Anti-neutrophil Cytoplasmic Antibody-associated Vasculitis
Superimposed on Post-streptococcal Acute Glomerulonephritis

Natsumi Kamijo¹, Akiko Mii¹, Sae Aratani¹, Tetsuya Kashiwagi¹, Takashi Oda², Akira Shimizu³ and Yukinao Sakai¹

Abstract:
A 44-year-old woman was admitted due to gross hematuria and progressive renal dysfunction. Poststreptococcal acute glomerulonephritis (PSAGN) was suspected due to her elevated anti-streptolysin O and anti-streptokinase titers and hypocomplementemia. A renal biopsy showed crescent formation and endocapillary hypercellularity with neutrophil infiltrate. An immunofluorescence analysis showed granular immunoglobulin G and C3 deposition, suggesting immune-complex-type glomerulonephritis. However, myeloperoxidase anti-neutrophil cytoplasmic antibody (ANCA) was positive, and peritubular capillaritis was observed. Furthermore, citrullinated histone H3-positive neutrophils were detected as markers for neutrophil extracellular trap formation. Therefore, she was diagnosed with ANCA-associated vasculitis superimposed on PSAGN that was the main contributor to her progressive renal injury.

Key words: ANCA-associated vasculitis, MPO-ANCA, neutrophil extracellular trap, post-streptococcal acute glomerulonephritis

Introduction
Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is necrotizing vasculitis involving small blood vessels (capillaries and small arterioles). Renal involvement of AAV is characterized by extensive glomerular crescent formation without immunoglobulin and complement deposition, which is classified as pauci-immune glomerulonephritis (1). Clinically, the prognosis is poor in patients with AAV, and the mortality rate is high. Therefore, aggressive immunosuppressive therapy is warranted.

Post-streptococcal acute glomerulonephritis (PSAGN), caused by group A β-hemolytic streptococci, is an infection-related glomerulonephritis (IRGN). This disease typically affects children, but it also affects some adults. Although PSAGN has a good prognosis, rare cases have become severe or progressed to chronic renal insufficiency. A disease concept of streptococcal infection-related nephritis (SIRN), which includes C3 glomerulopathy, membranoproliferative glomerulonephritis, immunoglobulin A (IgA) vasculitis, AAV, and PSAGN, was recently proposed (2). Infection is closely related to the development of various glomerular diseases, and its pathogenesis has further expanded. Therefore, managing this condition can be difficult.

In this case, laboratory test results and glomerular deposition of immune complexes (ICs) suggested the development of PSAGN. However, our patient was also positive for myeloperoxidase (MPO)-ANCA. Furthermore, crescent formation with necrosis, peritubular capillaritis (PTC-itis), and tubulitis were observed in the renal tissue, suggesting AAV superimposed on PSAGN as the primary pathogenesis of progressive renal insufficiency.
Table. Laboratory Data.

| WBC       | 3,000 /μL | Glucose   | 103 mg/dL | Urinalysis |
|-----------|-----------|-----------|-----------|------------|
| Neu       | 58.7 %    | HbA1c     | 5.3 %     | PH         |
| Lymph     | 36.9 %    | CRP       | 3.12 mg/dL| SG         |
| RBC       | 377 ×10^9/μL | IgG        | 1,340 mg/dL| Protein (3+) |
|           |           | IgA       | 152 mg/dL | Occult blood (3+) |
|           |           | IgM       | 351 mg/dL | RBC >100 /HPF |
| Hemoglobin| 8 g/dL    | IgE       | 78 mg/dL  | Red blood cell cast 5-9 /WF |
| Platelet  | 25 ×10^9/μL| C3        | 67 mg/dL  | Poikilocyte (+) |
| AST       | 13 U/L    | C4        | 10 mg/dL  |            |
| ALT       | 7 U/L     | CH50      | 19 U/mL   | β2MG 3,300 μg/L |
| LDL-C     | 104 mg/dL | MPO-ANCA  | 39.4 IU/mL|            |
| TG        | 143 mg/dL | Anti-GBM Ab | <7.0 U/mL |            |
| TP        | 5.4 g/dL  | IC-C1q    | <1.5 μg/mL|            |
| Albumin   | 2.3 g/dL  | ANA       | <×40      | Blood (-) |
| BUN       | 20.5 mg/dL| PR3-ANCA  | <2.0 IU/mL| Urine (-) |
| Cre       | 2.55 mg/dL| MPO-ANCA  | 39.4 IU/mL| Pharyngeal (-) |
| Uric acid | 8.0 mg/dL | ANA       | <7.0 U/mL |            |
| Na        | 137 mEq/L | ASO       | 223 IU/mL |
| K         | 4.5 mEq/L | ASK       | ×5,120    |
| Cl        | 106 mEq/L | HBs Ag    | (-)       |
| Ca        | 7.7 mg/dL | HBC Ab    | (-)       |
|           |           | HIV       | (-)       |
|           |           | TPLA      | (-)       |

WBC: white blood cells, Neu: neutrophils, Lymph: lymphocytes, RBC: red blood cells, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDL-C: LDL-cholesterol, TG: triglyceride, TP: total protein, BUN: blood urea nitrogen, Cre: creatinine, eGFR: estimated glomerular filtration rate, HbA1c: hemoglobin A1C, CRP: C-reactive protein, IgG: Immunoglobulin G, IgA: Immunoglobulin A, IgM: Immunoglobulin M, IgE: Immunoglobulin E, ANA: antinuclear antibody, PR3-ANCA: PR3-antineutrophil cytoplasmic antibody, MPO-ANCA: MPO-antineutrophil cytoplasmic antibody, anti-GBM Ab: anti-glomerular basement membrane antibody, ASO: anti-streptolysin O, ASK: anti-streptokinase, HBs Ag: hepatitis B virus antigen, HBC Ab: hepatitis C antibody, HIV: human immunodeficiency virus, TPLA: treponema pallidum latex agglutination

Case Report

A 44-year-old woman visited her local doctor due to a fever and skin rashes a month prior to her admission to our hospital. At that time, she was not aware of any symptoms of upper respiratory infection. She was prescribed antibiotics. However, her symptoms did not improve. Subsequently, she was referred to our hospital because her serum creatinine level increased from 0.86 mg/dL to 2.55 mg/dL within a week, accompanied by urinary abnormalities. She was diagnosed with rapidly progressive glomerulonephritis (RPGN) and admitted to our hospital.

On admission, the patient’s height, body weight, and body temperature were 158 cm, 56.8 kg, and 36.8°C, respectively. Blood pressure and pulse rates were 104/58 mmHg and 68 beats/min, respectively. A physical examination revealed mild bilateral edema of the lower legs. However, the skin rashes disappeared, and we were unable to detect any eruption. No redness or swelling of the pharynx was observed, either.

In addition to renal dysfunction, the clinical data (Table) showed hypocomplementemia (serum C3 level of 67 mg/dL, reference value: 73-138 mg/dL; serum C4 level of 10 mg/dL, reference value: 11-31 mg/dL; and serum CH50 level of 19 U/mL, reference value: 30-45 U/mL) as well as elevated titters of anti-streptolysin O (ASO) (223 IU/mL, reference value ≤160 IU/mL) and anti-streptokinase (ASK) (×5,120, reference value: ≤×2,560). C-reactive protein was slightly elevated, and MPO-ANCA was positive (39.4 U/mL, reference value <3.5 U/mL). A urinalysis revealed microscopic hematuria, and the urine protein-creatinine ratio was 1.0 g/gCr. Blood, urine, and pharyngeal culture tests were negative for infection. Version 3 of the Birmingham Vasculitis Activity Score at new or worse manifestations was 14/63 points due to the fever and RPGN (3).

Intravenous methylprednisolone pulse (500 mg/day for 3 days) as a treatment of RPGN was started immediately, followed by oral prednisolone (45 mg/day) (Fig. 1).

A renal biopsy was performed on day three after admission. Light microscopy (LM) revealed 19 glomeruli, including 7 exhibiting cellular or fibrocellular crescent formation and 6 comprising endocapillary proliferative lesions (Fig. 2A, B). Necrotic lesions with fibrin deposition were observed in four glomeruli. No sclerotic glomeruli were observed. The interstitium showed PTC-itis and tubulitis with...
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Figure 1. Patient’s clinical course. Methylprednisolone pulse therapy was administered followed by oral prednisolone every other day. Subsequently, intravenous cyclophosphamide therapy was administered, and steroid therapy was intensified. Initially, version 3 of the Birmingham Vasculitis Activity Score (BVAS v.3) at new or worse manifestations (new/worse) was 14/63 points due to the presence of a fever and RPGN. Thereafter, BVAS v.3 (new/worse) improved with treatment. However, BVAS v.3 (persistent) remained unchanged at 3/33 points due to persistent microhematuria. MP: methylprednisolone pulse, PSL: prednisolone, IVCY: intravenous cyclophosphamide, BVAS v.3: version 3 of the Birmingham vasculitis activity score, sCr: serum creatinine, U-P: urinary protein, MPO-ANCA: myeloperoxidase-anti-neutrophil cytoplasmic antibody.

Numerous neutrophils had infiltrated the glomeruli, PTC, and interstitium, presenting as capillaritis (Fig. 2). This neutrophil infiltration was therefore evaluated. In addition to hematoxylin and eosin staining, immunostaining for neutrophil markers (esterase and MPO) and neutrophil extracellular trap (NET) formation marker citrullinated histone H3 (H3 Cit) was performed in serial sections (Fig. 5A-D). H3Cit-positive neutrophils with necrotic lesions were found in the glomeruli, suggesting NET formation (Fig. 5D). Some infiltrating MPO-positive neutrophils were H3Cit-positive cells. These cells mainly infiltrated the necrotic lesions or endocapillary hypercellularity lesions. Furthermore, H3cit-positive neutrophils were also found in PTCs (Fig. 5E, F). Based on these findings, we considered the primary pathogenesis of our case to be AAV superimposed on PSAGN.

Cyclophosphamide was administered intravenously. Subsequently, intravenous methylprednisolone pulse (1 g/day for 3 days) was added because proteinuria and microhematuria persisted, resulting in a gradual improvement in the renal function and urinary findings (Fig. 1). The dosage of the steroids was then tapered.

Discussion

PSAGN was initially considered the primary pathology in this case because the patient had elevated titers of ASO and ASK. In addition, hypocomplementemia was observed, and an immunofluorescence study showed granular deposition of IgG and C3, especially C3 co-dominant, in the glomeruli. These findings suggested PSAGN. However, the glomerular deposition of NAP1r and plasmin activity as biomarkers for PSAGN were not documented. In addition, although small subepithelial deposits were noted, the typical hump-like de-
Figure 2. Renal biopsy findings. Renal biopsy shows cellular and fibrocellular crescent formation and endocapillary hypercellularity with neutrophil infiltration in glomeruli (A: Hematoxylin and Eosin staining, B: periodic acid methenamine silver staining). Peritubular capillaritis, tubulitis, and periglomerular inflammation are also observed (C, D: periodic acid-Schiff staining). A, B, D scale bar, 50 μm; C, scale bar, 100 μm.

Figure 3. Immunofluorescence study findings. Immunofluorescence study for immunoglobulin and complement (A, IgG; B, IgA; C, IgM; D, C1q; E, C3; F, C4). IgG and C3 are positive in glomerular mesangial and peripheral patterns. Scale bar, 50 μm. Ig: immunoglobulin

A few case reports of AAV have suggested involvement of streptococcal infections. However, the exact mechanism involved remains unclear. In addition, MPO-ANCA-positive AAV with streptococcal infection cases have rarely been reported (4). Previously, a case of PSAGN complicated by AAV in a nine-year-old boy was reported (5). He was initially diagnosed with PSAGN. However, his MPO-ANCA
Figure 4. Electron microscopic images. Electron microscopic images (A-C) show small subepithelial and paramesangial electron-dense deposits (arrows in B and C). A, scale bar, 5 μm; B, C, scale bar, 2 μm.

Figure 5. Citrullinated histone H3 (H3Cit)-positive neutrophils in the glomerulus and the interstitium. Many neutrophils infiltrate the glomerulus (A: Hematoxylin and Eosin staining). We perform immunohistochemical staining for neutrophil markers using serial sections. Esterase-and myeloperoxidase (MPO)-positive neutrophils are observed (B: esterase staining, C: MPO staining). Furthermore, citrullinated histone H3 (H3Cit)-positive neutrophils are also detected (D: H3Cit staining). MPO-positive neutrophils and H3Cit-positive neutrophils are also observed in the peritubular capillaries (E: MPO staining, F: H3Cit staining). Scale bar, A-D 50 μm; E, F 20 μm.
titer at the time of the diagnosis was weakly positive, and a kidney biopsy showed extensive crescentic glomeruli. Therefore, it was suggested that streptococcal infection and ANCA may have synergized to induce crescentic glomerulonephritis during the onset, exacerbating glomerulonephritis. In contrast, another case series showed that, among 210 PSAGN patients, 18 (9%) were positive for ANCA, and 4 were MPO-ANCA-positive with a poor prognosis (4). In the case of a 12-year-old boy diagnosed with ANCA-negative microscopic polyangiitis, suggesting involvement of streptococcal infection, detection of glomerular NAPlr and plasmin activity supported the diagnosis (6). There have also been several reports of PSAGN with ANCA-negative vasculitis (7-9).

AAV is commonly characterized by pauci-immune type glomerulonephritis. Recently, some reports have shown IC deposition in the glomeruli (10-13). Haas et al. evaluated 126 diagnosed AAV cases. They reported that 68 (54%) cases exhibited EDDs on EM, suggesting IC deposition (10). Among them, 58 patients (87%) showed immunoglobulin and complement deposition on immunofluorescent (IF) microscopy. In another report, 12 out of 28 (48%) MPO-ANCA-positive AAV patients showed IC deposition on IF and EM (11). Among the 53 AAV patients, 14 (26%) underwent IC deposition (12). IC deposition in AAV is common and has often been associated with infection. In addition, a recent report showed that 40 (41%) of 97 patients diagnosed with MPO-ANCA-associated glomerulonephritis had IC deposits based on a renal biopsy after excluding anti-glomerular basement membrane antibody nephritis, lupus nephritis, IgA nephropathy, drugs, and infections (13).

The mechanism underlying vasculitis, caused by ANCA, involves excessive neutrophil activation, leading to the release of inflammatory cytokines, reactive oxygen species, and lytic enzymes. In addition, excessive neutrophil activation by ANCA induces the formation of NETs (14). NET formation leads to a vicious cycle of ANCA production and inflammation (15). NETs are essential in capturing and killing microorganisms in extracellular spaces by disrupting their cell membrane (16). In NET formation, citrullination of histones is an important step. H3Cit-positive neutrophils were particularly prominent in necrotic AAV lesions. The presence of H3Cit-positive neutrophils was reportedly a disease-specific marker for AAV and its disease activity (17). In the present case, H3Cit-positive neutrophils were observed in the necrotic and endocapillary hypercellularity lesions. However, these neutrophils were not seen in the crescent lesions or segmental sclerotic lesions in the glomeruli. Furthermore, we also observed H3Cit-positive neutrophils in PTCs. Our previous study showed that H3Cit-positive cells were found in approximately 8% of MPO-positive cells in MPA. However, they are rarely seen in PSAGN (17). The presence of H3Cit-positive neutrophils is a specific marker for AAV. There are likely several neutrophil phenotypes, and some of these neutrophils may be associated with NET formation and cause severe vascular damage, such as necrosis or glomerular basement membrane (GBM) rupture.

Recently, Oda et al. proposed the entity of SIRN based on positivity for glomerular NAPlr deposition and plasmin activity in various glomerular diseases, including AAV (2, 18). ANCA-positive granulomatosis with polyangiitis with glomerular NAPlr deposition was also reported (19). In our case, glomerular NAPlr deposition was negative. About four weeks had already passed since the onset of the disease, and the NAPlr deposition may have become negative. Indeed, the localization of glomerular NAPlr in PSAGN patients was reportedly positive in all cases within two weeks after the disease onset and in half of cases at two to four weeks after the disease onset (20). Furthermore, LM showed focal segmental endocapillary hypercellularity, and EM also showed a small subepithelial deposit. These findings suggested the chronic phase of PSAGN. In addition, AAV is believed to have developed superimposed on PSAGN and caused progressive renal insufficiency.

We concluded that AAV was the main contributor to renal injury via neutrophil activation involved in NET formation and may be superimposed on PSAGN. The involvement of PSAGN in the development of MPO-ANCA-positive AAV has rarely been reported. More case studies are needed to further understand the pathogenesis of this disease.

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

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