.

Electron Microscopy

Ultrastructural examination of a glomerulus showed marked mesangial matrix expansion by numerous punctate-powdery electron-dense deposits (Figure 3a). Similar deposits were present throughout the thickened tubular basement membranes (Figure 3b), and segmentally within the glomerular basement membranes. There were no immune complex-type electron-dense deposits. Podocyte foot processes were mildly effaced.

Proteomic Analysis

A previously established LC-MS/MS method was used to analyze the extracellular glomerular deposits.[12] A 10-μm-thick section was cut from the formalin-fixed, paraffin-embedded biopsy tissue, mounted on a Director slide (Nantomics, Rockville, MD), stained with hematoxylin and eosin, and loaded on a laser microdissection apparatus (Leica, Wetzlar, Germany). Two replicate dissections, each configured to collect

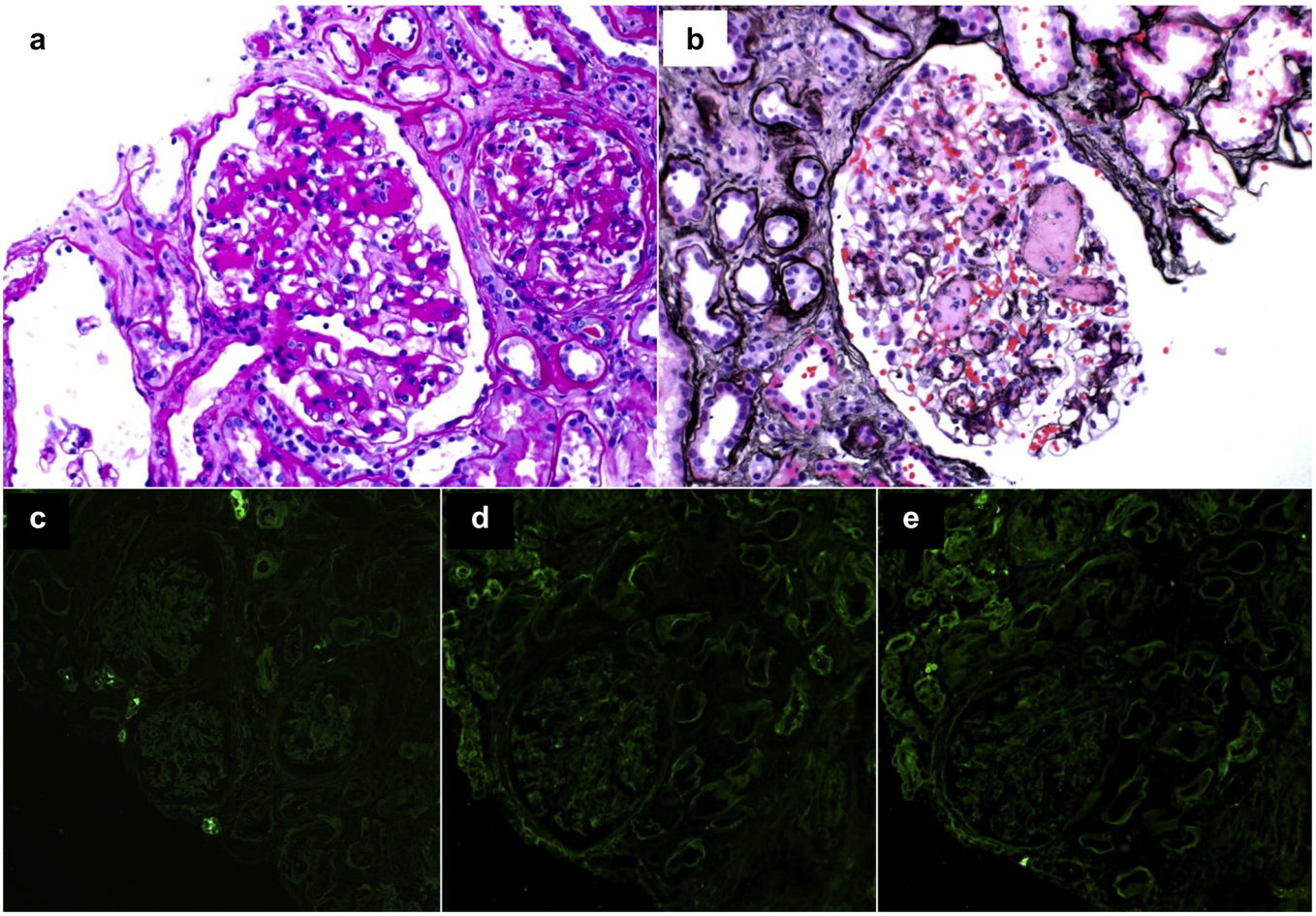

Figure 2. Heavy chain deposition disease. [a,b] Nodular glomerulosclerosis with periodic acid-Schiff–positive, non-argyrophilic mesangial nodules ([a] periodic acid-Schiff, original magnification ×200; [b] Jones methenamine silver, original magnification ×200). [c–e] Negative routine immunofluorescence ([c] IgA, direct immunofluorescence, original magnification ×100; [d] kappa light chain, direct immunofluorescence, original magnification ×200; [e] lambda light chain, direct immunofluorescence, original magnification ×200).

glomeruli from a total area of 60,000 μm^2, were performed.[13] Proteins were extracted from the fragments of each dissection and digested using trypsin as previously described.[13] Peptides were analyzed on a QExactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Protein identification was

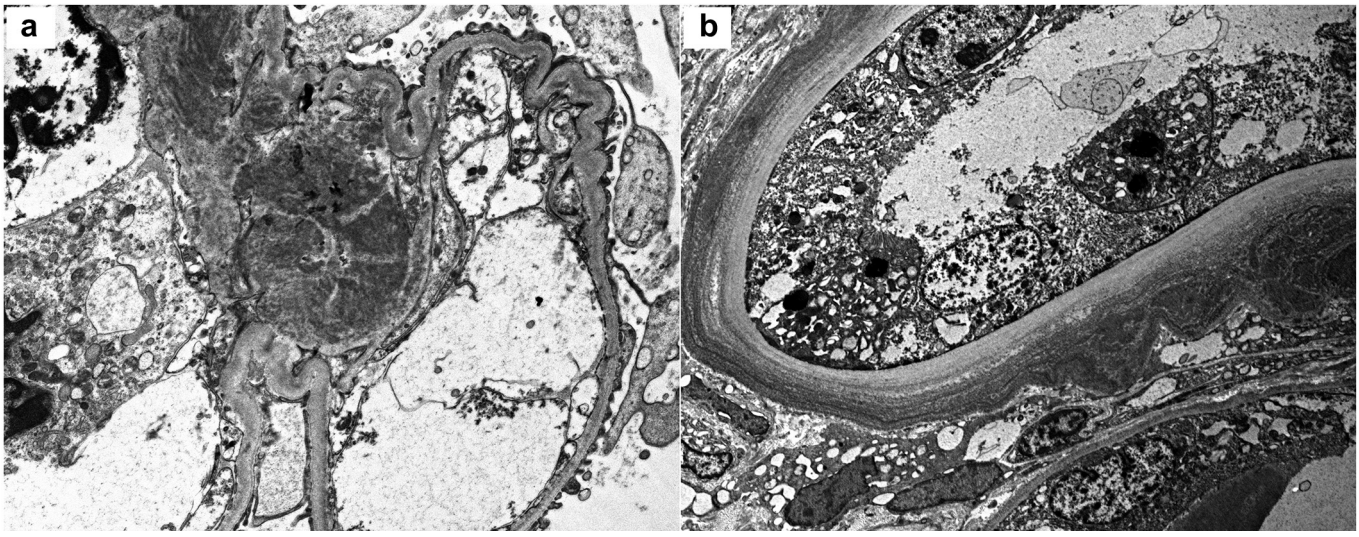

Figure 3. Electron microscopy. Punctate-powdery deposits within the mesangium ([a] original magnification ×8000) and tubular basement membranes ([b] original magnification ×3000).

accomplished by processing the MS data using a previously described bioinformatics pipeline.[14]

Abundant spectra for Igα-1 chain C region (IgA1 constant region) and apolipoprotein E were detected (Figure 4). No significant spectra for Ig kappa or lambda light chains, nor the amyloid chaperone proteins serum amyloid P component or apolipoprotein A4 were detected. The findings on electron microscopy and MS established the diagnosis of α-1 (IgA1) HCDD.

Probability Legend
over 95%
80% to 94%
50% to 79%
20% to 49%
0% to 19%

#	Visible?	Starred?	BioView: Identified Proteins (90) Including 4 Decoys	Accession Number	Molecular Weight	Protein Grouping Ambiguity	LMD#1	LMD#2
1	✓	★	Apolipoprotein E	APOE_HUM...	36 kDa		67	64
2	✓	★	Ig alpha-1 chain C region	IGHA1_HU...	38 kDa		46	47
3	✓	★	Serum amyloid P-component	SAMP_HUM...	25 kDa		5	5
4	✓	★	Apolipoprotein A-IV	APOA4_HU...	45 kDa		7	1
5	✓	★	Gelsolin	GELS_HUMAN	86 kDa		2	1
6	✓	★	Ig lambda-2 chain C regions	LAC2_HUM...	11 kDa		2	1
7	✓	★	Apolipoprotein A-I	APOA1_HU...	31 kDa		2	
8	✓	★	(CONTAMINANT) Ig gamma (C) Ra...	CONTAM_A...	20 kDa		1	1
9	✓	★	Ig kappa chain C region	IGKC_HUMAN	12 kDa		1	
10	✓	★	Ig gamma-2 chain C region	IGHG2_HU...	36 kDa		1	
11	✓		(ENZYME) Trypsin precursor	ENZYME_TR..	24 kDa	★	2399	2631
12	✓		Actin, cytoplasmic 1	ACTB_HUM...	42 kDa	★	187	173
13	✓		Vimentin	VIME_HUM...	54 kDa		114	98
14	✓		Hemoglobin subunit beta	HBB_HUMAN	16 kDa	★	72	69
15	✓		Serum albumin	ALBU_HUM...	69 kDa		88	52
16	✓		Hemoglobin subunit alpha	HBA_HUMAN	15 kDa		69	70
17	✓		Vitronectin	VTNC_HUM...	54 kDa		60	61
18	✓		Alpha-actinin-4	ACTN4_HU...	105 kDa		49	50
19	✓		Histone H2B type 1-B	H2B1B_HU...	14 kDa		53	51
20	✓		Myosin-9	MYH9_HUM...	227 kDa		43	34
21	✓		Trypsin-2	TRY2_HUM...	26 kDa	★	30	33
22	✓		Histone H2AX	H2AX_HUM...	15 kDa		42	30
23	✓		Histone H1.4	H14_HUMAN	22 kDa		30	27
24	✓		Clusterin	CLUS_HUM...	52 kDa		29	25

Figure 4. Mass spectrometry. Scaffold software display of most abundant proteins and Ig-related proteins identified within the deposits by liquid chromatography/tandem mass spectrometry. The 2 columns on the right (LMD#1, LMD#2) represent the separate micro-dissected samples run in duplicate. The numbers within the columns represent the total number of mass spectra identified that correspond to the listed proteins on the left. The color of the box reflects the probability that the spectra are correctly assigned to the identified protein. In this case, there were abundant spectra for Ig alpha-1 chain constant region and apolipoprotein E, whereas insignificant numbers of spectra for serum amyloid P component, apolipoprotein A4, Ig lambda, Ig kappa, and Ig gamma were observed.

Table 1. Teaching Points

1	Rare cases of monoclonal Ig deposition disease (MIDD) show negative immunofluorescence staining, potentially due to a deposition of truncated Ig.
2	Proteomic analysis of glomeruli can confirm the diagnosis of MIDD and determine the Ig component deposited in the kidney.
3	MIDD should remain in the differential diagnosis of nodular glomerulosclerosis with negative immunofluorescence, and electron microscopy and monoclonal protein studies should be performed.

Follow-up

The patient received 16 weeks (4 cycles) of initial therapy based on bortezomib-cyclophosphamide-dexamethasone, followed by autologous stem cell transplantation. The posttransplant course has been uncomplicated. At 2 months posttransplant, the serum IgA levels decreased to 106 mg/dl, the serum protein electrophoresis/urine protein electrophoresis with immunofixation electrophoresis showed absence of a monoclonal Ig, and the free serum kappa/lambda ratio was normal. The patient was subsequently started on maintenance therapy with bortezomib.

DISCUSSION

HCDD, although rare, is the second most common form of MIDD, involving the kidney, slightly more frequent than light and heavy chain deposition disease.[1,3] In the vast majority of cases, the heavy chain identified by immunofluorescence is of the γ-class (IgG).[1,3,9] To the best of our knowledge, only 14 cases of α-heavy chain (IgA) deposition disease have been reported.[1,3–5,8,9] As in the case of γ-heavy chain (IgG) deposition disease, the involved α-chains have partial or complete deletion of the first constant domain (CH1), which allows secretion of free unassembled heavy chain by plasma cells.[5,9] Although the morphology is similar to other forms of MIDD, α-chain (IgA) deposition disease is more frequently a crescentic pattern of glomerular injury.[4,5] Although light and electron microscopy may strongly suggest the presence of heavy and/or light chain deposits, definitive determination of the involved monoclonal Ig is made by immunofluorescence. Here, we present a case of nodular glomerulosclerosis with punctate-powdery deposits by electron microscopy, highly suspicious for MIDD, but with negative immunofluorescence, in a patient with known IgA-κ monoclonal gammopathy of undetermined significance. The differential diagnosis included either deposition of a truncated protein not detected by commercially available antibodies or deposition of an Ig not tested by routine immunofluorescence. A recent publication reported a similar case in which a diagnosis of δ-heavy chain (IgD) deposition disease was made using LC-MS/MS.[6]

LC-MS/MS has been primarily used in the past to assist in the diagnosis and typing of amyloidosis, particularly in situations in which immunofluorescence or immunohistochemistry staining is equivocal, or to identify rare and new types of amyloidosis.[15–18] More recently, however, the utility of this technology has expanded to study various forms of immune-complex–mediated diseases, diseases caused by abnormalities in regulation of the alternative pathway of complement, and diseases caused by deposition of organized deposits.[13,16] LC-MS/MS was performed in this case and showed a peptide profile supportive of α1-heavy chain (IgA1) deposition disease. The reason for the negative immunofluorescence staining in this patient remains to be determined. It could be due to a deposition of truncated monoclonal protein in which the Fc portion of α1-heavy chain that contains the epitopes recognized by the commercially available anti-α1-heavy chain antibodies used in the present case are missing leading to false-negative staining. This phenomenon has been previously reported in cases of light chain amyloidosis and heavy chain amyloidosis.[19,20] Alternatively, because clonal plasma cells in the bone marrow in this patient expressed IgA, this phenomenon could be due to posttranslational modifications that may have altered the tertiary structure of protein, rendering the antigenic sites for anti-α1-heavy chain antibodies inaccessible. Still, the fact that monoclonal plasma cells in this patient express IgA does not exclude a secretion of truncated IgA heavy chain, because a production of both truncated heavy chain and full-length monoclonal heavy chain by monoclonal plasma cells is common in HCDD.[9,21] Heavy chain sequencing of bone marrow material could have potentially provided further insight into the underlying molecular mechanism by which IgA antiserum is failing to react with the deposited IgA heavy chain; however, the lack of stored bone marrow cells in our patient precluded such investigation. The patient's plasma-cell burden was low (8% kappa-restricted plasmacytosis in the bone marrow) and, therefore, it is very unlikely that there are sufficient peripheral blood clonal elements (ie, circulating monoclonal plasma cells and/or B cells) for heavy chain sequencing on the peripheral blood. Because a custom curated database for Ig heavy chains is very difficult to construct, LC-MS/MS cannot recognize absence of a portion of Ig. Direct sequence analysis of kidney deposits requires large amounts of fresh renal tissue, such as nephrectomy tissue, which was not available in our patient.

Of note, we detected abundant spectra for apolipoprotein E (but not serum amyloid P component or apolipoprotein AIV) in the glomeruli by LC-MS/MS in this case, as is usually observed in MIDD.[6,22] It is currently unknown if apolipoprotein E plays a

pathologic role in MIDD or it is simply a reflection of increased matrix deposition, because it is also commonly seen in glomeruli of diabetic nephropathy and idiopathic mesangial sclerosis.[22]

Immunofluorescence is essential for the diagnosis of monoclonal gammopathy of renal significance, as it establishes the monotypic nature of Ig deposits. It also determines the specific component of monoclonal protein deposited in the kidney (ie, Ig heavy and light chains, Ig light chain only, or Ig heavy chain only) and its distribution in the kidney compartments.[9] However, false-negative results may occur, such as in renal Ig-related amyloidosis in which immunofluorescence fails to diagnose 8.5% of cases and in these cases LC-MS/MS is critical for establishing a tissue diagnosis of Ig-related amyloidosis before initiation of chemotherapy and/or stem cell transplantation.[23] In this report, we show that false-negative immunofluorescence staining (both on frozen and paraffin tissue) also may occur in HCDD, and that the identity of deposits can be determined by LC-MS/MS.

CONCLUSION

Nodular glomerulosclerosis due to other etiologies, such as diabetes mellitus and smoking, is one of the most common diagnoses encountered on routine renal pathology practice. Keeping a high index of suspicion in cases of nodular glomerulosclerosis with negative immunofluorescence in patients with a known monoclonal Ig, and use of ancillary studies, such as LC-MS/MS, is essential to establish the correct diagnosis in these patients (Table 1).[24,25]

DISCLOSURE

All the authors declared no competing interests.

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