Table 1 Characteristics of subjects

Category eGFR (ml/min/1.73 m^2) Urinary protein (g/gCr)	1 ≥60 <0.3	2 <60 <0.3	3 ≥60 ≥0.3	4 <60 ≥0.3	Total	*P*
n (% of category)	74 (55.6)	43 (32.3)	9 (6.8)	6 (4.5)	133	
Age (years)	52.7 ± 17.9	56.8 ± 18.5	49.0 ± 17.3	64.7 ± 9.2	54.4 ± 17.9	N.S.
Sex (% male)	24 (32.4)	1 (2.3)	2 (22.2)	0 (0)	27 (20.3)	<0.001
BMI (kg/m^2)	22.5 ± 3.8	22.2 ± 5.5	21.2 ± 4.2	21.8 ± 3.7	22.3 ± 4.4	N.S.
Albumin (g/dl)	3.97 ± 0.54	4.11 ± 1.45	3.86 ± 0.89	3.28 ± 0.82	3.97 ± 0.97	N.S
Creatinine (mg/dl)	0.66 ± 0.14	1.02 ± 0.31	0.69 ± 0.09	1.37 ± 0.92	0.81 ± 0.34	<0.001
eGFR (ml/min/1.73 m^2)	86.2 ± 24.7	48.7 ± 9.3	81.3 ± 22.7	40.5 ± 17.9	71.6 ± 28.2	<0.001
Urinary protein (g/gCr)	0.06 ± 0.06	0.07 ± 0.06	1.22 ± 0.07	0.94 ± 1.39	0.18 ± 0.5	<0.001
Hb (g/dl)	12.6 ± 2.3	11.6 ± 1.9	11.0 ± 2.6	9.27 ± 2.2	12.0 ± 2.3	N.S.
BNP (pg/ml) (*n* of >20 pg/ml)	21.4 ± 49.2 (8)	27.2 ± 68.5 (7)	10.2 ± 5.8 (0)	18.4 ± 16.8 (1)	22.9 ± 55.0 (16)	N.S.
The presence of comorbidity						
Diabetes (%)	12 (17.6)	4 (9.3)	1 (11.1)	1 (16.7)	18 (13.5)	
Hypertension (%)	6 (8.1)	2 (4.7)	1 (11.1)	1 (16.7)	10 (7.5)	
Connective tissue disease (%)	25 (33.8)	19 (44.2)	2 (22.2)	1 (16.7)	47 (35.3)	
Haematologic disease (%)	13 (17.6)	3 (7.0)	2 (22.2)	2 (33.3)	20 (15.0)	
Cardiac disease (%)	5 (6.8)	2 (4.7)	0 (0)	0 (0)	7 (5.3)	
Cancer (%)	14 (18.9)	8 (18.6)	3 (33.3)	1 (16.7)	26 (19.5)	
Other diseases[a] (%)	8 (10.8)	10 (23.3)	2 (22.2)	1 (16.7)	21 (15.8)	

Data are presented as means ± standard deviation (SD)

N.S. not significant

[a] Depression, vitreous haemorrhage, cataract, tonsillitis, sleep apnoea syndrome, thyroiditis, chronic hepatitis B, chronic hepatitis C, ileus, condyloma acuminatum, toxic eruption

Table 2 Comparison between PFT parameters in each category (renal function)

Category eGFR (ml/min/1.73 m^2) Urinary protein (g/gCr)	1 ≥60 <0.3	2 <60 <0.3	3 ≥60 ≥0.3	4 <60 ≥0.3	Total
%VC	109.2 ± 18.6	107.2 ± 15.7	111.3 ± 17.8	101.5 ± 14.2	108.3 ± 17.4
%FEV1	109.5 ± 20.4	114.2 ± 24.6	110.7 ± 12.9	105.9 ± 10.4	110.9 ± 21.1
FEV1/FVC	81.2 ± 7.2	80.1 ± 7.6	84.1 ± 7.7	76.7 ± 6.1	80.8 ± 7.4
%TLC	99.4 ± 14.0	99.2 ± 13.9	102.2 ± 12.1	96.1 ± 10.9	99.4 ± 13.6
%RV	94.2 ± 22.6	95.2 ± 20.4	94.3 ± 22.9	93.7 ± 15.9	94.6 ± 21.4
Hb-adjusted $\%DL_{CO}$	75.8 ± 18.9	63.6 ± 17.8*	62.5 ± 15.7	46.2 ± 7.5*	69.6 ± 18.9
VE (l/min)	9.37 ± 3.33	8.35 ± 2.53	8.53 ± 2.57	8.86 ± 1.67	9.00 ± 3.00

Data are presented as means ± standard deviation (SD)

* $P < 0.05$, compared with category 1

pulmonary function in participants undergoing examination for eGFR, proteinuria, and PFTs. Our data indicated that decreased eGFR (<60 ml/min/1.73 m^2) along with increased urinary protein (≥0.3 g/gCr) were associated with decreased Hb-adjusted $\%DL_{CO}$. These results suggest that patients with CKD may have impaired pulmonary capacity.

Our data indicated that increased urinary protein was associated with decreased Hb-adjusted $\%DL_{CO}$. Urinary protein is one of the surrogate markers for vascular damage in the kidney [16]. In addition, urinary protein is also a surrogate marker of interstitial fibrosis in the kidney [17]. Various studies demonstrated that increased serum levels of cytokines and chemokines in patients with end-stage renal disease (ESRD) promote kidney fibrosis [10, 17–19]. These increased serum cytokine and chemokine levels would affect lung fibrosis in a similar way to the kidney. Vascular damage (impaired capillary vessels in lung

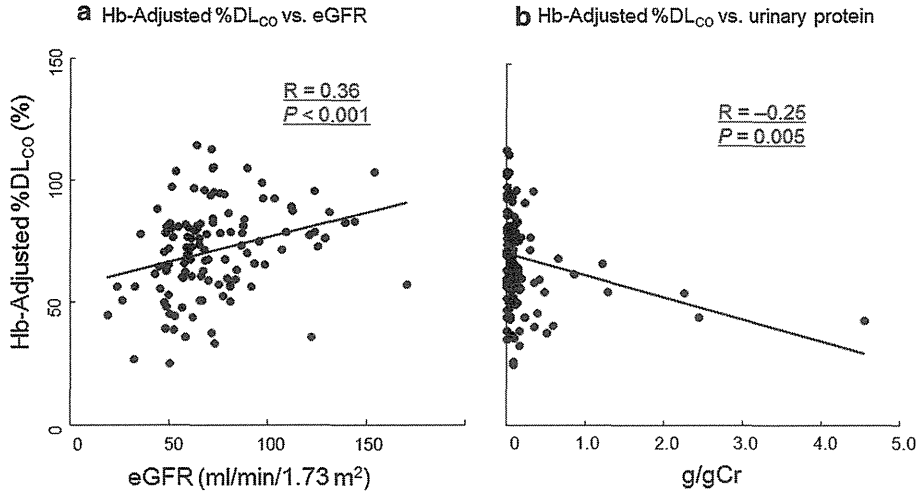

Fig. 2 Relationships between Hb-adjusted $\%DL_{CO}$ and kidney function (eGFR and urinary protein)

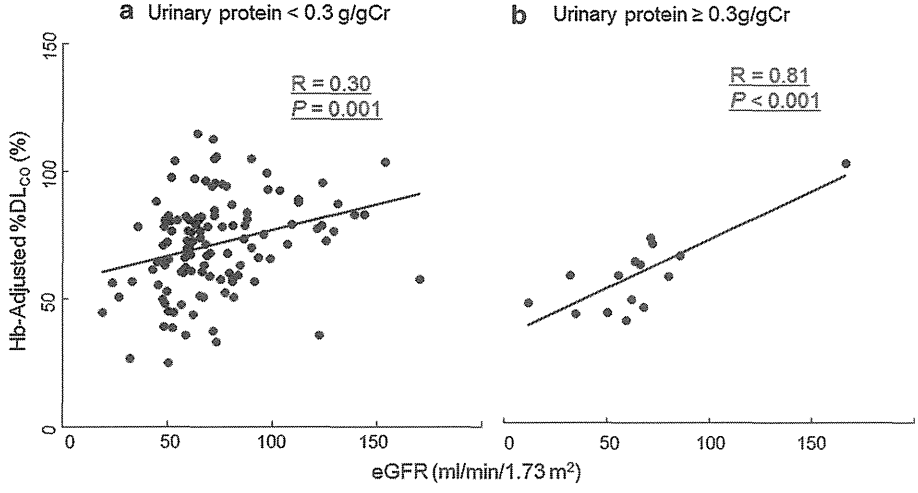

Fig. 3 Relationships between Hb-adjusted $\%DL_{CO}$ and eGFR depending on urinary protein

Table 3 Multiple linear regression analysis with Hb-adjusted $\%DL_{CO}$ as the dependent variable and age, gender, VE, eGFR, urinary protein, and the presence or absence of DM and hypertension as independent variables in all patients examined

Coefficient	Partial regression Coefficient		Standard partial Regression coefficient	
Model	B	SE (B)	β	*P*
Constant	71.643	9.300	−0.142	
Age (years)	−0.149	0.111	0.037	0.182
Gender (male)	1.643	5.196	0.037	0.753
VE (l/min)	14.253	11.040	0.135	0.200
eGFR (<60 ml/min/1.73 m^2)	−10.260	4.422	−0.260	0.023*
Urinary protein (≥0.3 g/gCr)	−10.019	5.922	−0.169	0.095
Diabetes (yes)	−0.111	6.085	−0.001	0.985
Hypertension (yes)	13.609	7.764	0.182	0.083

Regression analysis was adjusted for each factor

* $P < 0.05$

alveoli) and interstitial fibrosis (impaired diffusion from lung alveoli to the lumens of capillary vessels) are associated with decreased Hb-adjusted $\%DL_{CO}$ [20–27]. These mechanisms may be associated with the correlation between urinary protein and Hb-adjusted $\%DL_{CO}$.

Our data also indicated that decreased eGFR was associated with decreased Hb-adjusted $\%DL_{CO}$. Decreased eGFR enhances the risk of atherosclerosis [28–30]. Patients with CKD are more likely to be complicated by whole-body atherosclerosis caused by many factors, such as elevation of blood pressure, impaired glucose tolerance, activation of the renin–angiotensin system, abnormalities of calcium homeostasis, or deposition of calcium in the vessels [31–33]. Furthermore, Kumar et al. [34] reported that patients with CKD and ESRD are more likely to be complicated by pulmonary embolism. Vascular damage (impaired capillary vessels in lung alveoli) is associated with decreased Hb-adjusted $\%DL_{CO}$ [20–25]. These findings may have an impact on the contribution to the correlation between eGFR and Hb-adjusted $\%DL_{CO}$.

Our data further revealed that both urinary protein and decreased eGFR were associated with decreased Hb-adjusted $\%DL_{CO}$. The coexistence of urinary protein and decreased eGFR may result in a greater increase in vascular damage (impaired capillary vessels in lung alveoli) [20–25] and more severe interstitial fibrosis (impaired diffusion from lung alveoli to the lumens of capillary vessels) [26, 27] compared to the presence of urinary protein or decreased eGFR alone. Thus, they can have a synergetic effect on decreasing Hb-adjusted $\%DL_{CO}$. The mechanisms underlying the association between Hb-adjusted $\%DL_{CO}$ and decreased eGFR with increased urinary protein may be complicated. Therefore, further studies, such as longitudinal studies and basic research, are needed to clarify the detailed mechanisms underlying this association between the lungs and kidneys.

This study had some limitations, including sampling bias. The participants included in this study were limited to patients treated at Kanazawa University Hospital. As this is an advanced medical institution, our participants had an increased likelihood of having various complications. Therefore, it is possible that participants with normal pulmonary function did not undergo PFTs in this study, leading to overestimation of the relationships between renal dysfunction and pulmonary dysfunction. The other major limitation of this study may be confounding factors. Other factors have been reported to affect $\%DL_{CO}$. Reduction of haemoglobin concentration and impairment of lung alveolus are two major factors that affect $\%DL_{CO}$ [15, 35–38]. Reduction of haemoglobin concentration was not excluded in this study, because the influence of anaemia on $\%DL_{CO}$ is adjusted by haemoglobin. Impairment of lung alveoli is mainly associated with smoking habit and chronic obstructive pulmonary disease. Therefore, these patients were excluded from the study population. Moreover, diffusing capacity of the lung is influenced not only by Hb and inflammatory status (e.g., profibrotic cytokines), but also by oedematous condition (the presence of heart failure even in occult status) [39]. CKD patients with advanced stages may be complicated with mild or subclinical heart failure. Further studies are required to overcome these limitations.

In conclusion, the present study indicated that eGFR and urinary protein excretion were associated with pulmonary function (Hb-adjusted $\%DL_{CO}$). Further studies, such as longitudinal studies and basic research, are needed to confirm the present results and to clarify the detailed mechanisms underlying these observations.

Acknowledgments We wish to thank the staff of the Clinical Laboratory at Kanazawa University Hospital for their technical assistance in this study. This study was supported in part by Grant-in-Aids for Diabetic Nephropathy Research and for Diabetic Nephropathy and Nephrosclerosis Research, from the Ministry of Health, Labour and Welfare of Japan and by the Ministry of Education, Science, Sports and Culture, Japan.

Conflict of interest All authors declare that they have no conflicts of interest associated with this study.

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Sada et al. Arthritis Research & Therapy 2014, 16:R101
http://arthritis-research.com/content/16/2/R101

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RESEARCH ARTICLE Open Access

Classification and characteristics of Japanese patients with antineutrophil cytoplasmic antibody-associated vasculitis in a nationwide, prospective, inception cohort study

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Abstract

Introduction: We investigated the clinical and serological features of patients with antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) in Japan using data from a nationwide, prospective, inception cohort study.

Methods: In total, 156 Japanese patients with newly diagnosed AAV were classified according to the European Medicines Agency (EMEA) algorithm with exploratory surrogate markers for AAV-related non-granulomatous pulmonary lesions, predefined as alveolar haemorrhage and interstitial lung disease (ILD), and their clinical and serological features were evaluated.

Results: Using the EMEA algorithm, we identified 14 patients (9.0%) with eosinophilic granulomatosis with polyangiitis (EGPA), 33 (21.2%) with granulomatosis with polyangiitis (GPA), 78 (50.0%) with microscopic polyangiitis and renal-limited vasculitis (MPA/RLV), and 31 (19.9%) with unclassifiable vasculitis. The average ages of patients with EGPA (male/female, 5/9), GPA (12/21), and MPA/RLV (35/43) and unclassifiable (9/22) were 58.0, 63.6, 71.1, and 70.6 years, respectively. Myeloperoxidase (MPO)-ANCA and proteinase-3 ANCA positivity was 50.0% and 0% for EGPA, 54.6% and 45.5% for GPA, 97.4% and 2.6% for MPA/RLV, and 93.5% and 3.2% for unclassifiable, respectively. According to the Birmingham Vasculitis Activity Score (BVAS), cutaneous (71.4%) and nervous system (92.9%) manifestations were prominent in EGPA and ear, nose, and throat manifestations (84.9%) and chest manifestations (66.7%) in GPA. Renal manifestations developed frequently in MPA/RLV (91.0%) and GPA (63.6%). The average serum creatinine levels were 0.71 mg/dL for EGPA, 1.51 mg/dL for GPA, 2.46 mg/dL for MPA/RLV, and 0.69 mg/dL for unclassifiable. The percentages of patients with ILD were 14.3% for EGPA, 9.0% for GPA, 47.4% for MPA/RLV, and 61.3% for unclassifiable. Patients with ILD ($n = 61$) had significantly lower BVAS ($P = 0.019$) with fewer ear, nose, and throat and cardiovascular manifestations than patients without ILD ($n = 95$).

Conclusions: MPO-ANCA-positive MPA/RLV is the most common form of AAV in Japanese patients, and one-half of patients with GPA were positive for MPO-ANCA. ILD is an important clinical manifestation in Japanese patients with AAV. Unclassifiable vasculitis with MPO-ANCA positivity and ILD may represent a novel variant of MPA.

Trial Registration: The University Hospital Medical Information Network Clinical Trials Registry: UMIN000001648. Registered 28 February 2009.

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Introduction

Microscopic polyangiitis (MPA), granulomatosis with polyangiitis (Wegener's granulomatosis) (GPA), and eosinophilic granulomatosis with polyangiitis (Churg–Strauss syndrome) (EGPA) are the major categories of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), a multisystem autoimmune disease characterised by ANCA production and small-vessel inflammation [1,2]. Despite the overlapping clinicopathologic characteristics between the component diseases, the disease evolution, organ involvement, prognosis, and other clinical characteristics differ substantially among them. In addition, there are interesting geographic and ethnic differences in their relative incidence and myeloperoxidase (MPO)-ANCA or proteinase-3 (PR3)-ANCA positivity [3].

In 2007 Watts and colleagues proposed an AAV classification algorithm, the European Medicines Agency (EMEA) algorithm, with consensus of a group of European physicians interested in the epidemiology of vasculitis [4]. This stepwise algorithm incorporated both the American College of Rheumatology (ACR) criteria for EGPA and GPA and the Chapel Hill Consensus Conference (CHCC) definition of EGPA, GPA, and MPA [2]. In the EMEA algorithm, surrogate markers of granulomatous inflammation for GPA and those of renal vasculitis for renal-limited vasculitis (RLV), an organ-limited variant of MPA, were defined [4]. This algorithm is useful for classifying patients with AAV because no overlapping diagnoses occur and fewer patients are considered to have unclassifiable vasculitis [5], and has been used as the standard method for classification of AAV diseases in recent studies [6,7].

Only two reports have validated the algorithm in other ethnicities outside Europe using a good-quality database. Studies from China [5] and Japan [3] applied the EMEA algorithm to their patient populations and found that MPO-ANCA-positive MPA was the most common form of AAV. These studies, however, were retrospective and evaluated clinical data of patients from a small number of hospitals.

The lung is one of the organs frequently involved in AAV, and pulmonary granuloma, alveolar haemorrhage, and interstitial lung disease (ILD) are representative pulmonary lesions. Among these, only pulmonary granuloma is included in the EMEA algorithm. ILD in AAV is associated with MPO-ANCA and is more common in Asian countries [8-10] than in western countries [11,12], and some patients with MPO-ANCA and ILD subsequently develop typical MPA [13]. To understand the nature of AAV and classify the disease from a global perspective, it is essential to more precisely delineate the clinical implications of ILD in AAV in Asian countries.

To characterise the clinical and laboratory features, effectiveness, and safety of the remission-induction therapy used, as well as the prognosis of Japanese patients with AAV, the Research Committee on Intractable Vasculitides of the Ministry of Health, Labour and Welfare of Japan implemented a nationwide prospective cohort study of Remission Induction Therapy in Japanese Patients with ANCA-associated Vasculitides (RemIT-JAV). In this study, we classified Japanese patients with newly diagnosed AAV enrolled in the RemIT-JAV study according to the EMEA algorithm and compared their phenotypes across the AAVs. We also investigated the clinical relevance of ILD in the patient population.

Methods

Database

Twenty-two tertiary care institutions (university hospitals and referring hospitals) participated in this study (See Appendix) and enrolled consecutive patients with newly diagnosed AAV from April 2009 to December 2010. The criteria for enrolment in this study included receiving a diagnosis of AAV from the site investigators, fulfilling the criteria for primary systemic vasculitis proposed by the EMEA algorithm [4], and requiring immunosuppressive treatment based on the discretion of the site investigators. The exclusion criteria were age younger than 20 years, recurrent AAV, serological evidence for hepatitis B virus or hepatitis C virus infection, and a history of malignancies because this may influence treatment selection and prognosis of patients with AAV. We conducted this study according to the Declaration of Helsinki and the Ethical Guidelines for Epidemiological Research in Japan. Written informed consent was obtained from each participant, and the study protocol was approved by the ethics committee at each participating hospital (refer to Acknowledgements). This study was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN000001648).

Data collection

Each patient's baseline data included demographic information, general performance categorised using scales of the World Health Organization performance status except category 5 (death) [14], comorbidities, laboratory data, disease activity scored using the Birmingham Vasculitis Activity Score (BVAS) 2003 [15], imaging data (for example, chest radiograph, thoracic computed tomography, and magnetic resonance imaging of the head), and respiratory function data. The World Health Organization performance status runs from 0 to 5, with 0 denoting perfect health and 5 denoting death (0, asymptomatic; 1, symptomatic but completely ambulatory; 2, symptomatic, <50% in bed during the day; 3, symptomatic, >50% in bed, but not bedbound; 4, bedbound; 5, death).

Patients were evaluated at months 3, 6, 12, 18, and 24 and at relapse, and the following data were collected: vital status, BVAS 2003, laboratory data, treatments, and

adverse events. The Vascular Damage Index score was recorded at months 6, 12, and 24. Chest radiography, thoracic computed tomography, arterial blood gas analysis, and respiratory function data were collected at months 12 and 24 in patients with pulmonary involvement. Observation was completed in March 2013. Only the baseline data are included in this study; the results from analyses of follow-up data will be reported separately.

The site investigators completed and sent the electronic case report form for each patient to the RemIT-JAV data centre at the Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.

EMEA classification algorithm for AAV

The enrolled patients were classified using the stepwise EMEA algorithm as described previously [2,4]. Briefly, the ACR criteria and Lanham criteria for EGPA were applied first. Patients who did not fulfil the criteria for EGPA were classified as having GPA if they met the ACR criteria for GPA or the CHCC histological definition for GPA or if they showed histology compatible with the CHCC definition for MPA or ANCA positivity with either of the EMEA-defined GPA surrogate markers. The remaining patients were classified as having MPA if they had clinical features and histology compatible with small-vessel vasculitis without the GPA surrogate markers. In addition, ANCA-positive patients who had the EMEA-defined surrogate markers for renal vasculitis were classified as having RLV, a variant form of MPA. The rest of the patients without histology compatible with the CHCC definition of classic polyarteritis nodosa or typical angiographic features of classic polyarteritis nodosa were categorised as having unclassifiable vasculitis.

To identify a subset of unclassifiable vasculitis with AAV-related nongranulomatous pulmonary lesions, we defined exploratory surrogate markers for alveolar haemorrhage and ILD and then applied them to the EMEA-defined unclassifiable patient population. Surrogate markers for these conditions were as follows: haemoptysis or alveolar haemorrhage evaluated by bronchoscopic examination; or ILD diagnosed by chest X-ray or thoracic computed tomography.

Disease severity

The disease severity of the enrolled patients was classified as localised, early systemic, generalised, or severe according to the European League Against Rheumatism recommendation for conducting a clinical study in systemic vasculitis [16]. Organ failure, classified as severe disease, was defined by the presence of any of the following BVAS manifestations: massive haemoptysis/alveolar haemorrhage, respiratory failure, congestive cardiac failure, ischaemic abdominal pain, or stroke. Threatened vital organ function, classified as generalised disease, was defined by the presence of any of the following BVAS manifestations: sudden visual loss, blurred vision, retinal changes (vasculitis/thrombosis/exudates/haemorrhage), conductive deafness, sensorineural hearing loss, ischaemic cardiac pain, cardiomyopathy, peritonitis, bloody diarrhoea, meningitis, organic confusion, seizures, cord lesion, cranial nerve palsy, sensory peripheral neuropathy, or motor mononeuritis multiplex. Serum creatinine levels were also used to classify disease severity as localised and early systemic (<120 μmol/l (1.3 mg/dl)), generalised (<500 μmol/l (5.5 mg/dl)), and severe (≥500 μmol/l (5.5 mg/dl)) [16].

Statistical analysis

We used the baseline data of the patients enrolled in this study for statistical analysis. The primary purpose of this analysis was to determine the demographic and clinical characteristics of Japanese patients with AAV. Categorical variables were compared using Fisher's direct probability test, and continuous variables were compared using Student t test or the Mann–Whitney U test depending on data distribution. $P < 0.05$ was considered significant for statistical analyses between two categories. When comparing among three categories, statistical significance was determined by $P < 0.05/3$ using Bonferroni correction to avoid multiplicity. All statistical analyses were performed by a biostatistician using the Statistical Package of JMP for Windows software (version 8.0.2; SAS Institute Inc., Cary, NC, USA).

Results

Classification of 156 Japanese patients with AAV according to the EMEA algorithm

In total, 159 patients with AAV were initially enrolled in the RemIT-JAV study. Three patients were then excluded; two patients did not undergo treatment, and one patient had been diagnosed as having AAV and experienced a relapse at the time of enrolment. As a result, 156 patients with newly diagnosed AAV were enrolled in the study.

Using the EMEA algorithm, we identified 14 patients with EGPA, 33 patients with GPA, 78 patients with MPA/RLV, and 31 patients who were unclassifiable (Figure 1). The average ages of the patients with EGPA (male/female, 5/9), GPA (male/female, 12/21), and MPA/RLV (male/female, 35/43) were 58.0, 63.6, and 71.1 years, respectively (Table 1). Patients with MPA/RLV were significantly older at the time of presentation than those with EGPA and GPA ($P < 0.017$ for both), and there was a female predominance for all AAV diseases. MPO-ANCA was detectable in 50.0% of patients with EGPA, in 54.6% of those with GPA, and in 97.4% of those with MPA/RLV. In contrast, PR3-ANCA was detectable in none of the patients with EGPA, in 45.5% of those with GPA, and in 2.6% of those with MPA/RLV.

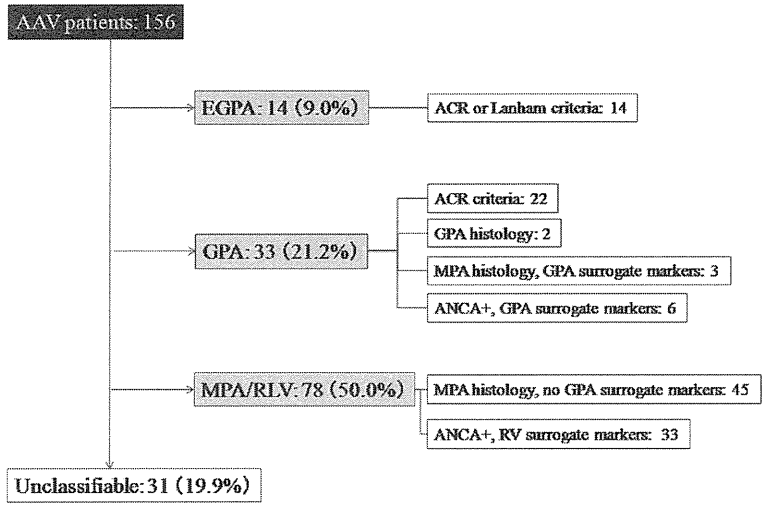

Figure 1 Classification of patients with antineutrophil cytoplasmic antibody-associated vasculitis according to the EMEA algorithm in this cohort study. AAV, antineutrophil cytoplasmic antibody-associated vasculitis; ACR, American College of Rheumatology; ANCA, antineutrophil cytoplasmic antibody; EGPA, eosinophilic granulomatosis with polyangiitis (Churg–Strauss syndrome); EMEA, European Medicines Agency; GPA, granulomatosis with polyangiitis (Wegener's granulomatosis); MPA, microscopic polyangiitis; RLV, renal-limited vasculitis; RV, renal vasculitis.

Surrogate markers involved in the classification according to the EMEA algorithm

Of the patients classified with GPA, 22 patients fulfilled the ACR criteria, two patients showed CHCC-defined GPA histology, three patients showed CHCC-defined MPA histology in the presence of EMEA-defined GPA surrogate markers, and six patients were positive for ANCA in the presence of GPA surrogate markers. Consequently, nine patients were classified by the presence of GPA surrogate markers, mostly by the presence of

Table 1 Comparison of demographics and disease states among AAV diseases

	EGPA ($n = 14$)	GPA ($n = 33$)	MPA/RLV ($n = 78$)	Unclassifiable ($n = 31$)
Male/female	5/9	12/21	35/43	9/22
Mean (median) age (years)[a,c]	58.0 ± 16.9 (62)	63.6 ± 12.6 (61)	71.1 ± 10.0 (73)	70.6 ± 11.8 (73)
MPO-ANCA[a,c]	7 (50.0)	18 (54.6)	76 (97.4)	29 (93.5)
PR3-ANCA[b,c]	0 (0)	15 (45.5)	2 (2.6)	1 (3.2)
ANCA-negative[a,b]	7 (50.0)	3 (9.1)	1 (1.3)	2 (6.5)
Serum creatinine (mg/dl)[a]	0.71 ± 0.39	1.51 ± 1.32	2.46 ± 2.18	0.69 ± 0.23
Disease severity[c]				
Localised	0 (0)	4 (12.1)	0 (0)	0 (0)
Early systemic	1 (7.1)	5 (15.1)	15 (19.2)	15 (48.4)
Generalised	13 (92.9)	18 (54.6)	47 (60.3)	13 (41.9)
Severe	0 (0)	6 (18.2)	16 (20.5)	3 (9.7)
General performance[d]				
0/1/2/3/4	1/7/2/4/0	8/11/2/11/1	10/29/17/16/6	3/11/7/9/1
Nongranulomatous pulmonary involvement				
Interstitial lung disease[a]	2 (14.3)	3 (9.0)	37 (47.4)	19 (61.3)
Alveolar haemorrhage	0 (0)	2 (6.1)	9 (11.5)	2 (6.5)

Values expressed as mean ± standard deviation or number (percentage) unless otherwise noted. AAV, antineutrophil cytoplasmic antibody-associated vasculitis; ANCA, antineutrophil cytoplasmic antibody; EGPA, eosinophilic granulomatosis with polyangiitis (Churg–Strauss syndrome); GPA, granulomatosis with polyangiitis (Wegener's granulomatosis); MPA, microscopic polyangiitis; MPO, myeloperoxidase; PR3, proteinase-3; RLV, renal-limited vasculitis. Comparisons between the EGPA, GPA, and MPV/RLV groups were made by Student *t* test or Mann–Whitney *U* test. Statistical significance was determined by $P < 0.05/3$ using Bonferroni correction: [a]EGPA versus MPA/RLV, [b]EGPA versus GPA, [c]GPA versus MPA/RLV. Unclassifiable AAV was not compared with other forms of AAV. [d]General performance was categorised according to the World Health Organization performance status except category 5 (death).

chronic sinusitis or otitis media (Table 2). Of the patients classified with MPA/RLV, 45 patients showed histology compatible with small-vessel vasculitis without GPA surrogate markers and 33 patients were positive for ANCA with EMEA-defined surrogate markers for renal vasculitis. Three patients with allergic rhinitis, but not asthma, who had eosinophilia of peripheral blood and tissue were considered to have unclassifiable vasculitis. The eosinophilic vasculitis was confirmed histologically in all three patients, and two of these three patients were MPO-ANCA-positive. The exploratory surrogate markers for AAV-related nongranulomatous pulmonary lesions were positive in 16 of the 31 unclassifiable patients; three patients had both markers (alveolar haemorrhage and ILD), and 13 patients had only ILD. These 16 patients were all positive for MPO-ANCA.

Organ involvement of 156 Japanese patients with AAV

Patterns of organ involvement defined by the BVAS 2003 scoring system were described and compared among patients with EGPA, GPA, and MPA/RLV (Table 3). Most patients with AAV presented with constitutional symptoms. Cutaneous and nervous system manifestations were most common in patients with EGPA (71.4% and 92.9%, respectively). Ear, nose, and throat manifestations and chest manifestations were characteristic of patients with GPA (84.9% and 66.7%, respectively). It is noteworthy that ILD is not included in the BVAS 2003 scoring system. Renal manifestations developed frequently in patients with MPA/RLV (91.0%) but also in patients with GPA (63.6%). The mean serum creatinine level and prevalence of ILD in patients with MPA/RLV was higher than that in patients with EGPA and GPA, with a significant difference between MPA/RLV and EGPA for both (Table 1). Unclassifiable patients had cutaneous (32.3%), renal (48.4%), and nervous system (48.4%) manifestations (Table 3).

Differences in clinical features between MPO-ANCA-positive and PR3-ANCA-positive AAV

We compared the demographic and clinical features of Japanese patients with AAV who had MPO-ANCA and those who had PR3-ANCA (Table 4). Patients with MPO-ANCA were significantly older at the time of presentation ($P = 0.012$) and had a higher rate of ILD ($P = 0.0015$). The mean serum creatinine level was numerically higher in patients with MPO-ANCA. According to the BVAS 2003 scoring system, MPO-ANCA-positive patients had more cutaneous ($P = 0.046$) and renal ($P = 0.010$) manifestations and fewer ear, nose, and throat manifestations ($P < 0.0001$) with statistical significance.

Of the 33 patients with GPA, 15 patients, 12 patients, and three patients were positive for MPO-ANCA alone, for PR3-ANCA alone, or for both ANCAs, respectively, but three patients were negative for ANCA. Patients with GPA who had MPO-ANCA had a numerically higher rate of renal disease (86.7%) than those with PR3-ANCA (41.7%). The mean serum creatinine level of patients with MPO-ANCA-positive GPA (2.05 ± 0.35 mg/dl) was also numerically higher than that of patients with PR3-ANCA-positive GPA (1.03 ± 0.39 mg/dl).

Clinical features of patients with or without interstitial lung disease

We compared the demographic and clinical characteristics of the patients with and without ILD (Table 5). MPO-ANCA was found significantly more frequently ($P < 0.001$) and PR3-ANCA was found less frequently ($P = 0.038$) in patients with ILD. These patients also tended to have more early systemic diseases and less generalised or severe diseases ($P = 0.059$) and had significantly lower BVAS ($P = 0.019$). The mean serum creatinine level and rates of patients with constitutive symptoms were similar between the two subgroups. The patients with ILD also had statistically fewer ear, nose, and throat ($P = 0.006$) and cardiovascular ($P = 0.012$) manifestations.

Table 2 Surrogate markers in nine patients who were classified with granulomatosis with polyangiitis using these markers

	Number of patients (MPO-ANCA/PR3-ANCA[a])
X-ray evidence of fixed pulmonary infiltrates, nodules, or cavitations present for >1 month	1 (1/0)
Bronchial stenosis	0 (0/0)
Bloody nasal discharge and crusting for 1 month or nasal ulceration	1[b] (0/1)
Chronic sinusitis, otitis media, or mastoiditis for >3 months	7[c] (4/5)
Retro-orbital mass or inflammation (pseudotumor)	0 (0/0)
Subglottic stenosis	1 (0/1)
Saddle nose deformity/destructive sinonasal disease	1 (0/1)

ANCA, antineutrophil cytoplasmic antibody; MPO, myeloperoxidase; PR3, proteinase-3. [a]MPO-ANCA/PR3-ANCA, anti-myeloperoxidase antibody-positive versus anti-proteinase-3 antibody-positive patients. [b]One patient had bloody nasal discharge and crusting and chronic sinusitis, and another patient had subglottic stenosis and saddle nose deformity. [c]Five patients with chronic sinusitis, three patients with otitis media, and no patients with mastoiditis.

Table 3 Comparison of disease activity and organ involvement among AAV diseases

	EGPA (*n* = 14)	GPA (*n* = 33)	MPA/RLV (*n* = 78)	Unclassifiable (*n* = 31)
BVAS	16.1 ± 7.7	19.9 ± 7.4	18.4 ± 7.3	12.1 ± 7.6
General	10 (71.4)	23 (69.7)	56 (71.8)	29 (93.6)
Cutaneous[a,b]	10 (71.4)	3 (9.1)	16 (20.5)	10 (32.3)
Mucous membranes/eyes	1 (7.1)	8 (24.2)	9 (11.5)	2 (6.5)
Ear, nose, and throat[a,b,c]	6 (42.9)	28 (84.9)	7 (9.0)	1 (3.2)
Chest[c]	5 (35.7)	22 (66.7)	30 (38.5)	6 (19.4)
Cardiovascular	2 (14.3)	4 (12.1)	6 (7.7)	0 (0)
Abdominal	0 (0)	1 (3.0)	0 (0)	1(3.2)
Renal[a,b]	2 (14.3)	21 (63.6)	71 (91.0)	15 (48.4)
Nervous system[a,b]	13 (92.9)	14 (42.4)	33 (42.3)	15 (48.4)

Values expressed as mean ± standard error or number (percentage). Disease activity and patterns of organ involvement were defined by the BVAS 2003 scoring system. AAV, antineutrophil cytoplasmic antibody-associated vasculitis; BVAS, Birmingham Vasculitis Activity Score; EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis (Wegener's granulomatosis); MPA, microscopic polyangiitis; RLV, renal-limited vasculitis. Comparisons between the EGPA, GPA, and MPV/RLV groups were made by Student *t* test or Mann–Whitney *U* test. Statistical significance was determined by $P < 0.05/3$ using Bonferroni correction: [a]EGPA versus GPA, [b]EGPA versus MPA/RLV, [c]GPA versus MPA/RLV. Unclassifiable AAV was not compared with other forms of AAV.

Discussion

This is the first study to apply the EMEA algorithm to prospectively collected and high-quality data of AAV patients outside Europe and to elucidate the clinical phenotypes of the disease. In this study, 156 Japanese patients with newly diagnosed AAV were enrolled from major universities and referring hospitals across Japan and classified according to the EMEA algorithm. The results clearly indicated that MPO-ANCA-positive MPA/RLV was the most common form of AAV in the Japanese population, and more than one-half of the patients with EMEA algorithm-classified GPA showed MPO-ANCA positivity. In addition, we showed that ILD was a common manifestation in Japanese patients with AAV, especially in those with MPA.

The predominance of MPA/RLV and MPO-ANCA positivity in the Japanese population is in marked contrast to the results of studies previously reported from European countries and the United States [3,17-19]. Watts and colleagues validated the EMEA algorithm using 80 paper cases that were originally written for evaluation of the BVAS system for systemic vasculitis with some modifications, representing the relative frequency of AAV in their communities as follows: GPA > MPA > EGPA [4]. It is therefore indispensable and important to evaluate the utility of the EMEA algorithm in ethnicities outside Europe, as we did in this study. We found some difficulties in the classification between GPA and MPA with the EMEA algorithm; for example, of the nine patients classified as having GPA owing to the presence of GPA surrogate markers, five had chronic sinusitis in which granulomatous inflammation was not proven by histology. Because chronic sinusitis is a common disease and because fixed pulmonary infiltrates and otitis media are sometimes observed in AAV diseases other than GPA, classification of AAV using GPA surrogate markers should be cautiously applied in the countries or regions where MPA is more prevalent than GPA.

Within the spectrum of AAV, there are interesting geographic differences in the relative incidence of GPA versus MPA as well as of MPO-ANCA versus PR3-ANCA positivity [20]. In European countries, the incidence of GPA is approximately 4.9 to 10 per million, depending on the geographic location, with higher incidences reported in more northern countries and lower incidences in more southern countries [21,22]. A similar inverse relationship between GPA and MPA has been observed in the Southern Hemisphere [22]. A higher incidence of MPA/RLV than GPA and the predominance of MPO-ANCA found in the Japanese and Chinese AAV populations [3,5] could be related to the lower latitude of these countries.

GPA and MPA are heterogeneous entities with overlapping phenotypes. Recent studies have indicated that the classification system based on ANCA specificity (that is, MPO-ANCA versus PR3-ANCA) may better reflect the phenotypic spectrum of AAV. Cluster analysis of patients with newly diagnosed GPA and MPA from five clinical trials showed that the ANCA specificity classification may be more strongly associated with outcomes such as death and relapse rate than the traditional GPA-MPA separation [23]. Moreover, compared with the CHCC definition and the EMEA algorithm, ANCA specificity was more predictive of relapse in patients with biopsy-proven AAV; patients with PR3-ANCA were almost twice as likely to experience a relapse as those with MPO-ANCA [24]. In this regard, it is intriguing that a genome-wide association study of a European population revealed the presence of genetic distinctions between GPA and MPA that are associated with

Table 4 Comparison of demographics and disease manifestations in MPO-ANCA-positive and PR3-ANCA-positive patients

	MPO-ANCA (*n* = 125)	PR3-ANCA (*n* = 13)	*P* value
Male/female	47/78	6/7	0.56
Mean (median) age (years)	70.0 ± 1.04 (73)	61.3 ± 3.2 (61)	0.012
Serum creatinine (mg/dl)	1.94 ± 0.17	1.22 ± 0.53	0.19
Interstitial lung disease	57 (45.6)	0 (0)	0.0015
Alveolar haemorrhage	11 (8.8)	1 (7.7)	0.89
Disease severity			0.26
Localised	2 (1.6)	1 (7.7)	
Early systemic	33 (26.4)	2 (15.4)	
Generalised	68 (54.4)	8 (61.5)	
Severe	22 (17.6)	2 (15.4)	
General performance[a]			0.26
0/1/2/3/4	16/44/26/31/8	4/6/1/2/0	
BVAS[b]			
BVAS	17.5 ± 0.71	17.5 ± 2.2	0.99
General	95 (76.0)	10 (76.9)	0.94
Cutaneous	30 (24.0)	0 (0)	0.046
Mucous membranes/eyes	16 (12.8)	4 (30.8)	0.08
Ear, nose, and throat	22 (17.6)	12 (92.3)	<0.0001
Chest	49 (39.2)	7 (53.9)	0.31
Cardiovascular	8 (6.4)	2 (15.4)	0.23
Abdominal	2 (1.6)	0 (0)	0.65
Renal	98 (78.4)	6 (46.2)	0.010
Nervous system	55 (44.0)	4 (30.8)	0.36

Values expressed as mean ± standard error or number (percentage) unless otherwise noted. Five patients who were double-positive for both ANCAs were excluded from this analysis. ANCA, antineutrophil cytoplasmic antibody; BVAS, Birmingham Vasculitis Activity Score; MPO, myeloperoxidase; PR3, proteinase-3. [a]General performance was categorised according to the World Health Organization performance status except category 5 (death). [b]Disease activity and patterns of organ involvement were defined by the BVAS 2003 scoring system.

Table 5 Comparing patients with or without interstitial lung disease

	Patients with ILD (*n* = 61)	Patients without ILD (*n* = 95)	*P* value
Male/female	28/33	33/62	0.16
Mean (median) age (years)	69.3 ± 1.6 (71)	67.3 ± 1.3 (71)	0.26
MPO-ANCA	60 (98.3)	70 (73.7)	<0.001
PR3-ANCA	3 (4.9)	15 (15.8)	0.038
Serum creatinine (mg/dl)	1.61 ± 0.23	1.83 ± 0.19	0.45
Disease severity			0.059
Localised	1 (1.7)	3 (3.1)	
Early systemic	21 (34.4)	15 (15.8)	
Generalised	31 (50.8)	60 (63.2)	
Severe	8 (13.1)	17 (17.9)	
General performance[a]			
0/1/2/3/4	11/25/11/12/2	11/33/17/28/6	0.47
BVAS[b]			
BVAS	15.4 ± 1.0	18.4 ± 0.8	0.019
General	49 (80.3)	69 (72.6)	0.27
Cutaneous	13 (21.3)	26 (27.4)	0.39
Mucous membranes/eyes	5 (8.2)	15 (15.8)	0.17
Ear, nose, and throat	9 (14.8)	33 (34.7)	0.006
Chest	22 (36.1)	41 (43.2)	0.38
Cardiovascular	1 (1.7)	11 (11.6)	0.012
Abdominal	0 (0)	2 (2.1)	0.25
Renal	44 (72.1)	65 (68.4)	0.62
Nervous system	28 (45.9)	47 (49.5)	0.66

Values expressed as mean ± standard error or number (percentage) unless otherwise noted. ANCA, antineutrophil cytoplasmic antibody; BVAS, Birmingham Vasculitis Activity Score; ILD, interstitial lung disease; MPO, myeloperoxidase; MPA, microscopic polyangiitis; PR3, proteinase-3. [a]General performance was categorised according to the World Health Organization Performance Status except category 5 (death). [b]Disease activity and patterns of organ involvement were defined by the BVAS 2003 scoring system.

ANCA specificity [25]. Because of the limited number of patients with PR3-ANCA in our RemIT-JAV cohort, we were not able to perform cluster analysis within this database. We are currently implementing another large-scale cohort study of Japanese patients with AAV, and the combined database will enable us to clarify an association between ANCA positivity and clinical characteristics of AAV in the Japanese population.

MPO-ANCA may contribute to the severity of chronic renal injury and the prevalence of ILD in patients with AAV. Studies of renal biopsy specimens from patients with AAV have demonstrated a higher prevalence and/or severity of renal lesions in MPO-ANCA-positive patients compared with PR3-ANCA-positive patients [26]. These reports are in line with our findings that the mean serum creatinine level of MPO-ANCA-positive patients was numerically higher than that of PR3-ANCA-positive patients (1.94 versus 1.22 mg/dl).

A number of case reports and small case series have indicated that ILD developed more frequently in patients with MPO-ANCA-positive AAV, mainly in those with a diagnosis of MPA, compared with patients with PR3-ANCA-positive AAV [27,28]. A high ratio of MPO-ANCA positivity to PR3-ANCA positivity and a high prevalence of ILD have been reported in Asian countries [8-10], and *vice versa* in northern European countries; ILD was reported in 7.2% of all patients with MPA in the United Kingdom and in less than 10% in other European

countries [11,12]. In this study, we confirmed a high prevalence of ILD in Japanese patients with AAV. These patients were categorised as having a milder form (that is, more early systemic and less generalised or severe diseases) and lower disease activity according to the BVAS (Table 5), partially because ILD is not included in these definitions. Investigation of the clinical courses and prognoses of patients with ILD will shed more light on the relevance of ILD in the severity and activity of AAV.

We identified 16 unclassifiable AAV patients with ILD who were eligible for the EMEA algorithm because they were MPO-ANCA-positive, had symptoms and signs compatible with AAV such as general symptoms, and could not be diagnosed as having other diseases. A previous study reported that MPO-ANCA seroconversion from negative to positive occurred in 10% of patients with ILD in their clinical courses and that some patients with MPO-ANCA and ILD eventually developed typical MPA [29]. On the other hand, vasculitis was proven in five of 15 biopsy specimens of MPO-ANCA-positive patients with pulmonary fibrosis [13]. These data indicate that patients with unclassifiable AAV and ILD could be classified as having MPA. Further investigation is required to pursue this possibility.

This study has some limitations. The number of patients evaluated was limited, and the patient data were collected from the university and referral hospitals in large cities in Japan, which might cause tertiary care biases for the relative frequency of AAV diseases.

Conclusions

MPO-ANCA-positive MPA/RLV is the most common component of AAV in the Japanese population, and more than one-half of patients with GPA are also positive for MPO-ANCA. ILD is an important clinical manifestation in Japanese patients with AAV. Unclassifiable vasculitis with MPO-ANCA positivity and ILD may represent a novel variant of MPA. These data confirm the substantial difference in clinical and ANCA serological features of AAV between western countries and Asian countries, including Japan, and indicate that further investigation and discussion are required from a global perspective for a better AAV classification system that can be applied to all geographic areas and ethnicities.

Consent

This study was approved by the following ethical committees: Ethics Committee of the Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences; Medical Research Ethics Committee of Tokyo Medical and Dental University; Kyoto University Ethics Committee Review Board; Ethics Committee of Kagawa University; Ethics Committee of Juntendo University School of Medicine; Ethics Committee University of Tsukuba Hospital; Ethics Committee of St. Marianna University School of Medicine; Kanazawa University Ethical Committee; Ethics Committee of the University of Tokyo; Ethics Committee of Kyorin University School of Medicine; Saitama Medical Center Hospital Ethics Committee; Research Ethics Committee of the University of Miyazaki; Local Ethics Committee of Toho University; Ethics Committee of Kobe University Hospital; Ethics Committee of Kitano Hospital, The Tazuke Kofukai Medical Research Institute; Shimane University Institutional Committee on Ethics; Ethics Review Committee of Nagoya City University Graduate School of Medical Sciences; Ethics Committee of Ehime University Graduate School of Medicine; Ethics Committee of Jichi Medical University; Ethics Committee of Kyoto Prefectural University School of Medicine; Ethics Committee of Tokyo Medical University Hachioji Medical Center; Ethics Committee of Kitasato University Hospital; and Ethics Committee of Hamamatsu University School of Medicine.

Appendix

Research Committee of Intractable Vasculitis Syndrome of the Ministry of Health, Labour, and Welfare of Japan: in addition to the authors, the following investigators and institutions participated in this study: Department of Rheumatology and Clinical Immunology, Saitama Medical Center, Saitama Medical University (Koichi Amano); Department of Nephrology, Faculty of Medicine, University of Tsukuba (Kunihiro Yamagata); Department of Hemovascular and Artificial Organs, Faculty of Medicine, University of Miyazaki (Shouichi Fujimoto); Department of Respiratory Medicine, Toho University Omori Medical Center (Sakae Homma); Department of Clinical Pathology and Immunology, Kobe University Graduate School of Medicine (Shunichi Kumagai); Center for Nephrology and Urology, Division of Nephrology and Dialysis, Kitano Hospital, Tazuke Kofukai Medical Research Institute (Eri Muso); Department of Rheumatology, Shimane University Faculty of Medicine (Yohko Murakawa); Division of Rheumatology, Department of Medical Oncology and Immunology, Nagoya City University Graduate School of Medical Science (Shogo Banno); Department of Bioregulatory Medicine, Ehime University Graduate School of Medicine (Hitoshi Hasegawa); Division of Nephrology, Department of Internal Medicine, Jichi Medical University (Wako Yumura); Department of Cardiovascular Medicine, Kyoto Prefectural University School of Medicine (Hiroaki Matsubara); Division of Nephrology, Tokyo Medical University Hachioji Medical Center (Masaharu Yoshida); Department of Dermatology, Kitasato University School of Medicine (Kensei Katsuoka); and Third Department of Internal Medicine, Division of Immunology and Rheumatology, Hamamatsu University School of Medicine, Hamamatsu (Noriyoshi Ogawa).

Abbreviations
AAV: antineutrophil cytoplasmic antibody-associated vasculitis; ACR: American College of Rheumatology; ANCA: antineutrophil cytoplasmic antibody; BVAS: Birmingham Vasculitis Activity Score; CHCC: Chapel Hill Consensus Conference; EGPA: eosinophilic granulomatosis with polyangiitis (Churg–Strauss syndrome); EMEA: European Medicines Agency; GPA: granulomatosis with polyangiitis (Wegener's granulomatosis); ILD: interstitial lung disease; MPA: microscopic polyangiitis; MPO: myeloperoxidase; PR3: proteinase-3; RemIT-JAV: Remission Induction Therapy in Japanese Patients with ANCA-associated Vasculitides; RLV: renal-limited vasculitis.

Competing interests
MH has received research grants and/or honoraria from Abbott Japan Co., Ltd, Astellas Pharma Inc., Bristol-Myers Squibb K.K., Chugai Pharmaceutical Co., Ltd, Eisai Co., Ltd, Janssen Pharmaceutical K.K., Mitsubishi Tanabe Pharma Co., Santen Pharmaceutical Co., Ltd, Takeda Pharmaceutical Co., Ltd, Teijin Pharma, Ltd, and Pfizer Japan Inc. TF has received research grants from Abbott Japan Co., Ltd, Astellas Pharma Inc., Bristol-Myers Squibb K.K., Chugai Pharmaceutical Co., Ltd, Daiichi-Sankyo Pharmaceutical Co. Ltd, Eisai Co., Ltd, Mitsubishi Tanabe Pharma Co, Takeda Pharmaceutical Co., Ltd, and Pfizer Japan Inc. HM serves as a consultant to AbbVie Inc., Astellas Pharma Inc., and Teijin Pharma Ltd; received honoraria from Astellas Pharma Inc., MSD K.K., Takeda Pharmaceutical Co., Ltd, and Mitsubishi Tanabe Pharma Co.; and received research funding from Astellas Pharma Inc., Daiichi Sankyo Inc., Dainippon Sumitomo Pharma Co., Ltd, MSD K.K., Novo Nordisk Pharma Ltd, and Takeda Pharmaceutical Co., Ltd.

Authors' contributions
KS was responsible for conception and design, data collection and analysis, and manuscript writing. MY, MH, and TF were responsible for conception and design, data collection and analysis, and critical revision. HD, YT, SI, HY, TW, and JH were responsible for data collection and interpretation, and critical revision. YA and HM were responsible for conception and design, data collection and analysis, and critical revision. All authors read and approved the final manuscript.

Acknowledgements
This work was supported by grants from Research on Rare and Intractable Diseases, the Ministry of Health, Labour and Welfare, Japan (nannti-ippann-004). The authors thank Keiko Hongo, Kumiko Muraki, Eri Katsuyama, Takayuki Katsuyama, Haruki Watanabe, Mariko Narazaki, Noriko Toyota, Yoshinori Matsumoto, Ryutaro Yamanaka, and Kouichi Sugiyama for their great assistance in data management.

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Received: 16 August 2013 Accepted: 9 April 2014
Published: 23 April 2014

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doi:10.1186/ar4550
Cite this article as: Sada *et al.*: Classification and characteristics of Japanese patients with antineutrophil cytoplasmic antibody-associated vasculitis in a nationwide, prospective, inception cohort study. *Arthritis Research & Therapy* 2014 **16:**R101.

Clin Exp Nephrol
DOI 10.1007/s10157-014-0987-9

ORIGINAL ARTICLE

Relapse and its remission in Japanese patients with idiopathic membranous nephropathy

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Received: 15 January 2014 / Accepted: 12 May 2014

Abstract
Background The prognosis in patients with idiopathic membranous nephropathy (IMN) is diverse. However, the prognosis after relapse and factors affecting relapse remain unclear.
Methods A total of 146 Japanese patients with IMN who had been followed up for at least 3 years, or until end-stage renal failure or death were enrolled in this retrospective study. The initial clinicopathological factors were examined between the patients with and without relapse. The patients were assigned to two groups based on the electron microscopic findings: homogeneous type with synchronous electron-dense deposits and heterogeneous type with various phases of dense deposits.
Results A total of 105 of the 146 patients (72 %) achieved complete remission (CR) or incomplete remission (ICR) I after initial treatment. Twenty-six of the 105 patients relapsed after CR or ICR I (25 %). There were no differences in initial clinical findings or data between the patients with and without relapse, except for the higher degree of proteinuria at onset in patients with relapse. The relapse rate of the heterogeneous group (43 %) was higher than that in the homogeneous group (20 %). There were no significant associations between relapse rate and immunosuppressive therapy at onset. Eleven of 26 patients showing relapse (42 %) achieved CR or ICR I, which was lower than the rate for patients with initial remission.
Conclusion Our results suggest that patients with relapse achieved CR or ICR I and that electron microscopic findings demonstrating heterogeneous type indicated susceptibility to relapse.

Keywords MN · Relapse · Remission · Electron-dense deposit

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Introduction

Idiopathic membranous nephropathy (IMN) remains the most common cause of adult onset nephrotic syndrome. Overall, 40 % of Japanese nephrotic patients, and one-third to one half of the patients in other countries, develop end-stage renal disease (ESRD) within 20 years of onset or die from complications, such as infection, cardiovascular incidents, or malignancy [1–5]. On the other hand, about 30 % of patients with IMN achieve spontaneous remission, suggesting that IMN may be a heterogeneous disease. Moreover, some patients relapse after achieving remission. However, there is little information on the clinical characteristics and prognosis of IMN after remission, and the clinical manifestations of relapsing IMN remain unclear. Conflicting results have been reported regarding the

Published online: 24 June 2014

therapeutic effects of various treatments due to the inability to demarcate the different prognostic groups [4, 5].

Recent studies emphasized the prognostic value of tubulointerstitial changes (cellular infiltration and fibrosis) [6, 7], glomerular changes with focal segmental sclerotic lesions [8], and advanced histological stage of the electron-dense deposits [9] (III–IV according to the criteria of Ehrenreich and Churg [10, 11]). Interestingly, we reported previously that synchronous heterogeneous type dense deposits represent an independent poor prognostic factor in IMN patients compared to patients with homogeneous type [12]. Moreover, we reported previously differences in clinical responses to high-dose intravenous immunoglobulin therapy among subtypes of IMN defined by their pattern of subepithelial electron-dense deposits [13]. Rosen et al. [14] previously delineated four groups according to their morphological and clinical patterns; short with one generation of deposits, short/repeated, long/rapid, and long/slow. We simplified this classification by combining these with one generation of deposits, short/repeated, and long/rapid into the homogeneous type and designating the long/slow cases as the heterogeneous type on the basis of synchronicity and clinical course [12].

In this study, we retrospectively analyzed the clinicopathological characteristics in 146 Japanese patients with IMN who were followed up for at least 3 years after the initial renal biopsy. Predisposing clinicopathological features of patients with relapse after remission regarding the electron-dense deposition, especially the significance of heterogeneous pattern, were noted. In addition, relapsing patients reached re-remission.

Subjects and methods

Patients and treatments

A total of 146 Japanese patients (85 men and 61 women, aged 11–80 years, mean 46.2 years) with IMN admitted to Kanazawa University Hospital, or its affiliated hospitals, between 1965 and 2009 were enrolled in this retrospective study. These patients were followed up for at least 3 years, or until end-stage renal failure (ESRF) or death (from 2 to 420 months, mean 134.7 months). Diagnosis was confirmed in all patients by percutaneous needle renal biopsy with informed consent. Patients with secondary membranous nephropathies, such as lupus nephritis, or membranous nephropathies related to hepatitis B virus or malignancy, according to the clinical and laboratory findings were excluded from the study. The patients were treated non-randomly, depending on the judgment of the doctor in charge of each case, with either no immunosuppressant or with supportive therapy ($n = 53$); corticosteroid (steroid) alone ($n = 17$); cyclophosphamide with steroid ($n = 23$); high-dose intravenous immunoglobulin with or without steroid ($n = 28$); cyclosporine with or without steroid ($n = 22$); or mizoribine with steroid ($n = 3$). Informed consent was obtained for all renal biopsies and treatments. The use of antihypertensive drugs, diuretics, angiotensin-converting enzyme inhibitors (ACEi)/angiotensin II receptor blockers (ARB), and statins was allowed during the follow-up period as required. This study was approved by the ethics committee of Kanazawa University (IRB approval number: 846).

Response to treatment

Clinical status was assessed according to the Japanese clinical categories employing the criteria of nephrotic state, the presence of marked proteinuria >3.5 g/day or 3 + (300 mg/dL) to 4 + (1,000 mg/dL) by Multistics (Miles, Tokyo, Japan) and hypoalbuminemia (<30 g/L); incomplete remission type II (ICR II) (i.e., normal serum albumin levels, >30 g/L) with a mean 24-h proteinuria of 1.0–3.5 g for 7 consecutive days or 2 + (100 mg/dL) to 3 + (300 mg/dL); incomplete remission type I (ICR I) (i.e., normal serum albumin levels, >30 g/L) with a mean daily proteinuria of <1.0 g for 7 consecutive days or + (30 mg/dL); and complete remission (CR) (i.e., daily proteinuria <0.3 g with normal serum albumin levels). Remission was defined as CR or ICR I with normal or stable renal function. Patients who did not achieve remission after initial treatments were defined as non-responders, and relapse was defined as a rise in proteinuria over 1.0 g/day after remission. ESRD was defined as the need for hemodialysis, peritoneal dialysis, or renal transplantation.

Histopathological studies

Electron microscopic examination

A total of 168 specimens were fixed with glutaraldehyde and osmium tetroxide, embedded in Epon 812 (Oken Shoji Co., Tokyo, Japan), sliced into sections 0.1 μm thick, double-stained with uranyl acetate and lead citrate, and examined under an electron microscope (Hitachi H-600; Hitachi Co., Tokyo, Japan). For this study, specimens were examined with emphasis on the phase and synchronicity of subepithelial and intramembranous electron-dense deposits, as reported previously [11]. The classification of Rosen, Tornroth, and Bernard [14] was modified and specimens showing synchronous electron-dense deposits with a single phase were arbitrarily classified as homogeneous type, and others having various phases of electron-dense deposits were classified as heterogeneous type (Fig. 1). In subanalyses for secondary outcome, seven patients of

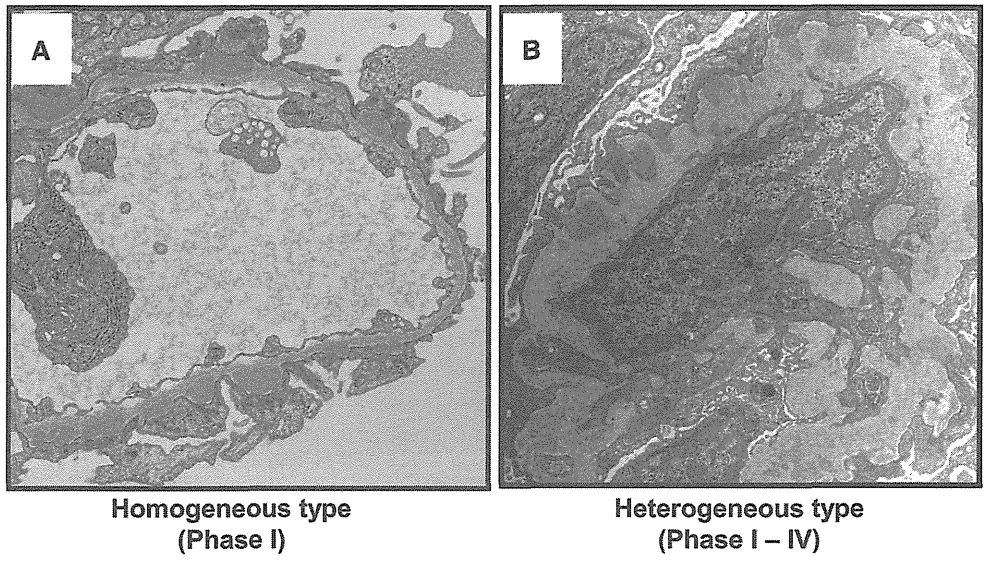

Fig. 1 Subtypes of idiopathic membranous nephropathy. **a** Homogeneous type (original magnification ×3,000). **b** Heterogeneous type (original magnification ×3,000)

Table 1 Clinical findings and data of all patients at onset

	Total	Remission (CR + ICR I)	Non-remission (ICR II + NS)
No. of patients	146	105	41
Age (y.o)	46.2 ± 15.5	44.8 ± 16.0	49.7 ± 13.9
Female/Male	61/85	46/59	15/26
Follow-up period (months)	152 ± 90	176 ± 88	89 ± 61*
Systolic BP (mmHg)	124.1 ± 19.1	120.8 ± 21.0	131.2 ± 11.9
Diastolic BP (mmHg)	76.2 ± 8.8	73.3 ± 9.0	82.3 ± 4.3*
Proteinuria (g/day)	4.8 ± 3.2	4.6 ± 2.6	5.7 ± 5.3
Nephrotic syndrome (%)	71.9	66.7	85.4*
Serum creatinine (mg/dL)	0.95 ± 0.42	0.89 ± 0.34	1.08 ± 0.59
Serum total protein (g/dL)	5.00 ± 1.14	5.08 ± 1.12	4.72 ± 1.25
Serum albumin (g/dL)	2.55 ± 0.88	2.65 ± 0.86	2.19 ± 0.86
Serum cholesterol (mg/dL)	314 ± 98	313 ± 88	317 ± 127

* $P < 0.05$ versus Remission group

homogeneous type were further classified as a deep subgroup due to large phase II depositions with prolonged nephrotic state (similar to Rosen's long/rapid type).

Statistical analysis

Statistical analyses were performed using the chi-square test, analysis of variance (ANOVA), the Kruskal–Wallis test, Pearson's and Spearman's correlation coefficient for analyses of parametric and nonparametric data, the Mantel–Cox log-rank test, and the Kaplan–Meier life-table method. Values are expressed as mean ± standard error of the mean (SEM). IBM SPSS 19 software was used for the statistical calculations. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

Results

Patient backgrounds and outcomes

At the time of renal biopsy, 105 patients (71.9 %) were in a nephrotic state and 41 (18.1 %) were in a non-nephrotic state. During the follow-up period, 15 patients (10.3 %) developed ESRD and 24 patients (22.9 %) died from non-renal diseases. A total of 105 of the 146 patients (72 %, 59 men and 46 women) achieved CR or incomplete ICR I after initial treatments (Table 1). Eighty-three percent of the homogeneous group and 48 % of the heterogeneous group achieved the criteria of remission. Twenty-six of the 105 patients relapsed after CR or ICR I (25 %, 14 men and 12 women; mean age, 44.5 years) (Table 2). The mean duration of relapse was 114 months, and the longest duration of remission was 456 months (Fig. 2).

Patient with heterogeneous type immune complex deposition tended to relapse

Next, predisposing characteristics for relapse were examined. Clinical parameters in patients with or without relapse were studied. There were no differences in initial clinical findings or data between the patients with and without relapse, with the exception of the higher degree of proteinuria at onset in patients with relapse (Table 1).

Table 2 Clinical findings and data at onset of relapse or non-relapse patients achieving CR

	Relapse	Non-relapse	*P* value
No. of patients	26	79	
Age (y.o)	44.5 ± 15.0	44.8 ± 16.4	NS
Female/Male	12/14	34/45	
Follow-up period (months)	151 ± 111	138 ± 79	NS
Initial treatment to remission (months)	33.4 ± 19.4	30.9 ± 20.4	NS
Systolic BP (mmHg)	119.5 ± 21.7	124.6 ± 21.6	NS
Diastolic BP (mmHg)	73.0 ± 4.8	73.9 ± 10.4	NS
Proteinuria (g/day)	6.4 ± 4.1	4.1 ± 2.3	0.02
Nephrotic syndrome (%)	75.0	64.5	NS
Serum creatinine (mg/dL)	0.90 ± 0.20	0.90 ± 0.31	NS
Serum total protein (g/dL)	4.65 ± 1.42	5.27 ± 1.03	NS
Serum albumin (g/dL)	2.60 ± 0.97	2.71 ± 0.80	NS
Serum cholesterol (mg/dL)	299 ± 55	314 ± 90	NS

NS Not significant

The relapse rate of the heterogeneous group (43 %) was higher than that of the homogeneous group (20 %). Therefore, a pattern of electron-dense deposition based on EM showing heterogeneous type may be a risk factor for relapse. On the other hand, there was no significant change in clinical findings and date at onset of according to the type of immune complex deposition (Table 3).

Patterns of immune complex deposition in re-biopsy specimens

During the follow-up period, nine relapsing patients underwent second renal biopsy. These re-biopsy specimens at relapse were examined to determine whether patterns of electron-dense deposits may change. Interestingly, the pattern of electron-dense deposits remained unchanged in most cases from onset to relapse (Fig. 3).

Correlation of relapse with immunosuppressive therapy

There were no significant associations between relapse rate and immunosuppressive therapy at onset (steroid alone, intravenous immunoglobulin, steroid with cyclophosphamide, or cyclosporine with or without steroid). Furthermore, 11 of 26 patients who showed relapse (42 %) achieved CR or ICR I, which was lower than the rate observed in patients with initial remission (74 %) (Table 4). The observation period of the relapses (86 months) was shorter than that of the initial remission (135 months).

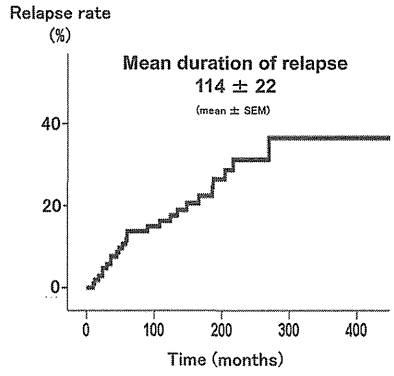

Fig. 2 Remission duration to relapse. The mean remission duration was 114 months, and the longest duration of remission was 456 months

Table 3 Clinical findings and data at onset of according to the type of immune complex deposition

	Homogeneous	Heterogeneous	*P* value
No. of patients	71	46	
Age (y.o)	49.6 ± 15.9	47.0 ± 13.7	NS
Female/Male	28/43	19/27	
Follow-up period (months)	125 ± 74	155 ± 92	NS
Systolic BP (mmHg)	121.4 ± 20.0	133.3 ± 12.6	NS
Diastolic BP (mmHg)	75.7 ± 9.2	78.1 ± 7.6	NS
Proteinuria (g/day)	4.9 ± 3.5	4.7 ± 2.3	NS
Nephrotic syndrome (%)	67.6	84.8	NS
Serum creatinine (mg/dL)	0.94 ± 0.43	0.98 + 0.43	NS
Serum total protein (g/dL)	4.86 ± 1.14	5.42 ± 1.09	NS
Serum albumin (g/dL)	2.41 ± 0.88	2.94 ± 0.76	NS
Serum cholesterol (mg/dL)	330 ± 101	259 ± 62	NS

NS Not significant

Discussion

In this study, the clinicopathological features of patients with relapse after remission were analyzed. There were no differences in initial clinical findings or data between the patients with and without relapse, with the exception of the higher degree of proteinuria at onset in patients with relapse. The relapse rate of the heterogeneous group was higher than that of the homogeneous group. In addition, patients with relapse achieved CR or ICR I. Finally, electron microscopic findings demonstrating heterogeneous type were associated with susceptibility to relapse.

This study indicated that 23 % of IMN relapsed within the period examined. There is little evidence regarding the rate of relapse of IMN after remission. In Europe and the

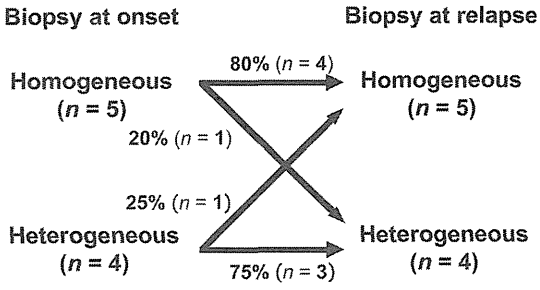

Fig. 3 Pathological findings after relapse. Type of electron-dense deposits did not change in most cases from onset to relapse

Table 4 Remission rate in patients with relapse

	Initial treatment	After relapse
No. of patients	146	26
Follow-up period (months) (mean ± SEM)	135 ± 7	86 ± 16
Remission (CR + ICR I)	74 %	42 %
ICR II	14 %	15 %
Nephrotic syndrome	4 %	31 %
End-stage renal disease	8 %	12 %

USA, 30–50 % of IMN patients relapse after remission [15–18]. However, the clinical manifestations of relapsing IMN in Japan remain unclear. Eriguchi et al. [19] reported relapse in 30 of 90 Japanese patients (33 %) with IMN who had achieved remission. Yuan et al. [20] reported relapse in 5 of 32 Chinese patients (19.2 %) with nephrotic IMN who were treated with i.v. pulse cyclophosphamide together with steroid and achieved remission. Based on these results together with those of the present study, the relapse rate in Japan may be lower than those in Europe and the USA. However, given the lack of evidence, further studies are required to compare the relapse rates among various races and their effects on long-term prognosis.

Our clinicopathological study revealed that the heterogeneous pattern on electron microscopy was a distinct risk factor for relapse. We reported previously that synchronous heterogeneous type dense deposition in IMN patients was an independent poor prognostic factor compared with the homogeneous type [12]. These findings suggest that electron microscopic examination of the immune complex deposition pattern may be a useful tool for estimating disease activity and clinical outcome. Ehrenreich et al. [10] first described the evolution of glomerular capillary lesions in terms of four stages: initial subepithelial dense deposits (stage I); a subsequent basement membrane spike response (stage II); eventual incorporation of the deposits within the glomerular basement membrane (stage III); and finally formation of a markedly thickened basement membrane (stage IV). Patients with single generation of deposits showed restoration of a normal glomerular basement membrane with intramembranous lucencies, e.g., IV, in complete remission. Alternatively, the present study indicated that the heterogeneous pattern of electron microscopic findings is a risk factor for relapse. Detailed molecular mechanism involved in this process remains investigated so far. The mechanism may be speculated that the patients of heterogenous type had some underlining factors for repeated depositions to glomerulus basement membrane, which may keep damage glomeruli subclinically even after patients achieved remission in clinical settings. In this condition, some certain triggering stimuli might re-activate glomerular damage. These underlying molecular mechanisms will be examined in the future.

Analyses of the present data indicated that there were no significant differences between the patients with and without relapse in terms of age, time to remission, initial creatinine level, or serum albumin level. However, proteinuria was more evident in the patients with relapse. Bohdan et al. [21] reported relapses in 16 of 55 IMN patients with CR, in whom the severity of proteinuria was greater than in the no-relapse group. These data may support our suggestion that a lower level of proteinuria at onset of IMN is a factor involved in maintenance of remission. Further, the contents of immunosuppressive therapy may be related to relapse. Surprisingly, our data revealed no significant associations between relapse rate and immunosuppressive therapy at onset (steroid alone, intravenous immunoglobulin, steroid with cyclophosphamide, or cyclosporine with or without steroid). Further studies are required to determine the clinical characteristics involved in relapse.

Importantly, 11 of 26 patients with relapse (42 %) achieved CR or ICR I, which was lower than the rate observed among patients with initial remission (74 %). This was partly because the observation period of the relapse (86 months) was shorter than that of the initial remission (135 months). In support of our suggestion, some papers have reported the rate of second remission. Manos et al. reported relapse in 8 of 15 IMN patients who had achieved remission. Of these eight patients, three (38 %) went into second remission [15]. Ponticelli et al. [17] reported the relapses of non-nephrotic in 22 of 74 patients of IMN who had achieved CR. Twelve of the 22 patients (55 %) went into second remission. Taken together with the results of the present study, re-remission after relapse is anticipated, although studies of re-remission rate are required.

In conclusion, the findings of the present study suggest that IMN patients with relapse achieved CR or ICR I and that electron microscopic findings demonstrating

heterogeneous type indicated susceptibility to relapse. Thus, our results suggest the value of a prompt diagnosis based on electron microscopic findings in patterns with IMN.

Acknowledgments This study was supported in part by a Grant-in-Aid for Progressive Renal Diseases Research, Research on Rare and Intractable Disease, from the Ministry of Health, Labour and Welfare of Japan.

Conflict of interest The authors have declared that no Conflict of interest exists.

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