Anti-neutrophil cytoplasmic antibody specificity determines a different clinical subset in granulomatosis with polyangiitis

S. Monti[1,2], M. Felicetti[3,4], P. Delvino[1,2], R. Padoan[3], A. Berti[4], G. Paolazzi[4], G. Brunori[5], F. Schiavon[3], R. Caporali[6], C. Montecucco[1]

[1]Department of Rheumatology, IRCCS Policlinico S. Matteo Fondazione, University of Pavia, Pavia, Italy; [2]PhD in Experimental Medicine, University of Pavia, Italy; [3]Operative Unit of Rheumatology, Department of Medicine DIMED, University of Padova, Italy; [4]Department of Rheumatology, and [5]Department of Nephrology, Santa Chiara Hospital, Trento, Italy; [6]Rheumatology Department, Istituto Pini, Milan, Italy.

Sara Monti, MD
Mara Felicetti, MD
Paolo Delvino, MD
Roberto Padoan, MD
Alvise Berti, MD
Giuseppe Paolazzi, MD
Giuliano Brunori, MD
Franco Schiavon, MD
Roberto Caporali, MD, PhD
Carlomaurizio Montecucco, MD

Please address correspondence to:
Sara Monti,
Dipartimento di Reumatologia,
Università di Pavia,
Policlinico San Matteo
IRCCS Foundazione,
Piazzale Golgi 2,
27100 Pavia, Italy.
E-mail: sara.saramonti@gmail.com

Received on February 3, 2021; accepted in revised form on April 21, 2021.

Clin Exp Rheumatol 2021; 39 (Suppl. 129): S107-S113.

Key words: Anti-neutrophil cytoplasmic antibody, ANCA-associated vasculitis, granulomatosis with polyangiitis, microscopic polyangiitis, clinical presentation, outcome

Competing interests: R. Caporali has received honoraria and speakers fee from AbbVie, BMS, Celltrion, Fresenius-Kabi, Galapagos, Lilly, UCB, Sanofi, Sandoz, Pfizer and Novartis. The other co-authors have declared no competing interests.

ABSTRACT

Objective. *It has been suggested that anti-neutrophil cytoplasmic antibody (ANCA) specificity, rather than clinical diagnosis influences the phenotype and course of ANCA-associated vasculitis (AAV). However, preliminary evidence suggests that further combined levels of categorisation might be of clinical relevance. The aim of this study was to investigate differences in clinical presentation at disease onset and outcomes based on clinical diagnosis and ANCA specificity.*

Methods. *Newly diagnosed patients with GPA or MPA assessed in three referral centres between 2000 and 2016 were included. Patients were grouped as MPO-ANCA-positive granulomatosis with polyangiitis (MPO-GPA), PR3-ANCA-positive-GPA (PR3-GPA), and MPO-ANCA-positive microscopic polyangiitis (MPO-MPA).*

Results. *Of the 143 AAV patients included (female 52%), 87 were categorised as PR3-GPA, 23 as MPO-GPA, and 33 as MPO-MPA. Patients with MPO-GPA were significantly younger than MPA patients (age 49±15 versus 63±10; p<0.001). MPO-GPA had significantly more frequent subglottic stenosis compared to PR3-GPA. Ear, nose, throat involvement was significantly more frequent in both GPA groups compared to MPA. Type of pulmonary involvement differed between both GPA groups and MPA with diffuse pulmonary haemorrhage being significantly more frequent in the latter (7% in PR3-GPA, 0% in MPO-GPA, 27% in MPO-MPA; p<0.001). Renal involvement was more frequent in MPO-MPA compared to both MPO-GPA and PR3-GPA (impaired renal function in 84%, 39%, and 36%, respectively; p<0.001). PR3-GPA relapsed significantly more than the other two groups. After adjusting for age, MPO-GPA was a significant risk factor for mortality [HR 4.44 (95%CI 1.46-13.52), p=0.009].*

Conclusion. *ANCA specificity identifies specific subsets of disease characterised by different clinical presentation and outcome within the clinical diagnosis of GPA.*

Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) are a spectrum of diseases characterised by necrotising vasculitis predominately affecting small vessels, often associated with antibodies directed against myeloperoxidase (MPO)-ANCA or proteinase 3 (PR3)-ANCA. AAV comprise three main conditions: granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA) (1). Traditionally, AAV, and particularly GPA and MPA, have been regarded as similar pathological entities sharing overlapping clinical and histopathological features. This assumption, together with the rarity of AAV and the initial lack of specific classification criteria has led to the inclusion of GPA and MPA into the same randomised controlled trials informing common treatment strategies (2, 3). Nevertheless, distinctive clinical features in terms of frequency of specific organ involvement and type of ANCA positivity between different forms of AAV have long been known (4). Increasing interest has recently grown towards the classification of AAV based on ANCA specificity rather than clinical diagnosis (5). Such an approach, supported by the findings of distinct genetic backgrounds coming from genome-wide association analysis (6) has found preliminary confirmation in terms of relapse rates, outcome and response to treatment (7, 8). A deeper insight into the role and impact of

ANCA specificity has prompted further investigations on the existence of clinically distinct entities based on both the diagnostic and serological characterisation of AAV. The analysis of the distinctive features between classical PR3-positive GPA (PR3-GPA), the rarer MPO-positive GPA (MPO-GPA), and MPO-positive MPA (MPO-MPA) is currently based on limited case series and retrospective studies and it is still not fully acknowledged. We therefore investigated differences in clinical presentation at disease onset, outcome, relapse rates and mortality between PR3-GPA, MPO-GPA and MPO-MPA in a multicentric Italian cohort of patients with AAV.

Methods

A collaborative initiative was started on 1st October 2016 among three third level Rheumatology Centres in Northern Italy specialised in the management of systemic vasculitides. Charts of consecutive patients with a new diagnosis of AAV followed at the Rheumatology departments of the University of Pavia, University of Padova and Trento Hospital between January 2000 and May 2016 were retrospectively reviewed and data collected in a common electronic database. All MPA and GPA clinical diagnoses were revised by physicians expert on vasculitis, according to the 2007 European Medical Agency (EMA) algorithm (9). Patients with a clinical diagnosis of GPA or MPA, and available data on ANCA specificity (with PR3 or MPO specificity confirmed by ELISA test) were included in the analysis to generate three groups: PR3-GPA, MPO-GPA and MPO-MPA. Patients were included from diagnosis and followed prospectively at fixed timepoints (at least every 6 months in the first year and yearly thereafter). Details on demographics, comorbidities, disease onset manifestations, treatment, relapses, outcome and mortality were collected. End stage renal disease (ESRD) was defined as a sustained eGFR <15 ml/min/1.73m^2, or the need of chronic kidney replacement therapy (dialysis or transplantation). Major relapses were defined when at least one major item of BVAS v. 3 was detected due to disease activity attributable to active inflammation requiring an increase in the dosage of glucocorticoids (GC) and a modification of the immunosuppressive treatment (10). Patients were treated according to European League Against Rheumatism (EULAR) recommendations for the management of primary small and medium vessel vasculitis (11), from the time they first became available, and subsequent updates (3). Generally, the standardised and shared treatment strategy included an induction phase with high dose GC and cyclophosphamide (Cyc) or rituximab (RTX) for generalised or organ/life-threatening manifestations (according to standardised definitions). Plasma-exchange was added to selected patients with diffuse alveolar haemorrhage or severe renal disease. Methotrexate (MTX), azathioprine (AZA), mycophenolate or RTX were prescribed as maintenance treatment or for induction of limited/non-organ or life-threatening disease. Disease activity was assessed with the Birmingham Vasculitis Activity Score (BVAS) v. 3 (12, 13) and damage with the Vasculitis Damage Index (VDI) (14).

Statistical analysis

Descriptive statistics were used to summarise data using mean and standard deviation (SD) or median and 25th-75th percentiles if continuous and with counts and percent if categorical.

For comparisons between groups, we performed ANOVA or Kruskal-Wallis for continuous variables and chi square test for categorical variables. In presence of significant differences, we applied a *post hoc* analysis with independent t-test or Mann-Whitney test for continuous variables and chi square test for categorical ones.

10-year mortality rate and 10-year relapse rate between the 3 groups was compared with Kaplan-Meier curve and then age-adjusted with Cox regression. The results are reported as hazard ratios (HRs) and 95% confidence interval (95%CI). *p*-values less than 0.05 were considered to be significant.

Results

Overall, 143 patients (female 52%) were included in the analysis, of these, 87 (61%) patients were categorised as PR3-GPA, 23 (16%) as MPO-GPA, and 33 (23%) as MPO-MPA. The general characteristics of the population are shown in Table I.

Age at diagnosis ≥65 years was significantly higher in the MPO-MPA group (59% of patients) compared to MPO-GPA (35%) and PR3-GPA (16%), $p<0.001$.

Clinical manifestations according to type of diagnosis and ANCA specificity

The clinical presentation was significantly different according to the type of diagnosis and ANCA specificity. ENT involvement was significantly more frequent in both PR3-GPA (67% of patients) and MPO-GPA (70%) patients compared to MPA (15%).

Pulmonary involvement was mainly represented by nodules in PR3-GPA, and alveolar haemorrhage and respiratory failure in patients MPA. Renal involvement defined as signs of active urinary sediment, 24-hours proteinuria, and impaired renal function was significantly more frequent in MPA compared to the other two groups. Renal function was reduced (GFR <60 ml/min) at diagnosis in 84% of patients with MPA compared to 39% of patients with MPO-GPA and 36% of PR3-GPA, $p<0.001$. There were no other significant differences among the three groups regarding type of organ-systems involvement. Moreover, there were no differences in the levels of disease activity measured with BVAS at baseline amongst the three groups of patients (19±8.7 in PR3-GPA *vs*. 16±7.8 in MPO-GPA *vs*. 19±5.8 in MPO-MPA, $p=0.39$).

The type and prevalence of the different organ-system manifestations are shown in Table I and Figure 1.

Treatment and outcomes

The remission induction treatment was not different in the three groups. All patients received glucocorticoids (GC). Cyc was prescribed to 67% of patients with PR3-GPA, 47% of MPO-GPA and 65% of patients with MPA. The remission maintenance treatment consisted of low-dose GC in all patients. RTX was significantly more prescribed to patients with PR3-GPA. MTX was more

Table I. Demographics and clinical characteristics at onset of disease in the three groups of patients: PR3-GPA, MPO-GPA, MPO-MPA.

	PR3-GPA (A) (n=87)	MPO-GPA (B) (n=23)	MPO-MPA (C) (n=33)	*p*
Female (n; %)	40 (46%)	13 (57%)	22 (67%)	0.12
Age at diagnosis (mean ± SD)	50 ± 15	55 ± 19	63 ± 11	***p*<0.001**
				A *vs*. C *p*<0.001
Diagnostic delay, months (median, Q1-Q3)	4 (1-6)	4 (1-15)	2 (1-10)	0.82
Hospitalisation at disease onset	68 (80%)	17 (74%)	28 (85%)	0.66
Systemic symptoms	57 (66%)	16 (70%)	27 (82%)	0.25
Fever	41 (48%)	10 (43%)	20 (63%)	0.29
Weight loss	18 (21%)	4 (17%)	10 (32%)	0.37
Musculoskeletal	40 (47%)	8 (35%)	13 (31%)	0.55
ENT	58 (67%)	16 (70%)	5 (15%)	**<0.001**
				A *vs*. C *p*<0.001
				B *vs*. C *p*<0.001
Nasal crusting/bloody discharge/ulcers	36 (42%)	7 (30%)	3* (10%)	**0.007**
				A *vs*. C *p*=0.005
Chronic sinusitis	35 (41%)	9 (39%)	2 (7%)	**0.002**
				A *vs*. C *p*=0.001
				B *vs*. C *p*=0.004
Otitis	18 (21%)	6 (26%)	1 (3%)	**0.05**
				A *vs*. C *p*=0.02
				B *vs*. C *p*=0.01
Sensorineural hearing loss	7 (8%)	0	1 (3%)	0.26
Subglottic involvement	0	2 (9%)	0	**0.006**
Lung	61 (72%)	15 (65%)	22 (67%)	0.76
Infiltrates	33 (39%)	12 (52%)	10 (31%)	0.29
Nodules	29 (34%)	3 (13%)	0	**0.001**
				A *vs*. C *p*=0.003
Diffuse alveolar haemorrhage	6 (7%)	0	9 (27%)	**0.001**
				A *vs*. C *p*=0.006
Respiratory failure	2 (2%)	2 (9%)	7 (21%)	**0.003**
				A *vs*. C *p*=0.004
Other pulmonary	16 (19%)	2 (9%)	11 (34%)	0.06
Skin	27 (31%)	5 (22%)	3 (9%)	0.05
Purpura	19 (22%)	2 (9%)	2 (7%)	0.07
Other	12 (14%)	3 (13%)	1 (3%)	0.26
Eyes	18 (22%)	2 (9%)	2 (6%)	0.07
Cardiovascular	3 (4%)	2 (9%)	1 (3%)	0.51
Gastrointestinal	4 (5%)	1 (4%)	0	0.47
Renal	54 (63%)	13 (56%)	32 (97%)	**<0.001**
				A *vs*. C 0.001
				B *vs*. C <0.001
Active urinary sediment	52 (61%)	13 (56%)	27 (87%)	**0.02**
				A *vs*. C *p*=0.03
				B *vs*. C *p*=0.04
Impaired renal function (GFR <60 ml/min)	31 (36%)	9 (39%)	26 (84%)	**<0.001**
				A *vs*. C *p*<0.001
				B *vs*. C *p*=0.003
Baseline eGFR (ml/min)	72.29 ± 38.19	57.27 ± 39.99	33.05 ± 22.39	**P<0.0001**
				A *vs*. C *p*<0.0001
				B *vs*. C *p*=0.005
Proteinuria > 0.5 g/24 hours	34 (49%)	7 (41%)	16 (64%)	0.29
Nervous system	28 (33%)	7 (30%)	11 (36%)	0.92
Peripheral nervous system	26 (31%)	7 (30%)	11 (38%)	0.83
CNS	0	0	0	na
BVAS (baseline)	19 ± 8.7	16 ± 7.8	19 ± 5.8	0.39

GPA-PR3: granulomatosis with polyangiitis-anti-proteinase 3 positive; GPA-MPO: granulomatosis with polyangiitis-anti-myeloperoxidase positive; MPA: microscopic polyangiitis-anti-myeloperoxydase-positive; SD: standard deviation; ENT: ear-nose-throat; eGFR: estimated glomerular filtration rate; CNS: central nervous system; BVAS: Birmingham Vasculitis Activity Score.
*mild episodes of epistaxis.

frequently prescribed to patients with GPA, regardless of the ANCA status, while AZA was prescribed more often as maintenance treatment in patients with MPA (Table II).

The mean follow-up was 73.25±67.43 months. During follow-up BVAS at 2 and 5 years was significantly higher in the MPO-GPA and MPA groups compared to PR3-GPA.

There were no differences between the three groups in terms of damage accrual, calculated with the VDI, up to 5 years from diagnosis, however, the rate of end-stage renal disease (ESRD) was significantly higher in the MPO-MPA group (21%) compared to PR3-GPA (7%) and MPO-GPA (13%), p=0.04 (Table III). Relapses were significantly more frequent in the PR3-GPA group (Supplementary Fig. S1).

The majority of patients experienced relapses of signs/symptoms that had already been present at diagnosis after having achieved a period of stable remission. Patients who had new manifestations of the disease during follow-up due to relapse of the disease were all PR3-GPA patients, mainly experiencing new articular (n=3) or ENT (n=7) manifestations. More severe, new manifestations during follow-up (compared to the type of organ involvement at baseline) were the occurrence of DAH or pulmonary infiltrates (n=4) and renal involvement (n=3). The eye was involved with new-onset episcleritis or optic neuritis in 4 patients.

In the cohort there were a total of 17 deaths (12%). Six (7%) patients died in the PR3-GPA group, 7 (33%) in the MPO-GPA group, and 4 (12%) in the MPA group, p=0.005.

At univariate analysis, MPO-GPA conferred a significantly higher risk of death (HR 4.77; 95%CI 1.58–14.39, p=0.06). After adjusting for age, having a diagnosis of MPO-GPA conferred a mortality risk of 4.44 (1.46–13.52), p=0.009 compared to GPA-PR3 (Fig. 2 and Table IV).

Differences within patients with a diagnosis of GPA according to ANCA specificity

Patients with a diagnosis of GPA did not display differences in terms of sex

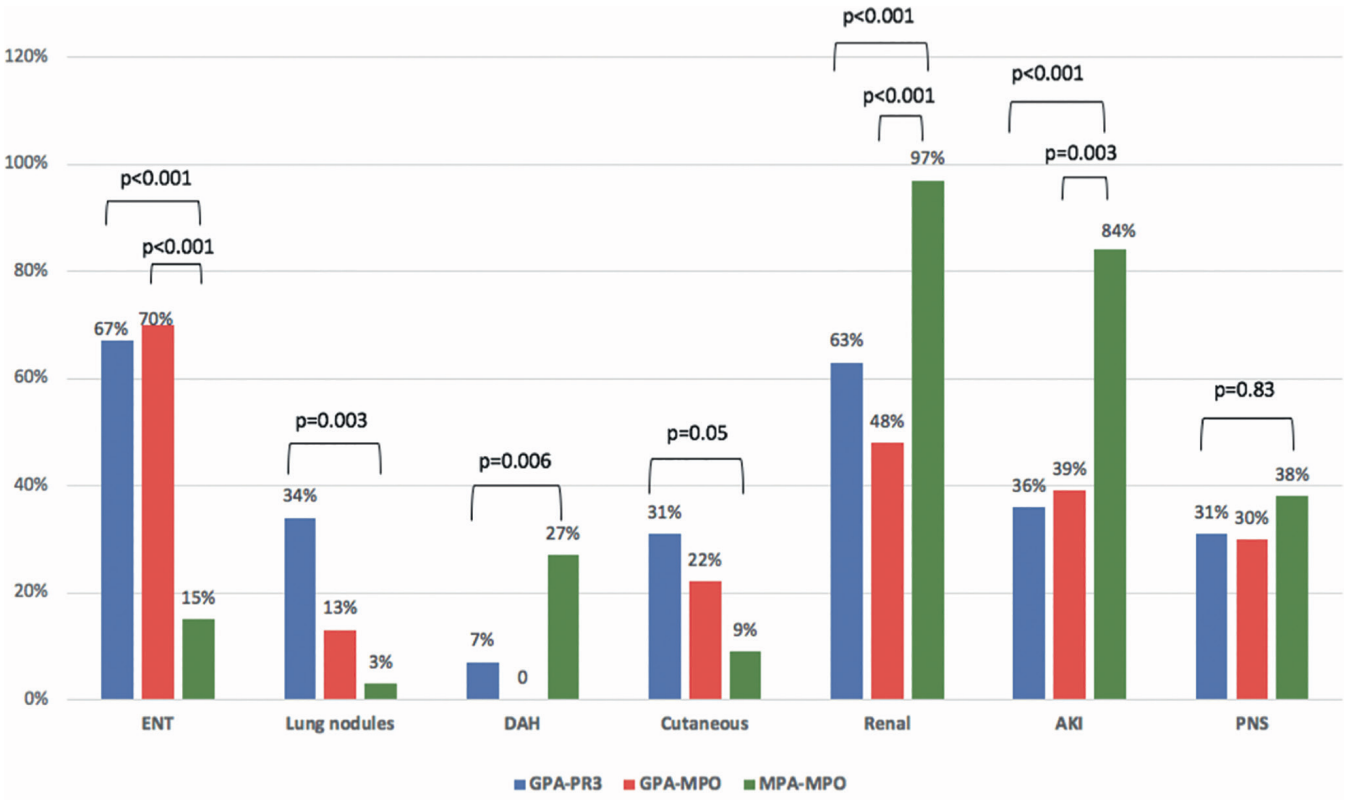

Fig. 1. Distribution of the different organ-system manifestations at baseline according to type of diagnosis and ANCA status.
ENT: ear, nose, throat; DAH: diffuse alveolar haemorrhage; AKI: acute kidney injury; PNS: peripheral nervous system.

Table II. Treatment strategies in the three groups of patients: PR3-GPA, MPO-GPA, MPO-MPA.

	PR3-GPA (A) (n=87)	MPO-GPA (B) (n=23)	MPO-MPA (C) (n=33)	*p*
Remission induction treatment				
GC	87 (100%)	23 (100%)	33 (100%)	1
GC Pulses	38 (49%)	7 (35%)	18 (62%)	0.17
Cyclophosphamide	54 (67%)	10 (47%)	86 (65%)	0.12
Rituximab	4 (5%)	3 (14%)	0	0.12
PEX	7 (9%)	1 (5%)	4 (14%)	0.51
Azathioprine	7 (9%)	3 (14%)	7 (22%)	0.12
Methotrexate	14 (17%)	5 (24%)	1 (3%)	0.12
Mycophenolate	1 (1%)	0	0	0.12
Remission maintenance treatment				
Low dose GC	87 (100%)	23 (100%)	33 (100%)	1
Rituximab	12 (15%)	1 (5%)	1 (4%)	0.04
Azathioprine	36 (46%)	10 (43%)	15 (58%)	0.2
Methotrexate	24 (27%)	5 (26%)	2 (8%)	0.04
Mycophenolate	5 (6%)	3 (16%)	5 (20%)	0.04

GPA-PR3: granulomatosis with polyangiitis-anti-proteinase 3 positive; GPA-MPO: granulomatosis with polyangiitis-anti-myeloperoxidase positive; MPA: microscopic polyangiitis-anti-myloperoxydase-positive; GC: glucocorticoids; PEX: plasma-exchange.

and age-group distribution according to ANCA specificity (mean age at diagnosis 50±15 in the PR3-GPA group *vs.* 55±19 in the MPO-GPA group). There were no significant differences for diagnostic delay between the two groups, with a median of 4 months since the onset of the first symptoms. The majority of patients (80% in the PR3-GPA group and 74% in the MPO-GPA group) were hospitalised at the time of diagnosis to ensure a thorough evaluation of the organ-systems involved and the management of acute organ- or life-threatening manifestations. Systemic symptoms were frequent at disease onset, occurring in up to 70% of patients, regardless of the ANCA specificity. Fever was present in approximately half of patients in both groups. Similarly, there were no differences in the frequency of musculoskeletal or cutaneous involvement according to ANCA specificity within the diagnosis of GPA. ENT and pulmonary manifestations were the most frequent types of organ-systems affected by the disease (Table I). Chronic sinusitis followed by nasal crusting/ulcers and otitis were the most frequent manifestations without significant differences in both groups.

The most significant difference found between patients with a diagnosis of GPA based on ANCA specificity was the occurrence of subglottic stenosis mainly observed in patients with MPO-GPA compared to PR3-MPA (p=0.004). Pulmonary infiltrates were recorded in 39% of PR3-GPA and 52% of MPO-GPA, lung nodules occurred at disease onset in 34% of patients with PR-GPA and 13% of MPO-GPA (p=0.05). DAH mainly occurred in patients with PR3-GPA (7% *vs.* 0).

During follow-up, mean BVAS values were significantly higher for the MPO-

Table III. Follow-up characteristics in the three groups of patients: PR3-GPA, MPO-GPA, MPO-MPA.

	PR3-GPA (A) (n=87)	MPO-GPA (B) (n=23)	MPO-MPA (C) (n=33)	*p*
BVAS (12 months from diagnosis)	1.00 ± 3.55	1.80 ± 3.53	1.78 ± 3.53	0.43
BVAS (24 months from diagnosis)	0 ± 5.20	2.41 ± 5.15	2.37 ± 5.18	0.03
BVAS (60 months from diagnosis)	0 ± 4.48	1.81 ± 3.83	2.00 ± 4.48	0.04
VDI at 1 year	2.18 ± 1.27	2.18 ± 1.31	2.17 ± 1.27	0.99
VDI at 2 years	2.50 ± 1.50	2.51 ± 1.55	2.48 ± 1.50	0.99
VDI at 5 years	2.95 ± 1.65	3.00 ± 1.71	2.95 ± 1.65	0.99
ESRD	6 (7%)	3 (13%)	7 (21%)	0.04
				A *vs*. C *p*=0.02

GPA-PR3: granulomatosis with polyangiitis-anti-proteinase 3 positive; GPA-MPO: granulomatosis with polyangiitis-anti-myeloperoxidase positive; MPA: microscopic polyangiitis-anti-myeloperoxydase-positive; BVAS: Birmingham Vasculitis Activity Score; VDI: Vasculitis Disease Index; ESRD: end-stage renal disease.

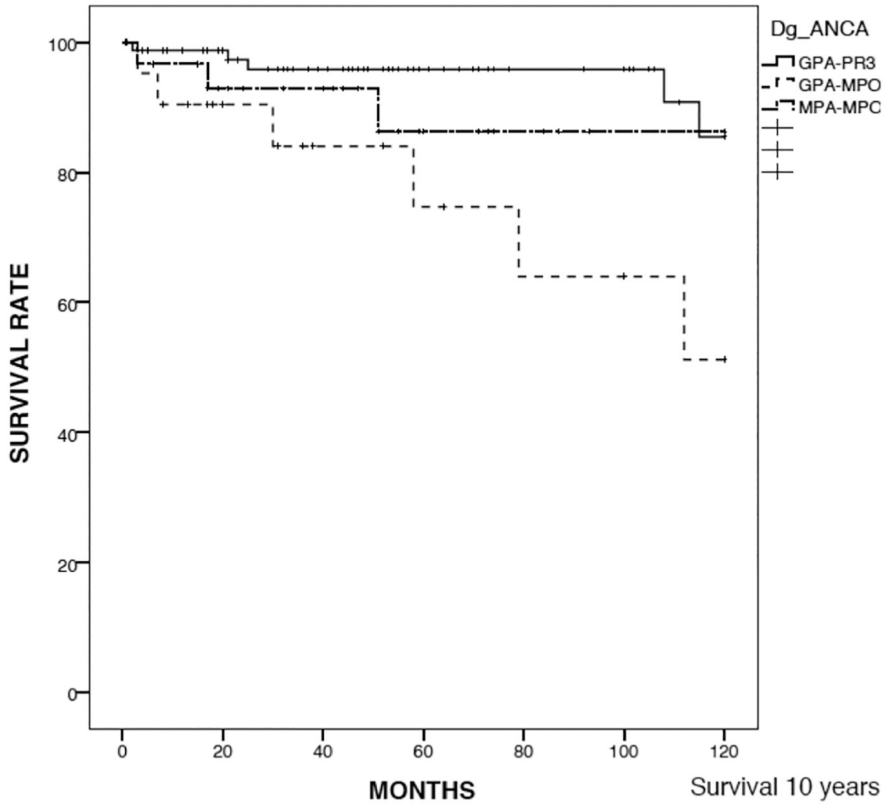

Fig. 2. Kaplan-Meier survival curves according to clinical diagnosis and ANCA type.
GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiitis; PR3: proteinase 3; MPO: myeloperoxidase.

GPA group compared to the PR3-GPA group (BVAS at the 2-year follow-up: 2.41±5.15 in MPO-GPA *vs*. 0±5.2 in PR3-GPA; BVAS at 5 years: 1.81±3.83 *vs*. 0±4.48, respectively). PR3-GPA relapsed significantly more than MPO-GPA (p=0.017) (Suppl. Fig. S1). As shown in the survival analysis, MPO-GPA was significantly associated with a higher mortality risk compared to PR3-GPA.

Discussion

The results of this study demonstrate that ANCA specificity is associated with different disease subsets and outcomes in MPO-GPA compared to PR3-GPA and MPO-MPA. We have demonstrated that mortality rate within a clinical diagnosis of GPA is significantly influenced by the presence of MPO-ANCA positivity. The literature has only recently focused on the value of categorising AAV based on ANCA type, however the clinical impact of such approach is still incompletely understood and has led to contradictory findings (5). Accumulating evidence supports the concept that PR3-ANCA and MPO-ANCA can more homogenously distinguish groups of patients with different genetic background, epidemiology and clinical phenotype. Although genetic differences exist between both ANCA serotypes and clinical diagnosis of GPA and MPA, the subdivision of AAV based on ANCA (PR3-ANCA and MPO-ANCA) seems to have stronger genetic basis than the subdivision based on clinical diagnosis (6, 15). Nevertheless, genetic variants within the same clinical diagnosis of GPA based on the ANCA serotype have never been explored (16). Global ethnic differences in the distribution of ANCA specificity have been demonstrated in patients with GPA with MPO-ANCA being more common in Japanese, Chinese and Southern Europeans, while PR3-ANCA more common in other ethnic groups, leading to differences in the type and severity of organ-systems involvement (17, 18).

To date, there have been only two studies specifically assessing the phenotype of GPA patients based on ANCA type (19, 20) which led to contradictory results. In the first study, Schirmer *et al*. (19) performed a case-control study including 59 patients with MPO-ANCA positive GPA compared to PR3-ANCA positive GPA and MPA. The authors reported a milder and more localised disease in MPO-GPA. A second study by Miloslavsky *et al*. (20) pooled the data from 321 patients with GPA included in the WGET and RAVE clinical trials but did not identify significant differences in the clinical manifestations or relapse rates based on the ANCA type. It is possible that the selection criteria for the enrolment into the randomised clinical trials might have introduced some bias. The results of our study contribute to clarify the previous controversial findings by adding important information with relevant clinical implications. We confirmed the significantly more frequent observation of subglottic stenosis in patients with MPO-GPA compared

Table IV. Cox-regression analysis of mortality risk according to disease/ANCA type category, adjusted for age.

	HR	95% CI	*p*
GPA-PR3	1.00		
GPA-MPO	4.44	1.46-13.52	0.009
MPA-MPO	1.22	0.31-4.73	0.777
Age≥ 65	5.02	1.63-15.49	0.005

GPA-PR3: granulomatosis with polyangiitis-anti-proteinase 3 positive; GPA-MPO: granulomatosis with polyangiitis-anti-myeloperoxidase positive; MPA: microscopic polyangiitis-anti-myloperoxydase-positive; HR: hazard ratio; CI: confidence interval.

to PR3-GPA as observed by Schirmer *et al*. (19). Given the severity and often refractory course of subglottic stenosis, this novel association with MPO-GPA requires a higher degree of attention and strict follow-up in this subset of patients. Both GPA groups were significantly different than MPO-MPA for the higher frequency of ENT involvement, the presence of pulmonary involvement in the form of nodules rather than diffuse alveolar haemorrhage, and the lower frequency and severity of renal involvement. This is a surprising finding potentially challenging the concept of disease categorisation exclusively based on ANCA type. Renal involvement is the hallmark of MPA which is typically MPO-ANCA positive. Moreover, the pathogenetic knowledge on AAV has previously demonstrated the pathogenicity *in vivo* of MPO-ANCA on the development of severe pauci-immune necrotising glomerulonephritis (21). It is possible that animal models differ from the human pathophysiology and that further unexplained mechanisms might lead to different effects of MPO-ANCA in different clinical conditions despite the common antibody positivity. We confirmed the well-known epidemiological feature of MPO-MPA patients being significantly older at diagnosis compared to both MPO-GPA and PR3-GPA patients. Whether ageing might have an influence on the organ-system targets of the pathological process in the presence of MPO-ANCA is still unknown (22-24).

We demonstrated that categorisation by diagnosis and ANCA type influences outcomes. Mortality analysis adjusted for age showed that MPO-GPA is a risk factor for higher mortality rates, while ESRD (an established prognostic factor) was significantly more frequent in MPO-MPA compared to both GPA groups, in line with previous observations (25). The interpretation of the reduced survival in MPO-GPA despite a lower frequency of severe organ-threatening manifestations might be explained by the observation of significantly higher disease activity during follow-up in MPO-GPA and MPO-MPA compared to PR3-GPA. Patients with MPO-GPA were less frequently treated with potent drugs such as rituximab in our cohort and this might have influenced the achievement of long-term sustained remission. Moreover, a recent analysis of the causes of mortality in AAV based on ANCA type has demonstrated that MPO-ANCA exposes patients to the risk of excessive cardiovascular mortality compared to PR3-ANCA (26). The early development of new cardiovascular risk factors has been shown to be higher in patients with MPA-MPO since the early phases of the disease regardless of age and renal involvement (27); similar studies should be conducted to clarify whether the same occurs in subsets of patients with MPO-GPA.

Disease subcategorisation based on cluster analysis suggested that further stratification, particularly based on renal involvement might be the clinically most relevant one and might add significant information in terms of outcomes. PR3-ANCA positive patients with renal involvement have been described as the group with the lowest mortality but the highest relapse rate compared to renal AAV with MPO-ANCA (or ANCA negative) (28). In our cohort, approximately half of the patients with MPO-GPA had renal involvement at baseline and, albeit not reaching statistical significance, MPO-GPA patients had a 2-fold increase in ESRD compared to PR3-GPA and this might have contributed to the reduced survival. Finally, MPO-ANCA have more frequently been associated with the development of fibrosis and this has been described as a confounding factor for mortality in previous cohorts (19).

The study limitations are those inherent to its retrospective design, including the lack of more detailed clinical information that could have been helpful in interpreting the results, and the relatively small number of patients albeit in the context of rare conditions. Furthermore, it is possible that categorisation based on diagnosis and ANCA type is still too simplistic to capture the complexity of factors influencing the clinical manifestations and outcome of AAV. Nevertheless, this study adds valuable information to the limited evidence available to date that distinct subsets of disease in AAV can be better clarified by combining clinical evaluation with ANCA serotypes.

In conclusion, this study supports the concept that AAV categorisation based on both clinical diagnosis and ANCA type has clinical and prognostic implications.

References

1. JENNETTE JC, FALK RJ, BACON PA *et al*.: 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013; 65: 1-11.
2. YATES M, WATTS R: ANCA-associated vasculitis. *Clin Med* (Lond) 2017; 17: 60-4.
3. YATES M, WATTS RA, BAJEMA IM *et al*.: EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. *Ann Rheum Dis* 2016; 75: 1583-94.
4. WATTS RA, MAHR A, MOHAMMAD AJ, GATENBY P, BASU N, FLORES-SUÁREZ LF: Classification, epidemiology and clinical subgrouping of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis. *Nephrol Dial Transplant* 2015; 30 (Suppl. 1): i14-22.
5. CORNEC D, CORNEC-LE GALL E, FERVENZA FC, SPECKS U: ANCA-associated vasculitis - clinical utility of using ANCA specificity to classify patients. *Nat Rev Rheumatol* 2016; 12: 570-9.
6. LYONS PA, RAYNER TF, TRIVEDI S *et al*.: Genetically distinct subsets within ANCA-associated vasculitis. *N Engl J Med* 2012; 367: 214-23.
7. SANDERS J-SF, HUITMA MG, KALLENBERG CGM, STEGEMAN CA: Prediction of relapses in PR3-ANCA-associated vasculitis by assessing responses of ANCA titres to treat-

ment. *Rheumatology* 2006; 45: 724-9.
8. UNIZONY S, VILLARREAL M, MILOSLAVSKY EM *et al*.: Clinical outcomes of treatment of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis based on ANCA type. *Ann Rheum Dis* 2016; 75: 1166-9.
9. 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: 222-7.
10. HELLMICH B, FLOSSMANN O, GROSS WL *et al*.: EULAR recommendations for conducting clinical studies and/or clinical trials in systemic vasculitis: focus on anti-neutrophil cytoplasm antibody-associated vasculitis. *Ann Rheum Dis* 2007; 66: 605-17.
11. MUKHTYAR C, GUILLEVIN L, CID MC *et al*.: EULAR recommendations for the management of primary small and medium vessel vasculitis. *Ann Rheum Dis* 2009; 68: 310-7.
12. LUQMANI RA, BACON PA, MOOTS RJ *et al*.: Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. *QJM* 1994; 87: 671-8.
13. MUKHTYAR C, LEE R, BROWN D *et al*.: Modification and validation of the Birmingham Vasculitis Activity Score (version 3). *Ann Rheum Dis* 2009; 68: 1827-32.
14. EXLEY AR, BACON PA, LUQMANI RA *et al*.: Development and initial validation of the Vasculitis Damage Index for the standardized clinical assessment of damage in the systemic vasculitides. *Arthritis Rheum* 1997; 40: 371-80.
15. RAHMATTULLA C, MOOYAART AL, VAN HOOVEN D *et al*.: Genetic variants in ANCA-associated vasculitis: a meta-analysis. *Ann Rheum Dis* 2016; 75: 1687-92.
16. FORDHAM S, MUKHTYAR C: Are PR3 positive and MPO positive GPA the same disease? *Int J Rheum Dis* 2019; 22 (Suppl. 1): 86-9.
17. PEARCE FA, CRAVEN A, MERKEL PA, LUQMANI RA, WATTS RA: Global ethnic and geographic differences in the clinical presentations of anti-neutrophil cytoplasm antibody-associated vasculitis. *Rheumatology* (Oxford) 2017; 56: 1962-9.
18. FURUTA S, CHAUDHRY AN, ARIMURA Y *et al*.: Comparison of the Phenotype and Outcome of Granulomatosis with Polyangiitis Between UK and Japanese Cohorts. *J Rheumatol* 2017; 44: 216-22.
19. SCHIRMER JH, WRIGHT MN, HERRMANN K *et al*.: Myeloperoxidase-antineutrophil cytoplasmic antibody (ANCA)-positive granulomatosis with polyangiitis (Wegener's) is a clinically distinct subset of ANCA-associated vasculitis: a retrospective analysis of 315 patients from a German vasculitis referral center. *Arthritis Rheumatol* 2016; 68: 2953-63.
20. MILOSLAVSKY EM, LU N, UNIZONY S *et al*.: Myeloperoxidase-antineutrophil cytoplasmic antibody (ANCA)-positive and ANCA-negative patients with granulomatosis with polyangiitis (Wegener's): distinct patient subsets. *Arthritis Rheumatol* 2016; 68: 2945-52.
21. HILHORST M, PAASSEN P VAN, TERVAERT JWC *et al*.: Proteinase 3-ANCA vasculitis versus myeloperoxidase-ANCA vasculitis. *J Am Soc Nephrol* 2015; 26: 2314-27.
22. BERTI A, CAPORALI R, MONTECUCCO C, PAOLAZZI G, MONTI S: Aging in primary systemic vasculitis: implications for diagnosis, clinical manifestations, and management. *Drugs Aging* 2019; 36: 53-63.
23. BERTI A, FELICETTI M, MONTI S *et al*.: Disease and treatment-related morbidity in young and elderly patients with granulomatosis with polyangiitis and microscopic polyangiitis. *Semin Arthritis Rheum* 2020; 50: 1441-8.
24. MONTI S, CRAVEN A, KLERSY C *et al*.: Association between age at disease onset of anti-neutrophil cytoplasmic antibody-associated vasculitis and clinical presentation and short-term outcomes. *Rheumatology* (Oxford) 2021; 60: 617-28.
25. CHANG D-Y, LI Z-Y, CHEN M, ZHAO M-H: Myeloperoxidase-ANCA-positive granulomatosis with polyangiitis is a distinct subset of ANCA-associated vasculitis: A retrospective analysis of 455 patients from a single center in China. *Semin Arthritis Rheum* 2019; 48: 701-6.
26. WALLACE Z, FU X, HARKNESS T, STONE J, ZHANG Y, CHOI H: Causes of death in ANCA-associated vasculitis According to ANCA Type [Abstract]. *Arthritis Rheumatol* 2019; 71 (Suppl. 10). https://acrabstracts.org/abstract/causes-of-death-in-anca-associated-vasculitis-according-to-anca-type/
27. MONTI S, ROBSON J, KLERSY C *et al*.: Early development of new cardiovascular risk factors in the systemic vasculitides. *Clin Exp Rheumatol* 2020; 38 (Suppl. 124): S126-34.
28. MAHR A, KATSAHIAN S, VARET H *et al*.: Revisiting the classification of clinical phenotypes of anti-neutrophil cytoplasmic antibody-associated vasculitis: a cluster analysis. *Ann Rheum Dis* 2013; 72: 1003-10.