**Table 2** The multivariate cox proportional hazard model for remission ($n = 22$)

| Predictors | Model 1[a] | | | Model 2[b] | | |
|---|---|---|---|---|---|---|
| | HR | 95 % CI | *P* value | HR | 95 % CI | *P* value |
| Age (per 5-year interval) | 1.35 | 0.76–2.40 | 0.299 | 1.64 | 0.64–4.17 | 0.299 |
| Female (vs. male) | 2.76 | 0.75–10.1 | 0.125 | 6.02 | 0.49–73.9 | 0.160 |
| eGFR (per 10 ml/min/1.73 $m^2$) | 1.56 | 0.96–2.54 | 0.071 | 2.01 | 0.82–4.91 | 0.124 |
| uPCR (per 1.0 g/gCre) | – | – | – | 0.95 | 0.72–1.27 | 0.771 |
| PLA2R-related (vs. -unrelated) | 1.24 | 0.29–5.20 | 0.759 | 1.41 | 0.30–6.55 | 0.659 |
| IgG4-dominant (vs. non-dominant) | 0.08 | 0.01–0.51 | 0.007* | 0.02 | 0.00–0.91 | 0.044* |
| Ehrenreich–Churg stage | – | – | – | 0.74 | 0.21–2.60 | 0.643 |
| Immunosuppressive therapy (vs. supportive therapy) | 3.39 | 1.20–47.6 | 0.140 | 7.58 | 1.20–47.6 | 0.030* |

*HR* hazard ratio, *CI* confidence interval, *PLA2R* M-type phospholipase A2 receptor, *uPCR* urine protein/creatinine ratio

[a] Model 1: adjusted for age, sex, eGFR, relevance to PLA2R, IgG4 dominancy, and treatment

[b] Model 2: model 1 with additional adjustment for uPCR and Ehrenreich–Churg stage

* *P* values of less than 0.05

**Table 3** Change of serum anti-PLA2R antibody positivity in primary MN patients whose serum antibodies were positive at baseline ($n = 11$)

| Patient | Baseline | | | | Treatment | Remission | Follow-up | |
|---|---|---|---|---|---|---|---|---|
| | uPCR (g/gCre) | Serum PLA2R | Biopsy PLA2R | IgG4 dominancy | | | uPCR (g/gCre) | Serum PLA2R |
| A | 3.21 | (+) | (+) | Dominant | Supportive | Yes | 0.16 | (+) |
| B | 19.31 | (+) | (+) | Dominant | IS | Yes | 0.05 | (+) |
| C | 5.82 | (+) | (+) | Dominant | Supportive | Yes | 0.06 | (−) |
| D | 4.7 | (+) | (+) | Non-dominant | Supportive | Yes | 0.45 | (+) |
| E | 9.88 | (+) | (+) | Dominant | IS | Yes | 0.18 | (+) |
| F | 2.15 | (+) | (+) | Non-dominant | IS | Yes | 0.25 | (−) |
| G | 7.82 | (+) | (+) | Dominant | IS | No | 10.12 | (+) |
| H | 12.38 | (+) | (+) | Dominant | Supportive | No | 8.07 | (+) |
| I | 6.84 | (+) | (+) | Dominant | Supportive | No | 5.79 | (+) |
| J | 21.34 | (+) | (−) | Dominant | IS | Yes | 0.15 | (−) |
| K | 10.19 | (+) | (−) | Non-dominant | IS | Yes | 0.03 | (−) |

*PLA2R* M-type phospholipase A2 receptor, *uPCR* urine protein/creatinine ratio, *IS* immunosuppressive therapy

remission of proteinuria [4, 5]. Furthermore, anti-PLA2R antibodies appeared prior to recurrence of MN in a transplant recipient [20]. In this study, the findings that serum antibodies still positive after remission of proteinuria might predict recurrence of proteinuria in the near future, or suggest that their antibodies lost nephritogenicity. For instance, it is possible that the antibodies changed their affinity or avidity, their subclass switched to non-complement-activating subclass or, in IgG4 antibodies, their degalactosylation was modified.

Finally, having PLA2R-related MN was not a predictor for remission of proteinuria in this study. However, Hoxha et al. [26] found that high anti-PLA2R antibody titer measured by ELISA was an independent risk factor for not achieving remission. For the purpose of evaluating the clinical outcome, such as remission of proteinuria, quantitative analysis by ELISA might be better than qualitative analysis by western blotting or assessing PLA2R in glomeruli.

In conclusion, assessments of both serum anti-PLA2R antibody and PLA2R antigen in glomeruli at the same time are a more sensitive method for the diagnosis of PLA2R-related primary MN at present. However, there are discrepancies between the presence of serum antibody and the detection of glomerular antigen. In further investigation, on a larger sample of patients, we need to classify PLA2R-related MN patients into three groups according to serum antibody positivity/negativity and glomerular antigen positivity/negativity, and compare their clinicopathological characteristics and clinical outcomes. As expected, IgG4 antibody is an important clue to elucidate the pathogenesis of PLA2R-related MN. Further investigations are necessary to resolve the issue of serum antibodies positivity after remission.

**Acknowledgments** We are grateful to the staff members of the Division of Nephrology for their cooperation and support. This study was supported in part by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science; (C) No.25461237 (HY), (B) No.24406029 (HY), and (B) No.2530502 (HY, SM), and a Grant-in-Aid for Progressive Renal Disease Research and Intractable Renal Disease Research, Research on Rare and Intractable Diseases, and Health and Labour Sciences Research Grants from the Ministry of Health, Labour, and Welfare of Japan.

**Conflict of interest** None of the authors has any competing interests.

## References

1. Glassock RJ. The pathogenesis of idiopathic membranous nephropathy: a 50-year odyssey. Am J Kidney Dis. 2010;56:157–67.
2. Beck LH Jr, Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009;361:11–21.
3. Hofstra JM, Debiec H, Short CD, et al. Antiphospholipase A2 receptor antibody titer and subclass in idiopathic membranous nephropathy. J Am Soc Nephrol. 2012;23:1735–43.
4. Hofstra JM, Beck LH Jr, Beck DM, et al. Anti-phospholipase A(2) receptor antibodies correlate with clinical status in idiopathic membranous nephropathy. Clin J Am Soc Nephrol. 2011;6:1286–91.
5. Beck LH Jr, Fervenza FC, Beck DM, et al. Rituximab-induced depletion of anti-PLA2R autoantibodies predicts response in membranous nephropathy. J Am Soc Nephrol. 2011;22:1543–50.
6. Hoxha E, Kneissler U, Stege G, et al. Enhanced expression of the M-type phospholipase A2 receptor in glomeruli correlates with serum receptor antibodies in primary membranous nephropathy. Kidney Int. 2012;82:797–804.
7. Svobodova B, Honsova E, Ronco P, et al. Kidney biopsy is a sensitive tool for retrospective diagnosis of PLA2R-related membranous nephropathy. Nephrol Dial Transpl. 2013;28:1839–44.
8. Shiiki H, Saito T, Nishitani Y, et al. Prognosis and risk factors for idiopathic membranous nephropathy with nephrotic syndrome in Japan. Kidney Int. 2004;65:1400–7.
9. Yokoyama H, Goshima S, Wada T, et al. The short- and long-term outcomes of membranous nephropathy treated with intravenous immune globulin therapy. Nephrol Dial Transpl. 1999;14:2379–86.
10. Yoshimoto K, Yokoyama H, Wada T, et al. Pathologic findings of initial biopsies reflect the outcomes of membranous nephropathy. Kidney Int. 2004;65:148–53.
11. Ehrenreich T, Porush JG, Churg J, et al. Treatment of idiopathic membranous nephropathy. N Engl J Med. 1976;295:741–6.
12. Qin W, Beck LH Jr, Zeng C, et al. Anti-phospholipase A2 receptor antibody in membranous nephropathy. J Am Soc Nephrol. 2011;22:1137–43.
13. Oh YJ, Yang SH, Kim DK, et al. Autoantibodies against phospholipase A2 receptor in Korean patients with membranous nephropathy. PLoS One. 2013;8:e62151.
14. Debiec H, Ronco P. PLA2R autoantibodies and PLA2R glomerular deposits in membranous nephropathy. N Engl J Med. 2011;364:689–90.
15. Larsen CP, Messias NC, Silva FG, et al. Determination of primary versus secondary membranous glomerulopathy utilizing phospholipase A2 receptor staining in renal biopsies. Mod Pathol. 2013;26:709–15.
16. Prunotto M, Carnevali ML, Candiano G, et al. Autoimmunity in membranous nephropathy targets aldose reductase and SOD2. J Am Soc Nephrol. 2010;21:507–19.
17. Qu Z, Liu G, Li J, et al. Absence of glomerular IgG4 deposition in patients with membranous nephropathy may indicate malignancy. Nephrol Dial Transpl. 2012;27:1931–7.
18. Ohtani H, Wakui H, Komatsuda A, et al. Distribution of glomerular IgG subclass deposits in malignancy-associated membranous nephropathy. Nephrol Dial Transpl. 2004;19:574–9.
19. Imai H, Hamai K, Komatsuda A, et al. IgG subclasses in patients with membranoproliferative glomerulonephritis, membranous nephropathy, and lupus nephritis. Kidney Int. 1997;51:270–6.
20. Debiec H, Hanoy M, Francois A, et al. Recurrent membranous nephropathy in an allograft caused by IgG3kappa targeting the PLA2 receptor. J Am Soc Nephrol. 2012;23:1949–54.
21. Salant DJ. Genetic variants in membranous nephropathy: perhaps a perfect storm rather than a straightforward conformeropathy? J Am Soc Nephrol. 2013;24:525–8.
22. Kuroki A, Iyoda M, Shibata T, et al. Th2 cytokines increase and stimulate B cells to produce IgG4 in idiopathic membranous nephropathy. Kidney Int. 2005;68:302–10.
23. Aalberse RC, Stapel SO, Schuurman J, et al. Immunoglobulin G4: an odd antibody. Clin Exp Allergy. 2009;39:469–77.
24. Huang CC, Lehman A, Albawardi A, et al. IgG subclass staining in renal biopsies with membranous glomerulonephritis indicates subclass switch during disease progression. Mod Pathol. 2013;26:799–805.
25. Rahimi S, Qian Z, Layton J, et al. Non-complement- and complement-activating antibodies synergize to cause rejection of cardiac allografts. Am J Transpl. 2004;4:326–34.
26. Hoxha E, Thiele I, Zahner G, et al. Phospholipase A2 receptor autoantibodies and clinical outcome in patients with primary membranous nephropathy. J Am Soc Nephrol. 2014;25:1357–66.

Clin Exp Nephrol (2014) 18:475–480
DOI 10.1007/s10157-013-0848-y

ORIGINAL ARTICLE

# Overestimation of the risk of progression to end-stage renal disease in the poor prognosis' group according to the 2002 Japanese histological classification for immunoglobulin A nephropathy

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Received: 19 April 2013 / Accepted: 21 July 2013 / Published online: 10 August 2013


**Abstract**
*Background* The current (2012) histological classification of immunoglobulin A nephropathy was established using a case–control study of 287 patients. However, the risk of progression to end-stage renal disease (ESRD) has not been validated for the previous (2002) classification. This study aimed to determine whether the previous classification could identify the risk of long-term renal outcome through re-analysis of the 2012 cohort.
*Methods* On the basis of the 2002 classification, namely 'good prognosis', 'relatively good prognosis', 'relatively poor prognosis', and 'poor prognosis', we examined the clinical data at the time of biopsy, the correlation between the 2002 classification and long-term renal outcomes, and a patient-by-patient correlation between the 2002 and 2012 classification systems. This was performed by analyzing samples from the 287 patients used to establish the 2012 classification.
*Results* The rate of decline of estimated glomerular filtration rate was greater and the odds ratio of progression to ESRD was higher in the 'poor prognosis' group. In contrast, the odds ratio for renal death was comparable between the groups described as 'relatively poor prognosis' and 'relatively good prognosis' in the 2002 classification. Many patients in the 2002 classification were classified with a lower histological grade in the current classification, but none were classified with a higher grade.

For the Special IgA Nephropathy Study Group.

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*Conclusions* The 2002 classification could also identify the risk of progression to ESRD. However, it was overestimated for patients in the 'poor prognosis' group in the 2002 classification, as that group included patients with milder histological damage.

**Keywords** Current histological classification · IgA nephropathy classification · Tuft adhesion · Multicenter · Case–control study · EGFR decline

## Introduction

In 2012, a new histological classification of immunoglobulin (Ig) A nephropathy was proposed by the Special IgAN Study Group of the Progressive Glomerular Diseases Study Committee, organized by the Ministry of Health, Labour and Welfare of Japan [1]. The classification was developed by performing a nationwide multicenter case–control study involving 287 patients at university and community hospitals. The study identified the independent pathological variables predicting progression to end-stage renal diseases (ESRD) as global sclerosis, segmental sclerosis, cellular crescents, fibrocellular crescents, and fibrous crescents. Depending on the percentage of glomeruli exhibiting these variables, 4 histological grades (HG) were established. Thus, HG1, HG2, HG3, and HG4 correspond to <25 %, 25–49 %, 50–74 %, >75 % of glomeruli exhibiting the above histological variables, respectively. Multivariate analysis revealed that the classification could identify the magnitude of the risk of progression to ESRD in patients with IgA nephropathy [1].

A number of histological grading systems have been proposed for predicting the renal outcome of IgA nephropathy [2–8]. Among them, the histological classification, previously established by the joint committee of the Japanese Society of Nephrology and the Special IgAN Study Group of the Progressive Glomerular Diseases Study Committee, organized by the Ministry of Health, Labour and Welfare of Japan in 2002 [9], has been widely used in Japan for the past 10 years. The classification had been determined on the basis of the percentage of glomeruli showing global sclerosis, crescents, or tuft adhesions. This classification comprised 4 groups, namely 'good prognosis', 'relatively good prognosis', 'relatively poor prognosis', and 'poor prognosis'. Thus, the 'good prognosis', 'relatively good prognosis', 'relatively poor prognosis', and 'poor prognosis' groups correspond to 0 %, <10 %, 10–29 %, and >30 % of glomeruli, respectively, showing global sclerosis, crescents, or tuft adhesions. The contribution of this classification was significant, in that medical staff could estimate the degree of histological damage and the predicted renal outcome for patients with IgA nephropathy having various histological lesions. For example, patients classified as having 'poor prognosis' and 'relatively poor prognosis' were defined as those who would need to undergo dialysis within 5 years and 5–20 years, respectively. However, the classification was established mainly based on empiric approaches by experts in nephrology and renal pathology, but did not include evidence-based methods. To date, renal outcomes based on this classification system have not been validated. Therefore, verification of whether the degree of renal progression could be discerned using the previous classification of IgA nephropathy is important, as many patients who previously underwent renal biopsy were classified according to the 2002 classification. In view of the chronic nature and lack of fundamental therapy for IgA nephropathy, an invalid classification may be misleading and result in inadequate therapy (overtreatment or undertreatment) for these patients.

Therefore, in the present study, we examined whether the 2002 histological classification effectively identified the risk of long-term renal outcome by conducting an analysis of the 287 patients involved in the development of the 2012 classification. We also examined the correlation between the 2002 and 2012 classifications for each patient in this cohort to clarify the relationship between the 2 classification systems.

## Methods

This study was performed according to the Declaration of Helsinki. The study protocol was approved by the ethics committee at each participating center (approval number from the Institutional Review Board at the Jikei University School of Medicine, 16–173). Informed consent was obtained from each patient included in this study.

### Patients

The inclusion criteria and data collection were previously described [1]. Patients who had available biopsy sections for light microscopy that contained at least 10 glomeruli,

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those who required renal replacement therapy or were followed for at least 5 years after renal biopsy without the need for renal replacement therapy, and those who had available information on the clinical course and therapies before and after renal biopsy were included in the present study. The data collected included age, gender, date of onset, blood pressure, serum creatinine levels, and urinary protein excretion at the time of biopsy and at the end of follow-up. The estimated glomerular filtration rate (eGFR) was calculated by the equation modified for the Japanese population [10].

### Pathological examination

The methods were described in our previous report [1]. The percentages of glomeruli having global glomerulosclerosis, segmental sclerosis, cellular crescents, fibrocellular crescents, fibrous crescents, and tuft adhesions were previously assessed for each patient. The 6 histological lesions were defined according to the Oxford classification of IgA nephropathy [11]. The classification was performed on the basis of the percentage of the total number of glomeruli showing global glomerulosclerosis, crescents, or tuft adhesions. The groups demonstrating 'good prognosis', 'relatively good prognosis', 'relatively poor prognosis', and 'poor prognosis' corresponded to 0 %, <10 %, 10–29 %, and >30 % of glomeruli in the specimens [9]. In the present study, the groups with 'good prognosis', 'relatively good prognosis', 'relatively poor prognosis', and 'poor prognosis' were designated as group 1, group 2, group 3, and group 4, respectively.

### Outcomes

The study outcome was the progression to ESRD requiring renal replacement therapy. The rate of decline in eGFR was also estimated by fitting a straight line thorough the available data using the principle of least squares.

### Statistical analysis

SPSS version 11.0 for Windows (SPSS, Chicago, IL, USA) was used for the statistical analyses. Normally distributed variables were expressed as mean ± standard deviation. Non-parametric variables were expressed as medians and ranges, and compared using the Kruskal–Wallis test. The risk of progression to ESRD was assessed using multivariate logistic regression analysis.

## Results

### Previous histological classification and clinical data at the time of biopsy

Clinical data at the time of biopsy and the renal outcomes of the overall cohort have been indicated in a previous study [1]. In the present study, the 287 patients were divided into the 2002 classification-defined groups, namely group 1, group 2, group 3, and group 4. Clinical data at the time of biopsy for each of the 4 groups are shown in Table 1. The patients in group 4 showed a more advanced age ($P < 0.01$) and greater mean arterial pressures and 24-h urinary protein excretion rates ($P < 0.01$), while also showing a lower eGFR ($P < 0.01$), when compared to the other groups. After biopsy, the percentages of patients who underwent treatment with renin–angiotensin system inhibitors (RASi), corticosteroids, and tonsillectomy were 38, 10, and 0 % in group 1; 67, 23 and 15 % in group 2; 75, 29 and 8 % in group 3; and 87, 51 and 2 % in group 4, respectively.

**Table 1** Clinical data at the time of renal biopsy in patients with IgA nephropathy

| | 2002 classification | | | | *P* value |
|---|---|---|---|---|---|
| | Good prognosis (group 1) | Relatively good prognosis (group 2) | Relatively poor prognosis (group 3) | Poor prognosis (group 4) | |
| Age (years) | 23 (7–63) | 29 (5–75) | 34 (12–67) | 40 (7–78) | <0.01 |
| Gender (male/female) | 16/14 | 24/20 | 49/42 | 58/64 | ns |
| Duration from onset (months) | 12 (1–225) | 14 (0–364) | 36 (1–300) | 43 (1–384) | <0.01 |
| MAP (mmHg) | 88.0 (65.3–116.7) | 84.0 (56.7–120.0) | 92.7 (66.0–156.7) | 98.0 (70.0–156.7) | <0.01 |
| eGFR (mL/min/1.73 $m^2$) | 100.7 (62.1–133.5) | 92.1 (26.3–166.3) | 83.8 (39.3–168.9) | 63.9 (7.6–195.7) | <0.01 |
| UPE (g/day) | 0.32 (0–7.6) | 0.40 (0–7.5) | 1.00 (0.05–6.2) | 1.8 (0.2–8.5) | <0.01 |

*MAP* mean arterial pressure, *eGFR* estimated glomerular filtration rate, *UPE* 24-h urinary protein excretion, *ns* not significant

Values are expressed as medians (ranges)

**Table 2** Odds ratio for progression to end-stage renal disease (ESRD) in each group, based on the 2002 histological classification

| Histological group | Patients | | Logistic regression analysis | | |
|---|---|---|---|---|---|
| | Total | ESRD | Odds ratio | 95 % CI | *P* value |
| Good prognosis (group 1) | 30 | 0 (0 %) | ND | ND | ND |
| Relatively good prognosis (group 2) | 44 | 4 (9 %) | 0.239 | 0.080–0.717 | <0.05 |
| Relatively poor prognosis (group 3) | 91 | 9 (10 %) | 0.262 | 0.119–0.578 | <0.01 |
| Poor prognosis (group 4) | 122 | 36 (30 %) | 1 | – | – |

*ND* not determined; *95 % CI* 95 % confidence interval

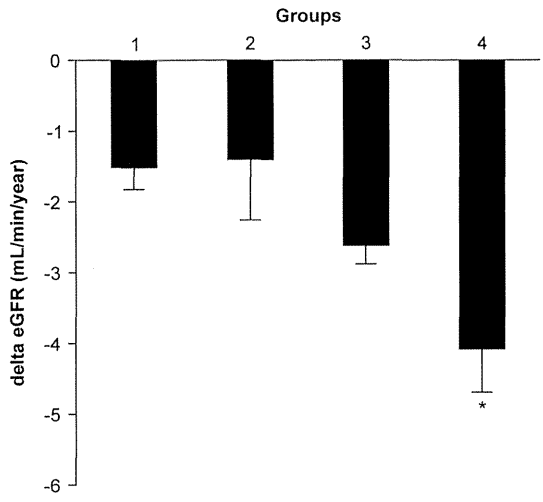


**Fig. 1** The rate of decline in the estimated glomerular filtration rate, per year, for each histological group included in the 2002 classification. Groups 1, 2, 3, and 4 correspond to 'good prognosis', 'relatively good prognosis', 'relatively poor prognosis', and 'poor prognosis', respectively. *Asterisk* denotes a significant difference ($P < 0.05$) versus groups 1 and 2. There is no significant difference among groups 1, 2, and 3

## Previous histological classification and renal outcome

The percentage of patients exhibiting progression to ESRD who required renal replacement therapy in each group is shown in Table 2. None of the group 1 patients progressed to ESRD. This is in agreement with the results from the current histological classification, which indicated that only 7 % progressed to ESRD in the HG1 class [1]. In the 2002 classification, however, the percentage of patients who required dialysis was comparable between groups 2 and 3, in contrast to the results from the 2012 classification that showed that more patients underwent dialysis in the HG3 group than in the HG2 group [1]. Multivariate logistic regression analysis was also performed, using group 4 as a reference, and the same findings were obtained—the risk of progression to ESRD was comparable between groups 2 and 3 according to the 2002 classification (Table 2).

The slopes of the eGFR declines were compared among the 4 groups in the 2002 classification (Fig. 1). The rate of eGFR decline, per year, was significantly higher in group 4 ($-4.09 \pm 6.68$ mL/min/year) than in groups 1 and 2 ($-1.52 \pm 1.63$ mL/min/year and $-1.41 \pm 5.64$ mL/min/year; respectively; $P < 0.05$, Kruskal–Wallis test), but not significantly higher than in group 3 ($-2.63 \pm 2.38$ mL/min/year). However, there were no significant differences among groups 1, 2, and 3.

## The relationship between the 2002 and 2012 histological classifications

The 4 groups from the 2002 classification were expected to correspond to the 4 HGs in the 2012 classification, i.e., group 1 to HG1, etc. In the present study, therefore, each patient who had been classified using the 2012 classification method [1] was re-classified using the 2002 classification system, and the relationship between the previous 'group' and current HG was examined for each patient (Table 3). All of the patients belonging to group 1 were also classified into HG1. Surprisingly, all of the 44 patients in group 2 were also classified into HG1 together with 91 patients from group 3; 71 and 20 patients were classified as HG1 and HG2, respectively. In addition, 6, 55, and 42 patients of the 122 patients in group 4 were classified as HG1, HG2, and HG3, respectively. Therefore, a large number of individuals were classified in a higher grade, according to the 2002 classification, including patients with milder histological damage than anticipated.

We then compared the histological findings using the criteria of the 2002 classification, i.e., glomerulosclerosis, crescents, and tuft adhesions, among patients in the four 2002 classification groups; these patients had also been classified as HG1 in the new 2012 classification (Table 4). The percentage of glomerulosclerosis and tuft adhesions, but not crescents, significantly increased in the groups with a higher grade and with a higher number. In contrast, the rate of eGFR decline was comparable among the 4 groups. A similar result was obtained when comparing patients between groups 3 and 4, who had been classified into HG2 (Table 4). Thus, the results suggested that the presence or absence of tuft adhesions in the evaluation criteria can contribute to the relationships between the 2002 and 2012 classification groups.

## Discussion

Many of the patients with IgA nephropathy who underwent renal biopsy during the past 10 years were classified according to the method empirically proposed in 2002 [9]. However, to date, the renal outcomes, on the basis of this classification, have not been validated. The invalid classification may be misleading for both physicians and patients, and incorrect recognition of the renal injury severity may lead to continuous, inappropriate therapy for patients with IgA nephropathy. Therefore, in the present study, we examined whether the histological classification proposed in 2002 could appropriately discern renal outcomes [9] by re-analysis of the cohort examined to establish the 2012 histological classifications [1]. We found that the 2002 classification could identify the risk of long-term renal outcome. Thus, age, mean arterial pressure, and 24-h urinary protein excretion were greater, whereas eGFR was lower in the 'poor prognosis' group, indicating that the 2002 classification correlates well with the clinical features that predict the renal outcome of IgA nephropathy (Table 1). Moreover, the rate of eGFR decline was greater in the 'poor prognosis' group than in other groups (Fig. 1). The odds ratio of progression to ESRD was also higher in the 'poor prognosis' group than in the other groups (Table 2).

However, several issues exist with the 2002 classification. First, although the odds ratio of renal death increases in a stepwise manner in the higher grades of the current classification, the odds ratios were not different between the 'relatively poor prognosis' and 'relatively good prognosis' group in the 2002 classification. Thus, the long-term renal outcome could not be distinguished between these 2 groups on the basis of the 2002 classification. Second, the slopes of the eGFR decline per year in the 'poor prognosis' group and HG4 were −4 mL/min/year and −8 mL/min/year, respectively. This suggests that the group with the greatest histological damage in the 2002 classification included more patients with mild histological damage, when compared to the corresponding grade in the 2012 classification. Indeed, many of the patients, classified according to the 2002 classification, were re-classified to a lower HG, based on the 2012 classification system, but none were classified to a higher grade (Table 3). Therefore, the severity in the 'poor prognosis' group was lessened due to the inclusion of the patients with milder histological damage. In the 2002 classification, 3 histological findings—global sclerosis, crescents, and tuft adhesions—were used for histological evaluation. In contrast, the 2012 classification specifies global sclerosis, segmental sclerosis, fibrous crescents, and cellular/fibrocellular crescents as significant pathological variables that contribute to the renal outcomes, whereas tuft adhesions were considered insignificant. Therefore, the presence or absence of tuft adhesions in the evaluation criteria is believed to be responsible for the differences in the discernment of renal outcomes between the 2012 and 2002 classifications. Indeed, the percentage of tuft adhesions significantly increased in a stepwise manner in the groups with a higher grade according to the 2002 classification, and were classified into HG1 according to the 2012 classification (Table 4). A similar finding was also shown in the analysis of patients from groups 3 and 4, who had been in the HG2 classification group.

**Table 3** Relationship between the 2002 and 2012 classifications

| 2002 classification | 2012 classification | Number of patients |
|---|---|---|
| Good prognosis (group 1) | HG1 | 30 |
| Relatively good prognosis (group 2) | HG1 | 44 |
| Relatively poor prognosis (group 3) | HG1 | 71 |
| | HG2 | 20 |
| Poor prognosis (group 4) | HG1 | 6 |
| | HG2 | 55 |
| | HG3 | 42 |
| | HG4 | 19 |

*HG* Histological grade

**Table 4** Comparison of histology and delta eGFR among patients in the2002 prognosis groups who were classified into HG1 or HG2 according to the 2012 classification

| | HG1 | | | | HG2 | |
|---|---|---|---|---|---|---|
| | Group 1 | Group 2 | Group 3 | Group 4 | Group 3 | Group 4 |
| Glomerulosclerosis (%) | 0 | 0 (0–9.1) | 8.3 (0–23.5)$^{*,\#}$ | 13.2 (0–20)$^{*,\#}$ | 13.8 (0–26.3) | 21.1 (0–46.2)$^{\$}$ |
| Crescents (%) | 0 | 0 (0–9.1) | 2.3 (0–20)$^{*}$ | 0 (0–10) | 2.9 (0–23.5) | 4.2 (0–36.4) |
| Tuft adhesions (%) | 0 | 0 (0–9.1) | 4.5 (0–20)$^{*,\#}$ | 18.8 (10–30)$^{*,\#,\&}$ | 6.7 (0–17.6) | 11.8 (0–40)$^{\$}$ |
| Delta eGFR/year (mL/min/year) | −1.52 ± 1.63 | −1.41 ± 5.64 | −2.75 ± 2.51 | −1.88 ± 1.92 | −2.23 ± 1.79 | −2.75 ± 3.24 |

*eGFR* estimated glomerular filtration rate

A significant difference ($P < 0.05$) versus group 1 (*), group 2 (#), group 3 (&) in HG1, and group 3 in HG2 ($)

There are several limitations to this study. First, a selection bias may have existed due to the retrospective nature of the study [1]. Second, although histological lesions were defined in accordance with the Oxford classification of IgA nephropathy [11], distinguishing the lesions with tuft adhesions, focal glomerular sclerosis, or small fibrous crescents was difficult. The precise determination of these features is important for both the 2002 and 2012 classifications. In addition, we did not refer to the Oxford classification proposed by the Working Group of the International IgA Nephropathy Network and the Renal Pathology Society in 2009 [12], as this was beyond the scope of this study. Finally, the treatment methods were different among the 4 groups, which may have affected renal outcomes. The percentage of patients receiving corticosteroids and RASi increased in the groups with a higher grade, indicating that intense therapy was indeed performed due to the severity of the histological damage. Of note, the percentage of patients who had undergone tonsillectomies was quite low, overall, in this cohort. Evidence has been amassed indicating that tonsillectomies, plus steroid pulses, impact the induction of clinical remission and attenuates the progression of renal injury [13–16]. Recent nationwide surveys have reported that approximately half of the institutes have performed tonsillectomies in conjunction with steroid pulse therapy [17, 18]. Therefore, validation of the current 2012 classification, using the cohort of ongoing prospective study performed by the Special IgA Nephropathy Study Group (Ministry of Health, Labor and Welfare of Japan), is important.

In conclusion, the previously proposed (2002) classification could identify the risk of long-term renal outcomes in IgA nephropathy by analyzing the same cohort used to establish the 2012 classification. However, the 2002 classification could not definitely discern the renal outcomes among the 4 groups, as the odds ratio of ESRD was not different between the groups with 'relatively poor prognosis' and 'relatively good prognosis'. In addition, the 'poor prognosis' group in the 2002 classification included patients with milder HG according to the current classification. Therefore, the risk of progression to ESRD is believed to be overestimated in patients in the 'poor prognosis' group of the 2002 classification.

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

**Conflict of interest** None.

## References

1. Kawamura T, Joh K, Okonogi H, Koike K, Utsunomiya Y, Miyazaki Y, et al. A histologic classification of IgA nephropathy for predicting long-term prognosis: emphasis on end-stage renal disease. J Nephrol. 2012;26:350–7.
2. Lee SM, Rao VM, Franklin WA, Schiffer MS, Aronson AJ, Katz AI. IgA nephropathy: morphologic predictors of progressive renal disease. Hum Pathol. 1982;13:314–22.
3. Lee HS, Koh HI, Lee HB, Park HC. IgA nephropathy in Korea: a morphological and clinical study. Clin Nephrol. 1987;27:131–40.
4. Johnston PA, Brown JS, Braumholz DA, Davison AM. Clinicopathological correlations and long-term follow-up of 253 United Kingdom patients with IgA nephropathy: a report from MRC Glomerulonephritis Registry. Q J Med. 1992;84:619–27.
5. Haas M. Histologic subclassification of IgA nephropathy: a clinicIpathologic study of 244 cases. Am J Kidney Dis. 1997;29:829–42.
6. Lee HS, Lee MS, Lee SM, Lee SY, Lee ES, Lee EY, et al. Histological grading of IgA nephropathy predicting renal outcome: revisiting H. S. Lee's glomerular grading system. Nephrol Dial Transplant. 2005;20:342–8.
7. Wakai K, Kawamura T, Endoh M, Kojima M, Tomino Y, Tamakoshi A, et al. A scoring system to predict renal outcome in IgA nephropathy: from a nationwide prospective study. Nephrol Dial Transplant. 2006;21:2800–8.
8. Manno C, Strippoli GF, D'Altri C, Torres D, Rossini M, Schena FP. A novel simpler histological classification for renal survival in IgA nephropathy: a retrospective study. Am J Kidney Dis. 2007;49:763–75.
9. Tomino Y, Sakai H. Special Study Group (IgA Nephropathy) on Progressive Glomerular Disease. Clinical guideline for immunoglobulin A (IgA) nephropathy in Japan, 2nd version. Clin Exp Nephrol. 2003;7:93–7.
10. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis. 2009;53:982–92.
11. Working Group of the International IgA Nephropathy Network and the Renal Pathology Society, Robert IS, Cook HT, Troyanov S, Alpers CE, Amore A, Barratt J, et al. The Oxford classification of IgA nephropathy: Pathology definitions, correlations and reproducibility. Kidney Int. 2009;76:546–56.
12. Working Group of the International IgA Nephropathy Network and the Renal Pathology Society, Cattran DC, Coppo R, Cook HT, Feehally J, Roberts IS, Troyanov S, et al. The Oxford classification of IgA nephrolaty: rationale, clinicopathological correlations, and classification. Kidney Int. 2009; 76:534–45.
13. Hotta O, Miyazaki M, Furuta T, Tomioka S, Chiba S, Horigome I, et al. Tonsillectomy and steroid pulse therapy significantly impact on clinical remission in patients with IgA nephropathy. Am J Kidney Dis. 2001;38:736–43.
14. Xie Y, Nishi S, Ueno M, Imai N, Sakatsume M, Narita I, et al. The efficacy of tonsillectomy on long-term renal survival in patients with IgA nephropathy. Kidney Int. 2003;63:1861–7.
15. Komatsu H, Fujimoto S, Hara S, Sato Y, Yamada K, Kitamura K. Effect of tonsillectomy plus steroid pulse therapy on clinical remission of IgA nephropathy: a controlled study. Clin J Am Soc Nephrol. 2008;3:1301–7.
16. Hirano K, Kawamura T, Tsuboi N, Okonogi H, Miyazaki Y, Ikeda M et al. The predictive value of attenuated proteinuria at 1 year after steroid therapy for renal survival in patients with IgA nephropathy. Clin Exp Nephrol (in press).
17. Miura N, Imai H, Kikuchi S, Hayashi S, Endoh M, Kawamura T, et al. Tonsillectomy and steroid pulse (TSP) therapy for patients with IgA nephropathy: a nationwide survey of TSP therapy in Japan and an analysis of the predictive factors for resistance to TSP therapy. Clin Exp Nephrol. 2009;13:460–6.
18. Matsuzaki K, Suzuki Y, Nakata J, Sakamoto N, Horikoshi S, Kawamura T, et al. Nationwide survey on current treatments for IgA nephropathy in Japan. Clin Exp Nephrol. (in press).

NDT Advance Access published January 22, 2015

Nephrol Dial Transplant (2015) 0: 1–11
doi: 10.1093/ndt/gfu385

*Original Article*

# Pulmonary involvements of anti-neutrophil cytoplasmic autoantibody-associated renal vasculitis in Japan

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## ABSTRACT

**Background.** Pulmonary involvement is one of the hallmark lesions of anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) as well as rapidly progressive glomerulonephritis (RPGN). However, the pulmonary involvement of AAV patients seems to differ between Europe and Japan, as does the ANCA serotype.

**Methods.** This retrospective and prospective multicenter cohort study collected the clinical data of the features and outcomes of 1772 RPGN patients treated from 1989 to 2007 in Japan. Based on this nationwide RPGN survey, we analyzed the cases of 1147 AAV patients.

**Results.** We found that 52.3% of the AAV patients had pulmonary involvements: 15.4% of the AAV patients had alveolar hemorrhage (AH), 26.2% had interstitial lung disease (ILD), 2.8% had bronchial asthma, 2.4% had pulmonary granuloma and 12.8% had a chest X-ray abnormality without AH, ILD or pulmonary granuloma. Patient survival was significantly different among the following six groups: the 5-year survival rate was 41.5% in the patients with AH, 50.2% in those with ILD, 67.9% in those with bronchial asthma, 62.5% in those with pulmonary granuloma, 55.8% in those with chest X-ray abnormality and 73.3% in those without pulmonary involvement. AH was one of the predictors of 1- and 5-year mortality for patient survival in AAV, and ILD was added as one of the predictors of 5-year mortality.

**Conclusion.** In these AAV patients, not only AH but also ILD was frequently observed. AH was associated with the prognosis, but ILD was associated with the long-term prognosis of AAV.

**Keywords:** alveolar hemorrhage, ANCA-associated vasculitis, interstitial lung disease, mortality

## INTRODUCTION

Pulmonary involvement is one of the hallmark lesions of anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) [1]. The features of the pulmonary involvement vary according to the type of vasculitis [2]. In eosinophilic granulomatosis with polyangiitis (EGPA), bronchial asthma is the major pulmonary lesion. The pulmonary manifestation of granulomatosis with polyangiitis (GPA) is necrotizing granulomatous inflammations, including nodules, masses with a cavity, airway stenosis and alveolar hemorrhage (AH). AH is also the most frequent pulmonary manifestation of microscopic polyangiitis (MPA). The incidence of AH is 7–45% in GPA

and 10–30% in MPA, but AH is rare in EGPA [3]. Among AAV patients with AH, the most common clinical diagnosis was MPA (52%), followed by GPA (41%) and EGPA (6%) [4].

Differences in the incidence and ANCA serotype of AAV patients were observed between studies conducted in Europe and Japan [5]. Although the data regarding the overall occurrence of renal vasculitis were similar in Europe and Japan, PR3-ANCA-associated vasculitis (or GPA) seemed to be much less common in Japan. Moreover, differences between studies from Europe and Japan were observed concerning the pulmonary involvement of AAV. Among patients with AAV, interstitial lung disease (ILD), such as pulmonary fibrosis and interstitial pneumonitis, was found to be rare (2–3%) in Europe [6, 7], but much more frequent in Japan (29–39%) [8, 9].

Among the ILD patients who were positive for myeloperoxidase (MPO)-ANCA in Japan, 45% demonstrated glomerulonephritis [10]. In an analysis of sequential cohorts of patients with MPA and renal-limited vasculitis collected from European and Japanese centers, the MPA patients in Japan had a significantly higher age at onset, more frequent MPO-ANCA positivity, lower serum creatinine and more frequent ILD compared with the MPA patients in Europe [11].

To clarify the patient characteristics and prognosis according to the pulmonary lesions of AAV in Japan, in this study we retrospectively analyzed the cases of 1147 AAV patients with rapidly progressive glomerulonephritis (RPGN) based on the previous Japan RPGN registry obtained via a questionnaire survey [12].

ORIGINAL ARTICLE

## MATERIALS AND METHODS

### Subjects

We retrospectively collected the records of patients with RPGN treated in Japan from 1989 to 1998 and prospectively collected the clinical records of RPGN patients treated from 1999 to 2007 by sending a questionnaire annually by post to 343 nephrology departments of tertiary hospitals in Japan (the names of all of departments are given in the Supplementary data S1). This study was approved by the Medical Ethics Committee at the Graduate School of Comprehensive Human Sciences, University of Tsukuba, in accordance with the guidelines for epidemiological research issued by the Ministry of Health, Labor and Welfare of Japan.

The definition of RPGN was based on the clinical findings of rapidly progressing renal failure over several weeks to a few months, accompanied by the following nephritic urinary abnormalities: hematuria, proteinuria, and red blood cell cast or granular cast in urine sediment. In total, 171 (49.9%) of the 343 nephrology departments responded, and 1772 patients with RPGN were registered [12].

Among the 1772 RPGN cases, 1147 ANCA-associated renal vasculitis patients were investigated in the present study. ANCA-associated renal vasculitis was diagnosed by each nephrology department, based on characteristic clinical and histological features of small-vessel vasculitis as defined by the Chapel Hill Consensus Conference 1994 [13], clinical evidence of RPGN and a positive test for ANCA. Patients with other types of systemic small-vessel vasculitis, such as Henoch–Schönlein purpura, essential cryoglobulinemic vasculitis, drug-induced vasculitis, systemic lupus erythematosus, rheumatoid arthritis and malignancy-associated vasculitis were excluded. The cases of patients with anti-glomerular basement membrane (GBM) antibody disease, which was defined as the presence of serum anti-GBM antibody or a linear binding of IgG as detected by direct immunofluorescence, were also excluded.

### Investigated parameters

Baseline characteristics including age, gender, height, weight, blood pressure, clinical symptoms, laboratory findings (blood cell counts, biochemical, serological and urinary features) and renal histological findings at presentation were obtained from the patients' clinical records. 'Pulmonary involvement' indicates AH, bronchial asthma, pulmonary granuloma, ILD or the existence of a chest X-ray abnormality. The initial dose of oral prednisolone, the duration of the initial dose, immunosuppressive treatment and plasma exchange (or plasmapheresis) were also recorded. Follow-up clinical data including survival outcome, dialysis dependence, initial date of dialysis therapy, final follow-up date and cause of death were recorded.

### Statistical analysis

Continuous variables are presented as mean ± SD, and categorical variables are presented as numbers with percentages. We used an analysis of variance (ANOVA) to assess differences among subject groups, and *post hoc* comparisons were made using the Bonferroni/Dunn test. Differences in categorical variables were checked using the $\chi^2$ test, and *post hoc* comparisons (Bonferroni correction) were performed to detect differences between the groups. Survival rates were estimated with the Kaplan–Meier method, and differences in estimated survival curves were assessed using the log-rank test. End points for patient survival were death, loss to follow-up and closure of study. We tested the parameters of patient characteristics, symptoms and laboratory data (including histological findings) in a univariate Cox analysis, and then we estimated the hazard ratios (HRs) of patient outcomes in a multivariate Cox regression analysis (using the covariates that were significantly associated with the event in the univariate analysis). The relative risk of death is expressed as a HR with 95% confidence interval. Statistical significance was defined as a P-value of <0.05, but in multiple comparisons, the critical P-value ($\alpha$) was divided by the number of comparisons being made (when one category among six groups was analyzed, the significance threshold was placed at $\alpha = 0.05/15 = 0.033$). All statistical analyses were performed using PASW Statistics software, version 18 (IBM Japan, Tokyo) for Windows.

## RESULTS

### Pulmonary involvements

Among the 1172 RPGN patients, 1147 patients had AAV, and the mean age at onset was 64.3 ± 14.4 years old. Females were more common (male:female = 1:1.18). Among the 1147 AAV subjects, 1088 AAV patients had MPO-ANCA and 114

had PR3-ANCA (55 AAV patients had both MPO- and PR3-ANCA).

Among the 1147 AAV subjects, 600 AAV patients had pulmonary involvement, 528 patients had AAV without pulmonary involvement and further information ~19 AAV patients was not available. Among the AAV subjects with pulmonary involvement, 177 patients had AH, 301 had ILD, 32 had bronchial asthma and 27 had pulmonary granuloma. Among the patients without AH, ILD or pulmonary granuloma, the existence of a chest X-ray abnormality was observed in 147 patients. We found that 508 patients had a single pulmonary involvement, 79 had two involvements and 6 had three involvements. Fifty patients had both AH and ILD, six had both AH and pulmonary granuloma, four had both AH and bronchial asthma, five had both ILD and pulmonary granuloma, seven had both ILD and bronchial asthma and seven had both bronchial asthma and chest X-ray abnormality. Among the six patients with three involvements, two patients had AH, ILD and bronchial asthma; two had AH, pulmonary granuloma and bronchial asthma; one had AH, ILD and pulmonary granuloma and one had ILD, pulmonary granuloma and bronchial asthma.

Among the 1088 MPO-AAV subjects, 494 patients had AAV without pulmonary involvement, 167 patients had AH, 291 had ILD, 31 had bronchial asthma, 20 had pulmonary granuloma and 143 had a chest X-ray abnormality without AH, ILD or pulmonary granuloma. Among the 114 AAV subjects with PR3-ANCA, 58 patients had AAV without pulmonary involvement, 18 patients had AH, 20 had ILD, 2 had bronchial asthma, 9 had pulmonary granuloma and 16 had a chest X-ray abnormality.

Lastly, we divided the AAV patients into six groups: the patients with AH (the AH group), patients with ILD (the ILD group), those with bronchial asthma (the BA group), those with pulmonary granuloma (the PG group), those with chest X-ray abnormalities (the X-ray group) and those without pulmonary involvement (the None group). The data of the AAV patients with two or more pulmonary involvements were included in each pulmonary involvement group.

### Clinical features and symptoms

Among the six AAV groups, there were no significant differences in gender, height or weight, but the mean age was significantly lower in the None group compared with the other three groups (versus the BA group, $P = 0.0032$; versus the ILD group, $P < 0.0001$; versus the X-ray group, $P < 0.0001$). The mean age in the AH group was significantly lower than that in the ILD group ($P = 0.0001$) and the X-ray group ($P = 0.0005$).

The baseline characteristics and symptoms of the patients in the six groups are given in Table 1. General symptoms such as fever, weight loss, fatigue and appetite loss were frequently observed in the ILD group. The frequencies of arthritis, episcleritis, ear–nose–throat (ENT) symptoms and nasal granuloma were significantly higher in the PG group than that in the None group. Hemosputum was significantly more common in the AH group compared with the X-ray, ILD and None groups. The percentages of bleeding tendency and purpura in the AH group were also significantly higher than those in the X-ray, ILD and None groups. The frequencies of dyspnea, heart disease and peripheral neuropathy were significantly higher in the BA group compared with the None group.

### Laboratory data

Table 2 provides the baseline laboratory and histological findings of the six patient groups. On laboratory examination, the white blood cell count in the None group was significantly lower than that in the other groups except for the PG group, and the serum C-reactive protein level in the None group was significantly lower than that in the other five groups. Hypereosinophilia was highly observed in the BA group. Severe anemia and more deteriorated renal function (blood urea nitrogen and serum creatinine levels) were observed in the AH group. MPO-ANCA was less detected and PR3-ANCA was highly detected in the PG group, but these findings were not significant. There were no significant differences in the urinary examination findings (i.e. urinary protein, urinary occult blood and cellular casts) among the six groups. There were no significant differences in kidney size or histological findings among the six groups.

### Treatments

Oral corticosteroids were administered to 95.3% of the patients (1076/1129), but immunosuppressants were used in 46.4% (516/1112) and plasmapheresis (including plasma exchange) was performed in 13.5% (151/1116) of these AAV patients with RPGN in Japan. The treatments administered to the patients in the six groups are given in Table 3. There was no significant difference in the frequency of the use of oral corticosteroids among the six groups, but the initial dose of oral corticosteroid, the frequency of use and the daily dose of methylprednisolone pulse therapy in the AH group were all significantly higher than that in the ILD, X-ray and None groups.

There were no differences in the uses of immunosuppressants, the use of oral or intravenous cyclophosphamide, or the doses or times of intravenous cyclophosphamide among the six groups. However, the percentage of patients who received plasma exchange therapy was significantly higher in the AH group than in the ILD, X-ray and None groups.

### Outcomes

The cumulative patient survival rate at 1 year for all of the AAV patients was 73.5%, and that at 5 years was 60.1%. There was no significant difference in prognosis among the patients classified by ANCA status (Figure 1A). Patient survival was significantly different among the six groups: the 1-year survival was 56.2% in the AH group, 69.9% in the ILD group, 82.1% in the BA group, 69.6% in the PG group, 70.8% in the X-ray group and 82.1% in the None group, whereas the 5-year survival rates were 41.5, 50.2, 67.9, 62.5, 55.8 and 73.3%, respectively (Figure 1B). Patient survival in the MPO-ANCA-positive AAV patients was significantly different among the six groups (Figure 1C) and similar to that in the total AAV patients, but there was no significant difference in patient survival among the PR3-ANCA-positive AAV patients (Figure 1D) because of the small numbers of patients.

**Table 1. Characteristics and symptoms of patients with ANCA-associated renal vasculitis**

| | Alveolar hemorrhage ($n = 177$) | Interstitial lung disease ($n = 301$) | Bronchial asthma ($n = 32$) | Pulmonary granuloma ($n = 27$) | Chest X-ray abnormality ($n = 147$) | None ($n = 528$) |
|---|---|---|---|---|---|---|
| No. of pulmonary involvements | 1.4 ± 0.5[a] | 1.2 ± 0.5[a] | 1.9 ± 0.7[a] | 1.7 ± 0.7[a] | 1.0 ± 0.2[a] | 0.0 ± 0.0 |
| Age (years) | 62.8 ± 15.7 | 67.8 ± 11.3[a,c] | 69.5 ± 11.3[a] | 64.9 ± 10.2 | 68.1 ± 10.0[a,c] | 61.6 ± 16.1 |
| Gender (male) | 44.6% (78/175) | 47.6% (140/294) | 40.6% (13/32) | 44.4% (12/27) | 49.7% (72/145) | 43.4% (225/519) |
| Height (cm) | 156.0 ± 9.3 | 156.0 ± 9.5 | 155.2 ± 10.1 | 157.0 ± 10.0 | 157.0 ± 9.2 | 156.4 ± 9.6 |
| Weight (kg) | 51.9 ± 10.3 | 53.3 ± 11.1 | 51.0 ± 8.6 | 50.8 ± 10.4 | 52.2 ± 9.6 | 52.4 ± 10.7 |
| Mean blood pressure (mmHg) | 98.3 ± 14.3 | 96.1 ± 14.8 | 95.7 ± 14.7 | 94.5 ± 12.2 | 96.5 ± 15.4 | 98.0 ± 15.9 |
| Arthritis | 19.0% (32/168) | 18.6% (54/291) | 16.1% (5/31) | 36.0% (9/25)[c] | 10.6% (15/142) | 18.2% (94/517) |
| Fever | 65.7% (115/175)[a] | 58.6% (173/295)[a] | 65.6% (21/32) | 69.2% (18/26) | 63.0% (92/146)[a] | 43.0% (226/525) |
| Weight loss | 26.7% (44/165) | 37.2% (106/285)[a] | 28.1% (9/32) | 37.5% (9/24) | 37.0% (51/138)[a] | 22.3% (115/516) |
| Fatigue | 68.0% (115/169) | 71.2% (208/292)[a] | 81.3% (26/32) | 68.0% (17/25) | 72.0% (103/143) | 58.5% (305/521) |
| Appetite loss | 56.5% (96/170)[a] | 60.8% (178/293)[a] | 67.7% (21/31) | 56.0% (14/25) | 61.3% (87/142)[a] | 42.8% (223/521) |
| Bleeding tendency | 19.6% (33/168)[a,b,d] | 5.9% (17/288) | 15.6% (5/32)[a] | 16.0% (4/25)[a] | 4.9% (7/143) | 2.9% (15/513) |
| Purpura | 16.6% (28/169)[a,b] | 9.3% (27/290) | 12.9% (4/31) | 20.0% (5/25)[b] | 2.8% (4/143) | 6.4% (33/516) |
| Rashes | 4.1% (7/169) | 6.6% (19/290)[a] | 9.7% (3/31) | 16.0% (4/25)[a] | 3.5% (5/143) | 1.9% (10/519) |
| Raunaud phenomenon | 3.0% (5/168) | 4.8% (14/290) | 6.7% (2/30) | 12.0% (3/25) | 2.8% (4/144) | 2.9% (15/516) |
| Gangrene | 0.6% (1/169) | 0.3% (1/291) | 0.0% (0/31) | 0.0% (0/25) | 0.0% (0/141) | 0.2% (1/517) |
| Mouth ulcer | 3.0% (5/169) | 2.1% (6/291) | 0.0% (0/31) | 0.0% (0/24) | 0.7% (1/143) | 1.7% (9/517) |
| (Epi)scleritis | 5.4% (9/167) | 3.5% (10/286) | 0.0% (0/31) | 23.1% (6/26)[a,b,c,d] | 5.7% (8/141) | 2.7% (14/512) |
| ENT symptoms | 46.8% (80/171)[a] | 37.7% (109/289)[a] | 50.0% (16/32)[a] | 72.0% (18/25)[a,d] | 46.2% (66/143)[a] | 22.2% (115/517) |
| Nasal granuloma | 3.5% (6/170) | 1.7% (5/291) | 3.2% (1/31) | 25.0% (6/24)[a,c,d] | 0.0% (0/142) | 1.5% (8/517) |
| Hearing loss | 6.5% (11/168) | 4.8% (14/290) | 10.7% (3/28) | 8.0% (2/25) | 2.9% (4/140) | 4.5% (23/513) |
| Hemosputum | 39.5% (68/172)[a,b,d] | 14.5% (42/289)[a] | 26.7% (8/30)[a] | 20.0% (5/25)[a] | 13.0% (19/146)[a] | 2.3% (12/518) |
| Dyspnea | 32.7% (56/171)[a] | 23.3% (67/288)[a] | 50.0% (16/32)[a,b,d] | 32.0% (8/25)[a] | 19.9% (28/141)[a] | 6.2% (32/516) |
| Heart disease | 10.1% (17/168) | 9.4% (27/287) | 30.0% (9/30)[a,c,d] | 8.0% (2/25) | 13.2% (19/144) | 6.0% (31/519) |
| Arrhythmia | 6.5% (11/170) | 7.2% (21/290) | 10.0% (3/30) | 4.0% (1/25) | 3.5% (5/142) | 4.8% (25/519) |
| Nausea | 25.1% (43/171) | 23.0% (66/287) | 35.5% (11/31) | 32.0% (8/25) | 28.2% (40/142) | 17.9% (93/520) |
| Abdominal pain | 10.1% (17/168) | 4.5% (13/289) | 9.4% (3/32) | 16.7% (4/24) | 8.4% (12/143) | 6.4% (33/517) |
| Peptic ulcer | 11.2% (19/169)[a] | 5.9% (17/290) | 9.7% (3/31) | 16.0% (4/25) | 6.4% (9/141) | 4.6% (24/518) |
| Bloody diarrhea | 15.6% (26/167)[a,d] | 5.9% (17/290) | 16.7% (5/30)[a] | 16.0% (4/25)[a] | 5.6% (8/144) | 3.5% (11/514) |
| Hypertension | 29.4% (50/170) | 36.2% (106/293) | 45.2% (14/31) | 32.0% (8/25) | 34.3% (49/143) | 32.8% (170/518) |
| Edema | 42.1% (72/171) | 43.8% (128/292) | 53.1% (17/32) | 46.2% (12/26) | 36.1% (52/144) | 35.3% (183/518) |
| Oliguria | 16.7% (28/168)[a] | 13.6% (39/287) | 21.9% (7/32) | 24.0% (6/25) | 7.7% (11/143) | 8.3% (43/516) |
| Gross hematuria | 13.8% (23/167) | 8.0% (23/286) | 28.1% (9/32)[a,d] | 16.0% (4/25) | 9.7% (14/144) | 10.2% (53/518) |
| Chance proteinuria/ hematuria | 38.4% (63/164) | 44.3% (127/287) | 71.0% (22/31)[c] | 42.3% (11/26) | 59.3% (83/140)[c] | 48.1% (248/516) |
| Nephrotic syndrome | 7.1% (12/168) | 7.9% (23/290) | 22.6% (7/31)[a] | 16.0% (4/25) | 8.5% (12/142) | 7.0% (36/516) |
| Uremia | 16.9% (28/166) | 12.4% (35/283) | 25.8% (8/31) | 12.0% (3/25) | 9.9% (14/142) | 9.7% (50/513) |
| CNS symptoms | 9.5% (16/168) | 6.6% (19/290) | 9.7% (3/31) | 8.0% (2/25) | 4.9% (7/142) | 4.0% (21/519) |
| Peripheral neuropathy | 15.5% (26/168) | 16.1% (46/285) | 25.8% (8/31)[a] | 16.0% (4/25) | 10.5% (15/143) | 10.1% (52/515) |

CNS, central nervous system.
[a]$P < 0.0033$ versus none.
[b]$P < 0.0033$ versus chest X-ray abnormality.
[c]$P < 0.0033$ versus alveolar hemorrhage.
[d]$P < 0.0033$ versus interstitial lung disease.

In the univariate Cox regression analysis for patient survival in AAV (all results of the univariate Cox regression analysis for patient survival in AAV are given in the Supplementary data S2), we found that age, pulmonary involvement, seven symptoms and 12 laboratory data were predictors of 1-year mortality (Table 4). However, AH, age, bleeding tendency, peripheral neuropathy and serum creatinine level were predictors of 1-year mortality in the multivariate Cox regression analysis for patient survival in AAV. As predictors of 5-year mortality, AH, age and serum creatinine level remained. Moreover, ILD and platelet count were newly added to these three parameters.

Regarding the causes of death (Table 5), infectious diseases accounted for 41.0% of the deaths (160/390); pulmonary involvements due to AAV were 13.8% (54/390), and the incidence of both diseases together was 20.0% (78/390). Regarding pulmonary involvements due to AAV, the percentage in the AH group was relatively higher (28.9%) than that of the other five groups. Infectious diseases were relatively common in every group, and infectious diseases (including both infection and vasculitis) were relatively more common in the ILD group (68.6%).

## DISCUSSION

One of the frequent pulmonary involvements of AAV is AH [3, 4], and it is also one of the life-threatening manifestations of

**Table 2. Laboratory data of patients with ANCA-associated renal vasculitis**

| | Alveolar hemorrhage ($n$ = 177) | Interstitial lung disease ($n$ = 301) | Bronchial asthma ($n$ = 32) | Pulmonary granuloma ($n$ = 27) | Chest X-ray abnormality ($n$ = 147) | None ($n$ = 528) |
|---|---|---|---|---|---|---|
| Erythrocyte sed. rate (mm/h) | 100 ± 40 | 101 ± 36[a] | 100 ± 44 | 105 ± 54 | 104 ± 35[a] | 88 ± 41 |
| White blood cell (/mL) | 10 310 ± 4561[a] | 10 150 ± 4466[a] | 12 181 ± 5410[a] | 9657 ± 5611 | 11 042 ± 5963[a] | 8829 ± 4320 |
| Hypereosinophilia | 28.0% (26/93) | 17.5% (32/183) | 42.3% (11/26)[a,b,d] | 41.2% (7/17) | 17.9% (15/84) | 19.4% (54/279) |
| Hemoglobin (g/dL) | 8.1 ± 1.8[a,d] | 8.7 ± 1.8 | 9.1 ± 2.0 | 8.9 ± 2.1 | 8.5 ± 1.7 | 8.8 ± 1.7 |
| Platelet (/mL) | 26.6 ± 11.3 | 29.5 ± 12.2 | 28.4 ± 14.5 | 29.0 ± 14.0 | 31.3 ± 13.7[c] | 28.4 ± 11.8 |
| Total protein (g/dL) | 6.2 ± 0.7[a,d,e] | 6.5 ± 0.8 | 6.4 ± 0.8 | 6.8 ± 0.2 | 6.4 ± 0.8 | 6.4 ± 0.8 |
| Albumin (g/dL) | 2.8 ± 0.6[a] | 2.8 ± 0.6[a] | 2.8 ± 0.5 | 2.8 ± 0.6 | 2.7 ± 0.5[a] | 3.0 ± 0.6 |
| Urea nitrogen (mg/dL) | 61.7 ± 26.6[a,d] | 52.0 ± 25.6 | 55.5 ± 28.7 | 58.0 ± 32.2 | 53.0 ± 27.7 | 51.0 ± 26.3 |
| Creatinine (mg/dL) | 5.41 ± 2.91[a,d] | 4.58 ± 2.89 | 4.13 ± 2.38 | 4.73 ± 3.31 | 4.56 ± 2.98 | 4.51 ± 2.90 |
| Total cholesterol (mg/dL) | 160 ± 47[a] | 162 ± 46[a] | 155 ± 40 | 155 ± 33 | 164 ± 47 | 179 ± 50 |
| C-reactive protein (mg/dL) | 7.06 ± 8.34[a] | 6.13 ± 6.65[a] | 9.11 ± 9.55[a] | 9.93 ± 11.04[a] | 6.62 ± 6.42[a] | 3.99 ± 5.22 |
| MPO-ANCA* (EU) | 94.4% (167/177) | 96.7% (291/301) | 96.9% (31/32) | 74.1% (20/27) | 97.3% (143/147) | 93.6% (494/528) |
| | 527.4 ± 1206.9 | 322.2 ± 566.2 | 380.2 ± 340.2 | 257.9 ± 303.5 | 312.6 ± 312.4 | 363.9 ± 787.4 |
| PR3-ANCA* (EU) | 10.2% (18/177) | 6.6% (20/301) | 6.3% (2/32) | 33.3% (9/27) | 10.9% (16/147) | 11.0% (58/528) |
| | 25.4 ± 92.7 | 14.4 ± 70.9 | 3.6 ± 5.4 | 34.7 ± 83.7 | 6.7 ± 16.6 | 21.4 ± 97.0 |
| Complements | | | | | | |
| Elevated | 10.5% (17/162) | 20.4% (57/279) | 23.1% (6/26) | 20.8% (5/24) | 23.1% (31/134) | 18.1% (90/496) |
| Decreased | 14.8% (24/162) | 12.2% (34/279) | 7.7% (2/26) | 4.2% (1/24) | 10.4% (14/134) | 9.3% (46/496) |
| Hyperglobulinemia | 57.0% (65/114) | 64.2% (147/229) | 56.0% (14/25) | 82.6% (19/23) | 64.9% (72/111) | 57.9% (198/342) |
| Serum IgG levels (mg/dL) | 1661 ± 596 | 1863 ± 673[a] | 1804 ± 664 | 2160 ± 471 | 1852 ± 572 | 1672 ± 693 |
| Urinary protein | | | | | | |
| (±) | 3.1% (5/162) | 4.2% (12/285) | 0.0% (0/30) | 8.3% (2/24) | 2.7% (4/147) | 1.8% (9/513) |
| (1+) | 18.5% (30/162) | 17.9% (51/285) | 30.0% (9/30) | 25.0% (6/24) | 19.0% (28/147) | 15.2% (78/513) |
| (2+) | 30.9% (50/162) | 35.4% (101/285) | 36.7% (11/30) | 29.2% (7/24) | 32.7% (48/147) | 27.7% (142/513) |
| (3+) | 31.5% (51/162) | 28.4% (81/285) | 23.3% (7/30) | 20.8% (5/24) | 31.3% (46/147) | 36.6% (188/513) |
| (4+) | 14.8% (24/162) | 11.6% (33/285) | 10.0% (3/30) | 16.7% (4/24) | 10.2% (15/147) | 14.6% (75/513) |
| Amounts of urinary protein (g/day) | 1.51 ± 1.93 | 1.31 ± 1.33 | 1.21 ± 1.03 | 1.21 ± 1.09 | 1.36 ± 1.26 | 1.53 ± 1.73 |
| Urinary occult blood | | | | | | |
| (±) | 1.2% (2/165) | 1.0% (3/290) | 0.0% (0/30) | 0.0% (0/21) | 0.0% (0/146) | 0.2% (1/512) |
| (1+) | 1.8% (3/165) | 2.1% (6/290) | 0.0% (0/30) | 9.5% (2/21) | 3.4% (5/146) | 2.0% (10/512) |
| (2+) | 10.3% (17/165) | 15.2% (44/290) | 6.7% (2/30) | 23.8% (5/21) | 10.3% (15/146) | 15.0% (77/512) |
| (3+) | 43.0% (71/165) | 50.7% (147/290) | 73.3% (22/30) | 42.9% (9/21) | 50.0% (73/146) | 45.9% (235/512) |
| (4+) | 43.6% (72/165) | 30.0% (87/290) | 20.0% (6/30) | 23.8% (5/21) | 34.9% (51/146) | 35.7% (183/512) |
| Red blood cell cast | 32.4% (47/145) | 31.1% (83/267) | 27.6% (8/29) | 45.5% (10/22) | 27.9% (39/140) | 29.3% (139/474) |
| Granular cast | 69.5% (105/151) | 69.5% (189/272) | 63.3% (19/30) | 77.3% (17/22) | 66.2% (92/139) | 68.3% (327/479) |
| White blood cell cast | 14.1% (20/142) | 19.4% (51/263) | 13.8% (4/29) | 19.0% (4/21) | 13.2% (18/136) | 14.3% (67/469) |
| Kidney size | | | | | | |
| Atrophy | 10.3% (16/155) | 13.9% (38/274) | 15.4% (4/26) | 4.2% (1/24) | 16.7% (22/132) | 13.3% (64/481) |
| Enlarged | 12.3% (19/155) | 10.6% (29/274) | 15.4% (4/26) | 16.7% (4/24) | 18.9% (25/132) | 11.6% (56/481) |
| Percentages of crescents | 60.1 ± 26.8 | 56.2 ± 26.6 | 50.0 ± 31.8 | 66.5 ± 34.9 | 54.1 ± 29.2 | 59.5 ± 28.1 |
| Patients with 50% of crescents | 91.5% (118/129) | 88.6% (202/228) | 91.7% (22/24) | 95.0% (19/20) | 86.4% (102/118) | 91.5% (411/449) |
| Main types of crescents | | | | | | |
| Cellular | 41.5% (49/118) | 46.0% (93/202) | 63.6% (14/22) | 47.4% (9/19) | 53.9% (55/102) | 43.8% (180/411) |
| Fibrocellular | 49.2% (58/118) | 43.6% (88/202) | 22.7% (5/22) | 42.1% (8/19) | 39.2% (40/102) | 39.2% (161/411) |
| Tubulointerstitial damage | | | | | | |
| Mild | 18.2% (22/121) | 18.5% (39/211) | 38.1% (8/21) | 26.3% (5/19) | 17.9% (20/112) | 16.2% (68/421) |
| Moderate | 45.5% (55/121) | 40.3% (85/211) | 38.1% (8/21) | 42.1% (8/19) | 43.8% (49/112) | 42.5% (179/421) |
| Severe | 35.5% (43/121) | 40.8% (86/211) | 23.8% (5/21) | 26.3% (5/19) | 35.7% (40/112) | 39.9% (168/421) |

*The missing values were described as negative.
[a]P < 0.0033 versus none.
[b]P < 0.0033 versus chest X-ray abnormality.
[c]P < 0.0033 versus alveolar haemorrhage.
[d]P < 0.0033 versus interstitial lung disease.
[e]P < 0.0033 versus pulmonary granuloma.

AAV [14–17]. However, the mortality due to AH varies among studies. Although AH alone was not associated with increased mortality [18], AH increased the relative risk at death by 8.6 times in one study [15]. The 1-year survival rate of AAV patients with AH was 82% [16], but another group reported that 50% of similar patients were alive at their 1-year follow-up [17]. In the present study, the 1-year survival rate was 56.2% and the HR at death by AH was the highest variable; the HR

**Table 3. Treatments of patients with ANCA-associated renal vasculitis**

| | Alveolar hemorrhage ($n = 177$) | Interstitial lung disease ($n = 301$) | Bronchial asthma ($n = 32$) | Pulmonary granuloma ($n = 27$) | Chest X-ray abnormality ($n = 147$) | None ($n = 528$) |
|---|---|---|---|---|---|---|
| Corticosteroids | 96.5% (167/173) | 97.0% (289/298) | 100.0% (32/32) | 100.0% (26/26) | 97.9% (143/146) | 93.1% (486/522) |
| Initial dose of PSL (mg/day) | 44.9 ± 14.4[a,b,c] | 39.9 ± 13.4 | 41.5 ± 11.6 | 42.4 ± 12.3 | 40.2 ± 12.5 | 39.4 ± 11.2 |
| Initial periods (weeks) | 3.8 ± 2.7 | 4.0 ± 2.3 | 3.8 ± 2.1 | 4.3 ± 2.2 | 4.0 ± 2.1 | 4.4 ± 3.1 |
| Methylprednisolone pulse | 86.6% (149/172)[a,b,c] | 73.1% (215/294) | 71.9% (24/32) | 87.5% (21/24) | 69.2% (101/146) | 70.2% (363/517) |
| Daily doses of mPSL (mg) | 806.9 ± 266.5[b] | 735.7 ± 279.7 | 833.3 ± 240.8 | 664.3 ± 292.0 | 703.5 ± 259.4 | 739.2 ± 268.0 |
| Days per time | 3.0 ± 0.3 | 3.0 ± 0.2 | 3.0 ± 0.0 | 2.9 ± 0.4 | 3.0 ± 0.4 | 3.0 ± 0.2 |
| Times | 1.7 ± 1.1 | 1.6 ± 1.1 | 1.2 ± 0.4 | 1.7 ± 0.9 | 1.4 ± 0.6 | 1.7 ± 1.2 |
| Immunosuppressants | 45.7% (73/173) | 44.7% (132/295) | 48.4% (15/31) | 40.7% (11/27) | 41.4% (58/140) | 46.7% (240/514) |
| Cyclophosphamide | 45.7% (73/173) | 40.1% (118/294) | 35.5% (11/31) | 55.6% (15/27) | 37.1% (52/140) | 41.2% (212/514) |
| Intravenous cyclophosphamide | 22.8% (38/167) | 14.7% (42/285) | 23.3% (7/30) | 28.0% (7/25) | 9.9% (14/141) | 10.9% (56/516) |
| Doses/time of IVCY (mg) | 482.9 ± 133.9 | 492.1 ± 139.7 | 535.7 ± 149.2 | 562.5 ± 104.6 | 564.3 ± 217.0 | 537.8 ± 156.9 |
| Times | 2.5 ± 2.3 | 2.3 ± 1.9 | 2.9 ± 1.6 | 4.0 ± 2.8 | 1.9 ± 1.3 | 2.8 ± 2.2 |
| Oral cyclophosphamide | 33.7% (58/172) | 34.6% (101/292) | 31.0% (9/29) | 52.0% (13/25) | 34.3% (48/140) | 36.2% (184/508) |
| Azathioprine | 2.9% (5/172) | 1.7% (5/292) | 3.4% (1/29) | 4.0% (1/25) | 1.4% (2/140) | 2.4% (12/508) |
| Mizoribine | 7.0% (12/172) | 3.4% (10/292) | 6.9% (2/29) | 8.0% (2/25) | 3.6% (5/140) | 5.7% (29/508) |
| Cyclosporin A | 0.6% (1/172) | 2.7% (8/292) | 6.9% (2/29)[a] | 0.0% (0/25) | 0.0% (0/140) | 0.1% (5/508) |
| Apheresis | 30.3% (53/175)[a,b,c] | 16.0% (47/293) | 12.5% (4/32) | 15.4% (4/26) | 15.0% (21/140) | 8.7% (45/516) |
| Plasma exchange | 28.6% (50/175)[a,b,c] | 12.6% (37/293) | 9.4% (3/32) | 11.5% (3/26) | 12.9% (18/140) | 7.2% (37/516) |
| Plasma absorption | 2.9% (5/175) | 2.7% (8/293) | 3.1% (1/32) | 3.8% (1/26) | 2.1% (3/140) | 1.4% (7/516) |
| Intravenous immunoglobulin | 5.7% (3/53) | 2.8% (3/107) | 0.0% (0/13) | 0.0% (0/12) | 0.0% (0/48) | 3.0% (5/166) |
| Biologics | 2.0% (1/49) | 1.0% (1/103) | 0.0% (0/13) | 9.1% (1/11) | 2.1% (1/48) | 0.6% (1/162) |

PSL, prednisolone; mPSL, methylprednisolone; IVCY, intravenous cyclophosphamide.
[a]$P < 0.0033$ versus none.
[b]$P < 0.0033$ versus chest X-ray abnormality.
[c]$P < 0.0033$ versus interstitial lung disease.

for 1-year survival was ~3.7 and that for 5-year survival was 3.0. Although the treatment strength of the initial dose of corticosteroid, the percentage of methylprednisolone pulse therapy usage and the frequency of receiving a plasma exchange treatment in the AAV patients with AH were significantly stronger than those in the other pulmonary involvement groups, poor outcomes were observed in the AAV patients with AH.

It was recently demonstrated that renal failure and older age were associated with higher mortality among AAV patients with AH [19]. In the present study, age and serum creatinine level were predictive variables that increased the relative risk at death, as was AH. Moreover, all of the analyzed patients had RPGN (since this study was based on the RPGN survey); this contributed to the poor outcomes of the patients with AH.

AAV with ILD was first reported in 1990 [20], and several reports of ILD in AAV patients [6–9, 11, 21–32] and ANCA-positive ILD patients [10, 33–38] were published since then. ILD developed more frequently in AAV patients who were MPO-ANCA positive, in particular in those with a diagnosis of MPA, compared with patients with PR3-ANCA-positive AAV [6–11, 23–32], and a higher prevalence of ILD has been reported in Asian countries [8, 9, 11, 31, 32]. In the present study, 26% of the AAV patients had ILD and 97% of the AAV patients with ILD had MPO-ANCA, similar to the above-cited studies reporting that AAV patients with ILD had higher percentages of general symptoms (such as fever, weight loss and fatigue), as in previous reports [30, 32]. Regarding the laboratory data, elevated serum C-reactive protein and erythrocyte sedimentation rate levels and abnormal urinalysis and/or renal function were demonstrated in a previous study [32], but there were no significant differences in inflammatory parameters in that comparison of ILD patients and patients with other pulmonary involvements. A higher prevalence of ILD in this study was also associated with the recruited subjects who had demonstrated RPGN, because renal involvement was frequently observed in AAV patients with ILD [20, 23–26, 30, 32].

The prognosis of AAV patients with ILD is still unknown; it was reported that there was no significant difference in mortality among patients with and without ILD [7]. However, several studies contended that AAV patients with ILD have a poor prognosis [6, 26, 29, 30, 32]. In the present study, AH was the most life-threatening variable for 1- or 5-year survival, but ILD also increased the relative risk at death at 5 years by ~2-fold. Therefore, AH may be associated with the short-term prognosis, and ILD may be associated with the long-term prognosis of AAV.

The causes of ILD in AAV are still unclear. Several potential risk factors for ILD have been demonstrated: cigarette smoking, environmental exposures, microbial agents and microaspiration with gastroesophageal reflux [39]. However, unfortunately, we were not able to identify how these factors affected ILD

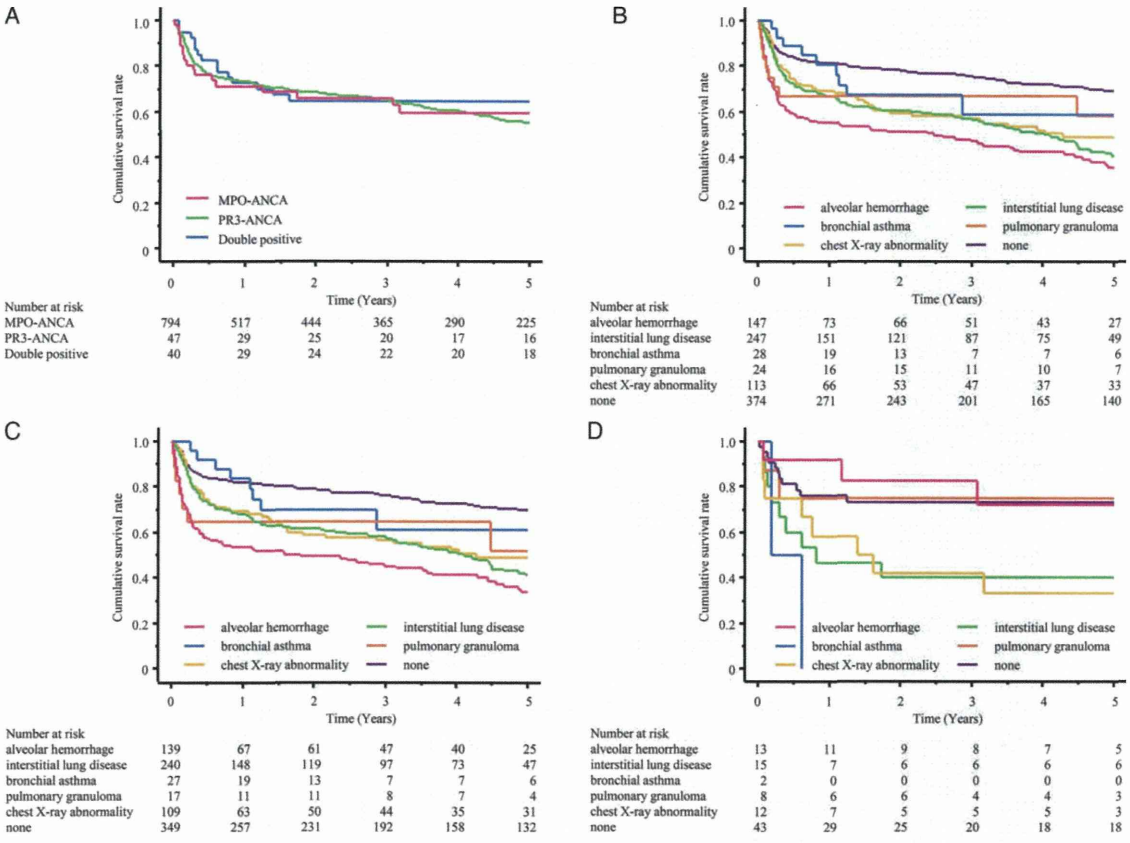


FIGURE 1: The 5-year patient survival curves for AAV patients (Kaplan–Meier method). The 5-year patient survival curves for AAV patients with differing ANCA status (**A**). Magenta line: MPO-ANCA-positive AAV patients; green: PR3-ANCA-positive AAV patients; blue: double ANCA-positive AAV patients. The 5-year patient survival curves for all AAV patients (**B**), MPO-AAV patients (**C**) and PR3-AAV patients (**D**) with differing pulmonary involvements. Magenta line: AAV patients without pulmonary involvement; pink line: patients with diffuse alveolar hemorrhage; green: interstitial lung disease; blue: bronchial asthma; orange: pulmonary granuloma; yellow: chest X-ray abnormalities.

in AAV, because we did not investigate environmental factors including smoking status. In previous studies of ILD in AAV or ANCA-positive ILD patients from Japan, 26–53% of the patients were nonsmokers [10, 34, 36, 38], and there may be other causes of ILD than smoking.

In the present study, 17% of the ILD patients (50/301) also presented AH. Ultrastructural and immunohistochemical analyses of AAV have demonstrated an association between the vascular injury and the progression of lung fibrosis [40], and subclinical AH was observed in patients with AAV or urticarial vasculitis [41–43]. It has thus been suggested that subclinical AH may play a role in the development of pulmonary fibrosis in AAV. However, MPO-ANCA itself may have a pathogenic role in the pulmonary tissue injury of AAV, as was suggested in a study using an animal model of anti-MPO-associated pulmonary vasculitis [44]. The binding of translocated MPO to the surface of neutrophils and circulating ANCA may cause neutrophil degranulation and the release of reactive oxygen species, resulting in pulmonary injury and consequent fibrosis [44, 45]. This hypothesis was supported by the association between MPO-ANCA and ILD in patients with rheumatoid arthritis [46], but this theory does not explain the pathogenesis of ANCA-negative pulmonary fibrosis. Although ANCA was negative at the diagnosis of ILD, ANCA-positive conversion occurred in several cases, and some of those cases developed AAV [33, 35, 38]. Therefore, further investigations into the pathogenesis of ILD in AAV are needed.

The AAV patients with a chest X-ray abnormality in the present study also had poor prognoses. This group may be heterogeneous; it might include AH patients who did not have hemosputum and/or were not examined by bronchoscopy, patients with pulmonary granuloma who could not be diagnosed histologically and patients with infectious pneumonia. Considering our finding that the major cause of death was infectious disease, infectious pneumonia may have been a complication at the time of diagnosis for some patients. Possible reasons for the poor prognosis of the AAV patients with chest X-ray abnormalities are an insufficient diagnosis and the complication of infectious pneumonia. To improve the outcome of AAV patients with pulmonary involvement, it is necessary to achieve the correct diagnosis of pulmonary lesions by active examinations such as bronchoscopy.

**Table 4. Cox regression analysis of predictors for outcome in patients with ANCA-associated renal vasculitis**

| | 1–year survival | | | | | | 5–year survival | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Univariate | | | Multivariate | | | Univariate | | | Multivariate | | |
| | Hazard ratio | 95% CI | P-value | Hazard ratio | 95% CI | P-value | Hazard ratio | 95% CI | P-value | Hazard ratio | 95% CI | P-value |
| Pulmonary involvements | | | <0.0001 | | | **0.0286** | | | <0.0001 | | | 0.0136 |
| None | ref. | – | – | ref. | – | – | ref. | – | – | ref. | – | – |
| Alveolar hemorrhage | 3.014 | 2.139–4.246 | <0.0001 | **3.084** | **1.612–5.900** | **0.0007** | 2.892 | 2.167–3.859 | <0.0001 | **2.743** | **1.588–4.738** | **0.0003** |
| Interstitial lung disease | 1.723 | 1.235–2.403 | 0.0014 | 1.379 | 0.771–2.534 | 0.2705 | 2.145 | 1.647–2.795 | <0.0001 | **1.674** | **1.066–2.630** | **0.0252** |
| Bronchial asthma | 0.954 | 0.384–2.366 | 0.9184 | 1.523 | 0.419–5.532 | 0.5226 | 1.336 | 0.675–2.644 | 0.4057 | 1.604 | 0.600–4.285 | 0.3460 |
| Pulmonary granuloma | 1.960 | 0.900–4.270 | 0.0902 | 1.299 | 0.291–5.790 | 0.7317 | 1.608 | 0.813–3.180 | 0.1725 | 1.315 | 0.446–3.883 | 0.6198 |
| Chest X-ray abnormality | 1.711 | 1.128–2.596 | 0.0116 | 1.240 | 0.590–2.607 | 0.5695 | 1.870 | 1.331–2.626 | 0.0003 | 1.360 | 0.754–2.452 | 0.3072 |
| Age | 1.032 | 1.020–1.045 | <0.0001 | **1.039** | **1.014–1.065** | **0.0020** | 1.036 | 1.026–1.047 | <0.0001 | **1.036** | **1.017–1.055** | **0.0002** |
| Bleeding tendency | 2.454 | 1.691–3.560 | <0.0001 | **3.155** | **1.383–7.196** | **0.0063** | 1.888 | 1.338–2.665 | 0.0003 | 1.844 | 0.910–3.737 | 0.0897 |
| Dyspnea | 1.396 | 1.034–1.885 | 0.0293 | 0.626 | 0.301–1.302 | 0.2101 | 1.612 | 1.269–2.049 | <0.0001 | 1.071 | 0.656–1.750 | 0.7835 |
| Bloody diarrhea | 2.341 | 1.597–3.432 | <0.0001 | 1.890 | 0.808–4.420 | 0.1421 | 1.887 | 1.331–2.674 | 0.0004 | 1.179 | 0.574–2.425 | 0.6535 |
| Oliguria | 1.775 | 1.284–2.453 | 0.0005 | 0.734 | 0.277–1.944 | 0.5333 | 1.515 | 1.140–2.014 | 0.0042 | 0.805 | 0.527–1.230 | 0.3161 |
| Uremia | 1.490 | 1.058–2.098 | 0.0225 | 0.782 | 0.364–1.679 | 0.5274 | 1.410 | 1.051–1.891 | 0.0220 | 0.838 | 0.471–1.493 | 0.5495 |
| CNS symptoms | 2.112 | 1.415–3.151 | 0.0003 | 1.849 | 0.802–4.265 | 0.1494 | 1.491 | 1.020–2.179 | 0.0391 | 1.126 | 0.492–2.575 | 0.7785 |
| Peripheral neuropathy | 0.517 | 0.324–0.826 | 0.0058 | **0.138** | **0.041–0.460** | **0.0013** | 0.774 | 0.563–1.064 | 0.1147 | – | – | – |
| White blood cell count | 1.000 | 1.000–1.000 | 0.0010 | 1.000 | 1.000–1.000 | 0.9015 | 1.000 | 1.000–1.000 | 0.0013 | 1.000 | 1.000–1.000 | 0.8001 |
| Hemoglobin concentration | 0.877 | 0.813–0.945 | 0.0006 | 1.111 | 0.946–1.306 | 0.2002 | 0.917 | 0.864–0.974 | 0.0051 | 1.084 | 0.959–1.226 | 0.1959 |
| Platelet count | 0.974 | 0.962–0.986 | <0.0001 | 0.982 | 0.960–1.004 | 0.1075 | 0.977 | 0.968–0.986 | <0.0001 | **0.978** | **0.962–0.996** | **0.0138** |
| Serum total protein level | 0.689 | 0.582–0.815 | <0.0001 | 0.951 | 0.673–1.343 | 0.7748 | 0.738 | 0.645–0.845 | <0.0001 | 0.922 | 0.710–1.197 | 0.5403 |
| Serum albumin level | 0.622 | 0.493–0.786 | <0.0001 | 0.797 | 0.455–1.396 | 0.4276 | 0.603 | 0.499–0.728 | <0.0001 | 0.791 | 0.520–1.205 | 0.2753 |
| Blood urea nitrogen level | 1.016 | 1.011–1.020 | <0.0001 | 1.009 | 0.996–1.021 | 0.1726 | 1.013 | 1.009–1.017 | <0.0001 | 1.008 | 0.998–1.018 | 0.0975 |
| Serum creatinine level | 1.166 | 1.125–1.207 | <0.0001 | **1.173** | **1.039–1.325** | **0.0099** | 1.144 | 1.111–1.178 | <0.0001 | **1.132** | **1.024–1.251** | **0.0154** |
| Total cholesterol level | 1.002 | 1.001–1.002 | <0.0001 | 0.995 | 0.990–1.001 | 0.1048 | 0.992 | 0.989–0.995 | <0.0001 | 0.998 | 0.995–1.000 | 0.0830 |
| Serum C-reactive protein level | 1.024 | 1.007–1.041 | 0.0050 | 1.040 | 0.997–1.086 | 0.0697 | 1.018 | 1.003–1.032 | 0.0142 | 1.024 | 0.988–1.061 | 0.1874 |
| Serum complement level | | | <0.0001 | | | 0.1111 | | | <0.0001 | | | 0.1891 |
| Normal | ref. | – | – | ref. | – | – | ref. | – | – | ref. | – | – |
| Decreased | 1.721 | 1.231–2.407 | 0.0015 | 1.259 | 0.669–2.370 | 0.4748 | 1.680 | 1.268–2.228 | 0.0003 | 1.208 | 0.735–1.985 | 0.4553 |
| Elevated | 0.472 | 0.302–0.734 | 0.0009 | 0.521 | 0.260–1.043 | 0.0657 | 0.568 | 0.409–0.791 | 0.0008 | 0.679 | 0.412–1.119 | 0.1288 |
| Percentages of crescents | 1.007 | 1.001–1.013 | 0.0133 | 1.002 | 0.993–1.011 | 0.6389 | 1.009 | 1.005–1.014 | <0.0001 | 1.006 | 0.999–1.013 | 0.0740 |

P < 0.05 in the multivariate analysis indicates bold values.
CI, confidence interval; CNS, central nervous system.

**Table 5. Causes of death in patients with ANCA-associated renal vasculitis**

| | Alveolar hemorrhage ($n = 177$) | Interstitial lung disease ($n = 301$) | Bronchial asthma ($n = 32$) | Pulmonary granuloma ($n = 27$) | Chest X-ray abnormality ($n = 147$) | None ($n = 528$) |
|---|---|---|---|---|---|---|
| Pulmonary involvements due to vasculitis | 28.9% (28/97)[a,b] | 17.8% (21/118)[a,b] | 12.5% (1/8) | 18.2% (2/11)[a] | 2.1% (1/48) | 0.9% (1/108) |
| Both pulmonary involvements and infectious diseases | 29.9% (29/97)[a,b] | 35.6% (42/118)[a,b] | 12.5% (1/8) | 27.3% (3/11)[a] | 4.2% (2/48) | 0.9% (1/108) |
| Infectious diseases | 30.9% (30/97)[a] | 33.1% (39/118)[a] | 50.0% (4/8) | 36.4% (4/11) | 52.1% (25/48) | 53.7% (58/108) |
| Infectious pneumonia | 47.4% (46/97) | 48.3% (57/118) | 62.5% (5/8) | 45.5% (5/11) | 45.8% (22/48) | 29.6% (32/108) |
| Others | 10.3% (10/97)[a,b] | 13.6% (16/118)[a,b] | 25.0% (2/8) | 18.2% (2/11) | 41.7% (20/48) | 44.4% (48/108) |

[a]$P < 0.0033$ versus none.
[b]$P < 0.0033$ versus chest X-ray abnormality.

Among the present AAV patients with pulmonary granuloma, symptoms of GPA such as arthritis, episcleritis, ENT symptoms and nasal granuloma were frequently observed, and PR3-ANCA was frequently detected. The numbers of patients with these two diseases in this study were relatively small. The reason for a low GPA may be much less common for PR3-ANCA-associated vasculitis in Japan compared with Europe [5]. In the present study, most of the AAV patients with bronchial asthma had specific symptoms of EGPA such as heart disease, peripheral neuropathy and ENT symptoms, and eosinophilia was frequently observed. It would have been appropriate to analyze AAV patients excluded EGPA because of the different prognoses from MPA or GPA. However, we were unfortunately unable to clearly exclude EGPA in this study, because some data were missing. Concerning EGPA, the incidence was similar between Europe and Japan [47], but renal involvement was observed in 20% of patients with EGPA [48], and thus, the number of EGPA patients in the present RPGN survey was small. Considering the findings mentioned above, we suspect that most of the investigated subjects had MPA. However, we were unable to clarify the classification of AAV in this study. The classification of AAV has been dependent on the various treating facilities; moreover, the time at which the present study started was before the classification algorithm was published by the European Medicines Agency [49], and thus, the classification of AAV may be imperfect. On the other hand, in the present study, 50.6% (267/528) of the AAV patients without pulmonary involvement did not have symptoms of other organ involvement; 23.3% (267/1147) of the AAV patients without pulmonary involvement had renal-limited vasculitis. In comparison with Europe, in which 14–25% of AAV patients with renal involvement had renal-limited vasculitis [50, 51], the frequency of renal-limited vasculitis in Japan is similar.

In the present study, the data of over 1000 AAV patients from across Japan were collected, but part of this study was retrospective and some data were missing because of the questionnaire aspect of the study. Therefore, the time points of the onset of pulmonary involvement in this patient population were not identified, because the collected data were insufficient for this analysis, and the classification for AAV was not perfect. Moreover, the severity of the pulmonary involvements could not be assessed by specific examinations such as blood gas analysis, respiratory function, high-resolution computed tomography and bronchoscopy. In addition, the treatment strength in the AAV patients with AH was significantly stronger. Investigations of the incidence of pulmonary involvements and the accurate outcomes should be conducted as prospective studies with larger numbers of AAV patients.

A prospective study of AAV in Japan demonstrated that 20 (41.7%) of 48 AAV patients had RPGN or pulmonary-renal syndrome [52]. On the other hand, another Japanese prospective study of 156 newly diagnosed AAV patients in Japan showed that 109 (69.9%) of those patients had renal involvements, and 61 (39.1%) of those patients had ILD [11]. That ILD frequency in that study was similar to that of the present study. Moreover, there was no significant difference in the frequency of renal involvement between the AAV patients with ILD and those without ILD in that prospective study [11]. However, AAV patients without renal involvement were not included in the present study, because the study was a part of a nationwide survey of patients with RPGN. Moreover, we did not include cases of AAV patients without ANCA positivity (such as those with ANCA-negative EGPA) or drug-induced AAV patients (such as those with antithyroid agent-associated vasculitis). It is desirable to investigate all patients with ANCA vasculitis, including patients without renal involvement.

In summary, we found that approximately one-half of the AAV patients with RPGN had pulmonary involvement, and that not only AH but also ILD was frequently observed in these AAV patients in Japan. AH was associated with the prognosis, and ILD was associated with the long-term prognosis of AAV.

## SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

## ACKNOWLEDGEMENTS

This work was part of the nationwide RPGN survey in Japan, which was supported by a grant-in-aid from the Research Fund for the Special Study Group on Progressive Glomerular

Disease, the Ministry of Health, Labor and Welfare, Japan. We acknowledge all of the people who participated in this survey and the members of the RPGN Clinical Guidelines Committee of Japan as follows: Dr S. Fujimoto, Dr S. Hirawa, Dr S. Horikoshi, Dr T. Hosoya, Dr T. Itoh, Dr O. Inaguma, Dr S. Kaname, Dr T. Kawamura, Dr K. Kimura, Dr S. Maruyama, Dr N. Nakabayashi, Dr M. Ogura, Dr T. Saitoh, Dr T. Tabei, Dr Y. Taguma, Dr K. Tsuruya, Dr T. Watanabe, Dr S. Yasunaga, Dr M. Yoshida, Dr W. Yumura, Dr Y. Yuzawa and Dr K. Sada.

## CONFLICT OF INTEREST STATEMENT

None declared.

## REFERENCES


1. Lane SE, Watts RA, Shepstone L *et al.* Primary systemic vasculitis: clinical features and mortality. Q J Med 2005; 98: 97–111
2. Manganelli P, Fietta P, Carotti M *et al.* Respiratory system involvement in systemic vasculitides. Clin Exp Rheumatol 2006; 24: S48–S59
3. Thickett DR, Richter AG, Nathani N *et al.* Pulmonary manifestations of anti-neutrophil cytoplasmic antibody (ANCA)-positive vasculitis. Rheumatology 2006; 45: 261–268
4. West S, Arulkumaran N, Ind PW *et al.* Diffuse alveolar haemorrhage in ANCA-associated vasculitis. Intern Med 2013; 52: 5–13
5. Watts RA, Scott DG, Jayne DR *et al.* Renal vasculitis in Japan and the UK—are there differences in epidemiology and clinical phenotype? Nephrol Dial Transplant 2008; 23: 3928–3931
6. Hervier B, Pagnoux C, Agard C *et al.* Pulmonary fibrosis associated with ANCA-positive vasculitides. Retrospective study of 12 cases and review of the literature. Ann Rheum Dis 2009; 68: 404–407
7. Arulkumaran N, Periselneris N, Gaskin G *et al.* Interstitial lung disease and ANCA-associated vasculitis: a retrospective observational cohort study. Rheumatology 2011; 50: 2035–2043
8. Uezono S, Sato Y, Hara S *et al.* Outcome of ANCA-associated primary renal vasculitis in Miyazaki Prefecture. Intern Med 2007; 46: 815–822
9. Sada KE, Yamamura M, Harigai M *et al.* Classification and characteristics of Japanese patients with antineutrophil cytoplasmic antibody-associated vasculitis in a nationwide, prospective, inception cohort study. Arthritis Res Ther 2014; 16: R101
10. Homma S, Matsushita H, Nakata K. Pulmonary fibrosis in myeloperoxidase antineutrophil cytoplasmic antibody-associated vasculitides. Respirology 2004; 9: 190–196
11. Furuta S, Chaudhry AN, Hamano Y *et al.* Comparison of phenotype and outcome in microscopic polyangiitis between Europe and Japan. J Rheumatol 2014; 41: 325–333
12. Yamagata K, Usui J, Saito C *et al.* ANCA-associated systemic vasculitis in Japan: clinical features and prognostic changes. Clin Exp Nephrol 2012; 16: 580–588
13. Jennette JC, Falk RJ, Andrassy K *et al.* Nomenclature of systemic vasculitides. Proposal of an international consensus conference. Arthritis Rheum 1994; 37: 187–192
14. Haworth SJ, Savage CO, Carr D *et al.* Pulmonary haemorrhage complicating Wegener's granulomatosis and microscopic polyarteritis. Br Med J 1985; 290: 1775–1778
15. Hogan SL, Nachman PH, Wilkman AS *et al.* Prognostic markers in patients with antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. J Am Soc Nephrol 1996; 7: 23–32
16. Gallagher H, Kwan JT, Jayne DR. Pulmonary renal syndrome: a 4-year, single-center experience. Am J Kidney Dis 2002; 39: 42–47
17. Lauque D, Cadranel J, Lazor R *et al.* Microscopic polyangiitis with alveolar hemorrhage. A study of 29 cases and review of the literature. Groupe d'Etudes et de Recherche sur les Maladies 'Orphelines' Pulmonaires (GERM'O'P). Medicine 2000; 79: 222–233
18. Kostianovsky A, Hauser T, Pagnoux C *et al.* Alveolar haemorrhage in ANCA-associated vasculitides: 80 patients' features and prognostic factors. Clin Exp Rheumatol 2012; 30: S77–S82
19. Hruskova Z, Casian AL, Konopasek P *et al.* Long-term outcome of severe alveolar haemorrhage in ANCA-associated vasculitis: a retrospective cohort study. Scand J Rheumatol 2013; 42: 211–214
20. Nada AK, Torres VE, Ryu JH *et al.* Pulmonary fibrosis as an unusual clinical manifestation of a pulmonary-renal vasculitis in elderly patients. Mayo Clin Proc 1990; 65: 847–856
21. Gal AA, Salinas FF, Staton GW, Jr. The clinical and pathological spectrum of antineutrophil cytoplasmic autoantibody-related pulmonary disease. A comparison between perinuclear and cytoplasmic antineutrophil cytoplasmic autoantibodies. Arch Pathol Lab Med 1994; 118: 1209–1214
22. Gaudin PB, Askin FB, Falk RJ *et al.* The pathologic spectrum of pulmonary lesions in patients with anti-neutrophil cytoplasmic autoantibodies specific for anti-proteinase 3 and anti-myeloperoxidase. Am J Clin Pathol 1995; 104: 7–16
23. Akikusa B, Kondo Y, Irabu N *et al.* Six cases of microscopic polyarteritis exhibiting acute interstitial pneumonia. Pathol Int 1995; 45: 580–588
24. Becker-Merok A, Nossent JC, Ritland N. Fibrosing alveolitis predating microscopic polyangiitis. Scand J Rheumatol 1999; 28: 254–256
25. Guillevin L, Durand-Gasselin B, Cevallos R *et al.* Microscopic polyangiitis: clinical and laboratory findings in eighty-five patients. Arthritis Rheum 1999; 42: 421–430
26. Hiromura K, Nojima Y, Kitahara T *et al.* Four cases of anti-myeloperoxidase antibody-related rapidly progressive glomerulonephritis during the course of idiopathic pulmonary fibrosis. Clin Nephrol 2000; 53: 384–389
27. Mansi IA, Opran A, Sondhi D *et al.* Microscopic polyangiitis presenting as idiopathic pulmonary fibrosis: is anti-neutrophilic cytoplasmic antibody testing indicated? Am J Med Sci 2001; 321: 201–202
28. Souid M, Terki NH, Nochy D *et al.* Myeloperoxidase anti-neutrophil cytoplasmic autoantibodies (MPO-ANCA)-related rapidly progressive glomerulonephritis (RPGN) and pulmonary fibrosis (PF) with dissociated evolution. Clin Nephrol 2001; 55: 337–338
29. Eschun GM, Mink SN, Sharma S. Pulmonary interstitial fibrosis as a presenting manifestation in perinuclear antineutrophilic cytoplasmic antibody microscopic polyangiitis. Chest 2003; 123: 297–301
30. Tzelepis GE, Kokosi M, Tzioufas A *et al.* Prevalence and outcome of pulmonary fibrosis in microscopic polyangiitis. Eur Respir J 2010; 36: 116–121
31. Ahn JK, Hwang JW, Lee J *et al.* Clinical features and outcome of microscopic polyangiitis under a new consensus algorithm of ANCA-associated vasculitides in Korea. Rheumatol Int 2012; 32: 2979–2986
32. Huang H, Wang YX, Jiang CG *et al.* A retrospective study of microscopic polyangiitis patients presenting with pulmonary fibrosis in China. BMC Pulm Med 2014; 14: 8
33. Foulon G, Delaval P, Valeyre D *et al.* ANCA-associated lung fibrosis: analysis of 17 patients. Respir Med 2008; 102: 1392–1398
34. Nozu T, Kondo M, Suzuki K *et al.* A comparison of the clinical features of ANCA-positive and ANCA-negative idiopathic pulmonary fibrosis patients. Respiration 2009; 77: 407–415
35. Bhanji A, Karim M. Pulmonary fibrosis-an uncommon manifestation of anti-myeloperoxidase- positive systemic vasculitis? NDT Plus 2010; 3: 351–353
36. Tanaka T, Otani K, Egashira R *et al.* Interstitial pneumonia associated with MPO-ANCA: clinicopathological features of nine patients. Respir Med 2012; 106: 1765–1770
37. Eleftheriou D, Katsenos S, Zorbas S *et al.* Pulmonary fibrosis presenting as an early manifestation of microscopic polyangiitis. Monaldi Arch Chest Dis 2012; 77: 141–144
38. Ando M, Miyazaki E, Ishii T *et al.* Incidence of myeloperoxidase antineutrophil cytoplasmic antibody positivity and microscopic polyangitis in the course of idiopathic pulmonary fibrosis. Respir Med 2013; 107: 608–615
39. Raghu G, Collard HR, Egan JJ *et al.* An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011; 183: 788–824

40. Gindre D, Peyrol S, Raccurt M *et al.* Fibrosing vasculitis in Wegener's granulomatosis: ultrastructural and immunohistochemical analysis of the vascular lesions. Virchows Arch 1995; 427: 385–393
41. Schnabel A, Reuter M, Csernok E *et al.* Subclinical alveolar bleeding in pulmonary vasculitides: correlation with indices of disease activity. Eur Respir J 1999; 14: 118–124
42. Al Riyami BM, Al Kaabi JK, Elagib EM *et al.* Subclinical pulmonary haemorrhage causing a restrictive lung defect in three siblings with a unique urticarial vasculitis syndrome. Clin Rheumatol 2003; 22: 309–313
43. Birnbaum J, Danoff S, Askin FB *et al.* Microscopic polyangiitis presenting as a 'pulmonary-muscle' syndrome: is subclinical alveolar hemorrhage the mechanism of pulmonary fibrosis? Arthritis Rheum 2007; 56: 2065–2071
44. Foucher P, Heeringa P, Petersen AH *et al.* Antimyeloperoxidase-associated lung disease. An experimental model. Am J Respir Crit Care Med 1999; 160: 987–994
45. Cambridge G, Williams M, Leaker B *et al.* Anti-myeloperoxidase antibodies in patients with rheumatoid arthritis: prevalence, clinical correlates, and IgG subclass. Ann Rheum Dis 1994; 53: 24–29
46. Little MA, Smyth L, Salama AD *et al.* Experimental autoimmune vasculitis: an animal model of anti-neutrophil cytoplasmic autoantibody-associated systemic vasculitis. Am J Pathol 2009; 174: 1212–1220
47. Sada KE, Amano K, Uehara R *et al.* A nationwide survey on the epidemiology and clinical features of eosinophilic granulomatosis with polyangiitis (Churg-Strauss) in Japan. Mod Rheumatol 2014; 24: 640–644
48. Comarmond C, Pagnoux C, Khellaf M *et al.* Eosinophilic granulomatosis with polyangiitis (Churg-Strauss): clinical characteristics and long-term follow up of the 383 patients enrolled in the French Vasculitis Study Group cohort. Arthritis Rheum 2013; 65: 270–281
49. Watts R, Lane S, Hanslik T *et al.* Development and validation of a consensus methodology for the classification of the ANCA-associated vasculitides and polyarteritis nodosa for epidemiological studies. Ann Rheum Dis 2007; 66: 222–227
50. Booth AD, Almond MK, Burns A *et al.* Outcome of ANCA-associated renal vasculitis: a 5-year retrospective study. Am J Kidney Dis 2003; 41: 776–784
51. Weidner S, Geuss S, Hafezi-Rachti S *et al.* ANCA-associated vasculitis with renal involvement: an outcome analysis. Nephrol Dial Transplant 2004; 19: 1403–1411
52. Ozaki S, Atsumi T, Hayashi T *et al.* Severity-based treatment for Japanese patients with MPO-ANCA-associated vasculitis: the JMAAV study. Mod Rheumatol 2012; 22: 394–404

*Received for publication: 28.6.2014; Accepted in revised form: 19.11.2014*

Clin Exp Nephrol
DOI 10.1007/s10157-014-1054-2

ORIGINAL ARTICLE

# Prevalence of anti-phospholipase A2 receptor antibodies in Japanese patients with membranous nephropathy

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Received: 25 September 2014 / Accepted: 31 October 2014


**Abstract**
*Background* Membranous nephropathy (MN) is the leading cause of nephrotic syndrome in adults. Anti-M-type phospholipase A2 receptor (anti-PLA2R) antibodies are found in most patients with idiopathic MN (iMN) worldwide, but the prevalence of anti-PLA2R antibodies among Japanese patients with MN is unknown. In this study, we determined the prevalence of anti-PLA2R antibodies in Japanese patients with MN.
*Methods* The study population of our retrospective cross-sectional consisted of 131 patients with biopsy-proven MN who had not received any immunosuppressive treatments at time of both renal biopsy and serum sample collection. Of these, 100 had iMN and 31 had secondary MN (sMN). The circulating anti-PLA2R antibodies were analyzed using a highly sensitive Western blot analysis. Analysis was performed under non-reducing conditions with a human glomerular extract at serum dilutions of 1:25, 1:10, and 1 as the primary antibody.
*Results* Anti-PLA2R antibodies were detected in 53 (53 %) of 100 patients with iMN and 0 (0 %) of 31 patients with sMN. The prevalence of anti-PLA2R antibodies was higher in patients with nephrotic syndrome (61 %) than in patients without nephrotic syndrome (43 %). The number of patients with serum albumin ≤3.0 g/dL was significantly higher in those with anti-PLA2R antibodies (92 %) than that in those without them (68 %).
*Conclusions* Anti-PLA2R antibodies were found in Japanese patients with iMN; however, the prevalence was lower than that of any other Asian country. This may indicate that the presence of other pathogenic antigens plays a significant role in Japanese patients with iMN.

**Keywords** Phospholipase A2 receptor · Antibody · Membranous nephropathy · Prevalence · Japan · Western blot

## Introduction

Membranous nephropathy (MN) is a leading cause of nephrotic syndrome in adults. It is classified as either idiopathic (iMN) or secondary (sMN) depending on its etiology. Overall, 40 % of Japanese patients with nephrotic syndrome and 33–50 % of patients with nephrotic syndrome in other countries either develop end-stage renal disease within 20 years of onset. Mortality from MN is high due to complications, such as infection, cardiovascular events, or malignancy [1–6]. The pathogenesis of iMN was not understood until the landmark study by Beck et al. in 2009, which demonstrated that the major target antigen of autoantibodies in iMN is an M-type phospholipase A2 receptor (PLA2R) expressed on podocytes [7].

The anti-PLA2R antibody specifically and accurately recognizes a 3-dimensional epitope supported by intramolecular disulfide bonds in the PLA2R protein. *N*-glycosylation of the PLA2R protein is not necessary for the interaction between the anti-PLA2R antibody and the PLA2R protein [7]. The major subclass of anti-PLA2R antibody in serum samples from patients with iMN is

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Published online: 21 November 2014