Diffuse Alveolar Hemorrhage Associated with Proteinase 3-Specific Anti-Neutrophil Cytoplasmic Antibodies

Masayuki KIKAWADA, Yuichi ICHINOSE, Kazushige MINEMURA, Akira KUNISAWA,
Hiroshi KUSUMOTO, Seiichi OKABE, Makoto YONEMARU, Makoto OGURA*,
Toshiyuki NAKAO*, Yoshiro EBIHARA** and Keisuke TOYAMA

A 31-year-old man was referred to our hospital for the management of progressive diffuse alveolar hemorrhage associated with renal dysfunction. Leukocytoclastic vasculitis was shown by skin biopsy and crescentic glomerulonephritis was also detected, in addition to positivity for proteinase 3-specific anti-neutrophil cytoplasmic antibodies (PR3-ANCA). The patient was diagnosed as a rare case of PR3-ANCA-positive pulmonary-renal vasculitic syndrome without granulomatous lesions. There was a good response to combination therapy with steroids and cyclophosphamide.

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Key words: alveolar hemorrhage, crescentic glomerulonephritis, anti-neutrophil cytoplasmic antibodies, Wegener's granulomatosis, microscopic polyangiitis

Introduction

When diffuse alveolar hemorrhage occurs in association with renal dysfunction, the well-known causes are Goodpasture's syndrome, systemic lupus erythematosus, and immune complex disease (1–3). Recently, it is considered that the most common cause of alveolar hemorrhagic syndrome is microscopic polyarteritis associated with anti-neutrophil cytoplasmic antibodies (ANCA) (4).

ANCA can be divided into two subsets by indirect immunofluorescent microscopy (5): one displays a cytoplasmic staining pattern (c-ANCA) and the other displays a perinuclear pattern (p-ANCA). The major target antigen of p-ANCA is myeloperoxidase (MPO-ANCA), while other antigens include neutrophil granule-associated elastase, lactoferrin, and undefined nuclear antigens. The major target antigen of c-ANCA is a 29 kD serine proteinase known as proteinase 3 (PR3-ANCA) (6). The presence of PR3-ANCA in a patient with pulmonary-renal vasculitic syndrome is suggestive of WG or microscopic polyarteritis. We report a male patient with PR3-ANCA-positive vasculitis who presented with diffuse alveolar hemorrhage, crescentic glomerulonephritis, and other abnormalities.

Case Report

A 31-year-old man developed cough, low-grade fever, and arthralgia on February 5, 1996. He was treated with antibiotics and acetylsalicylic acid for these symptoms at a local hospital. Seven days later, he noticed hemoptysis and congestion of the bilateral bulbar conjunctivae. A chest X-ray film showed alveolar infiltrates in the right lung field. He was diagnosed as having pneumonia and was admitted to hospital on February 13. Despite antibiotic therapy, his symptoms did not improve. Instead, dyspnea and diffuse bilateral alveolar infiltrates developed and he was referred to our hospital for the management of progressive dyspnea. He had a history of sinusitis at the age of 18 years and asthma during infancy.

On admission, his temperature was 37.9°C. He had bilateral congestion of the bulbar conjunctivae, bilateral pallor of the palpebral conjunctivae, and inflammation of the nasal cavity. His blood pressure was 112/50 mmHg and the pulse rate was 72/min (regular). Breath sounds were diminished in the right lower lung field and discontinuous sounds were heard at the bilateral bases. Petechiae were presented on the lower legs. There were no abnormal abdominal or neurological findings.

The abnormal laboratory findings were as follows (Table 1): red blood cell count, $2.61 \times 10^6/\text{mm}^3$; hemoglobin concentration, 6.8 g/dl; platelet count, $40.4 \times 10^4/\text{mm}^3$; total protein, 6.8 g/

From the First Department of Internal Medicine, *the Department of Nephrology and **the Second Department of Pathology, Tokyo Medical College, Tokyo
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Reprint requests should be addressed to Dr. Masayuki Kikawada, the First Department of Internal Medicine, Tokyo Medical College, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160

Alveolar Hemorrhage Associated with PR3-ANCA

Table 1. Laboratory Data on Admission

Hematology		T-cell subsets (peripheral)	
Red blood cell count	261×10 ⁴ /μl	CD4	38.3%
Hemoglobin	6.8 g/dl	CD8	36.9%
Hematocrit	21.5%	CD4/8	1.04
White blood cell count	8,600/μl	Legionella antibody	(-)
Stab neutrophils	5.2%	Mycoplasma (CF)	(-)
Segmented neutrophils	70.0%	Chlamydia (CF)	(-)
Lymphocytes	14.8%	Purified protein derivative	
Eosinophils	5.2%	skin test	0×0 mm
Platelets	40.9×10 ⁴ /μl		
Blood chemistry		Urinalysis	
Aspartate aminotransferase	66 IU/l	Protein	(+)
Alanine aminotransferase	119 IU/l	Sugar	(-)
Total protein	6.8 g/dl	Red blood cell	100/F
Albumin	3.2 g/dl	Granular casts	1-2/F
Alkaline phosphatase	231 IU/l	Arterial blood gas (room air)	
Lactic acid dehydrogenase	437 IU/l	pH	7.464
Choline esterase	0.5 ΔpH	PaCO ₂	40.2 Torr
Total bilirubin	1.0 mg/dl	PaO ₂	74.0 Torr
Blood urea nitrogen	12.4 mg/dl	HCO ₃ ⁻	28.8 mEq/l
Creatinine	0.77 mg/dl	SaO ₂	92.0%
Na	139 mEq/l	Bronchoalveolar	
K	5.1 mEq/l	lavage fluid (BALF)	
Cl	101 mEq/l	Total cell count	3.5×10 ⁵ /ml
Erythrocyte sedimentation rate	135 mm/h	Cell differential	
Serology		macrophages	51.0%
C-reactive protein	15.3 mg/dl	lymphocytes	6.0%
IgG	2,222 mg/dl	neutrophils	20.0%
IgA	144 mg/dl	eosinophils	23.0%
IgM	83.8 mg/dl	CD4/8	0.54
IgE	824 IU/ml		
Rheumatoid factor	(-)		
Complement 3	73 mg/dl		
Complement 4	20 mg/dl		
50% hemolytic unit of complement	38.7 U/dl		
Antinuclear antibody	(-)		
Immunocomplex	(-)		
Lupus anti coagulant	(-)		
Antiglomerular basement			
membrane antibody	(-)		
Myeloperoxidase specific anti-neutrophil			
cytoplasmic antibodies	(-)		
Proteinase 3 specific anti-neutrophil			
cytoplasmic antibodies	300 EU		

PaCO₂: partial pressure of carbon dioxide; PaO₂: partial pressure of oxygen, HCO₃⁻: bicarbonate, SaO₂: saturation of oxygen.

dl; aspartate aminotransferase (AST), 66 U/l; alanine aminotransferase (ALT), 119 U/l; gamma-glutamyl transpeptidase (γ-GTP), 108 U/l; erythrocyte sedimentation rate, 135 mm/h; and C-reactive protein, 15.3 mg/dl. No elevation of viral antibody titers was detected. Urinalysis revealed proteinuria, red blood cells, and white blood cells. Arterial blood gases (on

room air) were as follows: PaO₂, 74.0 Torr; PaCO₂, 40.2 Torr; and pH, 7.46. Rheumatoid factor, antinuclear antibody, the lupus erythematosus test, lupus anticoagulant, and immune complexes were all negative. Complement studies [CH50, C₃, and C₄] gave results within normal limits. Anti-glomerular basement membrane antibody and MPO-ANCA were negative,

Figure 1. Chest X-ray film and chest CT scan on admission showing alveolar infiltrates.

but PR3-ANCA analyzed by enzyme-linked immunosorbent assay (ELISA: nephrocolor C-ANC, EURO-DIAGNOZTICA AB, Malmö, Sweden) was positive (300 EU). The chest X-ray film and chest computed tomography (CT) scan showed alveolar infiltrates, especially in the right lung field (Fig. 1). Bronchoscopy revealed bleeding in almost all of the bronchi. Bronchoalveolar lavage (BAL) was performed (right B⁴) and the bronchoalveolar lavage fluid (BALF) was bloody, containing many hemosiderin-laden macrophages. The total cell count of lavage fluid was $3.5 \times 10^5/\text{ml}$, with 20% neutrophils, 23% eosinophils, and 51% alveolar macrophages. Elevation of the eosinophil ratio in BALF suggested immune complex disease or systemic vasculitis.

At 6 days after admission, the alveolar infiltrates had diffusely extended to involve the bilateral lung fields on chest X-ray films and many granular casts were detected on urinalysis. Steroid pulse therapy was started on day 5 for respiratory failure and was followed by maintenance therapy with prednisolone (60 mg/day) from March 10. At the same time, renal, skin, and nasal mucosal biopsy, and transbronchial lung biopsy (TBLB) were performed.

The number of glomeruli obtained by renal biopsy was 34, and histological examination revealed that 50% of the glomeruli were severely damaged. Crescent formation was detected in 30% of the observed glomeruli and global sclerosis (often with necrotizing lesions) was seen in the remainder. The interstitium was partially infiltrated with lymphocytes and a few plasma cells, and showed fibrotic changes. Deposits of immunoglobulin G, immunoglobulin M, C₃, and C₄ were not detected by immunofluorescence studies. Vasculitis and granulomatous changes also were not detected. The renal findings were consistent with a diagnosis of crescentic glomerulonephritis (Fig. 2).

Figure 2. Renal biopsy showing crescentic glomerulonephritis (HE stain, $\times 400$).

Skin biopsy at a site with petechiae showed small vessel leukocytoclastic vasculitis (Fig. 3), while lung biopsy showed a nonspecific intra-alveolar organizing process without granulomas or vasculitis (Fig. 4). Nasal biopsy showed only nonspecific inflammation and no granulomas or vasculitis were observed.

After steroid therapy for two weeks, his symptoms and alveolar infiltrates improved rapidly. However, proteinuria and granular casts were still found on urinalysis. Cyclophosphamide (100 mg/day) was added to prednisolone on April 1. About one month later, renal biopsy was performed again. The crescents had undergone fibrosis, and there was no active inflammation in the interstitium. In addition, the PR3-ANCA

Figure 3. Skin biopsy at a site with petechiae showing small vessel leukocytoclastic vasculitis (HE stain, $\times 100$).

Figure 4. Lung biopsy showing a nonspecific intra-alveolar organizing process without granulomas or vasculitis (HE stain, $\times 20$).

level fell to within the normal range.

Discussion

WG is generally characterized by necrotizing granulomatous vasculitis of the upper and lower respiratory tract, the kidneys, and other organs (7). The present patient had alveolar hemorrhage, crescentic glomerulonephritis, skin vasculitis, conjunctival congestion, and nasal inflammation in addition to PR3-ANCA positivity. These findings were highly suggestive of WG, but the final diagnosis must be based on the histological findings obtained by examination of biopsy specimens. No granulomas were found on biopsy and no nodular changes (suggestive of granulomas) were seen on X-ray films or CT scans despite a whole body survey. The leukocytoclastic vasculitis detected by skin biopsy was not enough for a diagnosis of WG. It is of course possible that the small amount of lung tissue

obtained or the presence of hemorrhage prevented the detection of granulomas (8), but biopsy material from the skin, upper respiratory tract, or kidney is usually enough to make a final diagnosis. On the other hand, Hoffman et al reported that 8% of WG patients were not diagnosed correctly for a very long period (5–16 years) (9), thus long-term follow-up of this case is important.

Since Davies et al (10) detected ANCA in a few patients with segmental necrotizing glomerulonephritis in 1982, these antibodies have been reported in many cases of systemic vasculitis. Because PR3-ANCA are frequently recognized in patients with WG (11), these antibodies are generally considered to be a sensitive and specific marker for this disease. It has also been reported that a few WG patients present with pulmonary hemorrhage as the initial manifestation (12–14). The specificity of PR3-ANCA for active WG was initially considered to be more than 90% (15). However, Kallenberg et al (16) subsequently reported that PR3-ANCA was far from specific for WG and there have been some cases of non-WG vasculitis with PR3-ANCA positivity.

Most systemic vasculitides are not well known or completely understood with regard to etiology and pathogenesis, so their classification is based on a combination of clinical and pathological findings. The nomenclature and classification of systemic vasculitis that is universally acceptable has not been established yet. The American College of Rheumatology (ACR) described criteria for the classification of vasculitis in 1990 (17), and concluded that criteria based on clinical findings which identify the disease and distinguish it from others are not appropriate to use in the diagnosis of individual patient. The Chapel Hill Consensus Conference attempted to classify systemic vasculitis based on the affected vessels (18). The conference also defined a new disease, termed as microscopic polyangiitis, in which necrotizing small vessel vasculitis affects the arterioles, venules, and capillaries. Since it may be difficult to differentiate whether an ANCA-associated pulmonary-renal vasculitic syndrome is caused by WG or microscopic polyangiitis due to the overlapping features of these diseases, the diagnostic terms used for vasculitis will change over time in some patients. The present case is considered to be suggestive of WG or microscopic polyangiitis with respiratory tract involvement according to the Chapel Hill Consensus Conference criteria, and may most likely be WG due to the lack of necrotizing small vessel vasculitis in the arterioles, in addition to positivity for PR3-ANCA. However, since Lie has doubt for a definitive term of “microscopic polyangiitis” as a clinicopathologic entity (19), it is expected that more precise criteria for classifying systemic vasculitis will be established.

Since combined therapy with steroids and cyclophosphamide was introduced, the prognosis of vasculitis has improved markedly (20). It is important to start treatment as soon as possible before irreversible organ damage occurs. If the treatment of alveolar hemorrhage is delayed or not performed, the patient's condition will become progressively worse. In some cases, a diagnosis of vasculitis is only made at autopsy (21). At present, ANCA provides useful information for the early diag-

nosis and treatment of pulmonary-renal vasculitic syndrome.

In summary, we have described a PR3-ANCA-positive patient whose initial manifestations were diffuse alveolar hemorrhage and crescentic glomerulonephritis. He showed a good response to combination therapy with steroids and cyclophosphamide.

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