

Clinical Report

Coexistence of ANCA-associated glomerulonephritis and anti-phospholipase A₂ receptor antibody-positive membranous nephropathy

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Abstract

Antibodies to myeloperoxidase (MPO) and proteinase 3 (PR3) have been demonstrated to mediate anti-neutrophil cytoplasmic antibody (ANCA)-associated disease. For membranous nephropathy, antibodies to the podocyte-expressed phospholipase A₂ receptor (anti-PLA₂R) are highly associated with disease activity and have been reported in at least 70% of patients with idiopathic membranous nephropathy (IMN). We present a case of a 56-year-old male with a 1 year history of hypertension, leg edema, and proteinuria, who presented with advanced renal failure and was found to have both ANCA-associated glomerulonephritis (GN) and IMN on kidney biopsy. Consistent with the idea that this is due to the chance occurrence of two independent diseases, we found both anti-MPO and anti-PLA₂R antibodies in the patient's sera. Treatment with methylprednisolone, plasmapheresis, and cyclophosphamide resulted in improvement in kidney function and proteinuria, together with the simultaneous decrease in both auto-antibodies. This is the first demonstration of two pathogenic antibodies giving rise to ANCA-associated GN and IMN in the same patient. It confirms the importance of classifying disease based upon the underlying mechanism, in addition to renal histopathology, to both optimize therapy and predict prognosis.

Keywords: ANCA vasculitis; antiphospholipase; membranous nephropathy

Introduction

Research over the past several years has demonstrated the importance of classifying disease based upon underlying mechanism, in addition to renal histopathology, to both optimize therapy and predict prognosis.

Antibodies to myeloperoxidase (MPO), proteinase 3 (PR3) and lysosomal-associated membrane protein, initially thought to be only biomarkers for anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (GN), have now been demonstrated to mediate disease in a number of different animal models and in patients [1, 2]. Studies by Beck *et al.* [3] have identified antibodies to the phospholipase A₂ receptor (anti-PLA₂R) expressed on podocytes as the likely mediator of idiopathic membranous nephropathy (IMN); anti-PLA₂R antibodies have been reported in ~70% of patients with IMN.

Case history

A 56-year-old African American male was initially seen by his primary care provider with complaints of bilateral lower

extremity edema. He was found to have Stage II hypertension and a serum creatinine 1.3 mg/dL (114.9 μmol/L), increased from 0.9 mg/dL (79.59 μmol/L) 3 years earlier. Urinalysis revealed large blood and 300 mg/dL (3 g/L) of protein. The patient was treated with furosemide and lisinopril. An evaluation of the hematuria and proteinuria was not initiated at this time. Over the next 6 months, he was intermittently compliant with medications, had persistence of the lower extremity edema and was sent to the emergency room (ER) when, on one of his follow-up visits, he was found to be severely hypertensive with advanced renal failure.

In the ER, his blood pressure (BP) was 206/134 mmHg, he had bilateral 2+ lower extremity pitting edema, scrotal edema and mild pulmonary congestion. Laboratory results showed a hemoglobin level of 7.3 g/dL (73 g/L), blood urea nitrogen of 85 mg/dL (30.34 μmol/L) and creatinine of 13.4 mg/dL (1184.56 μmol/L). Urinalysis revealed >1000 mg/dL (10 g/L) of protein, 19–24 WBC/high power field, numerous red blood cells and no casts. There were 14 g of protein on a 24-h urine collection. An ultrasound showed normal-sized kidneys without evidence of hydronephrosis.

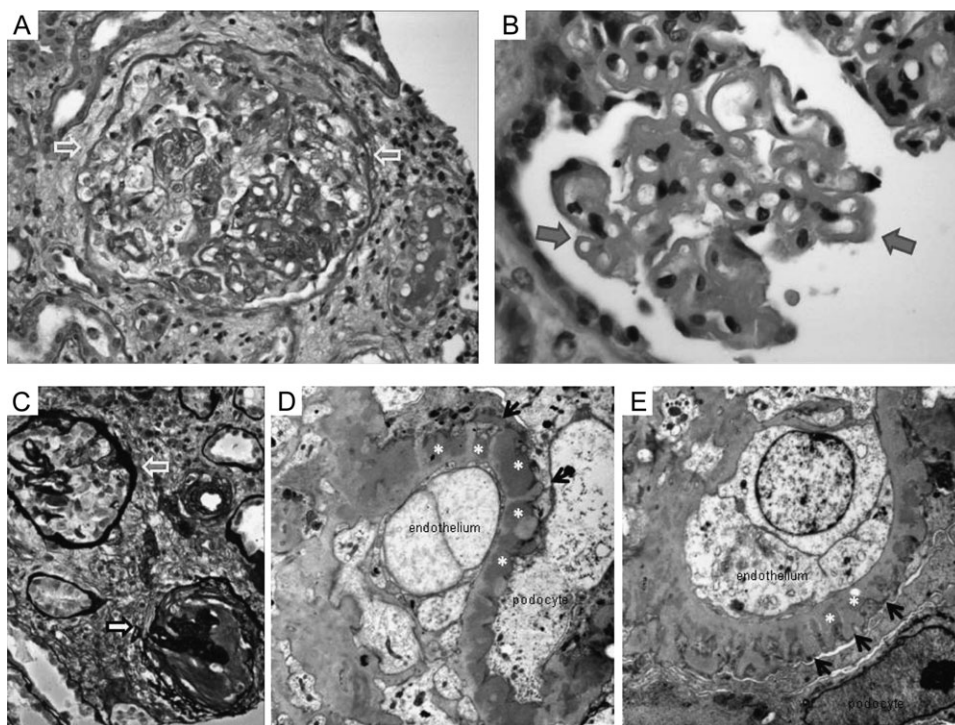


Fig. 1. Kidney specimens light microscopy (A–C). Cellular crescents were seen in some of the glomeruli (grey arrow with white outline) (A and C) (Periodic Acid Schiff stain $\times 40$ and silver $\times 20$) and were occasionally accompanied by sclerotic glomeruli with fibrous crescents (white arrow) (C) (Silver $\times 20$). The glomeruli not affected by extracapillary proliferation revealed diffuse thickening of the basement membranes (B) (hematoxylin and eosin $\times 40$). Electron microscopy (D, E): ultra structural analysis revealed diffuse and global distribution of subepithelial electron-dense deposits (asterisks), most of them partially surrounded by extracellular matrix forming spikes (black arrow heads).

Serological results were significant for elevated perinuclear ANCA (pANCA) titers 1:160, high anti-MPO antibodies 31.5 U/mL (3150 U/L), normal complements, negative anti-nuclear antibodies and the absence of antibodies to PR3, hepatitis C and hepatitis B. A renal biopsy confirmed the presence of crescentic GN (Figure 1), consistent with the positive anti-MPO antibodies in the serum. The renal biopsy contained up to eight glomeruli per level with focal circumferential cellular crescents and occasional necrosis of the glomerular tuft. Rare fibrous crescents were present as well. In non-affected glomeruli, the glomerular basement membranes appeared mildly thickened (Figure 1B). Immunofixation showed granular C3 staining in the glomerular capillary loops. Unfortunately, the sample tested for remaining immunofluorescence did not contain glomeruli. Electron microscopy revealed numerous subepithelial deposits, suggesting membranous nephropathy (Figure 1D).

Treatment with methylprednisolone, plasmapheresis and cyclophosphamide was initiated, with steady improvement in kidney function, proteinuria and resolution of hematuria over the next 3–4 weeks. Serum creatinine decreased from 14 mg/dL (1237.6 μ mol/L) on Day 2 of admission to 2.3 mg/dL (203.32 μ mol/L) on hospital Day 41 when the patient was discharged. In addition, proteinuria decreased from 14 g on admission to 4 g at 3 months.

To address whether anti-PLA₂R antibodies were present in patient's serum and accounted for the membranous lesion, immunoblots were performed on glomerular protein extracts and recombinant PLA₂R using the patient's serum prior to and following plasmapheresis and treatment. High titers of anti-PLA₂R antibodies were detected in the serum obtained at the time of admission and on the plasma removed from the initial plasmapheresis, which

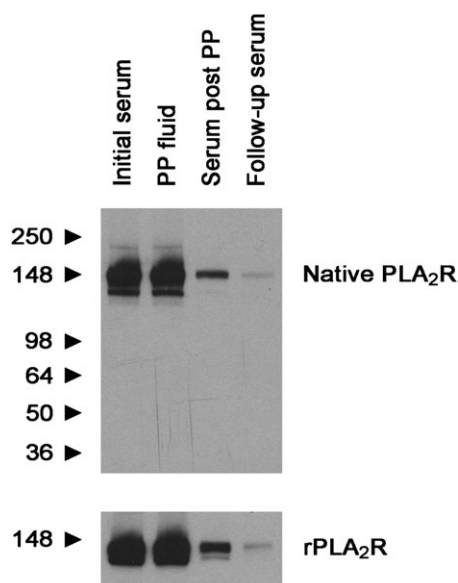


Fig. 2. Presence of anti-PLA₂R antibodies. Protein extracts from human glomeruli (upper panel; native PLA₂R) or recombinant PLA₂R (rPLA₂R; lower panel) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotted with serum from patient as indicated. PP, plasmapheresis.

decreased progressively after plasmapheresis and 4 weeks of treatment (Figure 2). Antibodies to MPO also decreased and became undetectable 3 months after starting treatment.

Discussion

We describe a patient with crescentic GN, rapidly progressive renal failure, and >14 g proteinuria who had both a crescentic GN and IMN. The clinical presentation of marked proteinuria, unusual in ANCA-associated GN, together with acute renal failure with active urinary sediment are key features in this case that suggested more than one underlying renal disease. Our demonstration of relevant antibodies for both diseases provides molecular support for the presence of two independent diseases in this patient. Based on the initial presentation of marked proteinuria, hypoalbuminemia, and relatively preserved renal function 6 months prior to admission, we would propose that the patient initially presented with IMN that arose secondary to anti-PLA₂R antibodies [3]. The subsequent acquisition of anti-MPO antibodies leading to a crescentic GN could account for the development of rapidly progressive renal failure leading to the current hospitalization.

Limited data are currently available to guide treatment for patients with both ANCA-associated GN and IMN. Since ANCA-associated GN most likely contributed to the rapidly progressive decline in glomerular filtration rate in our patient, we initiated plasmapheresis and cyclophosphamide, based on the evidence from the MEPEX trial and the randomized controlled trial by Szpirt *et al.* [4, 5]. While the CYCLOPS trial has shown equal efficacy of intravenous versus oral cyclophosphamide in ANCA-associated GN, albeit with higher relapse in the intravenous group [6], we elected to initiate treatment with daily oral cyclophosphamide due to the severity of the disease and the presence of poor prognostic markers including age, African American race, degree of proteinuria and elevated creatinine at presentation. In addition, oral cyclophosphamide has been shown to be efficacious in IMN [7]. Rituximab, which has shown efficacy in both ANCA-associated GN and IMN, was also considered [8, 9]. However, we elected cyclophosphamide as treatment for this patient due to increased toxicity and cyclophosphamide's proven record in the treatment of a severe disease.

Identification of the pathogenic antibody that mediates disease provides the potential to diagnose the disease non-invasively, monitor the response to treatment and identify patients at risk for relapse. Recently, Beck *et al.* demonstrated that anti-PLA₂R antibodies were present at initial presentation in patients with IMN and nephrotic range proteinuria, absent in many during remission and to recur in those patients who relapse [3, 10]. Consistent with the idea that anti-PLA₂R antibodies may be a good biomarker for disease activity and be useful in assessing response to treatment, we found that titers of anti-PLA₂R antibodies markedly decreased with treatment and clinical response in our patient [11]. The residual proteinuria in this patient despite the virtual disappearance of autoantibody likely reflects persistent structural changes in the glomerular filtration barrier. In contrast, studies addressing the role for following anti-MPO or anti-PR3 antibody titers in ANCA-associated GN to predict response to treatment, to time the switch to maintenance therapy or to predict relapse, have shown an imperfect correlation [12, 13]. This finding, together with the toxicity associated with treatment, has led most investigators to discourage use of making treatment decisions based solely on changes in ANCA titers.

Why would an individual develop two independent pathogenic autoantibodies? ANCA-associated GN has also been

associated with anti-GBM antibodies [14]. While the etiology of this association is unknown, one hypothesis proposed is that damage induced by ANCA leads secondarily to the development of anti-GBM antibodies. Based on a statistical review of their kidney biopsies between 2000 and 2008 at Columbia, Nasr *et al.* [15] have argued that the coexistence of ANCA-associated GN and IMN is likely due to chance alone. This analysis is further supported by recent genome-wide association studies demonstrating distinct genetic predisposition of patients with IMN to develop disease; IMN is associated with single-nucleotide polymorphisms in the genes HLA-DQA1 and PLA₂R1 [16]. Environmental triggers of ANCA-associated GN have been identified and are most closely associated with drugs as well as Gram-positive and Gram-negative infections [1, 2]. We would speculate that patients, such as the one described in our case report, have an underlying genetic susceptibility to develop IMN and subsequently are exposed to an environmental insult leading to ANCA-associated GN. Undoubtedly, future studies will likely identify both environmental triggers of IMN as well as the genetic susceptibility to develop ANCA.

Conclusions

We present a case of a 56-year-old male who was found to have both ANCA-associated GN and IMN on kidney biopsy. To the best of our knowledge, this is the first demonstration of ANCA and anti-PLA₂R antibodies occurring together in the same patient, which is most consistent with the simultaneous occurrence of two independent diseases.

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Conflict of interest statement. L. H. B. Jr and D. J. S. are co-inventors on the patent application 'Diagnostics in Membranous Nephropathy' and have received research support and consulting fees from Questcor Pharmaceuticals, Inc.

(See related article by Ronco and Debiec. Pathophysiological lessons from rare associations of autoimmune diseases. *Clin Kidney J* 2012; 5: 91–93)

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