ANCA-positive and ANCA-negative phenotypes of eosinophilic granulomatosis with polyangiitis (EGPA): outcome and long-term follow-up of 50 patients from a single Polish centre

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ABSTRACT

Objective. *The aim of the study was to compare the course of the disease and treatment outcomes in ANCA-positive and ANCA-negative eosinophilic granulomatosis with polyangiitis (EGPA) patients from one Polish tertiary referral centre.*

Methods. *Retrospective and prospective cohort study carried out on 50 patients treated in our department between 1998 and 2012. EGPA diagnosis was based on the American College of Rheumatology (ACR) criteria. Treatment protocol was based primarily on the predictive Five Factor Score (FFS) scale. Clinical characteristics of the patients, general symptoms, organ involvement, treatment regimen, and follow-up outcomes were evaluated according to ANCA status.*

Results. *Fifteen ANCA-positive patients and 35 ANCA-negative patients were enrolled. At the time of diagnosis ANCA-positive patients had a higher incidence of renal involvement (53% vs. 7.7%; p<0.001), skin involvement (93.3% vs. 57.1%; p=0.03), and peripheral neuropathy in the form of mononeuritis multiplex (60% vs. 25.7%; p=0.021). ANCA-negative patients had significantly more frequent cardiac manifestations, but only with regard to the entire period of follow-up (68.6% vs. 33.3%; p=0.021). Patients in both groups were under the same treatment regimens, however steroid dose necessary to maintain remission of the disease was significantly higher in the group of ANCA-positive patients (9±2.5 vs. 7.4±1.9 mg/day of methylprednisolone; p=0.023). The presence of ANCA did not affect the frequency of relapses.*

Conclusion. *Our results confirm the differences in clinical disease presentation based on ANCA status and indicate that ANCA-positive patients should be treated more aggressively.*

Introduction

Eosinophilic granulomatosis with polyangiitis (EGPA), previously named Churg-Strauss syndrome (CSS), is a rare (with an annual incidence of 0.5–6.8 cases/10^6 inhabitants), systemic, necrotising, small and medium vessel vasculitis (1-4). Despite growing knowledge about the disease and development of specific therapeutic schemes, it is still considered to be a severe and often life-threatening disease (1, 5, 6). Although its pathogenesis still remains unclear (7), ANCA (anti-neutrophil cytoplasmic antibodies) are present in about 40% of cases, suggesting an underlying autoimmunological pathogenesis (1). The primary treatment of EGPA includes corticosteroids with or without additional immunosuppressant, depending on specific indications (8, 9). Because ANCA are not always present recently attention has been drawn to the fact that there are two clinically relevant phenotypes of EGPA, namely ANCA-positive and ANCA-negative (8, 10-13). Due to the rarity of the disease until now only a few case series were published and they often contained clinical assessment only at diagnosis, without further follow-up and outcome, and often without taking into account ANCA-status (13-20).

The first case series regarding EGPA was published in 1951 and reported of 13 deaths which occurred in patients with necrotising vasculitis, severe asthma, fever, blood hypereosinophilia and eosinophilic infiltrates in tissues (14). Subsequent case series usually contained over a dozen patients from one centre and most of them were published before 2002 (15-20). The two

largest case series about EGPA were published in 2012, of which one reports on 386 patients with regard to their ANCA-phenotypes from several dozen centres associated with the French Vasculitis Study Group (21) and the second on 150 patients with EGPA comes from one German centre and contains outcomes based on an original treatment scheme but without division into ANCA-phenotypes (22). In a few articles concerning ANCA-phenotypes published to date differences in organ involvement at the time of diagnosis were reported, but it is still unclear whether it has a significant impact on the disease course or prognosis and whether the treatment regimen should be adapted to reflect the ANCA-phenotype (10-12). In this article, we present a case series of 50 EGPA patients from one centre with division into ANCA-phenotypes, and the entire course of the disease and outcomes, outlining our observations from the last 14 years and our own experience in the treatment of EGPA.

Subjects and methods

The study was conducted in the 2nd Department of Internal Medicine, Jagiellonian University Medical College in Krakow. Since 2006 we thoroughly searched our medical records in order to identify new patients with EGPA diagnosis and then we followed them in our outpatient clinic and regularly entered their data into an EGPA registry. In addition, we searched archived data in order to identify patients with EGPA diagnosed between 1998 to 2006 that remain in the care of our department. These patients were also enrolled in our study. Only patients with a comprehensive history of the disease enabling its precise assessment, and only those of them who were regularly followed-up within our outpatient clinic were included in the study. Based on this, we were able to assess the clinical data, both retrospectively and prospectively. In all patients the diagnosis of EGPA was verified prior to entry into our database. Due to the lack of diagnostic criteria for EGPA, the diagnosis was each time confirmed based mainly on applying the American College of Rheumatology (ACR) classification criteria from 1990 (23). According to them the diagnosis of EGPA is established when at least four out of six criteria are fulfilled: asthma, peripheral blood eosinophilia >10%, mono or polyneuropathy, chronic paranasal sinusitis, extravascular eosinophils revealed on biopsy, and migratory infiltrates in the lungs. The demographic characteristics and all available medical documents were reviewed, including clinical, radiologic, and laboratory results, as well as histological findings. In addition, each patient was questioned during follow-up visits concerning the course of the disease and treatment. A retrospective and prospective analysis was performed on the EGPA patients' data entered into our EGPA registry between 1998 and 2012. Furthermore, since 2006, regular training of patients with EGPA on the nature of their disease as well as how to identify and treat exacerbations was initiated. Each patient could contact our centre in case of any alarming symptoms and was given medical attention immediately. Our centre has developed a treatment protocol based primarily on the FFS predictive scale (Five Factor Score – one point for each of the following: severe vasculitis-related gastrointestinal involvement and/or cardiac involvement, serum creatinine level >150 umoles/litre, age older than 65, and/or absence of ear, nose, and throat [ENT] symptoms) (24). Patients with FFS=0 were treated with steroids only and patients with FFS ≥1 were treated with steroids and an immunosuppressive agent. An additional immunosuppressant was also included in the treatment of the patients diagnosed with peripheral nervous system involvement, as well as in those in which during steroid dose reduction (<10 mg of methylprednisolone/day) we were not able to achieve permanent remission of the disease.

The method of treatment was entered into the database and the minimum dose of steroids sufficient for controlling the disease was determined for each patient.

Based on laboratory results performed at the time of EGPA diagnosis, patients were divided into two study groups: ANCA-positive and ANCA-negative, which was defined as the documented absence of ANCA by using an IF (immunofluorescence) assay. Positive ANCA status was defined as the documented presence of anti-MPO (against myeloperoxidase) or anti-PR3 (against proteinase-3) on ELISA (enzyme-linked immunosorbent assay), regardless of the staining pattern on IF. A positive IF assay result for ANCA without confirmation of a positive anti-MPO or anti-PR3 result was considered indeterminate. Additionally, ANCA test results were analysed during the period of further observation and were re-perfomed during EGPA relapses and remissions.

The same parameters were evaluated in both groups. In each case we identified the exact time and age of diagnosis of EGPA as well as the time of initiation of treatment and its scheme, drugs administered before the diagnosis, and the period of time when asthma was present before the diagnosis was established. Additionally, history of atopy was assessed, with atopy being defined as a positive allergic history accompanied by positive skin prick tests for standard inhaled allergens. Moreover, we determined the order of appearance of the symptoms included in the classification criteria for EGPA. We retrospectively assessed the severity of asthma at the time of asthma diagnosis according to the guidelines of the National Heart, Lung and Blood Institute/National Asthma Education and Prevention Program (2007) in both groups (25). Asthma severity was assessed on the basis of complete medical documentation (received from the pulmonology clinics formerly treating each patient), which included a detailed description of clinical symptoms, spirometry results, and course of asthma treatment. In the course of further observation the degree of asthma control was assessed using current GINA (The Global Initiative for Asthma) guidelines. We also estimated (retrospectively in patients diagnosed before 2006 and during the first visit in patients diagnosed after 2006) the activity of EGPA according to the Birmingham Vasculitis Activity Score *(BVAS)* version 3 (26) and the prognosis based on the revisited Five Factor Score (24). Furthermore, we assessed general

symptoms and organ involvement at the moment of diagnosis and during the entire observation as well as organ involvement during the last follow-up visit. General symptoms were defined as fever ≥38.0°C, weight loss of >3 kg over 3 months preceding diagnosis, and general malaise. As for organ involvement, the following were investigated: heart, lung, kidney, gastrointestinal system (GIS), sinuses, central nervous system (CNS), peripheral nervous system (PNS), and skin. Cardiac involvement was diagnosed based on clinical findings and available ECG, echocardiography, MRI, and troponin I test results, only if present abnormalities could not be explained by any other cause. Lung involvement was diagnosed when chest x-ray and/or computed tomography revealed non-fixed pulmonary infiltration suggestive of eosinophilic pneumonia or eosinophilic and aseptic pleural effusion. The kidney was regarded as being involved if a patient had proteinuria ≥1 g/day and/or ≥10 red blood cells per high power field (hpf) in urinalysis or the patient's serum creatinine increased by ≥30%. GIS involvement was diagnosed based on the following symptoms: abdominal pain, diarrhea, gastrointestinal bleeding, perforation of the intestine during active period of disease and after exclusion of other causes, or confirmed by histopathological examination. Sinusitis was diagnosed based on clinical features and CT scan results. PNS involvement was diagnosed if new mononeuropathy or multiple mononeuropathies occurred, which coincided with the presence of eosinophilia or other systemic symptoms. CNS involvement was diagnosed when new symptoms of CNS damage occurred and were confirmed by means of computed tomography and/or magnetic resonance imaging together with an opinion of the consulting neurologist. Skin involvement was diagnosed if during the active period of EGPA new skin lesions occurred and could not be explained by any other cause, or confirmed by histopathological examination. When atypical disease symptoms were present they were classified as manifestation of EGPA only if no alternative cause was apparent. In addition, blood eosinophil count was examined at the moment of diagnosis just before initiation of immunosuppressive treatment. We evaluated the frequency of relapses per year. Relapse was defined as either the recurrence or worsening of EGPA manifestation following a stable remission, the need for the addition of immunosuppressive drugs, or an increase in steroid dosage of more than twice the previous dosage, resulting in a total dosage of >30 mg/day. An increase in the eosinophil count without any other clinical EGPA manifestation, or only isolated asthma exacerbation or sinusitis or rhinitis exacerbation with or without blood eosinophilia was not considered a relapse. Additionally, the scheme of immunosuppressive therapy during the entire course of the disease in all patients was analysed. For every patient we assessed the minimum dosage of glucocorticoids needed to maintain remission and whether additional immunosuppressive therapy was necessary to achieve that remission. At the last follow-up the number of patients in stable remission was evaluated. Remission was defined as the period of disease without symptoms of active disease, *i.e.* BVAS of 0 for at least 3 consecutive months. Moreover at the last follow-up the symptoms of persistent organ involvement were assessed. Studied outcomes were also termination of treatment with glucocorticoids, death and its causes, and cancers occurring during the follow-up period. Prior to inclusion in the study, all participants gave an informed consent to participate. The study protocol complied with the Helsinki Declaration and was approved by the Jagiellonian University Ethics Committee.

Statistical analysis

Statistical analysis was performed using StatSoft, Inc. (2009). STATISTICA (data analysis software system), version 9.0. www.statsoft.com. Data was checked for normality and was presented as the mean ± SD or median with interquartile range, when applicable. The follow-up time in the study was presented as the median in months along with the the 25th and 75th percentiles of this value. The statistical analysis was performed with Student's *t*-test, Mann-Whitney-U test, and Fisher's exact test (with Yates correction if applicable). *p*-values less than 0.05 were considered statistically significant.

Results

Fifty-two EGPA patients were identified in our hospital's medical database. All patients met ACR classification of EGPA (CSS) criteria. Two patients were excluded from further analysis because they lacked ANCA testing documentation from the EGPA diagnosis period. Additionally, one of them had not had follow-up visits in our hospital. In the end, we included 50 patients (34 women, 16 men, mean age at diagnosis of 41.2±14.7 years). Fifteen (30%) of them were ANCA-positive by IF with four (26.7%) positive for cANCA and ten (66.7%) positive for pANCA. One patient (6.6%) was both c/pANCA-positive. MPO was identified by ELISA in fourteen patients, and PR3 in one. Each patient included had medical records sufficient for analysis and underwent follow-up visits at our institution. Moreover, all of them have completed all questionnaires supplied during the follow-up visits. In the course of further observation 38 patients (76%) were retested for ANCA during EGPA relapse, but also during remission of the disease. The results of these tests did not influence the patients' phenotype categorisation; no ANCA antibodies appeared during the period of observation in patients who were originally ANCA-negative. Clinical characteristics at diagnosis are presented in Table I. We found that the order of appearance of subsequent symptoms was similar in the majority of patients (n=46; 92%): sinusitis at the beginning, followed by asthma, eosinophilia, and pulmonary infiltrates. At EGPA diagnosis, patients in both groups did not differ significantly in disease activity (BVAS score) or prognosis based on the FFS scale. In all patients during the course of the disease, asthma and eosinophilia were reported. At the time of EGPA diagnosis, the ANCA-negative patient group had a higher level of eosinophilia in the peripheral blood as compared to the ANCA-positive patient group. Further-

Table I. Clinical characteristics of the EGPA patients at the time of diagnosis.

	Group 1 ANCA-positive (n=15)	Group 2 ANCA-negative (n=35)	*p*-value
Sex, no. (female/male)	9/ 6	25/ 10	NS
Age at the diagnosis, mean ± SD [years]	49.9 ± 15.5	40 ± 13.4	0.048
EGPA duration, mean ± SD [months]	54.6 ± 38.6	74.6 ± 43.6	NS
Number of ACR criteria fulfilled	4.87 ± 0.6	4.74 ± 0.7	NS
Four: n (%)	4 (26.7)	14 (40)	
Five: n (%)	9 (60)	16 (45.7)	
Six: n (%)	2 (13.3)	5 (14.3)	
BVAS 23 ± 6.1	23 ± 6	NS	
FFS	0.4 ± 0.5	0.34 ± 0.5	NS
0: n (%)	9 (60)	23 (65.7)	
1: n (%)	6 (40)	12 (34.3)	
≥: n (%)	0	0	
Maximal blood eosinophilia, median [25-75 percentile] [cell/μl]	4833 [2318–7800]	8000 [5000–11000]	0.015
General symptoms			
weight loss, n (%)	11 (73.3)	28 (80)	NS
fever, n (%)	13 (86.7)	31 (88.6)	NS
Atopy, n (%)	6 (40)	21 (60)	NS
Asthma, n (%)	15 (100)	35 (100)	NS
Asthma duration before EGPA diagnosis, median [25-75 percentile] [months]	24 [12–48]	29 [12–50]	NS
Asthma severity at the time of diagnosis:	2.6 ± 0.6	3.8 ± 0.4	<0.001
Step 1, n (%)	1 (6.7%)	0	
Step 2, n (%)	4 (26.7%)	0	
Step 3, n (%)	10 (66.6%)	8 (22.9%)	
Step 4, n (%)	0	27 (77.1%)	

Data expressed as mean ± standard deviation, median [25-75 interquartile range] or n (%).
ACR: American College of Rheumatology; BVAS: Birmingham Vasculitis Activity Score; EGPA: Eosinophilic Granulomatosis with Polyangiitis; FFS: Five Factor Score; ANCA: anti-neutrophil cytoplasmic antibodies.

more the ANCA-negative patient group had a higher percentage of severe asthma prior to EGPA (77% *vs*. 0%) (see Table I). At the time of asthma diagnosis all ANCA-negative patients (100%) were being treated with large doses of inhaled steroids and long-acting beta-adrenoceptor agonists (LABA). In the ANCA-positive group, 10 patients were receiving large doses of inhaled steroids, 4 patients were receiving medium doses of inhaled steroids, 1 patient was receiving small doses of inhaled steroids, and 12 patients (80%) were receiving LABA. Furthermore, 19 ANCA-negative and 4 ANCA-positive patients were being treated with leukotriene receptor antagonists (montelukast). During the course of further observation, despite treatment before EGPA diagnosis both groups experienced frequent asthma exacerbations. At the time of EGPA diagnosis, asthma was uncontrolled in every patient, with the exception of one ANCA-positive patient. Moreover, at the time of EGPA diagnosis 15 patients (43%) in the ANCA-positive group and 5 patients (30%) in the ANCA-negative group additionally required oral steroids to control their asthma symptoms. Interestingly, after EGPA diagnosis and initiation of treatment, asthma symptoms were alleviated and a gradual decline in symptoms was observed. At the time of the last follow-up, in both groups of EGPA patients asthma was well controlled, only 30 patients total (60%) required treatment with inhaled steroids, of which 20 patients (40%) were on small doses and 10 patients (20%) were on medium doses. There was no significant difference in the required dosage of inhaled steroids observed between ANCA groups. Clinical characteristics of organ involvement at the time of EGPA diagnosis and organ involvement at any time during the entire course of EGPA are presented in Table II. Median time of the entire observation for all patients was 60 [24-96.5] months. In diagnosing organ involvement the following medical imaging tests were used: all patients underwent chest x-ray, chest CT, echocardiogram, and 43 patients underwent MRI.

At the time of diagnosis patients in both groups differed in the incidence of renal, skin, and peripheral nervous system involvement, but only in the case of mononeuritis multiplex in the latter. During follow-up, when relapses were observed, subsequent organs and systems were involved except for the skin and ENT. Significant differences in heart involvement in both groups emerged, being more frequent in the ANCA-negative group. We also observed that peripheral neuropathy was significantly more resistant to treatment and the symptoms did not subside as easily in the ANCA-positive group when compared to ANCA-negative patients. At the last follow-up visit in all ANCA-positive patients mononeuritis multiplex was still present, whereas in the ANCA-negative group it withdrew in all but one person (60% *vs*. 2.8%, $p<0.001$). Also in this group the symptoms of renal involvement subsided with more difficulty. During the last follow-up visit, symptoms of renal involvement were still present in two patients (13.35%) in the ANCA-positive group but in none of the ANCA-negative group ($p=0.016$). Furthermore, at the last follow-up, all patients were in remission except one ANCA-positive patient and two ANCA-negative patients. They were diagnosed with exacerbation of the disease, but did not fulfill the criteria for relapse. The most common patient complaint during the last follow-up visit were ENT-related symptoms with almost no complaints related to asthma.

All patients in both groups were treated accordingly to the current standards. Corticosteroids treatment was initiated shortly after establishing the diagnosis in all patients. There were no significant differences between initial doses of corticosteroids in all subjects. Additional immunosuppressive therapy was started in all patients with FFS ≥1

Table II. Clinical characteristics of organ involvement in the course of EGPA at diagnosis and organ involvement at any time during entire observation.

Characteristic	Group 1 ANCA-positive (n=15)	Group 2 ANCA-negative (n=35)	*p*-value	Group 1 ANCA-positive (n=15)	Group 2 ANCA-negative (n=35)	*p*-value
	At the time of EGPA diagnosis			Organ involvement at any time during entire observation		
ENT manifestation	15 (100)	34 (97.1)	NS	15 (100)	34 (97.1)	NS
Rhinitis, n (%)	14 (93.4)	34 (97.1)	NS	14 (93.4)	34 (97.1)	NS
Sinusitis, n (%)	15 (100)	34 (97.1)	NS	15 (100)	34 (97.1)	NS
Polyposis, n (%)	7 (46.7)	17 (48.6)	NS	7 (46.7)	17 (48.6)	NS
Lung manifestation	15 (100)	35 (100)	NS	15 (100)	35 (100)	NS
Lung infiltrates, n (%)	14 (93.3)	35 (100)	NS	14 (93.3)	35 (100)	NS
Pleural effusion, n (%)	5 (33.3)	13 (37.1)	NS	5 (33.3)	13 (37.1)	NS
Alveolar haemorrhage, n (%)	2 (13.3)	2 (5.7)	NS	3 (20)	3 (8.6)	NS
Cutaneous manifestation	14 (93.3)	20 (57.1)	0.03	14 (93.3)	20 (57.1)	0.03
Purpura, n (%)	4 (26.7)	3 (8.6)	NS	4 (26.7)	3 (8.6)	NS
Pseudo-urticarial rush, n (%)	4 (26.7)	3 (8.6)	NS	4 (26.7)	3 (8.6)	NS
Subcutaneous nodule(s), n (%)	1 (6.7)	4 (11.4)	NS	1 (6.7)	4 (11.4)	NS
Livedo reticularis, n (%)	1 (6.7)	0	NS	1 (6.7)	0	NS
Erythema, n (%)	1 (6.7)	0	NS	1 (6.7)	0	NS
Maculopapular rash, n (%)	3 (20)	10 (28.6)	NS	3 (20)	10 (28.6)	NS
Neurologic symptoms	10 (66.7)	16 (45.7)	NS	10 (66.7)	17 (48.6)	NS
Peripheral neuropathy, n (%)	10 (66.7)	16 (45.7)	NS	10 (66.7)	17 (48.6)	NS
Mononeuritis multiplex, n (%)	9 (60)	9 (25.7)	0.021	9 (60)	10 (28.6)	NS
CNS involvement, n (%)	1 (6.7)	0	NS	1 (6.7)	3 (8.6)	NS
Cardiac manifestations	5 (33.3)	22 (62.9)	NS	5 (33.3)	24 (68.6)	0.021
Endocarditis, n (%)	0	2 (5.7)	NS	0	2 (5.7)	NS
Myocarditis, n (%)	5 (33.3)	12 (34.3)	NS	5 (33.3)	14 (40)	NS
Pericarditis, n (%)	4 (26.7)	15 (42.8)	NS	4 (26.7)	16 (45.7)	NS
Gastrointestinal involvement	5 (33.3)	12 (33.3)	NS	6 (40)	15 (42.8)	NS
Abdominal pain, n (%)	4 (26.7)	8 (22.9)	NS	5 (33.3)	11 (31.4)	NS
Diarrhea, n (%)	4 (26.7)	6 (17.1)	NS	5 (33.3)	9 (25.7)	NS
Gastrointestinal bleeding, n (%)	3 (20)	2 (5.7)	NS	3 (20)	3 (8.6)	NS
Renal manifestations	8 (53.3)	2 (5.7)	< 0.001	11 (73.3)	3 (8.6)	< 0.001
Proteinuria >1g/24 hours, n (%)	3 (20)	1 (2.9)	NS	4 (26.7)	1 (2.9)	0.04
Haematuria, n (%)	5 (33.3)	1 (2.9)	0.01	6 (40)	1 (2.9)	0.002
Increased level of creatinine, n(%)	0	0	NS	1 (6.7)	1 (2.9)	NS
OTHER:	At the time of diagnosis			During last follow-up visit		
Frequency of EGPA relapses per year], median [25-75 percentile]	–	–	–	0.7 [0.3–2]	1 [0.7–1.5]	NS
Minimal doses of oral corticosteroids converted to methylprednisolone needed for maintenance of remission [mg/day], mean ± SD	NA	NA	NA	9 ± 2.5	7.4 ± 1.9	0.023
History of additional immunosupressants, n (%)	10 (66.7)	16 (45.7)	NS	8 (53.3)	17 (48.6)	NS

Data expressed as mean ± standard deviation, median [25-75 interquartile range] or n (%).
EGPA: Eosinophilic Granulomatosis with Polyangiitis; ANCA: anti-neutrophil cytoplasmic antibodies, ENT: ears, nose, and throat; CNS: central nervous system; GIS: gastrointestinal system.

(except for one patient where additional immunosuppression was impossible due to recurrent severe infections) and in patients with severe symptoms of peripheral neuropathy. One patient with FFS=0 was also treated with an extra immunosuppressant, because it was impossible to safely reduce oral steroids. During follow-up observations, we noticed that steroid dose (converted to methylprednisolone) necessary to sustain remission of the disease was significantly higher in the group of ANCA-positive patients. The presence of ANCA did not affect the frequency of relapses.

The outcome analysis revealed that in none of the patients was steroid therapy discontinued. In the ANCA-negative group one patient died due to pulmonary embolism while in remission. In the same group, two patients were diagnosed with cancer (basal cell skin carcinoma and colon cancer) and both were

effectively treated. The 5-year survival among those who have completed five years of follow-up was 96% (22 out of 23 patients; 1 death in the 4th year of observation due to pulmonary embolism) in the ANCA-negative group and 100% in the ANCA-positive group (4 patients). All ten patients who completed the 10-year follow-up are still alive.

Discussion

The presented description of a single-centre case series of 50 EGPA patients, is to our knowledge one of the largest monocentric studies (13, 15, 16, 19, 20, 22), and the largest which compares the outcomes of patients based on their ANCA-phenotype. As indicated by our study, when the IF screening test for the presence of ANCA is positive, it should always be additionally verified using ELISA, which determines the type of antibodies more precisely and accurately. In three of four of our patients with cANCA or atypical ANCA we identified MPO antibodies which are more typical for EGPA – similar observations were done in previous studies (22). Comparably to other reports, ANCA-positive patients in our cohort had more frequent renal and skin involvement and symptoms of peripheral neuropathy (limited however to mononeuritis multiplex) when compared to ANCA-negative patients (10, 13, 21). In the latter group, during the follow-up period but not at the beginning of the disease, heart involvement was more frequent. On the contrary to the biggest retrospective study published (21), we were unable to confirm a higher incidence of ENT symptoms in the ANCA-positive group. Although the general trends remain the same, our study shows higher percentages of certain organ involvement (especially heart, ENT and neurologic involvement) when compared to the previous studies (19-22, 27, 28). These differences may be probably explained by the fact, that our EGPA cohort is based on patients mostly diagnosed in the past 15 years. During this time more sophisticated diagnostic techniques such as MRI in heart assessment or ENG/EMG in CNS involvement and CT for the ENT symptoms have been made more easily available. Additionally many of our EGPA patients were screened for heart involvement with the MRI even without clinical symptoms being present (27).

Our observation that peripheral neuropathy in ANCA-positive patients was much more resistant to treatment than in ANCA-negative patients – even despite the fact that peripheral neuropathy was an independent indication for additional immunosuppression – seems to be new. A similar treatment strategy was applied by Moosig *et al*. (22), but they did not analyse outcomes according to the ANCA status.

While eosinophilic infiltrates in EGPA easily withdraw after the use of steroids, damage due to vasculitis seems to be more permanent. In our study maximal eosinophilia was higher in the ANCA-negative group. They also suffered from more severe asthma. The course of asthma characteristic for EGPA - severe symptoms before diagnosis and almost complete recovery during follow-up - might suggest eosinophilic infiltration as the main pathomechanism of the disease, with a prompt response to steroid therapy (9, 29). All these observations seem to confirm theories pointing at vasculitis as the dominant mechanism of organ involvement in the ANCA-positive group, while in the ANCA-negative group eosinophilic infiltration probably prevails (1). This may explain differences in renal involvement and difficulties in treating neuropathies in our patients. Results of recent studies indicated that relapses of the disease were statistically more frequent in ANCA-positive patients (13, 21, 30). The same authors also described lower maximum eosinophilia at the time of diagnosis as an independent risk factor for the relapse (21, 30). This is in agreement with the above mentioned theory and our observation of a lower maximum eosinophilia in the ANCA-positive group at the time of diagnosis. However, we were unable to observe more frequent relapses in ANCA-positive patients. Most probably this was due to the more frequent use of additional immunosuppressants in the ANCA-positive group. This approach in our ANCA-positive patients was mainly based on the previous observation that peripheral neuropathy is resistant to corticosteroids (31, 32). Better outcomes of a similarly aggressive immunosuppression was recently demonstrated in a retrospective analysis by Moosig *et al*. (22).

Recent studies indicate that, if well treated, prognosis in EGPA is relatively good. In the study of Samson *et al*., 7-year follow-up survival was 90% (30), and in that of Moosig *et al*. 5- and 10-year survival was 97% and 89% respectively (22). The analysis made by Comarmonda *et al*. indicates 5 and 10-year survival rates, depending on the presence of ANCA, to be 94.9% and 78.6%, respectively for ANCA-positive patients, and 88.8% and 76% respectively for ANCA-negative ones (21). Our results concur with those findings. In our study we observed clinical remission in 47 out of 50 patients during the last follow-up visit, and only one death in the course of the entire observation period.

Disease severity at diagnosis expressed by FFS and the age of patients in our group appears to be similar to other case series descriptions (21, 22). Lower mortality in our cohort may be due to a slightly shorter follow-up period and/or more aggressive immunosuppressive therapy (21). A relatively short delay between EGPA diagnosis and initiation of treatment, the importance of which we have shown previously (33), could also influence the outcomes. Comprehensive education of our patients and regular visits in a specialised referral centre might have also proved beneficial. However, our results also suggest that keeping the disease in remission requires chronic use of steroids in both ANCA groups, with an extra immunosuppressant needed especially in ANCA-positive patients. Hopefully this way a substantial part of the permanent damage to organs can be prevented.

Undoubtedly our study has several limitations. One limitation is that asthma severity and level of control that are based on symptoms may be biased by the partially retrospective character of this study. The second and main limitation was the number of analysed EGPA patients. As in any other study of rare diseases, numbers limit the statistical

performance. However in our opinion, the fact that our observations are monocentric and patients followed the same treatment regimen, should increase the power of the study. Furthermore, this study is partially prospective, based on many accessible research instruments (*e.g.* registry, clinical observations, patient feedback, etc.), which in our opinion improves the observation yield. Nonetheless, our observations should be repeated in larger groups in a multicentre cohort, and in a joint cluster analysis.

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