First-year cumulative myeloperoxidase-ANCA titres are associated with all-cause mortality in patients with microscopic polyangiitis

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Abstract

Objective

We investigated whether first-year cumulative myeloperoxidase (MPO)-antineutrophil cytoplasmic antibody (ANCA) and proteinase 3 (PR3)-ANCA titres were associated with all-cause mortality and relapse during follow-up in patients with microscopic polyangiitis (MPA) and gran

Methods

Altogether, 74 patients with MPA and 40 with GPA were included in this study. Their clinical data at diagnosis were collected. First-year cumulative ANCA titres were defined as the area under the curve (AUC) of ANCA titres during the first year after MPA or GPA diagnosis, which was obtained using the trapezoidal rule. All-cause mortality and relapse were considered poor outcomes of MPA and GPA.

Results

The median ages of patients with MPA and GPA were 65.5 and 60.5 years, respectively. No significant correlation was observed between ANCA titres at diagnosis and concurrent MPA and GPA activity or the inflammatory burden. First-year cumulative MPO-ANCA titres exhibited a significant AUC for all-cause mortality during follow-up in patients with MPA. The optimal cut-off of first-year cumulative MPO-ANCA titres for all-cause mortality was determined as 720.8 IU/mL using receiver operating characteristic curve analysis. MPA patients with first-year cumulative MPO-ANCA titres ≥720.8 IU/mL exhibited a significantly higher risk for all-cause mortality than those without (relative risk 13.250). Additionally, MPA patients with first-year cumulative MPO-ANCA titres ≥720.8 IU/mL exhibited a significantly lower cumulative patients' survival rate than those without.

Conclusion

This is the first study to demonstrate the association between first-year cumulative MPO-ANCA titres and all-cause mortality during follow-up in patients with MPA.

Key words
cumulative titres, antineutrophil cytoplasmic antibody, myeloperoxidase, microscopic polyangiitis, mortality

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Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a small-vessel vasculitis and is categorised into three subtypes as follows: microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), and eosinophilic GPA (EGPA) (1, 2). In the pathogenesis of AAV, the critical role of circulating ANCA in initiating AAV has been elucidated. Circulating ANCA may participate actively in AAV pathogenesis by binding to the cell surface or secretory ANCA antigens and eliciting ANCA-mediated activation of primed neutrophils. Consequently, ANCA-mediated neutrophil activation may trigger the production of reactive oxygen radicals and accelerate the degranulation of neutrophils, thus leading to bulky inflammation of the vessel walls and adjacent tissues (3-5). Additionally, in terms of AAV diagnosis, the classification criteria for AAV proposed in 2022 assigned relatively high scores to the items of ANCA positivity, emphasising the critical role of ANCA in the classification of MPA and GPA (6-10).

To date, the generally accepted theories are that ANCA titres may not directly reflect the current activity of AAV and that serial ANCA titres may predict relapse, although these are controversial (11-14). However, a previous study that the high cumulative anti-cyclic citrullinated peptide (anti-CCP) antibody titres over the first 2 years were significantly associated with accelerated radiographic progression in patients with rheumatoid arthritis (15). Considering the important role of anti-CCP antibodies in the pathogenesis of rheumatoid arthritis, it could be reasonably assumed that the early cumulative ANCA titres may be associated with poor outcomes of MPA and GPA, such as all-cause mortality, and relapse. However, owing to the different pathophysiology of EGPA from those of MPA and GPA, the probability of the association between the early cumulative ANCA titres and poor outcomes in patients with EGPA was expected to be lower than that of patients with MPA and GPA (16). Hence, in the present study, we investigated whether first-year cumulative MPO-ANCA and PR3-ANCA titres were associated with all-cause mortality and relapse during follow-up in patients with MPA and GPA, excluding those with EGPA.

Patients and methods

Patients

In the present study, we selected 74 patients with MPA, and 40 patients with GPA from the Severance Hospital ANCA-associated VasculitidEs (SHAVE) cohort, a prospective and observational AAV cohort, and retrospectively reviewed their medical records. The inclusion criteria were as follows: i) patients who were diagnosed with MPA, and GPA at the Division of Rheumatology, Department of Internal Medicine, Yonsei University College of Medicine, Severance Hospital from November 2016 to September 2022, according to the diagnostic algorithm for AAV proposed by the European Medicine Agency in 2007, and the revised Chapel Hill Consensus Conference nomenclature of vasculitides proposed in 2012 (1, 2); ii) patients who also met the 2022 American College of Rheumatology and the European Alliance of Associations for Rheumatology classification criteria for MPA, GPA, and EGPA (6, 7, 9); iii) patients who had the test results for myeloperoxidase (MPO)-ANCA and proteinase 3 (PR3)-ANCA at several times from diagnosis to more than one year after diagnosis (17); iv) patients who have been currently followed up at this hospital to accurately determine all-cause mortality and relapse; vi) patients who have been followed up ≥12 months; and vii) patients who had well-written medical records to collect clinical data at AAV diagnosis. The exclusion criteria were as follows: i) patients who had the only results of ANCA detected by an indirect immunofluorescence assay for perinuclear (P)-ANCA and cytoplasmic (C)-ANCA; ii) patients who had concomitant serious medical conditions mimicking AAV at diagnosis such as malignancies or infectious diseases (6, 7); and iii) patients who had received immunosuppressive drugs within 4 weeks before AAV diagnosis.

Ethical disclosure

This study was approved by the Institutional Review Board (IRB) of Sever-

ance Hospital, Seoul, Republic of Korea (approval number: 4-2016-0901) and conducted according to the Declaration of Helsinki. Written informed consent was obtained from all patients at the time of blood sampling for AAV diagnosis. The IRB waived the need for additional written informed consent since it had been previously obtained upon entry into the SHAVE cohort.

Data at diagnosis

Data at diagnosis were presented for each AAV subtype, MPA, and GPA. The demographic data included age, sex, smoking history, and body mass index (BMI). AAV-related variables, AAV subtypes, ANCA positivity and titres, and AAV-specific indices such as the Birmingham Vasculitis Activity Score (BVAS), and the Five-Factor Score (FFS) were obtained (18, 19). The results of routinely performed laboratory tests including erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels were also collected. Type 2 diabetes mellitus (T2DM), hypertension, and dyslipidaemia were recorded as comorbidities (20).

First-year cumulative ANCA titres

First-year cumulative ANCA titres were defined as the area under the curve (AUC) of ANCA titres during the first year after AAV diagnosis and were obtained using the trapezoidal rule (21). The units of the first year (X-axis) and ANCA titres (Y-axis) were set to months and IU/mL, respectively. When the ANCA test was performed ≥1 year after diagnosis, the AUC was calculated using ANCA titres; however, an AUC of up to 12 months was defined as first-year cumulative ANCA titres. The predictive potential of first-year cumulative ANCA titres for poor outcomes of MPA and GPA was investigated.

Follow-up data

All-cause mortality, and relapse were considered poor outcomes of MPO and GPA in the present study. The follow-up duration based on each poor outcome was defined as the period from AAV diagnosis to the occurrence of each poor outcome in patients with the corresponding poor outcome. Meanwhile, it was defined as the period from AAV diagnosis to the last visit in patients without the corresponding poor outcome. In terms of medications, the number of patients who received glucocorticoids and immunosuppressive drugs after AAV diagnosis was counted.

Method and frequency of ANCA measurement

In our institute, we measured MPO-ANCA and PR3-ANCA titres using a novel anchor-coated highly sensitive (hs) Phadia ELiA (Thermo Fisher Scientific/Phadia, Freiburg, Germany) and human native antigens, on a Phadia250 analyser. To calculate the AUC of ANCA titres according to the trapezoidal rule, both an initial (or baseline) value of ANCA titre at diagnosis and at least more than one value of ANCA titre during the first year after diagnosis are required. Therefore, all patients had at least two measurement values of ANCA titres including a baseline ANCA titre.

Statistical analysis

All statistical analyses were performed using SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, NY, USA). Continuous and categorical variables are expressed as medians with 25^{th} – 75^{th} quartiles and numbers (percentages). The correlation coefficient (r) between the two variables was obtained using the Pearson correlation analysis. The statistical significance of first-year cumulative MPO-ANCA and PR3-ANCA titres for all-cause mortality and relapse in patients with MPA and GPA, respectively, was verified using receiver operating characteristic (ROC) curve analysis. Additionally, the cut-off of first-year cumulative MPO-ANCA titres for all-cause mortality in patients with MPA was extrapolated by performing ROC curve analysis. The relative risk (RR) of first-year cumulative MPO-ANCA titre cut-off for all-cause mortality was analysed using contingency tables and the chi-squared test. The cumulative survival rates between the two groups were compared using the Kaplan Meier survival analysis with the log-rank test. Significant differences between the two categorical variables were analysed using the chi-square and Fisher's exact tests. The Mann-Whitney U-test was used to compare significant differences between two continuous variables. Statistical significance was set at $p<0.05$.

Results

Data at AAV diagnosis

The variables at AAV diagnosis among the 74 patients with MPA were as follows: median age, 65.5 years; 27 (36.5%) men; and 2 (2.7%) with smoking history. The median BMI was 22.1 kg/m^2. The median MPO-ANCA and PR3-ANCA titres were 61.3 and 0 IU/mL, respectively. The median BVAS, FFS, ESR, and CRP levels were 12.5, 2.0, 76.0 mm/h, and 14.0 mg/L, respectively. Of all patients with MPA, 18, 29, and 13 had T2DM, hypertension, and dyslipidaemia, respectively. The variables at diagnosis among the 40 patients with GPA were as follows: median age, 60.5 years; 17 (42.5%) men; and 2 (5.0%) ex-smokers. The median BMI and MPO-ANCA and PR3-ANCA titres were 22.7 kg/m^2, 0 and 2.5 IU/mL, respectively. The median BVAS, FFS, ESR, and CRP levels were 9.0, 1.0, 52.0 mm/h, and 13.2 mg/L, respectively. Of all the patients with GPA, 12, 15, and 8 patients had T2DM, hypertension, and dyslipidaemia, respectively (Table IA)

First-year cumulative ANCA titres

Using the trapezoidal rule, first-year cumulative MPO-ANCA and PR3-ANCA titres among the 74 patients with MPA were 251.2 IU/mL and 0 IU/mL, respectively. Meanwhile, first-year cumulative MPO-ANCA and PR3-ANCA titres among the 40 patients with GPA were calculated as 0 IU/mL and 7.7 IU/mL, respectively (Table IB).

Follow-up data

Among the 74 patients with MPA, 5 (6.8%) died and 15 (20.3%) experienced relapses. Of the patients, 71 (95.9%) received glucocorticoids, and the most frequently administered immunosuppressive drug was azathioprine (70.3%), followed by cyclophosphamide (56.8%). Among the 40 pa-

Table I. Characteristics of patients with MPA and GPA.

Variables	Values	
	Patients with MPA (n=74)	Patients with GPA (n=40)
A. Variables at AAV diagnosis		
Demographic data		
Age (years)	65.5 (53.0-72.0)	60.5 (46.5-68.0)
Male sex (n, (%))	27 (36.5)	17 (42.5)
Ex-smoker (n, (%))	2 (2.7)	2 (5.0)
BMI (kg/m^2)	22.1 (20.2-23.9)	22.7 (21.0-24.2)
ANCA titres		
MPO-ANCA (IU/mL)	61.3 (11.8-134.0)	0 (0-24.3)
PR3-ANCA (IU/mL)	0 (0-0)	2.5 (0-25.5)
AAV-specific indices		
BVAS	12.5 (7.0-17.3)	9.0 (5.0-14.8)
FFS	2.0 (1.0-3.0)	1.0 (0-2.0)
Acute phase reactants		
ESR (mm/hr)	76.0 (33.3-112.5)	52.0 (22.3-95.0)
CRP (mg/L)	14.0 (2.5-70.3)	13.2 (1.4-69.6)
Laboratory results		
White blood cell count (/mm^3)	8,335.0 (5,980.0-11,160.0)	9,490.0 (7,157.5-14,865.0)
Haemoglobin (g/dL)	10.2 (9.1-12.6)	11.6 (10.3-13.2)
Platelet count (× 1000/mm^3)	293.0 (213.5-395.0)	313.0 (224.5-425.0)
Fasting glucose (mg/dL)	100.5 (91.8-122.5)	93.0 (87.0-117.0)
Blood urea nitrogen (mg/dL)	21.2 (16.5-40.7)	16.5 (13.9-26.1)
Serum creatinine (mg/dL)	1.2 (0.7-2.7)	0.7 (0.6-1.1)
Serum total protein (g/dL)	6.8 (6.2-7.2)	6.8 (6.1-7.2)
Serum albumin (g/dL)	3.5 (3.1-4.0)	4.1 (3.2-4.4)
Comorbidities, n. (%)		
T2DM	18 (24.3)	12 (30.0)
Hypertension	29 (39.2)	15 (37.5)
Dyslipidaemia	13 (17.6)	8 (20.0)
B. First-year cumulative ANCA titres		
First-year cumulative MPO-ANCA titres (IU/mL)	251.2 (53.6-762.2)	0 (0-161.2)
First-year cumulative of PR3-ANCA titres (IU/mL)	0 (0-0)	7.7 (0-128.8)
C. Variables during AAV follow-up		
Poor outcomes, n, (%)		
All-cause mortali.y	5 (6.8)	4 (10.0)
Relapse	15 (20.3)	15 (37.5)
Follow-up duration based on each poor outcome (months)		
All-cause mortality	53.4 (30.6-72.2)	51.5 (27.5-65.1)
Relapse	40.3 (22.4-61.3)	24.7 (11.8-59.3)
Medications, n. (%)		
Glucocorticoids	71 (95.9)	39 (97.5)
Cyclophosphamide	42 (56.8)	27 (67.5)
Rituximab	15 (20.3)	13 (32.5)
Mycophenolate mofetil	25 (33.8)	10 (25.0)
Azathioprine	52 (70.3)	25 (62.5)
Tacrolimus	9 (12.2)	2 (5.0)
Methotrexate	7 (9.5)	5 (12.5)

Values are expressed as a median (25-75 percentile) or n (%).
MPA: microscopic polyangiitis; GPA: granulomatosis with polyangiitis; AAV: ANCA-associated vasculitis; ANCA: antineutrophil cytoplasmic antibody; BMI: body mass index; MPO: myeloperoxidase; P: perinuclear; PR3: proteinase 3; C: cytoplasmic; BVAS: Birmingham vasculitis activity score; FFS: five-factor score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; T2DM: type 2 diabetes mellitus.

tients with GPA, 4 (10.0%) died and 15 (37.5%) experienced relapses. The pattern of medication history was similar to that of patients with MPA (Table IC).

Correlation of ANCA titres with AAV activity or inflammatory burden at diagnosis

Based on the results of MPO-ANCA and PR3-ANCA titres at diagnosis in Table 1A, MPO-ANCA, and PR3-ANCA titres were separately investigated in patients with MPA and those with GPA, respectively. Among the 74 patients with MPA, MPO-ANCA titres at diagnosis were not significantly correlated with BVAS, FFS, ESR, or CRP. Similarly, among the 40 patients with GPA, no significant correlations were identified between PR3-ANCA titres at diagnosis and these variables (Fig. 1).

Association of first-year cumulative ANCA titres with all-cause mortality and relapse

When using ROC curve analysis, among the 74 patients with MPA, first-year cumulative MPO-ANCA titres exhibited a significant AUC for all-cause mortality during follow-up (AUC 0.812, 95% confidence interval [CI] 0.707, 0.916). However, no association between first-year cumulative MPO-ANCA titres and relapse during follow-up was identified. Among the 40 patients with GPA, first-year cumulative PR3-ANCA titres demonstrated no significant AUCs for all-cause mortality or relapse during follow-up (Fig. 2).

Optimal cut-off and RR of first-year cumulative MPO-ANCA titres for all-cause mortality in patients with MPA

The optimal cut-off of first-year cumulative MPO-ANCA titres for all-cause mortality in patients with MPA was 720.8 IU/mL (sensitivity and specificity were 80.0% and 76.8%, respectively) using ROC curve analysis. When we divided patients into two groups based on first-year cumulative MPO-ANCA titres ≥720.8 IU/mL, 20 of the 74 patients with MPA were assigned to the group with first-year cumulative MPO-ANCA titres ≥720.8 IU/mL. All-cause mortality was identified more frequently in MPA patients with first-year cumulative MPO-ANCA titres ≥ 720.8 IU/mL than those without (20.0% *versus* 1.9%, P=0.017). Furthermore, MPA patients with first-year cumulative MPO-ANCA titres ≥720.8 IU/mL exhibited a significantly higher risk for all-cause mortality than those without (RR 13.250, 95% CI 1.381, 127.167) (Fig. 3).

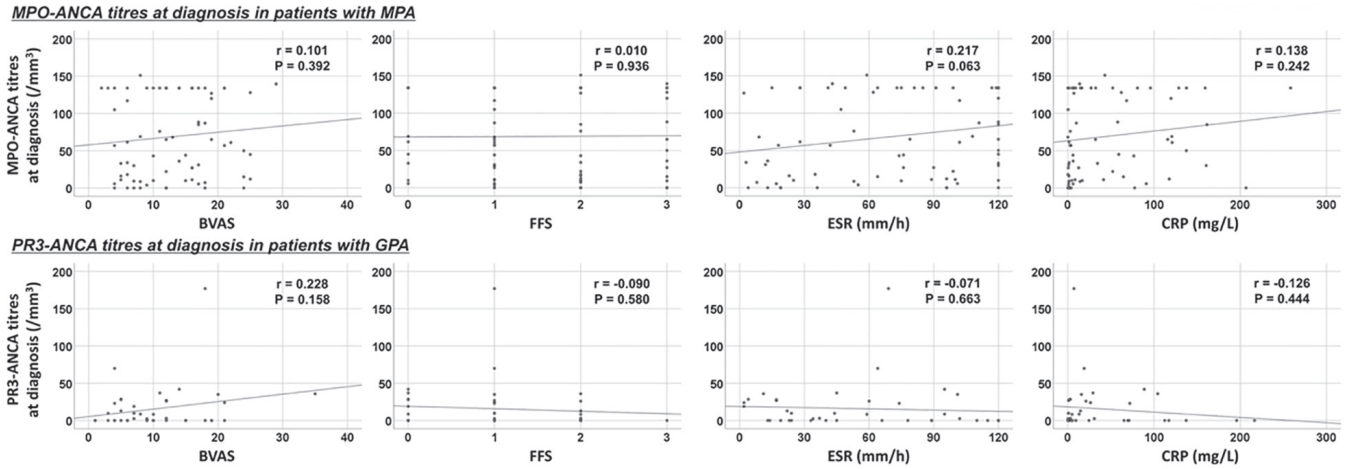

Fig. 1. Correlation of ANCA titres at diagnosis with cross-sectional AAV activity or inflammatory burden.
No significant correlations were identified between ANCA titres at diagnosis and cross-sectional BVAS, FFS, ESR, or CRP levels.
MPO: myeloperoxidase; ANCA: antineutrophil cytoplasmic antibody; MPA: microscopic polyangiitis; BVAS: the Birmingham Vasculitis Activity Score; FFS: the Five-Factor Score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; PR3: proteinase 3; GPA: granulomatosis with polyangiitis.

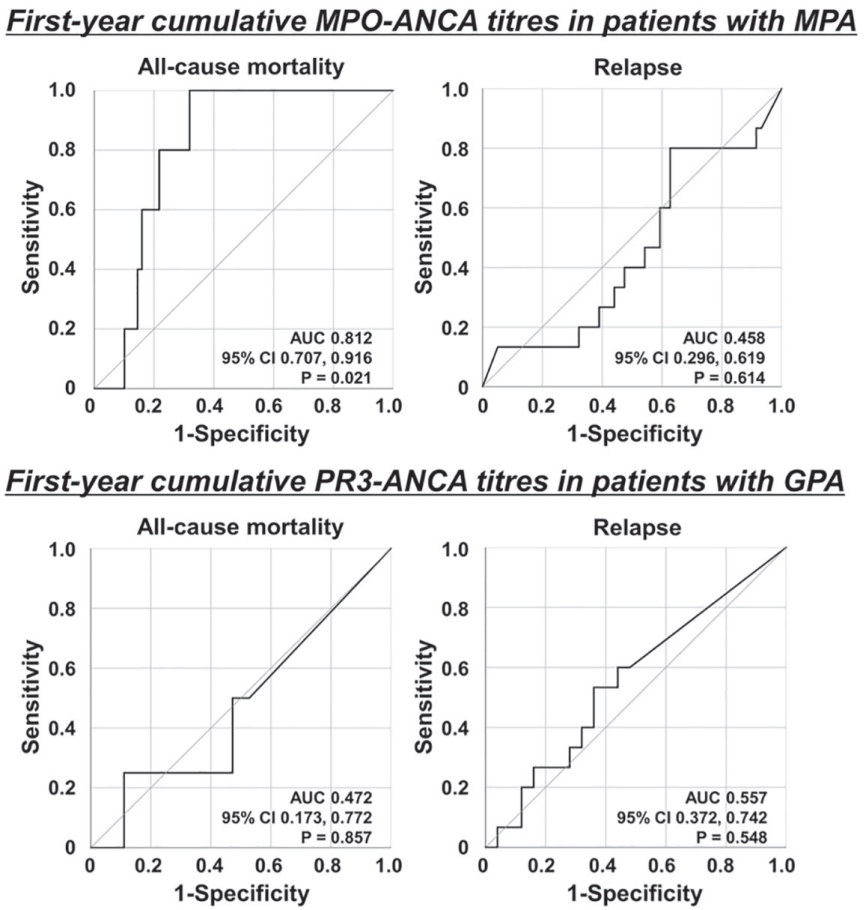

Fig. 2. Association of first-year cumulative ANCA titres with all-cause mortality and relapse.
Only first-year cumulative MPO-ANCA titres exhibited a significant AUC for all-cause mortality during follow-up in patients with MPA.
MPO: myeloperoxidase; ANCA: antineutrophil cytoplasmic antibody; MPA: microscopic polyangiitis; AUC: area under the curve; CI: confidence interval; PR3: proteinase 3; GPA: granulomatosis with polyangiitis.

Comparison of cumulative survival rates in patients with MPA

MPA patients with first-year cumulative MPO-ANCA titres ≥720.8 IU/mL exhibited a significantly lower cumulative patients' survival rate than those with first-year cumulative MPO-ANCA titres <720.8 IU/mL (p=0.013) (Fig. 4).

Discussion

In the present study, we investigated whether first-year cumulative ANCA titres might be associated with poor outcomes during follow-up in patients with MPA and GPA and obtained several findings. First, no significant correlation was observed between ANCA titres at diagnosis and concurrent levels of BVAS, FFS, ESR, and CRP levels. Second, in ROC curve analysis, first-year cumulative ANCA titres demonstrated no significant AUCs for relapse during follow-up in patients with MPA and GPA. Meanwhile, first-year cumulative MPO-ANCA titres exhibited a significant AUC for all-cause mortality during follow-up in patients with MPA. Third, when confined to patients with MPA, patients with first-year cumulative MPO-ANCA titres ≥720.8 IU/mL exhibited a significantly higher risk of all-cause mortality than those with first-year cumulative MPO-ANCA titres <720.8 IU/mL. Last, MPA patients with first-year cumulative MPO-ANCA titres ≥720.8 IU/mL exhibited a significantly lower cumulative patients' survival rate than those with first-year cumulative MPO-ANCA titres <720.8 IU/mL during follow-up. Therefore, we concluded that first-year cumulative MPO-ANCA titres were significantly associated with all-cause mortality in patients with MPA during the entire AAV follow-up period.

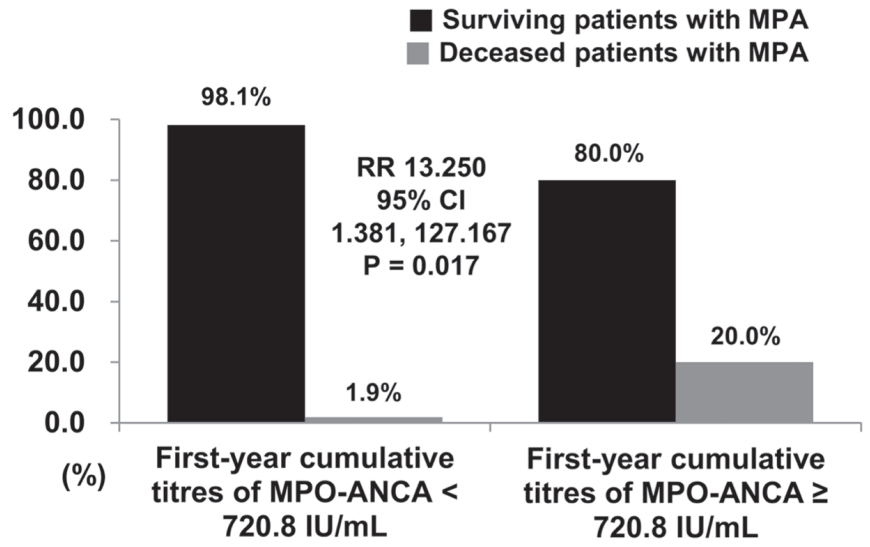

Fig. 3. Optimal cut-off and relative risk of first-year cumulative MPO-ANCA titres for all-cause mortality in patients with MPA.
The optimal cut-off of first-year cumulative MPO-ANCA titres for all-cause mortality was as 720.8 IU/mL. MPA patients with first-year cumulative MPO-ANCA titres ≥ 720.8 IU/mL exhibited a significantly higher risk for all-cause mortality than those without.
MPO: myeloperoxidase; ANCA: antineutrophil cytoplasmic antibody; MPA: microscopic polyangiitis; RR: relative risk; CI: confidence interval.

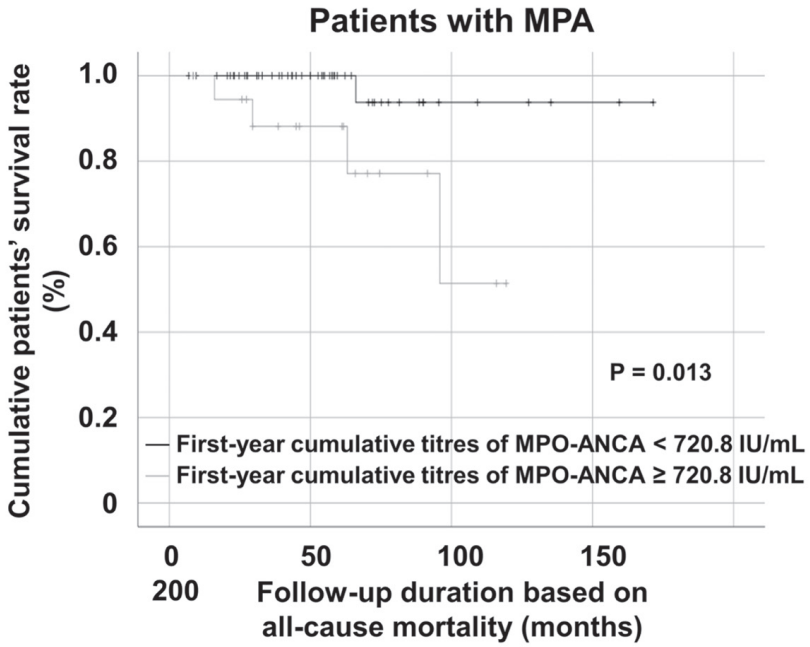

Fig. 4. Comparison of cumulative survival rates according to first-year cumulative MPO-ANCA titres ≥720.8 IU/mL.
MPA: microscopic polyangiitis; MPO: myeloperoxidase; ANCA: antineutrophil cytoplasmic antibody.

Whether the mechanism by which first-year cumulative MPO-ANCA titres were significantly associated with all-cause mortality during follow-up in patients with MPA was unclear. First, considering the probability that the indices reflecting AAV activity, such as BVAS and FFS, or those indicating the inflammatory burden including ESR and CRP levels might be risk factors for all-cause mortality during follow-up (22-24), we investigated whether first-year cumulative MPO-ANCA titres rather than MPO-ANCA titres at diagnosis were correlated with BVAS, FFS, ESR, and CRP levels at diagnosis. Among the four variables, only FFS at diagnosis was significantly correlated with first-year cumulative MPO-ANCA titres; however, it was an inverse correlation (r=-0.269, p=0.020). Therefore, the association between first-year MPO-ANCA titres and all-cause mortality during follow-up in MPA patients was not attributable to their significant association with MPA activities or the inflammatory burden at diagnosis.

Second, a previous study has demonstrated that the high vasculitis damage index (VDI) score was an independent factor associated with all-cause mortality in patients with AAV (25). Therefore, the initial high MPO-ANCA titres may enhance the ANCA-mediated neutrophil activation and accelerate the tissue infiltration of effector cells, resulting in the high extent of major organ damage at the time of AAV diagnosis which may increase the rate of all-cause mortality. We investigated the correlation between first-year cumulative MPO-ANCA titres and VDI score at diagnosis in patients with MPA; however, contrary to our assumption, no significant correlation was identified between the two variables (r=-0.034, p=0.775). Therefore, the association between first-year cumulative MPO-ANCA titres and all-cause mortality during follow-up in MPA patients was not attributable to the major organ damage that occurred in the early phase of MPA.

Third, we compared comorbidities at diagnosis, the initial clinical manifestations, and medications administered during follow-up between MPA patients with and those without first-year cumulative MPO-ANCA titres ≥720.8 IU/mL. However, no significant differences were observed between the two groups (Supplementary Table S1). In particular, the frequency of renal impairment or end-stage kidney disease which is a well-known mortality-predicting factor did not significantly differ between the two groups (p=0.377) (25). Therefore, the association between first-year cumulative MPO-ANCA titres and all-cause mortality during follow-up in MPA patients was not attributable to distinct differences in these risk factors for death.

We suggest several hypotheses regarding the association between first-year cumulative MPO-ANCA titres and

all-cause mortality during follow-up in MPA patients. The first hypothesis was on the conversion to pathogenic MPO-ANCA. High first-year cumulative MPO-ANCA titres may reflect the continuation of impaired T and B cell suppression, which can induce the conversion of natural MPO-ANCA to pathogenic MPO-ANCA, thereby contributing to the pathogenesis of MPA (26). The second hypothesis was on the persistent inflammatory burden and the numeric expansion of primed neutrophils. The increased secretion of inflammatory cytokines or chemokines may prime neutrophils, resulting in translocating MPO to their surface of or releasing them. Subsequently, impaired antigen-presenting cells may provoke the production of pathogenic MPO-ANCA by impaired T and B cells (3-5). The third hypothesis was on the increased levels of MPO-ANCA-mediated activation of neutrophils. Circulating MPO-ANCA can accelerate MPA activity by forming a dimer of primed neutrophils, in which the variable regions of MPO-ANCA may bind to MPO presented on the surface of neutrophils and its constant regions may bind to Fc gamma receptors. In theory, higher circulating MPO-ANCA may indicate frequent MPO-ANCA-mediated neutrophil activation (3-5). Therefore, all three hypotheses may have great potential to contribute to all-cause mortality by promoting the onset and worsening of MPA.

Considering the influence of the medications administered during the first year after MPA diagnosis on first-year cumulative MPO-ANCA titres, correcting first-year cumulative MPO-ANCA titres according to the corresponding medications was necessary. Glucocorticoids were provided to most patients with MPA. Meanwhile, oral immunosuppressive drugs were provided as maintenance therapeutic regimens after induction therapy to several patients, and even if started without induction therapy, their effect on first-year cumulative MPO-ANCA titres is less than that of induction therapeutic regimens. Therefore, we compared first-year cumulative MPO-ANCA titres according to the administration of rituximab or cyclophosphamide. No significant differences in the median first-year cumulative MPO-ANCA titres were observed between patients who received and who did not receive cyclophosphamide (256.4 IU/mL *vs*. 217.7 IU/mL, p=0.983) and rituximab (227.9 IU/mL *vs*. 278.6 IU/mL, p=0.586).

To the best of our knowledge, this is the first study to demonstrate the association between first-year cumulative MPO-ANCA titres and all-cause mortality during follow-up in patients with MPA. However, this study has several limitations. The number of patients with MPA was not sufficient to directly apply these results to clinical practice; in particular, the small proportion of deceased patients was also considered a limitation. Although the study participants were selected from a prospective observational cohort of AAV, the retrospective study design limited the collection of additional data for validating the mechanism of the association between first-year cumulative MPO-ANCA titres and all-cause mortality in patients with MPA. In addition, a close link was identified among several processes such as the aggravation of MPA, adverse events of immunosuppressive agents, of which the doses were escalated owing to MPA exacerbation, opportunistic infection related to immunosuppressive states, subsequent AAV aggravation due to recurrent infection, and progression to multi-organ failure. Therefore, as we could not clarify the causes of death, we used the term 'all-cause mortality' rather than 'death' in this study. Meanwhile, the number of patients receiving rituximab or cyclophosphamide was less than was expected considering the treatment recommendations. In real clinical settings in the Republic of Korea, this was because of not only limited coverage of rituximab by the National Health Insurance but also concerns regarding the adverse effects of cyclophosphamide. Therefore, we do not acknowledge that the results of this study can strongly assert the clinical significance of first-year cumulative MPO-ANCA titres in MPA patients from the clinical point of view. However, as a pilot study, we hope that this will serve as an opportunity to shift the perception on first-year cumulative MPO-ANCA titres in MPA patients in real clinical practice. Future prospective study involving more MPA patients and serial data on AAV-specific indices will provide more reliable and dynamic information on the clinical implications of first-year cumulative MPO-ANCA titres in patients with MPA.

In conclusion, in the present study, we demonstrated for the first time that first-year cumulative MPO-ANCA titres were associated with all-cause mortality during follow-up in patients with MPA, implying that they may have predictive potential for all-cause mortality. Therefore, if possible, we suggest serially measuring MPO-ANCA titres during the first year after diagnosis in patients with MPA and exploring proactive preventative methods to prevent all-cause mortality when first-year cumulative MPO-ANCA titres are higher than the cut-off derived based on the data of each cohort with different racial and regional characteristics.

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