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Appendix

Chair:
- Shoichi Ozaki, Division of Rheumatology and Allergy, Department of Internal Medicine, St. Marianna University School of Medicine

Members:
- Motomi Ando, Department of Cardiovascular Surgery, Fujita Health University
- Mitsuaki Isobe, Department of Cardiovascular Medicine, Tokyo Medical and Dental University
- Shigeto Kobayashi, Department of Internal Medicine, Juntendo University Koshigaya Hospital
- Naofumi Matsunaga, Department of Radiology, Yamaguchi University School of Medicine
- Tetsuro Miyata, Division of Vascular Surgery, Department of Surgery, Graduate School of Medicine, The University of Tokyo
- Kimimasa Nakabayashi, First Department of Internal Medicine, Kyorin University School of Medicine
- Yasuo Nakajima, Department of Radiology, St. Marianna University School of Medicine
- Masato Nose, Department of Pathogenomics, Ehime University Graduate School of Medicine
- Takashi Ohta, Department of Vascular Surgery, Aichi Medical University
- Hiroshi Shigematsu, Department of Surgery II, Tokyo Medical University
- Katsuo Sueishi, Fukuoka Higashi Medical Center
- Kazuo Tanemoto, Department of Cardiovascular Surgery, Kawasaki Medical School
- Akitoshi Yoshida, Department of Ophthalmology, Asahikawa Medical University
- Masaharu Yoshida, Renal Unit of Internal Medicine, Hachioji Medical Center, Tokyo Medical University
- Chikao Yutani, Department of Life Science, Okayama University of Science

Collaborators:
- Yoshihiro Arimura, First Department of Internal Medicine, Kyorin University School of Medicine
- Shusaku Fukaya, Division of Infectious Diseases and Rheumatology, Department of Internal Medicine, Fujita Health University
- Shingo Hamaguchi, Department of Radiology, St. Marianna University School of Medicine
- Hiroshi Hashimoto, Juntendo University Koshigaya Hospital
- Keiju Hiromura, Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine
- Akihiro Ishizu, Faculty of Health Sciences, Hokkaido University
- Takehisa Iwai, Tsukuba Vascular Center, Buerger Diseases Research Institute
- Kazunari Kaneko, Department of Pediatrics, Kansai Medical University Hirakata Hospital
- Hiroshi Kataoka, Department of Medicine II, Hokkaido University Graduate School of Medicine
- Seiji Kawana, Department of Dermatology, Nippon Medical School
- Issei Kida, Department of Internal Medicine, Juntendo University Koshigaya Hospital
- Yasuyuki Kobayashi, Department of Radiology, St. Marianna University School of Medicine
- Kimihiro Komori, Department of Vascular Surgery, Nagoya University Graduate School of Medicine
- Hisao Masaki, Department of Cardiovascular Surgery, Kawasaki Medical School
- Tsuneo Matsumoto, Department of Diagnostic Imaging, National Hospital Organization,Yamaguchi-Ube Medical Center
- Taiji Nagaoka, Department of Ophthalmology, Asahikawa Medical University
- Kohei Nagasawa, Department of Internal Medicine, Saga University Faculty of Medicine
- Yoshihisa Nojima, Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine
- Munemasa Okada, Department of Radiology, Yamaguchi University School of Medicine
- Takahiro Okazaki, Division of Rheumatology and Allergy, Department of Internal Medicine, St. Marianna University School of Medicine
- Ichiro Sakamoto, Department of Radiological Sciences, Graduate School of Biomedical Sciences, Nagasaki University
- Kunihiro Shigematsu, Division of Vascular Surgery, Department of Surgery, The University of Tokyo
- Norihiko Shiiya, First Department of Surgery, Hamamatsu University School of Medicine
- Atsushi Takahashi, Department of Ophthalmology, Asahikawa Medical University
- Hajime Takizawa, Forth Department of Internal Medicine, Teikyo University School of Medicine University Hospital
- Hidehiro Yamada, Division of Rheumatology and Allergy, Department of Internal Medicine, St. Marianna University School of Medicine
- Shunji Yoshida, Division of Infectious Diseases and Rheumatology, Department of Internal Medicine, Fujita Health University

Independent Assessment Committee:
- Tsuguya Fukui, St. Luke's International Hospital
- Minoru Horie, Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science
- Takao Koike, Second Department of Internal Medicine, Hokkaido University Graduate School of Medicine
- Shunichi Kumagai, Clinical Pathology and Immunology, Departments of Biomedical Informatics, Kobe University Graduate School of Medicine
- Tadahiro Sasajima, First Department of Surgery, Asahikawa Medical University

(The affiliations of the members are as of September 2010)

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ORIGINAL ARTICLE

Anti-glomerular basement membrane (anti-GBM) disease accompanied by vasculitis that was not positive for antineutrophil cytoplasmic antibodies to myeloperoxidase and proteinase 3: a report of two cases and the incidence of anti-GBM disease at one institution

Kimimasa Nakabayashi · Yasunori Fujioka · Yoshihiro Arimura · Toshihito Fukuoka · Tomohumi Marumo · Michiru Umino · Yasushi Kamiya · Takahiro Okai · Shigeru Tsurumaki · Toshihiko Nagasawa · Akira Yamada

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Abstract
Background Anti-glomerular basement membrane (anti-GBM) disease is thought to be distinct from vasculitis. In contrast, there have been several papers suggesting the presence of angiitis in cases that were positive for anti-GBM antibody (Ab), as well as for either myeloperoxidase (MPO)- or proteinase 3 (PR3)-anti-neutrophil cytoplasmic antibody (ANCA) (Group I). We experienced four patients who had anti-GBM Abs, but not MPO- and PR3-ANCA (Group II), and two of these patients were found to have vasculitis. Therefore, we performed an in-depth study on these two patients.
Methods The patients with anti-GBM disease were isolated from 578 cases whose renal tissues were examined, and they were categorized into two groups. We have already published the data about Group I. We then proceeded to study two vasculitic patients in Group II clinically, pathologically, and serologically. The anti-GBM Ab and ANCA levels were detected by enzyme-linked immunosorbent assays. Renal specimens were studied by routine staining as well as immunohistochemical investigations of CD31 and type IV collagen.
Results The total number of patients with anti-GBM disease was 7 (7/578 = 1.2%), with 3 patients belonging to Group I and 4 patients belonging to Group II. Two patients in Group II were diagnosed to have vasculitis, but the remaining 2 patients did not. One vasculitic patient was complicated by pulmonary hemorrhage, while the other vasculitic patient displayed peripheral neuropathy as well as a small cavity lesion in the lung. The latter patient was found to be positive for perinuclear (p)-ANCA, but not for any other ANCA subsets. The renal pathology in the two vasculitic patients showed crescentic glomerulonephritis (CSGN) and immunoglobulin (Ig) G linear deposits along the glomerular capillary loops. The former patient showed fibrinoid angiitis in an afferent arteriole as well as peritubular capillaritis. The latter patient demonstrated peritubular capillaritis. These peritubular capillaritides were diagnosed by the loss of CD31 and type IV collagen staining, the blurred appearance of peritubular capillary walls by periodic acid-Schiff staining, and the pericapillary infiltration of inflammatory cells.
Conclusion The incidence of anti-GBM disease was very low, and our patients were categorized into two groups (Groups I and II) based on whether or not they were positive for MPO- or PR3-ANCA. Two patients in Group II were found to have vasculitis. According to our results, we concluded that the anti-GBM disease of Group II could also be associated with vasculitis.

Keywords Anti-GBM disease · Goodpasture's syndrome · ANCA · Peritubular capillaritis · Vasculitis

K. Nakabayashi (✉) · Y. Arimura · T. Fukuoka · T. Marumo · Y. Kamiya · T. Nagasawa · A. Yamada
First Department of Internal Medicine,
Kyorin University School of Medicine,
6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan
e-mail: kiminaka@krd.biglobe.ne.jp

Y. Fujioka · M. Umino
Department of Pathology,
Kyorin University School of Medicine,
6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan

T. Okai
Department of Internal Medicine, Kawakita General Hospital,
1-7-3 Asagayakita, Suginami, Tokyo 166-8588, Japan

S. Tsurumaki
Division of Immunoserology in SRL Inc., 51 Komiya, Hachioji,
Tokyo 192-8535, Japan

Introduction

In 1919, Ernest Goodpasture [1] reported an autopsy case that showed rapidly progressive glomerulonephritis (RPGN) and pulmonary hemorrhage. This autopsy demonstrated crescentic glomerulonephritis (CSGN) and alveolar hemorrhage in the lungs. Later, RPGN associated with pulmonary hemorrhage was designated as Goodpasture's syndrome [2]. This syndrome was found to occur due to the development of an antibody (Ab) to the non-collagenous α3 NC1 domain of the basement membrane belonging to the lung or glomerulus [3]. This antibody is called anti-glomerular basement membrane (GBM) Ab and it binds to GBM in a linear fashion. Cases of RPGN arising due to anti-GBM Ab but without pulmonary hemorrhage have also been reported in the literature, and these cases were diagnosed as either "anti-GBM glomerulonephritis (GN) or anti-GBM disease, thereby differentiating them from Goodpasture's syndrome [4]. In addition, cases of anti-GBM disease, which are accompanied by either myeloperoxidase (MPO)- or proteinase 3 (PR3)-anti-neutrophil cytoplasmic antibody (ANCA), have previously been described in various journals over the past 20 years including our studies [5–14]. The cases with dual antibodies are different from the cases of Goodpasture's syndrome as well as anti-GBM disease with regard to the clinical manifestations of vasculitis, patient prognosis, age of the patients, and the response to treatment [6–8, 14–16]. At our institution, we experienced four patients who showed clinical features compatible with anti-GBM disease, but lacking positivity for MPO-ANCA and PR3-ANCA. Two of them demonstrated vasculitis in renal specimens. Therefore, we surveyed the incidence of anti-GBM disease at our institution, and proceeded to perform in-depth studies of the clinical, serological, and renal pathological findings in these two patients. Here we describe their characteristics and discuss the renal pathologies even though these patients were diagnosed with anti-GBM disease.

Materials and methods

Between 1998 and 2007, 555 biopsies and 23 autopsies for renal tissues were performed and examined at our institution. Only 7 anti-GBM Ab-positive cases were found, and one additional anti-GBM Ab-positive case was demonstrated serologically, but no pathological findings were obtained. Four of these 8 anti-GBM Ab-positive cases were also positive for MPO-ANCA, whereas the remaining 4 cases were not positive for MPO- and PR3-ANCA. The former group is categorized as having anti-GBM disease with either MPO- or PR3-ANCA (Group I), while the latter group is categorized as having anti-GBM disease without MPO- and PR3-ANCA (Group II). A manuscript about Group I consisting of 4 patients was accepted for publication in the *Journal of Clinical and Experimental Nephrology* (in press [14]). Therefore, we studied the cases of Group II and identified two patients who demonstrated vasculitis. These patients are presented in this article. The remaining two patients belonging to Group II were not found to have vasculitis, and a satisfactory serological investigation has not yet been performed at the present time. Therefore, these two patients were not included in the present case reports. The renal biopsies and autopsies were performed after the patients or family members provided their informed consent. The specimens of these biopsies were applied for routing staining as well as immunohistochemical staining for CD31 and type IV collagen. CD31 is a marker of the vascular endothelial cell surface, whereas type IV collagen is a marker of various basement membranes including vessels, tubuli, glomerulus, and Bowman's capsule. The procedures for staining CD31 and type IV collagen were described in our previous article [17] using monoclonal antibodies to each molecule (Dako, Glostrup, Denmark). The glomerular and tubulointerstitial (TI) lesions, including tubulitis [18] and peritubular capillaritis, were studied extensively. Peritubular capillaritis was identified by the destruction of peritubular capillary walls with periodic acid–Schiff (PAS) staining as well as loss of CD31 and type IV collagen staining in addition to the perivascular infiltration of inflammatory cells [17]. The serum anti-GBM Ab levels were determined by enzyme-linked immunosorbent assay (ELISA) (Euro-Diagnostica, Malmö, Sweden). The presence of MPO-ANCA and PR3-ANCA was tested using an ELISA kit from Nipro Company (Shiga, Japan). Immunofluorescence (IF) of the ANCA was examined using fluoro-ANCA test slides from Medical and Biological Laboratories (Nagoya, Japan). The other ANCAs, including azurocidine, BPI, cathepsin G, elastase, lactoferin, and lysozome, were investigated using ELISA from the Wieslab ANCA panel kit (Euro-Diagnostica).

Case reports

Case 1

A 44-year-old female, who had no history of smoking, experienced flu symptoms in late December. She developed dyspnea and edema in her legs, and was admitted to our affiliated hospital for treatment on December 28. On physical examination, dyspnea, pale face, and edema were observed in addition to having blood pressure (BP) 170/100 mmHg, pulse 74/min, and temp 37.5°C.

The laboratory data showed hemoglobin (Hb) 9.1 g/dl, white blood cells (WBCs) 11,900/μl, platelets 36.6 × 10^4/μl, urinary protein (+++) with numerous red blood cells (RBCs) in the sediment, C-reactive protein (CRP) >25 mg/dl, serum creatinine (Cr) 14.0 mg/dl, blood urea nitrogen (BUN) 99 mg/dl, anti-nuclear antibody (ANA) <40×, and rheumatoid arthritis (RA) factor 6.7 IU/ml. MPO-ANCA <10 EU, PR3-ANCA <10 EU, and anti-GBM Ab 763 EU/ml were confirmed 2 weeks later. A chest X-ray demonstrated bilateral pleural effusions and fine reticular shadows in both lung bases. She was started on daily hemodialysis (HD) and subsequently recovered from the dyspnea, but continued to have elevated CRP and required frequent blood transfusions. A renal biopsy was performed to confirm the diagnosis of renal failure on the 11th day after admission. The biopsy specimens showed necrotizing CSGN with fibrinoid angiitis in an afferent arteriole, severe TI nephritis, peritubular capillaritis, and immunoglobulin (Ig) G linear deposits along the glomerular capillary loops (GCLs) (Fig. 1a–f) (details in the "Results"). Anti-GBM Ab positivity and renal histopathology suggested a diagnosis of anti-GBM disease associated with fibrinoid angiitis. Because of the severe renal lesions, the patient was treated conservatively at first, but later developed pulmonary hemorrhage with hemoptysis 5 weeks after admission. She was transferred to our hospital for treatment on February 12. A chest X-ray and computed tomography (CT) scan showed shadows of ground glass opacification in the right lower lung field. Full methylprednisolone (mPSL) pulse therapy (1.0 g × 3 days) followed by oral PSL 60 mg per day was started for the treatment of the pulmonary hemorrhage. She recovered from the pulmonary hemorrhage, but not from renal failure, even though the titers of anti-GBM Ab decreased as shown at Fig. 2. She was discharged from the hospital on April 12 on 20 mg PSL daily. She has been followed at a HD clinic for 13 years, but no additional episode of hemoptysis or vasculitic manifestations has been noted while she has been receiving 5 mg PSL daily.

Case 2

A 65-year-old female, who had no past history of smoking or exposure to toxic solvents, developed swollen legs and arthralgia of ankle joints in early January. She was diagnosed with RA because of elevated serum matrix metalloproteinase-3 (MMP-3) levels, although she was not positive for RA factor. Methotrexate 4 mg per week and PSL 5 mg per day were prescribed. Even with these treatments, the patient demonstrated increased CRP levels and intermittent pyrexia of 38°C persisting for more than a month. She went to another hospital, and was diagnosed to have polymyalgia rheumatica, and her PSL was increased to 15 mg per day. However, the swollen legs and arthralgia of the ankles continued, and she was eventually transferred to our hospital on March 7. She was suspected to have deep vein thrombosis in both legs and seronegative RA at that time. The laboratory tests were negative for anticardiolipin IgG Ab, anti-β_2-glycoprotein I Ab, lupus anticoagulant, ANA, RA factor, anti-galactose-deficient IgG Ab, MPO-ANCA, and PR3-ANCA. The other tests disclosed Hb 12.6 g/dl, WBCs 10,600/μl, platelets 42 × 10^4/μl, CRP 1.9 mg/dl, serum Cr 0.8 mg/dl, CH_{50} 49.1 U/ml, MMP-3 128.9 ng/ml (normal range <60), negative proteinuria, and RBCs 1–4 per high-power field (HPF) in the urinary sediment. She continued to have a similar physical status as well as urinary findings with daily PSL 10–5 mg until July 10, when she noticed hematuria without oliguria, lumbago, and flu-like symptoms. She was admitted to our hospital on July 14 for evaluation. On physical examination, BP 170/98 mmHg, pulse 63/min, respiration rate 18/min, and temp 36.8°C were noted. The other findings included bilateral swollen joints in proximal interphalanx, hand, and ankle. Hypoesthesia of the palms and soles was also demonstrated (this finding was later confirmed by nerve conduction tests). The laboratory data on admission were as follows: urinalysis showed (++) proteinuria and numerous RBCs/HPF with 1–4 RBC casts/whole field in the sediment, Hb 8.1 g/dl, WBCs 13,400/μl, platelets 50.7 × 10^4/μl, CRP 10.9 mg/dl, serum Cr 1.8 mg/dl, MMP-3 374.4 ng/ml, anti-GBM Ab 96 EU/ml, MPO-ANCA <10 EU, and PR3-ANCA <10 EU. The chest X-ray and CT findings did not show any abnormalities. Because of the appearance of urinary abnormalities, increased serum Cr, and positivity for anti-GBM Ab, a renal biopsy was performed to confirm the diagnosis of renal lesions on July 18. The renal biopsy specimens showed necrotizing CSGN, TI nephritis, peritubular capillaritis, and IgG linear deposits along the GCLs (Fig. 3a–e) (details in the "Results"). Based on these data, the patient was diagnosed with anti-GBM disease accompanied by peritubular capillaritis, and full mPSL pulse therapy was instituted and was followed by daily plasma exchange (PE) (3 liter exchange per day). Even with these treatments, she progressed to renal failure and was started on intermittent HD on July 25. PSL 60 mg per day was given to her after pulse therapy, and PE was performed a total of twenty-one times, resulting in the stabilization of renal function around serum Cr 2.2 mg/dl. HD was finally discontinued on August 28. During these treatments, the patient developed a cavity lesion in the right upper lobe on August 11 when her anti-GBM Ab level was already less than 10 EU (Figs. 3f, 4). Again, no positivity for MPO- and PR3-ANCA was confirmed, nor were there any positive findings of tuberculosis, atypical mycobacterium, *Aspergillus*,

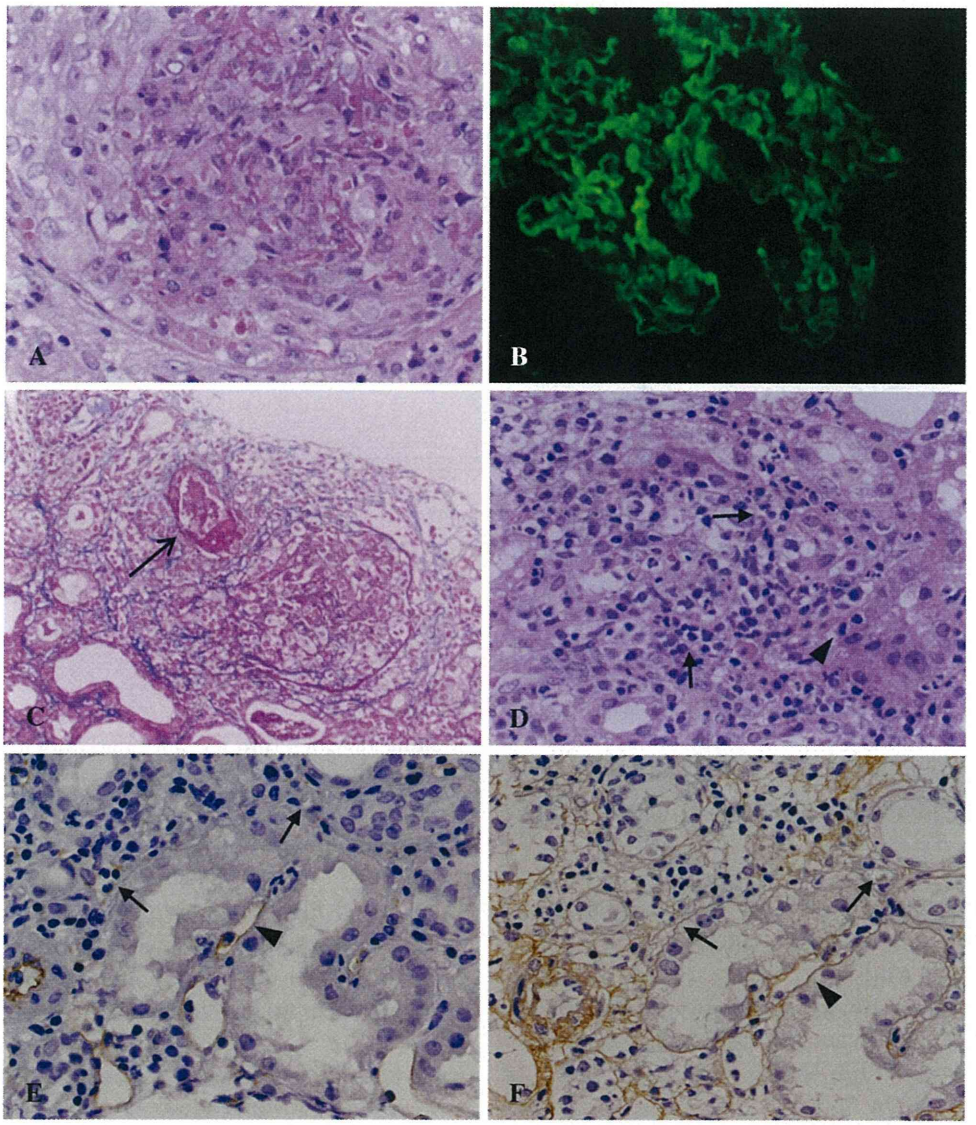


Fig. 1 Case 1. **a** A renal biopsy performed 2 weeks after the initial symptoms, shows necrotizing glomerulonephritis, cellular crescent, and infiltration of inflammatory cells in a glomerulus as well as the periglomerular area (PAS staining, ×400). **b** The IF study shows IgG linear deposits along the glomerular basement membrane (anti-IgG Ab staining, ×400). **c** The necrotizing angiitis of an arteriole (an *arrow*) is seen with partial cellular crescent formation in a glomerulus (Masson's trichrome staining, ×200). **d** The tubulointerstitium demonstrates remarkable infiltration of inflammatory cells among the destroyed tubuli as well as tubulitis (an *arrowhead*). The peritubular capillaries are not clearly observed because of the destruction of the basic interstitial architecture, thus suggesting the presence of peritubular capillaritis accompanied by the infiltration of inflammatory cells including neutrophils (*arrows*), leukocytoclasia, and mononuclear cells (PAS staining, ×400). **e** The CD31 staining discloses the loss of staining on peritubular capillaries in the inflammatory area (peritubular capillaritis) (*arrows*), but staining remains in the unaffected area (an *arrowhead*) (anti-CD31 Ab staining, ×600) **f** Type IV collagen staining, which is almost the same area as that presented in **e**, shows either a diminished appearance or the absence of the peritubular capillary walls in the peritubular capillaritides (*arrows*), but not in the unaffected ones (an *arrowhead*) (anti-type IV collagen Ab staining, ×600)

Candida or *Cryptococcus* in the sputum cultures and by polymerase chain reactions. With the continuation of steroid treatment, the pulmonary cavity lesion disappeared and the patient demonstrated almost normal urinary findings with (±) proteinuria and RBCs 1–4/HPF in the sediment as well as persistent negativity for CRP. She was subsequently discharged from our hospital 5 months after admission and was maintained on 12.5–5.0 mg PSL daily. No hemoptysis or pulmonary hemorrhage was observed for the following 3.5 years. Detection for IF ANCA and other ANCAs by ELISA were performed later with deeply frozen serum, which was obtained on admission, and it was found to be positive for p-ANCA but negative for the other ANCA subsets (Fig. 3g).

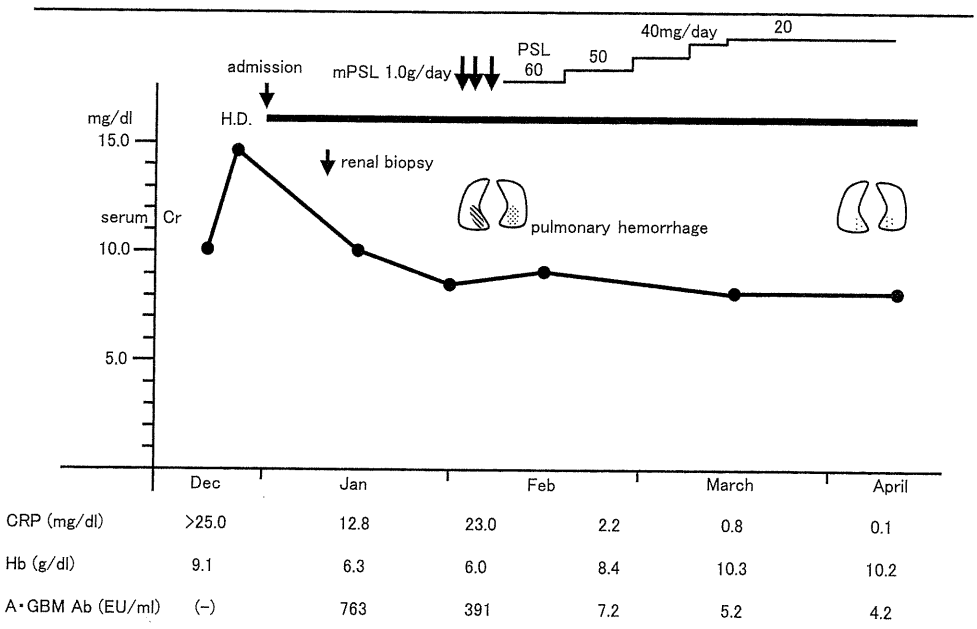


Fig. 2 Case 1. 44y/o ♀ Anti-GBM disease complicated by pulmonary hemorrhage

Results

The incidence of anti-GBM disease and its features at our institution

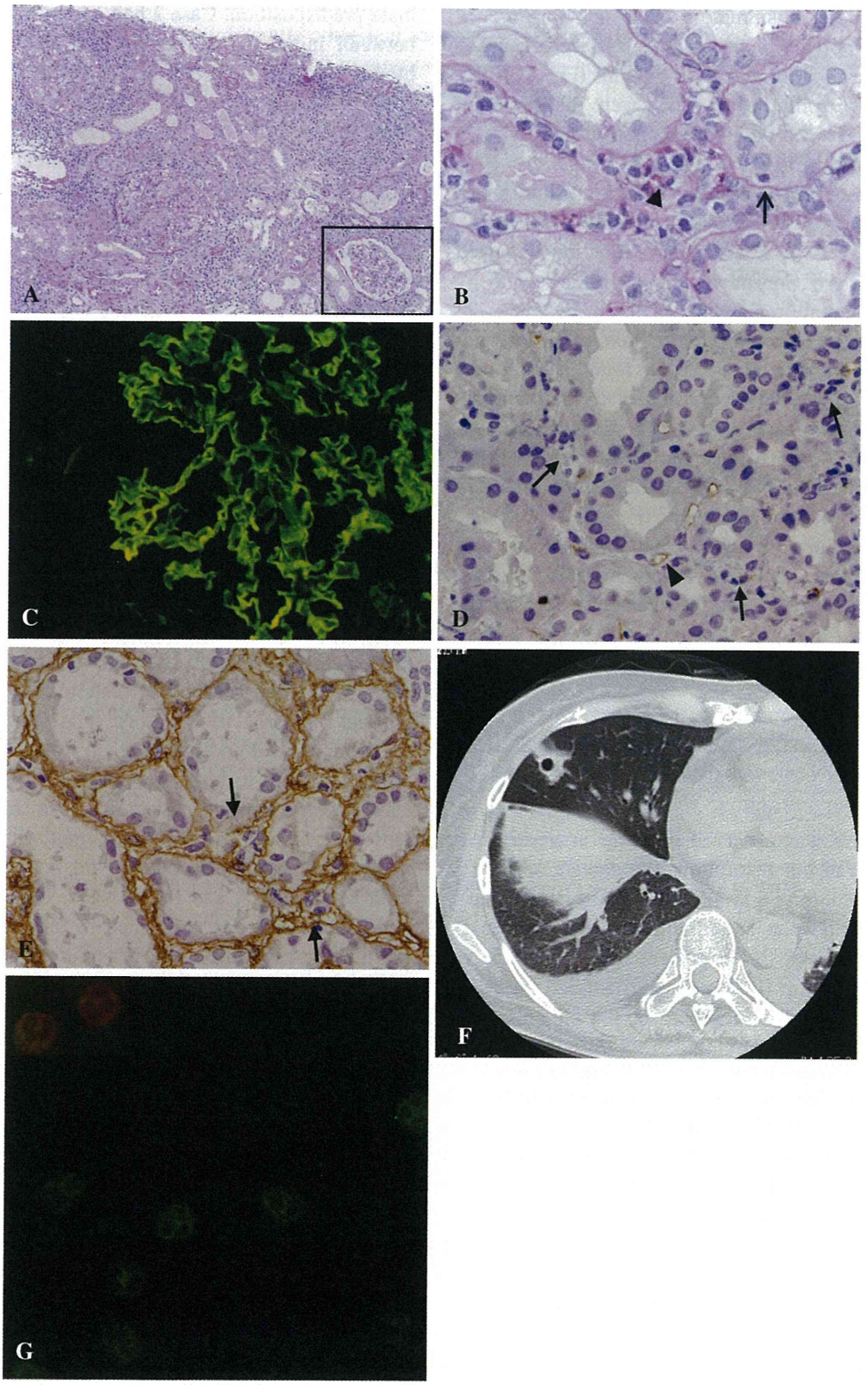
Five hundred and seventy-eight renal tissue samples were examined between 1998 and 2007, and only 7 cases were identified as having anti-GBM disease (7/578 = 1.2%). Three of these 7 cases were also positive for MPO-ANCA (Group I) whereas the remaining four cases were not positive for MPO- and PR3-ANCA (Group II). Group I cases were complicated by pulmonary hemorrhage, as well as prior pulmonary fibrosis (data not shown, see reference 14). Group II cases showed different features from dual Ab-positive cases, namely that two cases were complicated by pulmonary hemorrhage and the remaining two cases were not. In addition, the patients in Group II did not demonstrate prior pulmonary fibrosis.

Our present Case 1 was diagnosed as having Goodpasture's syndrome because of RPGN and pulmonary hemorrhage, whereas Case 2 was diagnosed with anti-GBM disease because of RPGN and no pulmonary hemorrhage. The remaining two patients with anti-GBM disease were not complicated by any other organ symptoms. Table 1 shows the major clinical features of the present two cases who were both female. With regard to their initial symptoms, one patient experienced flu symptoms, and the other had swollen legs and arthralgia in ankles. The intervals between the initial symptoms and the admission to the hospital were 1.5 and 28 weeks, respectively. Both patients demonstrated hypertension, anemia, raised serum Cr, and markedly elevated CRP, but no high fever.

Renal pathology of Cases 1 and 2

Table 2 and Figs. 1 and 3 present the main renal pathological features of these two patients. Case 1 had 12 glomeruli which revealed a circumferential crescent with segmental necrotizing lesion in 9 and a partial crescent

Fig. 3 Case 2. **a** Low magnification of renal specimens from a biopsy ▶ that was performed 28 weeks after the initial symptoms. The biopsy specimens show different degrees of crescent formation from a completely destroyed glomerulus to an almost intact glomerulus as well as the infiltration of inflammatory cells in the interstitium. The *inset* reveals an almost intact glomerulus. No necrotizing angiitis is observed (PAS staining, ×100). **b** The tubulointerstitium reveals infiltration of inflammatory cells among the tubuli as well as tubulitis (an *arrow*) and obscured peritubular capillary walls (an *arrowhead*) (PAS staining, ×600). **c** The IF study demonstrates IgG linear deposits along the GBM (anti-IgG Ab staining, ×400). **d** The CD31 staining shows positive visualization of vascular walls on the peritubular capillary in the intact area (an *arrowhead*), but staining is lost in the areas of peritubular capillaritis (*arrows*). Peritubular capillaritis is also manifested by the pericapillary infiltration of inflammatory cells (anti-CD31 Ab staining, ×400). **e** Type IV collagen staining reveals the loss of staining on peritubular capillary walls (*arrows*) as well as the tubular basement membrane in the affected area (anti-type IV collagen Ab staining, ×400). **f** Chest X-ray shows a cavity lesion with the surrounded reaction in the right upper lung field and interlobular pleural effusion. **g** The IF study for ANCA detection using fluoro-ANCA test slides discloses p-ANCA pattern on the ethanol-fixed neutrophils by the patient's serum at ×20 dilution, but a completely negative pattern on formalin-fixed neutrophils in the same diluted serum (*inset*) (anti-immunoglobulin Ab staining, ×600)



with minor necrotizing lesion in 3. Eight circumferential crescents were associated with ruptured Bowman's capsules. Case 2 included 15 glomeruli. They showed a circumferential crescent in 3, a partial crescent in 6, and non-crescentic glomerulus in 6. Two circumferential crescentic glomeruli in Case 2 were associated with segmental necrotizing lesion and the rupture of Bowman's capsule. TI findings in these two cases were associated with

severe infiltration of inflammatory cells including mononuclear cells and polymorph neutrophils, and were found to have peritubular capillaritis as well as tubulitis (Figs. 1d, 3b). These peritubular capillaritides, which were demonstrated by the loss of CD31 and type IV collagen staining as well as the blurred appearance of capillary walls by PAS staining, were associated with the pericapillary infiltration of inflammatory cells including neutrophils (Figs. 1e, f, 3d, e). They were observed in areas distant from the damaged glomeruli and were presumed not to be affected by glomerular inflammation and/or ischemia; however, TI nephritis, which was thought to be due to glomerular inflammation and/or ischemia, was also found in these two cases. Furthermore, Case 1 demonstrated fibrinoid angiitis of an afferent arteriole (Fig. 1c). The IF study demonstrated IgG linear deposits along the GCLs in both cases, but did not showed any significant deposition of other immunoglobulins or complements (Figs. 1b, 3c). The serological evaluations for MPO- and PR3-ANCA were negative in both cases, but Case 2 demonstrated the presence of IF p-ANCA (Fig. 3g). The detection test for the other ANCAs in Case 2 was negative. Both cases were found to be negative for hepatitis B antigen and hepatitis C Ab.

Discussion

Anti-GBM disease was observed to occur at a very low incidence (1.2%) at our institution. We categorized the 8 identified patients into two groups which consisted of dual Ab-positive cases (Group I) and single Ab-positive cases (Group II). The purpose of the present study was to focus on the 4 cases in Group II. When we performed the pathological study on renal tissues, two of the patients were identified to demonstrate vasculitis. Therefore, we studied these two patients clinically, pathologically, and serologically. Both patients revealed positivity for anti-GBM Ab in the serum as well as the IgG linear deposits along GCLs in the biopsy specimens. They were diagnosed to have anti-GBM disease and had RPGN and a RPGN-like course. Both patients did not reveal MPO-ANCA or PR3-ANCA positivity. Case 1 was complicated by pulmonary hemorrhage around the disease onset, whereas Case 2 did not show any hemoptysis or suggestive pulmonary shadows for hemorrhage in the X-ray and CT even during the follow-up. Of interest, Case 2 developed hypoesthesia of the palms and soles as well as a cavity lesion in the lung, suggesting the presence of vasculitis.

The main clinical profile of the patients is summarized in Table 1 and "Results". Compared with the typical cases of Goodpasture's syndrome and anti-GBM disease, both patients were female. This is in contrast to the generally male predisposition. Case 2 had a long interval (28 weeks) between initial symptoms and the development of full-blown symptoms of RPGN. Therefore, the present two cases had some atypical clinical features of Goodpasture's syndrome or anti-GBM disease [19, 20]. The reasons why these two patients had different clinical courses are not clear. However, it may be presumed that: (1) Case 1 had a high percentage of circumferential crescents, whereas Case 2 had a low percentage, (2) Case 1 had a rapid onset of illness, while Case 2 had a long prodromal onset of the disease, and (3) steroid treatment and PE were started very early during the RPGN course in Case 2. These factors resulted in the two patients having different clinical courses as described in the previous articles [19, 21, 22]. The renal pathology is presented in Table 2, "Results", and Figs. 1, 3. Both cases demonstrated CSGN and IgG linear deposits along the GCLs. In the tubulointerstitium, Case 1 showed marked inflammatory cell infiltration accompanied by peritubular capillaritis and tubulitis, as well as fibrinoid angiitis in an afferent arteriole. Case 2 also revealed similar findings of peritubular capillaritis and tubulitis, but did not show any arteritis/arteriolitis. These peritubular capillaritides were presumed to occur as a primary event, because they were observed to be scattered in the interstitium without any association with the damaged glomeruli. Based upon the clinical and pathological data, Case 1 could have been diagnosed to have Goodpasture's syndrome whereas Case 2 would have been diagnosed with anti-GBM disease. However, Case 1 had fibrinoid angiitis in the renal specimens even though the patient did not show positive for both MPO-ANCA and PR3-ANCA. Unfortunately, the IF ANCA test was not performed in this case. Case 2 with peritubular capillaritis had tests for IF ANCA and other ANCAs by ELISA. These tests showed positivity for p-ANCA but negativity for the known other ANCAs.

There has been much debate about the existence of vasculitis in Goodpasture's syndrome or anti-GBM disease except for glomerular and/or pulmonary capillaritis [19, 20, 23–25]. To date, these capillaritides have not been generally recognized as vasculitis [19, 20, 23–27]; however, several cases in the literature, that described the presence of angiitis in Goodpasture's syndrome or anti-GBM disease on the pathological findings, were reported before the development of assays for ANCA detection [23–30]. Only a few papers have shown photographs of necrotizing angiitis [23, 24, 26], but these pathological findings might have been due to vasculitis which resulted from unrecognized ANCA at the time. In contrast, there are several articles which described the presence of vasculitic manifestations but did not show the pathological findings in the cases positive for both anti-GBM Ab and MPO- or PR3-ANCA [5–7, 10–14]. In fact, only three recent reports of cases demonstrating anti-GBM Ab and MPO-ANCA positivity

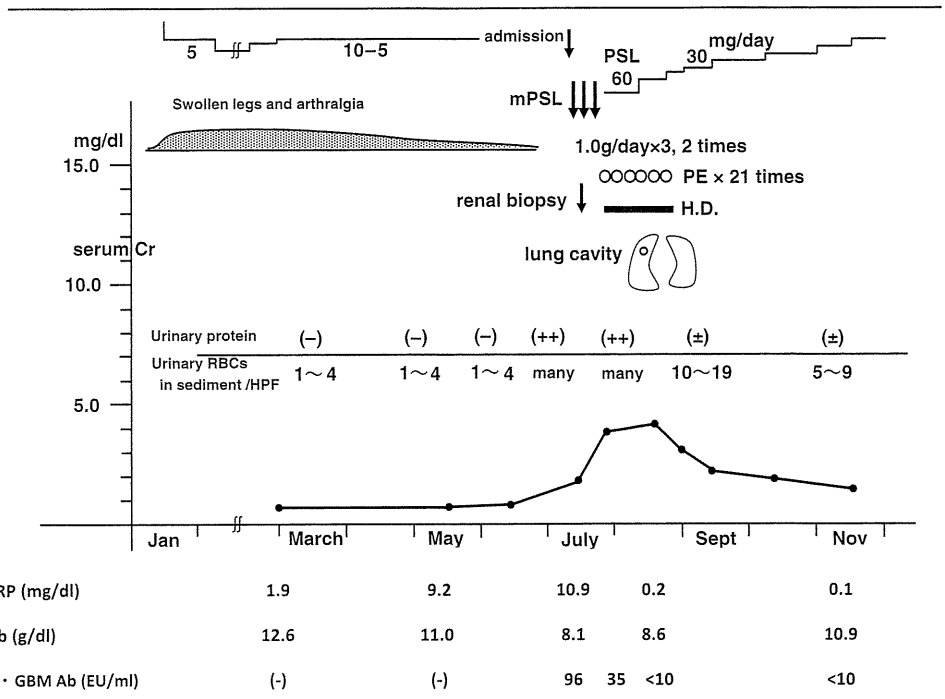


Fig. 4 Case 2. 65y/o ♀ Anti-GBM disease complicated by peripheral neuropathy and a lung cavity

Table 1 Clinical features of anti-GBM disease without MPO- and PR3-ANCA in two cases

	Age	Sex	Initial symptoms	Interval[a] (weeks)	On admission				
					BP (mmHg)	Temp (°C)	Hb (g/dl)	Serum Cr (mg/dl)	CRP (mg/dl)
Case 1	44	♀	Flu	1.5	170/100	37.5	9.1	14.0	>25.0
Case 2	65	♀	Swollen legs arthralgia	26	170/98	36.8	8.1	1.8	10.9

[a] Time interval between the initial symptoms and the admission to the affiliated hospital or our university hospital

Table 2 Pathological findings of anti-GBM disease without MPO- and PR3-ANCA in two cases

	Glomerulus			TI lesion	Vasculitis	IF
Case 1	Cf cellular crescent	9/12[a]	(75%[b])	MNC (+)[c], PMN (+)[c] markedly destroyed tubuli	Vas afference	IgG linear depositis along GCL
	Partial cellular crescent	3/12	(25%)		PTCitis	
	Bowman's rupture[d]		(+)	PTCitis, tubulitis		
Case 2	Cf cellular crescent	3/15	(20%)	MNC (+), PMN (+)	PTCitis	IgG linear deposits along GCL
	Partial cellular crescent	6/15	(40%)	Slight tubular atrophy		
	Almost normal	6/15	(40%)	PTCitis, tubulitis		
	Bowman's rupture		(+)			

Cf circumferential, *PTCitis* peritubular capillaritis, *GCL* glomerular capillary loop

[a] Numbers of pathological glomeruli/total observed glomeruli

[b] Percentage of circumferential or partial crescent or almost normal glomerus in the total observed glomeruli

[c] Infiltrating cells in the interstitium (MNC; mononuclear cells, PMN; polymorph neutrophils)

[d] Bowman's capsule rupture

presented with pathological findings of vasculitis [8, 9, 31]. They consisted of a case with fibrinoid angiitis in the kidney and two cases with angiitis not accompanied by fibrinoid degeneration in the kidney or muscle, respectively. In our own institution, we experienced one additional case which demonstrated angiitis without fibrinoid degeneration and

was positive for anti-GBM Ab as well as MPO-ANCA [14]. Therefore, it should be considered whether cases of Goodpasture's syndrome or anti-GBM disease associated with vasculitis are positive for MPO-ANCA, PR3-ANCA, other ANCA subsets, or IF ANCA [6–9, 11, 12, 14–16]. Both cases in our present study challenge the current notion as to whether Goodpasture's syndrome or anti-GBM disease without both MPO- and PR3-ANCA can demonstrate vasculitis. Case 2 was presumed to have an unknown ANCA which might induce peritubular capillaritis. Case 1 could also have had a similar factor that might have induced fibrinoid arteriolitis and peritubular capillaritis even though the other ANCA subsets and IF ANCA test were not performed. Therefore, patients with anti-GBM disease, including Goodpasture's syndrome, who are not associated with MPO- and PR3-ANCA positivity, should be carefully evaluated for the presence of vasculitic symptoms and pathology, as well as for the expression of various ANCAs including MPO, PR3, and other neutrophil proteolytic enzymes using an ELISA and IF test. Based on these studies, the question of whether anti-GBM disease without ANCA positivity can itself induce vasculitis is expected to be clarified in the near future.

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Conflicts of interest None.

Note added in proof One patient without vasculitis, who was evaluated after the submission of the manuscript, did not show positive for immunofluorescence ANCA as well as the other ANCAs. The other patient without vasculitis was not available for further investigation.

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ORIGINAL ARTICLE

Dual myeloperoxidase-antineutrophil cytoplasmic antibody- and antiglomerular basement membrane antibody-positive cases associated with prior pulmonary fibrosis: a report of four cases

Kimimasa Nakabayashi · Yasunori Fujioka · Toshihiko Nagasawa · Tsuneo Kimura · Kaoruko Kojima · Yasuhiro Arimura · Akira Yamada

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Abstract
Background Both myeloperoxidase-associated antineutrophil cytoplasmic antibody (MPO-ANCA) and antiglomerular basement membrane antibody (anti-GBM Ab) positivity have been demonstrated in patients with rapidly progressive glomerulonephritis (RPGN), either with or without pulmonary hemorrhage; however, the implications of these antibodies in such patients have not yet been elucidated. The cases with dual positive antibodies were studied clinically, serologically, and pathologically, and the implications of antibodies are discussed here.
Patients and methods Four patients with prior pulmonary fibrosis, who subsequently developed RPGN and pulmonary hemorrhage, were studied clinically, serologically, and pathologically. The clinical data were reviewed extensively and the dual positive antibodies were detected by enzyme-linked immunosorbent assays. Pathological studies were performed with a renal biopsy in one patient, a gastric biopsy in another patient, and autopsy materials in the remaining 2 patients.
Results All 4 patients had prior pulmonary fibrosis before the symptoms of RPGN when the dual positivity of MPO-ANCA and anti-GBM Ab was detected. Three cases were accompanied by pulmonary hemorrhage around the time of RPGN whereas the remaining case demonstrated pulmonary hemorrhage a few years later. Renal tissue specimens in 3 cases showed circumferential crescents and linear immunoglobulin G deposits along the glomerular capillary loops in glomeruli. Two autopsy specimens revealed vasculitis of the small arteries and arterioles of the kidney, and one of them showed similar vasculitic findings in both the gastrointestinal tract walls and the adipose tissues of the adrenal glands. Additionally, a case with pulmonary hemorrhage occurring after remission was associated with re-elevated MPO-ANCA levels but without anti-GBM Ab positivity. A gastric biopsy was unremarkable and non-contributory for the diagnosis, but this case showed vasculitic symptoms of peripheral neuritis and retinal hemorrhage. Taken together, all 4 cases demonstrated prior pulmonary fibrosis and dual positivity of MPO-ANCA as well as anti-GBM Abs at the time of RPGN, and were associated with either pulmonary hemorrhage or its occurrence thereafter.
Conclusion Four cases that showed prior pulmonary fibrosis as well as subsequent RPGN and pulmonary hemorrhage were both MPO-ANCA- and anti-GBM Ab-positive at the time of RPGN. The glomeruli disclosed features compatible with anti-GBM Ab disease, but the clinical and pathological vasculitic manifestations, including prior pulmonary fibrosis that might be an early manifestation of ANCA disease, suggested the occurrence of MPO-ANCA-associated vasculitis. Furthermore, 1 case subsequently showed repetitive pulmonary hemorrhage with re-elevated MPO-ANCA positivity but without anti-GBM Ab positivity, and this event was possibly due to MPO-ANCA-associated alveolar capillaritis. As anti-GBM Ab disease is generally thought not to manifest the clinical and pathological features of vasculitis excluding the kidney, MPO-ANCA might be a key factor regarding the occurrence of this dual positive disease.

K. Nakabayashi (✉) · T. Nagasawa · T. Kimura · Y. Arimura · A. Yamada
First Department of Internal Medicine,
Kyorin University School of Medicine,
6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan
e-mail: kiminaka@krd.biglobe.ne.jp

Y. Fujioka · K. Kojima
Department of Pathology,
Kyorin University School of Medicine, Mitaka, Japan

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Introduction

Antiglomerular basement membrane antibody (anti-GBM Ab)-mediated disease was initially described by Goodpasture [1] who presented a case complicated by rapidly progressive glomerulonephritis (RPGN) and hemoptysis. Subsequently, cases with anti-GBM Ab disease without pulmonary hemorrhage (hemoptysis) were reported and were referred to as anti-GBM Ab disease [2]. On the other hand, antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis was described and was commonly manifested as RPGN with symptoms of various organ involvement including pulmonary hemorrhage [3, 4]. The ANCA-associated RPGN and various organ involvement were caused by vasculitis [5, 6]. ANCA has two major subsets, myeloperoxidase (MPO)-ANCA and proteinase 3 (PR3)-ANCA. MPO-ANCA is commonly related to RPGN without any other organ involvement (renal limited vasculitis) or with pulmonary hemorrhage (pulmo-renal disease) whereas PR3-ANCA is generally referred to as Wegener's granulomatosis [7]. MPO-ANCA-associated vasculitis occurs more commonly than Wegener's granulomatosis in Japan [8]. Goodpasture syndrome (RPGN and pulmonary hemorrhage) typically affects male smokers or males with lung exposure to chemical agents, while MPO-ANCA-associated vasculitis tends to occur in elderly individuals [9, 10]. Over the last 15 years, dual positive cases with anti-GBM Ab and ANCA have been described in the literature and the implication of these antibodies has been discussed [11–29]. Several reports suggested that ANCA-associated disease occurred initially and damaged the alveolar or glomerular basement membrane (BM), thus resulting in the release of alveolar or glomerular BM antigen (α_3 NC1 antigen) into the circulation [12, 13, 26, 27]. These two BM antigens are presumed to have cross-reactivity and consequently lead to anti-GBM Ab production; however, a few cases initially occurred as anti-GBM Ab-mediated disease without ANCA positivity, and eventually developed into ANCA positivity accompanied by vasculitic manifestations [11, 28, 29]. Reverse cases, which showed only ANCA first and thereafter progressed to anti-GBM Ab disease without ANCA positivity, have never been previously presented in the literature. Regarding ANCA in dual positive cases, some cases were PR3-ANCA [11, 12, 15, 18, 22, 26] whereas most of the cases were MPO-ANCA [12–17, 19–29]. Since the implication of dual positivity in the cases has not yet been elucidated, this study evaluates 4 dual positive cases clinically, serologically, and pathologically, while also discussing the significance of dual positivity.

Materials and methods

From 1998 to 2007, 67 MPO-ANCA-positive patients were admitted to our hospital [30]. Four of these patients were also identified as positive for anti-GBM Ab. One patient underwent renal biopsy and is now alive on chronic hemodialysis. Of the other three patients who eventually died, 2 underwent an autopsy. The clinical, serological, and pathological features of these 4 patients were studied extensively. All MPO-ANCAs were determined using enzyme-linked immunosorbent assay (ELISA) kits of the Nipro Company (Shiga, Japan). Anti-GBM Ab in Cases 1, 3, and 4 was detected using ELISA kits of Euro-Diagnostica (Malmö, Sweden) and Case 2 (the earliest case) was examined using ELISA kits of SmithKline Beecham Clinical Laboratories (CA, USA). The findings of the latter test were only expressed as positive or negative without titration. The renal biopsy and autopsy were performed with the agreement of the patients or their family members.

Case reports

Case 1

A 78-year-old male had smoked about 20 cigarettes per day for over 50 years, but did not disclose any prior history of hypertension or diabetes mellitus. He was diagnosed with pulmonary fibrosis during a health examination at the age of 70. At that time, he developed flu-like symptoms with a cough, a fever of 38.4°C, and chills. He was diagnosed as having right-sided pneumothorax and pulmonary fibrosis, and therefore was admitted to the respiratory department of our hospital. On admission, a physical examination disclosed decreased breath sounds in the right chest and slight crackles in the left chest. The laboratory data were as follows: hemoglobin (Hb) 13.0 g/dl, white blood cells (WBCs) 10,800/μl, platelets 31 × 10^4/μl, C-reactive protein (CRP) 8.9 mg/dl, erythrocyte sedimentation rate (ESR) 91 mm (60 min), serum creatinine (Cr) 0.8 mg/dl (normal range <1.3), and urinary protein (+) with red blood cells (RBCs) 20–29/high power field (HPF), and few RBC casts/whole field (WH) in the sediment. MPO-ANCA and anti-GBM Ab were not examined on this admission. Methylprednisolone (mPSL) pulse therapy and air suction from the right-side chest were administered, and provided a satisfactory improvement of both pneumothorax and pulmonary fibrosis. The patient was discharged from the

hospital 2 months later; however, he developed edema in the legs with an increased serum Cr level of 1.3 mg/dl, positive proteinuria (+++) associated with RBCs 50–99/HPF in the sediment, and an increased urinary β_2-microglobulin level of 27,967 μg/l over the following 4 weeks. Four months after the first admission, he developed severe dyspnea and was re-admitted to hospital with blood pressure (BP) 138/70 mmHg, pulse 100/min, respiration 36/min, and temperature 37.4°C. The laboratory data were as follows: Hb 9.9 g/dl, WBCs 18,300/μl, platelets 26.8×10^4/μl, CRP 8.9 mg/dl, serum Cr 16.1 mg/dl, serum KL-6 998 U/ml, PaO_2 54.5 Torr, and urinary protein (+++) and RBCs 50–99/HPF in the sediment. The chest X-ray and computerized tomography (CT) findings revealed ground glass opacifications associated with an air bronchogram in the right lower lung and left middle lung fields, in addition to reticular shadows in the entire lung field (Fig. 1a). MPO-ANCA and anti-GBM Ab were examined and were 74 EU (normal range <10) and 231 EU (normal range <10), respectively; however, PR3-ANCA was less than 10 EU (normal range <10). Based on these findings, he was diagnosed with both MPO-ANCA- and anti-GBM Ab-associated RPGN and was treated with mPSL pulse therapy, plasma exchange, hemodialysis, and mechanical ventilation. Although no hemoptysis was observed, the chest X-ray, physical examination, and laboratory data suggested pulmonary hemorrhage because of the sudden onset of marked dyspnea, apyrexia, ground glass shadows of cloudy shape, progression of anemia, and the positivity of MPO-ANCA and anti-GBM Ab. The patient recovered once from respiratory failure with negativity of both MPO-ANCA and anti-GBM Ab, but subsequently showed worsening of his symptoms with *Pneumocystis jirovecii*-associated pneumonia, and he died of respiratory failure 3 months after the second admission. Permission was obtained for an autopsy to be performed.

Case 2

A 64-year-old male, who had smoked about 40 cigarettes per day for 30 years, developed exertional dyspnea and was admitted to a hospital. He had a prior history of pulmonary tuberculosis (Tbc) at the age of 31, and experienced repetitive episodes of lung infections thereafter. The patient's chest X-rays on admission showed a bilateral honeycomb lung with pulmonary fibrosis and pleural thickening. The laboratory data were negative for proteinuria but 3–5 RBCs/HPF in the urinary sediment, and

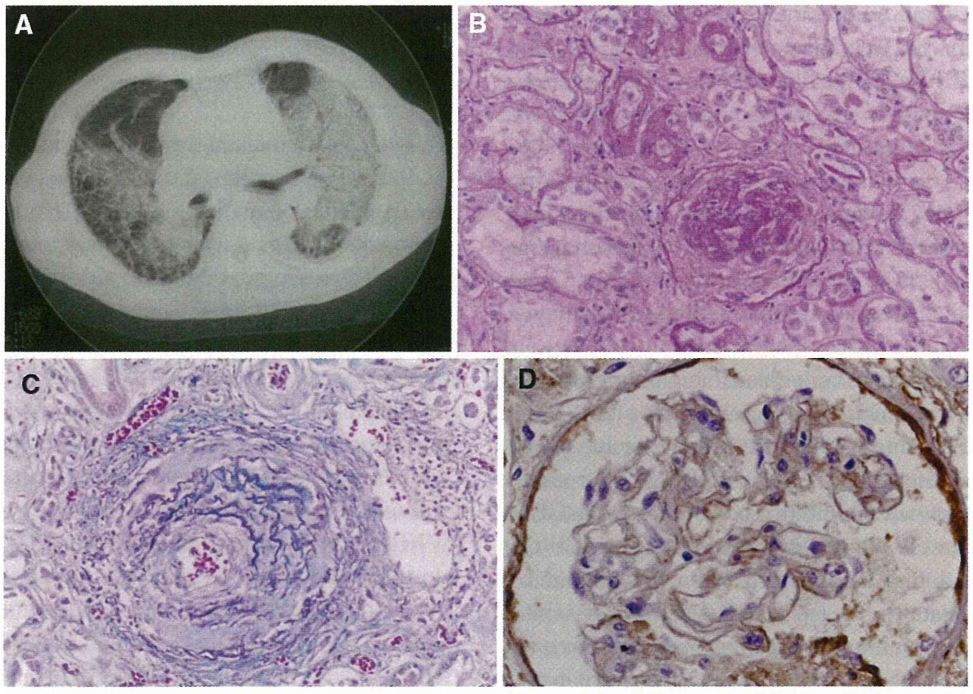

Fig. 1 Case 1: **a** CT scan of the chest on admission shows ground glass opacifications associated with the air bronchogram in the right lower lung and left middle lung in addition to reticular shadows in the remaining field. **b** The kidney shows a circumferential fibrocellular crescent in one glomerulus with interrupted Bowman's capsule, some periglomerular cell infiltration, and detached tubular epithelial cells in the tubular lumen. Three arterioles in this area have an almost normal structure (PAS staining, ×200). **c** An artery in the kidney, which reveals an interrupted lamina elastica interna with elastosis, marked intimal thickening, degenerated smooth muscle cells of the media, and perivascular inflammatory cell infiltration, thus suggesting a diagnosis of vasculitis (Elastica Masson's trichrome staining, ×200). **d** IgG staining of one non-crescentic glomerulus demonstrates positive linear deposits along the glomerular capillary loop (anti-IgG Ab staining by peroxidase-antiperoxidase method, ×400)

serum Cr 1.1 mg/dl. However, hemoptysis occurred 2 months later in association with an elevated serum Cr level of 2.0 mg/dl, positive proteinuria, and several RBCs in the sediment. With these episodes, his symptoms worsened with increased dyspnea and edema in the face as well as in the extremities. He was transferred to our hospital for a confirmative diagnosis and treatment. A physical examination on admission showed facial and extremity edema, BP 120/80 mmHg, pulse 106/min, and coarse crackles in the chest. The laboratory examination revealed the following: urinalysis showed proteinuria (+++) and numerous RBCs in the sediment, Hb 8.5 g/dl, WBCs 9,900/μl, platelets 18.3 × 10^4/μl, serum Cr 14.4 mg/dl, CH_{50} 30.6 U/ml, ANA 160× speckled pattern, anti-DNA antibody (RIA assay) 14 U/ml (normal range <8.0), CRP 4+, RA factor negative, MPO-ANCA 74 EU/ml, anti-GBM Ab positive, and immune complex by C_1q binding assay <1.5 μg/ml (<4.0). The immunofluorescence (IF) analysis of ANCA illustrated a perinuclear ANCA (p-ANCA) staining pattern without the appearance of cytoplasmic ANCA (c-ANCA) staining. The chest X-ray revealed ground glass opacifications in both the middle lung fields as well as a honeycomb appearance and pleural thickening of both sides. Based on these data, the patient was diagnosed as having MPO-ANCA- and anti-GBM Ab-associated RPGN, pulmonary fibrosis with a honeycomb appearance, and pulmonary hemorrhage. The patient was treated with oral prednisolone 60 mg/day and hemodialysis; however, respiratory failure progressed and the patient died 3 months after admission. An autopsy was subsequently performed.

Case 3

A 72-year-old male had a prior history of pulmonary Tbc at the age of 22, smoked about 20 cigarettes per day for 45 years, and had a family history of rheumatoid arthritis (RA) as well as pulmonary Tbc. He developed myalgia, arthralgia, and a low-grade fever lasting over 4 weeks. He was diagnosed as having RA by a physician and subsequently experienced two episodes of hemoptysis, thus suggesting the recurrence of pulmonary Tbc. As a result of these symptoms, and numbness of his left sole in addition to hemoptysis, he visited our hospital for an evaluation. A physical examination revealed slight tenderness on both calf muscles, mild joint pain with swollen fingers, hypoesthesia of the left foot, and purpura on both legs. Routine laboratory tests showed that the urine was negative for proteinuria but revealed 20–29 RBCs/HPF in the sediment, Hb 10.2 g/dl, serum Cr 1.2 mg/dl, CRP 8.6 mg/dl, negative RA test, and negative ANA. A chest X-ray and CT disclosed the appearance of right apical pleural thickening with bilateral fibrosis in the lung bases in addition to a moderate honeycomb lung (Fig. 2a). Therefore, only a non-steroidal anti-inflammatory drug was prescribed. During the following month, he lost 5.0 kg in body weight, and experienced another hemoptysis, bloody stool with abdominal pain, continued hypoesthesia of the left sole, and positive proteinuria accompanied by increased serum Cr to 1.8 mg/dl. Accordingly, he was admitted to our hospital for diagnosis and treatment. On admission, he was 167 cm in height and 54.5 kg in weight (previously 60.5 kg). BP was 140/80 mmHg, pulse 68/min, respiration 26/min, temperature 36.5°C, crackles audible over the lower chest, and decreased thermal pain sensations in the left foot. The laboratory data showed the following: Hb 7.0 g/dl, WBCs 1,800/μl, platelets 25 × 10^4/μl, serum Cr 16.0 mg/dl, CRP 12.0 mg/dl, positive stool for occult blood, PaO_2 60 Torr, and numerous non-deformed RBCs in the sediment with (+++) proteinuria. The chest X-ray and CT findings revealed butterfly-shaped central shadows associated with ground glass opacifications in the left lower lung field in addition to bilateral basal fibrosis. These data suggested the occurrence of RPGN when hemodialysis was initiated. Subsequently, dual positivity of MPO-ANCA (210 EU) and anti-GBM Ab (38 EU) was found, but PR3-ANCA was less than 10 EU. mPSL semi-pulse therapy (500 mg/day) was administered twice for 3 successive days, 4 packs of RBC blood transfusions were infused, and plasma exchange of 2.0 l was performed 3 times, but the patient had continued chronic hemodialysis. An additional examination, which was performed 4 weeks after pulse therapy, showed neither pulmonary hemorrhagic lesions by bronchofiberscopy nor gastrointestinal (GI) ulcers by gastro- and colonoscopies. A sputum culture demonstrated the growth of atypical mycobacteria not belonging to either avium or intracellular types. An examination of the optic fundi disclosed a small retinal hemorrhage of the right eye and the velocity test for sensory nerve in the left foot showed a delay. Taken together, RPGN, GI bleeding, retinal hemorrhage, peripheral neuritis, purpura, and positive MPO-ANCA were suggestive of MPO-ANCA-associated vasculitis, whereas RPGN and positive anti-GBM Ab suggested anti-GBM Ab disease, and the pulmonary shadows were compatible with either MPO-ANCA-associated vasculitis or anti-GBM Ab disease in addition to cardiac congestion. Therefore, the final diagnosis was both MPO-ANCA-associated vasculitis and anti-GBM Ab disease. Although aggressive treatment was instituted for the kidney as well as lung symptoms and both antibodies to MPO and GBM became negative, the patient was not weaned off chronic hemodialysis. Finally, he developed symptoms of atypical mycobacterium lung with multiple thin-walled cavities and myelodysplastic syndrome with pancytopenia (Hb 6.3 g/dl, WBCs 1,300/μl, platelets 1 × 10^4/μl), and died of myeloblastic leukemia after

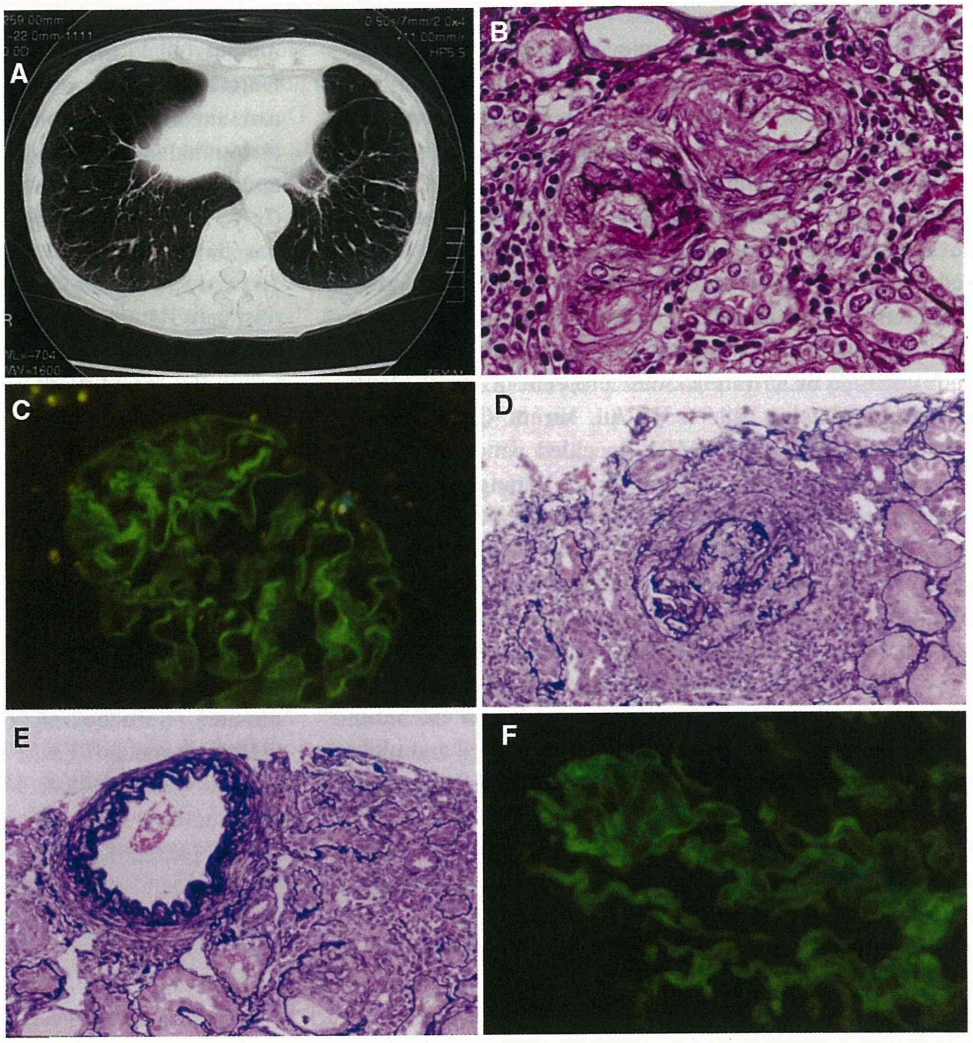

Fig. 2 Case 3: **a** CT sacn of the chest taken prior to RPGN shows fine reticular shadows in both posterior lung bases and a honeycomb appearance in the entire field. Case 2: **b** Small arteries at the bifurcation of the kidney, which disclose the destruction of the vessel wall with interrupted lamina elastica interna, degenerated smooth muscle cells of the media, and marked perivascular inflammatory cell infiltration, indicate the presence of vasculitis [periodic acid methenamine (PAM) silver staining, ×400]. **c** An immunofluorescence analysis of the kidney demonstrates linear IgG deposits along the glomerular capillary loop, but not on the tubular basement membrane (anti-IgG Ab staining, ×400). Case 4: **d**, **e** The kidney shows a semi-circumferential crescent in one glomerulus with ruptured Bowman's capsule and periglomerular cell infiltration (PAM staining, ×200), but the artery does not reveal any significant endothelial cell proliferation (PAM staining, ×200). **f** An IF analysis of the kidney reveals linear IgG deposits along the glomerular capillary loop (anti-IgG Ab staining, ×600)

6 years of pulmo-renal manifestations. No tissues from the kidney or lung were obtained from the patient throughout the illness, but gastric tissues were biopsied for the diagnosis of early cancer 8 months prior to death.

Case 4

A 42-year-old female, who had a history of Raynaud's phenomenon over the previous 20 years but did not smoke, developed swollen fingers, leg edema, proteinuria, and occult blood in the urine. She was admitted to our hospital for an evaluation. A physical examination revealed swollen fingers, sclerodactylia, digital ulcer scars on the finger tips, contracture of small lingual ligamentum, edema in both legs, and BP 122/84 mmHg; however, no cutaneous telangiectasia or proximal scleroderma was observed. The laboratory data on admission showed the following: proteinuria (0.5 g/day), RBCs 20–29/HPF, WBCs 20–29/HPF, hyaline cast 5–9/WF, Hb 10.7 g/dl, WBCs 6,300/μl, platelets 43.7 × 10^4/μl, serum Cr 1.6 mg/dl, CRP 5.2 mg/dl, ANA 80× speckled pattern, negative anti-ribonuclease protein (RNP) Ab, anti-scleroderma 70 Ab ×4, negative anti-centromere Ab, anti-DNA Ab (RIA assay) 1.6 IU/ml, MPOANCA 660 EU, PR3-ANCA <10 EU, anti-GBM Ab 406 EU, CH_{50} 43.5 U/ml, immune complex by C1q binding assay 2.7 μg/ml, and plasma renin activity 4.1 ng/ml/h. Chest X-rays and CT findings revealed slight pulmonary fibrosis in both lung bases. Based on these findings, she was diagnosed as having systemic sclerosis and a renal biopsy was performed for the pathological diagnosis. The pathological finding, which is described in the "Results", suggested the diagnosis of crescentic glomerulonephritis (CSGN) due to anti-GBM Ab and/or pauci-immune CSGN due to MPO-ANCA, but not scleroderma kidney disease. As a result of the diagnosis, the patient was treated with mPSL semi-pulse therapy (500 mg) for 3 successive days followed by 30 mg oral prednisolone as well as 50 mg oral cyclophosphamide per day. Although no plasma exchange was performed, MPO-ANCA and anti-GBM Ab titers became negative 6 weeks after the treatment; however, her serum

Cr level increased to 7.2 mg/day and hemodialysis was administered 4 times. While she was off hemodialysis for 5 weeks with serum Cr levels around 5 mg/dl, she eventually went into chronic hemodialysis. During the 8-year follow-up after this episode, she experienced 3 episodes of hemoptysis that were associated with the progression of anemia and re-elevated MPO-ANCA titers (512, 89, and 162 EU), but were not associated with positivity of anti-GBM Ab or increased CRP levels. The titers of MPO-ANCA remained positive for 8 subsequent years while on medication with a low dose of prednisolone of 7.5–5 mg/day.

Results

Characteristics of clinical and laboratory findings

All 4 cases had previously demonstrated pulmonary fibrosis in the lower lung fields but tests for anti-GBM Ab and ANCA were not performed at those times (Tables 1, 2); however, they showed minor urinary abnormalities, elevated CRP, and mild anemia, although one case was not available for the data of CRP and Hb (Table 1). Subsequently, three of the cases (Cases 1, 2, and 3) presented with almost simultaneous RPGN and pulmonary hemorrhage. The remaining case (Case 4) initially developed RPGN with pulmonary fibrosis but eventually developed hemoptysis with lung infiltration shadows. In addition, Case 3 presented with symptoms of peripheral neuritis, which were confirmed by the velocity test for the sensory nerve. All 4 cases demonstrated both positive anti-GBM Ab and MPO-ANCA at the time of RPGN and were diagnosed as having dual positive disease. These cases were treated with steroid therapy as well as with hemodialysis at the time of RPGN, and plasma exchange was administered in 2 patients (Cases 1 and 3). Even with these treatments, 2 patients (Cases 1 and 2) died during the acute phase of the disease (within 6 months after the diagnosis of dual positive disease), the third patient (Case 3) died of acute leukemia from myelodysplastic syndrome 4 years after the diagnosis of the disease, and the fourth patient (Case 4) is still alive and on chronic hemodialysis associated with repetitive hemoptysis.

Pathological findings

Pathological tissue specimens were observed from 2 autopsy cases, 1 renal biopsy, and 1 gastric biopsy (Table 2; Figs. 1, 2).

The autopsy tissue specimens from Case 1 were examined extensively. The kidney showed total circumferential cellular or fibrocellular crescents in 95% of the glomeruli and partial cellular crescents in the remaining 5% of the glomeruli. Tubulointerstitium (TI) disclosed periglomerular cell infiltrations with disrupted Bowman's capsules and vasculitis of the small arteries associated with interrupted lamina elastica interna, marked intimal thickening, degenerated smooth muscle cells of the media as well as perivascular inflammatory cell infiltrations, and cellular infiltration along the stripped areas (Fig. 1b, c). Similar vasculitic lesions of the small arteries were demonstrated in the gastric and intestinal walls as well as in the capsular adipose tissues of the adrenal glands. The lung revealed large phagocytic cells of hemosiderin in the alveoli and mononuclear cell infiltrations in the alveolar septa. An immunohistochemical study on the kidney showed linear immunoglobulin G (IgG) staining along the glomerular capillary loops (GCLs) (Fig. 1d) and focal alveolar septa.

Autopsy tissue specimens from Case 2 were also studied. The kidney showed total circumferential fibrocellular crescents in all glomeruli and remarkable cellular infiltrations in the interstitium. Small arteries and arterioles in the

Table 1 Clinical and laboratory data at the diagnosis of pulmonary fibrosis as well as other main features prior to rapidly progressive glomerulonephritis

	Years old	Sex	At the diagnosis of Pf in our hospital					Other main features prior to RPGN
			UA		Cr (mg/dl)	CRP (mg/dl)	Hb (g/dl)	
Case 1	78	♂	Proteinuria	(+)				Pyrexia, pneumothorax
			RBCs	20–29/HPF	0.8	8.9	13.0	
Case 2	64	♂	Proteinuria	(−)				(Pulmonary Tbc), repetitive bronchitis, hemoptysis
			RBCs	3–5/HPF	1.1	NA	NA	
Case 3	72	♂	Proteinuria	(−)				(Pulmonary Tbc), myalgia, arthralgia, numbness, hemoptysis
			RBCs	20–29/HPF	1.2	8.6	10.2	
Case 4	42	♀	Proteinuria	0.5 g/day				Raynand, SSc findings
			RBCs	20–29/HPF	1.6	5.2	10.7	

Pf pulmonary fibrosis, *UA* urinalysis, *RBCs* O–O/HPF in the urinary sediment, *NA* not available, *(pulmonary Tbc)* old past history

Table 2 Clinical and pathological features in both MPO-ANCA and anti-GBM Ab-positive cases

			Clinical data on admission				Pathology			Survival
			MPO-ANCA (EU/ml)	Anti GBM Ab (EU/ml)	Lung	Serum Cr (mg/dl)	Kidney	Lung	Angiitis	
Case 1	78	♂	74	231	pf → p.h., hemoptysis	16.1	CC 95% PC 5% IgG linear	p.h.	Kidney GI Adrenal	Death
Case 2	64	♂	74	(+)	pf → p.h., hemoptysis	14.4	CC 100% IgG linear	p.h.	Kidney	Death
Case 3	72	♂	210	38	pf → p.h., hemoptysis	16.0	NA	NA	[Neuritis, purpura GI][a]	Death (MDS)
Case 4	42	♀	660	406	pf → p.h., hemoptysis	1.6	CC 70% PC 30% IgG linear	NA	NA	Alive (on HD)

CC 95%, PC 5% indicate percentage of a circumferential or partial crescent in the total glomeruli

pf Pulmonary fibrosis, *p.h.* pulmonary hemorrhage, *CC* circumferential crescent, *PC* partial crescent, *NA* not available, *GI* gastrointestinal tract, *Death* (*MDS*) death due to MDS, not vasculitic death

[][a], only clinically

interstitium disclosed disrupted lamina elastica interna, degenerated smooth muscle cells of the media or complete destruction of the vessel wall, and perivascular inflammatory cell infiltrations (Fig. 2b). The lung demonstrated many RBCs in the alveoli and thickened alveolar septa accompanied with cell infiltrations or fibrosis. An IF study revealed linear IgG staining along the GCLs (Fig. 2c) and the focal alveolar septa of the lungs. In addition, pauci-granular C3 and C1q staining were also observed along the GCLs and some scattered C3 granular deposits on the tubular basement membranes. The patient's serum examination in the normal kidney specimens demonstrated IgG linear staining along the glomerular BM as well as on the tubular BM in addition to nuclear positivity.

The biopsy tissue specimens from the stomach in Case 3 did not show any vasculitic lesions in the specimens.

The pathology of Case 4 was evaluated in renal biopsy tissue obtained at the onset of RPGN. This specimen contained 6 glomeruli, 2 small arteries, and the tubulointerstitium. All 6 glomeruli showed 50–100% cellular crescent formation with ruptured Bowman's capsules and periglomerular inflammatory cell infiltrations. The small arteries and arterioles did not reveal any significant endothelial cell proliferation, interrupted lamina elastic interna, or fibrinoid degeneration (Fig. 2d, e). The TI tissue specimens did not demonstrate any vascular thrombosis in the arterioles and capillaries. An IF study showed linear IgG deposits along the GCLs (Fig. 2f) as well as pauci-granular C3 deposits on the GCLs of the semi-collapsed glomeruli and the intima of arterioles. Electron microscopy did not show any significant electron-dense deposits regarding the observation area.

Cases 1 and 2 were diagnosed as having anti-GBM Ab disease as well as MPO-ANCA-associated vasculitis. CSGN was presumed to be partly due to anti-GBM Ab and/or partly due to MPO-ANCA-associated vasculitis. Case 4 was also diagnosed as having anti-GBM Ab disease, and MPO-ANCA-associated CSGN was also suggested by the findings of pauci-granular C3 deposits on the capillary walls of the semi-collapsed glomeruli and the intima of arterioles, but not scleroderma kidney.

Discussion

Both MPO-ANCA-associated vasculitis and renal diseases have been studied clinically, pathologically, and serologically by our group [8, 14, 17, 30–32]. Sixty-seven MPO-ANCA-positive patients were evaluated from 1998 to 2007 and only 4 of these patients demonstrated dual positive disease. To date, the remaining 63 patients, who were treated with immunosuppressive drugs, have not developed the dual positive disease. The 4 cases presented in this study were characterized by dual positivity of MPO-ANCA and anti-GBM Ab at the time of RPGN, but tests for the antibodies were not performed before the onset of RPGN. All 4 cases had prior pulmonary fibrosis, and subsequently in 3 cases hemoptysis associated with pulmonary hemorrhage occurred almost simultaneously around the onset of RPGN. The remaining case (Case 4), who had systemic sclerosis before the onset of RPGN, was also thereafter complicated by hemoptysis and pulmonary hemorrhage. Three cases, except for Case 3, were confirmed to have linear IgG deposits along the GCLs, and in addition,