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CASE REPORT

A case presenting with the possible relationship between myeloperoxidase–antineutrophil cytoplasmic antibody-associated glomerulonephritis and membranous changes of the glomerular basement membrane

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Abstract A 72-year-old woman exhibited elevated serum myeloperoxidase–antineutrophil cytoplasmic antibody (MPO-ANCA) levels since 2006. Her serum creatinine (sCr) levels increased from 0.5 to 1.62 mg/dl in a stepwise pattern with proteinuria and hematuria up to January 2011. Renal biopsy indicated global sclerosis (14 %), fibrocellular crescents (28 %), and Swiss cheese-like appearance of the glomerular basement membrane (GBM) on light microscopy. IgG4 staining was negative. Immunofluorescent examination indicated granular staining with IgG and C3 along the GBM. MPO-ANCA-associated glomerulonephritis with membranous nephropathy (MN) was diagnosed. As chronic changes were relatively evident in the renal biopsy specimen without acute augmentation of renal function, immunosuppressive therapy was not administered. Thereafter, rapidly progressive renal dysfunction occurred (sCr, 3.67 mg/dl in May 2011) with proteinuria (~2 g/day), hematuria, and elevated serum MPO-ANCA levels. Therefore, a second renal biopsy was performed in May 2011, indicating global sclerosis (42 %) and cellular crescents (35 %) on light microscopy. Electron microscopy indicated electron-dense deposits in the GBM and mesangial lesions. Steroid therapy was subsequently initiated, and the patient's renal function partially improved. MPO-ANCA levels decreased to within normal limits and hematuria disappeared. MPO-ANCA-associated glomerulonephritis with MN is a rare dual glomerulopathy. However, complication should be considered when urinary protein appears in large amounts. Secondary MN was suspected due to the lack of IgG4 staining and distribution of electron-dense deposits to the mesangial lesion. Renal dysfunction occurring in a stepwise pattern may be attributed to intermittent augmentation in MPO-ANCA-associated glomerulonephritis.

Keywords MPO-ANCA-associated glomerulonephritis · Membranous nephropathy · Renal dysfunction in a stepwise pattern

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Introduction

Pauci-immune necrotizing and crescentic glomerulonephritis is characterized by glomerular necrosis and crescent formation in the presence of no more than a paucity of glomerular immune complex deposits. The majority of pauci-immune necrotizing and crescentic glomerulonephritis cases show circulating antineutrophil cytoplasmic antibody (ANCA), whose target antigen is mainly myeloperoxidase (MPO) [1]. MPO-ANCA-associated glomerulonephritis patients typically present with rapidly progressive glomerulonephritis and active urine sediment with red blood cell casts [2]. Although proteinuria is generally detected, massive proteinuria ranging up to nephrotic levels is rare.

On the other hand, membranous nephropathy (MN) is the most common cause of nephrotic syndrome in adults [3]. Pathologically, MN is characterized by the formation of subepithelial immune complex deposits with resultant changes to the glomerular basement membrane (GBM). Approximately 75 % of cases of MN are believed to

represent the primary disease, whereas the remaining 25 % of cases represent secondary forms of MN, most commonly related to systemic lupus erythematosus, infection, malignancy, or drugs [4].

A dual presentation of MPO-ANCA-associated glomerulonephritis with MN is rare [5–11]. We report here a rare case of MPO-ANCA-associated glomerulonephritis with MN suspected to be a secondary form.

Case report

Elevated serum MPO-ANCA levels in a 72-year-old woman were detected by chance in 2006. Because she had no subjective or objective symptoms, she was followed up without immunosuppressive therapy. In 2006, her serum creatinine (sCr) level was 0.5 mg/dl, with urinary protein and urinary occult blood being 2+ and 1+, respectively. Thereafter, sCr level increased in a stepwise pattern (0.88 mg/dl in April 2009, 1.21 mg/dl in August 2009, 1.25 mg/dl in April 2010, and 1.63 mg/dl in August 2010). In January 2011, she was referred to our hospital for evaluation of renal dysfunction (sCr, 1.62 mg/dl), with significant proteinuria (3+) and occult blood (2+) (Fig. 1). At the time of first admission, her blood pressure was 158/70 mm Hg with a regular pulse (83 beats/min). Her body weight was 63 kg and her body temperature was stable (36.4 °C). The physical examination findings were unremarkable. Laboratory data for serum examinations were as follows: hemoglobin, 10.6 g/dl; urea nitrogen, 29.2 mg/dl; sCr, 1.57 mg/dl; C-reactive protein (CRP), 0.04 mg/dl; and MPO-ANCA, 18 U/ml. All the serological examinations, including antinuclear antibody, proteinase-3 ANCA, antiglomerular basement membrane antibody, immunoglobulins, and complements, were within normal limits, with the exception of the elevated MPO-ANCA level. Urine test results were as follows: protein, 3+; daily urinary protein excretion, 1.35 g; occult blood, 1+; urinary sediment of red blood cells, 131/μl; and creatinine clearance, 33.3 ml/min. Renal biopsy was performed in January 2011; light microscopic examination indicated global sclerosis and fibrocellular crescents in 14 and 28 % of glomeruli, respectively. Although collapsed glomerular capillaries were observed, fibrinoid necrosis was not detected (Fig. 2a). A segmental increase in the mesangial matrix was observed in the glomerulus without crescentic formation (Fig. 2b). Tubular atrophy and interstitial fibrotic change were observed in 40 % of tubulointerstitial lesions. In addition, a Swiss cheese-like appearance in the GBM was noted (Fig. 2c, d). IgG4 staining was negative. Immunofluorescence microscopic examination showed granular 2+ staining with immunoglobulin G (IgG) and C3 along the capillary walls and in the mesangial lesions (Fig. 2e). However, other immunoglobulins and complements were not detected. Electron microscopic examination revealed electron-dense deposits in the subepithelial lesions (Fig. 2f). A case of MPO-ANCA-associated glomerulonephritis with MN was diagnosed. Acute histological change including fibrinoid necrosis was not observed, except for fibrocellular crescent in 28 % of glomeruli and chronic changes were relatively evident. The patient's serum creatinine level in the first renal biopsy in January 2011 was similar to the level in August 2010 without acute augmentation of renal function. Systemic inflammation was not so severe, because the CRP level was within normal limits (0.04 mg/dl) and her body temperature was stable (36.4 °C). These observations indicate that the disease activity was not flared in the situation of the first renal biopsy. In addition, she was 77 years old at the time of the first admission to our hospital, and we were afraid that the elderly patient would be a compromised host by the immunosuppressive therapy. Therefore, she was closely followed up without immunosuppressive therapy.

Thereafter, in our outpatient clinic, a rapidly progressive increase in sCr levels occurred (3.67 mg/dl on May 10, 2011) with urinary protein (3+) and occult blood (3+), with concurrently elevated serum MPO-ANCA levels. Therefore, she was admitted to our hospital on May 12, 2011 (Fig. 1). At the time of the second admission, her blood pressure was 158/74 mm Hg with a regular pulse (73 beats/min). Her body weight was slightly decreased (58.4 kg) and her body temperature was stable (36.8 °C). Except for slight conjunctival anemic appearance, the physical examination findings were unremarkable. Laboratory data for serum examinations were as follows: hemoglobin, 9.6 g/dl; urea nitrogen, 53.6 mg/dl; sCr, 4.05 mg/dl; CRP, 0.41 mg/dl; and MPO-ANCA, 38 U/ml. Urine test results were as follows: protein, 3+; daily urinary protein excretion, 2.28 g; occult blood, 3+; urinary sediment of red blood cells, 131/μl; urinary α1-microglobulin, 82.5 mg/l; and creatinine clearance, 9.58 ml/min.

A second renal biopsy was performed on May 13, 2011, which indicated global sclerosis and cellular crescents in 42 and 35 % of glomeruli, respectively. Capillary necrosis with large crescentic formation was extensively spread in almost all of the glomeruli. Therefore, it is difficult to estimate the difference in mesangial change between the first and second renal biopsies. In addition, the Swiss cheese-like appearance was observed in the GBM (Fig. 3a). Widespread tubular atrophy and interstitial fibrotic change was present with diffuse mononuclear cell infiltration, and tubular cells were detached from tubular basement membranes. The findings of vasculitis were not observed. IgG4 staining remained negative. Immunofluorescent microscopic examination showed granular staining for IgG and C3 along the capillary walls, similar to that in

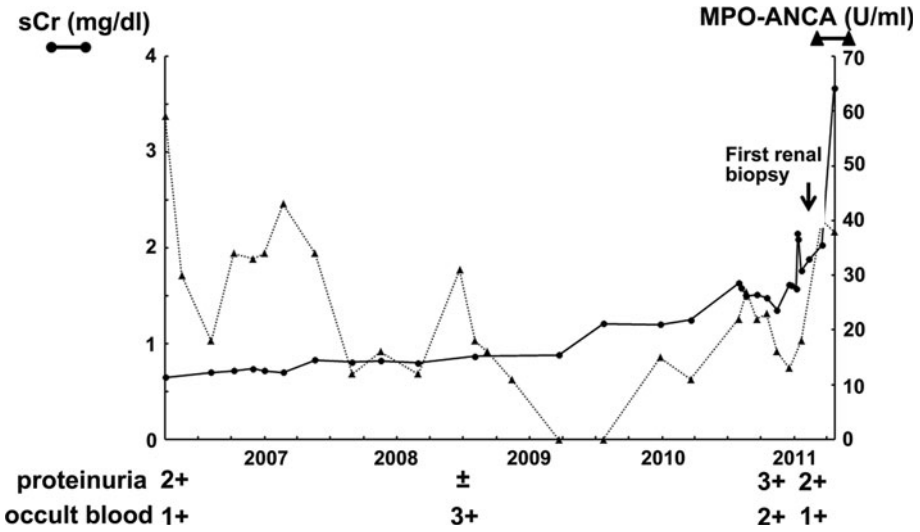

Fig. 1 Time course of disease activity up to the second hospital admission. The *solid line* with *dots* indicates serum creatinine (sCr) levels, and the *dotted line* with *triangles* indicates serum myeloperoxidase–antineutrophil cytoplasmic antibody (MPO-ANCA) levels

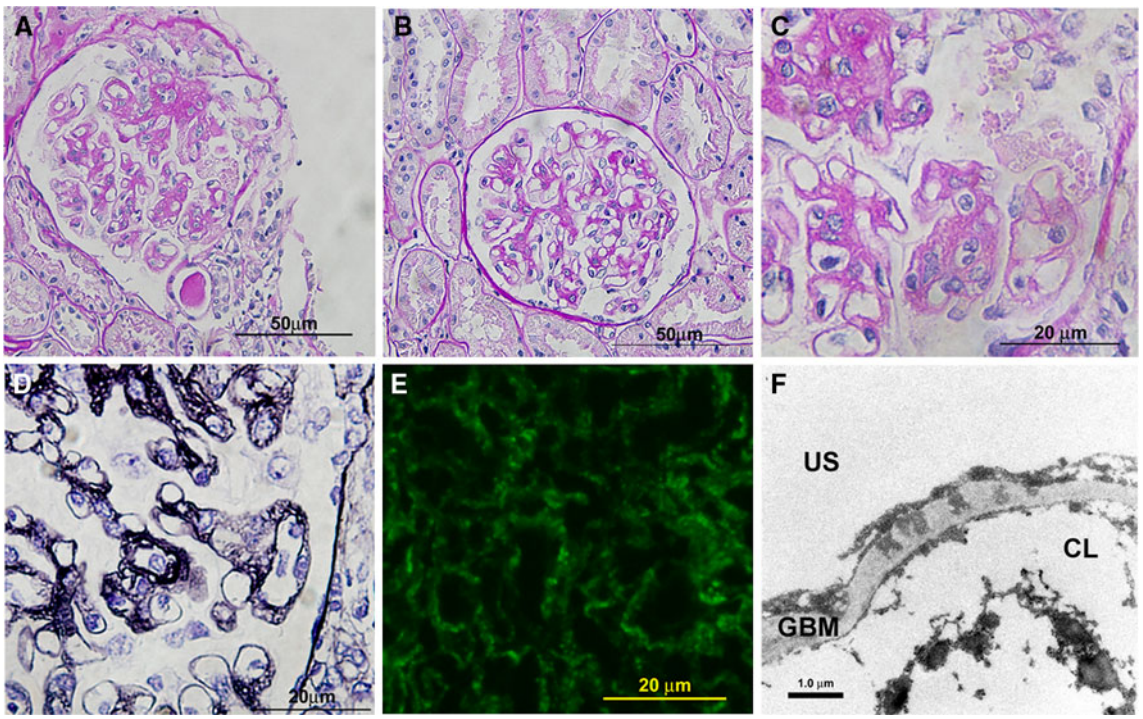

Fig. 2 Light and immunofluorescence microscopic examinations of the first renal biopsy. Light microscopic examination of the first renal biopsy specimen indicated fibrocellular crescents in a quarter of the glomerulus along the Bowman's capsule and collapsed glomerular capillaries (periodic acid-Schiff stain; ×400) (**a**). A segmental increase in the mesangial matrix was observed in the glomerulus without crescentic formation (periodic acid-Schiff stain; ×400) (**b**). A Swiss cheese-like appearance was detected in certain parts of the glomerular basement membrane (periodic acid-Schiff stain; ×1000, **c** and periodic acid silver methenamine stain; ×1000, **d**). Immunofluorescence microscopic examination showed granular 2+ staining for immunoglobulin G (IgG) (×1000) along the capillary walls and in mesangial lesions (**e**). Electron microscopic examination revealed electron-dense deposits mainly in the subepithelial lesions (**f**). *US* urinary space, *GBM* glomerular basement membrane, *CL* capillary lumen

the first renal biopsy. Electron microscopic examination revealed electron-dense deposits mainly in the GBM and slightly in the mesangial lesions. Based on the above findings, MPO-ANCA-associated glomerulonephritis was considered to be activated, and steroid therapy was initiated (500 mg daily of methylprednisolone pulse therapy for 3 consecutive days followed by 40 mg daily of prednisolone). Subsequently, MPO-ANCA levels decreased to within normal limits and hematuria disappeared; however, the patient's renal function remained at a level of partial improvement and daily urinary protein excretion (about 1 g) persisted (Fig. 4).

Discussion

It is known that, in vitro, ANCA can activate primed neutrophils to release lytic enzymes and reactive oxygen species, and damage and lyse endothelial cells [12], and that glomerular necrosis and crescent formation is caused by the presence of no more than a paucity of glomerular immune complex deposits in MPO-ANCA-associated glomerulonephritis [1]. Two mechanisms are suggested in immune complex glomerulonephritis; first, autoantibody deposition from circulation as complexes, and, second, in situ immune complex formation in which antibody reacts with an intrinsic GBM antigen or an exogenous planted antigen. The first mechanism leads mainly to subendothelial or mesangial deposits, which cause diffuse proliferative glomerulonephritis, and the second mechanism leads mainly to subepithelial deposits seen in MN. Because the mechanism of disease onset differs for these clinical entities, the occurrence of a complication involving both MPO-ANCA-associated glomerulonephritis and MN is rare. Thus far, only 6 case reports describing 10 patients of MPO-ANCA-associated glomerulonephritis with MN are available [5–10]. However, Hanamura et al. reported that six (35 %) of the biopsy samples from 17 cases with ANCA-associated glomerulonephritis showed granular deposition of IgG along the glomerular capillary walls. They demonstrated that double immunofluorescence using Alex Fluor 594-labeled anti-MPO antibody and fluorescein isothiocyanate-labeled anti-IgG antibody revealed partial colocalization of MPO and IgG within the GBM and mesangium [13]. These results indicate that, in some cases of MPO-ANCA-associated glomerulonephritis, MPO may form immune complexes and develop MN-like lesions. Recently, Matsumoto et al. [14] speculated that, in a case of MPO-ANCA-associated glomerulonephritis with MN, MPO is the antigen that causes MN in the immune complex, because anti-MPO antibody staining in a diffuse granular pattern was observed along the capillary walls by immunofluorescent examination. Positive IgG4 staining, which indicates selective significance in idiopathic MN [15], was not observed in our case. Electron microscopic examination also indicated electron-dense deposits in the GBM and mesangial lesions. These findings indicated that, in the present case, MN may not have been idiopathic but secondary in nature. It is, therefore, possible that MN was caused by the precedent MPO-ANCA-associated glomerulonephritis in our case. The relationship between MPO and the anti-MPO antibody immune complex is highly interesting in the pathogenesis of MN present in our patient.

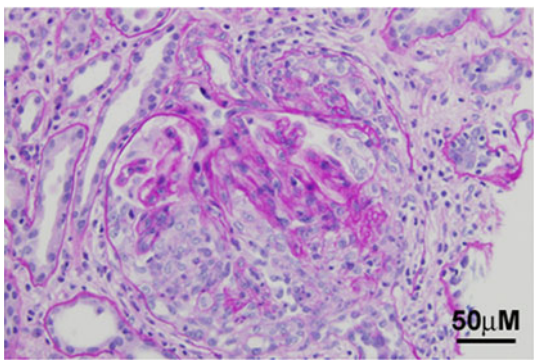

Fig. 3 Light microscopic examination of the second renal biopsy specimen indicated cellular crescents in more than half of the glomeruli. Necrotic changes were observed in the remaining glomerular capillaries, which were further compressed by the cellular crescents (periodic acid-Schiff stain; ×400)

Nasr et al. have reported the clinical features, pathologic findings, and outcomes in 14 patients with MPO-ANCA-associated glomerulonephritis concurrent with MN. They reported that the clinical presentation of patients with MN and ANCA-associated glomerulonephritis is relatively similar to ANCA-associated glomerulonephritis alone, except for the presence of more severe proteinuria, with a mean 24-h urine protein level of 6.5 g [11]. In the present case, the daily urinary protein levels were ∼2 g prior to steroid treatment, which was relatively low as compared to their report. However, the daily urinary protein levels in our case were much higher than the mean daily urinary protein levels of MPO-ANCA-associated glomerulonephritis alone; further, they were similar to those described for MN and ANCA-associated glomerulonephritis together [5, 6, 8, 9].

The MPO-ANCA levels decreased to within normal limits and hematuria disappeared following steroid therapy in our patient. However, the improvement in renal function was partial. The improvement of MPO-ANCA levels and hematuria indicates that renal disease activity was suppressed by steroid therapy. On the other hand, urinary protein excretion shows renal damage by both MPO-ANCA-associated glomerulonephritis and MN. As Cattran et al. [16] reported that it takes a relatively long time for remission of MN by steroid therapy, even though MPO-ANCA-associated glomerulonephritis achieves remission, it is possible that the levels of urinary protein excretion is not remarkably decreased due to MN. Therefore, it is difficult to estimate the renal disease activity using urinary protein excretion. Because global sclerosis in 42 % of glomeruli and widespread tubular atrophy and interstitial fibrotic change were observed before the steroid treatment, there is a high possibility that the improvement in renal function is partial, even though MPO-ANCA-associated glomerulonephritis achieves remission. Therefore, it is also difficult to estimate the renal disease activity using renal function. In addition to the difficulties of disease activity, Nasr et al. [11] reported that 50 % of the patients with

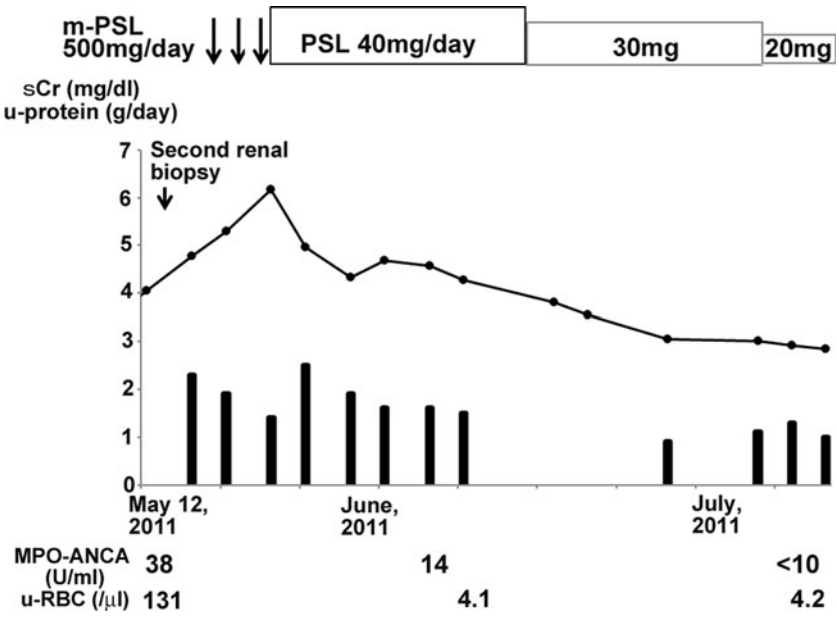

Fig. 4 Time course of disease activity after the second hospital admission. The *solid line* with *dots* indicates serum creatinine (sCr) levels and the *bars* indicate the daily urinary protein excretion. *m-PSL* methylprednisolone, *PSL* prednisolone, *u-protein* urinary protein, *MPO-ANCA* myeloperoxidase–antineutrophil cytoplasmic antibody, *u-RBC* urinary red blood cell

MPO-ANCA-associated glomerulonephritis and MN reached the endpoints of death or end-stage renal failure. We need to provide close follow-up in our outpatient clinic so as not to reach the endpoints of death or end-stage renal failure.

A pattern of stepwise significant declines in renal function was observed from 2006 onwards [between April 2009 (sCr; 0.88 mg/dl) and August 2009 (sCr; 1.21 mg/dl), between April 2010 (sCr; 1.25 mg/dl) and August 2010 (sCr; 1.63 mg/dl), and between January 2011 (sCr; 1.62 mg/dl) and May 2011 (sCr; 3.67 mg/dl)] (Fig. 1). Two serial renal biopsy findings indicated that the activity of MPO-ANCA-associated glomerulonephritis was augmented over time and that the MPO-ANCA-associated glomerulonephritis exacerbated from January 2011 to May 2011, i.e., the percentage of global sclerosis increased from 14 % in the first renal biopsy to 42 % in the second renal biopsy, and although 28 % of glomeruli showed fibrocellular crescents in the first renal biopsy, 35 % of glomeruli showed cellular crescents in the second renal biopsy. Fibrocellular crescents in the first renal biopsy may reflect the augmentation of disease activity between April 2010 and August 2010. The renal dysfunction occurring in a stepwise pattern may possibly be attributed to the intermittent augmentation of disease activity in MPO-ANCA-associated glomerulonephritis.

It is possible that the immunofluorescence staining depends on MPO-ANCA-associated glomerulonephritis alone and not the complication with MN. Certainly, it is reported that many patients have staining for immunoglobulin in pauci-immune crescentic glomerulonephritis [17]. Savage et al. [18] also reported glomerular IgG in 15 % of 20 renal biopsies from patients with microscopic polyangiitis. However, there is an inverse relationship between the amount of staining for immunoglobulin in a specimen with crescentic glomerulonephritis and the frequency of ANCA positivity, and 92 % with no immunofluorescence staining for immunoglobulin were ANCA-positive, 82 % with trace to 1+ staining were ANCA-positive, 23 % with 2+ staining were ANCA-positive, and 8 % with 3+ staining were ANCA-positive [17]. As in other glomerular diseases, areas of glomerular sclerosis have nonspecific irregular staining in MPO-ANCA-associated glomerulonephritis. The staining for IgM is most frequent and it is usually confined to or predominantly in the mesangium [19]. However, although it may be difficult to evaluate the staining intensity quantitatively, we determined the staining intensity of IgG as the 2+ level. The staining for IgG and C3 were globally distributed to capillaries and the mesangium without areas of glomerular sclerosis. In addition, the staining was confined to IgG and C3. These results indicate that this case is MPO-ANCA-associated glomerulonephritis with membranous nephropathy, and not MPO-ANCA-associated glomerulonephritis alone.

In conclusion, we have reported here a rare case of MPO-ANCA-associated glomerulonephritis with MN that was suspected to be secondary in nature rather than idiopathic or coincidental. MPO-ANCA-associated glomerulonephritis complicated with MN should be considered as a diagnosis when urinary proteins are present in large amounts. Renal dysfunction occurring in a stepwise pattern may be attributed to intermittent augmentation in MPO-ANCA-associated glomerulonephritis.

Conflict of interest The authors declare no conflict of interest.

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