Seronegative anti-GBM Disease with Coexistent ANCA Positivity

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Abstract

Anti-glomerular basement membrane disease has been reported to coexist with anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis. Seronegative anti-GBM disease has been previously described and mostly blamed for the relative insensitivity of earlier serologic assays.

A 58-year-old male was transferred to our facility for acute kidney injury. Prior to his hospital admission, the patient had a 2 week history of progressive fatigue, fevers, anorexia, vomiting, decreased urine output, sinus congestion, and non-productive cough. His creatinine reached 13 mg/dL.

P-ANCA was positive, anti GBM antibody was negative twice, and urinalysis showed hematuria. Chest x-ray demonstrated diffuse opacities, concerning for pulmonary hemorrhage.

Renal biopsy showed a severe necrotizing and crescentic glomerulonephritis with circumferential crescents. There was bright linear glomerular basement membrane staining with IgG consistent with anti-GBM disease.

Given these findings, the patient was started on oral cyclophosphamide (160 mg daily), in addition to pulse dose methylprednisolone. He was also initiated on therapeutic plasma exchange. Due to worsening renal function, hemodialysis was started.

The patient was discharged from the hospital and completed a course of treatment with cyclophosphamide and prednisone but remains oligo-anuric and hemodialysis dependent at 150 days since presentation.

This case highlights the importance of tissue diagnosis in situations similar to this.

Anti-glomerular basement membrane disease (anti-GBM disease) and anti-neutrophil cytoplasmic antibody (ANCA) vasculitis can coexist. In this setting, patients have a worse prognosis than ANCA isolated conditions, with a clinical course more similar to pure anti-GBM disease.

ANCA vasculitis and anti GBM disease can both present as a pulmonary-renal syndrome. Renal histology frequently demonstrates evidence of a crescentic glomerulonephritis. In ANCA vasculitis, there is negative immunoglobulin and complement staining on immunofluorescence (IF) and a necrotizing and crescentic glomerulonephritis with or without larger vessel vasculitis on light microscopy. There are variable tubulointerstitial findings typically with lymphocytic infiltrates in the interstitium and evidence of acute tubular injury. Anti-GBM disease has similar features on light microscopy, with the addition of positive linear basement membrane staining with IgG in the glomeruli.

Anti-GBM disease is a diagnosis that is made when there is a presence of circulating anti-GBM antibodies in the serum. Goodpasture’s syndrome is more specifically the finding of glomerulonephritis, pulmonary hemorrhage, and anti-GBM antibodies.

Seronegative anti-GBM disease has been previously described and mostly blamed for the relative insensitivity of earlier serologic assays. In these cases, the patients pres-
ent predominantly with pulmonary manifestations. Kidney affectionation is not common.

Here we present a case of seronegative anti-GBM disease with a positive p-ANCA serology. There is paucity of reports of similar cases in the literature.

**Case**

A 58-year-old male was transferred to our facility for acute kidney injury. Prior to hospital admission, the patient reported 2 weeks of progressive fatigue, subjective fevers, anorexia, decreased urine output, sinus congestion, and non-productive cough. He denied skin rash, arthralgias, epistaxis, hemoptysis, or shortness of breath.

His past medical history was significant for type I diabetes mellitus controlled on an insulin pump, hypertension, and dyslipidemia.

At the local hospital, before transfer to our institution, creatinine reached 13 mg/dL (normal 0.8 to 1.3 mg/dL), and urinalysis showed hematuria. Further evaluation showed positive p-ANCA with myeloperoxidase (MPO) antibody titer of 3.5 U. Anti GBM serology was negative.

Upon admission to our institution, labs were obtained as shown in Table 1. Urinalysis showed a 24-hour predicted protein of 2,026 mg, 51 to 100 RBCs/HPF with less than 25% dysmorphic cells, no RBC casts, and 1 to 3 renal epithelial cells. Further serologic evaluation revealed positive p-ANCA with MPO titer of 3 U.

Studies for c-ANCA, anti-proteinase 3 (PR3), anti-GBM, cryoglobulins, complement levels, anti-nuclear antibody, rheumatoid factor, hepatitis A, B, and C, and creatinine kinase were normal.

A chest x-ray showed interstitial opacities in the right lung (Fig. 1).

On hospital day 2, due to worsening renal function, the patient underwent an ultrasound-guided biopsy of the left kidney.

Renal ultrasound revealed bilateral, normal cortical echotexture and thickness with no masses or hydronephrosis. The patient was initiated on intermittent hemodialysis. Due to a high clinical suspicion of glomerulonephritis, treatment with methylprednisolone was initiated.

Renal biopsy showed severe necrotizing and crescentic glomerulonephritis with a generally circumferential nature of the crescents. There was bright linear glomerular basement membrane staining with IgG consistent with anti-GBM disease (Fig 2).

Given these findings and the changes on chest x-ray concerning for pulmonary hemorrhage, the patient was started on 160 mg daily of oral cyclophosphamide, in addition to pulse dose of methylprednisolone. Seven daily plasma exchange sessions were prescribed as well. Anti-GBM serology was repeated and again found to be negative. No episodes of hemoptysis were noted; nonetheless, the hemoglobin trended downward to a nadir of 7 gm/dL on

<table>
<thead>
<tr>
<th>Lab Test</th>
<th>Value</th>
<th>Normal Range</th>
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</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>10.2*</td>
<td>13.5-17.5 g/dL</td>
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<tr>
<td>Leukocytes</td>
<td>10.5</td>
<td>3.5-10.5 x10⁹/L</td>
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<tr>
<td>Platelets</td>
<td>369</td>
<td>150-450 x10⁹/L</td>
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<td>INR</td>
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<tr>
<td>Sodium</td>
<td>139</td>
<td>135-145 mmol/L</td>
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<tr>
<td>Potassium</td>
<td>5.6*</td>
<td>3.6-5.2 mmol/L</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>7.1*</td>
<td>2.5-4.5 mg/dL</td>
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<tr>
<td>Glucose</td>
<td>167*</td>
<td>70-140 mg/dL</td>
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<tr>
<td>Creatinine</td>
<td>13.1*</td>
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<tr>
<td>Erythrocyte sedimentation rate</td>
<td>63*</td>
<td>0-22 mm/h</td>
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<td>Calcium (ionized)</td>
<td>4.36*</td>
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<tr>
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<td>99*</td>
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<td>1.9</td>
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<tr>
<td>Blood urea nitrogen</td>
<td>85*</td>
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<tr>
<td>Albumin</td>
<td>2.5*</td>
<td>3.5-5 g/dL</td>
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<tr>
<td>P-ANCA</td>
<td>Positive*</td>
<td>Negative</td>
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<td>C-ANCA</td>
<td>Negative</td>
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<tr>
<td>Myeloperoxidase Ab</td>
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<tr>
<td>Anti GBM</td>
<td>&lt; 0.2</td>
<td>&lt; 1 U</td>
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</table>

*Abnormal values.
hospital day 7, requiring blood transfusion. CT scan of the chest showed interstitial and ground-glass infiltrates in the right lung. Bronchoscopy was not performed in this case, as the radiological, clinical, and pathological changes were attributed to a pulmonary renal syndrome (Fig. 1).

The patient was discharged from the hospital on day 8. He completed a course of treatment with cyclophosphamide and prednisone but remains oligo-anuric and hemodialysis dependent at 150 days since presentation.

Discussion

The incidence of anti-GBM disease is reported to be approximately one per million. In this disease, there is formation and deposition of antibodies against the alpha-3 chain of type IV collagen along glomerular and alveolar basement membranes.

The combined pulmonary-renal form of the disease is known as Goodpasture’s syndrome. Pulmonary hemorrhage occurs in around 50% of the patients. Also, there is rapidly progressive crescentic glomerulonephritis (RPGN).

About one third of all anti-GBM positive serum samples have detectable ANCA. Most of these patients have anti-MPO specific p-ANCA. On the other hand, 5% of all ANCA-positive serum samples are positive for anti-GBM antibodies. The coexistence of these antibodies can be explained by a general loss of immune tolerance. Another theory is that patients might develop an ANCA-related illness resulting in glomerular damage that would provoke an immune response against the exposed and damaged basement membrane.

The prognosis in mixed ANCA and anti-GBM disease is similar to that of pure anti-GBM disease.

In anti-GBM disease, the likelihood of renal recovery correlates with the serum creatinine and need of dialysis at initial presentation. Very few patients who have advanced
renal disease at presentation recover renal function despite immunosuppression and plasma exchange. In comparison, patients with pure ANCA-related disease (without anti-GBM antibodies) with renal failure recover renal function in more than 70% of the cases, after appropriate immunosuppres-

Cases of isolated pulmonary involvement and seronega-
tive anti-GBM disease were described previously. In renal biopsies, anti GBM antibody deposition was demonstrated. Older studies used indirect immunofluorescent (IIF) and radioimmunoassay (RIA) techniques. These techniques are considered to be less sensitive than currently available en-
zyme linked immunosorbent assays (ELISA) based assays.

Cases in which no circulating anti-GBM antibodies were detectable in serum by well-established enzyme-linked immuno-
sorbent assay or Western blotting techniques have been also described.11

The treatment of anti-GBM disease focuses on a combi-
nation of plasma exchange, cyclophosphamide, and pred-
onsense. Early diagnosis is of paramount importance since recovery of kidney function is rare after dialysis initiation. Plasmapheresis removes circulating anti-GBM antibodies and other mediators of inflammation, such as complement, and the immunosuppressive agents minimize new antibody formation.

The present case is unique for two reasons. First, the negative anti-GBM assays in the setting of new and more sensitive test methodologies in two different institutions is notable. The patient presented with rapidly progressive renal failure requiring dialysis. One would expect high antibody titers in this situation, which in this case were not detected, even before initiation of plasmapheresis.

False negative results may be due to low anti-GBM antibody titers, alterations in the epitopes and structure of the antigens not recognized by ELISA, deposition of large amounts of anti-GBM in the kidney resulting in undetect-
able levels in the serum, disease caused by non-IgG immu-
noglobulins, or alterations in the dissociation rate of the antibodies during the washing period.11 Regardless of the case, it is important to consider that in patients with anti-

Second, given the presence of positive p-ANCA, one could attribute mistakenly the renal disease entirely to a pauci-immune glomerulonephritis. As stated above, the renal prognosis is very different between these two diseases.

In conclusion, a high index of suspicion, rapid referral, and prompt tissue diagnosis are all crucial to diagnosing and treating cases of rapidly progressive glomerulonephritis.

If not for the renal biopsy, this would be a misleading case. Renal biopsy should be performed unless contrain-
dicated due to the variable sensitivity of available assays. Based on this, we suggest that despite negative serological test results, the diagnosis of anti-GBM disease should still be considered in the correct clinical context.

Disclosure Statement

None of the authors have a financial or proprietary interest in the subject matter or materials discussed, including, but not limited to, employment, consultancies, stock ownership, honoraria, and paid expert testimony.

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