Table 9 continued

Department of Internal Medicine, Yokosuka City Hospital	Department of Internal Medicine IV, Saitama Medical Center, Saitama Medical University	Department of Urology, Oita Medical University Hospital	Hyogo Prefectural Children's Hospital	Department of Invernal Medicine, Seirei Sakura Citizen Hospital	Internal Medicine II, Nihon University Hospital
Internal Medicine II, Yokohama City University Hospital	Department of Nephrology, Saitama Children's Medical Center	Department of Pediatrics, Oita Medical University Hospital	Department of Internal Medicine, Hyogo Prefectural Amagasaki Hospital	Department of Urinology, Seirei Sakura Citizen Hospital	Department of Pediatrics, Nihon University Surugadai Hospital
Department of Pediatric, Yokohama City University Medical Center	Department of Internal Medicine II, Sapporo Medical University Hospital	Department of Pediatrics, Yamato City Hospital	Department of Internal Medicine, Toyohashi City Hospital	Department of Invernal Medicine, National Cardiovascular Center	Department of Nephrology, Mito General Hospital
Department of Urology, Yokohama Minami Kyosai Hospital	Department of Pediatric, Sapporo Medical University Hospital	Takeshita Hospital	Internal Medicine II. National Defence Medical College	National Health Center for Children's Health and Development	Department of Nephrology, Hitachi General Hospital
Internal Medicine III, Okayama University Hospital	Department of Internal Medicine, Mitsui Memorial Hospital	Department of Nephrology, Tsukuba Gakuen Hospital	Department of Nephrology, Hokkaido Kinrosha Iryo Kyokai Chuo Hospital	Department of Internal Medicine, Sendai Red Cross Hospital	Department of Pediatrics, Hakodate Goryokaku Hospital
Department of Pediatrics, Okayama University Hospital	Department of Pediatrics, Mitsui Memorial Hospital	Department of Metabolism, Nakadori Sogo Hospital	Department of Pediatrics, Hokkaido University Hospital	Department of Nephrology, Senboku Kumiai Hospital	Department of Nephrology, Hashiro General Hospital
Department of Pediatrics, Okinawa Prefectural Chubu Hospital	Department of Internal Medicine I, Mie University Hospital	Department of Nephrology, Nakagami Hospital	Internal Medicine II, Hokkaido University Hospital	Department of Internal Medicine, Nishi Clinic	Internal Medicine II, Kochi Medical School
Department of Nephrology, Okinawa Prefectural Chubu Hospital	Department of Pediatric, Mie University Hospital	Department of Internal Medicine, Chubu Rosai Hospital	Department of Internal Medicine, Kitamatsu Central Hospital	Department of Nephrology, Nishikobe Medical Center	Department of Pediatrics, Kochi Medical School
Department of Internal Medicine, Okinawa Prefectural Chubu Hospital	Department of Internal Medicine, Saiseikan, Yamagata City Hospital	Department of Internal Medicine, Nakano Sogo Hospital	Department of Nephrology, Hokushin Sogo Hospital	Department of Nephrology, Shizuoka Children's Hospital	Department of Nephrology, Asahi Chuo Hospital
Internal Medicine II, Kansai Medical University	Department of Urology, Yamagata University Hospital	Internal Medicine II, Ngasaki University Hospital	Department of Nephrology, Kitazato University Hospital	Department of Nephrology, Shizuoka Saiseikai General Hospital	Department of Nephrology, Kasumigaura Medical Center
Department of Pediatrics, Kansai Medical University	Department of Pediatric, Yamaguchi University Hospital	Internal Medicine II, Nagasaki University Hospital	Department of Pediatrics, Kitazato University Hospital	Department of Nephrology, Shizuoka City Hospital	Department of Bacteriology, National Institute of Infectious Diseases

Table 9 continued

Department of Nephrology, Kansai Rosai Hospital	Department of Nephrology, Yamamoto Kumiai Sogo Hospital	Department of Pediatrics, Nagasaki University Hospital	Department of Nephrology, Hokuriku Central Hospital	Department of Nephrology, Sendai Shakai Hoken Hospital	Department of Internal Medicine I, Kanazawa Hospital
Kidney Dialysis Center, Kanto Hospital	Department of Pediatrics, Yamanashi Medical University Hospital	Department of Internal Medicine, Nagano Red Cross Hospital	Department of Nephrology, Horinouchi Hospital	Department of Nephrology, Chiba Children's Hospital	Department of Nephrology, Kure Medical Cemter,
Department of Pediatrics, Iwate Medical University	Cardiovascular Internal Medicine at Yamanashi Prefecural Central Hospital	Department of Internal Medicine II, Tottori University Hospital	Department of Nephrology, Honjo Daiichi Hospital	Department of Pediatrics, Medical Center East, Tokyo Women's Medical University	Department of Nephrology, Takasaki Hospital
Department of Pediatrics, Iwate Prefectural Central Hospital	Department of Internal Medicine, Yamanashi Red Cross Hospital	Department of Pediatrics, Tottori University Hospital	Department of Nephrology, Iizuka Hospital	Department of Internal Medicine, Tokyo Sembai Hospital	Department of Pediatrics, International Medical Center of Japan
Department of Pediatrics, Gifu University Hospital	Department of Nephrology, University of Occupational and Environmental Health, Japan Hospital	Department of Nephrology, Teikyo University Hospital	Department of Pediatrics, Mino City Hospital	Department of Nephrology, School of Medicine and Faculty of Medicine, The University of Tokyo	Department of Nephrology, Department of Pediatrics
Internal Medicine III, Kurume University Hospital	Department of Pediatric, University of Occupational and Environmental Health, Japan Hospital	Department of Pediatrics, Teikyo University Hospital	Department of Preventive Medicine, Nagoya University Hospital	Internal Medicine II, School of Medicine and Faculty of Medicine, The University of Tokyo	Internal Medicine II, Hirosaki University Hospital
Department of Pediatrics, Kurume University Hospital	Department of Internal Medicine I, University of Occupational and Environmental Health, Japan Hospital	Department of Internal Medicine III, Teikyo University Hospital	Internal Medicine III, Nagoya University Hospital	Department of Pediatrics, School of Medicine and Faculty of Medicine, The University of Tokyo	Department of Pediatrics, Hirosaki University Hospital
Internal Medicine I, Miyazaki Medical University Hospital	Department of Internal Medicine II, University of Occupational and Environmental Health, Japan Hospital	Department of Pediatrics, Tenri Yorozu Sodanjo Hospital	Department of Internal Medicine, Nagoya University Daiko Medical Center	Department of Urology, Tokyo University Branch Hospital	Department of Pediatrics, Faculty of Medicine, Kagawa University
Department of Pediatrics, Miyazaki Medical University Hospital	Department of Nephrology, Sapporo City Hospital	Department of Nephrology, Tenri Yorozu Sodanjo Hospital	Department of Pediatrics, Nagoya Daiichi Red Cross Hospital	Department of Nephrology, Tokyo Teishin Hospital	Department of Invernal Medicine, Faculty of Medicine, Kagawa University
Department of Pediatrics. Kyoto City Hospital	Department of Internal Medicine IV, Akita City Dogo Hospital	Department of Nephrology, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital	Department of Nephrology, Nagoya Daiichi Red Cross Hospital	Department of Nephrology, Tokyo Saiseikai Chuo Hospital	Department of Nephrology, Kagawa Prefectural Chuo Hospital

Table 9 continued

Cardiovascular Science and Medicine, School of Medicine, Kyoto University	Department of Pediatric, Itoigawa Sogo Hospital	Department of Nephrology Tokyo Metropolitan Kiyose Children's Hospital	Department of Nephrology, Nagoya Daini Red Cross Hospital	Department of Nephrology, Tokyo Fuchu Hospital	Department of Internal Medicine, Takaoka City Hospital
Department of Pediatrics, Onomichi City Hospital	Department of Internal Medicine, Teraoka Memorial Hospital	Department of Pediatrics, Metropolitan Bokutoh Hospital	Department of Pediatrics, Nagoya Daini Red Cross Hospital	Department of Internal Medicine, Tokyo Rosai Hospital	Department of Urology, Takamatsu City Hospital
Department of Pediatrics, School of Medicine, Kyoto University	Department of Internal Medicine III, Shiga University of Medical Science	Department of Internal Medicine, Metropolitan Bokutoh Hospital	Department of Pediatrics, Tachikawa Sogo Hospital	Department of Nephrology, Tokyo Medical University Hachioji Medical Center	Department of Pediatrics, Showa University Hospital
Kyoto University Health Service	Department of Pediatric, Shiga University of Medical Science	Department of Internal Medicine IV, Shimane Medical University	Internal Medicine III, Ryukyu University Hospital	Department of Nephrology, Tokyo Police Hospital	Department of Internal Medicine, Showa University Fuigaoka Hospital
Internal Medicine II, University Hospital, Kyoto Prefectural University of Medicine	Department of Nephrology, Jichi Medical University	Department of Pediatrics, Shimane Medical University Hospital	Department of Pediatrics, Ryukyu University Hospital	Internal Medicine II, The Jikei University	Department of Nephrology, Showa University Hospital
Department of Pediatrics, University Hospital, Kyoto Prefectural University of Medicine	Department of Pediatric, Jichi Medical University	Department of Nephrology, Shimada Memorial Hospital	Internal Medicine III, Wakayama Prefectural Medical University Hospital	Department of Pediatrics, The Jikei University	Department of Nephrology, Kamitsuga General Hospital
Internal Medicine III, Kinki University Hospital	Department of Nephrology, Saitama Medical Center, Jichi Medical University	Department of Nephrology, Tokai University Hospital	Department of Pediatrics, Wakayama Prefectural Medical University Hospital	Department of General Medicine, The Jikei University Kashiwa Hospital	Shinrakuen Hospital
Department of Pediatrics, Kinki University Hospital	Department of Pediatric, Kagoshima City Hospital	Department of Pediatrics, Tokai University Hospital	Department of Nephrology, Komatsu City Hospital	Internal Medicine IV, Tokyo Women's Medical University Hospital	Department of Pediatrics, Juntendo University Hospital
Department of Nephrology, Kanazawa Medical University	Department of Internal Medicine II, Kagoshima University Hospital	Department of Internal Medicine VII, Tokai University Oiso Hospital	Department of Pediatrics I, Dokyo Medical Unviersity Hospital	Department of Urology Kidney Center, Tokyo Women's Medical Unviersity	Department of Internal Medicine III, Gumma University Hospital
Department of Pediatrics, Kanazawa Medical University	Department of Pediatrics, Shakaihoken Chukyo Hospital	Department of Internal Medicine II, Tokyo Medical and Dental University	Department of Nephrology, Tsukuba University Hospital	Department of Internal Medicine, School of Medicine, Keio University	Department of Nephrology, Nihon Red Cross Medical Center

Table 9 continued

Internal Medicine I, Kanazawa University Hospital	Toride Kyodo Hospital	Department of Nephrology, Tokyo Medical University	Internal Medicine III, Hiroshima Red Cross Hospital & Atomic-Bomb Survivors Hospital	Department of Pediatrics, School of Medicine, Keio University	Department of Nephrology, Juntendo University Hospital
Department of Pediatrics, Kanazawa University Hospital	Department of Nephrology, Akita Kumiai Sogo Hospital	Department of Pediatrics, Tokyo Medical University	Internal Medicine II, Hiroshima University Hospital	Department of Pediatrics, Gunma University Hospital	Department of Pediatrics, Kumamoto Central Hospital
Department of Blood Purification Therapy, Medical School of Kanazawa University	Department of Internal Medicine III, Akita University Hospital	Department of Nephrology, Tokyo Medical University Kasumigaura Hospital	Department of Pediatrics, Hiroshima University Hospital	Department of Nephrology, Toranomon Hospital	Department of Pediatrics, Onomichi City Hospital
Internal Medicine II, Kyushu University Hospital	Department of Pediatrics, Akita University Hospital	Department of Nephrology, Kensei Sogo Hospital	Department of Nephrology, Showa University Fujigaoka Hospital	Department of Pediatrics, Toranomon Hospital	
Department of Pediatrics, Kyushu University Hospital	Department of Nephrology, Akita Rosai Hospital	Department of Nephrology, Hara Urological Clinic	Department of Nephrology, Matsuyama Red Cross Hospital	Department of Pathology I, Shinshu University School of Medicine	
Internal Medicine III, Kumamoto University Hospital	Department of Pediatrics, Sumitomo Hospital	Department of Nephrology, Koga Hospital	Department of Pediatrics, Matsuyama Red Cross Hospital	Department of Nephrology, Shizuoka City Hospital	
Department of Pediatrics, Kumamoto University Hospital	Department of Pediatrics, Shigei Medical Research Center Hospital	Department of Nephrology, Showa Hospital	Internal Medicine II, Shinshu University Hospital	Department of Nephrology, Sendai Shakai Hoken Hospital	
		Department of Pediatric Nephrology, Osaka Medical Center and Research Institute for Maternal and Child Health			

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ORIGINAL ARTICLE

Classification of clinical subtypes, patient survival, kidney prognosis, and relapse in patients with MPO-ANCA-associated vasculitis: a single-center experience

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Abstract Myeloperoxidase-type antineutrophil cytoplasmic antibody (MPO-ANCA)-associated vasculitis may manifest various organ symptoms. Treatment allows recovery from early, but severe, organ involvement. However, the relationship between the initial organ involvement and the eventual clinical course has not been studied in this disease. Therefore, the current study evaluated 30 patients who were hospitalized and then categorized into ten clinical subtypes based on organ involvement. The relationship of these subtypes to development of clinical features, patient survival, kidney prognosis, and relapse were evaluated over an average observation period of 4.3 years. During this study, the most common clinical features were lung and kidney involvement. Twenty-one patients already manifested clinical features around the time of admission and did not commonly present new symptoms as long as they were receiving the treatment for vasculitis. In contrast, as far as pulmonary involvement type at the initial time was concerned and in those not being treated for vasculitis, 7 of the 12 patients progressed to pulmo-renal involvement and 5 of them went onto renal failure. Progression to renal failure also occurred frequently in patients with pulmo-renal type manifesting at the initial time. Thirteen patients died, including three patients due to vasculitis of systemic type, seven due to infections, and three due to malignancy. Death due to vasculitis occurred in the early phase of treatment and was associated with either pulmonary hemorrhage or gastrointestinal bleeding. Infectious death occurred throughout the entire course of treatment, mostly in patients with pulmo-renal or pulmonary type, and tended to be associated with opportunistic organisms. Death with malignancy was observed after several years of treatment. Regarding renal prognosis, ten patients underwent hemodialysis. At initiation of hemodialysis, nine patients had pulmo-renal type and only one had renal type. A relapse was observed in ten patients, mainly in patients with pulmo-renal or pulmonary type, and it occurred after about 2.7 years, even with treatment. Such relapses manifested in a similar manner to their initial clinical subtypes. These results suggest that pulmo-renal type as well as pulmonary type have a high chance to progress to renal failure or systemic type, and they were fairly commonly associated with vasculitic or infectious death. Therefore, classification of clinical subtypes at the initial time and on admission is meaningful to some extent for predicting patient survival, kidney prognosis, and relapse, in addition to indicating the appropriate treatment regimen.

Keywords MPO-ANCA · Vasculitis · Prognosis · Relapse · Clinical subtype

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Introduction

MPO-ANCA-associated vasculitis mainly affects capillary and induces necrotizing capillaritis in lung and kidney involvement with high frequency [1, 2]. However, capillaries exist throughout all human organs, and capillaritis demonstrates various organ manifestations such as cardiac, gastrointestinal, ears, nose, throat, eyes, muscle/joint, cutaneous, and central nervous system (CNS) features as

well as peripheral neuropathy in addition to lung and kidney involvement [2, 3]. When the patients are seen at the time that MPO-ANCA-associated vasculitis is diagnosed, they do not necessarily present with the full-blown manifestations in all organs. The relationship between the clinical manifestations and patient survival in this disease has been described in some previous reports; for example, the important prognostic factors for patient survival have been described to be pulmonary hemorrhage, gastrointestinal bleeding or perforation, brain hemorrhage or thrombosis, and myocardial infarction [2, 4, 5]. Kidney prognosis has also been reported to be associated with percentage of crescent formation, increased number of sclerotic glomeruli, widening of tubulointerstitial fibrosis, and patient age [6–8]. However, these reports did not pay much attention to the relationship of early manifestation of the disease with the eventual clinical development as well as patient survival. Under these circumstances, hospitalized patients at our university hospital, who were thoroughly evaluated historically, physically, and based on laboratory investigations on admission, were categorized into ten clinical subtypes and then followed up over more than 2 years for eventual patient survival, kidney prognosis, and relapse. After making a diagnosis, the patients were treated with the recommended treatment as suggested by the Research Committee for Refractory Vasculitis Syndrome [9]. The results of the study disclosed some interesting new relationships between the classification of clinical subtypes and patient survival, kidney prognosis, as well as relapse in Japanese patients with MPO-ANCA-associated vasculitis.

Materials and methods

Study patients

Thirty patients, who were hospitalized in the First Department of Internal Medicine, Kyorin University, from 1 January 2000 to 31 December 2004 and were subsequently followed up for more than 2 years, were enrolled in this study. Any patients that died during the course of the study were excluded at that time, but their data prior to death were used for the clinical analysis. This study was ended on 31 December 2006. Patient ages ranged from 54 to 91 years old and their mean age was 69.2 years. The male-to-female ratio was 9:21. In addition to obtaining the precise history of their illness, urinalysis and serum creatinine level data were confirmed using health check sheets. Furthermore, if the patients had been previously treated by their family physicians, then all records of physical examination, chest X-ray, and blood chemistry were obtained for this study.

Diagnosis and the time interval for diagnosis

Diagnosis

Because of the difficulty in obtaining biopsy tissue specimens for the diagnosis of vasculitis from every patient, the diagnosis for MPO-ANCA-associated vasculitis was dependent on the positivity of MPO-ANCA as well as the existence of organ involvement due to vasculitis. The MPO-ANCA test was performed by the enzyme-linked immunoassay reagent produced by Nipro Company (Shiga, Japan) [10]. The common vasculitis symptoms and findings in each organ are listed in the Appendix. However, small-vessel vasculitic diseases of the connective tissues disorder such as systemic lupus erythematosus, Henoch-Schönlein purpara, Churg-Strauss syndrome, cryoglobulinemia, Wegener's granulomatosis, Sjögren syndrome, and Goodpasture syndrome were excluded from this study based on all of the diagnostic criteria as well as no apparent positivity of MPO-ANCA in these diseases.

The time interval for diagnosis

This was defined as the number of months or years between the onset of the initial organ symptoms and the diagnosis of MPO-ANCA-associated vasculitis. Because it was difficult to obtain a clear-cut history of the illness for most of the patients, the time interval after the first symptoms was estimated to be as follows: 0 months when the diagnosis was made less than 3 months later, 0.5 years when the diagnosis was made 4–9 months later, 1 year when the diagnosis was made 9–12 months later, and the required years when the diagnosis was made more than 1 year later.

Initial organ involvement and the definition of the observation period

Initial organ involvement was determined as the major clinical symptom and/or laboratory data which necessitated the patient to be seen by their physicians on at least three consecutive occasions. However, constitutional symptoms such as fever, fatigue or weight loss were excluded in the determination of initial organ involvement, because there were no organ-related symptoms. The definition of the observation period was the length of time that the patient was observed without receiving any appropriate treatment for vasculitis, calculated on a year(s) basis.

Clinical subtypes and their development during the observation period, and the clinical subtypes on admission

The clinical subtype of the patients was classified into pulmonary, renal, gastrointestinal, cardiovascular, CNS,

peripheral neuritis, musculo-articular, ear-nose-throat-eye (ENTE), dermatologic or systemic type. Systemic type included patients who had lesions in at least three vital organs. The vital organs included the lung, heart, kidney, gastrointestinal tract including the pancreas, gallbladder as well as liver, and CNS. As patients with both pulmonary and kidney involvement were frequently observed, the pulmo-renal type was tentatively added to the original ten clinical subtypes. If the patient manifested new clinical subtypes during the observation period, then this was defined as having developed a new type of disease. Clinical subtypes on admission were also evaluated and recorded for the study.

Patient survival and kidney prognosis

Patient survival was determined by the death of patients, and causes of death were analyzed by clinical features and laboratory data. Kidney prognosis was determined by reaching end-stage renal disease necessitating hemodialysis. The data regarding the patient survival are shown in the life table analysis.

Relapse

A relapse was defined as when at least one of the following occurred after the subsidence of initial organ symptoms with the treatment [5, 11, 12]:

(1) Repetitive urinalyses showing proteinuria and/or red blood cells (RBCs) in the sediment increased by more than two times, (2) a rapid rise in the serum creatinine level without any other cause for the deterioration of renal function, (3) hemoptysis, pulmonary hemorrhage or new expanding shadows by X-ray and/or computed tomography or (4) new appearance or reappearance of symptoms belonging to the remaining other organs described in the Appendix.

These symptoms were mostly accompanied by elevated C-reactive protein (CRP), but not always in the study. Infection-associated similar these symptoms were ruled out by the refractory findings to the treatment with various antibiotics, antifungal drugs, and antiviral agents.

Treatment

The recommended regimen was introduced to the patients based on the Research Committee of Refractory Vasculitis Syndrome sponsored by the Ministry of Health, Welfare, and Labor of Japan [9]. Specifically, steroid pulse therapy was introduced to patients complicated by either pulmonary hemorrhage, rapidly progressive glomerulonephritis, gastrointestinal bleeding (not occult bleeding), progressive pulmonary fibrosis or CNS involvement. Other patients were treated with 0.6–0.8 mg/kg body weight oral prednisolone per day. In addition, patients treated with steroid pulse therapy first were subsequently treated with the same dosage of oral prednisolone (PSL) as the other patients. The oral PSL dosage was gradually tapered off according to improvement of clinical symptoms and laboratory data. Oral immunosuppressants such as cyclophosphamide (CY), azathioprine (AZP), mizoribine (MZB), and methotrexate (MTX) were added according to the severity of the disease. However, none of these agents were intravenously administered during this study.

Results

The time interval for diagnosis

The time interval for diagnosis was 0 months for 12 patients, 0.5 years for 9 patients, and more than 2 years for 9 patients. The time intervals ranged from 0 months to 11 years, and the average interval was 2.6 years. The patients diagnosed to have MPO-ANCA-associated vasculitis more than 2 years after the initial organ involvement included eight with pulmonary fibrosis and one with nephritic urinalysis. These patients were observed for several years without a diagnosis of vasculitis.

Initial organ involvement and the observation period

Initial organ involvement was pulmonary type in 12 patients, renal type in 5 patients, pulmo-renal type in 7 patients, peripheral neuritis type in 3 patients (2 associated with additional slight musculo-articular symptoms and 1 associated with additional trace proteinuria and hematuria), musculo-articular type in 1 patient, ENTE type in 1 patient, and systemic type (lung, kidney, and gastrointestinal involvement) in 1 patient. No patients with dermatologic type, CNS type or cardiovascular type were observed in this study. The observation periods of the study patients ranged from 2 to 14 years and the average observation years was 4.3 years.

Development of clinical subtypes during the observation period and time of development (Table 1)

Two groups were observed in the study. One group continued to demonstrate the same clinical subtype whereas the other group showed new organ symptoms. The group demonstrating the same clinical subtype included 21 patients in total: 7 patients with pulmo-renal type, 5 patients with pulmonary type, 4 patient with renal type, 3 patients with peripheral neuritis type (2 patients with additional

Table 1 Classification of the initial clinical subtypes and their subsequent development during the observation period (observation more than 2 years, average 4.3 years)

Initial clinical subtype ($n = 30$)	Subsequent clinical subtype during the observation period [development $n = 9$, nondevelopment $n = 21$]
Pulmonary $n = 12$	Pulmonary $n = 5$
	Pulmo-renal $n = 5$
	Systemic $n = 2$
Renal $n = 5$	Renal $n = 4$
	Pulmo-renal $n = 1$
Pulmo-renal $n = 7$	Pulmo-renal $n = 7$
Peripheral neuritis + α $n = 3$	Peripheral neuritis + α $n = 3$
Musculo-articular $n = 1$	Musculo-articular $n = 1$
ENTE $n = 1$	*ENTE plus pulmo-renal $n = 1$*
Systemic $n = 1$	Systemic $n = 1$

Patients with nondevelopment $n = 21$ (70%)

Patients with development (italicized) $n = 9$ (30%)

α, musculo-articular or mild nephritis

musculo-articular symptoms and 1 patient with nephritic urinalysis), 1 patient with musculo-articular type, and 1 patient with systemic type. In contrast, the group demonstrating new organ symptoms included five patients with initial pulmonary type changing into pulmo-renal type, two patients with initial pulmonary type changing into systemic type, one patient with renal type changing into pulmo-renal type, and one patient with ENTE type changing into ENTE plus pulmo-renal type. The time of development ranged from 2 to 14 years and the average time was 5.1 years.

Classification of clinical subtypes, numbers of patients, and involved organs on admission (Fig. 1)

Our patients consisted of 5 patients with pulmonary type, 4 patients with renal type, 13 patients with pulmo-renal type, 3 patients with peripheral neuritis type (2 patients with additional musculo-articular symptoms and 1 patient with nephritic urinalysis), 1 patient with musculo-articular type, 1 patient with ENTE plus pulmo-renal type, and 3 patients with systemic type. These data and the total involved organs of all patients are shown in Fig. 1.

Patient survival (Figs. 2, 3)

Death occurred in 13 patients. The causes of death, as well as the incidence and time of death, are shown in Figs. 2 and 3. Of these patients, three were diagnosed as having died of vasculitis, of whom two had systemic type transformed from pulmonary type. Those two patients died due to either sigmoid bleeding or pulmonary hemorrhage. The third patient died of pulmonary hemorrhage from systemic type. The observed bleeding and hemorrhaging were due to the vasculitis itself with the following evidence. The sigmoid bleeding was confirmed by colon fiberscope which demonstrated fresh hemorrhaging from multiple deep small ulcers in the intestinal wall. Pulmonary hemorrhaging was diagnosed with hemoptysis as well as the findings of chest X-ray and computed tomography, which revealed tiny alveoli-filling high-density shadows. These findings are quite compatible with vasculitis in nature. These deaths occurred, respectively, at 2, 2, and 6 months after the diagnosis of vasculitis, even though steroid treatment was

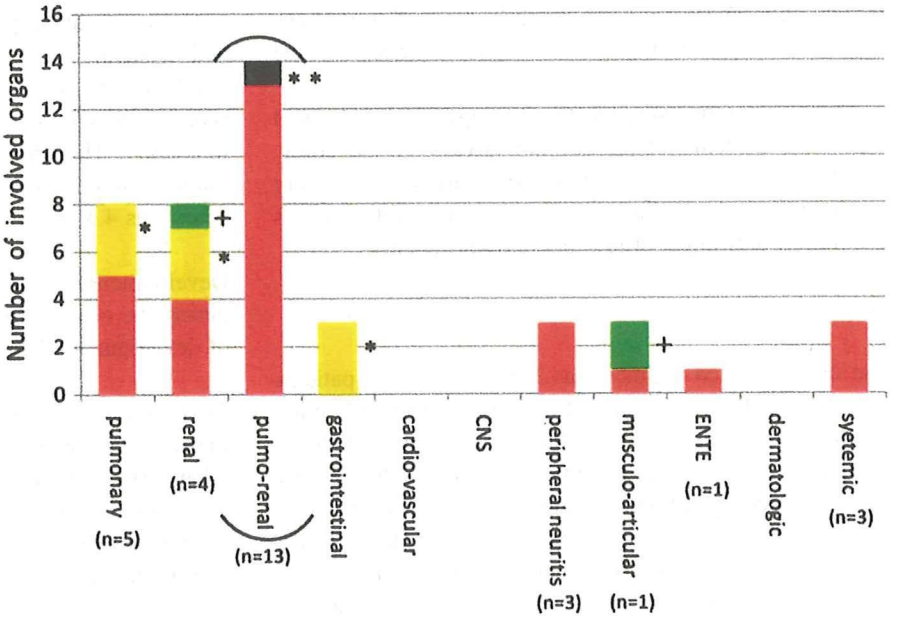

Fig. 1 Classification of clinical subtypes, number of patients, and the involved organs on admission. *Red colored columns* indicate the number of patients with classification of clinical subtypes. *Yellow colored columns* (*single asterisk*) mean the involved organs in systemic type. A *black colored column* (*double asterisks*) shows an additional organ involvement of pulmo-renal tissues in addition to ENTE type. *Green colored columns* (*plus symbols*) are an additional organ involvements of musculo-articular or nephritic symptoms in addition to peripheral neuritis type

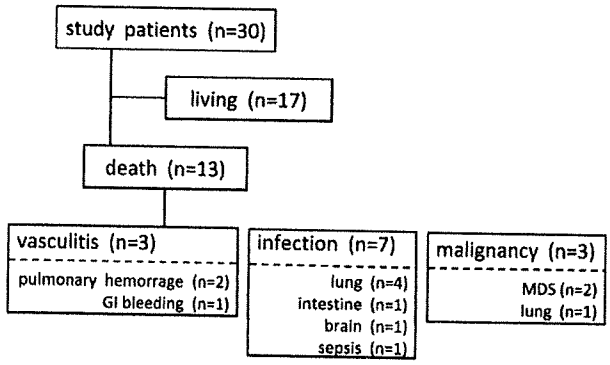

Fig. 2 Causes and number of deaths due to three causes

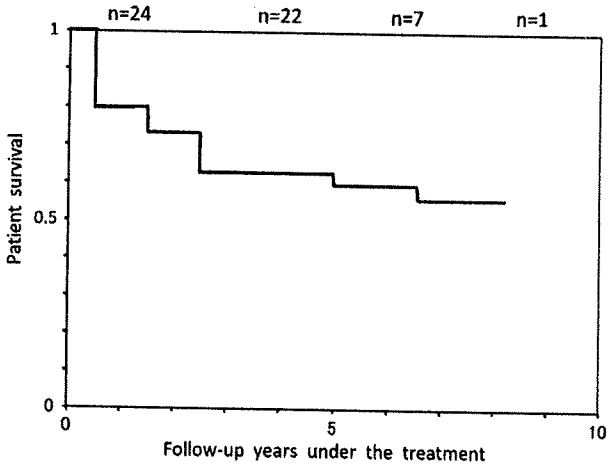

Fig. 3 Table analysis of patient survival. The numbers (for example, $n = 24$) by each *point* indicate the numbers available for analysis by that time

administered to the patients. Death due to infection was observed in seven patients, consisting of four patients with pulmo-renal type, two with pulmonary type, and one with renal type. The focus of these infections was the lungs in four patients, the gut in one, the brain in one, and sepsis in one. The pathogenetic microbes were cytomegalovirus in two patients, *Aspergillus* in two, *Pneumocystis jirovecii* in one, methicillin-resistant *Staphylococcus aureus* in one, *Pseudomonas aeruginosa* in one, and *Mycobacterium avium* in one. Of these infections, one patient had a complicated infection of both *Aspergillus* and cytomegalovirus. These infections occurred from 1 month to 5 years after the postdiagnosis treatment of vasculitis. The infections in three patients were demonstrated within 3 months, whereas in the remaining four patients the infections were detected after 6 months. All of these seven patients died 2–6 months after the occurrence of infections. Death due to malignancy was observed in three patients (two with myelodyscrasia syndrome and one with lung carcinoma), two of whom had pulmo-renal type and one pulmonary type. Two of these patients had received long-term administration of immunosuppressant drugs prior to diagnosis of malignancy, while the remaining patient did not receive any of these drugs.

Kidney prognosis

Ten patients developed chronic renal failure and required hemodialysis. The clinical subtypes of these patients when they underwent hemodialysis were pulmo-renal type in three patients, pulmonary type initially subsequently progressing to pulmo-renal type in five, renal type in one, and systemic type in one. The incubation period to hemodialysis after the diagnosis of vasculitis was within 3 months in nine patients, and 3 years in one patient. The interval from the initial organ involvement to hemodialysis was within 3 months in three patients, 1.5 years in one patient, and more than 3 years in six patients. Another 11 patients had kidney involvement but did not demonstrate end-stage renal disease at the end of the study.

Relapse

Ten patients relapsed, including six patients with pulmo-renal type, three patients with pulmonary type, and one patient with renal type. Time to relapse ranged from 1 to 5 years after diagnosis of vasculitis, and the average time was 2.7 years. All of these patients presented similar symptoms to the original clinical subtypes at time of relapse, except for one patient who showed additional symptoms of CNS involvement (meningitis).

Discussion

Thirty patients, who were hospitalized from 1 January 2001 to 31 December 2004 were observed to evaluate their initial organ involvement, development of clinical features, clinical manifestations on admission, patient survival, kidney prognosis, and relapse. The classification of ten clinical subtypes in addition to pulmo-renal type was applied to the study patients for the analysis of the above-mentioned events. The results disclosed that, when the initial organ involvement excluding constitutional manifestations were evaluated, pulmonary manifestations were found in 20 patients (66.7%), the most common symptom. Renal involvement was observed in 13 patients (43.3%) and was the second most common. The other initial organ involvements included peripheral neuritis in three patients (10.0%), muscle-articular ache in one patient (3.3%), ENTE features in one patient (3.3%), and systemic

vasculitis in one patient (3.3%). These data suggest that lung and kidney symptoms are the most common manifestation in this disease, even in the early phase of the disease, as partly described in the literature [1–3]. The time interval from the first organ symptoms to the diagnosis was within 3 months in 12 patients (40%), within 1 year in 9 patients (30%), and more than 2 years in 9 patients (30%). Accordingly, two-thirds of the patients were diagnosed within 1 year of the initial organ symptoms, whereas the patients with pulmonary fibrosis, but who were not associated with any other organ symptoms, were not diagnosed more than 2 years after the initial pulmonary manifestation. Therefore, when any type of pulmonary fibrosis is found, MPO-ANCA should be considered for the etiologic diagnosis.

Development of clinical subtypes during the observation period was also studied over an average period of 4.3 years. Twenty-one patients (70%) remained in the same clinical subtypes, whereas nine patients (30%) added new clinical symptoms during the observation period. The five patients with development showed pulmonary type initially and eventually progressed to pulmo-renal type. The remaining two patients, who manifested pulmonary type initially, subsequently progressed to systemic type, adding kidney and gastrointestinal symptoms. The other two patients, who had renal or ENTE type at first, eventually transformed to pulmo-renal type or ENTE plus pulmo-renal type, respectively. These developments of clinical features during the observation period mean that pulmonary type frequently progresses to pulmo-renal or systemic type, but the other types rarely go into the other types over approximately 4 years of observation. No reports have mentioned this kind of phenomenon in the literature to data, although a few articles discuss in which type of pulmonary involvement has poor prognosis for patient survival [2, 4, 12]. The clinical manifestations on admission were almost the same as the developmental findings of clinical features during the observation period, because all patients were hospitalized around the time of clinical development.

Patient death in this study was found to be related to vasculitis, infections or malignancy. Vasculitic death occurred in patients with systemic type and within 6 months and was due to pulmonary hemorrhage in two patients and gastrointestinal bleeding in one patient. Death due to infection was observed in seven patients; the pathogenetic organisms were opportunistic in nature. These infectious deaths were observed in four patients with pulmo-renal type and two patients with pulmonary type. These infections occurred within 3 months of vasculitis treatment or between 6 months and 5 years after the treatment. Death due to malignancy was observed in two patients with myelodyscrasia syndrome and one with pulmonary carcinoma. These deaths were in two patients with pulmo-renal type and one patient with pulmonary type. These data imply that early postdiagnosis death is associated with vasculitis itself, that infectious death occurring at various times is opportunistic and is associated with the steroid treatment as well as the old age of the patients, and that death due to malignancy is related to aging but not to treatment. These observations are closely consistent with previous reported findings [2, 4, 5, 12, 13]. However, these data were mostly reported from Europe or the USA, except for one article from our country [13].

Regarding kidney prognosis, one-third of the patients (ten patients) went onto hemodialysis and nine of them were of pulmo-renal type at the time of hemodialysis. The start of hemodialysis in nine patients was within 3 months after the diagnosis. However, after initial organ involvement, three patients needed hemodialysis within 3 months, while six patients required it after more than 3 years. These six patients, who did not require hemodialysis for more than 3 years, showed pulmonary fibrosis as the initial organ involvement and eventually developed to pulmo-renal type, resulting in end-stage renal failure. These features suggest that the pulmo-renal type has a high incidence of progression to renal failure, as is partly supported by the findings of a previous analytical article which was published in the *Journal of Clinical and Experimental Nephrology* by the Study Group for Rapidly Progressive Glomerulonephritis in Japan [14].

A relapse was observed in ten patients, who mainly had pulmo-renal or pulmonary type. Relapse occurred about 2.7 years after the diagnosis of vasculitis and most of the relapses were manifested with similar symptoms to the original clinical subtypes. The relapse time and rate were almost the same as in data described in previous articles [5, 11]. This similarity between relapse features and previous manifestations was also documented in other studies [2, 12]. These recurrent appearances of similar features in relapse are in accordance with the study of MPO epitopes analysis in MPO-ANCA vasculitis, which suggests the existence of different MPO epitopes in the different clinical subtypes [15].

Based on these analytical data, we can conclude that this classification of clinical subtypes at the initial time and on admission is meaningful to some extent for predicting patient survival, kidney prognosis, and relapse in the future as well as to help to identify the appropriate treatment.

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Appendix

Common vasculitis symptoms and findings in each organ.

1. Pulmonary: hemoptysis, pulmonary hemorrhage, interstitial pneumonitis, interstitial fibrosis
2. Renal: rapidly progressive glomerulonephritis, glomerulonephritis (proteinuria and/or RBCs in the sediment)
3. Gastrointestinal: abdominal pain with bloody stool or moderate—strong occult blood in the stool, epigastric pain or right upper quadrant pain associated with increased serum amylase and/or elevated liver enzymes
4. Cardiovascular: angina pectoris, myocarditis, myocardial infarction, pericarditis, aortitis
5. CNS: infarction, hemorrhage, meningitis
6. Peripheral neuritis: mononeuritis multiplex
7. Musculo-articular: muscle pain/tenderness, arthralgia, arthritis
8. ENTE: E, otitis media refractory to antibiotics, sudden onset of inner ear deafness; N, refractory rhinitis with epistaxis and/or nonpathognomonic bacterial growth purulent discharge; T, ulcer in the ororhinopharyx or larynx; E, episcleritis, iritis, uveitis, retinitis
9. Dermatologic: palpable purpura, ecchymosis, skin ulcer
10. Systemic type: involving more than three vital organs. Vital organs include lung, kidney, gastrointestinal tract, gallbladder, pancreas, liver, heart, CNS.

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ORIGINAL ARTICLE

Tubulointerstitial nephritis without glomerular lesions in three patients with myeloperoxidase-ANCA-associated vasculitis

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Abstract
Background Myeloperoxidase–antineutrophil cytoplasmic antibody (MPO–ANCA)-associated vasculitis frequently induces crescentic glomerulonephritis. However, a few cases have so far been reported to have only tubulointerstitial (TI) nephritis without any apparent glomerular lesions. We recently treated three similar cases. Therefore, their pathological features as well as clinical manifestations were studied in detail.
Methods The pathological study was performed with immunohistochemical staining using various antibodies to the vascular endothelial cell surface markers, von Willebrand factor, type IV collagen, cytokeratin, E-cadherin, and MPO in addition to the routine histochemical examination.
Results The study disclosed the loss of CD34 endothelial cell surface markers with and without the destruction of type IV collagen (capillary basement membrane) in the peritubular capillaries, even though the glomeruli showed good staining of these factors. Electron microscopy showed breaks in the capillary basement membrane. The loss of CD34 staining was associated with the infiltration of a few mononuclear cells and neutrophils in the lumen of peritubular capillaries and the surrounding interstitial tissues. The cytokeratin staining in the tubular epithelial cells was also diminished around these areas. Tubulitis was demonstrated with or without the destruction of the tubular basement membrane. The clinical manifestations of these three cases were only a few red blood cells and granular casts in the urinary sediment as well as slightly increased β2-microglobulin in the urine, but no proteinuria.
Conclusion Based on these findings, the loss of CD34 vascular endothelial markers occurs in the early phase of the disease because of the MPO, which is presumed to have burst out from the infiltrated, activated neutrophils. This MPO, which releases proteolytic enzymes and radical oxygen species, acts on tissue destruction, namely the lysis of endothelial cell membranes as well as vascular basement membranes in the peritubular capillary. This mechanism eventually proceeds to the destruction of the peritubular capillary walls (vasculitis). This pathogenesis is thought to play an important role in the pathogenesis of TI nephritis, which is associated with MPO–ANCA vasculitis.

Keywords Tubulointerstitial nephritis · MPO–ANCA · Peritubular capillaritis · Tubulitis

Introduction

Myeloperoxidase–antineutrophil cytoplasmic antibody (MPO–ANCA)-associated vasculitis is a disease that mainly affects the capillaries and commonly induces necrotizing vasculitis in the lung and kidney. The typical histopathology of the kidney is crescentic glomerulonephritis and is usually associated with the tubulointerstitial (TI) lesions to some extent. Most of these TI lesions in this

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disease are thought to be due to secondary phenomena resulting from the rupture of Bowman's capsule associated with crescent formation, arteriolitis/arteritis, and venulitis in the interstitium, tubulitis, or the release of cytokines by the infiltrated inflammatory cells in the interstitium [1, 2]. However, the exact role of vasculitis in the peritubular capillaries, which is presumed to occur in MPO-ANCA-associated vasculitis and to play an important role in the pathogenesis of TI lesions, has not been described in the literature outside of several cases complicated both by possible drug-induced MPO–ANCA positivity and TI nephritis [3–6]. These reports as well as the other presentations still did not describe the exact existence of peritubular capillaritis and its role in the pathogenesis of TI nephritis [1–8]. In addition, the lysis of the tubular basement membrane in the place of tubulitis, which is not accompanied by secondary TI nephritis due to the above-mentioned causes (the rupture of Bowman's capsule, etc.) in this disease, also has not been previously reported in the literature, except for one article [9]. Therefore, three cases that showed TI nephritis without any apparent glomemlar lesions in patients with MPO-ANCA positivity were studied. The tissue specimens obtained from kidney biopsies were investigated by immunohistochemical staining using antibodies (Abs) to the endothelial cell surface markers, von Willebrand factor, type IV collagen, cytokeratin, E-cadherin, and MPO in addition to the routine histochemical staining. The results suggest that peritubular capillaritis as well as tubulitis plays an important role in the pathogenesis of TI nephritis due to MPO-ANCA-associated kidney disease.

Case reports

Case 1

An 83-year-old female developed anorexia and low grade fever in late May 2006 and received antibiotics from her physician, but there was no improvement of the symptoms. Thereafter, increased CRP 10.2 mg/dl and leukocytosis 14,500/μl were found, and the patient was eventually admitted to our university hospital on 29 June 2006. A physical examination on admission showed her BP to be 159/60 mmHg, but there were no other positive findings. The laboratory data were as follows: hemoglobin (Hb) 7.9 g/dl, white blood cells (WBC) 22,800/μl, platelets 36.7 × 10^4/μl, C-reactive protein (CRP) 10.2 mg/dl, total protein 5.0 g/dl, serum albumin 2.0 g/dl, blood urea nitrogen 28.6 mg/dl, serum creatinine 1.6 mg/dl, MPO–ANCA 65 EU, ANA 320 × homogeneous pattern, CH_{50} 54.0 U/ml, and rheumatoid factor 550 IU/ml. Urinalysis showed negative proteinuria and red blood cells (RBCs) 0–1/high power field (HPF) in the sediment, but was accompanied by 30–49 granular casts in the whole fields. In addition, the urinary β_2-microglobulin at urine pH 7.0 was increased to 809 μg/l (<300 μg/l). The patient underwent a renal biopsy based on these abnormal urinary findings.

Case 2

A 73-year-old male noticed fever (38.6°C), cough, and sputum, and was diagnosed to have pneumonia by his family physician in February 2006. He received a bactericidal drug that relieved the symptoms. However, he developed anorexia, myalgia in his thighs and calves, ankle arthralgia, pyrexia (37–38°C), and weight loss (7.0 kg) in March 2006. These symptoms persisted, and at this time he was diagnosed to have polymyalgia rheumatica based on the above-described symptoms as well as increased CRP 8.2 mg/dl and negative ANA. Therefore, 20 mg per day of prednisolone was prescribed on 4 July 2006, but the patient showed positivity for MPO–ANCA 289 EU. He was admitted to our university hospital on 31 July 2006, because MPO-ANCA-associated vasculitis was suspected. A physical examination on admission showed his BP was 163/94 mmHg and body weight 54.0 kg (previously 61.0 kg), but there was no muscle tenderness or leg edema. His laboratory data were as follows: urinalysis was negative for proteinuria, RBCs 1–4/HPF and granular casts 1–4/HPF in the sediment, and β_2-microglobulin at urine pH 6.5 was 231 mg/l at 32 days after prednisolone therapy. The other blood tests revealed WBC 11,600/μl, serum creatinine 0.7 mg/dl, CRP 3.9 mg/dl, MPO–ANCA 140 EU, negative ANA, negative anti-Jo-1 antibody, CH_{50} 74.7 U/ml, rheumatoid factor 62 IU/ml, and KL-6 272 U/ml. The patient underwent a renal biopsy based on these abnormal urinary findings and the history of present illness.

Case 3

A 62-year-old female had been treated by her family physician with 1.25 mg/day glibenclamide for non-insulin-dependent diabetes mellitus since 1995. In August 2000, she developed a cough and low grade fever, and received medication from her physician, but had no improvement of these symptoms. Thereafter, she visited another hospital and was found to have leukocytosis 10,200/μl and CRP 15.8 mg/dl. Subsequently, she was admitted to this hospital, and several antibiotics were prescribed, but no improvement was observed in her symptoms or laboratory data. Her body temperature increased to 38–39°C and persisted. Therefore, she was transferred to our university hospital on 10 November 2000 for further evaluation. A physical examination on admission showed weight loss

from 59.0 to 53.0 kg, muscle tenderness in both the upper arms and shoulder areas as well as the calves, motion arthralgia in both elbow and shoulder joints, crackles in the bilateral chest bases, hypoparesthesia in both legs and the radial side of the right forearm, and leg edema. Laboratory data revealed the following: urinalysis showed negative proteinuria, (+) glucosuria, RBCs 1–4/HPF and granular casts 1–4/HPF in the sediment, and urinary β_2-microglobulin 407 μg/l at urine pH 6.5. The blood tests were Hb 7.7 g/dl, WBC 9,400/μl, platelet 30 × 10^4/μl, total protein 6.3 g/dl, serum albumin 2.3 g/dl, serum creatinine 0.7 mg/dl, creatine phosphokinase 32 IU/ml, CRP 9.4 mg/dl, CH_{50} 43.3 U/ml, rheumatoid factor 422 IU/ml, MPO–ANCA 43 EU, ANA ×320, negative anti-Jo-1 antibody, KL-6 257 U/ml, anti-ds DNA antibody 7 IU/ml, immune complex by C_1q binding assay 3.0 μg/ml, and HbA1c 4.7%. Further study disclosed simple diabetic retinopathy, negative gallium scintigraphy of the total body, and negative results for blood cultures for bacteria and fungi. MPO–ANCA-associated vasculitis was highly suspected because of her myalgia, arthralgia, weight loss, fever, peripheral neuropathy, increased CRP and MPO–ANCA, and negative findings for blood cultures and gallium scintigraphy. Therefore, the patient was persuaded to undergo a renal biopsy for evaluation of vasculitic manifestations as well as the severity of diabetic nephropathy.

The clinical data from all three patients are presented in Table 1.

Methods

The biopsy tissues of kidney were fixed in 4% buffered paraformaldehyde, embedded in paraffin, and sectioned 3–4 μm thick. These tissue specimens were stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS), and periodic acid-methenamine silver (PAM) for the routine histological examination. These paraffin-embedded tissues were also used for the immunohistochemical stainings, which were performed with antibodies to vascular endothelial cell surface markers (CD34: Nichirei, Tokyo), von Willebrand factor (Dako, Denmark), type IV collagen (Dako, Denmark), cytokeratin AE1/AE3 (Dako, Denmark), E-cadherin (BD Transduction Lab, California), and myeloperoxidase (Dako, Denmark). The antibodies to CD34, von Willebrand factor, type IV collagen, cytokeratin AE1/AE3, and E-cadherin are monoclonal antibodies to each human element, respectively. The antibody to myeloperoxidase (MPO) is a polyclonal antibody to human leukocyte MPO. The staining procedure was as follows: each mouse monoclonal antibody was diluted to CD34, ×50; von Willebrand factor, ×100; type IV collagen, ×100; cytokeratin AE1/AE3, ×100; E-cadherin, ×400, respectively. Polyclonal antibody to MPO was diluted to ×10,000. Antigen retrieval was performed by protease in type IV collagen, MPO, and cytokeratin AE1/AE3, but was by citrate buffer (pH 6.0) in von Willebrand factor and E-cadherin. No retrieval was done in CD34. Next, the diluted antibodies were applied to the renal tissue specimens for the first antibody under 4°C and an overnight reaction. After washing with phosphate-buffered saline (PBS), biotinylated goat anti-mouse antibody was reacted on the renal tissue as a second antibody for 60 min in the antibodies of CD34, von Willebrand factor, type IV collagen, and cytokeratin AE1/AE3. In the antibody of MPO, which is produced in rabbits, biotinylated goat anti-rabbit antibody was reacted on the renal tissue as a second antibody for 60 min. In both stainings, after washing with PBS, horseradish peroxidase-labeled streptoavidin was applied to these specimens for 60 min (Ventana system, Tucsan, AZ). However, in the antibody of E-cadherin, biotinylated goat anti-mouse IgG antibody labeled with horseradish peroxidase was reacted on the renal tissue as a second antibody for 60 min (Envision System, Dako, Kyoto). After performing all of these reactions and post-washing with PBS, 3-diaminobenzidine tetrahydrochloride (DAB) including

Table 1 Laboratory data on admission

Test	Normal value	Case 1	Case 2	Case 3
Urinary protein (mg/dl)	(–)	(–)	(–)	(–)
Urinary RBC in sediment (/HPF)	0–1	0–1	1–4	1–4
Urinary granular cast in sediment (/HPF)	0	30–49[a]	1–4	1–4
Urinary β_2-microglobulin (μg/l)	20–300	809	231[b]	407
CRP (mg/dl)	<0.4	10.2	3.9	12.0
MPO–ANCA (EU)	<20	65	140	43
Rheumatoid factor (IU/ml)	<20	550	62	422
Hemoglobin (g/dl)	11.5–15.5	7.9	13.9	7.7
WBC (/μl)	3,500–9,000	22,800	11,600	9,400
Platelet (×10^4/μl)	15.0–38.0	36.7	19.9	30.2
Serum creatinine (mg/dl)	0.2–0.8	1.6	0.9	0.7

[a] Means the numbers of urinary granular casts over the whole field in the sediment

[b] The value of 32 days after the treatment of prednisolone 20 mg/day

0.01% hydrogen peroxide was used for enzymatic visualization. These stained tissue specimens were then examined. For the staining of the serial sections using six different antibodies, the serial sections were successively applied to each staining in the arrangement of CD34, von Willebrand factor, type IV collagen, MPO, cytokeratin AE1/AE3, and E-cadherin according to the above-mentioned methods. An immunofluorescence study using frozen tissues was performed for the detection of immunoglobulin and complement deposits. An extensive electron microscopic examination of the peritubular capillaries was done to assess basement membrane breaks in addition to the usual observation.

Results

The pathological findings in each case

Routine study

Case 1: One kidney tissue specimen was obtained, and it contained six glomeruli that showed an almost normal appearance except for some infiltration of neutrophils in the lumens of the capillary loop. In the interstitium, only focal and minor infiltrations of neutrophils and mononuclear cells in the peritubular spaces as well as tubulitis were noted (Fig. 1a, b, c). However, no infiltration of eosinophils and plasma cells was found. High power magnification showed a few neutrophils and mononuclear cells in the peritubular capillaries with blurred, thickened, or disappearing capillary walls (Fig. 1c). The tubulitis was demonstrated by the infiltration of mononuclear cells in the tubular epithelium associated with blurred and thickened tubular basement membranes (Fig. 1c). No arteritis or arteriolitis was found. Immunofluorescence revealed no immunoglobulin or complement deposits. Electron microscopy showed that there were no dense deposits in the glomeruli or the capillaries. Extensive observation demonstrated breaks in the peritubular capillary basement membrane (Fig. 2).

Case 2: Two kidney specimens were obtained; one had six glomeruli, and the other had only medulla. All six glomeruli showed almost normal appearance, and only a few neutrophils were observed in their capillary lumens. In the interstitium, slight and focal infiltrations of neutrophils and mononuclear cells among peritubular tissues were noted in addition to tubulitis. High power magnification revealed few neutrophils and mononuclear cells in the peritubular capillaries with blurred capillary walls. Tubulitis was also noted around the peritubular capillaries, which were accompanied by inflammatory cells. It showed the partial disappearance of tubular basement membrane in addition to desquamating tubular epithelial cells, and this lesion was associated with the infiltration of neutrophils and mononuclear cells (Fig. 3a, b). Immunofluorescence showed no deposits of immunoglobulins and complements. No tissue was available for electron microscopic evaluations.

Case 3: Two kidney specimens were obtained, and they contained 15 glomeruli in total. Three glomeruli showed global sclerosis, and two glomeruli revealed fibrous thickening of Bowman's epithelial cells, with both slight wrinkling of the capillary loops and some widening of the mesangial matrix. None of these glomeruli showed any rupture of Bowman's capsule. The remaining ten glomeruli demonstrated almost normal appearance. In the interstitium, there were focal and slight infiltrations of neutrophils and mononuclear cells in the peritubular tissues except for the surrounding areas of sclerotic glomeruli, where a considerable number of mononuclear cells were noted. Scattered tubulitis was found, but it was rarely associated with the destruction of tubular basement membranes outside of the areas of sclerotic glomeruli. High power magnification revealed the infiltration of a few mononuclear cells in the lumens of the peritubular capillaries, and this was accompanied by the destruction of the capillary walls. Arterio-arteriolosclerosis was noted, but without fibrinoid degeneration. Immunofluorescence demonstrated faint linear staining with anti-IgG and IgA antibodies on the glomerular capillary walls. Electron microscopy showed both a slightly thickened glomerular basement membrane and some increased mesangial matrix, but no apparent breaks of the peritubular capillary basement membranes as far as we could determine based on our findings.

Immunohistochemical study

Vascular endothelial cell findings with anti-CD34 antibody The antibody to CD34 marker, which binds to the cell surface of vascular endothelial cells [10], was used for the detection of the vascular wall. In the preliminary staining, the antibody showed good visualization of the vascular walls on the peritubular capillary and glomerulus in normal subjects (Fig. 4a, inset) as well as the renal tissues of several glomerulonephritides (data not shown). All three cases showed an almost similar appearance. Namely, the glomeruli revealed good staining along the capillary walls (Fig. 4b). In contrast, a loss of staining was focally observed on the peritubular capillaries in the interstitium where the capillary walls were either destroyed or evenly preserved (Fig. 4a, b).

Vascular endothelial cell findings with anti-von Willebrand factor antibody The von Willebrand factor, some of which is known to be secreted from the endothelial cells, is restored beneath the vascular endothelial cells [11]. The preliminary study on the other glomerulonephritides showed increased

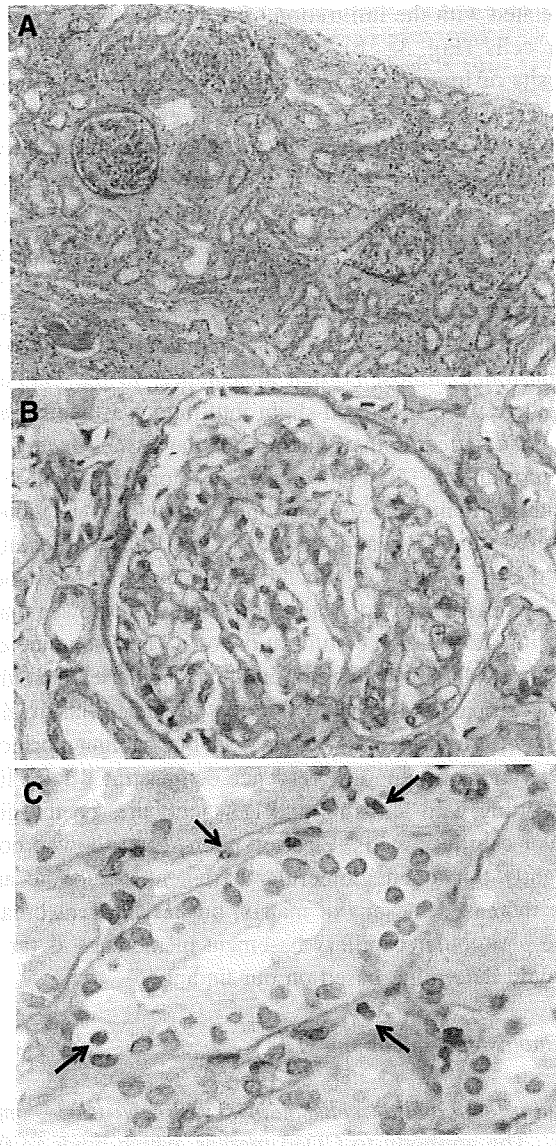


Fig. 1 Case 1: Light microscopy shows an almost normal appearance of glomeruli and slight infiltrations of cells in the interstitium. High magnification demonstrates the infiltration of a few neutrophils and/or mononuclear cells in the peritubular capillaries as well as tubular epithelial cells (*arrows*) (PAS staining, **a** ×100, **b** ×400, and **c** ×600)

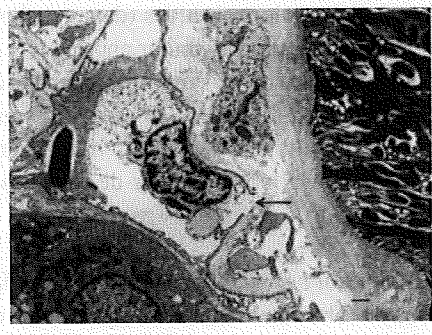


Fig. 2 Case 1: Electron microscopy reveals the rupture of peritubular capillary basement membrane (an *arrow*, ×8,000). A *bar* indicates 1 μm in length

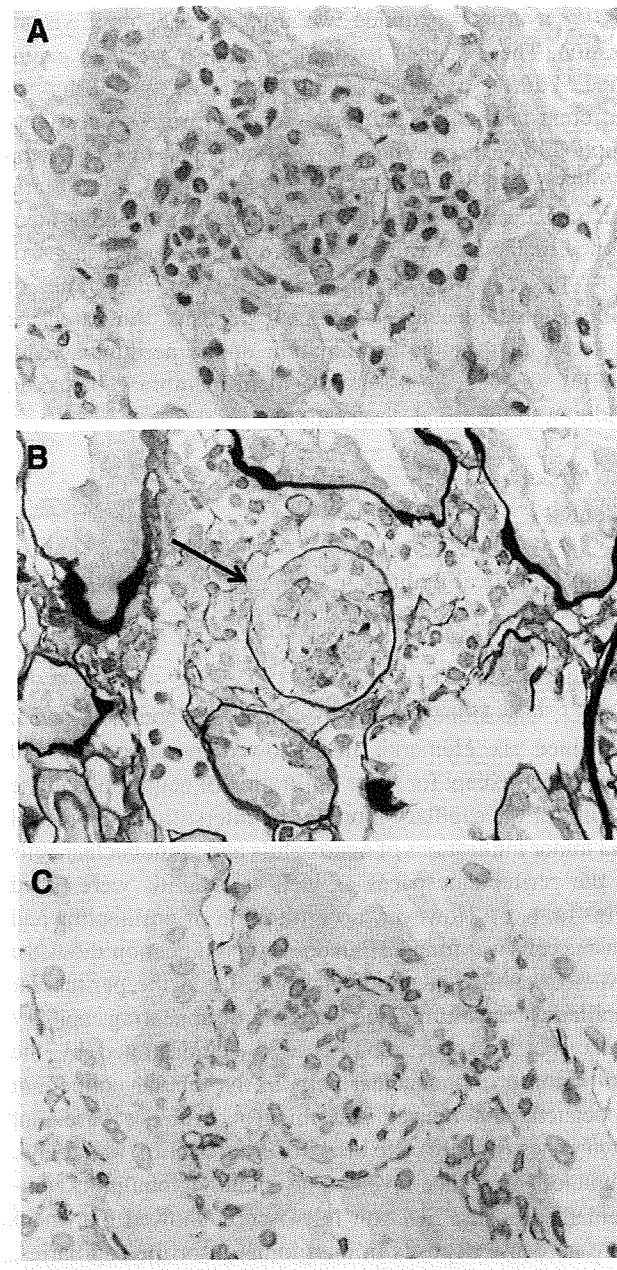
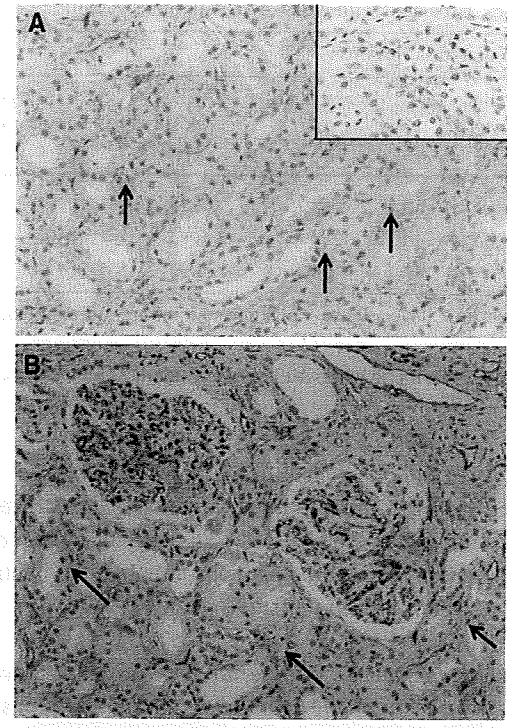


Fig. 3 Case 2: Serial sections. Tubulitis is associated with the desquamation of the tubular epithelial cells and infiltrating neutrophils as well as mononuclear cells, and shows partial lysis of the tubular basement membrane (*arrow*) (PAS staining, **a** ×600; PAM staining, **b** ×600). Anti-CD34 Ab staining shows good preservation of its positive finding on the surrounding peritubular capillary even in the area of tubulitis (**c** ×600)

or decreased staining along the damaged or non-damaged peritubular capillary walls, depending on the areas (data not shown). Accordingly, only supportive findings were obtained with this antibody in this study. In all three cases, some peritubular capillaries demonstrated increased positive staining, whereas most of them showed no staining of this factor (data not shown).



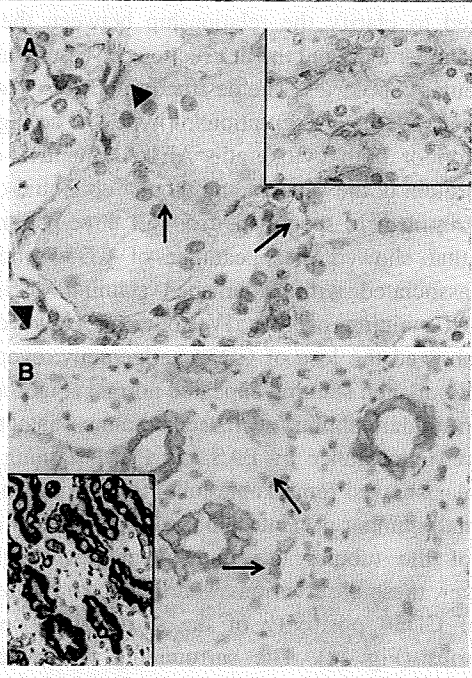


Fig. 5 Case 3: Anti-type IV collagen Ab staining of the renal tissue from a 65-year-old healthy male shows positive findings on tubular basement membrane and peritubular capillary walls (**a**, *inset* ×400). However, some tubular basement membranes and peritubular capillary walls of the patient show decreased staining (*arrows*), whereas the other area reveals its increased intensity with wrinkling or duplicating of the tissue (*arrowheads*) (**a**, ×600). Anti-cytokeratin AE1/AE3 Ab staining of the renal tissue from a 65-year-old healthy male shows homogenous positive findings of tubular epithelial cells. However, two tubuli probably belonging to the thick limb of Henle show negative staining (**b**, *inset* ×400). In contrast, the tubular epithelial cells of the patient show the loss of positivity to some extent, but not all of them (*arrows*) (**b**, ×400)

Fig. 4 Case 1: Anti-CD34 Ab staining of the renal tissue from a 65-year-old healthy male shows a positive finding of the peritubular capillary walls in the interstitium (**a**, *inset* ×400). In contrast, some peritubular capillary walls of the patients lose this marker (*arrows*), whereas the other area keeps the positivity of this maker (**a** ×400). The glomeruli and periglomerular capillary walls show positive findings, but some capillary walls in the interstitium lose this marker, too (*arrows*) (**b** ×400)

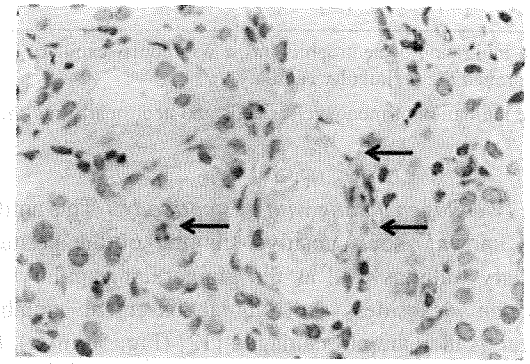


Fig. 6 Case 1: Anti-MPO Ab staining demonstrates trace amounts on a peritubular capillary wall and on the infiltrating neutrophils in the tubular lumen as well as a probable neutrophil in a peritubular capillary (*arrows*) (×600)

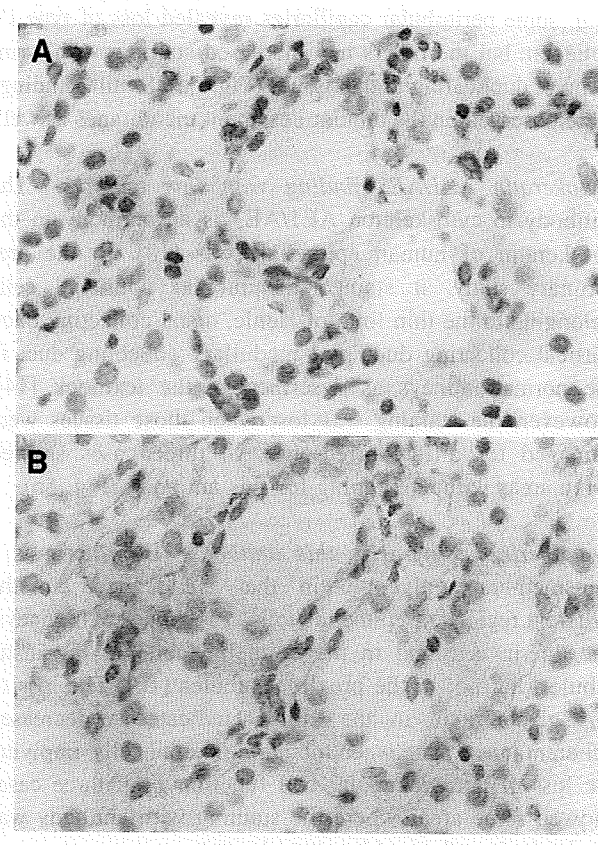


Fig. 7 Case 2: Serial sections. Anti-CD34 Ab and anti-type IV collagen Ab stainings demonstrate the apparent loss of CD34-positive findings, whereas type IV collagen staining is noted (anti-CD34 Ab staining, **a** ×600; anti-type IV collagen staining, **b** ×600)

Table 2 Tubulointerstitial nephritis without glomerular lesions in the three cases associated with MPO-ANCA-positive vasculitis

	Glomerular lesions				Tubulointerstitial lesions				
	Crescent	Fibrin in Bowman's space	Neutrophils in glomerular capillary loops	Increased cells in glomeruli	Peritubular capillaritis[a] (PTCitis)	TBM lysis	Tubulitis[b]	CD34 positivity on PTC	CD34 positivity on glomeruli
Case 1	(−)	(−)	(+)	(−)	(+)	(+)	(+)	(−)	(+)
Case 2	(−)	(−)	(+)	(−)	(+)	(+)	(+)	(−)	(+)
Case 3	(−)	(−)	(+)	(−)	(+)	(+)	(+)	(−)	(+)

[a] The infiltration of neutrophils in the peritubular capillary lumens, breaks of peritubular capillary walls, and the infiltration of inflammatory cells around the peritubular capillary

[b] The infiltration of mononuclear cells and neutrophils into the tubular epithelial cells with or without the lysis of tubular basement membrane

Type IV collagen finding with this antibody This antibody, as is known, shows positive staining of the peritubular capillary walls as well as the glomerular capillary loops, Bowman's capsules, and tubular basement membranes insofar as they are not damaged [12] (Fig. 5a inset). Damage was associated with a loss of staining in the complete destruction or increased staining with the wrinkling of tissues in the early phase of destruction. All three cases showed positive type IV collagen staining in the glomeruli and Bowman's capsules (data not shown). In the interstitium, some peritubular capillaries revealed loss of type IV collagen, but the others had increased staining with wrinkling or duplicating of the tissues (Fig. 5a). Similar findings were observed on the tubular basement membranes as well.

Cytokeratin AE1/AE3 finding with this antibody The antibody to cytoskeleton AE1/AE3 is an antibody to the cytokeratin of human epithelial cells [13]. In the preliminary study, it stained the tubular epithelial cells belonging to the thin limb of Henle, distal collecting duct, cortical collecting duct, and medullary collecting duct in the normal kidneys as described in the textbook [14]. However, the staining was lost when those tissues were damaged for different reasons. All three cases showed some areas without staining for this antibody (Fig. 5b).

E-cadherin finding with this antibody E-cadherin is a transmembrane glycoprotein that is localized in the adherent regions of epithelial cells [15]. In normal kidneys, E-cadherin is found in the epithelial cells of the distal tubules, but not in the proximal tubules [16]. The loss of this glycoprotein in the kidney indicates a decreased adherent junction between tubular epithelial cells, implying the loss of normal tubular cell functions. All three cases showed some areas where the staining with antibody was diminished or absent (data not shown).

Localization of myeloperoxidase with anti-MPO antibody In the experimental model and hypothesis of MPO-ANCA-associated vasculitis, MPO-ANCA exists on the vessel walls and induces the destruction of vessel walls [1]. MPO staining on the vessel walls was observed only in limited areas in the three patients, although the neutrophils were routinely stained positive for MPO (Fig. 6).

Immunohistochemistry in serial sections stained with the six above-mentioned antibodies Serial sections were observed to clarify the relationship between the loss of CD34 antigenicity and the destruction of vessel walls in the peritubular capillaries. The destruction of vessel walls was evaluated by the preservation of type IV collagen. The alteration of both cytokeratin AE1/AE3 and E-cadherin glycoprotein in the surrounding tubular epithelial cells was also examined. In specimens from all three patients, most areas that showed disappearance of CD34 antigenicity were associated with diminished staining or increased wrinkled staining of type IV collagen. However, some areas showed the loss of CD34 antigenicity, even though type IV collagen staining appeared normal (Fig. 7a, b), and the peritubular capillary wall seemed almost intact without a blurred appearance by PAS and PAM staining. Only a few areas showed remarkable tubulitis with the infiltration of a small number of neutrophil and mononuclear cells and lysis of the tubular basement membrane (Fig. 3a, b). However, these observations were not accompanied by the loss of CD34 positivity of the surrounding peritubular capillaries (Fig. 3c). The surrounding tubular epithelial cells diminished or lost cytokeratin AE1/AE3 and E-cadherin staining as well (Fig. 5b). The total immunohistochemical findings are summarized in Table 2.

Discussion

A total of 66 renal biopsy specimens were obtained from patients with MPO-ANCA-associated nephritis from 1998 to 2007 in our department. However, only three cases (4.5%) showed TI nephritis without any apparent

glomerular lesions, and this incidence is almost consistent with previous reports [1, 17]. TI nephritis in our three cases was presumed to result from peritubular capillaritis and tubulitis, because the kidney specimens showed almost normal glomeruli and no existence of arteritis, arteriolitis, or venulitis except in case 3. Case 3 had some sclerotic glomeruli and fibrous thickening of Bowman's epithelial cells and showed the infiltration of inflammatory cells around them, but this pathology was only secondary TI nephritis. The remaining tissue demonstrated identical features to cases 1 and 2. Peritubular capillaritis in the interstitium was diagnosed by loss of CD34 staining, type IV collagen wrinkling or loss of staining, and basement membrane breaks in the peritubular capillary walls as well as the infiltration of neutrophils and mononuclear cells in the peritubular capillary lumens and in the surrounding interstitium. Tubulitis was also diagnosed by the standard definition and based on inflammatory cell infiltration into the tubular epithelial cells [18]. Furthermore, tubulitis was associated with TBM lysis and desquamating tubular epithelial cells, which were observed with the infiltration of neutrophils and mononuclear cells into the tubular epithelial cells. The tubulitis was also accompanied by the infiltration of neutrophils and mononuclear cells in the surrounding peritubular tissue. However, the area of TI nephritis did not show the presence of eosinophils or plasma cells. Close observation of the peritubular capillaries revealed the loss of CD34 staining even with continued staining of type IV collagen and the intact appearance of the capillary wall on PAS staining. These lesions disclosed the loss of cytokeratin AE1/AE3 staining and diminished expression of E-cadherin staining in the surrounding tubular epithelial cells. Based upon these pathological findings, TI nephritis associated with peritubular capillaritis and tubulitis was diagnosed in the three cases. When the pathogenesis of peritubular capillaritis and tubulitis is considered, MPO-ANCA-associated vasculitis is highly suspected, but a few other pathogenetic mechanisms must be addressed. First, drug-induced TI nephritis associated with MPO–ANCA positivity must be suspected. However, most cases in the literature report the infiltration of mononuclear cells, including eosinophils and plasma cells, thus suggesting that the origin of TI nephritis is drug-induced [3–6]. The three current cases showed none of these cells. Second, TI nephritis associated with virus infections shows similar pathological features and the infiltration of mononuclear cells of lymphocytes, plasma cells, and histiocytes, but not neutrophils [19]. Third, autoimmune TI nephritis, such as Sjögren syndrome, systemic lupus erythematosus (SLE), tubulointerstitial nephritis–uveitis syndrome, rheumatoid arthritis with arteritis (RAA), and essential cryoglobulinemia should also be considered [20–22]. These types of autoimmune TI nephritis are reported to have the infiltration of mononuclear cells, but not neutrophils, in the interstitium (Sjögren syndrome) or depositions of immunoglobulins and complements on the capillary walls as well as the TBM (essential cryoglobulinemia, SLE, etc.), except for RAA. In RAA, the patients who manifest the necrotizing type of vasculitis usually show low levels of serum CH_{50} in addition to apparent joint manifestations of arthritis, such as swollen and deformed joints [22]. These findings were not observed in our three cases. In addition, capillaritis due to the necrotizing type of vasculitis has never been described until today [22]. Therefore, these pathogeneses were not considered in the three cases presented here. When the pathogenic processes are considered in regard to the present cases, the loss of CD34 cells in the peritubular capillaries occurred initially and relatively diffusely in the interstitium. This staining loss was noted in the areas of TI nephritis, but was also observed even in the areas with good type IV collagen staining. This observation suggests that MPO, which is included in the infiltrating neutrophils, bursts out from these cells, and releases proteolytic enzymes and radical oxygen species, induces the antigenic loss of vascular endothelial cells, and results in the initial damage of the peritubular capillary walls [1]. This process progresses to peritubular capillaritis and the infiltration of neutrophils and mononuclear cells in the surrounding interstitium [23]. However, similar processes were not observed in the glomeruli, which showed both preservation of CD34 and type IV collagen staining in all three cases. The discrepancy between these two findings in this study is not easily explained, but might be due to MPO epitope differences or different MPO affinities [24, 25]. In tubulitis, TBM lysis was noted with the infiltration of neutrophils and mononuclear cells into the tubular epithelial cells. This lesion was accompanied by the infiltration of these cells in the surrounding interstitium. This observation implies that tubulitis itself could induce TBM lysis as described by Akikusa et al. [9] in which the tubulorrhexis in systemic vasculitis was described.

This study described three cases with TI nephritis but without glomerular lesions in MPO-ANCA-associated vasculitis and early loss of CD34 antigenicity in the peritubular capillary. This early loss of CD34 antigenicity due to MPO and tubulitis played important roles in the pathogenesis of peritubular capillaritis and the lysis of tubular basement membrane, and induced TI nephritis in the present cases. These observations and these pathogenetic processes have not previously been described in MPO-ANCA-associated vasculitis as far as we could determine in the literature.