ORIGINAL ARTICLE

Impact of kidney function and urinary protein excretion on pulmonary function in Japanese patients with chronic kidney disease

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Abstract

Background Although the cardiorenal relationship in chronic kidney disease has been investigated, information about the lung–kidney relationship is limited. Here, we investigated the impact of kidney function and urinary protein excretion on pulmonary dysfunction.

Methods The data from pulmonary function tests and kidney function (estimated glomerular filtration rate [eGFR] and urinary protein) between 1 April 2005 and 30 June 2010 were selected from our laboratory database. Data were classified into 4 categories according to eGFR and proteinuria. Category 1, eGFR $\geq$60 ml/min/1.73 m^2 and urinary protein <0.3 g/gCr; category 2, eGFR <60 ml/min/1.73 m^2 and urinary protein <0.3 g/gCr; category 3, eGFR $\geq$60 ml/min/1.73 m^2 and urinary protein $\geq$0.3 g/gCr; and category 4, eGFR <60 ml/min/1.73 m^2 and urinary protein $\geq$0.3 g/gCr. Pulmonary function data were evaluated according to these 4 categories.

Results A total of 133 participants without major respiratory disease, abnormal computed tomography and smoking history were enrolled. Hemoglobin (Hb)-adjusted percentage carbon monoxide diffusing capacity (%DL$_{CO}$) in category 4 (46.2 $\pm$ 7.5) and category 2 (63.6 $\pm$ 17.8) were significantly lower than in category 1 (75.8 $\pm$ 18.9) ($P < 0.05$). In addition, Hb-adjusted %DL$_{CO}$ was weakly correlated with eGFR in participants with urinary protein <0.3 g/gCr ($R = 0.30$, $P = 0.001$). Hb-adjusted %DL$_{CO}$ was strongly correlated with eGFR in participants with urinary protein $\geq$0.3 g/gCr ($R = 0.81$, $P < 0.001$). Other pulmonary function test markers (percentage (%) vital capacity, % forced expiratory volume in one second (FEV1), FEV1/forced vital capacity, % total lung capacity, and % residual volume) were not significantly different between categories.

Conclusion This study suggests that decreased eGFR is associated with decreased %DL$_{CO}$ in proteinuric patients.

Keywords Kidney function · Pulmonary function tests · Chronic kidney disease · Urinary protein · DL$_{CO}$ (carbon monoxide diffusing capacity) · Diabetic nephropathy

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Background

The kidneys maintain whole-body homeostasis through regulation of body fluid, blood pressure, electrolytes and acid–base balance. Thus, the kidneys work in cooperation with the heart, liver, lungs and other organs. Various mechanisms participate in the organ-to-organ networks,

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including humoral factors, and neuronal network systems. Although several reports indicate a relationship between the kidneys and the heart [1, 2], limited information is available regarding crosstalk between the kidneys and lungs in chronic kidney disease (CKD).

In contrast to CKD, acute kidney injury (AKI) often leads to acute lung injury. These associations between the kidneys and lungs [3–6] result in deterioration of the general condition of patients in critical care. Recent animal studies indicate that AKI increases inflammatory cytokine levels in the injured kidney and serum, and these cytokines may induce lung injury [7–9]. However, inflammatory cytokine production is also detected in the injured kidneys in CKD as well as in AKI [10]. Therefore, it is reasonable to speculate that CKD may affect pulmonary function.

The present study was performed to examine the associations between parameters of CKD (estimated glomeular filtration rate [eGFR] and urinary protein) and the results of pulmonary function tests (PFTs).

Method

Subjects

The data from PFTs and kidney function parameters (eGFR and urinary protein) were selected from our laboratory database between 1 April 2005 and 30 June 2010. PFTs were evaluated by international criteria [11]. Vital capacity (VC), forced vital capacity (FVC), functional residual capacity (FRC), and carbon monoxide diffusing capacity (DL_{CO}) were measured using the PFT system CHESTAC-9800 (Chest M.I., Inc., Tokyo, Japan). Participants were coached regarding standard forced expiratory manoeuvres. Three technically acceptable blows were recorded and the best values were used as their data.

We selected participants with normal spirometry (both %VC >80 % and forced expiratory volume in one second (FEV1)/FVC >70 %) to evaluate the association between renal function and pulmonary function. Participants with abnormal spirometry, major respiratory disease (asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, interstitial pneumonia, neuromuscular disease, scleroderma, pulmonary resection, and pneumonedema) and abnormal computed tomography (CT) were excluded. Moreover, participants with a history of smoking were excluded to remove the influence of smoking on kidney [12] and lung fucntion [13]. PFT markers (%VC, %FEV1, % residual volume [RV], % total lung capacity [TLC], and $\%DL_{CO}$) were adjusted for age, height, and sex using prediction formula.

This study was approved by the ethics committee of Kanazawa University Hospital (Approval No. 907) and was conducted in accordance with the Declaration of Helsinki.

Method of DLco

We measured DLco in accordance with the recommendations [14]. In short, once the mouthpiece is in place, four to five tidal volumes are recorded to determine a regular end-expiratory baseline. The DLco manoeuvre then begins with exhalation to RV. At RV the subject's mouthpiece is connected to a source of test gas and the subject inhales rapidly to TLC. The volume of test gas inhaled is V_I. DL_{CO} should be measured near TLC.

Participants' lung and kidney data function

eGFR (ml/min/1.73 m^2) was calculated using the prediction formula 194 × creatinine (Cr)$^{-1.094}$ × age (year)$^{-0.287}$ (multiplied by 0.739 for females) developed by the Japanese Society of Nephrology. Urinary protein (g/gCr) was evaluated by spot urine protein–creatinine ratio.

Pulmonary function was evaluated using PFT markers of restrictive ventilatory impairment (%VC, %RV, and %TLC), PFT markers of obstructive ventilatory impairment (%FEV1 and FEV1/FVC), and PFT markers of pulmonary diffusing capacity ($\%DL_{CO}$). To avoid the effects of physical features (age, sex and height), we used the predicted value of all PFT markers. Because predicted value is corrected by physical features, PFT markers are not influenced by physical features [13]. Furthermore, to avoid the effects of renal anaemia on CKD, $\%DL_{CO}$ was corrected by haemoglobin (Hb-adjusted $\%DL_{CO}$). The compensation formula of DL_{CO} for males is DL_{CO} × (10.22 + haemoglobin)/(1.7 × haemoglobin) [14, 15]. For females, it is DL_{CO} × (9.38 + haemoglobin)/(1.7 × haemoglobin).

Participants were classified into 4 categories according to eGFR and urinary protein—category 1, eGFR ≥60 ml/min/1.73 m^2 and urinary protein <0.3 g/gCr, category 2, eGFR <60 ml/min/1.73 m^2 and urinary protein <0.3 g/gCr; category 3, eGFR ≥60 ml/min/1.73 m^2 and urinary protein ≥0.3 g/gCr; and category 4, eGFR <60 ml/min/1.73 m^2 and urinary protein ≥0.3 g/gCr. Participants with CKD had an eGFR <60 ml/min/1.73 m^2 or/and U-PCR >0.3 g/gCr for at least 3 months. Pulmonary function was compared using these 4 categories.

Statistics

One-way analysis of variance (ANOVA) and Dunnett's post hoc test were used to compare PFT markers in category 1 to those in other categories. The chi-squared test was used for categorical parameters. Spearman's rank correlation test and multiple linear regression analysis were used to examine relationships between PFTs (Hb-adjusted $\%DL_{CO}$) and renal function (eGFR and urinary protein).

Fig. 1 Inclusion and exclusion criteria

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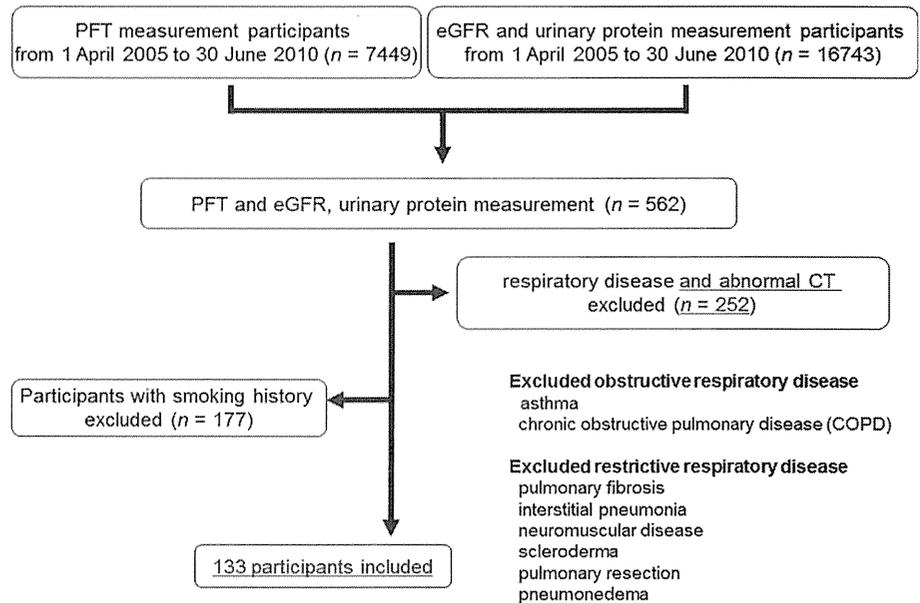
Stata 12 (Stata Corp, College Station, TX, USA) was used for all statistical analyses. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

Results

Participant selection and baseline characteristics

A total of 7,449 participants undergoing PFTs and 16,743 participants undergoing eGFR and urinary protein measurement were enrolled between 1 April 2005, and 30 June 2010 at Kanazawa University Hospital. A total of 562 participants underwent both PFTs and renal function tests. We excluded 252 participants with major respiratory disease and abnormal CT and 177 participants with smoking history. Thus, 133 participants were finally included in this study (Fig. 1).

Table 1 shows the characteristics of the subjects. Neither age, body mass index (BMI), Hb, brain natriuretic peptide (BNP) nor the presence of diabetes and hypertension were significantly different between categories. As we excluded participants with smoking history, females were predominant. The percentages of males in category 2 (2.3 %) and category 4 (0 %) were significantly lower than those in category 1 (32.4 %) and category 3 (22.2 %).

Changes in pulmonary function according to eGFR and urinary protein excretion

Table 2 shows a comparison between PFT parameters in each category. Hb-adjusted %DL$_{CO}$ in category 4 (46.2 ± 7.5) and category 2 (63.6 ± 17.8) were significantly lower than in category 1 (75.8 ± 18.9) ($P < 0.05$). Other PFT markers (%VC, %FEV1, FEV1/FVC, %TLC,

and %RV) were not significantly different between the categories.

Association between Hb-adjusted %DL$_{CO}$ and eGFR and urinary protein excretion

Figure 2 shows the results of single regression analysis between Hb-adjusted %DL$_{CO}$ and kidney function. Hb-adjusted %DL$_{CO}$ was significantly positively correlated with eGFR ($R = 0.36$, $P < 0.001$, Fig. 2a), but was significantly negatively correlated with urinary protein ($R = -0.25$, $P = 0.005$, Fig. 2b).

Figure 3 shows the results of single regression analysis between Hb-adjusted %DL$_{CO}$ and eGFR. Hb-adjusted %DL$_{CO}$ was weakly correlated with eGFR in patients with urinary protein <0.3 g/gCr ($R = 0.30$, $P = 0.001$, Fig. 3a), but was strongly correlated with eGFR in patients with urinary protein ≥0.3 g/gCr ($R = 0.81$, $P < 0.001$, Fig. 3b).

To examine the precise relationships between kidney function and Hb-adjusted %DL$_{CO}$, multiple linear regression analysis was conducted in all patients examined in this study. As shown in Table 3, eGFR was associated with Hb-adjusted %DL$_{CO}$ ($P = 0.023$) after adjusting for physical features (age and gender), expiratory minute volume (VE), and the presence or absence of DM and Hypertension. Urinary protein tended to be associated with Hb-adjusted %DL$_{CO}$ ($P = 0.095$) after adjusting for physical features (age and gender), VE, and the presence or absence of DM, and hypertension.

Discussion

This study was performed to examine the associations between kidney function or urinary protein excretion and

Table 1 Characteristics of subjects

Category eGFR (ml/min/1.73 m²) 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/m²)	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 m²)	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 m²) 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 m²) 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)
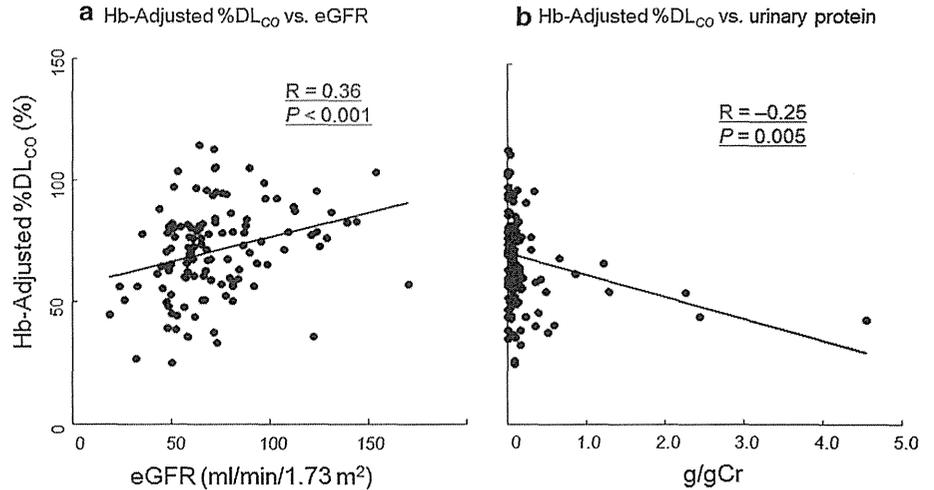

a Hb-Adjusted %DL_CO vs. eGFR

R = 0.36
P < 0.001

b Hb-Adjusted %DL_CO vs. urinary protein

R = −0.25
P = 0.005

Fig. 3 Relationships between Hb-adjusted %DL_CO and eGFR depending on urinary protein

a Urinary protein < 0.3 g/gCr

R = 0.30
P = 0.001

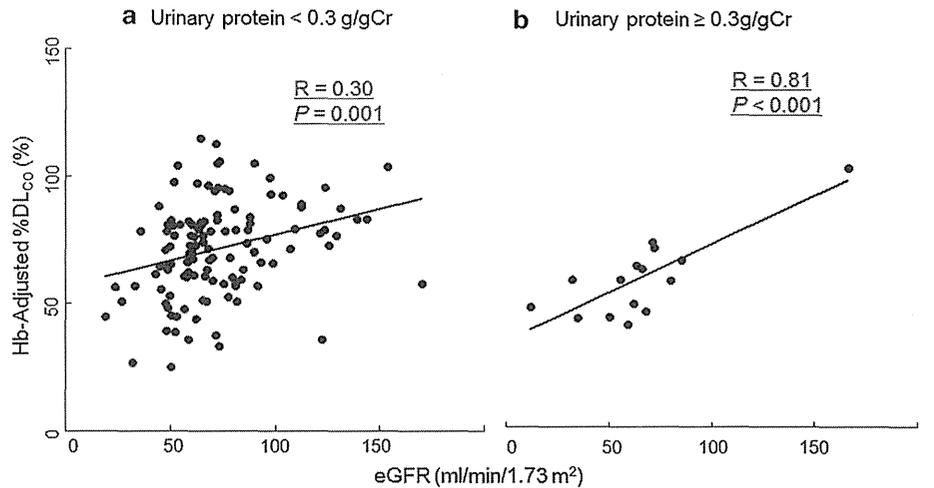**b** Urinary protein ≥ 0.3g/gCr

R = 0.81
P < 0.001

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.

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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.

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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].
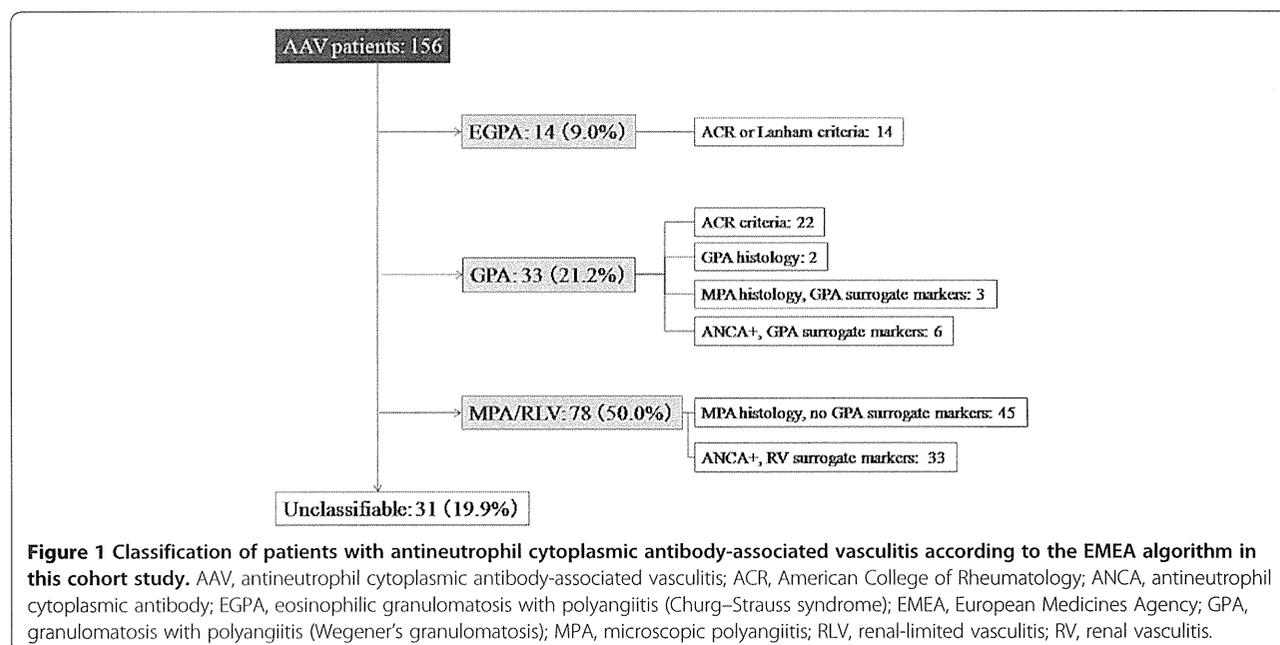
Statistical analysis

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.

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ORIGINAL ARTICLE

Relapse and its remission in Japanese patients with idiopathic membranous nephropathy

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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

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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).
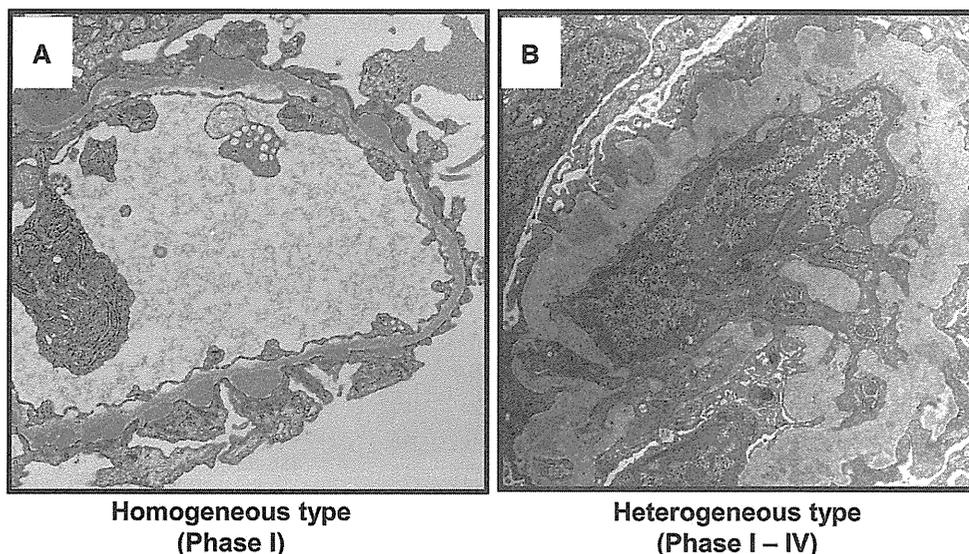**b** Heterogeneous type (original
magnification ×3,000)

Homogeneous type
(Phase I)

Heterogeneous type
(Phase I – IV)

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 sub-
group 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 dura-
tion 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 exam-
ined. 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).