Anti-neutrophil cytoplasmic antibody patterns can predict clinical relapse in ANCA-associated vasculitis: overall population and subgroups

C. Jiang, J. Liu, H. Wang, H. Liu, Z. Fu, M. Li, X. Liu

Department of Nephrology, The Affiliated Hospital of Qingdao University, Qingdao, China.

Abstract

Objective

In anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV), relapses are cause of concern as they are unpredictable and predictors of poor prognosis. We aimed to assess the characteristic and predictors of clinical relapse in AAV.

Methods

This retrospective study included 132 cases of AAV newly diagnosed from January 2016 through November 2021 in the Affiliated Hospital of Qingdao University. We reviewed clinical data of patients and analysed the risk factors for clinical relapse of overall population and subgroups by univariate and multivariate regression models and the K-M survival curve was plotted.

Results

The rate of relapse was highest in the positive conversion group than the others significantly ($p<0.001$). In overall population, ANCA patterns ($p<0.001$; persistent positive pattern: HR=3.352, 95%CI 1.463~7.678, $p=0.004$; positive conversion pattern: HR=4.760, 95%CI 2.094~10.820, $p<0.001$) and infections (HR =4.684, 95%CI 1.980~11.079, $p<0.001$) were significantly associated with clinical relapse. In myeloperoxidase (MPO)-AAV patients, ANCA patterns ($p=0.001$; persistent positive pattern: HR=4.495, 95%CI 1.508~13.396, $p=0.007$; positive conversion pattern: HR=7.404, 95%CI 2.652~20.671, $p<0.001$) and infections (HR=3.594, 95%CI 1.511~8.547, $p=0.004$) were significantly associated with clinical relapse. In renal involvement patients, ANCA patterns ($p=0.004$; persistent positive pattern: HR=3.618, 95%CI 1.364~9.592, $p=0.01$; positive conversion pattern: HR=4.492, 95%CI 1.778~11.352, $p<0.001$) and infections (HR=7.791, 95%CI 2.511~24.174, $p<0.001$) were significantly associated with clinical relapse, but were not in patients without renal involvement.

Conclusion

Persistently positive and re-positive ANCA and infections predict clinical relapse in AAV, especially in patients with MPO-AAV and renal involvement. Regular ANCA monitoring should be carried out in high-risk populations.

Key words

ANCA-associated vasculitis, microscopic polyangiitis, renal involvement, relapse

Chunhui Jiang, MM
Jun Liu, MM
Huifang Wang, MM
Hang Liu, MM
Zixuan Fu, MM
Min Li, MM
Xuemei Liu, MD

Please address correspondence to:
Xuemei Liu,
Department of Nephrology,
The Affiliated Hospital of
Qingdao University,
Qingdao 266000, Shandong, China.
E-mail: liuxuemei@qdu.edu.cn

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Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a heterogeneous group of disorders characterised by inflammation and destruction of small- and medium-sized blood vessels and the presence of circulating ANCA (1, 2). There are two main types of ANCA that can be detected in AAV patients. These ANCAs are defined by their own antigenic targets, namely leukocyte proteinase 3 (PR3) and myeloperoxidase (MPO) (3).

With the widespread use of glucocorticoid and immunosuppressants, AAV has transformed from a fatal disease to a chronic disease with a certain incidence and relapse rate in the past 2 decades (1). The annual incidence of AAV is about 20 per million population in Europe and North America (1). A number of cohorts and long-term follow-up of clinical trials have demonstrated relapse rates that vary between 21% and 89% at 5 years, and in order to prevent disease relapse the maintenance therapy is launched after remission (4). But the optimal duration of maintenance, which is depended on the underlying ANCA serology as well as treatment, hasn't reached a consensus (5, 6). While prolonged treatment with immunosuppressive drug may help reduce the incidence and severity of relapse, it also results in unnecessary treatment-related toxicity and an increased risk of adverse reactions. Therefore, it is crucial to identify predictors of relapse for stratify patients and individualised treatment regimens.

Until now, some risk factors, such as PR3-ANCA positivity, age>65, lower serum creatinine and cardiovascular system involvement, have been reported, but not confirmed by other studies. ANCA has been proposed as a predictor of relapse since its entry into clinical studies (7), but the prediction of relapse based on ANCA patterns remains controversial (8-13). Thai *et al*. confirmed clinical outcomes and ANCA levels coincided for only 60% of the granulomatosis with polyangiitis (GPA) patients (11). A recent study observed recurrent or persistent ANCA patterns are associated with a higher risk of clinical relapse in AAV patients (9). It should be noted that all these studies were performed in different included populations, and research results were influenced by disease heterogeneity. Besides, the current study showed that accurate phenotypic clustering based on the degree of organ involvement and patient demographics was essential for optimal management of AAV (14).

The aim of this study was to assess the clinical characteristic and frequency of clinical relapse and to evaluate independent predictors of clinical relapse, especially ANCA patterns. We also analysed predictors of relapse in the subgroup of MPO-AAV and renal involvement respectively.

Methods

Patients

This retrospective study was conducted at the Affiliated Hospital of Qingdao University between January 2016 and November 2021. Patients were eligible for inclusion if they met the following criteria: 1) age older than 18 years; 2) fulfilling the criteria proposed by the 2012 Chapel Hill Consensus Conferences Nomenclature of vasculitis; 3) had positive MPO- or PR3-ANCA positivity at least once, detected by both indirect immunofluorescence assay and antigen-specific ELISA; 4) had at least 3 consecutive ANCA tests, with an interval of about 1 month. The exclusion criteria were: 1) EGPA or secondary vasculitis; 2) comorbid kidney diseases, such as membranous nephropathy, IgA nephropathy, diabetic nephropathy, and anti-glomerular basement membrane glomerulonephritis; 3) the co-existence of another autoimmune disease, such as lupus nephritis, Sjögren's syndrome, rheumatoid arthritis; 4) immunocompromised or primary immunodeficiency, such as malignant tumour, transplantation; 5) patients who did not complete 6 months of follow-up. The study protocol was in accordance with the provisions of the Declaration of Helsinki and was approved by the Ethics Committee of Affiliated Hospital of Qingdao University (QYFY WZLL 27163).

Data collection

Baseline demographic data, clinical and laboratory findings, treatments, and follow-up data were collected from

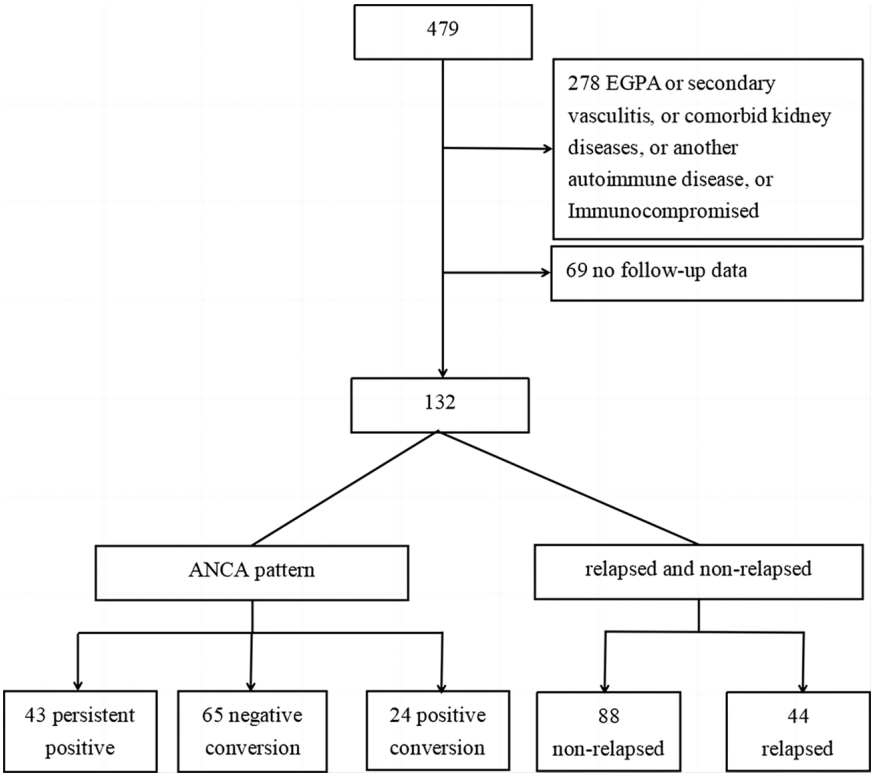

Fig. 1. Patient flow in the study.

the electronic medical record system in the hospital. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equations (CKD-EPI) formula (15, 16). We assessed the five factors score (FFS) revised (2009) form to predict the relapse and prognosis (17), including 4 risk factors (age>65 years, stabilised peak creatinine≥150 μmol/L, cardiac symptoms, gastrointestinal involvement) and 1 protective factor (ENT symptoms). The presence of each factor is one point.

Groups and definition

According to ANCA patterns during the follow-up process, the patients were divided into three groups: 1) persistent positive group: ANCA was consistently positive throughout follow-up; 2) negative conversion group: ANCA were turned into negative and remained negative; 3) positive conversion group: after completing the above definition of negative transformation, ANCA changes from negative to positive. The patients were divided into relapse group and non-relapse group according to clinical recurrence during follow-up. Relapse is defined as recurrence or new onset of clinical signs and symptoms caused by active vasculitis after clinical remission of the disease. Remission was defined as absence of clinical symptoms or absence of laboratory evidence for more than 1 month. Severe infections were defined as infections requiring more than 3 days of intravenous antimicrobial treatment and hospitalisation for at least 3 days (18-20), hereafter referred to as "infection". ESRD was defined as eGFR<15mL/min/1.73m^2 or dialysis dependence>3 months.

Statistical analysis

SPSS 25.0 software was used for statistical processing of the data. K-S test was used to verify the normality analysis of measurement materials. Descriptive statistics were expressed in mean ± standard deviation (SD) and median (interquartile range [IQR]) for continuous normal and non-normally distributed variables respectively. Categorical variables were expressed in number and frequency. T-test or the analysis of variance (ANOVA), Mann-Whitney U-test, Chi-square, or Fisher's test were used for inter-group comparison. The association between ANCA patterns and clinical relapses was analysed using Kaplan-Meier curves and Cox proportional hazards model. p-values <0.05 were considered statistically significant.

Results

Among the 479 AAV patients followed in our study, 132 met the inclusion criteria (Fig. 1). The clinical characteristics of the patients are shown in Table I. Of those, 67 were male and 65 were female. The median age at diagnosis was 66 years and median length of follow-up was 22.8 months. Among the 132 patients, 107 (81%) were positive for anti-MPO antibodies and 25 (19%) for anti-PR3 antibodies. During the follow up, 44 (33%) had at least one clinical relapse and 77 (58%) experienced infection events, of which 13/77 (17%) had two infections and 14/77 (18%) had three or more.

Comparison of patients based on ANCA patterns

Of the 132 patients, 65 patients (49%) were in the negative conversion group, 43 patients (33%) were in the persistent positive group, and 24 patients (18%) were in the positive conversion group. The differences in demographic, clinical characteristics, treatment and outcome of the three groups are shown in Table I. By the time of relapse, ANCA was re-positive in 19 patients, negative in 14 patients and persistently positive in 11 patients. The rate of relapse was highest in the positive conversion group than the others significantly (79% *vs.* 17% *vs.* 33%, p<0.001). However, relapse rates were similar in the latter two groups (p=0.059). There were no significant differences in renal involvement and ESRD at diagnosis among the three groups (renal involvement: 82% *vs.* 63% *vs.* 75%, p=0.092; ESRD: 26% *vs.* 23% *vs.* 21%, p=0.859 respectively).

Comparison of patients between relapsed and non-relapsed patients

The above clinical data were included in the analysis. Table II summarised the differences in clinical data between 44 relapsed patients and 88 non-relapsed. The median time to relapse was 23.8

Table I. Comparison of clinical characteristics according to ANCA patterns.

	Total (n=132)	Persistent positive (n=43)	Negative conversion (n=65)	Positive conversion (n=24)	*p*
Male, n (%)	67 (51)	19 (41)	36 (55)	12 (54)	0.356
Age, years (median, IQR)	66.0 (58.6, 70.0)	65.0 (57.0, 69.0)	67.0 (60.5, 72.0)	66.5 (56.0, 73.0)	0.186
Anti-MPO, n (%)	107 (81)	35 (81)	52 (80)	20 (83)	0.936
Follow-up, months (median, IQR)	22.8 (12.1, 38.1)	20.9 (11.5, 34.8)	24.3 (12.0, 40.5)	24.1 (15.7, 37.8)	0.517
Infections, n (%)	77 (58)	21 (49)	39 (60)	17 (71)	0.201
Laboratory					
WBC, 10^9 /L (median, IQR)	8.9 (7.1, 11.0)	9.3 (6.9, 10.8)	8.9 (7.1, 11.1)	8.5 (7.5, 12.4)	0.827
LY, 10^9 /L (median, IQR)	1.5 (1.0, 2.0)	1.5 (1.0, 2.3)	1.5 (1.2, 1.9)	1.3 (1.0, 2.0)	0.563
PTLs, 10^9 /L (mean, SD)	272. ± 102.6	263.5 ± 105.6	258.2 ± 94.3	327.1 ± 105.1	0.014
Hb, g/L (mean, SD)	102.6 ± 23.6	106.9 ± 24.3	99.4 ±2 4.3	103.7 ± 19.9	0.261
CRP, mg/L (median, IQR)	20.3 (7.8, 80.5)	14.7 (5.3, 35.2)	35.3 (10.5, 85.6)	55.9 (7.2, 94.9)	0.073
Creatinine, μmol/L (median, IQR)	140.0 (81.3, 298.5)	118 (74, 290)	158 (88, 319)	113 (78, 193)	0.171
Albumin, g/L (mean, SD)	31.3 ± 5.7	32.0 ± 5.1	31.4 ± 6.2	29.7 ± 4.8	0.268
eGFR (median, IQR)	39.6 (16.9, 78.3)	55.6 (18.0, 90.3)	35.4 (13.4, 69.8)	52.4 (26.9, 83.7)	0.215
FFS>2, n (%)	41 (31)	9 (22)	26 (39)	6 (25)	0.144
Organ involvement					
Renal, n (%)	98 (74)	27 (63)	53 (82)	18 (75)	0.092
Lung, n (%)	93 (71)	31 (72)	46 (71)	16 (67)	0.894
ENT, n (%)	28 (21)	7 (16)	13 (20)	8 (33)	0.247
GI, n (%)	6 (5)	1 (2)	4 (6)	1 (4)	0.643
Cardiovascular, n (%)	23 (17)	4 (9)	13 (20)	6 (25)	0.199
Treatment					
GC, n (%)	41 (31)	16 (37)	21 (32)	4 (17)	0.209
GC+CYC, n (%)	67 (51)	21 (49)	33 (51)	13 (54)	0.916
GC+MMF, n (%)	16 (12)	4 (9)	6 (9)	6 (25)	0.102
RTX, n (%)	10 (8)	4 (9)	5 (8)	1 (4)	0.747
GC pulse, n (%)	41 (31)	12 (28)	23 (35)	6 (25)	0.555
Plasmapheresis, n (%)	22 (17)	3 (7)	14 (22)	5 (21)	0.115
Outcome					
Clinical relapse, n (%)	44 (33)	14 (33)	11 (17)	19 (79)	<0.001
ESRD, n (%)	45 (34)	14 (34)	25 (37)	6 (25)	0.551
-ESRD at baseline, n (%)	32 (24)	10 (23)	17 (26)	5 (21)	0.859
-ESRD during follow-up, n (%)	13 (10)	4 (9)	8 (12)	1 (4)	0.514

ANCA: anti-neutrophil cytoplasmic antibody; MPO: myeloperoxidase; WBC: white blood count; LY: lymphocyte count; PTLs: platelets; Hb: haemoglobin; CRP: C reactive protein; eGFR: estimated glomerular filtration rate; FFS: five factors score; ENT: ear, nose and throat; GI: gastrointestinal; GC: glucocorticoids; CYC: cyclophosphamide; MMF: mycophenolate mofetil; RTX: rituximab; ESRD: end-stage renal disease.

months. Overall, the relapse group had more males, more frequent cardiovascular involvement, lower lymphocyte counts and higher CRP ($p<0.05$). In addition, there were also significant differences in ANCA patterns and incidence of infection events between the two groups ($p<0.001$). There were fewer patients with negative conversion and more with positive conversion in the relapse group than another, but there was no statistically significant difference between two groups in persistent positive ANCA pattern which were 12 of the 44 patients who relapsed and 29 of the 88 patients who did not relapse (27% *vs*. 33%, $p=0.506$). Infection events occurred in 36/44 patients in the relapse group, significantly higher than those in the non-relapse group (82% *vs*. 46%, $p<0.001$), and the frequency of infections was also higher than that in the non-relapse group ($p<0.001$). Among them, 28/44 (64%) patients had at least one infection within 1 month before relapse, and 81% were pulmonary infection. There was no significant difference in the treatment regimens between the two groups.

Predictors of relapse in AAV

All parameters entered into the univariate and multivariate analysis are shown in Table III. In the univariate analysis, in addition to ANCA patterns, male, infection events, cardiovascular involvement, FFS>2, low lymphocyte count and high C-reactive protein predicted an increased relapse rate ($p<0.05$). ANCA types were not associated with an increased risk of clinical relapse. In the cox multivariate analysis, ANCA patterns ($p<0.001$; persistent positive pattern: *HR*=3.352, 95%*CI* 1.463~7.678, $p=0.004$; positive conversion pattern: *HR*=4.760, 95%*CI* 2.094~10.820, $p<0.001$) and infections (*HR* =4.684, 95%*CI* 1.980~11.079, $p<0.001$) were independently associated with a higher risk of clinical relapse. As shown in Figure 2a, the Kaplan-Meier curve of relapse demonstrated the relationship between ANCA patterns and relapse ($p<0.001$). And positive conversion group was more likely to relapse than persistent positive group, which in turn was more likely to relapse than negative conversion group (Fig. 2a).

Predictors of relapse in the MPO-AAV subgroup

We have compared clinical characteristics according to ANCA specificity. The

Table II. Comparison of clinical characteristics between relapsed and non-relapsed patients.

	Relapsed (n=44)	Non-relapsed (n=88)	*p*
Male, n (%)	28 (64)	39 (44)	0.036
Age, years (median, IQR)	66.5 (60.7, 70.8)	66.0 (57.7, 69.8)	0.457
Anti-MPO, n (%)	32 (73)	75 (85)	0.084
Follow-up, months (median, IQR)	23.8 (11.9, 33.7)	22.3 (12.4, 41.3)	0.47
ANCA patterns			
(negative conversion/persistent positive/positive conversion), n	13/12/19	54/29/5	<0.001
Infection events			
Infections, n (%)	36 (82)	40 (46)	<0.001
Number of infections per person (0/1/2/≥3), n	7/25/7/5	48/25/6/9	<0.001
LY, 10^9/L (median, IQR)	1.3 (0.9, 1.8)	1.5 (1.2, 2.2)	0.025
CRP, mg/L (median, IQR)	40.6 (16.0, 94.3)	18.5 (4.9, 67.5)	0.013
Creatinine, μmol/L (median, IQR)	1450 (87, 252)	137 (77, 315)	0.662
eGFR (median, IQR)	35.9 (18.6, 81.5)	40.9 (13.9, 78.3)	0.954
FFS>2, n (%)	17 (39)	24 (27)	0.184
Cardiovascular involvement, n (%)	12 (27)	11 (13)	0.035

NCA: anti-neutrophil cytoplasmic antibody; MPO: myeloperoxidase; WBC: white blood count; LY: lymphocyte count; PTLs: platelets; Hb: haemoglobin; CRP: C-reactive protein; eGFR: estimated glomerular filtration rate; FFS: five factors score; ENT: ear, nose and throat.

Table III. Cox-regression analysis for clinical relapse in AAV patients(n=132).

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95%*CI*)	*p*	Hazard ratio (95%*CI*)	*p*
Male	1.871 (1.01, 3.468)	0.047	1.823(0.960, 3.459)	0.066
Age (year)	1.023 (0.996, 1.051)	0.098		
Anti-PR3	1.818 (0.935, 3.535)	0.078		
Infections	4.899 (2.169, 11.069)	<0.001	4.684 (1.980, 11.079)	<0.001
ANCA patterns		<0.001		<0.001
Negative conversion	1		1	
Persistent positive	2.319 (1.05, 5.12)	0.037	3.352 (1.463, 7.678)	0.004
Positive conversion	5.011 (2.372, 10.585)	<0.001	4.760 (2.094, 10.820)	<0.001
Laboratory				
LY	0.505 (0.305, 0.836)	0.008	0.712 (0.405, 1.251)	0.238
Hb	0.992 (0.979, 1.004)	0.181		
CRP	1.006 (1.001, 1.011)	0.016	0.997 (0.89, 1.005)	0.495
Creatinine	1.001 (0.999, 1.002)	0.338		
Albumin	0.958 (0.912, 1.007)	0.091		
eGFR	0.996 (0.987, 1.004)	0.335		
FFS>2	2.057 (1.103, 3.837)	0.023	1.779 (0.641, 4.939)	0.269
Organ involvement				
Renal	0.944 (0.477, 1.871)	0.869		
Lung	1.485 (0.761, 2.899)	0.247		
ENT	1.605 (0.839, 3.07)	0.153		
GI	2.805 (0.842, 9.344)	0.093		
Cardiovascular	2.445 (1.24, 4.822)	0.01	1.301 (0.491, 3.448)	0.596
Treatment				
GC	1.468 (0.777, 2.773)	0.237		
GC+CYC	0.725 (0.4, 1.313)	0.288		
GC+MMF	0.89 (0.35, 2.26)	0.806		
RTX	2.341 (0.534, 10.273)	0.26		
GC pulse	1.231 (0.659, 2.299)	0.515		
Plasmapheresis	0.908 (0.355, 2.322)	0.84		

ANCA: anti-neutrophil cytoplasmic antibody; PR3: proteinase 3; WBC: white blood count; LY: lymphocyte count; PTLs: platelets; Hb: haemoglobin; CRP: C reactive protein; eGFR: estimated glomerular filtration rate; FFS: five factors score; ENT: ear, nose and throat; GI: gastrointestinal; GC: glucocorticoids; CYC: cyclophosphamide; MMF: mycophenolate mofetil; RTX: rituximab.

results showed that PR3-AAV was male dominant (p=0.018), and no significant statistical differences in the other factors. Due to the small proportion of PR3-AAV patients in this study, cox-regression analysis was not performed for this subgroup. All above parameters were included in Cox regression analysis in MPO-AAV patients. In the univariate analysis, ANCA patterns, infection events and high CRP were associated with a higher risk of relapse in MPO-AAV (p<0.05), while after adjusting for potential confounders, ANCA patterns (p=0.001; persistent positive pattern: *HR*=4.495, 95%*CI* 1.508~13.396, p=0.007; positive conversion pattern: *HR*=7.404, 95%*CI* 2.652~20.671, p<0.001) and infections (*HR*=3.594, 95%*CI* 1.511~8.547, p=0.004) predicted clinical relapse independently. Characteristics of severe infection in MPO-AAV patients are shown in Table IV and Table V. ANCA patterns, especially positive conversion was more strongly related to relapse in patients with MPO-ANCA positive than entire cohort. As shown in Fig.2b, there was a significant difference in relapse among ANCA patterns in patients with MPO-ANCA positive (p<0.001).

Predictors of relapse in renal involvement group

In addition, we divided the cohort into 98 persons with renal involvement and 34 persons without renal involvement. Multivariate analysis showed that ANCA patterns (p=0.004; persistent positive pattern: *HR*=3.618, 95%*CI* 1.364~9.592, p=0.01; positive conversion pattern: *HR*=4.492, 95%*CI* 1.778~11.352, p<0.001) and infections (*HR*=7.791, 95%*CI* 2.511~24.174, p<0.001) predicted clinical relapse independently, but did not demonstrate predictive power in patients without renal involvement. As shown in Figure 2c, relapse rate was significantly different among patients with renal involvement among the three ANCA patterns (p<0.001).

Discussion

The results in our study showed infection, persistently positive and re-positive ANCA were associated with an in-

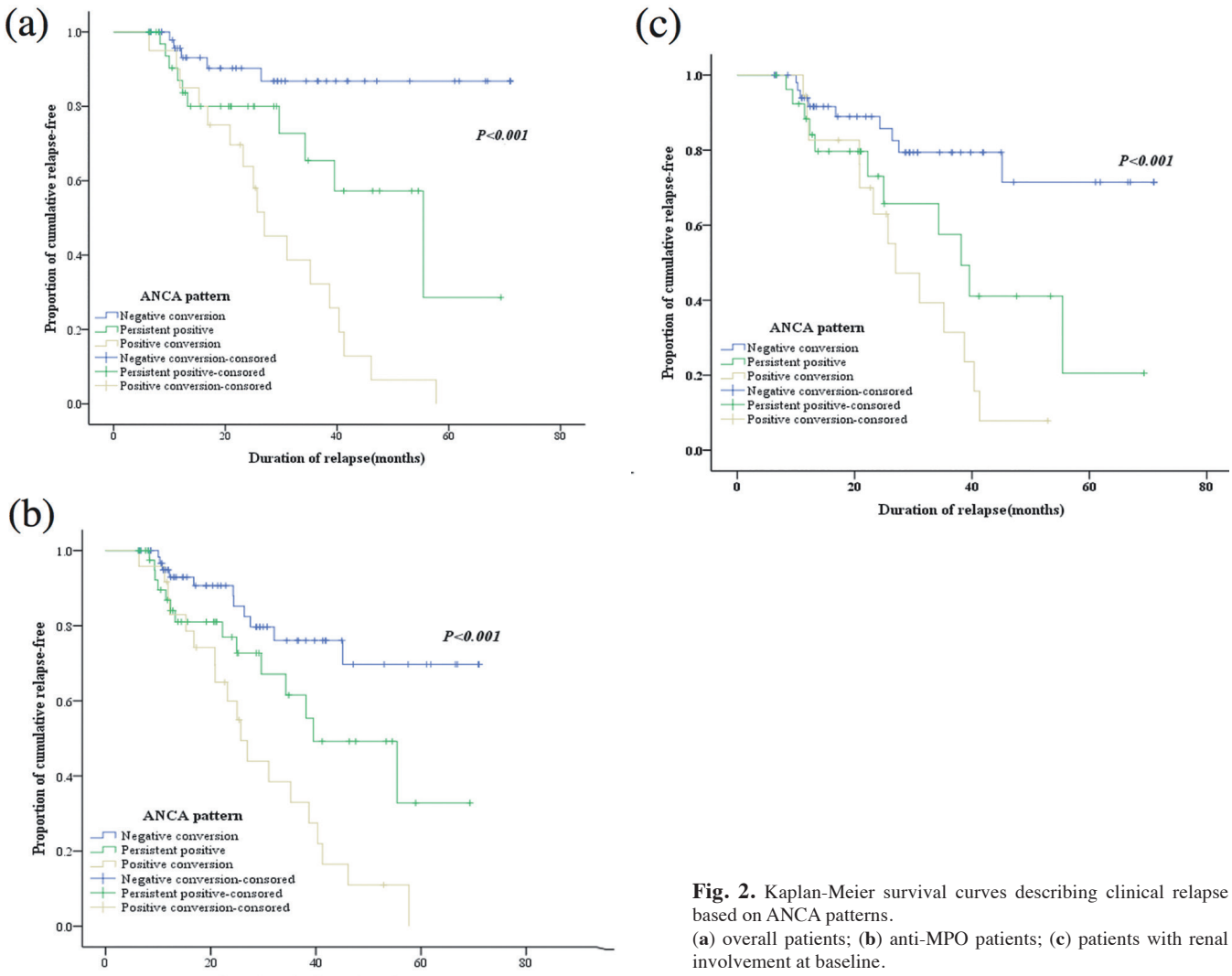

Fig. 2. Kaplan-Meier survival curves describing clinical relapse based on ANCA patterns.
(**a**) overall patients; (**b**) anti-MPO patients; (**c**) patients with renal involvement at baseline.

creased risk of clinical relapse whether in the overall or MPO-AAV and renal involvement subgroups, and positive conversion appeared to be more tend to predict subsequent relapse than persistently positive ANCA.

After ruling out statistical difference in follow-up time between relapse and non-relapse patients, some patients with persistently positive ANCA have not experienced a relapse in the cohort. This suggests that the presence of ANCA alone was not sufficient to cause relapse (21). Studies have showed that not all ANCA is pathogenicity, which may be determined by galactosylation and sialylation levels of IgG1 and epitope specificity (22-24). In addition, as demonstrated in the MPO-ANCA mouse model, the pro-inflammatory synergy of the infectious agent is also important before the patient truly develops a relapse (25, 26). The results of this study showed that infection was an independent risk factor for clinical relapse, and there were 28/44 (64%) patients with at least one infection within 1 month prior to clinical relapse in relapse group. Pro-inflammatory cytokines such as TNF and IL-1β are generated during infection and activate neutrophils to express target antigens (*i.e.* MPO and PR3) on their cell surface. ANCA binds to these antigens and activates an inflammatory response that destroys vascular endothelial cells (27). Therefore, when infection events occur during remission, antibiotics should be added in time and monitoring of disease recurrence should be strengthened. However, whether to adjust glucocorticoids or immunosuppressants should be combined with other clinical characteristics and laboratory tests to evaluate disease activity and relapse tendency.

Accumulating evidence suggests that AAV varies significantly according to ANCA types: PR3-AAV and MPO-AAV(6, 28). PR3-ANCA positivity has previously been reported to be associated with a higher rate of disease relapse (3, 29). However, our study was consistent with Oristrell *et al.* and Kemna *et al.*, failing to establish an association between ANCA types and relapse (9, 10), and perhaps is related to the small number of PR3-AAV participants. To minimise population heterogeneity (30), we analysed the risk factors for relapse in MPO-AAV patients separately, which showed that ANCA patterns were independent predictors of MPO-AAV relapse. Studies in the last decade have found that anti-MPO antibody levels are closely correlated with BVAS and disease sever-

Table IV. Characteristics and categories of severe infection in MPO-AAV patients.

Infections	MPO-AAV
Infection cases, n (%)	59(55)
Number of infections per person (0/1/2/≥3), n	48/36/10/13
Types of infection (total n=97)	
Pneumonia	79
Urinary tract infection	14
Bacteremia	3
Oral infections	1
Bacterial pathogens	
Pseudomonas aeruginosa	10
Escherichia coli	9
Staphylococcus aureus	6
Klebsiella sp.	4
Enterobacter cloacae	6
Acinetobacter baumannii, Stenotrophomonas maltophilia, Enterococci	3 each
Citrobacter, Raoult	2 each
Streptococcus pneumoniae, Serratia marcescens, Enterobacteraerogenes, Bacillus cereus, Haemophilus influenzae, Pseudomonas, Staphylococcus capitis, Nocardia, Clostridium difficile	1 each
Fungal pathogens	
Pseudomonas	15
Candica albicans	13
Pneumocystis jiroveci	1

ANCA: anti-neutrophil cytoplasmic antibody; MPO: myeloperoxidase.

Table V. Characteristics and categories of severe infection in MPO-AAV patients according to immunosuppressive treatment and steroid exposure.

Infections	GC	GC+CTX	GC+MMF	RTX
Infection cases, n (%)	20(61)	25(47)	8(57)	6(75)
Number of infections per person (0/1/2/≥3), n.	13/12/2/6	28/14/4/7	6/5/3/0	2/5/1/0
Types of infection				
Pneumonia	32	37	6	4
Urinary tract infection	9	5	2	3
Oral infections	1	1	3	1
Bacteremia	0	0	0	1

ANCA: anti-neutrophil cytoplasmic antibody; MPO: myeloperoxidase; GC: glucocorticoids; CYC: cyclophosphamide; MMF: mycophenolate mofetil; RTX: rituximab.

ity index, but the relationship between anti-PR3 antibody and disease activity has not been found (11, 31-33). Similarly, *in vitro* and i*n vivo* experiments confirmed the pathogenicity of MPO-ANCA, but the convincing evidence of PR3-ANCA has not been reported to date (25, 34-36). However, some authors reported that serial measurements of PR3-ANCA may have clinical utility in relapse prediction with limitations (12, 13, 37, 38). All these suggest that the ability of ANCA patterns to predict disease relapse should be managed hierarchically according to ANCA type. MPO-ANCA patterns, especially the re-positive MPO-ANCA is more likely to indicate that disease activity leads to clinical relapse, and in which regular testing of ANCA is of considerable benefit.

We have also found that persistent positive and re-positive ANCA were useful biomarkers for relapse in patients with renal involvement at baseline, but have no predictive value for relapse in patients without renal involvement at baseline. We agree monitoring ANCA helps predict clinical relapse in patients with renal involvement (10, 39), but continuous ANCA testing is not necessary in patients without renal involvement, especially in the absence of other manifestations of severe vasculitis. Unfortunately, we were unable to confirm the effect of the ANCA patterns on renal survival.

There are some limitations in our study. First, this study was a single-centre retrospective study with a relatively short follow-up time and a small sample size, which may lead to selection bias. Second, ANCA titer could not be evaluated due to the limitation of detection conditions. This was a retrospective study and samples could not be re-tested. Third, the number of PR3-AAV patients in our series is too small to directly compare the risk factors of relapse between PR3-AAV and MPO-AAV patients. The last, due to the high expenses and recommended level of rituximab treatment for AAV patients in China, only 10 patients received RTX in our cohort.

Conclusion

In our cohort, ANCA patterns, especially re-positive ANCA, and infection are independent predictors of clinical relapse in AAV patients, which are more significant in MPO-AAV and renal involvement patients. Our study suggests that regular ANCA monitoring should be carried out in high-risk populations.

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