MPO-ANCA-positive Microscopic Polyangiitis Following COVID-19 Infection

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Abstract:
Antineutrophil cytoplasmic antibody-associated vasculitis (AAV) is a systemic disease that causes vasculitis in various organs. Although the cause of the onset is unknown, infection has been reported to be a causative factor. The subsequent cytokine storm triggered by the immune response against SARS-CoV-2 infection has been reported to lead to symptoms being more severe. We herein report our experience with the onset of AAV following COVID-19 infection. We also report the course of anti-SARS-CoV-2 serum antibody titers following induction therapy, which suggests that vaccination and education concerning standard precautions are necessary in patients who require immunosuppressive therapy, even after COVID-19 infection.

Key words: vasculitis, MPO-ANCA, COVID-19, SARS-CoV-2, rapidly progressive glomerulonephritis

Introduction
Starting with reports of an epidemic outbreak of unexplained pneumonia in December 2019 in Wuhan, Hubei Province, China, coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) became a pandemic. The subsequent cytokine storm triggered by the immune response against SARS-CoV-2 infection has been reported to lead to symptoms being more severe.

Microscopic polyangiitis (MPA), granulomatosis with polyangiitis and eosinophilic granulomatosis with polyangiitis are three diseases classified under antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). AAV is a systemic disease that causes vasculitis in various organs, such as the kidneys, lungs, and nervous system, and although the cause of the onset is unknown, infection has been reported to be a causative factor. In some instances, the diagnosis is difficult, and if the degree of organ disorder is severe, the disease becomes refractory and is associated with a poor prognosis.

We herein report our experience with the onset of myeloperoxidase anti-neutrophil cytoplasmic antibody (MPO-ANCA)-positive MPA following COVID-19 infection. We also report the possibility of the onset of AAV following COVID-19 and the course of anti-SARS-CoV-2 serum antibody titers.

Case Presentation
The patient was a 61-year-old woman with a no significant medical history who was admitted for a fever and malaise. She had been in her usual state of health until three months prior to admission, when a family member of the patient was found to have COVID-19. Having been in close contact, polymerase chain reaction (PCR) testing was performed on the patient, revealing a positive result. She had no symptoms other than pyrexia, which resolved without treatment, and the patient became PCR-negative. The patient developed a fever again two months prior to admission, but a repeat PCR test for COVID-19 was negative. Thereafter, an intermittent fever (maximum temperature exceeding 38 °C) and bilateral gastrocnemius myalgia were observed. She took over-the-counter antipyretics, which were ineffective, and was eventually admitted to a local hospital 11 days prior to admission to our hospital.

Although she had high levels of inflammatory markers

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(C-reactive protein [CRP]: 14.2 mg/dL), an infectious cause was excluded by blood cultures and imaging tests, and empirical antibiotics (tazobactam/piperacillin) were also ineffective. Other blood tests, including for MPO-ANCA, were found to be positive (30.7 IU/mL); thus, the patient was transferred to our hospital for a further evaluation.

On admission, her vital signs were normal except for a mild fever (37.5 °C) and tachycardia (117/min). Her oxygen saturation was normal. She lost 8 kg in 2 months. Other physical examinations, including skin, joints, and neurological examinations, were normal; however, bilateral gastrocnemius myalgia and tenderness were noted.

Laboratory findings on admission are shown in Fig. 1. She had mild leukocytosis with predominance of neutrophils (82.6%), and anemia and hypoalbuminemia with albumin 2.9 g/dL were observed. Her renal function was normal, and there were no electrolyte abnormalities. Serological tests showed the following: CRP 13.6 mg/dL, rheumatoid factor 45 IU/mL, and MPO-ANCA 61.9 U/mL. A urinalysis showed negative protein but positive blood (2+) findings on qualitative tests. She had mild proteinuria of 0.25 g/gCr and positive N-acetyl-beta-D-glucosaminidase (43.1 U/L) and β2-microglobulin (1,094 μg/L) on quantitative tests. She had urinary sediment, red blood cells (RBC) 10-19/high power field, granular casts 5-9/whole field (WF), and RBC casts 1-4/WF. Imaging tests, including whole-trunk computed tomography, showed no active lesions or interstitial changes in the lungs and findings of mild sinusitis; however, a sinus biopsy did not reveal evidence of vasculitis. Unfortunately, we did not perform imaging or a biopsy of the gastrocnemius muscle.

Although she had a normal renal function without significant proteinuria, a detailed urinalysis showed glomerular hematuria with granular and RBC casts and high levels of tubulointerstitial protein; thus, a renal biopsy was performed.

The renal biopsy specimen (25 total glomeruli) revealed 1 glomerulus with global sclerosis and 8 with cellular crescents. Inflammatory cell infiltration mainly consisted of neutrophils and capillary loop necrosis, and immunofluorescence staining was pauci-immune type. Although renal tubulointerstitial damage was mild in severity (≤10%), infiltration of various inflammatory cells, including lymphocytes, neutrophils, and plasma cells, as well as tubulitis, tubular atrophy, peritubular capillaritis, and vascular wall destruction with fibrinoid necrosis in small arteries was noted, leading to a diagnosis of highly active nephritis associated with AAV (Fig. 2).

During detailed examinations over 1 week, her renal function decreased by at least 30%, and RPGN [rapidly progressive glomerulonephritis] was observed; thus, remission induction therapy was initiated with pulse steroid therapy followed by 1 mg/kg prednisolone and intravenous cyclophosphamide, ultimately resulting in remission (Fig. 1). When anti-SARS-CoV-2 serum antibodies were measured (SARS-CoV-2 IgM: SARS-CoV-2 IgM(IB), Fujirebio, [Tokyo], Japan; CLEIA; SARS-CoV-2 IgG: Elecsys Anti- SARS-CoV-2 RUO, Roche Diagnostics, [Tokyo], Japan; ECLIA), the levels were decreased compared with one month earlier, before steroid/immunosuppressant therapy (Table). The fever and gastrocnemius myalgia rapidly disappeared after the start of steroid treatment. Furthermore, the MPO-ANCA titer and CRP level were decreased, and the renal function and urinal abnormalities were improved.

**Discussion**

There has been a report of autoantibody positivity in COVID-19 patients without a history of autoimmune disease (1). In terms of the causal relationship between SARS-CoV-2 and AAV and the possible mechanism, angiotensin-
converting enzyme 2 (ACE2) receptors are known to be involved in the cell invasion of SARS-CoV-2, and since SARS-CoV-2 has a high affinity for ACE2 receptors, invasion into endothelial cells is observed, which reportedly causes vasculitis (2). Following infection, monocyte-derived macrophages and neutrophils are recruited, which further increase the inflammatory response, leading to cytokine storm and causing fibrinoid necrosis, abnormalities in the coagulation/fibrinolytic system, thrombotic microangiopathy, and endothelial cell damage (3). ANCA is produced by cytokines, activated neutrophils, and macrophages, and the induction of vasculitis by neutrophil extracellular traps, which have been said to be the cause of AAV, is assumed (4). The possibility that the virus directly damages renal tissues has also been reported (5-7), although a consensus has not been reached.

Furthermore, regarding the mechanism underlying the course from ANCA production to the onset of necrotizing crescentic glomerulonephritis (NCGN), it takes about 3 days to produce MPO-ANCA in mice with experimental nephritis, and the antibody titer rises even after 13 days. In addition, mice injected with ANCA developed necrotizing glomerulonephritis and deterioration of the renal function after six days (8).

The onset of AAV in the setting of COVID-19 has been reported in 5 patients (9-12). In all of these patients, AAV was diagnosed together with COVID-19, and serious respiratory failure and renal disorder were observed.

In our patient, since ANCA measurements and a urinalysis were not performed at the time of infection, whether or not AAV occurred concurrently is unclear; however, the patient had mild COVID-19 with only pyrexia. After the COVID-19 PCR test became negative, physical findings associated with vasculitis, such as pyrexia and myalgia, became prominent, and necrotizing crescentic nephritis was confirmed in renal tissue along with obvious nephritiform findings. COVID-19 infection cannot be ruled out as a trigger for the onset of AAV. The implication is that highly active AAV may occur after COVID-19 that was observably mild, in addition to the previously described AAV associated with severe infection. As animal models also show ANCA production and the pathogenic mechanism of AAV, the inflammatory response in the body may be prolonged and exacerbated following COVID-19 infection, with activated neutrophils and ANCA produced, MPO-MPA then develops due to symptoms such as a fever, myalgia, and RPGN.

In the previous longitudinal cohort study in healthcare workers (HCWs), the presence of anti-spike antibodies was associated with a substantially reduced risk of PCR-confirmed SARS-CoV-2 infection and the severity thereof over six months of follow-up (13). In the cohort study of working-age HCWs (n=452) who had infected SARS-CoV-2

| Table. SARS-CoV Serology Pre- and Post-immunosuppressive Treatment. |
|---------------------------------------------------------------|
| Prior to treatment (at the time of diagnosis of MPO-MPA) | one month after immuno suppressive treatment | Criteria |
|------------------------------------------------------------|---------------------------------------------|----------|
| SARS-CoV-2 IgM antibody (cutoff index [C.O.I.])             | 1.7                                         | <1.0     |
| SARS-CoV-2 IgG antibody (cutoff index [C.O.I.])             | 20.5                                        | <1.0     |

Figure 2. Light microscopy showing necrotizing glomerulonephritis and tubular interstitial nephritis. (a) Periodic-acid methenamine (PAM) staining, (b) Periodic acid Schiff (PAS) staining. These findings indicated necrotizing crescentic glomerulonephritis, tubulitis and peritubular capillaritis Cellular crescents, inflammatory cell infiltration and capillary loop necrosis are observed. There are infiltration of various inflammatory cells, as well as tubulitis, tubular atrophy and peritubular capillaritis.
treatment, including rituximab, glucocorticoid and other
which was at least partially explained by the underlying
moral response (31%) to the vaccine (mRNA vaccine),
cent report from Israel, AA V was associated with a low hu-
clear (13). This report further supports our proposal for vac-
munosuppressive therapy (three to four months after
COVID-19 infection), with IgG values decreasing by half.
Although another report has described antibodies after
COVID-19 persisting for about six months, even in dialysis
patients who are immunocompromised (15), the levels of
anti-SARS-CoV-2 serum antibodies rapidly decrease first,
and the humoral immunity against SARS-CoV-2 may not
persist for a long time (16), suggesting the need for vaccina-
tion to prevent reinfection and aggravation of COVID-19,
even in patients who have already been infected and in pa-
ients who require steroid immunosuppressive therapy.
Furthermore, the previous study reported that the neutralizing
antibody titer was significantly lower after the administra-
tion of a second dose of vaccine in previously uninfected
patients than the titer after only a single dose of a vaccine in
previously infected participants, although how the neutraliza-
ing antibody titers influence the ability of the host to trans-
mit the virus or to reduce the severity of infection is un-
clear (13). This report further supports our proposal for vac-
cination even after COVID-19 infection.

Regarding the efficacy of vaccination, according to a re-
cent report from Israel, AAV was associated with a low hu-
merosal response (31%) to the vaccine (mRNA vaccine),
which was at least partially explained by the underlying
treatment, including rituximab, glucocorticoid and other
therapies (17). Therefore, we need to educate patients who
receive immunosuppressive medications regarding the stan-
dard precautions to be taken, even after vaccination.

The patient received a third course of IVCY with a re-
duced dose due to leukopenia, and PSL was gradually re-
duced to 7.5 mg/day over 5 months after the diagnosis of
MPO-MPA. The activity of vasculitis remained quiescent
without increasing the ANCA titer. This is not significantly
different from the general course of ANCA-related vasculi-
tis. It is necessary to carefully follow the course of the pa-
tient to determine whether or not immunosuppressants can
be gradually reduced or discontinued and whether or not the
ANCA titer and vasculitis symptoms at the time differ from
cases without COVID-19.

In conclusion, following COVID-19, the onset of highly
active AAV may be observed even in mild COVID-19 cases.
In addition, since anti-SARS-CoV-2 serum antibody levels
gradually decrease even in patients who have already been
infected, it is suggested that vaccination and education con-
cerning standard precautions are necessary in patients who
require immunosuppressive therapy, even after COVID-19
infection.

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

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