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Significance of clinical-immunological patterns and diagnostic yield of biopsies in microscopic polyangiitis and granulomatosis with polyangiitis

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Short tittle: MPA/GPA patterns and diagnostic yield of biopsies

Abstract.

Background. Microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (GPA) are the two major antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV).

Objectives. To characterize a homogenous AAV cohort and to assess the impact of clinicopathological profiles and ANCA serotypes on clinical presentation and prognosis. Clinical differences in GPA patients according to ANCA serotype and the diagnostic yield for vasculitis of biopsies in different territories were also investigated.

Results. This retrospective study (2000-2021) included 152 patients with AAV (77 MPA/75 GPA). MPA patients (96.1% myeloperoxidase/MPO-ANCA and 2.6% proteinase 3/PR3-ANCA) presented more often with weight loss, myalgia, renal involvement, interstitial lung disease (ILD), cutaneous purpura and peripheral nerve involvement. Patients with GPA (44% PR3-ANCA, 33.3% MPO and 22.7% negative/atypical ANCA) presented more commonly with ear, nose, and throat (ENT) and eye/orbital manifestations, more relapses, and higher survival than patients with MPA. GPA was the only independent risk factor for relapse. Poor survival predictors were older age at diagnosis and peripheral nerve involvement. ANCA serotypes differentiated clinical features in a lesser degree than clinical phenotypes. A mean of 1.5 biopsies were performed in 93.4% of patients in different territories. Overall, vasculitis was identified in 80.3% (97.3% in MPA; 61.8% in GPA) of patients.

Conclusions. The identification of GPA presentations associated with MPO-ANCA and awareness of risk factors for relapse and mortality are important to guide proper therapeutic strategies in AAV patients. Biopsies of different affected territories should be pursued in difficult to diagnose patients based on their significant diagnostic yield.

Keywords: ANCA-associated vasculitis, microscopic polyangiitis, granulomatosis with polyangiitis, relapse, mortality, biopsy diagnostic yield.

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) encompasses microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), and eosinophilic granulomatosis with polyangiitis (EGPA). Patients with MPA, GPA and EGPA, especially those with ANCA positivity, share critical features, such as necrotizing glomerulonephritis and pulmonary capillaritis [1]. Regarding MPA and GPA, several manifestations contribute to differentiate them, such as granulomatous inflammation involving nose and throat (ENT) territories, lower airways and orbital structures, which are prominent features of GPA, absent in MPA [1, 2]. In addition, interstitial lung disease (ILD) has recently emerged as a relevant manifestation of AAV, mainly MPA, which may occur concomitantly or years before of an overt systemic vasculitis [3-6]. For this reason, ILD has been included as part of the key items of the 2022 American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) classification criteria for MPA [7].

MPA and GPA have geographic and ethnic epidemiological differences. While GPA is prevalent in Northern Europe, MPA occurs more frequently in southern European countries, and is the predominant AAV in Asian countries [8-10]. Proteinase 3 (PR3)-ANCA and myeloperoxidase (MPO)-ANCA are strongly associated with GPA and MPA, respectively. About 72-90% of individuals with GPA are positive for PR3-ANCA in European and Northern American cohorts [11-16]. Conversely, MPO-ANCA are detected in 30-60% of patients with GPA in Eastern Asian countries [17-20]. Although the role of ANCA type in disease extent, severity, relapses and mortality has been explored with controversial results, patients with MPA and MPO-ANCA have been reported as having worse global and/or renal survival in some large European and Northern American series [11-14, 21, 22], and PR3-AAV have been repeatedly associated with higher relapse rates [13, 16, 23-27].

Classification criteria in MPA and GPA are designed to differentiate among AAV and to standardize inclusion criteria for clinical trials and other studies [7, 28]. Nevertheless, a definite diagnosis of vasculitis is always desirable by proving histopathological vascular inflammation. In this sense, biopsies of ENT structures, kidney, lung, striated muscle, peripheral nerves and other territories have already proved to have a remarkable diagnostic yield of vasculitis in AAV [2, 29-36].

The present study aimed to retrospectively characterize a Spanish single reference center cohort of patients with AAV by describing different disease profiles (MPA and GPA) in order to identify prognostic factors for relapse and mortality. Clinical differences according to ANCA serotypes, GPA variability based on ANCA specificity, and the diagnostic yield of biopsies performed in the affected territories were also evaluated.

Material and Methods

Patients, inclusion and exclusion criteria, and ethical approval

From January 2000 to December 2021, all patients diagnosed with MPA and GPA followed at the Vasculitis Research Unit, Department of Autoimmune Diseases, Hospital Clinic de Barcelona, were included. Diagnoses were based on clinical and histological findings in accordance with the 2012 Chapel Hill Consensus Nomenclature [37], and the European Medicines Agency (EMA) algorithm [38]. Definitions for disease extent and activity, organ assessment, biopsy procedures and

treatments used are detailed in the Supplementary Methods. Although ANCA type was also determined by indirect immunofluorescence as perinuclear (P-ANCA) or cytoplasmic (C-ANCA) pattern, with the purpose of homogenizing analyses, ANCA specificity was assessed using enzyme-linked immunosorbent assay as MPO-ANCA, PR3-ANCA or atypical ANCA. Patients with ANCA and isolated ILD, renal limited vasculitis, EGPA or cocaine-induced AAV, and those with missing information regarding clinical presentation or follow-up were excluded.

This retrospective study was approved by the Research Ethics Committee of the Hospital Clínic de Barcelona (HCB/2021/1067). All procedures were performed in accordance with the ethical principles expressed in the 2013 Declaration of Helsinki.

Clinical variables

Demographic, clinical, laboratory, imaging and histological data were retrieved from patients' clinical charts and collected to an anonymized database. Clinical variables covered disease extent, disease activity, disease duration until diagnosis, constitutional symptoms and those manifestations derived from musculoskeletal, pulmonary, ENT, ocular, renal, cutaneous, neurological, cardiac, gastrointestinal and salivary gland involvement. Number of relapses, remission induction treatments (mainly focused on cyclophosphamide or rituximab, and plasma exchange), development of chronic kidney disease (CKD) and/or end-stage kidney disease (ESKD), and deaths during the study period were also recorded. Biopsies performed in different territories for AAV diagnosis and histopathological results providing definite diagnosis of vasculitis were documented.

All clinical parameters were compared between groups, according to clinicopathological presentation (GPA *vs.* MPA) and ANCA serotype (MPO-ANCA *vs.* PR3-ANCA). GPA patients were evaluated based on ANCA specificity. The diagnostic yield for vasculitis in every tissue in which biopsies were performed was also calculated.

Statistical analysis

Analysis was performed using STATA MP version 14 (StataCorp LLC®, Texas, USA). Categorical data was presented as frequency counts and percentages, and was analyzed with chi-square test and Fisher's exact test, as appropriate. Normality was tested with Kolmogorov-Smirnov test. The skewed distributions were described with medians and interquartile ranges (IQR) and were compared with the Mann-Whitney test. Normal distributions were described with means and standard deviations and were compared with the student's t-test. The two-sided alpha level was set at 0.05. The 95% confidence intervals (95%CI) were shown. The two outcomes of interest were focused on relapses and survival. Incidence rates of relapse and mortality were calculated using survival analysis and compared using the Poisson regression. To identify clinical predictors of relapse, subhazard ratios (sHR) were calculated using a competing-risk regression model, assuming deaths as competing risk events. Predictors of overall survival during the follow-up were assessed by hazard ratios (HR) calculated by Cox proportional hazard model. The univariable analysis was subsequently used to analyze the association between potential prognostic factors and disease outcomes, and results were expressed as the sHR/HR with 95%CI. Models used a forward-stepping covariate selection procedure in order to select the most significant predictors into multivariable models.

Results

Global series results

Main clinical characteristics of the entire series are listed in Table 1 and detailed features are included in Table S1. A total of 152 patients with AAV, 88 (57.9%) female and 132 (86.8%) Caucasians, were included. Median (IQR) age at diagnosis was 62 (47-72) years. Seventy-seven (50.7%) patients were diagnosed with MPA and 75 (49.3%) with GPA. Regarding ANCA serotype, 99 patients (65.1%) had MPO-ANCA, 35 (23%) PR3-ANCA, 4 (2.6%) atypical ANCA (2 cathepsin-G and 2 bactericidal/permeability-increasing protein) and 14 (9.2%) patients were ANCA-negative.

Biopsies were performed in 142 (93.4%) patients in at least one affected tissue and 57 (37.7%) of them, underwent ≥2 biopsies. The reason to perform repeated or additional biopsies in different territories was a previous biopsy revealing negative or inconclusive results (mostly unspecific inflammatory changes) not allowing a firm diagnosis of vasculitis. A total of 227 biopsies were carried out, with a mean (SD) of 1.5 (0.9) biopsies per patient [median (range)= 1 (0-4)] (Table 2).

Cyclophosphamide or rituximab was initially administered to 60 (39.5%) and 24 (15.8%) patients, respectively, and plasma exchange was used in 19 (12.5%) cases. At last follow-up, 45 (29.6%) patients had developed CKD, 6 (3.9%) had progressed to ESKD and 2 (1.3%) had received a kidney transplant (Table 1).

During a median (IQR) follow-up of 75 (35-144) months, 72 (47.4%) patients presented with relapses. Median (IQR) time to first relapse was 36 (14-69) months. The incidence rate of relapse was 0.10 (95%CI 0.083-0.13) per patient-year. Cumulative incidence of relapses at 1, 5 and 10 years were 13.7% (95%CI 9.1-20.4%), 49.1% (95%CI 40.6-58.3%), and 74.8% (95%CI 65.2-83.5%), respectively. In order of frequency, relapses occurred as renal involvement, with renal function impairment, active urinalysis and/or proteinuria (36%), lung/respiratory abnormalities (28%), asthenia (25%), fever (18%), and musculoskeletal (22%), ENT (21%), neurological (17%), ocular (15%), and skin (6%) manifestations. ANCA titers increased or become positive in 49% of cases, and CRP and ESR levels were elevated in 57% of patients with relapses.

Thirty-three (21.7%) patients deceased during the study period. Mortality rate was 0.028 (95%CI 0.020-0.040) per patient-year. Overall, survival rates at 1, 5 and 10 years were 95.3% (95%CI 90.4-97.7%), 88.5% (95%CI 81.5-93.0%), and 79.3% (95%CI 69.6-86.2%), respectively. Causes of death included infectious complications (n=16; 48.5%), cardiovascular disease (n=8; 24.2%), cancer (n=4; 12.1%), and vasculitis complications (n=4; 12.1%).

Relationship between disease phenotypes and ANCA serotypes

Among MPA patients, 74 (96.1%) had MPO-ANCA, 2 (2.6%) PR3-ANCA and 1 (1.3%) was ANCA negative. In GPA patients, 33 (44%) subjects had PR3-ANCA, 25 (33.3%) MPO-ANCA, 2 (2.7%) atypical ANCA, and 15 (20%) were ANCA negative. Patients with GPA and those with PR3-ANCA were more commonly associated with a limited disease. Patients with GPA and atypical/negative ANCA displayed more often a limited disease than patients with detectable MPO/PR3-ANCA (Table 3).

Patients with MPA and those with MPO-ANCA were older than patients with GPA and PR3-ANCA, respectively. Women predominated in the MPA group compared with GPA and significantly outnumbered men in MPO-ANCA group compared to PR3-ANCA. Initial BVAS was

higher in MPA than GPA patients, but it did not differ by ANCA serotype. Follow-up time was similar in all groups (Table 1).

Main clinical features differentiating MPA from GPA and MPO-ANCA from PR3-ANCA are summarized in Table 1, and listed in detail in Table S1. Limited disease was manifested in 34 (45.3%) patients with GPA. Disease duration until diagnosis was lower in patients with MPA than GPA. Clinical variables significantly more frequent in MPA included weight loss, musculoskeletal manifestations (mostly myalgia), ILD with usual interstitial pneumonitis (UIP) radiologic pattern, purpuric skin lesions, peripheral nerve involvement and kidney involvement. Contrarily, GPA presented more frequently with pulmonary nodules, ENT, ocular, orbital, and cutaneous lesions (other than purpura), and lacrimal gland inflammation. Central nervous system involvement, particularly pachymeningitis, tended to occur more frequent in GPA patients. Cutaneous, cardiac, and abdominal manifestations were similar in all AAV patients.

Regarding to ANCA serotype, patients with MPO-ANCA presented more frequently with myalgia, ILD (with UIP) and uveitis, and tended to manifest more often with weight loss and peripheral nerve involvement than patients with PR3-ANCA. Conversely, PR3-ANCA subjects had more often pulmonary nodules, ENT, ocular and orbital involvement, and tended to present more commonly with pachymeningitis and stroke. ANCA serotype did not discriminate in renal, cutaneous, cardiac, abdominal, or salivary gland manifestations (Table 1 and Table S1).

Either cyclophosphamide or rituximab were more frequently administered to patients with MPA. Impairment of the renal function during the follow-up was significantly higher in MPA than in GPA, without differences between ANCA groups (Table 1).

The incidence rate of relapse was higher in patients with GPA than in those with MPA and no differences were observed by ANCA serotype (Table 1 and Fig. 1A and 1B). The time elapsed to the first relapse was similar in all groups. Any type of lung involvement and ENT manifestations were associated with higher relapse rates (Fig. S1A and S1B).

Mortality was higher in MPA than GPA patients, and tended to be higher in MPO-ANCA than in PR3-ANCA patients (Table 1 and Fig. 1C and 1D). Survival was also lower in patients with ILD and peripheral nervous system involvement (Figs. S1C and S1D). Patients with ENT manifestations had better survival than those without ENT involvement (Fig. S1E). CKD development was not associated with higher mortality (Fig. S1F). No increased mortality was observed in patients with pulmonary hemorrhage.

Results from univariable and multivariable analyses are illustrated in Table 4. At the univariable analysis, suffering GPA was associated with a higher relapse incidence. Using the multivariable model, only GPA was also found to be a predictor of higher relapse rate (sHR 1.70 [95%CI 1.01-2.87; p=0.047]). The univariable analysis found poor survival associated with older age, MPA diagnosis and having ILD and peripheral nerve involvement, and having GPA was associated with better survival. The independent predictors of worse survival at the multivariable model included age at diagnosis ≥68 years (HR 3.87 [95%CI 1.73-8.65; p=0.001]) and peripheral nerve involvement (HR 2.29 [95%CI 1.11-4.71; p=0.024].

Differences in GPA according to ANCA serotype

Patients with GPA and PR3-ANCA were more frequently men, had shorter disease duration until diagnosis, and presented with alveolar hemorrhage and received cyclophosphamide or rituximab for induction remission more often than MPO-ANCA-GPA patients. No differences were found in age,

disease extent, and the remaining clinical manifestations (Table 3 and Table S2). The incidence rate of relapses was similar in both GPA groups (Table 3 and Fig. S2A). A trend to lower survival was observed in patients with GPA with MPO-ANCA compared to those with PR3-ANCA (Fig. S2B).

Compared to patients GPA with ANCA-PR3 or ANCA-MPO, patients with GPA and atypical or negative ANCA significantly had longer duration of symptoms until diagnosis, lower BVAS and presented less frequently with constitutional symptoms, fever, pulmonary infiltrates, and renal involvement (which was absent). Subglottic stenosis and paranasal inflammation occurred in a higher proportion in GPA patients with atypical/negative ANCA and no deaths occurred in this group (Table 3 and Table S2).

Performed biopsies and histopathological results

No differences were found between patients with MPA and GPA regarding the number of biopsies performed. Vasculitis was found in 65.2% (78.9% in MPA; 51.3% in GPA) and granulomatous findings in 11.5% (0% in MPA; 23% in GPA) of all samples. Overall, vasculitis was observed in 80.3% (97.3% in MPA; 61.8% in GPA) and granulomatous inflammatory lesions in 16.9% (0% in MPA; 35.3% in GPA) of patients in whom at least one biopsy was performed.

By territories, a kidney biopsy was performed in 74 (48.7%) patients (67.5% in MPA; 29.3% in GPA), with confirmation of vasculitis (pauci-immune glomerulonephritis) in 72 (97.3%) samples (100% in MPA; 90.1% in GPA). Lung biopsies were performed in 21 (13.8%; 7.8% in MPA; 20% in GPA) patients (16 transbronchial, 1 CT-guided transthoracic and 4 open lung biopsies), of whom vasculitis and/or granulomatous lesions were observed in 8 (38.1%) patients (all of them with GPA, in 4 transbronchial, 1 transthoracic and 3 open lung biopsies). ENT biopsies were carried out in 32 (21.1%) patients, all of them with GPA (42.7% of GPA patients). Nasal mucosa was biopsied in 26 (34.7%) patients, with 12 (46.2%) samples showing vasculitis and/or granulomas and 14 (53.8%) non-specific chronic inflammatory changes. Six (8%) patients underwent subglottic biopsies, and all of them (100%) had chronic inflammation without vasculitis or granulomatous changes. Periorbital structures and salivary glands were biopsied in 3 (2%) patients each (all with GPA), and vasculitis and/or granuloma were found in 100% of orbit and 66.7% of salivary gland samples. Skin was biopsied in 19 (12.5%) subjects, and 16 (84.2%) samples showed vasculitic and/or granulomatous inflammatory features. Twelve (83.3%) GPA patients exhibited all type of histological changes and 6 (85.7%) samples from MPA patients showed leukocytoclastic vasculitis.

Muscle biopsies, usually from deltoid or gastrocnemius muscle (the latter were performed together with a sural nerve biopsy) were carried out in 37 (24.3%) patients providing the diagnosis of small-to-medium vessel vasculitis in 27 (64.9%) cases. Muscles were biopsied more frequently in MPA than in GPA patients (31.2 *vs.* 17.3; p=0.047) and disclosed histological changes of vasculitis in 70.8% and 53.8% of MPA and GPA samples, respectively. Sural nerve was analyzed in 18 (11.8%) of cases and showed vasculitis in 66.7% of samples. Peripheral nerve biopsies were performed more commonly in MPA than GPA patients (18.2% *vs.* 5.3%; p=0.014), with nerve vasculitis confirmed in 64.3% and 75% of cases, respectively. A temporal artery biopsy was performed in 20 (13.2%) patients because of craniofacial manifestations in the setting of a suspected systemic vasculitis. Thirteen (65%; 54.5% of MPA and 77.8% of GPA) samples showed vasculitis involving adventitial small vessels or collateral vessels surrounding a non-inflamed temporal artery.

Table 2 provides detailed information about the biopsies performed in different territories and the histological results obtained in patients with AAV.

Discussion

MPA and GPA as clinicopathological AAV forms differentiate better clinical characteristics than the division by ANCA serotype. On one hand, compared to GPA subjects, our MPA patients were significantly older, had shorter disease duration until diagnosis, higher initial BVAS and presented more frequently with weight loss, myalgia, ILD (mainly UIP pattern), cutaneous purpuric lesions, and renal and peripheral nerve involvement. MPA patients also presented with less relapses, received more often intensive remission induction therapies, developed more frequently CKD and ESKD, and had higher mortality than GPA patients. On the other hand, our patients with GPA were younger and presented with longer disease duration, lower BVAS, and less often with manifestations predominating in MPA patients. ENT manifestations were exclusively observed in GPA, and orbital masses, ocular inflammatory lesions, and salivary gland involvement were more frequent in GPA patients. Indeed, GPA subjects had more relapses, lower use of cyclophosphamide or rituximab, lower development of CKD/ESKD and mortality than MPA patients. Compared to patients with MPA (with more aggressive disease onset), the pronounced diagnostic delay in GPA, probably due to the high proportion of patients with long-lasting smoldering ENT disease, was in turn associated with a lower frequency of systemic manifestations, such as weight loss and musculoskeletal symptoms.

As in our cohort, other large AAV series showed a predominance of older persons in MPA [11, 13-15], and similar clinical manifestations [3, 11, 13, 15, 39]. However, peripheral nerve involvement, which was previously found without differences between both AAV [11, 13, 15], occurred more frequent in our MPA patients. As in previous AAV cohorts [3, 4], ILD occurred more often in our patients with MPA and MPO-ANCA than in those with GPA and PR3-ANCA. In this sense, the presence of ILD, mainly UIP pattern, has been previously recognized as a poor prognostic factor associated with increased mortality [4].

In our patients with MPA, MPO-ANCA clearly prevailed over PR3-ANCA. Although PR3-ANCA have been reported in 22-40% of patients in MPA cohorts from multicenter European and Northern American studies [12-14, 16], the lower PR3-ANCA rate found in our cohort has been similarly found in other MPA populations [3, 15]. In our patients with GPA, MPO-ANCA were detected in a third of cases, and negative/atypical ANCA in almost a quarter of them. In this regard, a non-overlapping Spanish registry analyzing 167 MPA and 184 GPA patients found MPA associated with MPO-ANCA in 90.4% and PR3-ANCA in 9.6% of cases, and GPA with PR3-ANCA in 74%, MPO-ANCA in 15%, and ANCA negative in 11% of patients [11]. Our patients with GPA (with clear predominance of Caucasians) presented a higher MPO-ANCA proportion than this Spanish cohort [11], and also than larger international and multicenter studies showing 9-11% of MPO-ANCA in GPA patients [12-15]. Our GPA patients display a higher proportion of MPO-ANCA, indeed more characteristic of Eastern Asian countries [17-20].

Grouping by ANCA serotype did not discriminate in the development of renal and cutaneous involvement in our patients. Compared with PR3-ANCA, our patients with MPO-ANCA were older, more frequently women and presented more commonly with a systemic disease, myalgia, ILD (UIP pattern), fewer relapses, and tended to suffer more peripheral nerve damage. Conversely, our patients with PR3-ANCA were younger, more often male, and had more

commonly a limited disease, and ENT and ocular/orbital involvement than patients with MPO-ANCA. Similar results to ours have been reported by other authors [24]. However, those studies in which MPO-ANCA was identified as a marker of severity at disease presentation with poorer renal outcomes and higher mortality (not found in our series) included mainly renal AAV patients [12-14, 21, 22]. In addition, our results are aligned with previous studies showing the association between PR3-ANCA and an increased frequency of relapses [13, 16, 23-27]. Nevertheless, according to our results, most of the differences between patients with PR3-ANCA and MPO-ANCA seem to be determined mainly by the GPA/MPA phenotype, since patients with GPA exhibit lower clinical differences with regard to ANCA serotype, as detailed below and in Table 3.

Differences in our patients with GPA according to ANCA serotype were only observed in the predominance of women in MPO-ANCA patients, and shorter disease duration until diagnosis, higher frequency of alveolar hemorrhage, and more requirements of intensive therapies in those with PR3-ANCA (Table 3). As in our study, compared to GPA patients with PR3-ANCA, those with MPO-ANCA have been previously described to have a predominance of female patients [16, 26], higher frequency of alveolar hemorrhage [40], and lower requirements of aggressive immunosuppressive therapies [26]. Other GPA studies did not find differences in clinical manifestations and treatment requirements between ANCA serotypes [15, 24, 41].

Similarly to other studies [15, 21], our ANCA-negative-GPA patients had lower initial BVAS and less CKD development than GPA patients with detectable ANCA. In addition, compared with ANCA-positive GPA patients, our GPA patients with atypical or negative ANCA exhibited a longer disease duration until disease diagnosis, lower frequency of constitutional symptoms, fever, pulmonary infiltrates, renal involvement, higher proportion of subglottic stenosis, lower requirements of intensive remission induction regimens, and reduced mortality.

About half of our patients suffered relapses, which were more frequent in GPA than MPA. Relapses have been similarly reported in 35-38% of both AAV and around 50% of GPA patients [2, 13, 27, 42-44]. Several risk factors for suffering relapses in MPA and GPA have been identified and include an age ≤75 years [44], PR3-ANCA [45-47], estimated glomerular filtration rate (eGFR) ≥30 at disease onset [45], lung disease, and upper respiratory tract involvement [46]. As previously reported [24, 44], having GPA was identified in our series as the only risk factor associated with relapses in the multivariable analysis. However, ENT involvement was also associated with higher cumulative incidence of relapses. The finding of GPA as a predictor for relapse with PR3-ANCA having a neutral impact could be explained by the fact that a third of patients with GPA are MPO-ANCA positive in our population.

Compared with the general population, mortality is higher in patients with GPA and MPA in all ages despite newer therapeutic strategies [14]. The first year and long-term survival rates in our AAV patients were around 90% and 80%, respectively, which are in line with previous studies [12, 13, 48, 49]. Large MPA and GPA cohorts have found advanced age, male sex, higher initial BVAS, impaired renal function, and lower hemoglobin levels as risk factors associated with an increased mortality [12, 14, 44]. In accordance to our study, MPA has been associated with lower global and renal survival rates than GPA in large European and Northern American cohorts [11-14, 21, 22]. In the univariable analysis, having an advanced age, MPA diagnosis, ILD and peripheral nerve involvement were associated with higher mortality, and suffering GPA was associated with a lower mortality. Although patients with ENT manifestations had better survival curves than those without ENT involvement, only advanced age and having peripheral neuropathy were found independent predictors of poor survival. Therefore, older age seems to account for the significant worse survival

observed in MPA compared to GPA. Likewise, in MPA series, advanced age and ILD/UIP pattern (due to an increased susceptibility to infection and ILD progression) have been also associated with higher mortality [50]. As occurred in our patients, a German series of 144 MPA individuals also identified older age, and having ILD and peripheral nerve involvement as risk factors associated with higher mortality [3].

The diagnostic yield of biopsies in different territories for AAV diagnosis has been repeatedly explored. Renal biopsy in patients AAV has provided a vasculitis diagnosis in 86% of kidney samples [51], which was achieved 97.3% in our series. ENT biopsies offer a variable diagnostic yield (from 23% to 70% of samples [2, 29, 30, 51]), which was of 46.2% in nasal mucosa in our (only in GPA) patients. In subglottic lesions, changes of granulomatous inflammation and vasculitis have been reported as low as 5%, since nonspecific acute and chronic inflammation alone is common to most samples [2, 52], as it occurred in all our subglottic biopsies. Lung biopsies have provided higher diagnostic yield in GPA than in MPA [31], which was even higher in open lung than in transbronchial biopsies (91% *vs.* 7%) [2, 30]. In our AAV patients, diagnostic yield of lung biopsies (mostly transbronchial) was 38.1% (0% in MPA; 53.3% in GPA). Orbit biopsies in GPA have demonstrated vasculitis or granulomatous lesions in 75-85% of patients [53], and was of 100% in our GPA patients. Skin biopsies have been reported in 22-44% of AAV patients, mostly for purpuric lesions, achieving a vasculitis diagnosis in 68-94% of patients [39], being of 84.2% in our patients (without differences between AAV). Muscle biopsies have provided a vasculitis diagnosis in more than 50% of AAV patients [32, 33], and was of 64.9% (70.8% in MPA; 53.8 in GPA) in our series. Sural nerve biopsy in AAV has showed vasculitis in 28% of procedures [34]. In our AAV series, sural nerve provided the diagnosis of vasculitis in a higher proportion (66.7%) of patients (64.3% in MPA; 75% in GPA). As in our patients, other potential territories in which vasculitis can be diagnosed in AAV are salivary glands (in GPA) [54] and temporal arteries [55, 56]. Overall, our study demonstrated that the performance of more than one biopsy in different territories (when a previous procedure resulted negative or inconclusive) offers a higher diagnostic yield for vasculitis than that provided by isolated biopsies.

Several limitations of this study that may have contributed to misinterpret some results, including its retrospective nature, the limited sample size, and the fact that the large number of variables analyzed and the multiple comparisons performed may increase the risk of false positive values. The lower progression to CKD and ESKD in this study compared to previous AAV nephrology series may be related to the relatively reduced number of patients with renal involvement. However, the strengths of this study series rely in the fact that all patients were diagnosed and managed with homogenous standardized procedures used over time by the same multidisciplinary team of physicians.

Conclusions

In the present AAV series with a high number of patients with biopsy proven vasculitis, MPA and GPA as clinicopathological forms differentiate better clinical groups than ANCA serotypes. GPA was associated more often with a relapsing disease and higher survival rates than MPA. Having GPA was found an independent risk factor for relapse. Predictors of worse survival included older age at diagnosis and peripheral nerve involvement, which were indeed more frequent in patients with MPA and MPO-ANCA. A third of patients with GPA in our cohort presented with MPO-ANCA, which implies a high proportion comparable to that observed in Asian series. No major clinical differences were found in GPA patients regarding ANCA serotype. The diagnostic yield for

vasculitis of biopsies in different territories was remarkably high. Based on these results, the identification of GPA presentations associated to MPO-ANCA and the awareness for risk factors for relapse and mortality are important to anticipate an accurate diagnosis and guide proper therapeutic strategies in patients with MPA and GPA. In addition, in cases entailing a difficult diagnostic process, biopsies of different affected territories should be pursued given their potential diagnostic yield in AAV.

Online-only supporting information
Supplementary Methods: Definitions for disease assessment, biopsy procedures, and treatments used in patients with ANCA-associated vasculitis (AAV); Supplementary Tables: Table S1. Detailed clinical manifestations of the entire study population of AAV and by groups according to clinical diagnosis and ANCA serotypes. Table S2. Detailed clinical manifestations of patients with granulomatosis with polyangiitis (GPA) according to ANCA serotypes; Supplementary Figures: Figure S1. Estimated cumulative incidence of relapses and survival during the follow-up according to specific organ involvement; Figure S2. Estimated cumulative incidence of relapses and survival in GPA subgroups during the follow-up according to ANCA serotypes.

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Conflict of interest
MCC reports consulting fees from GSK, AbbVie, CSLVifor, Janssen and AstraZeneca; royalties from UpToDate, and a research grant and meeting travel support from Kiniksa Pharmaceuticals Corp. Participation in advisory boards for GSK, CSLVifor and Astrazeneca. The remaining authors declare no conflict of interest regarding this work.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement
The study was performed in accordance with the ethical principles expressed in the 2013 Declaration of Helsinki. The Research Ethics Committee of the Hospital Clínic de Barcelona approved the study (HCB/2021/1067).

Author contributions
João Fernandes-Serodio, Sergio Prieto-González, Maria C. Cid and José Hernández-Rodríguez designed the study. João Fernandes-Serodio, José Hernández-Rodríguez and Sergio Prieto-González were involved in data collection. José Hernández-Rodríguez and João Fernandes-Serodio were involved in data interpretation and manuscript preparation. All authors participated in the revision of the manuscript and approved the final version.

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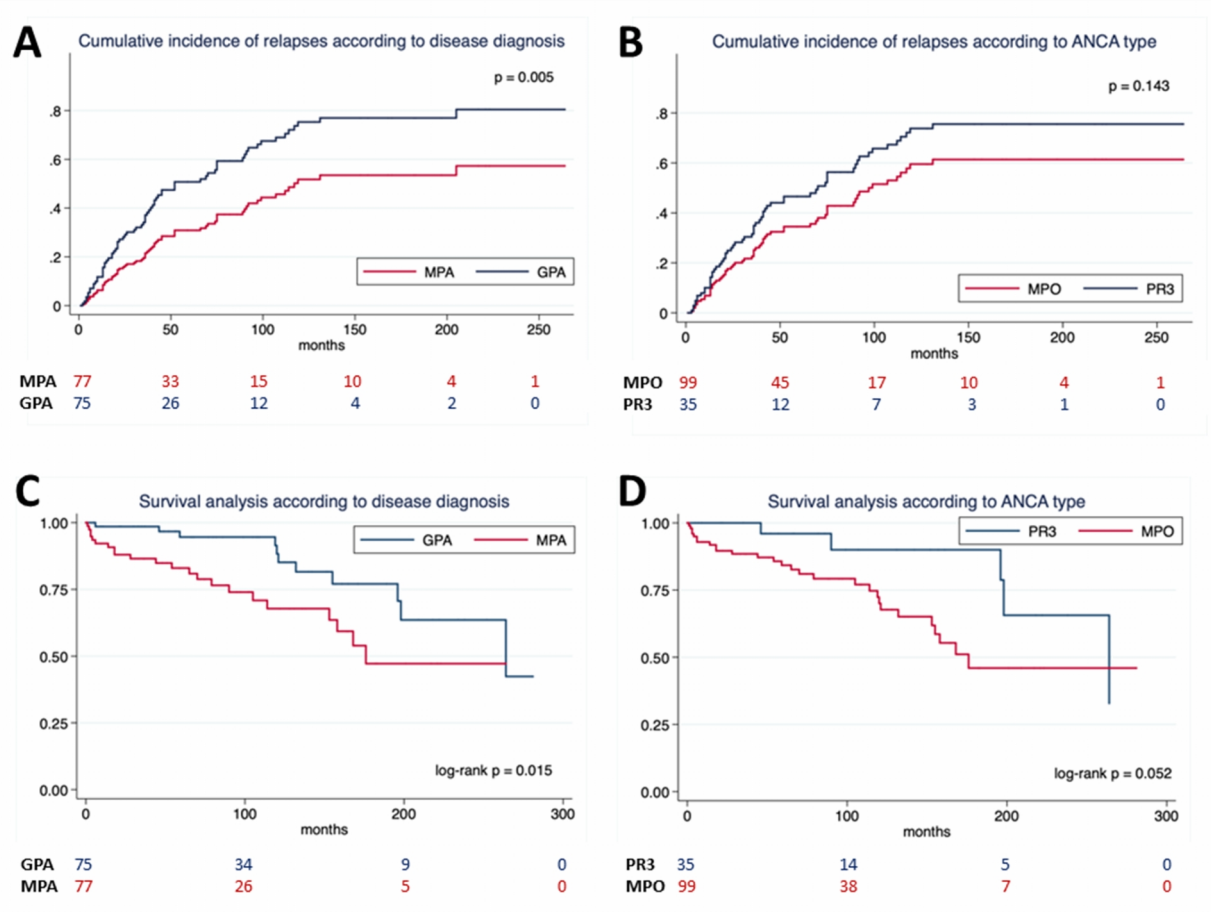
FIGURE LEGENDS

Figure 1. Estimated cumulative incidence of relapses and survival during the follow-up according to clinical diagnosis and ANCA serotypes.



TABLES

Table 1. Main general characteristics of the entire study population of AAV and by groups according to clinical diagnosis and ANCA serotypes

Characteristics	AVV patients	MPA	GPA	*p-value*	MPO-ANCA	PR3-ANCA	*p-value*
Number of patients	152	77 (50.7)	75 (49.3)		99 (73.9)	35 (26.1)	
Age at diagnosis (years); median (IQR)	62 (47-72)	**68 (57-74)**	**59 (41-70)**	**0.001**	**66 (55-74)**	**57 (42-70)**	**0.010**
Women; n (%)	88 (57.9)	49 (63.6)	39 (52)	0.146	**64 (64.6)**	**13 (37.1)**	**0.003**
Time from symptoms onset to diagnosis (months); median (IQR)	4 (3-9)	3 (2-6)	6 (3.5-9.5)	0.001	4 (2-7)	4 (3-8)	0.416
ANCA serotypes							
MPO-ANCA; n (%)	99 (65.1)	**74 (96.1)**	**25 (33.3)**	**<0.001**	NA	NA	NA
PR3-ANCA; n (%)	35 (23)	**2 (2.6)**	**33 (44)**	**<0.001**	NA	NA	NA
Negative/atypical ANCA; n (%)	18 (11.8)	**1 (1.3)**	**17 (22.7)**	**<0.001**	NA	NA	NA
Disease extent and severity							
Limited disease; n (%)	34 (22.4)	**0 (0)**	**34 (45.3)**	**<0.001**	**8 (8.1)**	**11 (31.4)**	**0.002**
Systemic disease; n (%)	118 (77.6)	**77 (100)**	**41 (54.7)**	**<0.001**	**91 (91.9)**	**25 (71.4)**	**0.002**
Initial BVAS; median (IQR)	18 (12-22)	**20 (15-23)**	**15 (9-21)**	**0.001**	19 (14-23)	19 (12-26)	0.881
Clinical manifestations							
Constitutional symptoms; n (%)	103 (67.8)	54 (70.1)	49 (65.3)	0.527	71 (71.7)	25 (71.4)	0.797
Musculoskeletal; n (%)	98 (64.5)	**57 (74)**	**41 (54.7)**	**0.013**	**71 (71.7)**	**19 (54.3)**	**0.039**
Renal involvement; n (%)	81 (53.3)	**56 (72.7)**	**25 (33.3)**	**<0.001**	61 (61.6)	19 (54.3)	0.447
Pulmonary involvement; n (%)	98 (64.5)	50 (66.2)	48 (62.7)	0.904	65 (65.7)	25 (71.4)	0.680
Ear nose and throat; n (%)	59 (38.8)	**0 (0)**	**59 (78.7)**	**<0.001**	**19 (19.2)**	**27 (77.1)**	**<0.001**
Eye and orbit; n (%)	39 (25.7)	**8 (10.4)**	**31 (41.3)**	**<0.001**	**18 (18.2)**	**15 (42.9)**	**0.005**
Cutaneous involvement; n (%)	39 (25.7)	22 (28.6)	17 (22.7)	0.405	26 (26.3)	8 (22.9)	0.691
Neurological							
Peripheral nerve; n (%)	47 (30.9)	**32 (41.6)**	**15 (20)**	**0.004**	28 (38.4)	8 (22.9)	0.080
Central nervous system; n (%)	12 (7.9)	3 (3.9)	9 (12)	0.064	7 (7.1)	4 (11.1)	0.448
Cardiac; n (%)	7 (4.6)	5 (6.5)	2 (2.7)	0.442	6 (6.1)	1 (2.8)	0.675
Gastrointestinal; n (%)	3 (2)	2 (2.6)	1 (1.3)	0.575	3 (3)	0 (0)	0.564
Salivary gland; n (%)	7 (4.6)	**0 (0)**	**7 (9.3)**	**0.006**	3 (3)	2 (5.7)	0.492
Relapses, initial treatment, and outcomes during the follow-up							
Relapse (any); n (%)	72 (47.4)	31 (40.3)	41 (54.7)	0.075	44 (44.4)	18 (50)	0.567
Relapse rate, per patient-year (95%CI)	0.10 (0.083-0.13)	0.076 (0.053-0.11)	0.14 (0.11-0.19)	**0.009**	0.092 (0.068-0.12)	0.13 (0.081-0.20)	0.239
Time to first relapse (months); median (IQR)	36 (14-69)	31 (14-66)	36 (14-74)	0.772	36 (16-72)	29 (13-75)	0.681
Induction with CYC/RTX ever; n (%)	84 (55.3)	**52 (67.5)**	**32 (42.7)**	**0.003**	66 (66.7)	25 (71.4)	0.160
Plasma exchange; n (%)	19 (12.5)	11 (14.3)	8 (10.7)	0.500	12 (12.1)	6 (16.7)	0.492
Chronic kidney disease*	45 (29.6)	**32 (41.6)**	**13 (17.3)**	**0.001**	34 (34.3)	10 (28.5)	0.472
End-stage kidney failure	6 (3.9)	**6 (7.8)**	**0 (0)**	**0.014**	6 (6.1)	0 (0)	0.131
Deaths; n (%)	35 (26.1)	**23 (29.9)**	**12 (16)**	**0.042**	28 (28.3)	7 (20)	0.338
Mortality rate, per patient-year (95%CI)	0.028 (0.020-0.040)	0.041 (0.027-0.062)	0.018 (0.01-0.032)	**0.024**	0.039 (0.027-0.057)	0.017 (0.007-0.041)	0.088
Lost-to-follow-up; n (%)	13 (8.6)	9 (11.7)	4 (5.3)	0.161	9 (9.1)	2 (5.7)	0.532
Follow-up (months); median (IQR)	75 (33-144)	70 (33-131)	82 (33-152)	0.287	72 (33-136)	82 (22-161)	0.588

* Three patients with MPA and MPO-ANCA underwent renal transplantation

Note: Statistically significant results are shown in bold

Abbreviations: AAV= ANCA-associated vasculitis; ANCA= antineutrophil cytoplasmic antibodies; BVAS= Birmingham Vasculitis Activity Score; CI= Confidence interval; CYC= cyclophosphamide; GPA= Granulomatosis with polyangiitis; IQR= interquartile ranges [percentiles 25th - 75th]; MPA: Microscopic polyangiitis; MPO= Myeloperoxidase; NA= Not applicable; PR3= Proteinase 3; RXT= rituximab.

Table 2. Territories and organs in which biopsies were performed according to the AAV disease and diagnostic yield of the histological findings.

Biopsies in AAV patients	**AVV patients**	**MPA**	**GPA**	***p-value***
Number of patients	152	77	75	
Biopsies performed; total number; mean (SD)	227; 1.5 (0.9)	114; 1.5 (0.9)	113; 1.5 (0.9)	0.722
Patients who underwent (any) biopsy; n (%)	142 (93.4)	74 (96.1)	68 (90.7)	0.176
Patients who underwent ≥2 biopsies; n (%)	57 (37.5)	24 (31.2)	33 (44)	0.102
Patients with granulomatous lesions in biopsies; n (%)	24/142 (16.9)	**0/74 (0)**	**24/68 (35.3)**	**<0.001**
Patients with vasculitis in biopsies; n (%)	114/142 (80.3)	**72/74 (97.3)**	**42/68 (61.8)**	**<0.001**
Biopsies disclosing granulomatous lesions; n (%)	26/227 (11.5)	**0/114 (0)**	**26/113 (23)**	**<0.001**
Biopsies disclosing vasculitis; n (%)	148/227 (65.2)	**90/114 (78.9)**	**58/113 (51.3)**	**<0.001**
Biopsies performed (by territory) / Histological changes				
Renal	74 (48.7)	**52 (67.5)**	**22 (29.3)**	**<0.001**
Pauci-immune glomerulonephritis	72 (97.3)	52 (100)	20 (90.1)	
No vasculitis	2 (2.7)	0 (0)	2 (9.9)	
Lung	21 (13.8)	**6 (7.8)**	**15 (20)**	**0.035**
Small vessel vasculitis	1 (4.8)	0 (0)	1 (6.7)	
Vasculitis and granuloma	4 (19)	0 (0)	4 (26.7)	
Granuloma	3 (14.3)	0 (0)	3 (20)	
Other non-vasculitic lesions	6 (28.6)	3 (50)	3 (20)	
No lesions	7 (33.3)	3 (50)	4 (26.7)	
Ear, nose and throat	32 (21.1)	**0 (0)**	**32 (42.7)**	**<0.001**
Nasal mucosa*	26 (17.1)	**0 (0)**	**26 (34.7)**	**<0.001**
Small vessel vasculitis	2 (7.7)	-	2 (7.7)	
Vasculitis and granuloma	2 (7.7)	-	2 (7.7)	
Granuloma	8 (30.8)	-	8 (30.8)	
Chronic inflammation	14 (53.8)	-	14 (53.8)	
Subglottis	6 (3.9)	**0 (0)**	**6 (8)**	**0.013**
Chronic inflammation	6 (100)	-	6 (100)	
Orbit structures	3 (2)	**0 (0)**	**3 (4)**	0.118
Vasculitis and granuloma	2 (66.7)	-	2 (66.7)	
Vasculitis	1 (33.3)	-	1 (33.3)	
Salivary glands**	3 (2)	**0 (0)**	**3 (4)**	0.118
Vasculitis and granuloma	2 (66.7)	-	2 (66.7)	
Chronic inflammation	1 (33.3)	-	1 (33.3)	
Skin	19 (12.5)	**7 (9.1)**	**12 (16)**	0.227
Leukocytoclastic vasculitis	8 (42.1)	6 (85.7)	2 (16.7)	
Lymphocytic small vessel vasculitis	3 (15.8)	0 (0)	3 (25)	
Vasculitis and granuloma formation	3 (15.8)	0 (0)	3 (25)	
Granulomatous inflammation	2 (10.5)	0 (0)	2 (16.7)	
Other non-vasculitic lesions	3 (15.8)	1 (14.3)	2 (16.7)	
Muscle	37 (24.3)	**24 (31.2)**	**13 (17.3)**	**0.047**
Small/medium vessel vasculitis	24 (64.9)	17 (70.8)	7 (53.8)	
No vasculitis	13 (35.1)	7 (29.2)	6 (46.2)	
Sural nerve	18 (11.8)	**14 (18.2)**	**4 (5.3)**	**0.014**
Small/medium vessel vasculitis	12 (66.7)	9 (64.3)	3 (75)	
No vasculitis	6 (33.3)	5 (35.7)	1 (25)	
Temporal artery	20 (13.2)	**11 (14.3)**	**9 (12)**	0.811
Collateral small-vessel vasculitis	9 (45)	6 (54.5)	3 (33.3)	
Adventitial small-vessel vasculitis	1 (5)	0 (0)	1 (11.1)	
Granulomatous vasculitis of collateral branch)	3 (15)	0 (0)	3 (33.3)	
No vasculitis	7 (35)	5 (45.5)	2 (22.2)	

Note: Statistically significant results are shown in bold

Abbreviations: AAV= ANCA-associated vasculitis; GPA= Granulomatosis with polyangiitis; MPA: Microscopic polyangiitis.

Table 3. Main clinical, therapeutic and outcomes characteristics of patients with granulomatosis with polyangiitis (GPA) according to ANCA serotypes.

Characteristics	GPA PR3-ANCA	GPA MPO-ANCA	*p-value*	GPA Negative/ Atypical ANCA	*p-value*
Number of patients	33	25		17	
Age at diagnosis (years); median (IQR)	57 (43-68)	63 (41-76)	0.200	45 (37-65)	0.194
Women; n (%)	**11 (33.3)**	**16 (64)**	**0.020**	12 (70.6)	0.081
Time from symptoms onset to diagnosis (months); median (IQR)	4 (3-8)	6 (4-11.5)	0.001	13 (7.5-24)	0.001
Disease extent and severity					
Limited disease; n (%)	10 (30.3)	8 (32)	0.890	16 (94.1)	<0.001
Systemic disease; n (%)	23 (69.7)	17 (68)	0.890	1 (5.9)	<0.001
Initial BVAS; median (IQR)	19 (12-25)	17 (14-24)	0.694	**9 (8-13)**	**<0.001**
Clinical manifestations					
Constitutional symptoms; n (%)	23 (69.7)	19 (76)	0.595	**7 (41.2)**	**0.017**
Musculoskeletal; n (%)	17 (51.5)	16 (64)	0.342	8 (47.1)	0.474
Renal involvement; n (%)	16 (48.5)	8 (32)	0.207	**0 (0)**	**0.001**
Pulmonary involvement; n (%)	23 (69.7)	16 (64)	0.647	9 (52.9)	0.280
Ear nose and throat; n (%)	26 (78.8)	18 (72)	0.550	15 (88.2)	0.273
Eye and orbit; n (%)	15 (45.5)	10 (40)	0.678	6 (35.3)	0.565
Cutaneous involvement; n(%)	7 (21.2)	6 (24)	0.801	4 (23.5)	0.923
Neurological					
Peripheral nerve; n (%)	6 (18.2)	8 (32)	0.223	1 (5.9)	0.167
Central nervous system; n (%)	4 (12.1)	4 (16)	0.154	1 (5.9)	0.674
Cardiac; n (%)	1 (3)	1 (4)	0.841	0 (0)	0.438
Gastrointestinal; n (%)	0 (0)	1 (4)	0.431	0 (0)	0.586
Salivary gland; n (%)	2 (6.1)	3 (12)	0.643	2 (11.8)	0.695
Relapses, initial treatments and outcomes during the follow-up					
Relapse (any); n (%)	17 (51.5)	14 (56)	0.735	10 (58.8)	0.695
Relapse rate, per patient-year (95%CI)	0.13 (0.079-0.20)	0.15 (0.089-0.24)	0.667	0.15 (0.076-0.31)	0.832
Time to first relapse (months); median (IQR)	35 (7-79)	37 (16-72)	0.504	21 (7-50)	0.296
Induction with CYC/RTX ever; n (%)	**23 (69.7)**	**7 (28)**	**0.003**	**2 (11.8)**	**0.004**
Plasma exchange; n (%)	6 (18.2)	1 (4)	0.101	1 (5.9)	0.674
Chronic kidney disease	9 (27.3)	4 (16)	0.358	**0 (0)**	**0.032**
End-stage kidney failure	0 (0)	0 (0)	0.999	0 (0)	0.999
Deaths; n (%)	6 (18.2)	6 (24)	0.588	**0 (0)**	**0.041**
Mortality rate, per patient-year (95%CI)	0.014 (0.005-0.038)	0.035 (0.017-0.073)	0.155	0 (0)	0.994
Lost-to-follow-up; n (%)	1 (3)	1 (4)	0.841	2 (11.8)	0.219
Follow-up (months); median (IQR)	82 (22-161)	80 (37-132)	0.950	77 (27-156)	0.835

Notes: Statistically significant results are shown in bold; Patients with GPA and negative or atypical ANCA were compared with patients with positivity for both PR3-ANCA and MPO-ANCA.

Abbreviations: AAV= ANCA-associated vasculitis; ANCA= antineutrophil cytoplasmic antibodies; BVAS= Birmingham Vasculitis Activity Score; CI= Confidence interval; CYC= cyclophosphamide; GPA= Granulomatosis with polyangiitis; IQR= interquartile ranges [percentiles 25th - 75th]; MPA: Microscopic polyangiitis; MPO= Myeloperoxidase; PR3= Proteinase 3; RXT= rituximab.

Table 4. Predictors of relapse incidence using competing-risk regression model and predictors of survival using Cox proportional hazards model in the entire series of patients with MPA and GPA.

		Univariable analysis		Multivariable analysis	
Endpoint	**Predictors**	**sHR (95% CI)**	**p-value**	**sHR (95% CI)**	**p-value**
Cumulative incidence of relapse	Age at diagnosis ≥ 68 years	0.70 (0.43-1.14)	0.143	0.76 (0.47-1.24)	0.276
	Female sex	0.75 (0.46-1.18)	0.213		
	GPA (vs. MPA)	**1.92 (1.21-3.04)**	**0.005**	**1.70 (1.01-2.87)**	**0.047**
	PR3-ANCA (*vs*. MPO-ANCA)	1.48 (0.87-2.51)	0.146		
	Systemic disease	0.67 (0.40-1.13)	0.116	0.89 (0.48-1.66)	0.722
	Kidney involvement*	0.91 (0.57-1.44)	0.690		
	Baseline creatinine > 2.2 mg/dL	0.59 (0.31-1.15)	0.112	1.62 (0.94-2.79)	0.083
	Lung involvement (any)	1.53 (0.92-2.55)	0.105	0.61 (0.31-1.18)	0.143
	Lung, - alveolar hemorrhage	0.73 (0.39-1.42)	0.359		
	Lung - interstitial lung disease	0.62 (0.39-1.21)	0.164		
	Peripheral nerve involvement	0.84 (0.51-1.39)	0.498		
Overall survival	Age at diagnosis ≥68 years	**5.42 (2.51-11.71)**	**<0.001**	**3.87 (1.73-8.65)**	**0.001**
	Female sex	0.69 (0.35-1.38)	0.299		
	GPA (vs. MPA)	**0.42 (0.19-0.86)**	**0.018**	0.66 (0.31-1.41)	0.283
	PR3-ANCA (*vs*. MPO-ANCA)	0.40 (0.15-1.04)	0.060		
	Systemic disease	0.48 (0.57-3.86)	0.418		
	Kidney involvement*	0.83 (0.41-1.67)	0.604		
	Baseline creatinine > 2.2 mg/dL	1.08 (0.47-2.49)	0.861		
	Lung involvement (any)	1.07 (0.52-2.21)	0.859		
	Lung - alveolar hemorrhage	0.59 (0.18-1.96)	0.393		
	Lung - interstitial lung disease	**2.94 (1.41-6.13)**	**0.004**	1.78 (0.84-3,78)	0.132
	Peripheral nerve involvement	**3.16 (1.58-6.39)**	**0.001**	**2.29 (1.11-4.71)**	**0.024**

Note: Statistically significant results are shown in bold

* Presence of active urinalysis (microhematuria and proteinuria > 500 mg/day) with glomerulonephritis confirmed by renal biopsy (when performed).

Abbreviations: ANCA= antineutrophil cytoplasmic antibodies; CI confidence interval; GPA= granulomatosis with polyangiitis; MPA: microscopic polyangiitis; MPO= myeloperoxidase; PR3= proteinase 3; sHR= subhazard ratio.

Significance of clinical-immunological patterns and diagnostic yield of biopsies in microscopic polyangiitis and granulomatosis with polyangiitis

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Supplementary Methods

Definitions for disease assessment, biopsy procedures, and treatments used in patients with ANCA-associated vasculitis (AAV)

Disease extent and severity were defined as ENT limited and systemic disease, in parallel with the subgroups limited and severe adopted by the Wegener's Granulomatosis Etanercept Trial (WGET) [1], which also respectively corresponded to localized (for limited) or early-systemic and generalized or severe renal (for systemic) of the European Vasculitis (EUVAS) Study Group categorization [2]. Disease activity was assessed using the Birmingham Vasculitis Activity Score (BVAS; version 3) [3]. Disease remission was considered when BVAS was 0 with no active symptoms related to the vasculitis and when a prednisone dose ≤ 10 mg/day could be maintained for at least two months. Relapse was defined as the re-occurrence or worsening of disease activity attributable to active inflammation requiring for its control an increase of glucocorticoids or an escalation of treatment that included the change or addition of a different immunosuppressive agent [4].

Renal involvement was defined by an active urinalysis as the presence of microhematuria and proteinuria > 500 mg/day, and confirmed by renal biopsy (when performed). Chronic renal disease (CRD) was defined as a residual estimated glomerular filtration rate (eGFR) <50 ml/min [5, 6]. Ear, nose and throat (ENT) examination and biopsies, as well as paranasal sinuses, subglottic and orbital imaging studies, were performed when needed. Lung involvement was classified according to the respiratory symptoms (such as coughing, chest pain and/or hemoptysis), bronchoscopy findings (cytology and transbronchial biopsies), and imaging abnormalities on chest X-ray or chest-CT. Radiologic changes included lung infiltrates, nodules, ground-glass opacities (suggesting capillaritis and/or alveolar hemorrhage), pleural effusion and interstitial lung disease (ILD). Patients with a suspected ILD were assessed with high-resolution chest-CT and classified as usual interstitial pneumonitis (UIP), non-specific interstitial pneumonitis (NSIP) and organizing pneumonia (OP). Myalgia, arthralgia and/or arthritis were considered musculoskeletal manifestations. An electromyography (EMG) was performed in those patients presenting with symptomatic peripheral neuropathy symptoms. A muscle biopsy (if myalgia) with nerve biopsy (if pathologic EMG results) was performed to explore muscle and peripheral nerve involvement. Some patients underwent temporal artery biopsy because of any craniofacial manifestation in the setting of a suspected systemic vasculitis, which is finally part of an AAV. Histological changes in this subset of AAV patients have been described as vasculitis involving adventitial and collateral small vessels surrounding a non-inflamed temporal artery [7].

Patients were treated according to the European Alliance of Associations for Rheumatology (EULAR) recommendations for the management of AAV [8] and our own clinical guidelines for the diagnosis and treatment of AAV, published and updated in 2020 [9]. Overall, high-dose glucocorticoids and cyclophosphamide or rituximab were used for induction of remission in severe cases. Maintenance of remission consisted of tapering glucocorticoid doses in association with an additional immunosuppressive agent (such as methotrexate, azathioprine, mycophenolate mofetil or rituximab). Plasma exchange was used in patients with life-threatening disease, usually those with rapidly progressive glomerulonephritis and/or diffuse lung hemorrhage. Limited disease was treated with glucocorticoids plus an additional immunosuppressive agent, preferably methotrexate. Rituximab was mainly used for all types of refractory patients who were initially treated with a different therapeutic line. Limited ENT, endotracheal, endobronchial and ophthalmic involvement were additionally managed by every specialist with local interventional therapies when appropriate [10].

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Supplementary Tables

Table S1. Detailed clinical manifestations of the entire study population of AAV and by groups according to clinical diagnosis and ANCA serotypes

Clinical manifestations	AVV patients	MPA	GPA	*p-value*	MPO-ANCA	PR3-ANCA	*p-value*
Number of patients	152	77 (50.7)	75 (49.3)		99 (73.9)	35 (26.1)	
Constitutional symptoms; n (%)	103 (67.8)	54 (70.1)	49 (65.3)	0.527	71 (71.7)	25 (71.4)	0.797
Fever >37ºC	83 (54.6)	41 (53.2)	42 (56)	0.733	57 (57.6)	22 (62.9)	0.715
Weight loss >10%	62 (40.8)	**38 (49.4)**	**24 (32)**	**0.030**	47 (47.5)	11 (31.4)	0.079
Musculoskeletal; n (%)	98 (64.5)	**57 (74)**	**41 (54.7)**	**0.013**	**71 (71.7)**	**19 (54.3)**	**0.039**
Arthralgia	73 (48)	39 (50.6)	34 (45.3)	0.512	50 (50.5)	16 (45.7)	0.533
Arthritis	33 (21.7)	17 (22.1)	16 (21.3)	0.911	23 (23.2)	7 (20)	0.840
Myalgia	61 (40.1)	**41 (53.2)**	**20 (26.7)**	**<0.001**	**48 (48.5)**	**9 (25.7)**	**0.011**
Renal involvement; n (%)	81 (53.3)	**56 (72.7)**	**25 (33.3)**	**<0.001**	61 (61.6)	19 (54.3)	0.447
Pulmonary involvement; n (%)	98 (64.5)	50 (66.2)	48 (62.7)	0.904	65 (65.7)	25 (71.4)	0.680
Alveolar hemorrhage	19 (12.2)	12 (15.6)	7 (9.3)	0.244	13 (13.1)	6 (16.7)	0.601
Nodules	33 (21.8)	**5 (6.5)**	**33 (44)**	**<0.001**	**16 (16.2)**	**21 (60)**	**0.002**
Infiltrates	58 (38.2)	32 (41.6)	28 (37.3)	0.382	39 (39.4)	18 (51.4)	0.270
Pleural effusion	9 (5.9)	5 (6.5)	4 (5.3)	0.762	6 (6.1)	3 (8.6)	0.640
Interstitial lung disease	29 (19.1)	**23 (29.9)**	**6 (8)**	**0.001**	**26 (26.3)**	**2 (5.6)**	**0.008**
UIP pattern	14 (9.2)	**14 (18.2)**	**0**	**<0.001**	**14 (14.1)**	**0**	**0.021**
NSIP/OP pattern	15 (9.9)	9 (11.7)	6 (8)	0.446	12 (12.1)	2 (5.6)	0.352
Ear nose and throat; n (%)	59 (38.8)	**0 (0)**	**59 (78.7)**	**<0.001**	**19 (19.2)**	**27 (77.1)**	**<0.001**
Nasal crusting	31 (20.4)	**0 (0)**	**30 (40)**	**<0.001**	**8 (8.1)**	**17 (48.6)**	**<0.001**
Paranasal inflammation	55 (36.2)	**0 (0)**	**54 (72)**	**<0.001**	**17 (17.2)**	**24 (68.6)**	**<0.001**
Nasal septum perforation	9 (5.9)	**0 (0)**	**9 (12)**	**0.002**	**1 (1)**	**6 (16.7)**	**<0.001**
Saddle nose	5 (3.3)	**0 (0)**	**5 (6.7)**	**0.021**	1 (1)	2 (5.6)	0.113
Serous otitis	23 (15.1)	**0 (0)**	**23 (20.7)**	**<0.001**	**9 (9.1)**	**9 (25.7)**	**0.016**
Subglottic stenosis	10 (6.6)	**0 (0)**	**10 (13.3)**	**0.001**	2 (2)	3 (8.6)	0.086
Eye and orbit; n (%)	39 (25.7)	**8 (10.4)**	**31 (41.3)**	**<0.001**	**18 (18.2)**	**15 (42.9)**	**0.005**
Orbital mass	10 (6.6)	**0 (0)**	**10 (13.3)**	**0.001**	3 (3)	4 (11.1)	0.061
Dacryocystitis	7 (4.6)	**0 (0)**	**7 (9.3)**	**0.006**	3 (3)	2 (5.6)	0.492
Keratoconjunctivitis	10 (6.6)	**2 (2.6)**	**8 (10.7)**	**0.045**	6 (6.1)	4 (11.1)	0.322
Scleritis/Episcleritis	17 (11.2)	**5 (6.5)**	**13 (17.3)**	**0.039**	**9 (9.1)**	**8 (22.9)**	**0.042**
Uveitis	2 (1.3)	0 (0)	2 (2.7)	0.149	**0 (0)**	**2 (5.6)**	**0.018**
Optic neuritis	7 (4.6)	**1 (1.3)**	**6 (8)**	**0.049**	4 (4)	3 (8.6)	0.320
Cutaneous involvement; n (%)	39 (25.7)	22 (28.6)	17 (22.7)	0.405	26 (26.3)	8 (22.9)	0.691
Purpura	31 (20.4)	**21 (27.3)**	**10 (13.3)**	**0.033**	22 (22.2)	6 (17.1)	0.525
Other skin lesions	8 (5.3)	**1 (1.3)**	**7 (9.3)**	**0.033**	4 (4)	2 (5.7)	0.651
Neurological							
Peripheral nerve; n (%)	47 (30.9)	**32 (41.6)**	**15 (20)**	**0.004**	28 (38.4)	8 (22.9)	0.080
Paresthesia	36 (23.7)	**25 (32.5)**	**11 (14.7)**	**0.024**	38 (28.3)	6 (16.7)	0.169
Abnormal EMG	47/120 (39.2)	**14/51 (27.5)**	**33/69 (47.8)**	**0.002**	37/87 (42.5)	8/23 (34.8)	0.502
Polyneuropathy	25 (16.4)	16 (20.8)	9 (12)	0.144	18 (18.2)	6 (16.7)	0.839
Mononeuritis multiplex	22 (14.5)	**17 (22.1)**	**5 (6.7)**	**0.007**	19 (19.2)	2 (5.6)	0.053
Central nervous system; n (%)	12 (7.9)	3 (3.9)	9 (12)	0.064	7 (7.1)	4 (11.1)	0.448
Cranial nerve palsy	6 (3.9)	2 (2.6)	4 (5.3)	0.386	5 (19.2)	0 (0)	0.169
Pachymeningitis	3 (2)	0 (0)	3 (4)	0.076	1 (1)	2 (5.6)	0.113
Stroke	2 (1.3)	0 (0)	2 (2.7)	0.149	**0 (0)**	**2 (5.6)**	**0.018**
Cardiac; n (%)	7 (4.6)	5 (6.5)	2 (2.7)	0.442	6 (6.1)	1 (2.8)	0.675
Gastrointestinal; n (%)	3 (2)	2 (2.6)	1 (1.3)	0.575	3 (3)	0 (0)	0.564
Salivary gland; n (%)	7 (4.6)	**0 (0)**	**7 (9.3)**	**0.006**	3 (3)	2 (5.7)	0.492

Note: Statistically significant results are shown in bold

Abbreviations: AAV= ANCA-associated vasculitis; ANCA= antineutrophil cytoplasmic antibodies; GPA= Granulomatosis with polyangiitis; MPA: Microscopic polyangiitis; MPO= Myeloperoxidase; NSIP= non-specific interstitial pneumonitis; OP= organizing pneumonia; PR3= Proteinase 3; UIP= Usual interstitial pneumonitis.

Table S2. Detailed clinical manifestations of patients with granulomatosis with polyangiitis (GPA) according to ANCA serotypes.

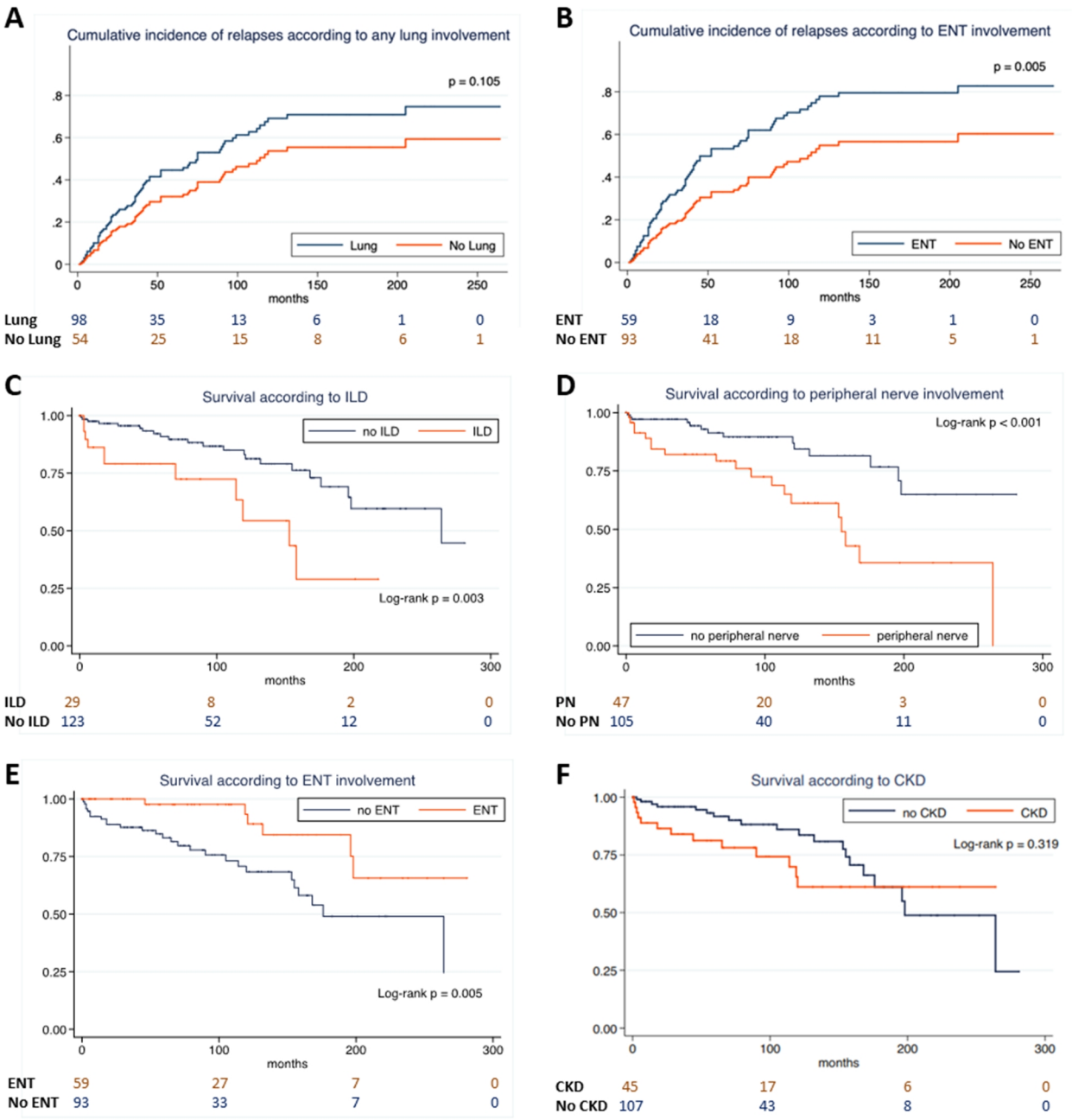
Clinical manifestations	GPA PR3-ANCA	GPA MPO-ANCA	*p-value*	GPA Negative/ Atypical ANCA	*p-value*
Number of patients	33	25		17	
Constitutional symptoms; n (%)	23 (69.7)	19 (76)	0.595	**7 (41.2)**	**0.017**
Fever >37ºC	20 (60.6)	17 (68)	0.562	**5 (29.4)**	**0.012**
Weight loss >10%	9 (27.3)	12 (48)	0.104	3 (17.6)	0.149
Musculoskeletal; n (%)	17 (51.5)	16 (64)	0.342	8 (47.1)	0.474
Arthralgia	14 (42.4)	12 (48)	0.672	8 (47.1)	0.871
Arthritis	5 (15.2)	8 (32)	0.128	3 (17.6)	0.673
Myalgia	8 (24.2)	8 (32)	0.513	4 (23.5)	0.739
Renal involvement; n (%)	16 (48.5)	8 (32)	0.207	**0 (0)**	**0.001**
Pulmonary involvement; n (%)	23 (69.7)	16 (64)	0.647	9 (52.9)	0.280
Alveolar hemorrhage	**6 (18.2)**	**0 (0)**	**0.024**	1 (5.9)	0.578
Nodules	15 (45.5)	10 (40)	0.678	8 (47.1)	0.773
Infiltrates	16 (48.5)	8 (32)	0.207	**2 (11.8)**	**0.040**
Pleural effusion	3 (9.1)	1 (4)	0.627	0 (0)	0.568
Interstitial lung disease	2 (6.1)	3 (12)	0.643	1 (5.9)	0.714
UIP pattern	0 (0)	0 (0)	0.999	0 (0)	0.999
NSIP/OP pattern	2 (6.1)	3 (12)	0.643	1 (5.9)	0.714
Ear nose and throat; n (%)	26 (78.8)	18 (72)	0.550	15 (88.2)	0.273
Nasal crusting	16 (48.5)	9 (36)	0.342	5 (29.4)	0.311
Paranasal inflammation	23 (69.7)	16 (64)	0.647	15 (88.2)	0.090
Nasal septum perforation	9 (27.3)	9 (36)	0.477	2 (11.8)	0.973
Saddle nose	2 (6.1)	1 (4)	0.726	2 (11.8)	0.317
Serous otitis	2 (6.1)	3 (12)	0.643	5 (29.4)	0.898
Subglottic stenosis	3 (9.1)	2 (8)	0.883	**5 (29.4)**	**0.027**
Eye and orbit; n (%)	15 (45.5)	10 (40)	0.678	6 (35.3)	0.565
Orbital mass	4 (12.1)	3 (12)	0.989	3 (17.6)	0.552
Dacryocystitis	2 (6.1)	3 (12)	0.643	2 (11.8)	0.695
Keratoconjunctivitis	4 (12.1)	4 (16)	0.671	0 (0)	0.186
Scleritis/Episcleritis	8 (24.2)	4 (16)	0.443	1 (5.9)	0.275
Uveitis	2 (6.1)	0 (0)	0.501	0 (0)	0.438
Optic neuritis	3 (9.1)	3 (12)	0.719	0 (0)	0.327
Cutaneous involvement; n(%)	7 (21.2)	6 (24)	0.801	4 (23.5)	0.923
Purpura	5 (15.2)	3 (12)	0.703	2 (11.8)	0.829
Other skin lesions	2 (6.1)	3 (12)	0.643	2 (11.8)	0.653
Neurological					
Peripheral nerve; n (%)	6 (18.2)	8 (32)	0.223	1 (5.9)	0.167
Paresthesia	5 (15.2)	5 (20)	0.628	1 (5.9)	0.438
Abnormal EMG	1 (3)	4 (16)	0.154	1 (5.9)	0.168
Polyneuropathy	5 (15.2)	3 (12)	0.730	1 (5.9)	0.674
Mononeuritis multiplex	1 (3)	4 (16)	0.154	0 (0)	0.582
Central nervous system; n (%)	4 (12.1)	4 (16)	0.154	1 (5.9)	0.674
Cranial nerve palsy	0 (0)	3 (12)	0.075	1 (5.9)	0.909
Pachymeningitis	2 (6.1)	1 (4)	0.726	0 (0)	0.339
Stroke	2 (6.1)	0 (0)	0.501	0 (0)	0.438
Cardiac; n (%)	1 (3)	1 (4)	0.841	0 (0)	0.438
Gastrointestinal; n (%)	0 (0)	1 (4)	0.431	0 (0)	0.586
Salivary gland; n (%)	2 (6.1)	3 (12)	0.643	2 (11.8)	0.695

Notes: Statistically significant results are shown in bold; Patients with GPA and negative or atypical ANCA were compared with patients with positivity for both PR3-ANCA and MPO-ANCA.

Abbreviations: AAV= ANCA-associated vasculitis; ANCA= antineutrophil cytoplasmic antibodies; GPA= Granulomatosis with polyangiitis; MPA: Microscopic polyangiitis; MPO= Myeloperoxidase; NSIP= non-specific interstitial pneumonitis; OP= organizing pneumonia; PR3= Proteinase 3; UIP= Usual interstitial pneumonitis.

SUPPLEMENTARY FIGURES

Figure S1. Estimated cumulative incidence of relapses and survival during the follow-up according to specific organ involvement.



Supplementary Figure S2. Estimated cumulative incidence of relapses and survival in GPA subgroups during the follow-up according to ANCA serotypes.