AJKD
Kidney Biopsy Teaching Case

Membranoproliferative Glomerulonephritis: The Role for Laser Microdissection and Mass Spectrometry

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Monoclonal gammopathy is increasingly recognized as a common cause of membranoproliferative glomerulonephritis (MPGN); however, establishing this diagnosis can be challenging. We report the case of a 58-year-old asymptomatic woman who presented with proteinuria with protein excretion of 5,000 mg/d, microscopic hematuria, and normal kidney function. Kidney biopsy was consistent with MPGN pattern of injury. Immunofluorescence studies were positive for nonspecific segmental immunoglobulin M (IgM) and C3 staining. Electron microscopy showed subendothelial, subepithelial, and mesangial electron-dense deposits. The workup excluded an infectious or autoimmune disease, but IgG κ monoclonal protein was detected in serum at a concentration of 0.4 mg/dL. Because there was a mismatch between the serum monoclonal protein (IgG κ) and immunofluorescence staining pattern (nonspecific IgM, no light chain restriction), laser microdissection and mass spectrometry were performed on the kidney biopsy tissue. This identified the deposits as monoclonal IgG κ, thereby leading to the diagnosis of monoclonal gammopathy–associated MPGN. Our case emphasizes the importance of searching for an underlying cause of MPGN, reviews the technique of laser microdissection–mass spectrometry, and highlights its application as a pathology tool for the evaluation of monoclonal gammopathy–related glomerulonephritis.
Am J Kidney Dis. 63(2):324-328.

INDEX WORDS: Membranoproliferative glomerulonephritis; monoclonal gammopathy; laser microdissection; laser-capture microdissection–mass spectrometry (LCM-MS); mass spectrometry; proteomics; nephrotic; proteinuria.

INTRODUCTION

Membranoproliferative glomerulonephritis (MPGN) is a glomerular pattern of injury characterized by mesangial hypercellularity, endocapillary proliferation, and capillary wall remodeling with formation of double contours. The clinical presentation is variable, with overlapping features of nephritic and nephrotic syndrome. Historically, it has been classified as type I, type II, or type III based on electron microscopy findings and distribution of the electron-dense deposits. Due to increased knowledge regarding the pathogenesis of MPGN, a new classification has been proposed based on immunofluorescence findings, dividing it into immune complex–mediated or complement-mediated MPGN.[1-3] The new classification helps guide appropriate evaluation and treatment.

Immune complex–mediated MPGN arises as a result of deposition of immune complexes in glomeruli, leading to activation of the classical complement pathway. Most often, the cause is chronic infections (such as hepatitis C), autoimmune disorders, or monoclonal gammopathies. Immunofluorescence microscopy shows staining for both immunoglobulin and complement. Complement-mediated MPGN may be secondary to inherited or acquired abnormalities of the alternative complement pathway. Complement, but not immunoglobulin, is detectable upon immunofluorescence microscopy. The MPGN pattern of injury also can be seen in the setting of thrombotic microangiopathies, with absent staining on immunofluorescence microscopy for both immunoglobulin and complement.

To show the potential of laser-capture microdissection–mass spectrometry (MS) in the evaluation of monoclonal gammopathy–related glomerulonephritis, we provide a detailed description of the diagnostic strategy for a patient with monoclonal gammopathy on serum analysis. The patient, whose case has been highlighted briefly in a review article,[4] underwent kidney biopsy for new-onset proteinuria and microscopic hematuria. The biopsy showed MPGN pattern of injury, but immunofluorescence studies for light chain staining were inconclusive.

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Received May 22, 2013. Accepted in revised form September 13, 2013. Originally published online October 21, 2013.

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0272-6386/$36.00
http://dx.doi.org/10.1053/j.ajkd.2013.09.007

CASE REPORT

Clinical History and Initial Laboratory Data

A 58-year-old asymptomatic woman was referred for evaluation of proteinuria and hematuria incidentally noted on routine urinalysis. She denied gross hematuria. Her medical history was significant for hypertension and hyperlipidemia, for which she was taking diltiazem, metoprolol, and simvastatin. There was no family history of kidney disease. On physical examination, blood pressure was 158/94 mm Hg. Cardiopulmonary examination findings were normal, but mild bilateral lower-extremity edema was noted.

Laboratory results are listed in Table 1. Kidney function was normal based on serum creatinine, serum urea nitrogen, and estimated glomerular filtration rate values. Serum albumin level was within the reference range. Urine dipstick showed protein (3+) and blood (3+). Urinalysis results from 6 months prior were unremarkable, with no protein or blood. Urine sediment showed 10 red blood cells per high-power field and rare granular casts, but no red blood cell casts. Subsequent 24-hour urinalysis showed proteinuria with protein excretion of 5,000 mg/d. Serologic workup for glomerular disease was negative for antinuclear antibody, serum complements, anti-neutrophil cytoplasmic antibody, anti–glomerular basement membrane antibodies, hepatitis panel, and cryoglobulins. Serum protein electrophoresis revealed an M spike, which was identified as immunoglobulin G (IgG) κ monoclonal protein, at a concentration of 0.4 mg/dL. Serum free light chain κ:λ ratio was elevated at 2.96 (reference range, 0.26-1.65). Urine protein electrophoresis showed nonselective glomerular proteinuria. Following this, the patient underwent bone marrow biopsy that showed 8% plasma cells that were positive for IgG κ. There was no evidence of lytic lesions, hypercalcemia, or anemia. Hence, the diagnosis of monoclonal gammopathy of undetermined significance (MGUS) was made. Thereafter, a native kidney ultrasound-guided biopsy was performed.

Table 1. Laboratory Results at Baseline

Test Name	Value	Reference Range
Hemoglobin (g/dL)	12.5	11.6-14.6
Hematocrit (%)	35.5	34.1-43.3
Serum urea nitrogen (mg/dL)	20	8-26
Creatinine (mg/dL)	0.8	0.5-1.4
Estimated GFR[a] (mL/min/1.73 m^2)	≥60	
Serum albumin (mg/dL)	3.8	3.4-5
Serum calcium (mg/dL)	10	8.4-10.2
Total creatine kinase (IU/L)	106	0-200
Hepatitis B antigen	Undetectable	
Hepatitis C antibody	Undetectable	
ANA titer	<1:80	<1:80
ASO (IU/mL)	<25	0-116
ANCA	Undetectable	
Rheumatoid factor	Undetectable	
Complement C3 (mg/dL)	124	79-152
Complement C4 (mg/dL)	15	16-38
Cryoglobulin screen	Negative	
Blood cultures	Negative	

Note: Conversion factors for international units: calcium in mg/dL to mmol/L, ×0.2586; creatinine in mg/dL, to μmol/L, ×88.4; serum urea nitrogen in mg/dL to mmol/L, ×0.357.

Abbreviations: ANA, antinuclear antibody; ANCA, anti-neutrophil cytoplasmic antibody; ASO, anti-streptolysin O; GFR, glomerular filtration rate.

[a]Calculated using the MDRD (Modification of Diet in Renal Disease) Study equation.

Kidney Biopsy

The kidney biopsy was performed and 2 cores were obtained. Material submitted for light microscopy contained 18 glomeruli, of which one was globally sclerosed. Glomeruli were enlarged and hypercellular and showed lobular accentuation of glomerular tufts (Fig 1A and B). The mesangium was expanded with increased matrix and cellularity. Glomeruli showed segmental endocapillary proliferation with mononuclear cells. Glomerular capillary walls were thickened, with many loops having double contours. Tubules contained protein reabsorption granules. There was no interstitial inflammation. Only mild (15%) tubular atrophy and interstitial fibrosis was present. Arteries showed moderate sclerosis of the intima.

Immunofluorescence studies showed mild segmental staining for IgM, C3, and C1q in a few glomerular capillary tufts. No light chain restriction pattern was noted. Electron microscopy showed subendothelial electron-dense deposits. Occasional intramembranous, subepithelial, and mesangial deposits also were present (Fig 1C). Substructures were not identified. There was mild focal effacement of the foot processes of visceral epithelial cells. Some loops also showed subendothelial expansion with cellular elements, electron-dense deposits, and new basement membrane formation resulting in double contours. Electron-dense deposits were not detectable along the tubular basement membranes. Congo Red stain for amyloid gave negative results.

Laser Microdissection and MS

Kidney biopsy findings were consistent with MPGN. Due to the differences between serum monoclonal protein (IgG κ) and immunofluorescence findings (IgM, no light chain restriction), it was difficult to conclude whether the patient's MPGN was related to the monoclonal gammopathy. Hence, further analysis was performed on the biopsy specimen. Laser microdissection (LMD)-MS was performed to determine the composition of the deposits noted on electron microscopy. LMD-MS analysis of amyloid has been described previously.[5,6] Briefly, 3 samples (each containing at least 2 glomeruli) were microdissected by using laser capture techniques. Peptides were extracted from the microdissected tissue and subjected to liquid chromatography and tandem MS. As described previously,[7] the resulting raw data files were queried using 3 different protein identification algorithms and results were combined and analyzed by Scaffold (Proteome Software Inc) proteomics software suite. Matches of experimentally derived peptide sequences to protein sequences found in databases were accepted if they could be established at >90.0% probability as specified by the Peptide Prophet algorithm.[8,9] This technique identified spectra corresponding to immunoglobulin κ chain C region and immunoglobulin λ1 chain C region, while immunoglobulin γ light chains were not detected (Table 2). Large numbers of spectra matching complement factors C4b and C3 also were noted, indicating activation and accumulation of components of the classical pathway of complement.

Diagnosis

Monoclonal gammopathy–associated MPGN.

Clinical Follow-up

The patient was treated with antihypertensive medications, including lisinopril, to achieve adequate blood pressure control and reduction in proteinuria. After consultation with hematology, it was decided that in order to halt the kidney disease, her underlying plasma cell dyscrasia should be treated. She was started