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Research Article

Systemic vasculitis associated with anti neutrophil cytoplasmic antibodies in Bulgaria – epidemiological, health-demographic and clinical-pharmacological real-world data

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Abstract

Systemic vasculitides are rare and heterogeneous diseases affecting different organs and systems with varying degrees of severity depending on the type of vessels affected. The etiology and pathogenesis are unclear. Immune mechanisms play a role in the pathogenesis: deposition of immune complexes, autoantibodies (anti-endothelial and anti-neutrophil cytoplasmic antibody, cellular and molecular responses, granulomas, and endothelial cell damage. All vasculitides are "rare diseases". ANCA- associated vasculitis has an incidence of 20 cases/1 million population. Without treatment, mortality is >90% within up to 5 years. We conducted a two-centre, retrospective, observational, non- interventional, epidemiological, health-demographic, clinical-pharmacological study to evaluate ANCA-associated vasculitis in Bulgaria. From 2018 to 2021, we screened 12 individuals with Wegener's granulomatosis. The analyzed population is approximately 60% of the patients in Bulgaria. The time from symptom onset to diagnosis is short, but the diagnosis is made at an advanced stage of the disease - the measured BVAS version 3 activity is moderate-severe.

Keywords

Systemic vasculitis, ANCA, Microscopic polyangiitis, Wegener granulomatosis, Churg- Strauss syndrome

Introduction

Vasculitides are characterised by inflammation of blood vessels that leads to organ or tissue damage or to necrosis. In some cases, the damage is inconsequential and may result in minor, often asymptomatic clinical manifestations, such as microhemorrhages. In severe forms of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), however, rapid onset of ischemia and blood vessel occlusion can lead to organ failure and

death. Any part of the vascular tree is potentially at risk from AAV. Vasculitis associated with antineutrophil cytoplasmic antibodies is a necrotizing vasculitis, with little or no immune deposition, affecting predominantly small vessels (i.e., capillaries, venules, and other arterioles and small arteries), and may be associated with myeloperoxidase (MPO) ANCA or proteinase 3 (PR3) ANCA. Not all patients have ANCA. Three types of AAV have been described: **1.** Microscopic polyangiitis (MPA) – necrotizing vasculitis, with little or no immune deposits, predominantly affecting small vessels (i.e., capillaries, venules, or arterioles); necrotizing glomerulonephritis is very common, pulmonary capillaritis is also common, no granulomatous inflammation is found. **2.** Granulomatosis with polyangiitis (GPA; Wegener granulomatosis) – necrotizing granulomatous inflammation, usually involving the upper and lower airways and necrotizing vasculitis, affecting mainly small to medium vessels (capillaries, venules, arterioles, arteries and veins); necrotizing glomerulonephritis is also common. **3.** Eosinophilic granulomatosis with polyangiitis (EGPA; Churg-Strauss) is an eosinophil-rich, necrotizing granulomatous inflammation involving the airways and necrotizing vasculitis of predominantly small to medium vessels associated with asthma and eosinophilia. ANCA antibodies are seen more frequently in patients with glomerulonephritis.

The etiology and pathogenesis of the disease are incompletely understood. ANCAs play a major role in the pathogenesis of these diseases. The pathogenic potential of MPO-ANCAs has been confirmed in animal models of vasculitis, yet the pathogenesis of this disease remains multifactorial in nature. There is a significant genetic predisposition to these diseases (Fujimoto et al. 2011; Watts et al. 2012). Patients with PR3-ANCA have human leukocyte antigen DP (HLA-DP) and genes encoding alpha-1-antitrypsin (SERPINA1) and proteinase 3 (PRTN3). In contrast, MPO-ANCA patients have an association with HLA-DQ. Other important factors are environmental influences, exposure to silica or certain strains of *Staphylococcus aureus*, combined with a lack of effective regulation of T-cells to prevent inflammation. Neutrophil leukocytes play an important role in the early stages of AAV, as their primary granules contain significant amounts of PR3 and MPO, which are expressed on the cell surface upon activation. Furthermore, they are responsible for early endothelial damage to vessels and, if activated extravascularly, can induce local tissue necrosis. Neutrophils generate neutrophil extracellular traps (NETS) that can trap proteinase PR3 and MPO, helping to disrupt immune tolerance and antibody formation. ANCA-activated neutrophils stimulate the alternative complement pathway. Subsequent damage leads to an innate immune response, with monocytes and T-cells accumulating in the inflammatory focus to replace neutrophils destroyed during the initial phase of inflammation. The monocytes transform into macrophages and multinucleated giant cells. Repeated cycles of this process result in necrotic lesions from the accumulation of lymphocytes, monocytes and macrophages in granulomatous inflammatory tissue (Fujimoto et al. 2011; Romero-Gomez et al. 2015). Eosinophilic granulomatosis with polyangiitis has two overlapping clinical phenotypes. The first is primarily associated with hypereosinophilia and the second is associated with the presence of ANCA. The pathogenesis of EGPA remains unclear. Interleukin-5 (IL-5) is one of the major cytokines responsible for eosinophil proliferation and has been implicated in disease complication as in hypereosinophilic syndrome (HES).

Despite increasing understanding and awareness of vasculitis as a clinical problem, patients with vasculitis are not always recognisable and are difficult to diagnose, especially in the early stages of the disease. The detection of ANCA antibodies and their association with vasculitis in small vessels has significantly improved the clinical recognition of this type of disease. Greater awareness of vasculitides combined with more widespread ANCA testing has improved the diagnostic process and increased the apparent incidence of vasculitides (Khoury et al. 2012). The incidence of AAV is 10 to 20 new cases per million per year in most parts of the world where epidemiological studies have been done. The number of MPA cases is higher than GPA cases in Southern Europe compared to Northern Europe. In the Far East, MPA is much more common than GPA. ANCA directed against MPO is the predominant antibody detected in AAV patients in Japan, but autoantibodies directed against PR3 are rarely seen in Japanese patients; however, they are the most common form of ANCA in Northern Europe (Mohammad et al. 2007). Eosinophilic granulomatosis with polyangiitis is rarer than GPA or MPA, with an incidence of about 0.6 per million per year. The epidemiology of EPGA is less well studied compared to that of MPA and GPA. EGPA is characterized by increased eosinophil counts in patients with late-stage asthma. About 50% of patients have ANCA, usually directed against MPO. There is potential overlap with HES and it is unclear whether some cases of HES are truly not cases of EGPA or vice versa. Indeed, if patients with suspected EGPA are negative for ANCA, distinguishing between the two conditions is very challenging (Bloch et al. 1990). Furthermore, as bronchospasm is a key feature of EGPA, it is possible that some patients with asthma (an extremely common condition) may actually have EGPA. This has been found with the use of the leukotriene inhibitor montelukast. Patients with difficult-to-treat asthma who are given montelukast sometimes develop EGPA. It has been suggested that these patients probably had a form of EGPA that was previously suppressed with systemic glucocorticoids, but when they were withdrawn from treatment, the characteristics of EGPA became more apparent. However, it is possible that some cases of EGPA are actually caused by leukotriene inhibition. Most patients with AAV are in their 60s or 70s (with an incidence more than twice that of the general population), but people of any age can be affected. In southern Sweden, the estimated incidence is 160 patients per 1 million population (95% confidence interval (CI), 114–206) for GPA, 94 (CI, 58–129) for MPA, and 14 (CI, 0.3–27) for EGPA (Stone et al. 2001). These

data are based on a relatively small population size and are higher than reported Spanish data (Mohammad et al. 2007; Romero-Gomez et al. 2015) in which the prevalence of all forms of AAV was less than 45 per million.

Large-scale systematic epidemiological studies have not been conducted in Bulgaria, and few scientific publications on the topic have been found. A description of the disease with the presentation of one clinical case, for the first time in Bulgaria, was made by prof. Dimitrov 2001). In Bulgaria, the largest study on Churg-Strauss vasculitis/syndrome (CSS) was performed by Krasimir Nikolov and Marta Baleva, and in three of the 7 patients with CSS (43%) studied by them, positive pANCA were found in the titer 1:64–1:128, determined by indirect immunofluorescence method. These results are consistent with some authors and the worldwide literature, but others find that 70% or more of CSS patients have positive pANCAs. The main clinical symptoms of the disease in the patients observed by Nikolov and Baleva were: allergic rhinitis, asthma, blood and tissue eosinophilia, and a rapidly transient (Löffler) pulmonary infiltrate detected radiologically (Baleva et al. 1994; Baleva et al. 1995). The authors found no evidence of mono- or polyneuritis, cutaneous, cardiac, gastroenterological or renal manifestations of the disease. In 2002, Manolova et al reported 1 patient with positive cANCA in CSS. People aged about 40–50 years are most affected, but the disease occurs at any age, although it is very rare in childhood.

There are many common clinical symptoms and syndromes in patients with AAVs with predominant involvement of the kidneys and lungs in MPA and GPA and predominant involvement of the upper and lower airways in GPA and EGPA. EGPA is characterized by eosinophilia, late-stage asthma, and neuropathy, but some patients may also develop cardiac involvement (Table 1).

The clinical symptoms in patients with AAV are highly variable, as vasculitis can affect any organ or system or multiple organs at different times, and progress simultaneously. Common manifestations such as discomfort, fever, weight loss or myalgia could be met in many other diseases and delay recognition of the disease. Patients with MPA may have isolated asymptomatic microscopic hematuria and hypertension with nonspecific systemic features. In GPA, patients usually have upper respiratory tract problems, secretions containing blood clots, sinusitis, or hearing loss. The clinical course of MPA is usually acute, but in GPA patients may remain undiagnosed for months or even years before symptoms from the upper and/or lower airways are recognized as being caused by vasculitis. Delayed diagnosis is a problem in all of these diseases. For all forms of rapidly worsening AAV, the differential diagnosis is made with infection, cancer, other inflammatory processes and drug toxicity. A cocaine snorting can cause local tissue necrosis, especially in the palate, leading to perforations, including of the nasal septum. In addition, cocaine can induce ANCA production. It has been suggested that levamisole, which is often mixed with cocaine, may also cause acute necrotizing vasculitis of the skin and extremities, further complicating the symptoms associated with cocaine use. Cessation of cocaine intake (especially if contaminated with levamisole) is a prerequisite for halting the further course and progression of the disease.

Diagnosis and differential diagnosis of ANCA-vasculitis is a serious challenge. Examination of patients with suspected AAV requires careful case history taking and status to determine the likely diagnosis. In acute disease, the differential diagnosis is very broad and the specific combination of clinical features should be sought. A key aspect in the diagnosis of ANCA vasculitis is polyorgan involvement. Many of the laboratory investigations can lead

Table 1. Clinical features of AAV by organs and systems.

Common symptoms	Muscle aches, joint pain, fever and weight loss are not exclusive to vasculitis
Skin	The skin is the most commonly affected organ in many forms of vasculitis, with small lesions such as infarction or purpura or more serious manifestations such as deep ulcerations and even gangrene.
Mucosal membranes and eyes	Oral (and rarely genital) ulcers appear; swelling of the salivary and lacrimal glands is less common; inflammatory eye diseases including scleritis, episcleritis, iritis and keratitis; retinal vasculitis may also occur in some patients.
Ears, nose, throat	Ear, nose and throat involvement is most commonly associated with GPA. Nasal blood clots, nasal crusts, sinusitis, subglottic stenosis or hearing loss (conductive or sensorineural) are found. Chronic rhinitis and polyps are most commonly found in EGPA but usually do not result in destructive nasal lesions; in comparison, patients with MPA usually do not have significant upper airway involvement.
Chest	EGPA patients may have a history of advanced asthma; hemoptysis and shortness of breath are common symptoms of lung involvement in all three diseases. Detailed examination may reveal the more extensive presence of nodules, cavities, infiltrates, and bronchial involvement. In extreme cases (usually MPA and GPA), massive hemoptysis caused by alveolar hemorrhage accompanied by severe respiratory failure may occur.
Cardiovascular disorders	Some patients develop pericarditis, valvular heart disease, ischemic heart pain, and heart failure caused by direct involvement of the heart muscle, especially with EGPA.
Gastrointestinal disorders	Patients may experience peritonitis, bloody diarrhea caused by ischemic colitis or intestinal ulcer, or ischemic abdominal pain associated with feeding.
Kidney disorders	This is the most important system for AAV; vasculitis is most often symptomatic and therefore it is important to monitor blood pressure, examine urine for the presence of blood and protein, and measure renal function by determining serum creatinine or glomerular filtration rate.
Neurological disorders	Peripheral nerve involvement occurs in about 15% of patients with GPA and MPA and is more common with EGPA. CNS involvement is much less common but includes aseptic meningitis and intracerebral infarcts or granulomas. These can lead to stroke or seizures.

to non-specific findings such as an elevated white blood cell count, platelet count or erythrocyte sedimentation rate, C-reactive protein is usually elevated. Patients may have anemia. Liver or kidney function may be impaired. The presence of hypereosinophilia (often >1500/mm^3) may suggest a diagnosis of EGPA, but there are other causes of hypereosinophilia – in particular drug reactions. It is important to assess renal function in all patients with suspected vasculitis. The presence of abnormal sediment on urinalysis, in combination with new- onset hypertension, suggests renal involvement in AAV. Detection of ANCA allows rapid diagnosis of vasculitis (Berden et al. 2010) with renal involvement and provides better management of the disease process. Antineutrophil cytoplasmic antibodies are detected in over 90% of patients with GPA and in 60%–80% of patients with MPA. Microscopic hematuria is found in almost all patients in whom the kidneys are affected. The presence of erythrocytic casts suggests active glomerulonephritis. Proteinuria can be detected in more than 90% of patients, but is usually less than 3.5 g/24 hours. Renal histology in patients with renal impairment is usually characterized by focal segmental thrombosis and necrotizing glomerulonephritis. Crescents are found in most renal biopsies. The severity of renal damage and the prognosis of renal function correlate with the presence of glomerular sclerosis, tubular involvement and active glomerular damage with crescent formation (Adu et al. 1987).

Histological examination remains the most important investigation when vasculitis is suspected, both for making an accurate diagnosis, assessing the volume of investigations, and also for excluding other causes and comorbidities. Although a histological examination of the airways may not have direct diagnostic value, such an examination will help to exclude the presence of cancer, sarcoidosis, tuberculosis, or immunoglobulin G4 (IgG4)-related disease whose clinical picture resembles upper or lower airway inflammation. Renal histology remains the "gold standard" for establishing glomerulonephritis and making an accurate diagnosis and prognosis (Adu et al, 1987).

Clinical evaluation of vasculitis is based on activity scales. The Birmingham Vasculitis Activity Score (BVAS) in adults (Luqmani et al. 1994; Stone et al. 2001; Mukhtyar et al. 2009) and the Paediatric Vasculitis Activity Score (PVAS) in children (Dolezalova et al. 2013) are the main tools for clinical assessment of disease activity in patients with AAV (Ponte et al. 2014). The two most recent forms of BVAS, BVAS version 3 and a BVAS specific version designed for patients with GPA, were used. The BVAS for GPA can also be used in other forms of vasculitis, including MPA, without further validation. The BVAS and PVAS are control questionnaires to identify clinical manifestations in vasculitis that are not always specific to vasculitis. A timeframe of 3 months is recommended for reassessment of disease activity – this is usually the timeframe that conventional immunosuppressive therapy (cyclophosphamide (CYC) and glucocorticoids) is expected to take effect. BVAS version 3 is a common test and can be used in all forms of AAV (Mukhtyar et al. 2009; Suppiah et al. 2010). It contains a structured list of clinical manifestations of active disease. However, these clinical manifestations are not strictly specific to vasculitis. To determine the severity of vasculitis, the organ and systemic damage and clinical symptoms in each individual patient must be taken into account. The Birmingham Vasculitis Activity Score (BVAS) is an instrument with 9 domains (general, cutaneous, mucous membranes/eyes, ears/nose/throat (ENT), chest, cardiovascular, abdominal, renal, and nervous system). Each BVAS item is scored if the sign or symptom started or worsened over the four weeks prior to the evaluation. The BVAS produces a summary score for overall disease activity that can range from 0 to 63. The summary score is composed of the sum of each organ domain specific scores. Although the BVAS and PVAS have a number of disadvantages, they are the most effective clinical tool for assessing the severity of vasculitis and monitoring the status of patients with or without treatment.

The Vasculitis Damage Index (VDI) is the most widely used and validated measure of vasculitis damage (Exley et al. 1997; Suppiah et al. 2010). Individual symptoms/injuries are scored if they have lasted for at least 3 months or occurred at least 3 months previously (in the case of single events over time, such as stroke or myocardial infarction) and should reflect a permanent change in the patient's condition. The severity of the impairment is cumulative and reflects the overall severity of the patient's illness. Each VDI scale item is scored with 1 point. A VDI scale score above 5 points at 6 months of illness carries a significantly increased risk of subsequent mortality compared with a lower VDI scale score at 6 months follow-up (Exley et al. 1997). The BVAS and VDI provide quantitative scores based on individual symptoms and are an effective tool for determining a patient's condition. Other measures such as the Five-Factor Score, which is an effective prognostic tool in AAV (Guillevin et al. 2011), are also used in practice; an earlier version was also effective in PAN (Guillevin et al. 1996). A Disease Extent Index (DEI) has been developed for patients with GPA (de Groot et al. 2001) and serves as a prognostic criterion but is not widely used.

Treatment of AAV is specific, usually involving glucocorticoids and one or more immunosuppressants (Table 2). However, a comprehensive treatment approach is mandatory – in addition to specific treatment, the therapeutic strategy should take into account: 1. Existing or new comorbidity (e.g. hypertension, diabetes mellitus, cardiovascular and other chronic diseases); 2. Limiting drug toxicity and interactions; 3. Operative interventions; 4. Past medical conditions, etc. Nowadays, common therapeutic practice is to give the patient induction therapy, which usually consists of intensive immunosuppression such as cyclophosphamide (CYC) or rituximab (RTX) combined with high doses of glucocorticoids, followed by maintenance treatment and a tapering course of steroids. Subsequently, the main goal is to withdraw immunosuppressive therapy, but there are no set rules as to how long after

Table 2. Medicinal products used for treatment of AAV.

Glucocorticoids	Combine with other immunosuppressants. The aim is to establish good disease control. High doses are used initially, usually 1 mg/kg/day prednisolone or equivalent or pulse therapy, and the dose is rapidly reduced to minimize toxicity.
Cyclophosphamide (CYC)	It was first used in systemic vasculitis in the 1970s and is still the most effective agent available for polyorgan AAV. Administered p.o. 2–3 mg/day significantly improves survival (Ntatsaki et al. 2014). The use of RTX has led to a reduction in the use of CYC, with increasing numbers of patients maintaining remission with RTX instead of CYC, especially when there is a contraindication to the use of CYC. Relapses are frequent, a single course of therapy rarely achieves long-lasting remission. Repeated cycles of treatment are usually required.
Azathioprine (AZA)	A cytostatic immunosuppressant, effective in combination with steroids, reduces overall mortality. It is administered p.o. at a dose of 2 to 2.5 mg/kg/day as maintenance therapy after remission has been achieved with another medicinal product.
Methotrexate (MTX)	Effective in the treatment of GPA. Dose between 20 and 25 mg/week.
Mycophenolate mofetil	Less effective than AZA as a maintenance agent in patients who have achieved remission. It is usually prescribed 2 to 3 g/day as an oral dose along with tapering courses of steroids.
Cyclosporine	It has been applied in a small number of patients. It is administered orally in doses of 2 to 3 mg/kg/day, its use is restricted due to renal toxicity.
Leflunomide	Data are uncertain – it has been applied to ANCA-associated vasculitis in studies with small populations.
Rituximab	The use of biologics for the treatment of vasculitis is a therapeutic novelty and it is of considerable clinical interest. For the first time, a biological medicinal product with therapeutic indications in the field of rheumatology received Marketing Authorization via Centralized procedure by the European Medicines Agency EMA) on 02.06.1998 – MabThera, with international non-proprietary name (INN) RTX**.** At the date of first authorization, RTX was indicated for the treatment of – Chronic Lymphocytic Leukemia (CLL), Non-Hodgkin's Lymphoma (NHL) and Rheumatoid Arthritis (RA) (EMA 2009). On 15 November 2018, The Committee for Medicinal Products for Human Use (CHMP) of the EMA adopted a favourable opinion and recommended to accept a variation to the terms of the Marketing Authorisation for MabThera to extend the therapeutic indications by: "granulomatosis with polyangiitis and microscopic polyangiitis; RTX, in combination with glucocorticoids, is indicated for the treatment of adult patients with severe active granulomatosis with polyangiitis (Wegener's granulomatosis) (GPA) and microscopic polyangiitis (MPA)" (EMA 2009). This recommendation was subsequently approved by the EC and thus MabThera became the first biologic containing monoclonal antibodies indicated for the treatment of systemic vasculitis associated with antineutrophil cytoplasmic antibodies (ANCA). The active substance, RTX, is a monoclonal antibody designed to recognize and bind to a protein called CD20 that is found on the surface of all B-lymphocytes. When it binds to CD20, RTX destroys B- lymphocytes. In patients with severe active granulomatosis with polyangiitis and microscopic polyangiitis, destruction of B-lymphocytes reduces the production of antibodies, which are thought to play an important role in attacking blood vessels and which cause inflammation. The new therapeutic indication is supported by two clinical trials. **1.** In a study of 198 patients with GPA or MPA, 64% of patients taking RTX were in complete remission after six months compared to 55% of patients taking cyclophosphamide. **2.** A second study in 117 patients looked at the proportion of patients who relapsed within 28 months. The study showed that only 5% of patients treated with RTX relapsed compared with 29% of patients treated with azathioprine (EMA 2009).
Belimumab	B-activating factor (BAFF) inhibitor of the tumor necrosis factor (tumor necrosis factor) family. Undergoing trials as maintenance therapy for AAV.
Mepolizumab	mAb directed against IL-5, a potent stimulator of eosinophil production. It has been used with success in hypereosinophilic syndrome and is currently being tested in EGPA – initial results in a small number of patients are promising (Moosig et al. 2014).
Intravenous immunoglobulins (IVIG)	They provide short-term control of AAV (Mukhtyar et al. 2009). Plasmapheresis can be successful as emergency therapy in patients with very aggressive forms. Their use is associated with improved renal function in patients with severe ANCA-associated vasculitis during the first 2 years. The benefit in patients whose initial renal function is impaired is severely limited.

treatment initiation this should occur. It is believed that if treatment is shorter than 2 years, the likelihood of relapse is higher compared with treatment lasting longer than 2 years. Current practice requires patients to be treated for at least 4 years, which includes 3 to 6 months of more intensive therapy followed by lower intensity treatment with medicinal products such as azathioprine (AZA) or methotrexate (MTX) (Yates et al. 2016).

The aim of the study was to collect and analyse the epidemiological, health-demographic and clinical- pharmacological characteristics of systemic vasculitis associated with antineutrophil cytoplasmic antibodies in Bulgaria.

Materials and methods

The study design was a two-centre, retrospective-prospective, observational, non-interventional trial. Planned number of study participants: minimum number of participants – not fewer than 6; expected number of participants – not more than 24. Only adults aged 18 to 75 years were included in the study and data analysed. Data collection period – 2018–2021. All respondents signed an informed consent form and were recruited in two centres – specialised rheumatology clinics in the city. Participants

were recruited from two sample groups of rheumatologists in Sofia. The information collected about the participants in the non-interventional observation included data on gender, age, disease, time since diagnosis, previous treatment, health status assessment using the Birmingham Vasculitis Activity Score (version 3), while maintaining complete anonymity of the respondents. The trial was conducted according to the Declaration of Helsinki and subsequent amendments, the Law on Medicinal Products in Human Medicine (Ministry of Health 2007), the Law of Health (Ministry of Health 2004) and Personal Data Protection Act (PDPA 2002).

Results and discussion

For the non-interventional observation period, we only accumulated data of patients diagnosed with granulomatosis with polyangiitis (Wegener's granulomatosis, Wegener's disease). The research team did not come across any patients diagnosed with microscopic polyangiitis and/or eosinophilic granulomatosis with polyangiitis. The analysis group included 12 individuals, 10 men and 2 women (Table 3).

Table 3. Distribution of patients by sex.

Patients with Wegener's granulomatosis (n/%)	Men (n/%)	Women (n/%)
12 (100)	10 (83.33)	2 (16.67)

We analysed the size of the study group as a proportion of the total number of patients in Bulgaria to determine the representativeness of our study. As already mentioned in the introduction, epidemiological data show that the incidence rates of newly diagnosed cases with GPA, MPA and EGPA are 2.1–14.4, 2.4–10.1 and 0.5–3.7 per million population in Europe, respectively, and the prevalence of all cases with AAV is estimated to be 46–184 per million population. All three diseases meet the definition of Orphan disease.

The incidence of Wegener's granulomatosis in Bulgaria is estimated as newly diagnosed cases between 14.7 and 100.80 with a median of 43.05. No literature or official data from national statistics on the actual total number of patients with Wegener's granulomatosis for the period 2018–2021 are available. We consider as a reasonable assumption the presence of no more than 40 to 60 patients with this disease in Bulgaria, of which no more than 20 are diagnosed or as a relative proportion no more than 30% of the total. The basis for this kind of assumption is provided by the most recently published data from the NHIF for 2017, which show that in the first, second and third quarters of that year the NHIF paid for the treatment of 18, 15 and 12 patients respectively, or an average of 15 patients per month (NHIF 2020). With 12 patients included in the study, we can assume that the analyzed population represents 81.6% of the total number of patients as newly diagnosed cases with regression to 11.9% of the maximum incidence. The size of the population we analyzed, calculated over the total number of patients with the above reasonable assumptions, is not fewer than 60% of all patients in Bulgaria. In Table 4, we present an analysis of the demographic characteristics of the study participants. The mean age of the followed men with Wegener's granulomatosis was 52.4 years, the median age was 53 years, and the most common age was 51 years with a standard deviation of 13.54 years. The mean age of the women followed up in the Wegener's granulomatosis registry was 55.5 years, the median and most common age could not be calculated as there were only two women in the study and they were not sufficient to derive statistical results (standard deviation 4.95 years).

Clinical characteristics of the studied group of patients

We analyzed a series of clinical characteristics of the study group of patients. We performed a retrospective analysis of the evolution of the disease in all patients by stratification as follows: mean disease duration, time from first symptoms of the disease to final diagnosis, time to initiation of treatment after diagnosis, duration of conventional treatment, and switch to biologic treatment (Table 5). The mean disease duration in our follow-up group ranged from 1 to 13 years with a median of 5 years. The time to diagnosis after the first symptoms was relatively short, despite the varied and complex clinical symptoms: in 58.3% (7 patients) of cases the diagnosis was made within 1 month, in 3 cases within half a year. In our sample, there were 2 cases with serious delays in the diagnosis: in 1 patient, more than 1 year was required, and in a second case, as many as 7 years elapsed – the patient was considered to have primary immune kidney disease. Despite the drastic deviation in both patients, the median and mode by this criteria were equal to 1 month. The median duration of conventional treatment from the date of diagnosis to the switch to biologic treatment was 3.5 years, with SD = 1.62.

Patients received conventional treatment as follows: immunosuppressants – INN Cyclophosphamide, Azathioprine, Methotrexate; systemic glucocorticoid therapy – INN Methylprednisolone, Prednisone, Betamethasone; immunoglobulins – INN Immunoglobulins, normal human. During follow-up, all patients underwent RTX treatment, which included 1 and/or 2 courses of treatment. Almost all patients were planned to continue treatment with additional courses of RTX – between 4 and 6, unfortunately these data were not included in our analyses.

We evaluated a series of additional clinical parameters such as: clinical manifestation of the disease, complications, comorbidities, severity of the disease assessed by the Birmingham Vasculitis Activity Score – version 3 (BVAS), conventional treatment – type and duration, presence of progression and/or remission, biological treatment – duration, effect of treatment, etc. A BVAS version 3 clinical scorecard was completed for each patient, containing the 9 sections and the 56 assessment items, the maximum achievable points for each section and the total number of points. BVAS activity was scored using constant points (BVAS –Persistent points) from 0 to 33 (Table 6).

Table 4. Demographic indicators.

Age	Men	Women	Total
18–45	2(16.66%)	0(0%)	2(16.66%)
46–65	7(58.33%)	2(16.66%)	9(75.0%)
Over 65	1(8.33%)	0(0%)	1(8.33%)
Number	10(83.33%)	2(16.67%)	12(100.0%)
Average value	52.4	55.5	4
Median	53	–	2
Mode	51	–	–
Standard deviation (SD)	13.54	4.95	4.36

Table 5. Disease evolution in the studied group of patients – stages.

Patient	Duration of the disease (years)	Time from first symptoms to diagnosis (months)	Time to treatment after placement of diagnosis (months)	Conventional treatment – Duration (years)	Treatment with RTX (number of infusions)
1	11	4	1	5	1
2	7	1	1	6	1
3	1	2	1	1	1
4	2	1	1	2	1
5	8	1	1	4	1
6	4	1	1	2	1
7	4	1	1	3	1
8	13	84	1	4	1
9	5	6	6	5	1
10	5	15	1	5	2
11	4	1	2	2	1
12	5	1	1	2	2
Average value	5.75	9.83	1.5	3.45	1.17
Median	5	1	1	3.5	1
Mode	4.5	1	1	2	1
Standard deviation (SD)	3.49	23.70	1.44	1.62	0.38

Table 6. Clinical characteristics of the studied group of patients – BVAS.

Patient №	Clinical disease activity*, (BVAS -Persistent points)**	Complications	Comorbidities
1	12	Renal failure	None
2	19	Chronic otitis media of the right ear Paresis of the right ulnar nerve	None
3	16	Renal failure Arterial hypertension Dyslipidaemia	Secondary anemic syndrome
4	19	Renal failure Arterial hypertension DIC syndrome (thromboses)	Chronic gastritis Secondary anemiae
5	18	Renal failure Infection of the right eye	Dry cough, shortness of breath, chest pain and tightness
6	7	Dry cough, shortness of breathing, chest pain and tightness	None
7	24	Renal failure Steroid diabetes	Dry cough, shortness of breathing, chest pain and tightness Chronic gastritis Secondary anemiae
8	33	Cerebral hemorrhage with subsequent craniotomy Bilateral abscess pneumonia Renal failure	Secondary anemiae
9	7	Pulmofibrosis	Osteoporosis Scoliosis
10	19	Arterial hypertension	Cushing's syndrome
11	7	Arterial hypertension	Generalized osteoporosis with fractures Gallstone disease
12	6	None	None

*Clinical manifestation of the disease was determined according to the criteria described in Table 1. Clinical presentation of AAV by organs and systems. **Disease manifestations were assessed in the presence of active vasculitis. Results for all manifestations of active (but not new or worsening) vasculitis were assessed as permanent. Constant scores can range from 0 to 33. All manifestations of new or worsening vasculitis are scored as new/worsening results. These can range from 0 to 63 (MDApp 2021).

Table 7. Frequency distribution of clinical disease activity of the persistent type as measured by the Birmingham Vasculitis Activity Scale (BVAS -Persistent points).

Level	Number	Relative share	Cumulative share
6	1	8.3%	8.3%
7	3	25.0%	33.3%
12	1	8.3%	41.7%
16	1	8.3%	50.0%
18	1	8.3%	58.3%
19	3	25.0%	83.3%
24	1	8.3%	91.7%
33	1	8.3%	100.0%

The initial clinical presentations in the analysed group of patients were extremely varied, with symptoms and syndromes from all 9 groups of the rating scale. Because of the average duration of diseases and retrospective collection of data, we have not compiled a detailed description of the initial symptoms – some of them are not described in the case histories and patients didn't remember them

clearly. The most common complication was the development of renal failure, in 50% of cases, followed by cases of arterial hypertension, 33.3%. Anamnestically, 1/3 of the patients reported upper respiratory tract symptoms as the most common accompanying disease – dry cough, shortness of breath, chest pain and tightness.

The minimum number of BVAS persistence points is 6 and the maximum is 33, with a median of 17. The group we analyzed was assessed as moderate-severe BVAS version 3 activity, bearing in mind that all patients had conventional treatment. The descriptive analysis of BVAS version 3 activity is shown in Table 8.

Table 8. Descriptive analysis of BVAS version 3 activity.

Parameters	
Number (n) / Absence (missing)	12/0
Average / Mean	15.6
Standard error (mean)	2.37
Median	17.0
Mode	7.00
Standard deviation	8.21
Dispersion / Variance	67.4
Range	27
Minimum value / Minimum	6
Maximum value / Maximum	33
Skewness	0.630
Standard error skewness	0.637
Shapiro-Wilk - p	0.172

Conclusion

The frequency of newly diagnosed patients in Bulgaria with Wegener's granulomatosis is low and corresponds with the epidemiological data of Europe. The probable absolute number of patients in Bulgaria is no more than 20 per year on average. The male/female sex distribution is 5:1. The average age is 53 years and the average duration of disease is 1 to 13 years. The diagnostic process is relatively short, but in view of the severity of the course and the high mortality in the absence of treatment, the time for diagnosis should be accelerated. Our measured disease activity in the Bulgarian population is moderate-severe BVAS version 3, not overlooking the fact that all patients have had conventional treatment and at least one infusion of RTX. Unfortunately, in Bulgaria RTX is reimbursed only in patients with GPA. Due to the high cost of treatment, the lack of reimbursement for biologic treatment of the other two diseases, MPA and EGPA, limits the access of patients with these diseases to biologic treatment. The treatment of vasculitis with RTX is a therapeutic innovation, represents significant clinical interest and has changed the current understanding of the treatment of ANCA-associated systemic vasculitis.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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