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Time-course of serum pro-inflammatory cytokines and chemokines levels observed in granulomatosis with polyangiitis: a case report

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Abstract. A 77-year-old man visited our hospital with chief complaints of difficulty in hearing, nasal discharge and fever. The patients was diagnosed with otitis media, and his fever continued at approximately 38°C despite the administration of clarithromycin. After that, dyspnea on exertion developed and chest X-ray examination indicated multiple infiltrative shadows. PR3-ANCA levels were high (238-fold of the normal levels), and granulomatosis with polyangiitis (GPA) was diagnosed on the basis of clinical symptoms, laboratory results, and pathological findings. Thus, remission induction therapy was initiated with prednisolone and cyclophosphamide, following which symptoms and imaging findings rapidly improved. Blood concentrations of tumor necrosis factor-α, interleukin-8, and growth-related oncogene that had been measured since before treatment decreased over time with the improvement of lesions following treatment. *(Sarcoidosis Vasc Diffuse Lung Dis 2016; 33: 407-412)*

Key words: granulomatosis with polyangiitis, interleukin 8, tumor necrosis factor-α, growth-related oncogene

Introduction

In granulomatosis with polyangiitis (GPA), a complex autoimmune small-vessel vasculitis frequently associated with chronic necrotizing granulomatous inflammation of the upper and lower respiratory tracts. The Chapel Hill classification, which divides vasculitis syndrome into the three categories based on large, medium, and small blood vessels, was revised as CHCC2012. GPA is defined as a disease exhibiting inflammation of small- to medium-sized blood vessels throughout the body and characterized by upper respiratory tract, lower respiratory tract, and renal lesions (1).

Approximately 80%–90% of the cases of GPA go into remission upon treatment initiation, and approximately half of these cases will exhibit subsequent recurrence. Here, we report a case of GPA who evaluated the changes over time in serum pro-inflammatory cytokine and chemokines with the improvement of lesions following treatment.

Case

A 77-year-old man presented with chief complaints of hearing difficulties and fever. His medical history included a diagnosis of pancreatitis at the age

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of 60 years, and his family history was unremarkable. The patient had smoked 20 cigarettes/day for 50 years, but refrained from smoking since the past 10 years.

Nasal discharge developed from late March 2013. His local doctor prescribed him common cold medication; however, his condition did not improve, and on April 4, coughing, sputum discharge, decreased hearing ability, and a slight fever developed. Later, temporary dizziness and a sudden exacerbation of the hearing difficulty developed. His condition did not improve despite the administration of clarithromycin, and an intermittent fever of ≥38˚C continued for 2 weeks. Thus, the patient visited our hospital, because hemosputum and gross hematuria were observed, with multiple nodular shadows with cavitary lesions in both lungs on imaging. On the basis of these findings, granulomatosis with polyangiitis (GPA) was suspected, and the patient was hospitalized.

Findings on admission: height, 164 cm; weight, 50 kg; blood pressure, 127/67 mm Hg; heart rate, 92 bpm, regular; body temperature, 38.1˚C; and SpO2, 93% (room air). Decreased hearing ability was observed on both sides. No obvious rash and no saddle nose were noted. His palpebral conjunctiva was anemic. Respiratory sounds in the lower right lung field were weakened according to chest auscultation. Neither pulmonary nor heart murmurs were observed. There were no abdominal findings of note and no edema in the lower limbs. No finger clubbing was noted.

Laboratory findings on hospitalization included a markedly elevated inflammatory response, with a white blood cell count of 14200/μL, a C-reactive protein (CRP) level of 20.47 mg/dL. Although mild impairment of liver function was observed, kidney function was within the normal range. Urine testing results revealed hematuria without proteinuria. The patient was positive for myeloperoxidase anti-neutrophil cytoplasmic antibody (MPO-ANCA), with a value of 19 IU/mL. Chest X-ray images revealed multiple mass-like shadows (Figure 1A), and computed tomography (CT) images of the chest region showed dispersion of multiple nodular shadows of varying sizes. The larger lesions exhibited cavitary interiors (Figure 1B). CT images of the paranasal cavity revealed findings suggestive of paranasal sinusitis in the right maxillary and ethmoidal sinuses (Figure 1C).

On hospitalization, upper respiratory tract symptoms included nasal discharge, sore throat, and otitis media, and pulmonary symptoms included coughing and bloody sputum. Occult hematuria was present, and the patient was positive for PR3-ANCA. Therefore, systemic GPA was strongly suspected. To enable the establishment of a definitive diagnosis, bronchoscopy was performed on day 3 of hospitalization. Although no clear granulomas were observed on bronchopulmonary biopsy of the upper right and left lobes, inflammatory cell infiltration by mainly neutrophil granulocytes was observed around the blood vessels at capillary level. Marked inflammatory changes were observed in the mucous membrane of the bronchus. Furthermore, on day 4 of hospitalization, analysis of a biopsy from the nasal septum indicated inflammatory cell infiltration, and multinucleated cells accompanied by fibrinoid necrosis were identified around the blood vessels. Thus, GPA was diagnosed and, on day 6 of hospitalization, treatment was initiated with 50 mg/day of prednisolone and 50 mg/day of cyclophosphamide. The fever quickly resolved after treatment initiation, and a reduction of the masses was observed on day 10 of hospitalization. Subsequently, the pulmonary lesions gradually began to disappear, and on day 20 of hospitalization, the prednisolone dose was reduced to 40 mg/day. The patient's prednisolone dose was steadily reduced thereafter. Subjective symptoms (lung, ear, and nasal symptoms), imaging findings, and blood test results improved, and on day 36 of hospitalization, the patient was discharged from hospital and was yet receiving 20 mg/day of prednisolone (Fig. 2).

Changes of serum TNF-α, IL-8, and GRO levels

To clarify how inflammatory cytokines and chemokines contribute to disease pathology, blood TNF-α, IL-8, GRO, and MCP-1 levels were measured over time before treatment with 50 mg/day of prednisolone and 50 mg/day of cyclophosphamide, 7 days after treatment, and 14 days after treatment. As Fig. 3 shows, blood levels of TNF-α, IL-8, and GRO rapidly decreased on treatment, whereas no changes were observed in blood MCP-1 levels.

Discussion

Our patient exhibited multiple cavitary lesions in the lungs. He was diagnosed with systemic GPA

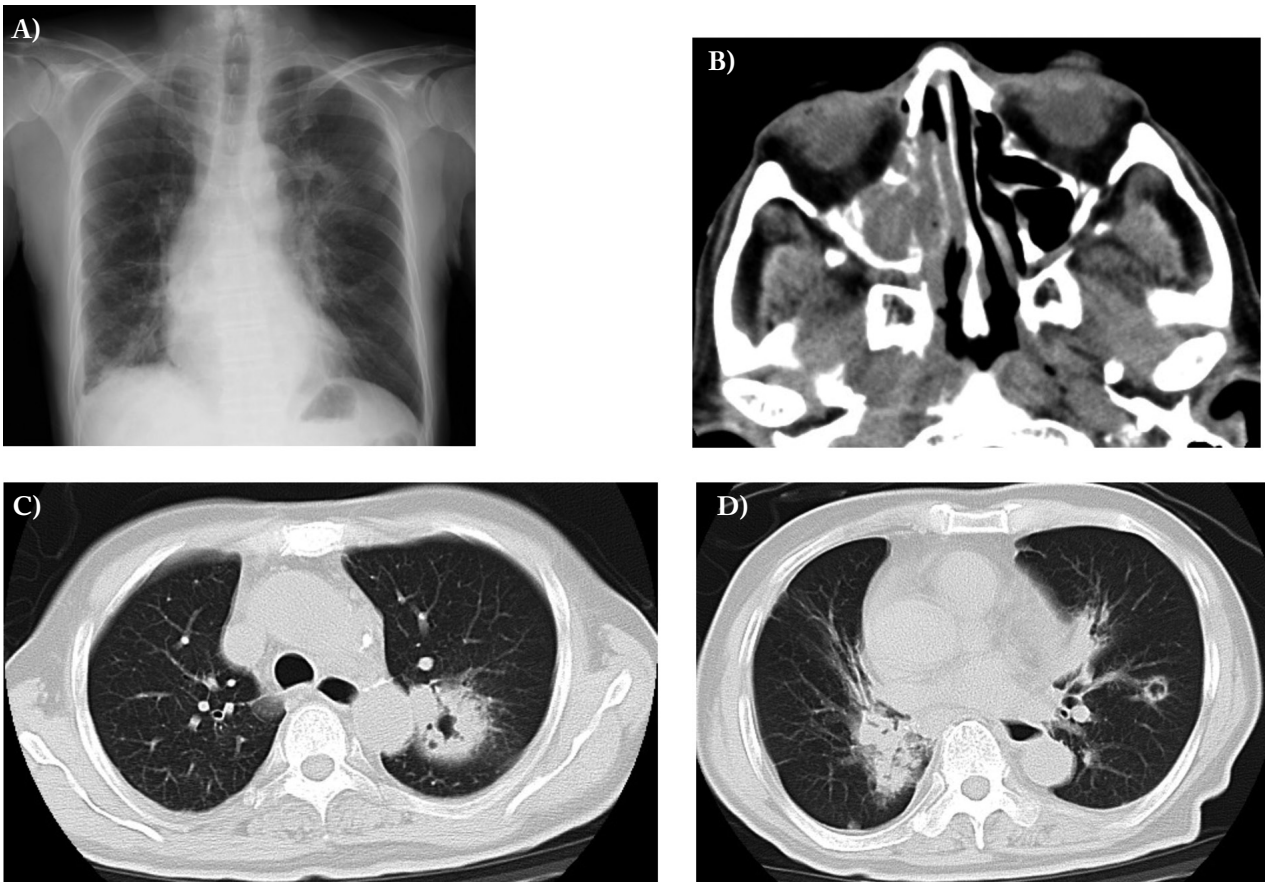

Fig. 1. Radiological findings. Chest X-ray (A), paranasal sinus computed tomography (CT) (B), and chest CT (C, D) and administered at admission

on the basis of high levels of PR3-ANCA, hematuria, multiple cavitary lesions in the lungs, otitis media, hearing difficulties, and renal impairment (2). Although the onset patterns of this disease may greatly vary (3), in similar with our patient, some patients may be detected because of the development of hearing difficulties. Approximately 80%–90% of the cases go into remission upon treatment initiation, and approximately half of these cases will exhibit subsequent recurrence. It has been reported that infections associated with steroids and immunosuppressors and progressing respiratory failure are associated with prognosis (4). Old age and kidney dysfunction are poor-prognosis factors (4).

The vasculitis activity is often evaluated using the Birmingham vasculitis activity score (BVAS) (5) and the degree of organ damage is evaluated using the vasculitis damage index (VDI) (6). In addition, treatment indices for GPA include CRP and PR3-ANCA. Because elevated PR3-ANCA levels often occur before the recurrence of vasculitis, it is recommended that PR3-ANCA levels are monitored during treatment. However, because some cases may not develop recurrence of vasculitis despite elevated PR3-ANCA levels, it is not recommended to intensify treatment on the basis of ANCA elevation alone (7, 8). It has been reported that IL-17 and IL-21 are useful serum markers of GPA activity (9, 10). Moreover, it has been reported that blood concentrations of ENREF_15, CXCL13, MMP-3, and TIMP-1 are useful as markers of GPA activity (11).

In the present case, we continuously measured serum concentrations of GRO, TNF-α, IL-8, and MCP-1 before treatment and at 6 days and 14 days after treatment initiation to reveal any association with the treatment course. In our patient, we

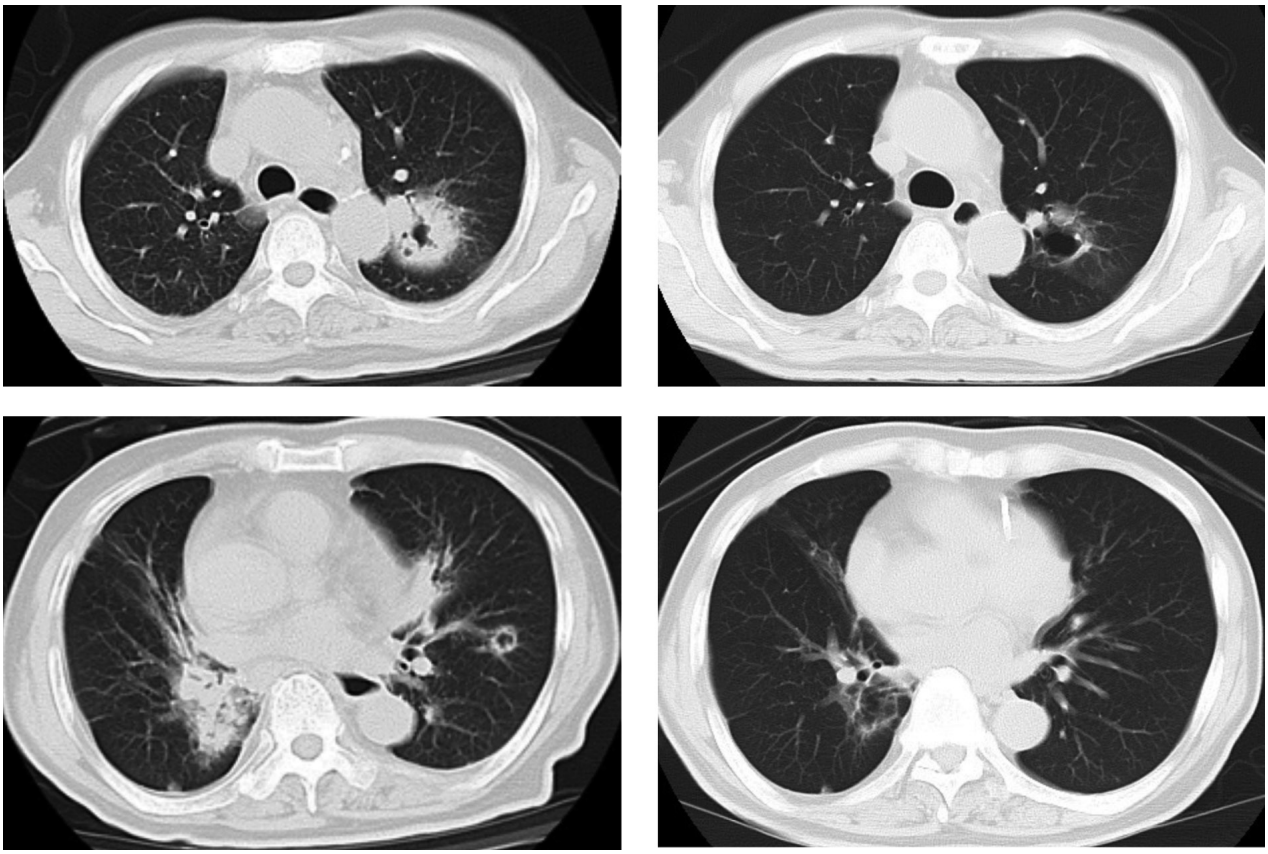

Fig. 1. Clinical course of chest computed tomography (CT) images before and after the treatment to granulomatosis with polyangiitis. Chest CT before the treatment (left upper and left bottom) and at day 21 after the treatment (right upper and right bottom)

observed clear decreases in blood TNF-α, IL-8, and GRO levels after treatment. No previous reports have indicated that serum GRO levels increase with GPA; however, there is a report stating that serum and bronchoalveolar lavage fluid (BALF) levels of neutrophil-related cytokines, including interleukin (IL)-8, granulocyte colony-stimulating factor (G-CSF), and IL-1 beta are increased in GPA patients (11). It has been reported that serum TNF-α levels increase in GPA patients (12, 13). Reports have stated that neutrophils that have been primed with TNF-α express proteinase 3 (PR3 antigen) on the cell surface, with the presence of anti-PR3 antibodies causing neutrophil activation, thereby suggesting that TNF-α is involved in pathophysiology (14). Furthermore, because of several cases of recurrence caused by staphylococcal infection and activation of 13, TLR2, and TLR9 are associated with GPA pathophysiology (15), it has been suggested that neutrophil activation are likely to be involved in the disease pathology. Therefore, because TNF-α, GRO, and IL-8 are typical inflammatory cytokines and chemokines induced by bacterial infection and TLR signal activation, elevation of serum NF-α, GRO, and IL-8 levels may be associated with GPA pathophysiology.

Because at least 3 months are required before the PR-3ANCA test is negative, a biomarker that can appropriately evaluate disease progression requires to be created. As a future prospect for GPA management, Tarzi *et al.* stated that neutrophilic inflammatory cytokine 15 is a useful marker (16). However, no previous reports have observed blood levels of inflammatory cytokines over time. In the present study, we observed serum TNF-α, GRO, and IL-8 levels over time before and after treatment and found that

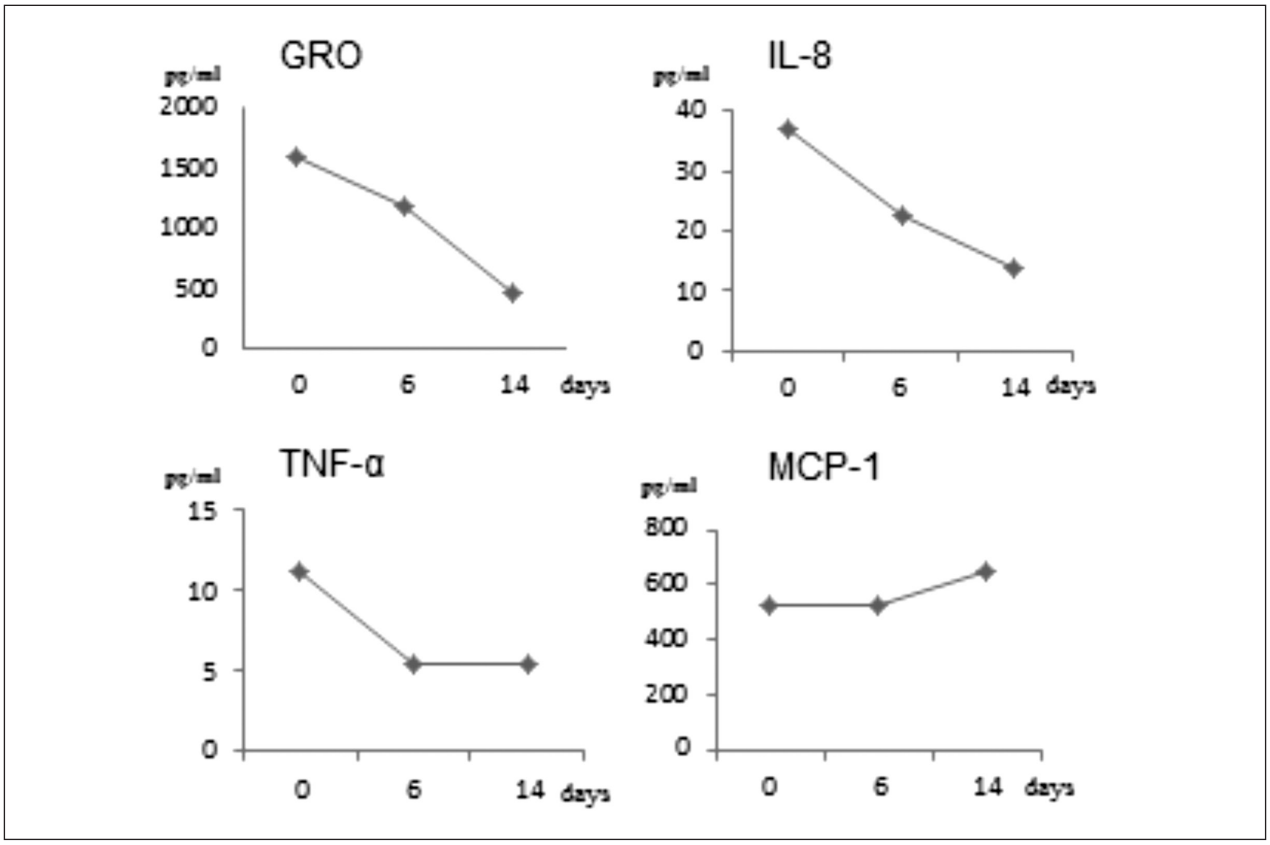

Fig. 3. Time course of serum TNF-α, IL-8, and GRO levels before or after the treatment

these substances rapidly decreased after treatment. Accordingly, our results suggested that cytokines and chemokines associated with neutrophilic inflammation could be measured as blood markers to evaluate disease activity. Studies with large samples of patients should be performed in the future to investigate the efficacy of such markers.

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