CASE REPORT

Myeloperoxidase Antineutrophil Cytoplasmic Antibody (MPO-ANCA) Associated Crescentic and Necrotizing Glomerulonephritis (GN) with Membranoproliferative GN Features

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Abstract

A 77-year-old man presented with a fever, non-productive cough, and edema formation. A laboratory analysis showed an elevated creatinine level (2.5 mg/dL), a high titer of myeloperoxidase (MPO)-antineutrophil cytoplasmic antibody (ANCA) (99 U/mL), positive reaction for antinuclear antibody (×320), hematuria, and massive proteinuria (3.33 g/day). A renal biopsy revealed crescentic and necrotizing glomerulonephritis (GN) with membranoproliferative GN features [double contour appearance of the glomerular basement membrane, granular deposition of immunoglobulin (Ig) G, IgM, and C3 along the capillary wall, subendothelial and subepithelial deposits with mesangial interposition]. A potential relationship between MPO-ANCA associated GN and membranoproliferative GN is discussed.

Key words: antinuclear antibody (ANA), antineutrophil cytoplasmic antibody (ANCA), crescentic glomerulonephritis, membranoproliferative glomerulonephritis, myeloperoxidase (MPO)

(Intern Med 55: 2043-2048, 2016)
(DOI: 10.2169/internalmedicine.55.6426)

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated crescentic glomerulonephritis (GN) is the leading cause of rapidly progressive GN in the elderly worldwide. Extra-capillary proliferation or “crescent” is a consequence of severe intra-glomerular inflammation. This condition is referred to as “pauci-immune” crescentic GN, since the deposition of immunoglobulins or complement components is not typically detected. The coexistence of ANCA-associated crescentic GN with membranoproliferative GN (MPGN) is rare, and information about this clinical condition is quite limited. We herein describe a case of myeloperoxidase (MPO)-ANCA associated crescentic GN associated with massive subendothelial and subepithelial deposits with circumferential mesangial interposition - the typical histological features of MPGN.

Case Report

A 77-year-old man experienced a high nocturnal fever and persistent non-productive cough since early July. Later in the month, he noticed edema in his bilateral lower extremities and macroscopic hematuria. Blood tests conducted by a family practitioner revealed an elevated level of creatinine (2.0 mg/dL), and the patient was referred to our department.

On this admission, his weight was 55.0 kg, and his height was 168 cm. The patient’s body temperature was 38.0°C, blood pressure 114/74 mmHg, and pulse rate 90 beats per minute. A physical examination showed pale conjunctivae, fine crackles in the base of the lungs, and marked pitting edema in the bilateral lower extremities. His heart sounds were normal without an audible murmur. Skin eruptions were absent, and no neurological abnormalities were ob-
A clinical analysis of the blood showed the following: white blood cells, 11,500/μL; hemoglobin, 12.2 g/dL; platelet count, 23.4x10⁴/μL; total protein, 6.1 g/dL; albumin, 1.83 g/dL; blood urea nitrogen, 37 mg/dL; creatinine, 2.5 mg/dL; and C-reactive protein (CRP), 6.96 mg/dL. Liver function was normal. The blood sample was found to be positive for ANA (at 320-fold dilution, showing a homogenous pattern), while anti-double stranded DNA antibody, anti-glomerular basement membrane (GBM) antibody, and cryoglobulin were absent. Viral serology tests for hepatitis B and hepatitis C were negative. Serum levels of IgG, IgA, and IgM were 2,156 mg/dL, 452 mg/dL, and 74 mg/dL, respectively. Serum IgG4 was slightly increased (117 mg/dL, normal range: 4.8-105), but did not meet the criteria of the diagnostic guideline for IgG4-related kidney disease (1). Complement levels were within the normal ranges (C3: 93 mg/dL, normal range: 65-135 mg/dL; C4: 28.3 mg/dL, normal range: 13-35 mg/dL). MPO-ANCA was positive [99 U/mL (ELISA), normal <10 U/mL], but proteinase 3 (PR3)-ANCA was negative. The serum level of KL-6 was 1,521 U/mL. A urinalysis demonstrated proteinuria (3+) and severe microscopic hematuria (>100 red blood cells/high power field) with hyaline, granular, and waxy casts. Protein excretion was 3.33 g/day. Urinary Bence Jones protein was negative. A CT scan revealed a bilateral reticular shadow and honeycomb appearance in the lungs, indicating interstitial pneumonia. The kidneys were nearly normal in shape, and obstruction in the urinary tracts was not apparent. The patient had been a smoker for more than 30 years (smoking approximately 40 cigarettes per day), but had quit smoking 10 years previously. He had no close relatives with renal disease. A kidney biopsy was performed on the 3rd admission day (Fig. 1, light microscopy; Fig. 2, immunofluorescence and electron microscopy, and Fig. 3, IgG subclass analysis). Light microscopy of the biopsy specimen revealed 12 glomeruli, with four showing a cellular crescent, and three of them showing fibrinoid necrosis. Mesangial matrix and cellular proliferation was moderate. Endocapillary proliferation was mild. The capillary loops were broadly thickened. The GBM showed a tram-tracking appearance with subendothelial deposits. Tubular damage with mild lymphocyte infiltration was observed. Fibrinoid necrosis was not found in the arterioles. On immunofluorescence microscopy, granular deposition of IgG, IgM, and C3 was observed in the glomerular capillary loops and partially in the mesangial lesion. Deposition of C1q was not detected. Electron microscopy showed massive, subendothelial electron dense deposits. Subepithelial and paramesangial dense deposits were observed.
Figure 2. Immunofluorescence and electron microscopy. Immunofluorescence (A-C; 200×). IgG (A), IgM (B), and C3 (C) showed granular deposition in the capillary walls. Electron microscopy (D, E). Massive subendothelial (black arrow) and subepithelial (arrowheads) electron dense deposits were observed (D). Circumferential mesangial interposition between the basement membrane was noted (E: black arrows).

Figure 3. Immunofluorescent study for IgG subclass. IgG1 (A) and IgG2 (B) showed strong positivity, whereas IgG3 (C) and IgG4 (D) deposition was faint.
occasionally observed. Podocyte foot process effacement was extensive, and circumferential mesangial interposition was observed. Tubuloreticular inclusions were not detected. An IgG subclass analysis by immunofluorescence revealed strong positivity in IgG1 and IgG2, whereas the deposition of IgG3 and IgG4 was faint (Fig. 3). Though the result of the IgG subclass analysis showed a potential complication of autoimmune disease, this patient did not meet the criteria of an autoimmune disease, such as systemic lupus erythematosus (SLE). The patient was diagnosed with MPO-ANCA associated crescentic GN with MPGN. He received intravenous methylprednisolone (500 mg per day for 3 days) therapy, followed by 40 mg prednisolone (oral administration). Seven days after steroid therapy, the CRP concentration decreased to less than 0.5 mg/dL. The titer of MPO-ANCA had been controlled to less than 10 U/mL, and serum KL-6 level had reduced to approximately 700 U/mL during the follow-up period. The creatinine level had decreased to less than 1 mg/dL. Six months later, urinary protein decreased to less than 0.5 g/g Creatinine, and the edema had improved dramatically. Oral prednisolone was gradually tapered over the next two years. The patient currently receives 7.5 mg of prednisolone daily, and remains free from relapse.

Discussion

We presented a case of MPO-ANCA associated crescentic GN with MPGN features. The coexistence of ANCA-associated GN with MPGN features is an extremely rare condition. To the best of our knowledge, only 9 cases of ANCA-positive GN with MPGN have been reported to date (Table). A majority of these cases had underlying diseases, such as eosinophilic granulomatosis with polyangiitis (EGPA) (2), rheumatoid arthritis (3), carbamazepine (CBZ)-induced autoimmune syndrome (4), shunt infection (5-7), periodic fever syndrome (8), and subacute bacterial endocarditis (9), and displayed features of type 1 MPGN (subendothelial immune deposits). Only one case report described a patient with features of type 3 MPGN (subendothelial and subepithelial deposits) (10). Our patient showed several similarities to this patient [positive ANA, type of ANCA (MPO), presence of interstitial pneumonia, and deposition of IgG, IgM, and C3 in the capillary loop].

MPGN has conventionally been classified into 3 categories according to the location of the dense deposits and the ultrastructural appearance, determined by electron microscopy. Type 1 MPGN is characterized by subendothelial immune deposits, which are considered to be a consequence of circulating immune complex deposition. Type 2 MPGN, or dense deposit disease, is characterized by intramembranous dense deposits, and type 3 by both subendothelial and subepithelial deposits. However, this classification based on morphological findings has several diagnostic limitations, as it does not reflect the pathogenesis of disease per se. Thus, a new classification of MPGN (i.e., “immune-complex-mediated MPGN” and “complement-mediated MPGN”) has been recently proposed (11). Although circulating immune complexes (assessed by a C1q assay) were not detected in this case, strong granular staining of IgG, IgM, and C3 was observed on the immunofluorescence analysis, indicating that this patient had “immune-complex-mediated” MPGN. Chronic viral infection, such as hepatitis C infection, bacterial infections, monoclonal gammopathy, and autoimmune diseases, such as SLE, rheumatoid arthritis (RA), and primary Sjögren’s syndrome (SjS), are known to be associated with “immune-complex-mediated” MPGN. In the present case, viral serology tests for hepatitis B, hepatitis C, and cryoglobulin were negative. Additionally, apparent symptoms or radiographic findings suggestive of chronic infection were not detected, and a urinary test for Bence Jones protein was negative. The high titer of ANA (×320) suggests the possibility of underlying unidentified autoimmune disease.

Whereas an IgG subclass analysis has been eagerly investigated in membranous nephropathy, that of MPGN is quite limited (12, 13). Imai et al. reported predominant IgG3 deposition in primary MPGN (IgG3 was positive in all MPGN cases in their study) (13). Since IgG3 is a strong activator of the complement pathway, they suggested that the subtype of IgG determines the histological feature of glomerulonephritis. However, this study investigated only 7 cases of MPGN. It is plausible to say that the significance of IgG subclass deposition in MPGN remains inconclusive thus far. The clinical importance of the deposition pattern of the IgG subclass in this patient (strong IgG1, IgG2 and faint IgG3, IgG4) remains undetermined. Thus, a careful follow-up is mandatory regarding the future development of malignancy or autoimmune diseases that can induce MPGN.

In fact, immunoglobulin deposition is occasionally observed in ANCA-associated crescentic GN (10, 14). The degree of proteinuria and kidney dysfunction was reported to be more severe in the subset of patients with ANCA-associated crescentic GN accompanied by immune deposits than in those lacking such deposits (14). The renal outcome also tends to be worse in ANCA-associated crescentic GN with immune deposits (14). The clinical feature of this patient is consistent with these reports, since the patient presented with heavy proteinuria (3.33 g/day) and impaired kidney function (creatinine 2.5 mg/dl). Though the titer of MPO-ANCA and level of CRP improved after the initiation of steroid therapy, the creatinine level remained high (1.4 mg/dL).

It is unclear whether immune deposits play a pathogenic role in ANCA-associated GN. It is conceivable that the deposition of immunoglobulins or complement in ANCA-associated GN is a mere coincidence and not a causal relationship. In a rat model of ANCA-associated GN, it was demonstrated that IgG and complement localized transiently along the GBM after immunization with MPO in the early phase, but detection of IgG became impossible after the development of severe intraglomerular inflammation (15). Xiao et al. reported the crucial role of complement in the pathogenesis of MPO-ANCA associated vasculitis using an in
<table>
<thead>
<tr>
<th>Age/gender</th>
<th>Complications</th>
<th>UP (g/day)</th>
<th>CRP (mg/dL)</th>
<th>ANA</th>
<th>MPO-ANCA</th>
<th>PR3-ANCA</th>
<th>HCV-Ab</th>
<th>Cryoglobulin</th>
<th>CIC</th>
<th>LM</th>
<th>IF</th>
<th>EM</th>
<th>Medication</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>62/M</td>
<td>EGPA, alveolar hemorrhage</td>
<td>Cr 2.4 mg/dL</td>
<td>10.4</td>
<td>8.2</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(1:1000)</td>
<td>(-)</td>
<td>(-)</td>
<td>MPGN without crescent</td>
</tr>
<tr>
<td>69/M</td>
<td>Rheumatoid arthritis</td>
<td>Cr 17 mL/min</td>
<td>5</td>
<td>2.9</td>
<td>(-)</td>
<td>(+)</td>
<td>(1:1200)</td>
<td>(+)</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>MPGN with severe nephrosclerosis</td>
<td>subendothelial deposits of IgG, IgM, C3, C1q</td>
</tr>
<tr>
<td>40/M</td>
<td>CBZ-induced autoimmune syndrome</td>
<td>Cr 250 μmol/L</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>(1:1200)</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(1:1000)</td>
<td>(-)</td>
<td>(-)</td>
<td>MPGN with cellular crescents</td>
</tr>
<tr>
<td>50/F</td>
<td>arachnoid cyst, cystoatrial shunt infection</td>
<td>Cr 85 μmol/L</td>
<td>2.1</td>
<td>*</td>
<td>(-)</td>
<td>*</td>
<td>39 IU/mL (&lt;20)</td>
<td>(-)</td>
<td>(+)</td>
<td>(1:1000)</td>
<td>(-)</td>
<td>(-)</td>
<td>MPGN without extracapillary lesions</td>
<td>pseudolinear deposition of IgA, IgM, C3</td>
</tr>
<tr>
<td>62/M</td>
<td>EGPA, alveolar hemorrhage</td>
<td>Cr 0.75 mg/dL</td>
<td>1.4</td>
<td>4.7</td>
<td>&lt;0.01</td>
<td>(-)</td>
<td>44 EU/mL (&lt;10)</td>
<td>(-)</td>
<td>(+)</td>
<td>(1:1000)</td>
<td>(-)</td>
<td>(+)</td>
<td>MPGN</td>
<td>IgG, IgM, C1q, C4 along capillary walls</td>
</tr>
<tr>
<td>55/M</td>
<td>Ventriculoatrial shunt infection</td>
<td>Cr 1.5 mg/dL</td>
<td>5.4</td>
<td>12.4</td>
<td>(-)</td>
<td>(-)</td>
<td>113.0 U/mL</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
<td>(+)</td>
<td>MPGN without extracapillary lesions</td>
<td>granular deposition of IgM, C1q, C3</td>
<td>subendothelial deposits</td>
</tr>
<tr>
<td>36/M</td>
<td>Periodic fever syndrome</td>
<td>Cr 1.71 mg/dL</td>
<td>5.7</td>
<td>1.43</td>
<td>(&lt;0.06)</td>
<td>(-)</td>
<td>44.1 U/mL (&lt;3.5)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>MPGN with fibrocellular crescents</td>
<td>mesangial and capillary wall staining of C3c and C1q, marginal segmental staining of IgG, IgA and IgM</td>
<td>mPSL pulse, oral PSL, CA, rituximab</td>
</tr>
<tr>
<td>68/M</td>
<td>Subacute bacterial endocarditis</td>
<td>Cr 2.16 mg/dL</td>
<td>(3+)</td>
<td>2.62</td>
<td>(&lt;0.06)</td>
<td>1:1000</td>
<td>102 EU (&lt;15)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>MPGN with crescents</td>
<td>granular staining of IgG, IgM, C1q, C3 along capillary walls</td>
<td>subendothelial deposits</td>
</tr>
<tr>
<td>78/M</td>
<td>CGN, interstitial pneumonia</td>
<td>Cr 8.3 mg/dL</td>
<td>3.17</td>
<td>*</td>
<td>(+)</td>
<td>536 EU (&lt;20)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>MPGN type 3 with cellular crescent</td>
<td>granular deposition of IgG, IgM, C3</td>
<td>subendothelial and transmembranous deposits</td>
<td>mPSL pulse, oral PSL, HD</td>
</tr>
<tr>
<td>77/M</td>
<td>Interstitial pneumonia</td>
<td>Cr 2.50 mg/dL</td>
<td>3.33</td>
<td>6.96</td>
<td>(&lt;0.06)</td>
<td>8.2</td>
<td>320</td>
<td>(&lt;10)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>MPGN with cellular and fibrocellular crescents</td>
<td>granular deposition of IgG, IgM, C3 along capillary loops</td>
</tr>
</tbody>
</table>

vivo experimental model (16). It is possible that the deposition of IgG and complement contributes to the initiation and development of inflammatory processes in ANCA-associated GN in the early phase, however, these deposits might become undetectable due to their degradation at the time of the renal biopsy in humans.

Continuous immune complex production as a consequence of chronic infection, autoimmune disease, or monoclonal gammopathy might exacerbate intraglomerular inflammation in ANCA-associated crescentic GN. Zachem et al. demonstrated, using a concanavalin A injection model (immune complex-induced GN model), that after the deposition of immune complexes, platelets adhere to glomerular endothelial cells and are activated to express P-selectin, mediating neutrophil accumulation and a subsequent inflammatory response (17). In the presence of ANCA, the inflammatory reaction triggered by activated neutrophils is intensified (18). Thus, persistent immune complex deposition could synergistically work with ANCA to aggravate intraglomerular inflammation, and this might explain the severity of proteinuria and renal dysfunction seen in ANCA-associated crescentic GN with immune deposits. Due to its rarity, additional experimental and clinical research is needed to clarify the characteristics of ANCA-associated crescentic GN with immune deposits.

We herein described a rare case of MPO-ANCA associated crescentic GN with MPGN accompanied by positivity for ANA and interstitial pneumonia. Continuous immune deposition secondary to underlying autoimmune dysregulation might exist in this case. Immune deposits might exacerbate the proteinuria and kidney dysfunction observed in ANCA-associated GN. A greater accumulation of case studies is necessary to elucidate the features of ANCA-associated GN with immune deposits.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

We thank Takuya Okamura and Mutsuo Jinmai for their technical assistance with the preparation of kidney biopsy samples, including those for immunofluorescence and electron microscopy.

References