Membranous Lupus Nephritis and Antineutrophil Cytoplasmic Antibody-associated Vasculitis Overlap Syndrome: A Case Report

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Lupus nephritis (LN) is a classic immune complex-mediated disease. Glomerular immune deposition results in complement activation, cytokine release, cellular proliferation, and in some cases, crescent formation and glomerular necrosis. Antineutrophil cytoplasmic antibodies (ANCA), mainly with the p-ANCA pattern, are detected in approximately 20% of patients with systemic lupus erythematosus (SLE); yet, the clinical significance is unclear [1]. The renal injury in ANCA-associated vasculitis (AAV) is usually typified by pauci-immune necrotizing and crescentic glomerulonephritis, with extensive fibrinoid necrosis in the absence of significant endocapillary proliferation. Recently, several studies have described a subset of patients demonstrating overlap of LN and AAV. Most patients are female, with ANCA positivity primarily directed at anti-MPO antibodies. Of note, in most reports, the predominant LN class on renal biopsy has been class IV-S (segmental) [1]. Class V LN has been reported infrequently.

Our patient presented with an established diagnosis of SLE with acute nephritic syndrome. Renal biopsy showed necrotizing, crescentic glomerulonephritis with no endocapillary proliferation. Immunofluorescence for IgG, IgM, IgA, C3, and C1q was negative. Electron microscopy revealed prominent subepithelial and intramembranous electron dense deposits (EDD) with scant subendothelial EDD. Serology was positive for p-ANCA by indirect immunofluorescence but negative for myeloperoxidase by enzyme-linked immunosorbent assay (ELISA). The findings are consistent with class V LN in conjunction with AAV. In necrotizing crescentic LN, biopsy findings showing no endocapillary proliferation and the virtual absence of subendothelial EDD should lead to a search for ANCA directed at more than the usual MPO and PR3 autoantibodies. Concurrent Class V LN should also be excluded.

PATIENT DESCRIPTION

A 67-year-old woman was admitted to the hospital in June 2019, due to new onset subnephrotic proteinuria and a rise in serum creatinine (SCr). In 2011, she was diagnosed with SLE, initially manifesting as arthralgia, malar rash, hypocomplementemia, and lymphopenia. She tested positive for antinuclear antibodies (ANA) and anti-dsDNA antibodies. Until this hospital admission, she showed no evidence of renal involvement (no hematuria, urine protein consistently < 150 mg/24 hours and stable SCr at 0.8 mg/dl). She had been maintained on daily 200 mg hydroxychloroquine and 5 mg prednisone.

Prior to her hospitalization, the patient did not experience any aggravation of arthralgias or rash. On examination, blood pressure was 180/80 mmHg. Laboratory data showed SCr 1.15 mg/dl (standard range 0.51–0.95 mg/dl), bland urine sediment, and proteinuria 1300 mg/24h. ANA titer was 1:640. Complement levels were low, with C3 49 mg/dl (range 83–193 mg/dl) and C4 9 mg/dl (range 15–57 mg/dl).

An ultrasound-guided kidney biopsy was performed. Light microscopy showed 10 glomeruli, 3 of which were globally sclerosed. Five of the 10 glomeruli showed fibrinoid necrosis with fibrocellular crescents, with only slight mesangial hypercellularity and no endocapillary proliferation [Figure 1A, Figure 1B]. Immunofluorescence was negative for all antibodies tested, including IgG, IgM, IgA, C3, and C1q. Electron microscopy showed extensive podocyte effacement, subepithelial, and intramembranous EDD, some of which were new and others with signs of partial resorption and scant subendothelial EDD [Figure 1C, Figure 1D]. Based on these findings, the renal biopsy was interpreted as membranous (class V) lupus nephritis combined with pauci-immune crescentic glomerulonephritis. Given the diagnosis based on the kidney biopsy, ANCA assay was performed. Indirect immunofluorescence (IIF) using both formaldehyde and ethanol-fixed substrates was positive for p-ANCA at a titer of 1/40. However, enzyme-linked

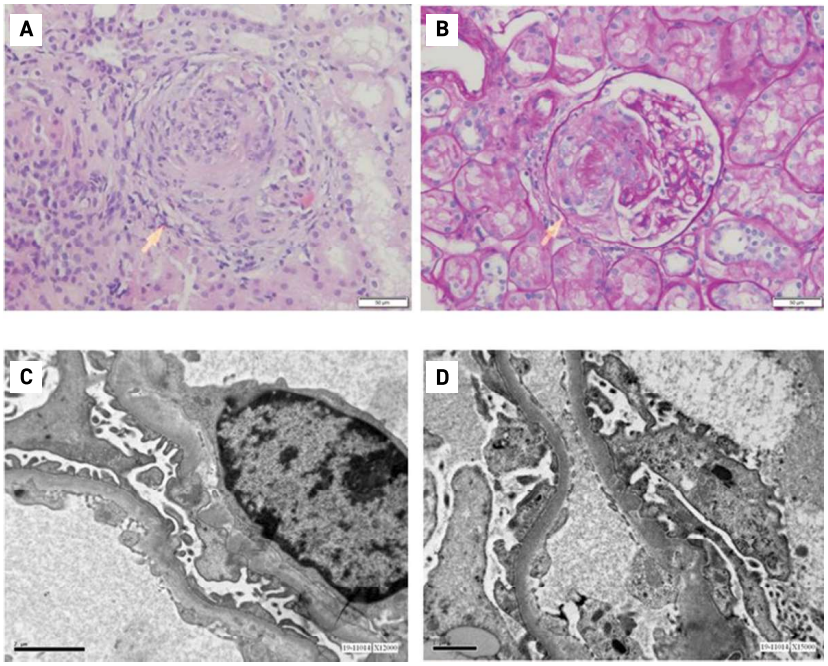
immunosorbent assay (ELISA) was negative for both myeloperoxidase (MPO) and proteinase 3 (PR3) antibodies. Of note, the patient was not taking any medication known to be related to AAV (hydralazine, propylthiouracil, isoniazide). Pulse solumedrol at a dose of 500 mg/day for 3 consecutive days was initiated. This treatment was followed by combined intravenous cyclophosphamide, 500 mg every 4 weeks for 6 sessions and 1 mg/kg prednisone with gradual tapering. The patient was assessed regularly at 4-week intervals during cyclophosphamide treatment. Adherence to prednisone treatment reported by the patient was satisfactory, with no major adverse events from the combined regimen. On this regimen, proteinuria decreased to 150 mg/day, blood pressure stabilized, and SCr decreased to 0.9 mg/dl. One month after the last cyclophosphamide session, she continued on maintenance treatment of 50 mg azathioprine twice daily and 5 mg/day prednisone.

Informed consent was obtained from the patient.

Figure 1. Renal biopsy

[A] Light microscopy showing a glomerulus with a fibrocellular crescent (arrow) within which a focus of fibrinoid necrosis is seen (HEX400). **[B]** Slightly thickened glomerular basement membrane in an otherwise normal structured glomerulus with a fibrous crescent (arrow); (PASX400). **[C,D]** Electron microscopy showing subepithelial electron dense deposits (asterisk)

HE = hematoxylin and eosin, PAS = periodic acid-Schiff



COMMENT

Most patients with SLE-induced renal injury have immune complex-mediated glomerular disease. A complex spectrum of vascular lesions can be seen in SLE patients, including thrombotic microangiopathy (usually in association with anti-phospholipid antibodies), arteriosclerosis, renal vein thrombosis, vascular immune deposits, and true inflammatory vasculitis. Vessel wall immune deposits are the most frequent. They are detected by immunofluorescence and electron microscopy but do not elicit an inflammatory response and do not compromise the vessel lumen. The combination of SLE and AAV is rare. Recently, however, reports of this overlap syndrome are increasing, suggesting that the association between them is more than just a coincidental occurrence of two unrelated diseases. The hallmark biopsy findings in these patients are pauci-immune necrotizing, crescentic glomerulonephritis with minimal or absent endocapillary proliferation, and a scarcity of subendothelial EDD [2].

Approximately 20% of patients with SLE are ANCA positive on IIF, mainly demonstrating a p-ANCA pattern [1]. Yet, this finding is usually without clinical significance, as most patients with SLE and ANCA positivity do not demonstrate clinicopathological features of vasculitis. This confounding effect could partially be explained by a cross reaction with ANA due to an artifact in ethanol fixation. Immunofluorescence assays using both formalin and ethanol-based substrates, as performed with our patient, should address this issue.

Although the target antigen for p-ANCA is most commonly MPO, other antigens may be encountered less frequently, mainly in SLE patients, including lactoferrin (LF), cathepsin G, lysozyme, elastase, bactericidal permeability-increasing protein (BPI), and others [1]. However, ELISA testing is usually directed only at anti-MPO or anti-PR3 antibodies. Jarrot and colleagues [3] documented eight cases of biopsy-proven SLE/AAV overlap syndrome, including a systematic review of 31 reported cases. ANCA positivity by IIF was shown in 30/31 cases, while only 21/26 patients were anti-MPO positive. None were positive for anti PR3. Thus, the prevalence of ANCA positivity in SLE differs between IIF and ELISA. There are conflicting reports on the significance of ANCA positivity in SLE patients. ANCA positivity was found to be more frequent in SLE patients with necrotizing features on kidney biopsy [2]. Although, there is no convincing evidence that it is associated with other histopathologic features of glomerular inflammation

and its prognostic relevance is unclear. A report on patients with biopsy-proven LN with necrotizing glomerular nephritis indicated that the most prominent LN class encountered was IV-S [1]. No direct correlation between ANCA positivity and clinical outcomes was observed; yet, it was suggested that either one of the two conditions (ANCA positivity or LN) may create fertile conditions for the second to develop. However, a recent study [4] found ANCA positivity to be an independent risk-factor for survival among patients with LN. It was also significantly associated with poorer renal function at the time of kidney biopsy and with a shorter time to renal replacement therapy. The percentage of biopsies with crescents did not differ between ANCA-positive and negative patients. Moreover, ANCA-positive patients experienced significantly higher remission rates. Thus, further studies are warranted to address the prognostic significance of ANCA positivity in patients with lupus nephritis.

Similar to our patient, class V (membranous) LN with necrotizing features has occasionally been documented. Nasr et al. [1] described 10 cases of biopsy-proven LN/AAV with necrotizing glomerular nephritis. Although most demonstrated proliferative features on kidney biopsy, three were described as class V (membranous) LN. All patients were ANCA-positive, and ELISA was positive for anti-MPO in all five patients tested. Less common target ANCA antigens were not investigated. The three patients with class V LN had no subendothelial deposits. It has been suggested that overlap syndrome might have a more aggressive course. It appears, however, to respond to vigorous immunosuppressive therapy. Most reports have used a treatment regimen of IV cyclophosphamide and steroids, as with our patient, followed by maintenance therapy with either azathioprine or mofetil mycophenolate.

Our patient presented is an illustrative case who was ANCA positive by IIF but negative for MPO and PR3 by ELISA. Other autoantibodies were not tested.

This case report has a few limitations. First, positive immunofluorescence staining for immunoglobulins, as generally occurs in lupus nephritis, would have been expected. Yet, in a review by Yu et al. [2], clinicopathological characteristics and outcomes in patients with lupus nephritis with and without crescentic glomerulonephritis, were assessed. It was shown that among patients with concurrent crescentic glomerulonephritis, the average intensity of IgG, IgM, IgA, C3, and C1q was lower. It has been suggested that crescentic glomerulonephritis in LN seemed more like a pauci-immune AAV. Cunningham and co-authors [5] reported that in pauci-immune glomerulonephritis, significant cell-mediated immunity develops via activated T cells. This T cell-mediated immune injury could be more important than the antibody immune complex-mediated glomerular injury. Thus, in overlap syndrome, alternate immune pathways may be at play, resulting in the lower intensity or absence of immunofluorescence staining, as seen in our patient. Second, as previously mentioned, immunoglobulins other than anti-MPO and anti-PR3 were not assessed.

CONCLUSIONS

We report an unusual case of overlap syndrome consisting of class V LN and AAV manifested as a pauci-immune necrotizing crescentic glomerulonephritis with positive p-ANCA and negative ELISA for anti-MPO and PR3. In necrotizing crescentic LN, biopsy findings showing no endocapillary proliferation and the virtual absence of subendothelial EDD should lead to a search for ANCA directed at more than the usual MPO and PR3 autoantibodies. It should also lead to a search for subepithelial and intramembranous EDD on electron microscopy suggesting an overlap of necrotizing glomerulonephritis and class V lupus nephritis.

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They know enough who know how to learn.
Henry Adams (1838–1918), American historian and teacher

The mark of the educated man is not in his boast that he has built his mountain of facts and stood on the top of it, but in his admission that there may be other peaks in the same range with men on the top of them, and that, though their views of the landscape may be different from his, they are nonetheless legitimate.
E.J. Pratt (1882–1964), Canadian poet