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**ANCA-associated renal vasculitis - epidemiology, diagnostics and treatment**

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## INTRODUCTION

### 1. Clinical characteristics of primary vasculitis

The primary systemic vasculitides are a group of heterogeneous disorders of unknown etiology characterized by more or less widespread inflammation of the vessel wall. A clinical classification of the various disease entities within this group has been proposed by the American College of Rheumatology (ACR) and is based on the presence of particular clinical symptoms and histopathological findings (1). These so-called ACR-criteria are widely used but have their drawbacks with regard to disease specificity and sensitivity. A more precise nomenclature and definitions for the primary vasculitides have therefore been proposed by a group of experts in this field in 1993 (table 1).

Large vessel vasculitis	Giant cell (temporal) arteritis
	Takayasu arteritis
Medium-sized vessel vasculitis	Polyarteritis nodosa
	Kawasaki disease
Small vessel vasculitis	Wegener's granulomatosis
	Churg-Strauss syndrome
	Microscopic polyangiitis
	Henoch-Schoenlein purpura
	Essential cryoglobulinemic vasculitis
	Cutaneous leukocytoclastic angiitis

Table 1. Names and definitions of vasculitides adopted by the Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis

These definitions for the nomenclature of the vasculitides are known as the Chapel Hill Concensus Conference (CHCC) definitions and are now widely used as diagnostic criteria, although they were not intended as such. Based on these definitions new diagnostic and classification criteria have to be developed (2).

Within the spectrum of the primary vasculitides renal involvement is common, particularly in the small-vessel vasculitides (3). Immunopathologically, Henoch-Schoenlein purpura and cryoglobulinemic vasculitis are characterized by immune deposits, which are considered to play a major and initiating role in the development of renal lesions. The remaining small-vessel vasculitides show paucity or absence of immune deposits. These pauci-immune vasculitides, that is Wegener's Granulomatosis (WG), Churg-Strauss Syndrome (CSS), microscopic polyangiitis (MPA) and its renal limited form, are strongly associated with the presence of anti-neutrophil cytoplasmic antibodies (ANCA). Their annual incidence in Europe is 10 to 20 per million per year and they account for at least 5% of the causes of end stage renal failure. There is an increased incidence with age, with a median age of 56 years in studies over the past 10 years. Males slightly predominate (4,5).

This work focuses on the ANCA-associated vasculitides (AAV) with renal involvement - their ethiopathogenesis, clinical presentation and treatment and the overall and renal outcome of patients with AAV.

## 2. ANCA-associated vasculitis

### 2.1. ANCA testing

As stated, the idiopathic pauci-immune necrotizing small-vessel vasculitides are strongly associated with ANCA. This finding was first described in 1982 (6). These antibodies were initially believed to be



associated with Ross River virus infections. By 1985, however, ANCA had been linked to WG (7). Within several more years, a relationship among ANCA, WG, MPA and renal limited vasculitis (RLV) was established (5, 8). ANCA testing currently plays a critical role in the diagnosis and classification of vasculitides, even as debate about their ultimate importance in the pathogenesis and pathophysiology of these conditions continues.

In vasculitis, ANCA are most often directed to either proteinase 3 (PR3-ANCA) or to myeloperoxidase (MPO-ANCA). Both PR3 and MPO are located in the azurophilic granules of neutrophils and the peroxidase-positive lysosomes of monocytes. PR3 is a cationic protein belonging to the trypsin family of serine proteases. MPO catalyses the production of hypochloric acid, which is effective in killing phagocytized bacteria and viruses.

Two types of ANCA assays are currently widely used – a more sensitive indirect immunofluorescence (IIA) assay and a more specific enzyme-linked immunosorbent assay (ELISA). To maximize diagnostic utility, current guidelines recommend dual testing, to screen with IIA and to confirm all positive results with ELISAs directed against the vasculitis-specific target antigens (mainly PR3 and MPO).

#### 2.1.1. Indirect immunofluorescence

When the sera of patients with AAV are incubated with ethanol-fixed human neutrophils, two major immunofluorescence patterns are observed. With the C-ANCA pattern (fig 1), the staining is diffuse throughout the cytoplasm (C-cytoplasmic type of immunofluorescence). In most cases, antibodies directed against PR3 (detected by ELISA) cause this pattern, but MPO-ANCA can occasionally be responsible (9,10).

The perinuclear or P-ANCA pattern (fig 1) results from a staining pattern

around the nucleus, which represents an artifact of ethanol fixation. With ethanol fixation of the neutrophil substrate, positively-charged granule constituents rearrange themselves around the negatively-charged nuclear membrane, leading to perinuclear fluorescence (5). The antibody responsible for this pattern (detected by ELISA) is usually directed against MPO (and occasionally PR3).

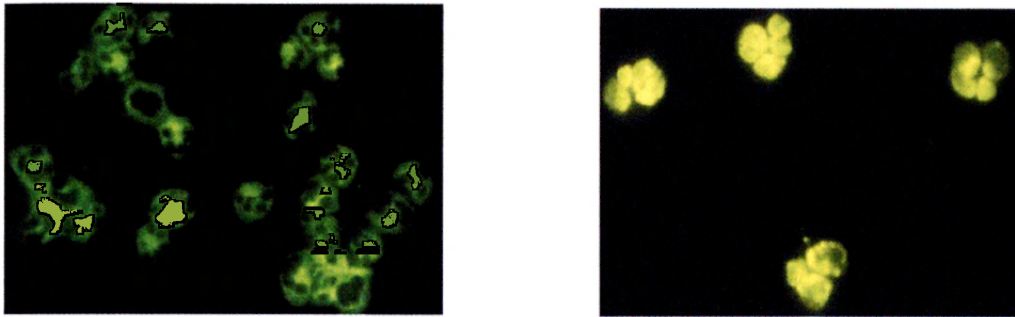


Figure 1. C-cytoplasmic type of immunofluorescence on the left and P-perinuclear type of immunofluorescence on the right

There are several reasons for caution in the interpretation of immunofluorescence results. First of all, they are highly dependent on the experience of the laboratory personnel. Furthermore, immunofluorescence results lack specificity (in one study positive C-ANCA were associated with vasculitis in only 50% of patients) (11). It is frequently difficult to distinguish the P-ANCA pattern of immunofluorescence from that caused by antinuclear antibodies (ANA). Individuals with ANA frequently have false-positive results on ANCA testing by immunofluorescence. The use of both formalin- and ethanol-fixed neutrophil substrates permits the distinction between P-ANCA and ANA, because formalin-fixed neutrophils prevent the rearrangement of charged cellular components around the nucleus. Moreover, ANCA do not react with lymphocytes, and

therefore the presence of lymphocytes on the test slides enables the observer to easily distinguish between the presence of ANA and ANCA. However, most commercially available slides for IIF-ANCA screening lack these control cells.

Although PR3 and MPO are the two most common targets for ANCA, an increasing number of cytoplasmic proteins has been identified so far as minor ANCA target antigens, including bactericidal/permeability-increasing protein (BPI), lactoferrin, azurocidin, cathepsin G, human elastase, or lysozyme. Some ANCA-targeted antigens are not the part of granules but are located directly in the cytoplasm, like  $\alpha$ -enolase and catalase, or even in the nucleus, e.g. non-histone chromosomal proteins HMG1 and 2 (high-mobility proteins). In IIA, atypical ANCA patterns may be confused with P-ANCA pattern (12).

### 2.1.2. Enzyme-linked immunoassay

Specific ELISAs for antibodies to PR3 and MPO (and in some centers for other antigens as well) are now available, and should be part of testing for ANCA. PR3-ANCA and MPO-ANCA are associated with substantially higher specificities and positive predictive values than the immunofluorescence patterns to which they usually correspond (C- and P-ANCA, respectively). There are, however, significant differences in sensitivity, specificity and predictive value among available commercial direct ELISA kits. The best practice includes both IIA and ELISA testing, because it has been shown that a C-ANCA combined with positive PR3-ANCA was 99% specific for AAV, and similarly, a P-ANCA combined with a positive MPO-ANCA was 99% specific for AAV (13).

### 2.1.3. Newer methods

Fluoroimmunoenzymatic assay (EliA) - a fully automated fluorescent assay performed on CAPs coated with the corresponding autoantigen. It has a high sensitivity (82.7%), a very good specificity (97.2%), and a good correlation with MPO and PR3 ELISAs. Its accuracy in association with the practicality of the automated EliA system make this method a useful tool for the diagnosis of AAV (14).

Capture ELISAs - for PR3-ANCA detection, capture ELISAs are reported to be superior to direct ELISAs. A better interlaboratory correlation in PR3-ANCA and detection of immunocomplexes have been described as their major advantages (15). However, standard capture ELISAs, in which PR3 is anchored by anti-PR3 monoclonal antibodies (the plate is precoated with a monoclonal antibody to capture the antigen), have two potential disadvantages. First, the capturing monoclonal antibodies (moAB) may compete for epitopes recognized by some PR3-ANCA, causing occasional false-negative results. Second, the capture of recombinant PR3 (rPR3) mutant molecules becomes unpredictable as modifications of specific conformational epitopes may not only affect the binding of PR3-ANCA, but also the affinity of the capturing anti-PR3 moAB. To overcome these difficulties a new capture ELISA for PR3-ANCA detection was developed (16). This new assay is based on the standardized capture of a variety of different carboxy-terminally c-myc tagged recombinant ANCA target antigens using anti-c-myc coated ELISA plates. Antigen used include c-myc tagged human rPR3 variants (mature and pro-form conformations), mouse mature rPR3 and human recombinant neutrophil elastase. The analytical sensitivity and specificity for PR3-ANCA positive serum samples were 93% and 100%, respectively, when rPR3 with mature conformation was used as the target antigen, and 83% and 100% when the pro-enzyme conformation was employed. In conclusion, this new anti-c-



myc capture ELISA compares favorably to our standard capture ELISA for PR3-ANCA detection, enables the unified capture of different ANCA target antigens through binding to a c-myc tag, and allows capture of rPR3 mutants necessary for PR3-ANCA epitope mapping studies.

## 2.2. Disease associations

### 2.2.1. Drug-induced vasculitis

ANCA are associated with certain drug-induced vasculitis syndromes. Most patients reported with drug-induced vasculitis have MPO-ANCA, often in very high titres, antibodies to elastase, lactoferrin or other minor antigens (17). Many cases of drug-induced AAV are associated with constitutional symptoms, arthralgias/arthritis, and cutaneous vasculitis. However, a full range of clinical features, including crescentic glomerulonephritis and alveolar hemorrhage, can also occur. The strongest links between medications and AAV occur in patients treated with propylthiouracil, methimazole, hydralazine, and minocycline. Other drugs occasionally implicated include penicillamin, allopurinol, sulfasalazine, procainamide, carbimazole, thiamazole, clozapine, and phenytoin (18-20).

### 2.2.2. Cystic fibrosis

ANCA directed against BPI are typical for a subgroup of patients suffering from cystic fibrosis and diffuse panbronchiolitis, especially patients with highly impaired pulmonary function and a lengthy period of colonization with *Pseudomonas aeruginosa*. BPI-ANCA have been found to inhibit neutrophil-mediated killing of *Pseudomonas aeruginosa* (21-23).

### 2.2.3. Autoimmune liver diseases

ANCA antibodies are found in some patients with autoimmune hepatitis,

sclerosing cholangitis and primary biliary cirrhosis, without the correlation with the disease status (24). In one study on almost 200 patients, ANCA were present in 74% of patients with autoimmune hepatitis, 26% of patients with primary biliary cirrhosis, and 60% of patients with primary sclerosing cholangitis. Major antigens were catalase,  $\alpha$ -enolase, and lactoferrin. The presence of ANCA as detected by indirect immunofluorescence was associated with the occurrence of relapses in autoimmune hepatitis, with decreased liver synthesis function in primary biliary cirrhosis and in primary sclerosing cholangitis, and with increased cholestasis in primary sclerosing cholangitis (25).

#### 2.2.4. Ulcerative colitis

In patients with inflammatory bowel diseases, the testing for both ANCA and anti-Saccharomyces cerevisiae antibodies (ASCA) enables clear-cut differential diagnosis of ulcerative colitis (UC) or Crohn's disease (CD) based on the high specificity (ANCA+ ASCA- 92.5% for UC, ANCA- ASCA+ 93.2% for CD). However, the presence of ANCA does not reflect any disease activity (26).

#### 2.2.5. Inflammatory rheumatic diseases

In patients with rheumatoid arthritis (RA), ANCA positivity ranges from 10% to 50% with the following target antigens: lactoferrin, MPO and others (27). ANCA have been reported with many other inflammatory rheumatic conditions, including systemic lupus erythematosus (SLE), ankylosing spondylitis, juvenile chronic arthritis, Sjögren's syndrome, inflammatory myopathies, and scleroderma. In one study, almost 400 patients with connective tissue diseases (CTD) were tested for the presence of ANCA. ANCA were detected in 13.9% of patients with SLE, 14.7% of

patients with RA, 9.5% of patients with systemic sclerosis and 13.1% of patients with Sjogren's syndrome. No ANCA were detected in healthy individuals (28).

#### 2.2.6. Infections

ANCA are found also in some infectious diseases, like subacute bacterial endocarditis (anti-PR3) and invasive amoebiasis, and HIV infection (anti-BPI, anti-MPO) (29-31).

#### 2.2.7. ANCA and anti-GBM double positivity

Between 10 and 40 percent of patients with anti-glomerular basement membrane (GBM) antibodies have ANCA, usually anti-MPO and about 10% of patients with ANCA antibodies have anti-GBM. The clinical significance of combined ANCA and anti-GBM antibodies is unclear. Outcomes in double positive patients with severe disease have generally been poor. In some, the titres of ANCA are low and there are no clinical manifestations of vasculitis. Others, however, present with disease features that are uncommon to anti-GBM antibody disease but quite typical of systemic vasculitis, including purpura, arthralgias, and granulomatous inflammation. The most likely explanation for the emergence of two autoantibodies is that ANCA-related glomerular damage subsequently mounts an immune response against the exposed and damaged glomerular basement membrane. In practice, patients with either ANCA-associated disease or anti-GBM disease, whether diagnosed serologically or by renal biopsy, should be tested for the second antibody. This approach should provide better prognosis and guide management (32, 33).

### 2.2.8. ANCA associated vasculitides

The clinical manifestation of WG, MPA, and CSS are extremely varied because they are influenced by the sites of involvement, and the activity versus the chronicity of the involvement. All three categories of vasculitis share features caused by the small vessel vasculitis, and patients with WG and CSS have additional features that define each of these syndromes. Generalized nonspecific manifestations of systemic inflammatory disease, such as fever, malaise, anorexia, weight loss, myalgias, and arthralgias, are often present in all the entities. Many patients trace the origin of their disease to a „flu-like“ illness.

#### 2.2.8.1. Wegener's granulomatosis

According to the CHCC nomenclature (2), WG is a systemic necrotizing vasculitis affecting small to medium-sized vessels (e.g. capillaries, venules, arterioles, and arteries). It typically produces granulomatous inflammation of the upper and lower respiratory tracts and necrotizing, pauci-immune glomerulonephritis in the kidneys. A „limited“ form, with clinical findings confined to the upper respiratory tract or the lungs, occurs in approximately one-fourth of cases. However, this subdivision is somewhat artificial because approximately 80 percent of such patients eventually have renal involvement.

The ACR proposed four clinical criteria for the classification of WG in 1990, before the routine availability of ANCA testing (1):

- nasal or oral inflammation (painful or painless oral ulcers or purulent or bloody nasal discharge)
- abnormal chest radiograph showing nodules, fixed infiltrates, or cavities
- abnormal urinary sediment (microscopic hematuria or red cell casts)
- granulomatous inflammation on biopsy of an artery or perivascular area.

The presence of two or more of these criteria yielded a sensitivity of 88%



and specificity of 92%.

At present, the diagnosis of WG is suggested from the clinical and laboratory findings and from the presence of ANCA that are more often directed against PR3 (70%) than to MPO (25%). About 5% are ANCA negative (34). As previously mentioned, renal disease is common (80%), manifested by acute renal failure and/or active urinary sediment with red cells, red cell and other casts, and proteinuria. Lung involvement is found in up to 90% of patients with WG, while E.N.T involvement also occurs in about 90%. Other organ systems that may become involved include (35, 36):

musculoskeletal system (myalgias, arthralgias, arthritis) – 60%

skin (vesicular, palpable purpuric, ulcerative, and hemorrhagic lesions, ischemic lesions, livedo reticularis) – 40%

nervous system (motor mononeuritis multiplex, sensory peripheral neuropathy, cranial nerve palsy, cord lesion, palsy, central nervous system mass lesions, organic confusion/dementia, seizures, hearing loss) – 30-50%

eyes (conjunctivitis, corneal ulceration, episcleritis, optic neuropathy, nasolacrimal duct obstruction, proptosis, diplopia, retinal vasculitis, and uveitis) – 15-30%

heart (pericarditis, myocarditis, coronary arteritis, cardiac valve involvement, arrhythmias, conduction disorders, congestive heart failure) – 5-50%

less commonly, the gastrointestinal tract, subglottis or trachea, lower genitourinary tract, parotid glands, thyroid, liver, or breast. Basically, any organ may be affected.

#### 2.2.8.2. Microscopic polyangiitis

According to the CHCC nomenclature (2), MPA is a necrotizing

vasculitis, with few or no immune deposits, affecting small vessels (capillaries, venules, or arterioles), although necrotizing arteritis involving small and medium-sized arteries may be present. Necrotizing glomerulonephritis is very common (90%). Pulmonary capillaritis often occurs (50%), but, by definition, patients with MPA do not have granulomatous respiratory tract lesions. Similarly, E.N.T. lesions may occur in MPA (35%), but they are caused by angiitis alone, without granulomatous inflammation. Destruction of bone, for example resulting in septal perforation and saddle nose deformity, appears to require necrotizing granulomatous inflammation (as in WG and CSS) and, therefore, does not occur in MPA. Nodular cutaneous lesions caused by dermal or subcutaneous arteritis and by the necrotizing granulomatous inflammation of WG and CSS, are very rare with MPA, other skin lesions occur often, in up to 40% of patients. Neurologic, musculoskeletal and other organ involvement is similar to those with WG, eye involvement is less frequent than in WG.

Patients with MPA have MPO-ANCA in 50%, PR3-ANCA in 40%, and are ANCA negative in 10% (36).

#### 2.2.8.3. Churg-Strauss syndrome

According to the CHCC nomenclature (2), CSS is a necrotizing vasculitis with eosinophil-rich and granulomatous inflammation affecting small to medium-sized vessels, involving the respiratory tract, and is associated with asthma and eosinophilia.

The following six criteria have been formulated by the ACR for the diagnosis of the CSS in patients with documented vasculitis (1):

- asthma
- eosinophilia of >10% on differential white blood cell count
- mononeuropathy (including multiplex) or polyneuropathy

- migratory or transient pulmonary opacities detected radiographically
- paranasal sinus abnormality
- biopsy containing a blood vessel showing the accumulation of eosinophils in extravascular areas

The presence of four or more of these criteria yields sensitivity of 85% and specificity of 99.7% for the CSS.

Patients with CSS have MPO-ANCA in 60%, PR3-ANCA in 10%, and they are ANCA negative in 30%. The vasculitis typically involves the arteries of the lung (70%) and skin (60%), but may be generalized. Renal involvement is less frequent in CSS (45%), E.N.T. involvement occurs in about 50% of patients. On the other hand, neurologic manifestation (usually with a mononeuritis multiplex) is most frequent in CSS compared to other AVV.

#### 2.2.8.4. Renal-limited vasculitis

RLV, or isolated (idiopathic) pauci-immune necrotizing/crescentic glomerulonephritis is distinguished from MPA and WG by the absence of extrarenal symptoms of vasculitis. It is more often MPO-ANCA positive and therefore considered a renal limited form of MPA.

### 2.3. Disease scoring

AAV is now viewed as chronic disease rather than a fatal condition due to improvement in survival by treating with immunosuppressants. Consequently, it is no longer appropriate to use death as the main study outcome. Therefore, the European Vasculitis Study Group (EUVAS) developed 3 assessment tools that are used for the outcome of clinical trials in vasculitis. Birmingham Vasculitis Activity Score (BVAS) is used to document current disease activity. It consists of a set of items divided

into nine organ based systems. The scoring sheet simply records the presence or absence of new or worsening features due to active vasculitis. Each item is weighted and the total score on all nine organ systems gives an indication of the disease activity in each patient at the time of scoring. Vasculitis Damage Index (VDI) is a scoring system to document those features, which are due to persistent systemic damage where there is no current disease activity. It is a cumulative assessment of organ dysfunction, damage or scarring. Finally, the Short Form 36 patient questionnaire is designed to assess the patient's perspective on the impact of the disease on their lives (37).

#### 2.4. Disease stages

The EUVAS group also defines disease stages of AAV based on clinical and pathologic considerations (table 2) (38):

Subgroup	Organ involvement	Constitutional symptoms	ANCA
Localised	Upper and/or lower respiratory tract	no	+/-
Early systemic	Any except renal/imminent organ failure	yes	usually +
Generalized	creatinine $\leq$ 500 $\mu$ mol/l and/or other imminent organ failure	yes	+
Severe renal	creatinine $>$ 500 $\mu$ mol/l	yes	+
Refractory	Progressive despite therapy	yes	+/-

Table 2. Clinical subgroups of AAV according to the definitions of the European Vasculitis Study Group (EUVAS). Constitutional symptoms are: fever, night sweats, weight loss, malaise, and fatigue. Imminent organ failure includes progressive lung, eye, nervous system, or gastrointestinal involvement.



## 2.5. Diagnostic tools

Routine laboratory tests are generally nonspecific in AAV. Common abnormalities include leukocytosis, thrombocytosis, marked elevation of the erythrocyte sedimentation rate (ESR) and C- reactive protein (CRP), and normochromic, normocytic anemia (35). Like ANCA, the markers of inflammation (ESR and CRP) fluctuate with vasculitis activity (39).

The diagnosis of AAV is generally confirmed by tissue biopsy at the site of active disease. Biopsy of a nasopharyngeal lesion is relatively noninvasive, but very often non-diagnostic, as the amount of tissue from this site is often small. Granulomatous inflammation is commonly present, but actual vasculitis is only detected in about one-third of cases. The granulomatous lesions of Wegener's granulomatosis are built up by CD4+ T-cells, CD8+ T-cells, histiocytes, CD20+ B-lymphocytes, neutrophil granulocytes, CD68+ macrophages, and CD68+ multinucleated giant cells surrounding a central necrosis. Scattered eosinophil granulocytes may be interspersed and do not necessarily automatically support the diagnosis of CSS. The central necrosis may be confluent or show an irregular serpiginous pattern coined "geographic" necrosis. A palisade of epitheloid histiocytes may arrange around the necrotic foci. The center of the necrosis is acellular or, in some instances, contains polymorphonuclear leukocytes. Apart from the respiratory tract granulomatous lesions may be found in virtually any organ, including for example retroorbital tissues or meninges. However, renal granulomas are rare.

If there is no lesion in the upper respiratory tract, the next step is biopsy of an affected organ such as kidney or lung. Renal biopsy is preferred because it is easier to perform, safer and often more diagnostic. To a certain extent, it provides us with additional information on renal prognosis (discussed later). Kidney biopsy typically reveals a segmental necrotizing glomerulonephritis with little or no immunoglobulin

deposition (pauci-immune) on immunofluorescence or electron microscopy. This finding is essentially diagnostic of an AAV. However, the histopathological features vary among patients from mild focal segmental extracapillary proliferation to diffuse crescentic necrotizing glomerulonephritis with granulomas and tubular intra-epithelial infiltrates. In some cases, extensive glomerulosclerosis is found (40).

If performed, the lung biopsy reveals vasculitis and granulomatous inflammation in WG. Special stains and cultures have to be performed to exclude the presence of infections that can produce granulomas, vasculitis or necrosis. Lung biopsy most often requires open or thoracoscopic lung biopsy. In a small number of cases (<10%), sufficient tissue for diagnosis can be obtained by transbronchial biopsy; however, negative result in this case does not exclude the diagnosis of vasculitis (41). Modern imaging techniques including computed tomography, magnetic resonance imaging, echocardiography and sometimes positron-emission tomography are essential tools for disease management.

Gallium scans are clinically helpful as a negative scan virtually excludes active WG. However, because of positive scans in cases of bacterial and viral infections, specificity is low (42). For research purposes, somatostatin receptor (SSTR) scintigraphy was used for the assessment of disease activity and extent in the upper and lower respiratory tract, and treatment efficacy. The specificity for active versus non-active disease was 96% for pulmonary disease and 100% for ENT involvement, while sensitivity was 86% and 68%, respectively (43).

Bronchoalveolar lavage (BAL) may disclose inflammatory neutrophilic (usually < 20%) or lymphocytic cell (median 40%) profiles suggestive of alveolitis and pulmonary hemorrhage. BAL will also be helpful in excluding opportunistic infections in patients treated with immunosuppressants (44, 45).

## 2.6. Etiology and pathogenesis of AAV

AAV is a complex, immune-mediated disorder in which tissue injury results from the interplay between an initiating inflammatory event and a highly specific pathogenic immune response to previously shielded epitopes of neutrophil granule proteins. This generates high titer ANCA directed against antigens within the primary granules of neutrophils and monocytes. These antibodies produce tissue damage via interactions with primed neutrophils and endothelial cells. Inflammatory cytokine production and adhesion molecule activation or upregulation are important determinants of the pathogenic inflammatory responses noted in vasculitis. The exact mechanisms by which ANCA arise and their role in the etiology of AAV remain unclear. The exact events leading to the initiation of the disease are obscure. Infectious, genetic, and environmental risk factors and combinations of all three have been entertained (46).

### 2.6.1. Etiology - environmental factors

#### 2.6.1.1. Infection

An infection is thought to be one of triggering factors in AAV. A seasonal variation of WG with the onset being the highest in winter supports this hypothesis (47,48) even if this has not been observed by other authors (35). The development of ANCA and vasculitis in a rat immunized with bacterial (*S. aureus* and *E. coli*) proteins supports the relationship between infections and ANCA (49). In WG, respiratory tract infections frequently precede or accompany initial symptoms. There is clear evidence that chronic nasal carriage of *Staphylococcus aureus* is approximately three times higher in Wegener's granulomatosis compared to healthy and disease controls and constitutes a risk factor for disease exacerbation of Wegener's granulomatosis (50). Prophylactic treatment with trimethoprim-

sulfamethoxazole reduces respiratory and non-respiratory tract infections and the risk of relapses in Wegener's granulomatosis and may also induce remissions in localized Wegener's granulomatosis (51). How *S. aureus* may induce (re)activation of disease activity has not been fully established. *S. aureus* produces a cationic protein, staphylococcal acid phosphatase, which can bind to endothelial cells, by charge interaction, in vitro (52), and has been demonstrated in glomeruli of some patients with WG. Antibodies to this phosphatase are present in the sera of patients with WG and controls, albeit at higher levels in the patients (53). It has been hypothesized that focal immune complex formation, possibly consisting, at least in part, of the phosphatase and its cognate antibodies, occurs in the vessel wall which attracts neutrophils. In the presence of ANCA, this focal inflammatory reaction is strongly enhanced resulting in necrotizing lesions and degradation of the immune complexes that were initially present. Indeed, immune complexes have been demonstrated in early skin lesions of patients with WG (54). Other *S. aureus*-related mechanisms of disease activation have been suggested as well, in particular autoreactive PR3-producing B-cells activation within granulomatous lesions of the respiratory tract resulting from stimulation with *S. aureus*-derived superantigens, such as staphylococcal protein A. Antigenic mimicry is suspected to be an important factor in triggering ANCA formation. *S. aureus* genome directly encodes a variety of serine proteases, which may be cross-reactive with C-ANCA (56). As mentioned above, a translocation of intracellular hidden antigens during nonspecific activation of neutrophils during any inflammation may also be involved. When released from the cell they may become easily accessible to ANCA, or may induce ANCA formation (57).

#### 2.6.1.2. Drugs and chemicals

The existence of drug-induced AAV has been already discussed. Given the frequency with which the first symptoms of WG occur in the respiratory tract, exposure to noninfectious agents or toxins is another possible inciting event. The possible candidates are silica dust and organic solvents. Since 1960, several patients with pulmonary silicosis have been described to develop pauci-immune necrotizing crescentic glomerulonephritis. Later it was reported that these patients have ANCAs that in most cases are directed to myeloperoxidase. The exposure to silica dust has been therefore repeatedly studied in patients with ANCA and AAV. It has been reported to be significantly higher than in healthy controls, lupus nephritis or other conditions. Recently, a case report was published of a patient who developed and died of MPA after silicone breast implantation (58). Silica is thus one of the first well-documented environmental triggers in these diseases, although the mechanisms by which silica may induce AAV are not well known. Silicon-containing compounds have a pronounced adjuvant effect on immune responses, and silica particles are potent stimulators of lymphocytes and monocytes or macrophages. Further, silica may induce apoptosis of monocytes or macrophages and possibly neutrophils (59-61).

Interestingly, the proportion of active smokers in AAV patients was significantly lower than in the entire population in one study on almost 200 patients. Cigarette smoking may be associated with a reduced risk of AAV (62).

#### 2.6.2. Etiology - genetic factors

WG is more predominant among Caucasians and the genetic predisposition appears quite complex. A number of familial cases of AAV have been described, and suggested candidate genes include, among others, cytotoxic

T-lymphocyte-associated protein 4 (CTLA-4), interleukin-1 receptor antagonist (IL-1ra), IL-10, and Fc $\gamma$ RII/Fc $\gamma$ RIII (63). Fc $\gamma$ -receptor polymorphism seems to be involved in disease expression (64). The PTPN22 620W allele is a risk factor for Wegener's granulomatosis (65). The PR3 gene itself shows polymorphism and an association was demonstrated for the A-564G polymorphism in the PR3 promoter, affecting a putative transcription factor binding site, with WG (66). It has been shown that, in some persons, PR3 is expressed on the cell surface of a varying percentage of resting peripheral blood neutrophils. This so-called bimodal expression of PR3 was more frequently seen in patients with WG as well as in their healthy relatives (67).

Mutations in the gene encoding  $\alpha$ -1 antitrypsin (AAT), the primary in vivo inhibitor of PR3, are found more frequently in patients with AAV (68). This observation suggests a potential pathogenic role in this disease for deficient PR3 clearance from the sites of inflammation. Decreased local concentrations of AAT caused by genetic polymorphisms or alterations in the enzyme's functionality induced by inflammation may therefore lead to protease/anti-protease imbalance in the disease microenvironment. AAT phenotypes leading to decreased levels of functional AAT have been associated with an increased risk of WG as well as with increased morbidity (69). Although unproven, these events may be responsible for generating immunogenic forms of PR3 in these patients. However, no case of AAV was found among a group of patients having a heterozygous mutation of this gene (70).

Suspected polymorphisms of the TNF $\alpha$  promoter were excluded as risk factors for the disease in a cohort of German patients (63), but the same authors described polymorphism in adhesion molecule CD18 to be associated with various forms of AAV (71). As to HLA class II, alleles DQw7 and DR4 haplotypes were found to be associated with the



persistence of ANCA in vasculitis (72). However, so far, no particular narrow HLA phenotype has been identified to convey susceptibility to Wegener's granulomatosis.

In summary, as in other autoimmune diseases, the etiology of AAV is heterogeneous and principally unknown. Different predisposing factors play probably differential etiopathogenic roles in various groups of AAV.

### 2.6.3. Pathogenesis

The pathogenesis of systemic vasculitis is complex and is likely to involve many mechanisms.

#### 2.6.3.1. Neutrophil activation and ANCA

Although ANCA antigens are normally in the cytoplasm of neutrophils and monocytes, preactivation (priming) of these cells, as occurs following exposure to low amount of pro-inflammatory cytokines such as TNF $\alpha$ , TNF $\beta$ , granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1, IL-8 results in the release of small amounts of ANCA antigens at the cell surface.

TGF $\beta$  is a multifunctional cytokine modulating onset and course of autoimmune diseases. The possible interactions of TGF $\beta$  with lysosomal enzymes identified as ANCA autoantigens (e.g. PR3) were studied. This included TGF $\beta$  effects on the translocation of the lysosomal enzymes to cell surface of polymorphonuclear cells (PMN), and the presumed activation of latent TGF $\beta$  by these enzymes. Flow cytometry analysis showed TGF $\beta$ 1 to be a potent translocation factor for PR3 comparable with other neutrophil activating factors such as IL-8. The PR3 membrane expression on primed PMN increased by up to 51% after incubation with TGF $\beta$ 1. PR3 itself was revealed as a potent activator of latent TGF $\beta$ , thus mediating bioeffect of this cytokine. Patients with various types of

systemic vasculitis showed marked TGF $\beta$  overexpression correlating with disease (73).

During priming the target antigens of ANCA, i.e. PR3 and MPO, are expressed at the cell surface and become accessible for interaction with ANCA.

ANCA-induced neutrophil activation involves not only binding of the antibodies via their F(ab')<sub>2</sub>-fragments to surface expressed PR3 or MPO, but also interaction of their Fc-fragments with Fc-receptors on neutrophils, particularly with Fc $\gamma$ RIIa-receptor (74, 75). It has been demonstrated that ligation of Fc $\gamma$ RIIa and Fc $\gamma$ RIIIb is necessary for ANCA-induced neutrophil activation, but that the signaling cascades used by ANCA were different from the signal pathways used by Fc $\gamma$ R engagement only, suggesting that ANCA require other not yet identified membrane cofactors for neutrophil activation than Fc $\gamma$ R engagement alone (76). The Fc $\gamma$ RIIa is the only Fc-receptor that interacts with IgG2 whereas this receptor also has a particular affinity for the IgG3 subclass. Interestingly, the increase in neutrophil activating capacity of serum IgG fractions from remission to relapse in patients with PR3-ANCA positive WG correlated with increases of levels of IgG3 subclass ANCA in those fractions and not with that of other subclasses (77). In addition, renal relapses of WG are particularly associated with increases of the IgG3 subclass of ANCA, although IgG1- and IgG4 subclasses of ANCA are present as well (78). It should be, however, stated that measuring the IgG3 subclass of PR3-ANCA concomitantly with the total IgG PR3-ANCA did not improve the predictive value of a rise in ANCA for ensuing relapses (79). Nevertheless, these data suggest that the IgG3 subclass of ANCA may play a particular role in neutrophil activation via Fc $\gamma$  receptor interactions.

The interaction of ANCA and target antigens is followed by activation of neutrophils.

It has been demonstrated on the neutrophils from MPO-deficient donors by the absence of superoxide anion production that the presence of MPO is a condition sine qua non for neutrophil activation by anti-MPO antibodies (80). In vitro, ANCA-IgG causes cytokine-primed neutrophils to undergo a respiratory burst and degranulation (81). The degranulation of neutrophils and release of chemoattractants and cytotoxic oxygen free-radicals causes tissue damage (82). The activation of neutrophils in AAV was established in vitro by reactive oxygen species (ROS) production and the ability of superoxide anion to reduce ferrocytochrome c (83). ANCA are able to induce stable adherence of rolling neutrophils to layers expressing adhesion molecules (84).

In addition, primed neutrophils not only damage endothelial cells, but attract additional neutrophils to the site of damage, thereby creating an auto-amplifying loop. The release of MPO, PR3, elastase and other proteases from activated neutrophils also contributes directly to the local inflammatory process. Released enzymes can bind to the surface of unprimed neutrophils, facilitating subsequent recognition by ANCA and amplifying ANCA-potentiated neutrophil activation. It has been demonstrated that not only ANCA, but also their F(ab')<sub>2</sub>-fragments, although to a lesser extent, could induce the release of lytic enzymes.

The activation of the neutrophils only occurs when they are adherent to a surface, a process in which beta2-integrins are involved (85). In vivo, this process is assumed to occur at the endothelial surface. Indeed, activated neutrophils, adherent to the epithelium, are observed in renal biopsies from patients with ANCA-associated necrotizing crescentic glomerulonephritis (86). ANCA-antigen complexes adsorb onto endothelial cells where they can participate in in situ immune-complex formation. Persistent ANCA binding to neutrophils on the endothelial surface can enhance the degree of vascular injury (87).

Endothelial cells are the target of the initial injury resulting in swelling, necrosis and deadherence of endothelial cells. Lysed neutrophil granulocytes are found within affected vessels. In the lung, capillaries, venules and arterioles are infiltrated by polymorphonuclear leukocytes. Pulmonary microvascular necrotizing vasculitis (capillaritis) is the cause of pulmonary hemorrhage. In the kidney, rupture of the basement membrane subsequent to neutrophil degranulation gives rise to glomerular capillary thrombosis followed by a cascade of events leading to focal segmental crescentic glomerulonephritis.

#### 2.6.3.2. Monocyte activation and ANCA

Besides neutrophils, also monocytes can be activated by ANCA. Affinity-purified PR3-ANCA and MPO-ANCA were able to induce monocytes to produce oxygen radicals. Blocking of the Fc $\gamma$ RIIa reduced the formation of oxygen radicals but F(ab')<sub>2</sub>-fragments of ANCA were still able to activate monocytes suggesting that activation occurs along several lines (88). The complex of PR3 and ANCA leads to an increased expression of CD18, CD14 and an elevated synthesis of cytokines and chemokines such as IL-1, IL-8 in monocytes. Patients with active WG have higher levels of CD4<sup>+</sup> T cell and monocytic activation markers (TNF $\alpha$ , INF $\gamma$ , IL-12) than patients in remission and healthy controls. IL-10 treatment (which inhibits Th1 pathway by impairing the production of IL-12) of mononuclear cells from active WG patients impairs the production of INF $\gamma$  in vitro (89-91).

In both monocytes and granulocytes apoptosis finally occurs (92).

#### 2.6.3.3. The role of T cells

There is growing evidence that T cells may contribute to the pathogenesis of AAV. In active AAV, the cellular infiltrates in kidney, lung, and nasal

tissue mainly consist of macrophages, T and B cells (immunohistochemical examinations of nasal biopsies from untreated patients with active WG revealed the presence of CD3+, CD4+, CD8+, CD20+, CD38+, CD68+ cells) (93-97). Patients with AAV have abnormal expansion of T cells, especially in CD4+ subset, compared with T-cell expansions in healthy individuals (98). Although the presence of T cells strongly suggests a pathogenic role, the antigenic specificity of the infiltrating T cells has not been identified.

T lymphocyte proliferation was observed by in vitro stimulation with an extract of human neutrophil alpha granules, containing PR-3 in patients with C-ANCA and active disease. However, at least some degree of proliferation has also also been observed in PR3-stimulated T cells from healthy controls. Thus, a pathogenic role for T cells in the effector phase of AAV, at least in WG, seems likely, but has not been fully established (99). The predominant IgG subclasses of ANCA in patients with WG are IgG1 and IgG4. Isotype switching from IgG1 to IgG4 depends on repeated antigenic stimulation and also on T-cell cytokines such as IL-4, which suggests that the production of the antibodies is T-cell-dependent (100-101). Furthermore, several studies found increased serum markers of T cell activation, including soluble IL-2 receptor, soluble CD4, and soluble CD8 in AAV. In patients with WG, the levels of soluble IL-2 receptor (sIL-2R) correlate well with disease activity and may indicate imminent relapse. Levels of sIL-2R increased in the moment of major relapse, correlated with CRP and with the disease activity score. The rise of the ANCA titer preceded the rise in sIL-2R by at least 1 month. The authors concluded that major relapses of WG were accompanied by systemic T cell activation, which, however, did not appear to precede the rise in ANCA titers (102). Percentages of activated CD4+ and CD8+ T cells in peripheral blood were higher in patients with active WG than in healthy individuals. However,

this increase was also observed in patients in remission, which might indicate that T cell activation persists during remission (103-105).

CSS was found to be associated, in its active state, with markedly increased levels of sIL2-R and eosinophil cationic protein (ECP) indicating T cell and eosinophil activation, and elevated soluble trombomodulin (sTM) as a sign of endothelial cell damage (102).

Based on their cytokine profile and related functions, CD4+ T-helper lymphocytes are classified into two distinct types: type 1 (Th1) and type 2 (Th2) (table 3). When no polarization of the response is present the resultant phenotype is designated as type 0 (Th0). The main inducer of the Th1 profile is macrophage-derived cytokine IL-12 (106). In general, Th1 cells characteristically produce IFN $\gamma$  and are involved in cell-mediated inflammatory reactions (107). Th2 cells, producing IL-4, IL-5 and IL-10, are associated with phagocyte-independent host responses, encouraging antibody production and enhancing eosinophil proliferation and function. Of the Th2-related cytokines, IL-4 promotes differentiation of naive CD4+ cells into Th2 cells, whereas IL-10 inhibits Th1 cytokine synthesis (108-110).

Property	Th1	Th2
Cytokine secretion		
IFN $\gamma$	++	-
TNF- $\alpha$	++	+
IL-2	++	+
IL-13	+	++
IL-10	+	++
IL-4	-	++
IL-5	-	++
Surface expression		
CD26	++	+/-
CCR5	++	+/-
CCR3	+/-	++
CD30	+/-	++



Property	Th1	Th2
Release in biological fluids		
sCD26	++	+/-
sCD30	+/-	++

Table 3. Th1 and Th2 characteristics (110).

In the granulomatous lesions of patients with Crohn's disease and tuberculosis, a clear-cut Th1 cytokine profile was found, with high numbers of CD4+ cells producing IFN $\gamma$ , but no IL-4 (111-112). In localized WG, T cells in nasal inflammatory infiltrates were found to express the Th1 marker CD26 and higher numbers of IFN $\gamma$ -positive cells than in generalized disease (113). This was accompanied by increased spontaneous IFN $\gamma$  and IL-10 production by peripheral blood mononuclear cells of patients with localized WG compared to generalized WG, whereas in nasal inflammatory infiltrates in generalized WG IL-4 mRNA was detected in higher amounts. Another study confirmed this Th2 environment in nasal granuloma in generalized WG. By immunohistochemistry, IL-4 was found to be upregulated, while IFN $\gamma$  was not detected in nasal biopsies of 10 patients with generalized active WG (114). Predominance of Th1- type chemokine receptor CCR5 expression on T-cells is seen in localized Wegener's granulomatosis and may favor stronger recruitment of Th1-type cytokine-secreting cells into inflammatory lesions in localized as compared to generalized Wegener's granulomatosis in response to its CCchemokine ligand RANTES (regulated on activation, normal T-cell expressed and secreted/CCL5). In contrast to localized Wegener's granulomatosis, a fraction of Th2-type CCR3+ T-cells is also seen in generalized Wegener's granulomatosis (115).

Activation of T cells involving cytokine production and proliferation requires at least one costimulatory signal. A subset of circulating T-cells lacking the co-stimulatory molecule CD28 is expanded in Wegener's granulomatosis. The expansion of CD28<sup>-</sup> T-cells starts early in the disease process and is already evident in localized Wegener's granulomatosis. The expansion of CD28<sup>-</sup> T-cells correlates with the organ involvement. Circulating peripheral blood as well as CD4<sup>+</sup>CD28<sup>-</sup> T-cells within granulomatous lesions are a major source of Th1-type cytokine secretion which is mainly restricted to TNF- $\alpha$  and IFN- $\gamma$ . The Th1-type CD28<sup>-</sup>T-cell subset displays features of effector memory T-cells. A lower Th1-type CCR5 expression on CD4<sup>+</sup>CD28<sup>-</sup> T-cells in generalized as compared to localized Wegener's granulomatosis suggests further differentiation of this T-cell subset during disease progression. These studies suggest that an aberrant Th1-type response might play a role during the initiation and progress of the disease process (116-118). On the other hand, plasma levels of sCD30 have been shown to be significantly increased and correlate with disease extent and activity in generalized WG. These findings suggest that sCD30 can act as a useful marker for evaluation of disease extent and activity, and that generalized WG may be associated with Th2-type immune response (119).

In summary, T cells appear to be involved in the pathogenesis of systemic vasculitis, but their specific role is still uncertain. The immunopathological process is T-cell-driven and ANCA production appears to be T-cell dependent. Peripheral blood T-cell responses to PR3-ANCA are seen in patients and to a lesser extent in controls. Selection of particular TCRs in patients with systemic vasculitis may suggest the existence of a specific vasculitis-associated T-cell antigen. Understanding the mechanisms resulting in loss of tolerance in patients with systemic vasculitis may be of importance for prognosis and the development of new immunotherapies

(120).

#### 2.6.3.4. The role of the endothelial cell

Endothelial cells may actively recruit inflammatory cells in the early stages of active disease, and enhance their adhesion to sites of vascular injury. They may synthesize PR3 (unproven), which could participate in in situ immunocomplex formation (121).

Endothelial cells may be targeted by anti-endothelial cell antibodies (AECA) and are central targets of numerous proinflammatory cytokines. To define mechanisms of vascular injury in WG and MPA, AECA were sought in serum from 168 patients, all of whom had ANCA. Using an ELISA with human umbilical vein endothelial cells (HUVEC), IgG AECA were demonstrated in 59% and IgM AECA in 68% patients. Pre-treatment of HUVEC with TNF, IL-1 or INF $\gamma$  led to increased binding. A widespread endothelial cell damage was suggested from a marked elevation of vWF antigen levels. These data suggest that patients with WG or MPA can develop AECA to constitutively expressed but cytokine modulated determinants on HUVEC. These antibodies did not appear to support complement-mediated cytotoxicity, but rather antibody-dependent cellular cytotoxicity, suggesting that they may contribute to vascular injury (122). The soluble endothelial leucocyte adhesion molecule-1 (ELAM-1) has been shown to act as a neutrophil chemoattractant and may also represent a specific marker of endothelial cell damage or activation. Nine patients with AAV were prospectively monitored for disease activity, serum ELAM-1, C-reactive proteins (CRPs), von Willebrand factor (vWF) and ANCA levels. The abnormally high ELAM-1 levels at presentation fell within normal limits a week following pulse methylprednisolone therapy. This preceded a fall in CRP, vWF and subsequent clinical remission. One patients relapsed with rising ELAM-1 levels. These findings suggest a

possible role of ELAM-1 in monitoring of AAV (123).

Since the expression of E-selectin and the production of IL-6 by endothelial cells is an early step in the sequence of events leading to vascular injury, the extent of HUVEC activation by ANCA-positive sera was measured by the lectin expression by flow cytometry and the production of IL-6 by ELISA. The positive results suggest that the activation of endothelial cells in patients with WG and MPA can be induced by circulating antibodies. Both ANCA and AECA can be responsible for this effect (124).

Whether endothelial cells themselves express ANCA-antigens such as PR3, has been a subject of controversy. Some data suggest that endothelial cells express PR3, particularly when activated, and that subsequently, ANCA can bind to surface expressed PR3 resulting in upregulation of adhesion molecules and further activation of those cells (125). Other authors, however, have not been able to confirm PR3 expression by endothelial cells but demonstrated that PR3 binds to endothelial cells via a specific receptor (126).

#### 2.6.3.5. Pathogenicity of ANCA

A direct evidence for pathogenicity of ANCA is a recently described case of a woman with a history of MPA who relapsed during pregnancy. Forty-eight hours after delivery the newborn developed pulmonary hemorrhage and renal abnormalities. The newborn's cord blood showed an immunoglobulin G MPO-ANCA level identical to that of the mother's serum, indicating passive transfer of the antibody to the neonate (127). A mouse model that shows that MPO-ANCA alone may induce vasculitis in mice, deficient in both T and B lymphocytes (Rag2<sup>-/-</sup>), was also described (128).

Indirect evidence lies in the correlation of ANCA (and surface expression

of PR3 on neutrophils) with the disease activity (39, 129), even though even situations when high titers of ANCA do not correlate with active disease are not rare (130). The different epitopes on PR3 and MPO recognized by ANCA and their relation to disease activity remain to be explained. The potential of ANCA to bind to target antigens and modify their physiologic function (induction of oxygen radical release, degranulation, inhibition of microbicidal function, defective apoptotic process), thereby contributing to tissue damage (82), or the potential of ANCA to bind to planted antigen on endothelial cell and induction of endothelial cell injury (131) are another indirect proofs of the pathogenicity of ANCA.

## 2.7. Treatment of ANCA-associated vasculitis

### 2.7.1. Natural History

Prior to the introduction of immunosuppressive therapy, the outcome of the patients with AAV was fatal, with most patients dying in less than a year due to a vital organ failure (36).

### 2.7.2. Induction therapy

#### 2.7.2.1. Cyclophosphamide

In the early 1980s, Fauci and Wolff introduced a regimen combining daily cyclophosphamide (CYC) therapy given for one year after remission with prednisone therapy initiated at a dose of 1 mg/kg/b.w./day and tapered on an alternate-day schedule. This treatment ("Fauci-scheme") has been found to induce remission in 80-100 % of patients and can result in long-term survival. It was an empirical regimen and therefore entailed considerable therapy-related morbidity and mortality. Furthermore, when therapy is tapered and discontinued, relapses are common (in up to 50% of cases). Although CYC treatment is effective in managing the relapses, repeated

courses of CYC are associated with bone marrow suppression, infection, cystitis, infertility, myelodysplasia, and transitional-cell carcinoma of the bladder and other secondary malignancies. As a result, the introduction of immunosuppressants has changed the natural history of an acute, progressive and life-threatening disease into a chronic, often grumbling one with progressive accumulation of tissue damage due to disease scars and adverse effects of the therapy (132).

For all the above mentioned reasons, a number of clinical trials aimed at improvement of induction and maintenance treatment of AAV has been launched. In Europe, EUVAS was created gradually during the first half of the 1990s and focused first on diagnostic role of ANCA, followed by the standardization of ANCA testing, histological assessment and classification of AAV. It was assumed that despite their different clinicopathological characteristics, AAV could be studied together, but, for treatment purposes, subclassified based on their severity at presentation (see Disease scoring) (133).

The optimal induction treatment of induction of remission in patients with generalised, but not immediately life-threatening AAV was tested in the CYCLOPS trial, where the standard daily oral CYC (2 mg/kg b.w./day for months 0-3 and 1.5 mg/kg b.w./day for months 3-6) was compared with pulsed CYC (10 iv pulses 15 mg/kg b.w. during 6 months), in both limbs with corticosteroids (CS), with the aim to reduce the cumulative dose of CYC and thereby the toxicity of the treatment. According to published preliminary results of CYCLOPS, the intermittent pulse administration of CYC proved to be as efficient as the pulse administration with significant reduction of cumulative dose and both short-term and long-term toxicity of CYC (134).



#### 2.7.2.2. Methotrexate

The NORAM trial compared the effect of methotrexate (MTX) (15-25 mg weekly) and CYC (at a standard dose 2 mg/kg b.w./day) on the remission rate in early AAV. At 6 months, the remission rate in patients treated with MTX (89.8%) was not inferior to that in patients treated with CYC (93.5%). In the MTX group, remission was delayed among patients with more extensive disease or pulmonary involvement. Relapse rates at 18 months were 69.5% in the MTX group and 46.5% in the CYC group. Leukopenia was less frequent in the MTX versus the CYC group, liver dysfunction was more frequent in the MTX group. The authors conclude that MTX can replace CYC for initial treatment of early AAV. The MTX regimen used in the present study was less effective for induction of remission in patients with extensive disease and pulmonary involvement and was associated with more relapses than the CYC regimen after termination of treatment. The high relapse rates in both treatment arms support the practice of continuation of immunosuppressive treatment beyond 12 months (135).

#### 2.7.2.3. Plasma exchange

The role of plasmapheresis has been studied in the MEPEX trial, where the patients with acute renal failure due to AAV were randomized to adjunctive therapy with either seven plasma exchange treatments (each 60 ml/kg b.w.) or three pulses of intravenous methylprednisolone (each 15 mg/kg b.w.). Although the mortality in both trial arms was the same, renal survival was much better in patients with plasma exchange (136). These data confirmed meta-analysis of several smaller studies and strongly suggest that plasma exchange should be used as an adjunctive treatment to CYC in patients with AAV with acute renal failure (137). Also patients presenting with hemoptysis and pulmonary infiltrates caused by diffuse

alveolar hemorrhage benefit from prompt initiation of plasmapheresis therapy coupled with aggressive immunosuppression (66).

#### 2.7.2.4. Recommendations for induction treatment

In summary, most physicians favor a CYC-CS combination regimen in the initial treatment of most patients with AAV. This is particularly indicated in those with life-threatening disease, including patients with a serum creatinine concentration above 177  $\mu\text{mol/l}$ , pulmonary involvement, CNS disease, and/or bowel perforation/infarction. Pulsed CYC will get preference over daily oral CYC as it is a comparably effective and safer alternative. The initial dose of CS is usually 1 mg/kg per day of oral prednisone and is gradually tapered. CYC and CS are continued until stable remission is induced (usually 3-6 months). A methotrexate-based regimen is an option in patients with active but not immediately life-threatening disease and normal or near normal renal function (with a serum creatinine concentration below 177  $\mu\text{mol/l}$ ). Prednisone alone is not recommended (139). Plasmapheresis should be added in patients with dialysis-dependent renal failure and life-threatening pulmonary hemorrhage at presentation, especially in those with high titer ANCA and/or anti-GBM antibodies.

#### 2.7.3. Maintenance therapy

##### 2.7.3.1. Azathioprine

The CYCAZEREM (cyclophosphamide vs azathioprine for early remission phase of vasculitis) examined whether azathioprine (AZA) was as effective as CYC in maintaining remission and preventing relapses in AAV, but with fewer side effects. One hundred and forty-one patients with threatened vital organ function and creatinine levels  $<500 \mu\text{mol/l}$  were randomized after induction of remission (with daily oral CYC + CS usually

for 3 months) to either treatment with continued oral CYC (1.5 mg/kg b.w./day) for 12 months, or AZA (2 mg/kg b.w./day). Both treatments were found to be equally effective (a comparable mortality, relapse rate, renal outcome, and short-term adverse effects). Given the known long-term safety profile of AZA compared with CYC, this trial has clearly established the superiority of AZA over CYC in preventing relapse after initial induction of remission in AAV. The results support the concept of aggressive treatment of active disease and lower-intensity therapy for the maintenance of remission. The possible need for further CYC treatment for late relapse adds to the importance of minimizing the initial level of exposure (140).

#### 2.7.3.2. Mycophenolate

Encouraged by a preliminary experience with mycophenolate (MMF) in smaller studies (141), the EUVAS has launched the IMPROVE trial comparing MMF and AZA as a maintenance therapy in patients in remission. MMF could have a place not only in the treatment of patients in remission, but also in patients with chronic active disease unresponsive to CYC, or in whom further courses of CYC would be inappropriate. It will also be assessed for induction treatment in patients with moderately extended disease.

#### 2.7.3.3. Methotrexate

MTX has been given for maintenance treatment of AAV with good success. The French collaborative vasculitis research group has compared low-dose MTX to AZA in a large randomized controlled trial which showed similar relapse rates in both arms. A trial comparing MTX to leflunomide by the German Rheumanet study group was terminated prematurely because of an unexpected high relapse rate in the MTX group.

However, the better results seen with leflunomide were offset by more adverse events (134). In one controlled study, MTX has been shown superior to trimethoprim-sulfamethoxazole in maintaining remission (142, 143).

#### 2.7.3.4. Trimetoprim-sulfamethoxazole (co-trimoxazole)

It has been shown in a double blind, placebo-controlled, multicentric trial, that treatment with co-trimoxazole (in a dose of 800 mg of sulfamethoxazole and 160 mg of trimethoprim given twice daily for 24 months) reduces the incidence of relapses in patients with AAV in remission. The reduction of the number of relapses was especially evident with respect to relapses involving the upper airways. In addition, fewer respiratory and non-respiratory tract infections were found in the treated group. These findings suggest that the drug exerts its protective effect by preventing infections. Given the reported association between nasal carriage of *Staphylococcus aureus* and an increased risk of relapse of WG, it is tempting to postulate that co-trimoxazole reduces the frequency of relapses by eliminating or reducing *S. aureus* in the upper airways. On the other hand, co-trimoxazole, through its antagonism of folic acid metabolism or other yet unknown mechanisms, may have immunosuppressive properties (144). Moreover, trimethoprim-sulfamethoxazole induces remissions in localized Wegener's granulomatosis.

#### 2.7.3.5. Cyclosporin A

Little data is available on the use of cyclosporin A. Haubitz et al. treated 7 patients for one year after inducing remission and none of the patients suffered a relapse during the year of follow-up. This is a very small and short-term study, but supports data showing low rates of relapse in patients

with vasculitis receiving cyclosporin A after renal transplantation (145).

#### 2.7.3.6. Recommendations for maintenance therapy

Once complete remission is achieved, CYC is discontinued and either MTX (which is an option only in those with a serum creatinine < 177  $\mu\text{mol/L}$ ) or AZA is initiated. Maintenance therapy is usually continued for 12 to 24 months. Slow tapering of CS is initiated once there is a significant response, which usually occurs after one month. A low-dose (5-10 mg per day of prednisone), possibly in an alternate day regimen, is maintained for as long as immunosuppressive therapy is continued.

#### 2.7.4. Treatment of relapse

Treatment of relapse is determined by severity and by whether or not the patient is still being treated. Among those with minor relapses determined clinically or by biopsy who are still receiving maintenance therapy, a trial of increasing the dose of CS and immunosuppressive agents can be considered. By comparison, reinstatement of the initial induction regimen is warranted in patients with more severe disease and in those who are no longer on immunosuppressive therapy (34). Thus, treatment of recurrent vasculitis is largely similar to that of the primary disease. Given the increased drug exposure, greater attention must be paid to potential toxicity. In terms of maintenance therapy, the duration after reinduction is prolonged to two years after remission. In addition, if relapse occurred while on maintenance therapy, a different drug should be used (eg. MMF rather than AZA in a patient with renal disease).

#### 2.7.5. Other therapeutic approaches

##### 2.7.5.1. Intravenous immunoglobulin

A number of studies have reported beneficial effects of intravenous

immunoglobulin (IVIG) in patients with chronic grumbling vasculitis despite more conventional treatments or in patients with acute disease. Jayne et al. conducted a small controlled trial of IVIG given as a single dose of 2 g/kg b.w. in patients with chronic active disease. Fourteen of 17 patients given IVIG showed improvement compared with only 6 of 17 given placebo, but the effect was short-lived (146). Schoenfeld et al. used monthly IVIG (2g/kg b.w.) given over 5 days in 13 patients with vasculitis, after other treatments had failed to control disease; 8 patients had a beneficial outcome, but relapses were common (147).

#### 2.7.5.2. Deoxyspergualin

The mechanism of action of deoxyspergualin (DSG) includes inhibition of IL-1 synthesis and anti-proliferative effects. It appears to be an effective and safe agent to treat patients with AAV refractory or with contraindications to standard immunosuppressants. The experience with this drug is very limited so far and further studies are warranted (and already under way) to investigate DSG as secondary or even primary agent in patients with AAV (148).

#### 2.7.5.3. Leflunomide

Leflunomide inhibits pyrimidine nucleotide synthesis, inhibits proliferation of activated lymphocytes, and reduces IL-2, TGF $\alpha$  and antibody production. Unlike in rheumatoid arthritis, in AAV there is only a single report of the use of leflunomide in the maintenance phase with very good results (149).

#### 2.7.5.4. Etoposide

According to a report by Danish authors and our unpublished experience with two patients, treatment with cyclic etoposid can result in complete



remission in patients resistant to and non-tolerant of standard immunosuppressive therapy (150).

#### 2.7.6. New therapeutic approaches

Lymphocyte depletion using monoclonal antibodies (CAMPATH 1H-anti-CD52 pan lymphocyte antigen, or anti-CD4) has been reported in a handful of patients with relapsing or persistent disease (151). Fifteen patients with histologically proven active refractory Wegener's granulomatosis were treated with anti-thymocyte globulin (ATG) by a protocol (SOLUTION protocol) designed by EUVAS. Thirteen of 15 patients showed a favorable response to ATG. During a follow-up of 21.8 months, seven patients relapsed. Two patients died, 1 and 3 days following the first dose of ATG, due to pulmonary hemorrhage and infection. Although further immunosuppressive treatment was required in all surviving patients, a less intensive regimen could be applied in 12. Beside fever and chills associated with the first gift of ATG, ATG was well tolerated, with infections being observed in five cases and serum sickness in two. The authors conclude that anti-T-cell-directed treatment with ATG may be a therapeutic option for severe refractory Wegener's granulomatosis (152).

Elimination of B cells by anti-CD20 chimeric antibody (rituximab) induced stable remissions in patients with AAV refractory to conventional therapy in several smaller studies. Safety did not appear to be a major problem, but continued vigilance is warranted. To prevent formation of antibodies to rituximab, an immunosuppressive agent has to be added or continued (153). At least 5 patients have been treated with an anti-CD18 monoclonal antibody (anti-adhesion molecule directed therapy) with clinical improvement in four (154). More specific approaches include co-stimulation blockade (with anti-CD40 ligand for example) to prevent

antigen-driven immune responses. The expansion of circulating TNF- $\alpha$ -producing Th1-type CD28<sup>-</sup> T-cell effector memory T-cells and their presence as Th1-type cytokine profile-driving cell population within granulomatous lesions provide the rationale for using TNF- $\alpha$ -blocking agents in refractory AAV. Both the chimeric monoclonal anti-TNF- $\alpha$  antibody infliximab and the human soluble p75 TNF- $\alpha$  receptor fusion protein etanercept have been successfully applied in refractory Wegener's granulomatosis and anti-TNF antibody therapy. In view of the importance of ANCA in the pathogenesis of AAV, semispecific removal of these antibodies has been attempted using L-tryptophan immunoabsorption (155), and more specifically with MPO-bound immunosorbent columns to remove anti-MPO ANCA (156). Finally, a few patients with severe disease have received immunoablation with autologous bone marrow stem cell transplant, with only short-term benefit (149).

#### 2.7.7. Supportive treatment

Together with an aggressive immunosuppression, a prophylaxis against corticosteroid-induced gastritis, fungal infection, and *Pneumocystis carinii* pneumonia is strongly recommended. Patients >50 years usually receive calcium and vitamin D tablets for bone protection. With high-dose CYC in iv pulses, uromitexan is used. Special attention is paid to those in fertile age before CYC is initiated. Trimethoprim-sulfamethoxazole and nasal mupirocin ointment are used in eliminating chronic nasal carriage of *Staphylococcus aureus*.

#### 2.8. PR3-ANCA and MPO-ANCA disease – is there a difference?

It has been noted that PR3-ANCA are predominantly found in patients with WG and MPO-ANCA in patients with MPA, its renal limited form, or CSS. Nevertheless, patients with WG incidentally have MPO-ANCA and

PR3-ANCA do occur in patients with MPA and RLV. These associations led to the question whether patients with PR3-ANCA differ from those with MPO-ANCA with respect to clinical presentation, histopathological findings and clinical outcome. Franssen et al. (157) retrospectively analyzed clinical features, pattern of pre-treatment renal function loss, renal morphology and outcome in a consecutive series of 46 patients with PR3-ANCA and 46 patients with MPO-ANCA. Patients with MPO-ANCA had a higher median age than patients with PR3-ANCA (63 and 56 years, respectively). The prevalence of renal involvement did not significantly differ between PR3-ANCA and MPO-ANCA positive patients (83% and 67%, respectively), but, prior to treatment, renal function deteriorated significantly faster in PR3-ANCA. Moreover, kidney biopsies showed a higher activity index (cellular and fibrocellular crescents, necrosis, insudation, infiltration by inflammatory cells) and a lower chronicity index (glomerulosclerosis, interstitial fibrosis, and tubular atrophy) than biopsies from patients with MPO-ANCA. However, although PR3-ANCA positive patients showed a more active renal disease, kidney survival did not differ between PR3-ANCA positive patients (73%) compared to MPO-ANCA positive patients (61%). The authors suggest that the more acute clinical presentation of patients with PR3-ANCA results in the earlier institution of immunosuppressive treatment explaining the comparable or even better renal outcome. These data are in agreement with other studies (158).

In order to explain the observed difference in inflammatory activity of the lesions between PR3-ANCA and MPO-ANCA positive vasculitis, Franssen et al. compared the neutrophil activating capacity of IgG fractions of PR3-ANCA and MPO-ANCA and indeed, PR3-ANCA positive IgG fractions induced more oxygen radical release from donor neutrophils and generated more neutrophil degranulation of  $\beta$ -glucuronidase than MPO-ANCA positive IgG fractions. These

observations may, at least in part, explain the clinical and histopathological differences between PR3- and MPO-ANCA positive patients (159).

Franssen et al. also evaluated their patients with PR3-ANCA and MPO-ANCA associated vasculitis for extrarenal involvement. They found that, at diagnosis, patients with PR3-ANCA had a higher vasculitis activity index and a higher number of affected organs. Involvement of both kidneys and the respiratory tract was far more common in patients with PR3-ANCA than in patients with MPO-ANCA (78.3% vs 23.9%). RLV occurred exclusively in patients with MPO-ANCA (160).

Taken together, PR3-ANCA and MPO-ANCA are markers of different disease entities within the spectrum of AAV.

## 2.9. Factors involved in relapse of AAV

AAV is a relapsing disease. The reported relapse rate differs from 16% in 18 months of follow-up (140) to up to 50% in long-term observations (50). It has been noted by several groups that PR3-ANCA relapse more frequently than patients with MPO-ANCA associated vasculitis (39, 157, 161, 162). The reason for this difference is not clear. In patients with PR3-ANCA persistence of (PR3-)ANCA after induction of remission is a risk factor for relapse. Longitudinal observations made by several groups (39, 163, 164) showed that relapses of WG were preceded by rises in ANCA titres. It has been even suggested that rising titers of ANCA may be used as a guideline for the institution of immunosuppressive therapy, but this has not been proven beneficial to the patient as the amount of cyclophosphamide required to prevent relapses may be harmful to the patient in the long term due to its toxicity (165). A prospective study of pre-emptive therapy with AZA and prednisone or no preemptive therapy once a rise in ANCA titer had occurred, was recently performed by Boomsma et al. (166) and proved, that early relapses could be prevented

with pre-emptive treatment but that late relapses occurred in many cases after stopping preventive treatment. Thus, rising titers of ANCA are frequently followed by relapses, but the use of an elevation in ANCA titer as the sole parameter to justify immunosuppressive therapy cannot be endorsed; the patients with rising titers should be followed closely for signs of clinical activity.

Besides rising titers of ANCA, persistence of ANCA after induction of remission in WG has also been identified as a risk factor for an ensuing relapse (144, 164, 167), which suggests that long-term maintenance treatment should be instituted in patients who are persistently positive for ANCA after induction of remission. On the other hand, a persistently ANCA-negative status is not an absolute proof of remission. This was illustrated in a report, where 8% of patients were ANCA negative at the time of relapse (168).

A second factor relevant for relapse in WG is chronic nasal carriage of *Staphylococcus aureus*. Stegeman et al. observed that 63% of patients with WG were chronic nasal carriers of *S. aureus* and that relapses occurred almost exclusively in these patients. In agreement with these data, maintenance treatment with co-trimoxazole resulted in a reduction of relapses in WG, as it was already previously mentioned (144). Several hypotheses of the mechanisms involved in relapse induction by nasal carriage of *S. aureus*, have been suggested – *S. aureus* derived superantigens may activate the immune system, *S. aureus* derived cationic proteins may adhere to (glomerular) basement membrane, induce a subclinical vasculitis/glomerulonephritis which, in the presence of ANCA, develops into clinically overt disease (169, 170).

Genetic risk factors have also been implicated in the occurrence of relapses. Patients with polymorphic forms of Fc-gamma receptors that exhibit low affinity for certain IgG-subclasses were more prone to relapses

in WG in the first 5 years after diagnosis (171); high membrane expression of PR3 on resting circulating neutrophils was associated with a significantly increased risk for relapse (172).

In conclusion, a frequent monitoring of ANCA levels, a closer follow-up of those with rising titers and an eradication of *S. aureus* are necessary. The increased relapse rate in PR3 disease should be probably taken into consideration in the length of maintenance treatment.

## 2.10. Outcome of patients with AAV

### 2.10.1. Morbidity and mortality

AAV is a life-threatening disease that requires prompt recognition and therapy. Prognosis is an especially important issue as the disease process is aggressive and the therapeutic options are inherently dangerous. Since the introduction of CYC and CS in the treatment of AAV, mortality has significantly decreased from 82% in 1 year to 59-95% in various patients groups during various follow-up periods, and remission rates have increased to up to 93% (39). Different authors have reported following survival periods in their cohorts of patients: Little et al. 85.5% at 1 year and 63% at 5 years, Aasard et al. 88% at 2 years and 74% at 5 years (173), Slot et al. 73% at 5 years and 62% at 10 years (174), Booth et al. 84% at 1 year and 76% at 5 years (175). At the time of disease presentation, clinicians are faced with several factors that may influence the outcome of the patients. The treatment used in AAV is toxic and carries the potential risk of life-threatening infection. Additionally, cytotoxic agents are associated with cancerogenesis, mutagenesis, infertility, and interstitial cystitis. Significant prognostic factors for mortality were found to be the entry age (162, 173-175), the serum creatinine level and dialysis-dependence at presentation (162, 173-175), the developing dialysis-dependence during follow-up (174), the multi-system manifestation and



the presence of pulmonary hemorrhage (176); however, the latter was not confirmed by others (174). Some authors reported higher mortality in C-ANCA disease and WG (176), whereas others did not confirm this finding (162, 173, 175). The male gender was sometimes (174) associated with increased mortality, again not confirmed by others (175). Factors unrelated to vasculitis, such as functional status (as quantified by Karnofsky score) and non-vasculitic co-morbidity were, not-surprisingly, found to be potent predictors of poor outcome (173). Some authors report low-intensity immunosuppression to be associated with a worse outcome (173, 176), whereas others stress the treatment-associated leukopenia and ensuing sepsis as an independent risk factor for death (175), which indicates the need for more effective better targeted therapy. Overall, the morbidity and mortality results from several factors. In the early phase of the disease it is associated with irreversible organ dysfunction because of inflammatory injury (within days), further on with aggressive immunosuppressive therapy and its short-term adverse effects, namely infections (within months), and long-term sequelae, such as secondary tumors, myelodysplastic syndrome, accelerated atherosclerosis etc. (162, 174).

#### 2.10.2. Renal outcome

The reported renal survival in patients with AAV with renal involvement differs according to the severity of renal disease at presentation, from 65% at 5 years and 51% at 10 years (174), to 44% at 48 months (176). The strongest predictors of long-term renal outcome are the entry serum creatinine level and dialysis-dependence and the occurrence of renal relapses (162, 174-176). Booth et al. found the older age to be associated with an increased renal death (175); Westman et al. (162) reported a worse renal survival in patients with very high titers of PR3-ANCA and in those with low blood thrombocytes and Slot et al. (174) in those with proteinuria

at diagnosis and during follow-up (only in MPO-ANCA). Proteinuria was confirmed as a determinant of poor renal prognosis in another study (177). Recently, the occurrence of an increased amount of IgM in urine at presentation was found to be a strong marker of poor renal outcome, as it is in other forms of glomerular diseases (178). Hogan et al. (176) stressed the importance of the race with a worse renal outcome in African Americans and arterial sclerosis on renal biopsy. Bajema et al. (179) evaluated the predictive value of renal biopsy findings for renal outcome in ANCA-associated necrotizing glomerulonephritis. The percentage of normal glomeruli in the biopsy was the best predictor for renal recovery and outcome. Reversely, glomerular sclerosis, diffuse interstitial infiltrates, tubular necrosis and atrophy were each associated with a worse recovery and outcome. Also, this study shows that the extent of chronicity, and not the activity of glomerular lesions, in the renal biopsy at presentation is the major factor for renal outcome. The latter, apparently, are largely reversible once adequate immunosuppressive therapy has been instituted.

## **OWN OBSERVATIONS - AIMS AND RESULTS**

1. An abridged version of the introduction to this thesis was the subject of "ANCA - associated renal vasculitis – epidemiology, diagnostics and treatment" published in Prague Medical Report (annex 1). A lot has changed in this field since the article was first published in 2004, and an update was therefore desirable. An increased understanding of the pathogenesis and a confirmation of the pathogenicity of ANCA has led to the use of a range of biologic therapies. There is now an unprecedented opportunity to develop novel therapies for AAV targeted at the pathophysiologic mechanism involved.

The predictive value of ANCA as markers of clinical activity of AAV is more and more controversial. The final decision concerning an introduction of immunosuppressive therapy has to be based on both clinical and laboratory markers. A search for new markers of imminent relapse is therefore underway. It is possible that, for instance, the differences in Th response might modify disease manifestations and the occurrence of relapses. A more frequent assessment of the Th1/Th2 balance in patients might lead to a better monitoring of disease activity and more adequate therapeutic strategies. One possible therapeutic approach of the Th response is the administration of IL-10, which has been proposed as a potential therapeutic cytokine in WG.

The results of the EUVAS studies demonstrated the benefits of tailoring therapy according to the stage of the disease and inherent risk factors of the patients such as their age. They warrant further attempts at an early diagnosis of AAV and early therapeutic intervention in order to preserve the function of organs, prolong the patients' survival and minimize exposure to CYC.

2. The diagnostics of AAV was described in "Multiple Extrarenal Complications of Wegener's Granulomatosis" published in Case Report and Clinical Practice Revue (annex 2). It is a case report of a patient with WG with an unusually high number of extrarenal complications. The patient had a two-year history of intermittent bloody nasal discharge; subsequently she developed a 'saddle' nose. After a febrile episode and arthralgias, the disease directly presented with a corneal perforation. The conjunctival, ENT and renal involvement were verified histologically. The peculiarities of this case included a double cardiac involvement - an acute myocardial infarction due to coronal arteritis and a vasculitis of the aortic valve - and gastrointestinal bleeding. Another relatively infrequent complication was the development of a very tight subglottic stenosis during the remission of the disease. Since all markers of pathologic activity were normal, we considered the development of stenosis as a result of reparative changes.

3. The etiologic factors of AAV were discussed in two papers - "Silica and Asbestos Exposure in ANCA-Associated Vasculitis with Pulmonary Involvement" published in Renal Failure (annex 3) and "Two Familial Cases of antineutrophil cytoplasmic antibody (ANCA)- associated vasculitis" published in Rheumatology (annex 4).

First we carried out a study to find out whether patients with pulmonary involvement attributable to AAV have been exposed to silicon-containing materials. We included a total of 31 patients with AAV with pulmonary and renal involvement diagnosed in our center between the years 1993 and 2002. The patients were asked to complete questionnaires designed by the occupational health physicians to evaluate their exposure to silica-containing chemicals and estimate their extent. Seven AAV patients (22.6%) had at one time been exposed to silicon-containing chemicals

(12.9% to SiO<sub>2</sub>, 9.7% to asbestos). The mean length of exposure was almost 4 years. In all cases the exposure was considered as low. We explored the possibility that the exposure was more likely to be associated with sex, smoking, and specific ANCA pattern or disease category. None of these was proven significant, although there was a tendency towards higher exposure in the MPO group. The results were compared to those of 30 age, sex, and residence-matched controls, where no patients were found to have any previous occupational exposure to silicon-containing materials. The difference was statistically significant ( $p < 0.05$ ). Exposure to smoking was comparable in the AAV and control groups (41.9% vs. 43.3% of smokers). It is largely accepted that AAV is genetically based but environmentally triggered, and there is an increasing evidence of a pathophysiologic role of silica in AAV, although the mechanisms by which silica may induce AAV are not well known. We were able to show a significantly higher anamnestic exposure to silicon-containing compounds in our group of AAV patients compared to the control group.

Moreover, the patterns of pulmonary involvement in AAV – not yet satisfactorily described in literature – were discussed in this paper. The majority of our patients had an obstructive disease on spirometry and either normal or reduced TLCO. However, as the tests were mostly performed in remission, these findings reflect the consequences of pulmonary AAV attributable to vasculitis damage, and not the vasculitis activity.

Subsequently, the role of a genetic predisposition to AAV was addressed in a description of two familial cases of AAV. In the first family, the father presented with an acute renal failure due to PR3-ANCA-associated disease three years before his daughter had exactly the same manifestation. However, at the time of her admission to our ward she had already had E.N.T. involvement, which was later attributed to Wegener's granulomatosis. The second family involved two sisters, who were

diagnosed with microscopic polyangiitis with a more gradual decline of renal function within four months of each other. The younger sister actually suggested the diagnosis of her sibling based on her symptoms and probably saved her a significant portion of renal function.

To our best knowledge, these are the first two familial cases of AAV from the Czech Republic described so far. They illustrate several very important aspects of the AAV from both theoretical and practical points of view. They show that the clinical manifestation of the different AAV are extremely varied because they are influenced by the sites of involvement and the activity versus the chronicity of the involvement. Generalized nonspecific manifestations of systemic inflammatory disease are often present in all entities. These constitutional and frequent E.N.T. or respiratory symptoms make the diagnosis difficult and lead mostly to a treatment with a variety of antibiotics. The detection of blood in the urine very often prompts another course of antibiotics for a presumed urinary infection and subsequently, as the finding does not resolve, an extended urologic work-up is done. Another tricky feature of the disease is the possibility of temporary spontaneous withdrawal of the symptoms, which further confuses and delays the diagnosis. As the disease reappears or persists, it may start to resemble a malignancy. We have had several patients in whom the diagnosis of WG was established from a kidney or lung lobe removed because of a mass in the organ, and morphological features of a necrotizing granulomatous inflammatory disease made the situation clear (unpublished data). Admittedly, there are patients with an isolated mass and no other symptoms in whom the invasive procedures seem to be justified, but they are rare. An erroneous clinical and histologic diagnosis of carcinoma with a subsequent oncologic treatment in the setting of a histological diagnosis of cutaneous vasculitis \_ as happened to the female patient of the first family \_ is a grave mistake.



However varied the AAV may be, their clinical presentation in the two families described was in some aspects remarkably similar. Both the father and daughter from the first case study had WG with ANCA directed against PR3. In both of them a history of E.N.T. involvement preceded the presentation with dialysis requiring renal failure. The renal biopsies in both had some corresponding features. The two sisters had both MPA with MPO-ANCA. Their clinical presentation with nonspecific constitutional symptoms and the histological and laboratory evidence of a rather slower decline in the renal function was the same. The disease in the older sister was detected earlier thanks to the younger one. Her morphological features were more active without chronic changes and her symptoms were therefore presumably lesser pronounced. We were unable to obtain additional objective data on their father's illness. Nevertheless, the daughters described a suggestive picture of AAV with pulmonary involvement that resembled that of WG. The difference in the disease presentation in the two families shows that PR3-ANCA and MPO-ANCA are markers of different disease entities within the spectrum of AAV.

Last, but not least, the presented case reports raise the question of a familial predisposition in AAV. Our patients within the two families shared a similar genetic background with the HLA haplotype A 01, B 57, Cw 06, DRB1 07/11 in the affected members of the first family and A 02/30, B 8/62, Cw 03, DRB1 04 in the two sisters. The healthy members of both families were thoroughly checked, including for ANCA, and no signs of AAV were found. Mutations in the gene encoding  $\alpha$ -1 antitrypsin (AAT), the natural in vivo inhibitor of PR3, are more frequently found in patients with AAV. All our patients had AAT levels within the normal range. The patients described in our study did not share the same environment. The fact that two members of the two families fell ill with the same disease and their similar HLA support the hypothesis that genetic

predisposition plays an important, although not yet fully understood role.

4. Inflammatory cytokine production and adhesion molecule activation or upregulation are important determinants of the pathogenic inflammatory responses noted in vasculitis. The role of T cells was addressed in "Regulatory Cytokines in ANCA-associated Vasculitis" published in proceedings from the 11th European Meeting on Cardioneurology (annex 5). We examined 48 peripheral blood samples of patients with AAV and 21 peripheral blood samples of age- and sex-matched healthy controls. Using flow cytometry, the following markers were assessed: T lymphocytes activation markers (HLA DR+, CD28+, chemokine receptors CXCR3 and CCR5), surface molecules CD4, CD8, CD3, CD19, costimulation molecule CD80 on B lymphocytes, and intracellular cytokines: interferon gamma (IFN $\gamma$ ), tumor necrosis factor alpha (TNF $\alpha$ ), interleukin 2 (IL-2), interleukin 4 (IL-4) in CD3+ T cells, and interleukin 10 (IL-10) and interleukin 12 (IL-12) in monocytes.

We found higher IL-2 and activation markers on T lymphocytes (DR+) and B lymphocytes (CD80+) in the patients when compared with healthy subjects. This activation of the immune system persisted even during remission. The IL-2 production was significantly lower in MPA when compared with WG suggesting less inflammation in MPA. On the other hand, the patients had lower IL-10 and IL-12, most probably as a result of previous or ongoing immunosuppressive treatment. Moreover, the patients had higher levels of IFN $\gamma$  and CCR5 when compared with healthy controls, which represents a significant shift towards Th1 population. The higher number of CD8 positive cells and lower number of CD4 positive cells indicates that cytotoxic T lymphocytes are involved in the pathogenesis of AAV. The number of DR positive activated T lymphocytes and TNF $\alpha$  production increased with advanced renal failure.

In conclusion, our study supported the hypothesis of AAV being a Th1 mediated disease. The difference in the IL-2 production between WG and MPA patients suggests a different cytokine regulation of immune reaction in these patients.

5. Although the incidence of AAV is increasing, it is still low and therefore necessitates multicentric studies on its treatment and prognosis to responsibly address these issues. We discussed the treatment of AAV in two papers. The subclassification of AAV based on the disease severity at presentation, and the two EUVAS waves of clinical trials were reviewed in "Current treatment strategies in ANCA-positive renal vasculitis - lessons from European randomized trials." The first wave of randomized clinical trials had the aim of optimizing the existing therapeutic regimens. The second wave concentrated on testing of some newer therapeutic approaches (annex 6).

Our centre closely cooperates with EUVAS and repeatedly includes a considerable number of patients in the studies. Between years 1995 and 2001 we contributed a total of 40 patients to the trials CYCAZAREM (cyclophosphamide versus azathioprine during remission for generalised vasculitis), MEPEX (plasma exchange versus methylprednisolone for severe renal vasculitis) and CYCLOPS (daily oral versus pulse cyclophosphamide during induction phase for generalised vasculitis). We continue to randomize our patients to subsequent studies such as the IMPROVE trial comparing mycophenolate and azathioprine as a maintenance therapy in patients in remission. So far, most of the patients have been included in the international randomized trial CYCLOPS, the aim of which was to optimise the treatment of induction of remission in patients with generalized, but not immediately life-threatening AAV, which is the stage of the disease where most of our patients are diagnosed. The intent was to reduce the toxicity of induction therapy by reducing the

overall dose of CYC during the induction period by using it in intermittent pulsed form.

We published the results of patients included in our center in "Daily oral versus pulse intravenous cyclophosphamide in the therapy of ANCA-associated vasculitis – preliminary single center experience" in Prague Medical Report (annex 7). Our center included 28 patients from Dec. 1999-Nov. 2001. Eighteen (12 women, 6 men) were evaluable at the moment of analysis. The mean age at presentation was 54.6 years. Seven patients were randomised to the pulse limb. Eleven patients were randomised to the oral limb. The cumulative dose of CYC in pulse limb was approximately 150 mg/kg body weight/6 months. In the oral limb the cumulative dose of CYC was more than two-fold, 315 mg/kg body weight/6 months. All the patients in the pulse limb (100%) achieved remission, contrary to only 55% of patients in the oral limb. During the whole period studied, there was only one relapse in the pulse limb, which occurred 5 months after the cessation of immunosuppressive treatment. The number of infectious complications in immunosuppressive treatment was comparable in both limbs (pulse 43% vs. oral 45%). However, there were only 14% \_ one patient \_ of serious (i.e. requiring hospitalization) events in the pulse limb. The patient had leukopenia ( $1,7 \cdot 10^9/L$ ) caused by CMV infection and was successfully treated with ganciclovir. In the oral limb, 27% of infectious complications \_ two patients \_ were severe and unfortunately resulted in the death of both. All three patients died during the induction treatment, even though CYC was always stopped as soon as leukopenia and infection were ascertained. The overall mortality was also higher in the oral limb (36%) compared to the pulse limb (14%). Apart from the three patients from the oral limb who died of infectious complications there was one more death in this group, not related to the diagnosis or therapy. This patient died in a local hospital due to bleeding

caused by an overdose of coumarine. In the pulse limb, only one death was recorded. This patient died of pulmonary embolism while on a maintenance dose of CS (prednisolone 10mg/day). We cannot confidently exclude that the therapy did not contribute to the fatal complication.

The final results of the multicentric randomized trial CYCLOPS have not been yet published. Our preliminary results of a small group of patients confirm the higher toxicity of oral CYC that resulted in higher morbidity and mortality in this group. Surprisingly, in our hands, the efficacy of pulse CYC seemed to be better. This was certainly due to the small number of patients and the high mortality in the oral limb. There was no early relapse in the followed-up period (18 months after enrollment). In the pulse limb, one patient relapsed later on. The preliminary results of the patients from all centers proved the intermittent pulse administration of CYC to be as efficient as the pulse administration with a significant reduction of the cumulative dose and both short-term and long-term toxicity of CYC.

6. The presenting features, response to therapy and the overall and renal survival of patients with AAV with renal involvement were studied in "Long-Term Outcome of Patients with Antineutrophil Cytoplasmic Autoantibody-Associated Vasculitis with Renal Involvement" published in *Kidney and Blood Pressure Research* (annex 8). This retrospective analysis involved patients who were diagnosed and followed in our center between 1986 and 1997. Special attention was paid to the impact of age, diagnostic subgroups, level of renal function and type of ANCA on the outcome of the patients.

Sixty-one patients were included in the study (54.1% had WG, 23% RLV, 16.4% MPA and 4.9% CSS). The median age was 54 years, and 60.7% were men. Patients with RLV and MPA were significantly older than

patients with WG. ANCA was detected in 85.2% (C-ANCA in 57.4%, P-ANCA in 42.6%). The median follow-up was 90.5 (range 1–168) months. All patients received homogenous induction treatment according to the treatment guidelines in the respective years. It consisted of oral continuous CYC and CS. Plasma exchange was used in 29.3% of patients. In 45.2% of patients CYC was switched to AZA after reaching stable remission. More than three quarters of the patients had generalized disease at presentation. By definition, all patients had a renal involvement, which was biopsy-proven in 87%. The median serum creatinine level of patients dialysis-independent at diagnosis was 221.5  $\mu\text{mol/l}$ , and 32.8% were dialysis-dependent. Initial renal function did not differ according to sex, age or C-ANCA/P-ANCA.

Remission was achieved in 87% of the patients, more often in patients with independent renal function at presentation. All the patients who did not achieve remission died. Relapses occurred in 44.7%. The median disease-free interval was 58 months, the median renal disease-free interval was 62.5 months. The relapse rate was higher in C-ANCA-associated disease when compared to P-ANCA, although the difference was of borderline significance. For the group of 61 patients, the estimated patient survival at 5 and 10 years was 78.3 and 62.2%, respectively. The estimated survival depended on age. It did not depend on sex, initial proteinuria, C-reactive protein or hemoglobin level. Patients who had to be initially hemodialyzed had significantly worse estimated survival compared to patients who were dialysis-independent. Pulmonary involvement did not have any impact on estimated survival, nor did the use of plasma exchange in the initial treatment. Nineteen of 61 patients (31%) died. The median time to death was 41.3 months. Six patients died of infectious complications related to immunosuppressive treatment; 7 patients due to cardiovascular events; 2 of exsanguination from a gastric ulcer; 2 of cancer; 1 of multi-organ failure,



and 1 cause of death is unknown. No patient died of active vasculitis. Nine patients presented with lung hemorrhage, and none of them died of this condition. The estimated renal survival at 5 and 10 years was 69.2 and 55.8%, respectively. None of the variable studied had any impact on the renal prognosis. Twenty-five of 61 patients (41%) suffered from adverse effects of their therapy. A severe bacterial infection requiring hospital admission occurred in 16 patients (26%). A severe viral infection requiring hospital admission occurred in 5 patients (8%). Solid tumors occurred in 3 patients. However, in only 1 of them (lung cancer 5 years after the diagnosis of WG) the malignancy may have been secondary to the immunosuppression.

In conclusion, our retrospective study comprised a high number of patients from one center treated in principle with a uniform induction regimen and followed for a long period of time. All patients had a relatively severe renal involvement, most of them were biopsied. The demographic data in our cohort confirmed that at presentation, patients with RLV and MPA were older compared to WG patients, possibly due to the more indolent course of the disease leading also to the higher serum creatinine level at presentation in the RLV group. AAV is a life-threatening disease that requires prompt recognition and therapy. Prognosis is an especially important issue as the disease process is aggressive and the therapeutic options are inherently dangerous. The cumulative 5- and 10-year patient survival in our cohort was 78.3 and 62.2%, respectively, which is comparable to the findings of other investigators. Mortality was associated with age over 50 and advanced renal failure at presentation. No patient died of active vasculitis. The causes of early mortality were related to the adverse effects and toxicity of the treatment (infections, gastrointestinal bleeding). Infections remained the main cause of morbidity as well. Late mortality was mainly due to cardiovascular events. In view of the severity

of the renal disease at presentation and the length of follow-up, the renal survival in our study was very satisfactory. In this study, a relapse rate of 44.7% was noted with the median renal disease-free interval of more than 5 years; the vast majority of patients were no longer on immunosuppressive treatment at the time of relapse. Renal relapses probably have a major impact on the loss of independent renal function in the course of the disease. However, some of our patients progressed to end-stage renal failure without experiencing any relapse. In these cases, the non-immunologic progression of renal disease was probably involved, which highlights the importance of optimal conservative care, in particular careful blood pressure control, preferentially using ACE inhibitors, or angiotensin-II antagonists. Consistent with previous reports we noticed an increased relapse rate in C-ANCA-associated disease, which should probably be taken into consideration, especially in the length of maintenance treatment.

## CONCLUSION

AAV is a multi-factorial disease with an increasing incidence. Although our knowledge of etiopathogenesis is increasing rapidly, the environmental and genetic factors involved in the etiology and the exact pathogenetic mechanisms remain to be elucidated. In a case-control study, we were able to show a significantly higher anamnestic exposure to silicon-containing compounds in our group of AAV patients with pulmonary involvement compared to the control group. In keeping with the published data, we found a tendency to higher exposure in the P-ANCA subgroup. None of the other factors studied (sex, diagnosis, smoking) were significant. The question of familial predisposition in AAV was raised in the first two familial cases of AAV from the Czech Republic described so far. The families shared a similar genetic background with the HLA haplotype A 01, B 57, Cw 06, DRB1 07/11 in the affected members of the first family and A 02/30, B 8/62, Cw 03, DRB1 04 in the second. The patients described in our study did not share the same environment. The fact that two members of the two families fell ill with the same disease and their similar HLA typing seem to favor the role of a genetic predisposition to AAV.

The issue of AAV pathogenesis was addressed in a study comparing cytokine profile in patients and healthy controls. The higher activation of the immune system observed in AAV patients persists even during remission. The IL-2 production was significantly lower in MPA when compared with WG suggesting less inflammation in MPA. The patients had higher levels of IFN $\gamma$  and CCR5 when compared with healthy controls, which represents a significant shift towards Th1 population. The higher number of CD8 positive cells and lower number of CD4 positive cells indicates that cytotoxic T lymphocytes are involved in the

pathogenesis of AAV.

The last two decades, with the advent of cytotoxic therapy, have brought greatly increased survival probability, but a significant risk of infective complications in particular and many other problems with the management of chronic grumbling and relapsing disease with accumulating morbidity and mortality. The vasculitides are relatively rare and are heterogeneous in their presentation; hence the importance of well conducted, multi-centre collaborative trials to identify promising new therapies and to maximize the benefit of existing treatment regimens. Our preliminary results of patients recruited for the CYCLOPS trial confirm the higher toxicity of daily oral CYC with a double cumulative dose of the drug when compared to the intermittent pulse administration. In our hands, the efficacy of pulse CYC was at least as good as in the oral limb.

In our outcome analysis of 61 patients with renal AAV we were able to demonstrate a difference in age and initial renal function between the patients with RLV, MPA and WG. The estimated cumulative 5- and 10-year patient survival in our cohort was 78.3 and 62.2%. Mortality was associated with age and renal failure at presentation. Infection were the main cause of early morbidity and mortality. Late mortality was mainly due to cardiovascular events. The estimated renal survival at 5 and 10 years was 69.2 and 55.8%, respectively. In this study, a relapse rate of 44.7% was noted with the median renal disease-free interval of more than 5 years. Consistent with previous reports we noticed an increased relapse rate in C-ANCA-associated disease, which should probably be taken into consideration, especially in the length of maintenance treatment. Our data confirm that despite effective induction treatment, the long-term outcome of patients with AAV remains unsatisfactory with a relatively high overall mortality (with standardized mortality ratio compared to the common population of about 2.5) and dialysis dependence due to a high relapse rate

and toxicity of current treatment. Search for newer, more effective and less toxic modes of treatment is therefore warranted. As the outcome in AAV was found to be related to age and the level of renal function at presentation, a diagnostic delay may have a major influence on the outcome. An increased awareness of AAV with subsequent rapid ANCA testing, recognition of the presence of organ involvement and a quick referral of the patient to a specialist is therefore warranted.

The study of AAV therefore remains a challenge in all aspects.

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## **LIST OF ANNEXES**

## ANCA – Associated Renal Vasculitis – Epidemiology, Diagnostics and Treatment

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**Abstract:** The pauciimmune small-vessel vasculitides are multisystem diseases with frequent renal involvement. They are strongly associated with the presence of anti-neutrophil cytoplasmic antibodies (ANCA).

In this review we have focused on the etiopathogenesis and the role of ANCA, clinical presentation and histopathologic findings of different ANCA – associated vasculitides (AAV). Current treatment strategies and the overall and renal outcome of patients with AAV are also discussed.

**Key words:** ANCA – Cyclophosphamide – Vasculitis – Wegener's granulomatosis

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### Primary vasculitides

The primary systemic vasculitides are a group of heterogeneous disorders of unknown etiology characterized by more or less wide-spread inflammation of the vessel wall. A clinical classification of the various disease entities within this group has been proposed by the American College of Rheumatology (ACR) and is based on the presence of particular clinical symptoms and histopathological findings [1]. These so-called ACR-criteria are widely used but have their drawbacks with regard to disease specificity and sensitivity. Therefore, a more precise nomenclature for the primary vasculitides has been proposed by a group of experts in this field in 1993 (Tab. 1) [2]. These definitions for the nomenclature of the vasculitides are known as the Chapel Hill Consensus Conference (CHCC) definitions and are now widely used as diagnostic criteria although they were not intended as such. Based on these definitions new diagnostic and classification criteria have to be developed.

Within the spectrum of the primary vasculitides (Tab. 1) renal involvement is common, particularly in the small-vessel vasculitides [3]. Immunopathologically, Henoch-Schoenlein purpura and cryoglobulinemic vasculitis are characterized by immune deposits which are considered to play a major and initiating role in the development of renal lesions. The remaining small-vessel vasculitides show paucity or absence of immune deposits. These pauci-immune vasculitides, that is Wegener's Granulomatosis (WG), Churg-Strauss Syndrome (CSS), microscopic polyangiitis (MPA) and its renal limited form, are strongly associated with the presence of anti-neutrophil cytoplasmic antibodies (ANCA). Their annual incidence in Europe is 10 to 20 per million per year and they account for at least 5% of the causes of end stage renal failure. There is an increased incidence with age, with a median age of 56 years in studies over the past 10 years. Males slightly predominate [4, 5].

**Table 1 – Names of vasculitides adopted by the Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis**

Large vessel vasculitis	Giant cell [temporal] arteritis Takayasu arteritis
Medium-sized vessel vasculitis	Polyarteritis nodosa Kawasaki disease
Small vessel vasculitis	Wegener's granulomatosis Churg-Strauss syndrome Microscopic polyangiitis Henoch-Schoenlein purpura Essential cryoglobulinemic vasculitis Cutaneous leukocytoclastic angiitis

### **ANCA-associated vasculitis**

#### *ANCA testing*

As stated, the idiopathic pauci-immune necrotizing small-vessel vasculitides are strongly associated with ANCA. This finding was first described in 1982 [6]. These antibodies were initially believed to be associated with Ross River virus infections. By 1985, however, ANCA had been linked to WG [7]. Within several more years, a relationship among ANCA, WG, MPA and renal limited vasculitis (RLV) had been established [5, 8]. ANCA testing currently plays a critical role in the diagnosis and classification of vasculitides, even as debate about their ultimate importance in the pathogenesis and pathophysiology of these conditions continues.

In vasculitis, ANCA are most often directed to either proteinase 3 (PR3-ANCA) or to myeloperoxidase (MPO-ANCA). Both PR3 and MPO are located in the azurophilic granules of neutrophils and the peroxidase-positive lysosomes of monocytes. Two types of ANCA assays are currently in wide use – a more sensitive indirect immunofluorescence (IIA) assay and a more specific enzyme-linked immunosorbent assay (ELISA). The optimal approach to clinical testing for ANCA is therefore to screen with IIA and to confirm all positive results with ELISAs directed against the vasculitis-specific target antigens (mainly PR3 and MPO).

Indirect immunofluorescence: When the sera of patients with AAV are incubated with ethanol-fixed human neutrophils, two major immunofluorescence patterns are observed:

- With the C-ANCA pattern (Fig. 1), the staining is diffuse throughout the cytoplasm (C-cytoplasmic type of immunofluorescence). In most cases, antibodies directed against PR3 (detected by ELISA) cause this pattern, but MPO-ANCA can occasionally be responsible [9, 10].
- The perinuclear or P-ANCA pattern results from a staining pattern around the nucleus (figure 2). With ethanol fixation of the neutrophil substrate, positively-charged granule constituents rearrange themselves around the negatively-charged nuclear membrane, leading to perinuclear fluorescence [5]. The antibody responsible for this pattern (detected by ELISA) is usually directed against MPO (and occasionally PR3).

There are several reasons for caution in the interpretation of immunofluorescence results:

- They are highly dependent on the experience of the laboratory personnel.
- Immunofluorescence results lack specificity (in one study positive C-ANCA were associated with vasculitis in only 50% of patients) [11].
- A frequent difficulty in distinguishing the P-ANCA pattern of immunofluorescence from that caused by antinuclear antibodies (ANA).

Individuals with ANA frequently have false-positive results on ANCA testing by immunofluorescence. The use of both formalin- and ethanol-fixed neutrophil substrates permits the distinction between P-ANCA and ANA, because formalin-fixed neutrophils prevent the rearrangement of charged cellular components around the nucleus.

- Although PR3 and MPO are the two most common targets for ANCA, an increasing number of cytoplasmic proteins has been identified so far as minor ANCA target antigens, including bactericidal/permeability-increasing protein (BPI), lactoferrin, cathepsin G, human elastase, or lysozyme. Some ANCA-targeted antigens are not the part of granules but are located directly in the cytoplasm, like  $\alpha$ -enolase and catalase, or even in the nucleus, e.g. non-histone chromosomal proteins HMG1 and 2 (high-mobility proteins). In IIA, atypical ANCA patterns may be confused with P-ANCA pattern [12].

Enzyme-linked immunoassays: Specific ELISAs for antibodies to PR3 and MPO (and in some centers for other antigens as well) are now available, and should be part of testing for ANCA. PR3-ANCA and MPO-ANCA are associated with substantially higher specificity and positive predictive values than the immunofluorescence patterns to which they usually correspond (C- and P-ANCA, respectively). There are, however, significant differences in sensitivity, specificity and predictive value among available commercial direct ELISA kits. The best practice includes both IIA and ELISA testing, because it has been shown that a C-ANCA combined with positive PR3-ANCA was 99% specific for AAV, and similarly, a P-ANCA combined with a positive MPO-ANCA was 99% specific for AAV. [13, 14].

#### Disease associations

ANCA are associated with many cases of WG, MPA, CSS, RLV, and certain drug-induced vasculitis syndromes. In these conditions, ANCA consistently have specificity for either PR3 or MPO, but almost never for both. Most patients reported with drug-induced vasculitis have MPO-ANCA, often in very high titre,

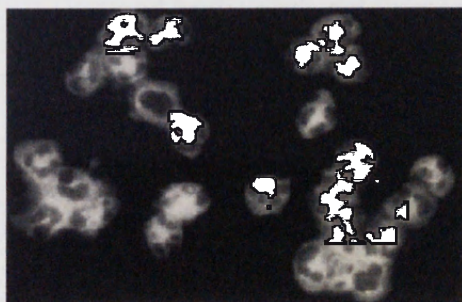


Fig. 1 – C-ANCA pattern - cytoplasmic type of immunofluorescence.



Fig. 2 – P-ANCA pattern – perinuclear type of immunofluorescence.



antibodies to elastase, lactoferrin or other minor antigens [15]. Many cases of drug-induced AAV are associated with constitutional symptoms, arthralgias/arthritis, and cutaneous vasculitis. However, the full range of clinical features, including crescentic glomerulonephritis and alveolar hemorrhage, can also occur. The strongest links between medications and AAV are with propylthiouracil, hydralazine, and minocycline. Other drugs occasionally implicated include penicillamin, allopurinol, procainamide, carbimazole, thiamazole, clozapine, and phenytoin [16–18]. The spectrum of diseases associated with ANCA is not limited solely to the above mentioned vasculitides. ANCA directed against BPI are typical for a subgroup of patients suffering from cystic fibrosis [19]. Additionally, anti-BPI or other ANCA antibodies are found in some patients with autoimmune hepatitis, ulcerative colitis, sclerosing cholangitis, without the correlation with the disease status [20]. In patients with rheumatoid arthritis, ANCA positivity ranges from 18% to 50% with the following target antigens: lactoferrin, MPO and others [21]. ANCA have been reported with many other inflammatory rheumatic conditions, including systemic lupus erythematosus, Sjögren's syndrome, inflammatory myopathies, scleroderma and others. ANCA are found also in some infectious diseases, like bacterial endocarditis and invasive amoebiasis, and in HIV infection [22–24].

Between 10 and 40 percent of patients with anti-glomerular basement membrane (GBM) antibody disease are ANCA-positive. The clinical significance of combined ANCA and anti-GBM antibodies is unclear. In some, the titre of ANCA is low and there are no clinical manifestations of vasculitis. Others, however, present with disease features that are uncommon to anti-GBM antibody disease but quite typical of systemic vasculitis, including purpura, arthralgias, and granulomatous inflammation, suggesting the concurrence of two disease processes [25].

### **ANCA-associated vasculitides**

The clinical manifestation of WG, MPA, and CSS are extremely varied because they are influenced by the sites of involvement, and the activity versus the chronicity of the involvement. All three categories of vasculitis share features caused by the small vessel vasculitis, and patients with WG and CSS have additional features that define each of these syndromes. Generalized nonspecific manifestations of systemic inflammatory disease, such as fever, malaise, anorexia, weight loss, myalgias, and arthralgias, are often present in all the entities. Many patients trace the origin of their disease to a "flu-like" illness.

**Wegener's granulomatosis:** According to the CHCC nomenclature [2], WG is a systemic necrotizing vasculitis affecting small to medium-sized vessels. It typically produces granulomatous inflammation of the upper and lower respiratory tracts and necrotizing, pauci-immune glomerulonephritis in the kidneys. A "limited" form, with clinical findings isolated to the upper respiratory tract or the lungs, occurs in approximately one-fourth of cases and represents often a diagnostic dilemma. It is

often misdiagnosed as an infection or tumor. However, this subdivision is somewhat artificial because approximately 80 percent of such patients eventually have renal involvement. The diagnosis of WG is suggested from the clinical and laboratory findings and from the presence of ANCA that are more often directed against PR3 (70%) than to MPO (25%). About 5% are ANCA negative [26]. As previously mentioned, renal disease is common (80%), being manifested by acute renal failure and/or active urinary sediment with red cells, red cell and other casts, and proteinuria. Lung involvement have up to 90% of patients with WG, E.N.T. involvement about 90% (Fig. 3), as well. Other organ systems that may become involved include [27, 28] musculoskeletal system, skin, nervous system, eyes, heart and less commonly gastrointestinal tract, subglottis or trachea, lower genitourinary tract, parotid glands, thyroid, liver, or breast.

**Microscopic polyangiitis:** According to the CHCC nomenclature [2], MPA is a necrotizing vasculitis, with few or no immune deposits, affecting small vessels, although necrotizing arteritis involving small and medium-sized arteries may be present. Necrotizing glomerulonephritis is very common (90%). Pulmonary capillaries (Fig. 4) frequently occur (50%), but, by definition, patients with MPA do not have granulomatous respiratory tract lesions. Similarly, E.N.T. lesions may occur in MPA (35%), but they are caused by angiitis alone, without granulomatous inflammation. Destruction of bone, for example resulting in septal perforation and

saddle nose deformity, appears to require necrotizing granulomatous inflammation (as in WG and CSS) and, therefore, does not occur in MPA. Nodular cutaneous lesions caused by dermal or subcutaneous arteritis and by the necrotizing granulomatous inflammation of WG and CSS, are very rare with MPA, other skin lesions occur often, in up to 40% of patients. Neurologic, musculoskeletal and other organ involvement is similar to those with WG, eye involvement is less frequent than in WG. Patients with MPA have MPO-ANCA in 50%, PR3-ANCA in 40%, and are ANCA negative in 10% [28].

**Churg-Strauss syndrome:** According to the CHCC nomenclature [2], CSS is a necrotizing vasculitis with eosinophil-rich and granulomatous inflammation affecting small to medium-sized vessels, involving the respiratory tract, and is associated with asthma and eosinophilia. In addition to that, neuropathy, migratory or transient pulmonary

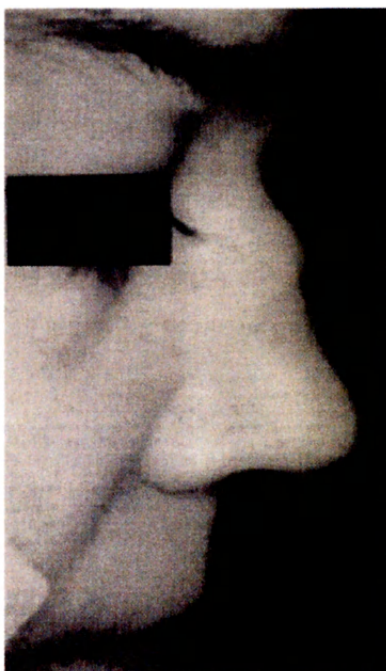


Fig. 3 – A typical saddle nose deformity in a patient with Wegener's Granulomatosis.

opacities detected radiographically, paranasal sinus abnormalities and a biopsy containing a blood vessel showing the accumulation of eosinophils in extravascular areas belong to the diagnostic criteria. Patients with CSS have MPO-ANCA in 60%, PR3-ANCA in 10%, and they are ANCA negative in 30%. The vasculitis classically involves the arteries of the lung (70%) and skin (60%), but may be generalized. Renal involvement is less frequent in CSS (45%), E.N.T. involvement occurs in about 50% of patients. On the other hand, neurologic manifestation (usually with a mononeuritis multiplex) is most frequent in CSS compared to other SVV.

Renal-limited vasculitis: RLV or isolated (idiopathic) pauci-immune necrotizing/crescentic glomerulonephritis is distinguished from MPA and WG by the absence of extrarenal symptoms of vasculitis. It is more often MPO-ANCA positive and therefore, considered a renal limited form of MPA.

#### *Routine laboratory tests and tissue biopsy*

Routine laboratory tests are generally nonspecific in AAV. Common abnormalities include leukocytosis, thrombocytosis, marked elevation of the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and normochromic, normocytic anemia [27]. As well as ANCA, the markers of inflammation (ESR and CRP) fluctuate with vasculitis activity [29].

The diagnosis of AAV is generally confirmed by tissue biopsy at a site of active disease. Biopsy of a nasopharyngeal lesion is relatively noninvasive, but very often non-diagnostic, as the amount of tissue from this site is often small. Granulomatous inflammation is commonly present but actual vasculitis is seen in only about one-third of cases. If there is no lesion in the upper respiratory tract, the next step is biopsy of an affected organ such as kidney or lung. Renal biopsy is preferred because it is easier to perform, safer and often more diagnostic. To a certain extent, it provides us with an additional information on renal prognosis (discussed later). Kidney biopsy typically reveals a segmental necrotizing glomerulonephritis

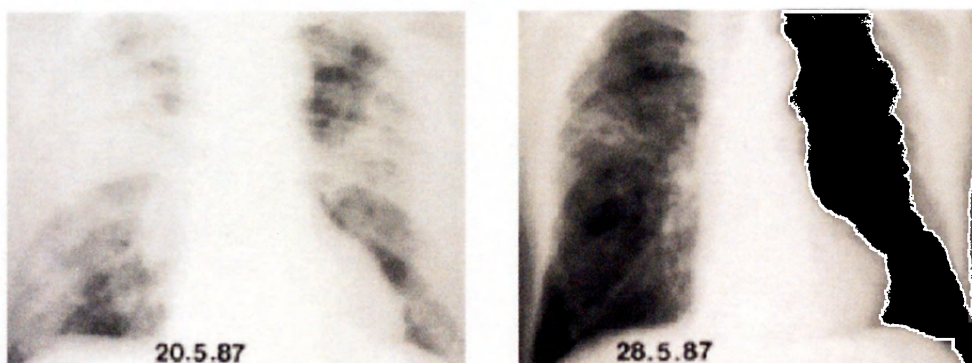


Fig. 4 – Chest x-ray – alveolar shadowing caused by pulmonary hemorrhage in a patient with microscopic polyangiitis, improved after immunosuppression and plasma exchange therapy



with crescents with little or no immunoglobulin deposition (pauci-immune) on immunofluorescence or electron microscopy (Fig. 5). This finding is essentially diagnostic of an AAV. However, the histopathological features vary among patients from mild focal segmental extracapillary proliferation to diffuse crescentic necrotizing glomerulonephritis with granulomas and tubular intra-epithelial infiltrates. In some cases, extensive glomerulosclerosis is found [30].

If performed, the lung biopsy reveals vasculitis and granulomatous inflammation in WG. Special stains and cultures have to be performed to exclude the presence of infections that can produce granulomas, vasculitis or necrosis. Lung biopsy most often requires open or thoracoscopic lung biopsy. In a small number of cases (<10%), sufficient tissue for diagnosis can be obtained by transbronchial biopsy; however, negative result in this case does not exclude the diagnosis of vasculitis [31].

#### *Etiology and pathogenesis of ANCA-associated vasculitis*

AAV is a complex, immune-mediated disorder in which tissue injury results from the interplay between an initiating inflammatory event and a highly specific pathogenic immune response to previously shielded epitopes of neutrophil granule proteins. This generates high titer ANCA directed against antigens within the primary granules of neutrophils and monocytes. These antibodies produce tissue damage via interactions with primed neutrophils and endothelial cells. The exact mechanisms by which ANCA arise, their role in the etiology of AAV, and the events leading to the initiation of the disease remain unclear. Infectious, genetic, and environmental risk factors and combinations of all three have been entertained [32].

#### *Etiology of AAV – environmental factors*

Infection: An infection is thought to be one of triggering factors in AAV. A seasonal variation in the onset of WG supports this hypothesis [33, 34] even if this has not

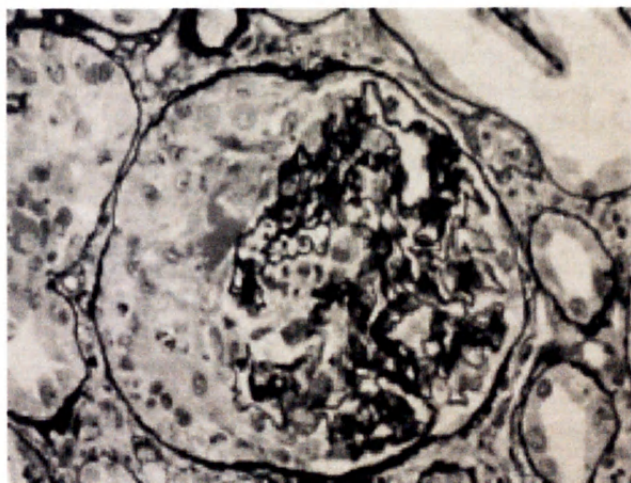


Fig. 5 – Kidney biopsy – cellular crescent in a glomerulus from a patient with Wegener's Granulomatosis.



been observed by other authors [27]. Coxsackie B3 and parvovirus B19 were implicated as infectious triggers for ANCA and/or WG [34]. An association of chronic nasal carriage of *Staphylococcus aureus* with higher relapse rate in WG was reported [35]. However, microbial pathogens in patients with new onset of WG have not been identified [36]. Antigenic mimicry is suspected to be an important factor in triggering ANCA formation. *S. aureus* genome directly encodes a variety of serine proteases, which may be cross-reactive with C-ANCA [37]. As mentioned above, a translocation of intracellular hidden antigens during nonspecific activation of neutrophils during any inflammation may also be involved. When released from the cell they may become easily accessible to ANCA, or may induce ANCA formation [38].

**Drugs and chemicals:** The existence of drug-induced AAV has been already discussed. Given the frequency with which the first symptoms of WG occur in the respiratory tract, exposure to noninfectious agents or toxins is another possible inciting event. The possible candidates are silica dust and organic solvents. The exposure to silica dust has been repeatedly reported to be significantly higher in patients with ANCA and AAV than in healthy controls, lupus nephritis or other conditions [39–41].

#### *Etiology of AAV – genetic factors*

A number of familial cases of WG have been described, and suggested candidate genes include, among others, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), interleukin-1 receptor antagonist (IL-1ra), IL-10, and FcγRII/FcγRIII [42]. Mutations in the gene encoding α-1 antitrypsin (AAT), the primary in vivo inhibitor of PR3, are found more frequently in patients with AAV [43]. This observation suggests a potential pathogenic role in this disease for deficient PR3 clearance from the sites of inflammation. Decreased local concentrations of AAT caused by genetic polymorphisms or alterations in the enzyme's functionality induced by inflammation may therefore lead to protease/anti-protease imbalance in the disease microenvironment. Although unproven, these events may be responsible for generating immunogenic forms of PR3 in these patients. However, no case of AAV was found among a group of patients having a heterozygous mutation of this gene [44].

Suspected polymorphisms of the TNFα promoter were excluded as risk factors for the disease in a cohort of German patients [42], but the same authors described polymorphism in adhesion molecule CD18 to be associated with various forms of AAV [45]. As to HLA class II, alleles DQw7 and DR4 haplotypes were found to be associated with the persistence of ANCA in vasculitis [46].

In summary, as in other autoimmune diseases, the etiology of AAV is heterogeneous and principally unknown. Different predisposing factors play probably differential ethiopathogenic roles in various groups of AAV

### *Pathogenesis*

**Mechanisms of ANCA production:** The autoantibody response that produces ANCA is probably generated against newly exposed epitopes of the target autoantigen. Following the production of ANCA, the antibody response may then generalize to the rest of the molecule or to other components of a macromolecular protein complex via the process of epitope spreading. This hypothesis is supported by a significant role of mononuclear cells in AAV: patients with active WG have higher levels of CD4+ T cell and monocytic activation markers (TNF-alpha, INF-gamma, IL-12) than patients in remission and healthy controls; IL-10 treatment (which inhibits Th1 pathway by impairing the production of IL-12) of mononuclear cells from active WG patients impairs the production of INF-gamma *in vitro* [47–49].

**Neutrophil activation and ANCA:** Once neutrophils are activated (primed) by cytokines, ANCA can bind relevant membrane-bound antigens, causing abnormal constitutive activation via either the crosslinking of MPO or PR3 or the binding of Fc receptors. Persistent ANCA binding to neutrophils on the endothelial surface can enhance the degree of vascular injury [50]. ANCA-antigen complexes adsorb onto endothelial cells where they can participate in *in situ* immune-complex formation. The degranulation of neutrophils and release of chemoattractants and cytotoxic oxygen free-radicals, also increased by ANCA, causes tissue damage [51]. In addition, primed neutrophils not only damage endothelial cells, but also attract additional neutrophils to the site of damage, thereby creating an auto-amplifying loop. The release of MPO, PR3, elastase and other proteases from activated neutrophils also contributes directly to the local inflammatory process.

**The role of the endothelial cell:** In AAV, endothelial cells may actively recruit inflammatory cells in the early stages of active disease, and enhance their adhesion to sites of vascular injury. They may synthesize PR3 (unproven), which could participate in *in situ* immune-complex formation [52].

**Pathogenicity of ANCA:** A direct evidence for pathogenicity of ANCA is a recently described mouse model, that shows that MPO-ANCA alone may induce vasculitis in mice, deficient in both T and B lymphocytes (Rag2<sup>-/-</sup>) [53]. Indirect evidence lies in the correlation of ANCA (and surface expression of PR3 on neutrophils) with the disease activity [29, 54], in the potential of ANCA to bind to target antigens and modify their physiologic function (induction of oxygen radical release, degranulation, inhibition of microbicidal function, defective apoptotic process), thereby contributing to tissue damage [51], or in the potential of ANCA to bind to planted antigen on endothelial cell and induction of endothelial cell injury [55]. The fact that passive transfer of ANCA to newborns through the placenta [56] or to experimental animals [57] does not induce vasculitis reflects the rather nondirect pathogenic potential of ANCA. Moreover, even situations when high titers of ANCA do not correlate with active disease are not rare [58].

### **Treatment of ANCA-associated vasculitis**

#### **Induction therapy**

Prior to the introduction of immunosuppressive therapy, the outcome of the patients with AAV was fatal, with most patients dying in less than a year due to a vital organ failure [28].

**Cyclophosphamide:** In the early 1980s, Fauci and Wolff introduced a regimen combining daily cyclophosphamide (CYC) therapy given for one year after remission was achieved with prednisone therapy initiated at a dose of 1 mg/kg/b.w./day and tapered on an alternate-day schedule. This treatment (“Fauci-scheme”) has been found to induce remission in 80–100 % of patients and can result in long-term survival. It was an empirical regimen and therefore entailed considerable therapy-related morbidity and mortality. Furthermore, when therapy is tapered and discontinued, relapses are common (in up to 50% of cases). Although CYC treatment is effective in managing the relapses, repeated courses of CYC are associated with bone marrow suppression, infection, cystitis, infertility, myelodysplasia, and transitional-cell carcinoma of the bladder and other secondary malignancies. As a result, the introduction of immunosuppressants has changed the natural history of the an acute, progressive and life-threatening disease into a chronic, often grumbling one with progressive accumulation of tissue damage due to disease scars and adverse effects of the therapy [59].

For all the above-mentioned reasons, a number of clinical trials aimed at improvement of induction and maintenance treatment of AAV has been launched. In Europe, the European Vasculitis Study Group (EUVAS) was created gradually during the first half of the 1990s and focused on first on diagnostic role of ANCA, followed by the standardization of ANCA testing, histological assessment and classification of AAV. It was assumed that despite their different clinicopathological characteristics, AAV could be studied together, but, for treatment purposes, subclassified based on their severity at presentation as follows:

- localized vasculitis (with serum creatinine < 120 mmol/L, no constitutional symptoms, no threat to any vital organ function and positive or negative ANCA)
- early systemic vasculitis (with serum creatinine < 120 mmol/L and constitutional symptoms, no threat to any vital organ function and positive or negative ANCA)
- generalized vasculitis (with serum creatinine < 500 mmol/L and constitutional symptoms, dysfunction of any vital organ function and positive ANCA)
- severe renal vasculitis (with serum creatinine > 500 mmol/L, constitutional symptoms and positive ANCA)
- refractory vasculitis (any serum creatinine, constitutional symptoms, threatened function of any vital organ and positive or negative ANCA) [60].

The optimal induction treatment of induction of remission in patients with generalized, but not immediately life-threatening AAV is currently tested in the

CYCLOPS trial, where the standard daily oral CYC (2 mg/kg b.w./day for months 0–3 and 1.5 mg/kg b.w./day for months 3–6) is compared with pulsed CYC (10 iv pulses 15 mg/kg b.w. during 6 months), in both limbs with corticosteroids (CS), with the aim to reduce the cumulative dose of CYC and thereby the toxicity of the treatment. The results are not available yet, but the results of some earlier smaller studies are promising [61, 62].

**Methotrexate:** The NORAM trial compared the effect of methotrexate (MTX) 15–25 mg weekly and CYC at a standard dose 2 mg/kg b.w./day on the remission rate in early AAV. According to the preliminary results [63], the remission rates were similar in both arms (more than 80% at 6 months), but the relapse rate was higher in the MTX group (69% vs 42%).

**Plasma exchange:** The role of plasmapheresis has been studied in the MEPEX trial, where the patients with acute renal failure due to AAV were randomized to adjunctive therapy with either seven plasma exchange treatments (each 60 ml/kg b.w.) or three pulses of intravenous methylprednisolone (each 15 mg/kg b.w.). Although the mortality in both trial arms was the same, renal survival was much better in patients with plasma exchange [64]. These data confirmed meta-analysis of several smaller studies and strongly suggest that plasma exchange should be used as an adjunctive treatment to CYC in patients with AAV with acute renal failure [65]. Also patients presenting with hemoptysis and pulmonary infiltrates caused by diffuse alveolar hemorrhage benefit from prompt initiation of plasmapheresis therapy coupled with aggressive immunosuppression [66].

**Recommendations for induction treatment:** In summary, most physicians favor a CYC-CS combination regimen in the initial treatment of most patients with AAV. This is particularly indicated in those with life-threatening disease, including patients with a serum creatinine concentration above 177 mmol/l, pulmonary involvement, CNS disease, and/or bowel perforation/infarction. CYC is given orally in a dose of 1.5 to 2 mg/kg b.w./day [and pulsed CYC will probably be a comparably effective and safer alternative], CS usually in an initial dose of 1 mg/kg per day of oral prednisone and gradually tapered. CYC and CS are continued until stable remission is induced (usually 3–6 months). A methotrexate-based regimen is an option in patients with active but not immediately life-threatening disease and normal or near normal renal function (with a serum creatinine concentration below 177 mmol/l). Prednisone alone is not recommended [67]. Plasmapheresis should be added in patients with dialysis-dependent renal failure and life-threatening pulmonary hemorrhage at presentation, especially in those with high titer ANCA and/or anti-GBM antibodies.

#### *Maintenance therapy*

**Azathioprine:** The CYCAZEREM trial examined whether azathioprine (AZA) was as effective as CYC in maintaining remission and preventing relapses in AAV, but with fewer side effects. One hundred and forty-one patients with threatened vital

organ function and creatinine levels  $< 500$  mmol/l were randomized after induction of remission (with daily oral CYC + CS usually for 3 months) to either treatment with continued oral CYC (1.5 mg/kg b.w./day) for 12 months, or AZA (2 mg/kg b.w./day). Both treatments were found to be equally effective (a comparable mortality, relapse rate, renal outcome, and short-term adverse effects). Given the known long-term safety profile of AZA compared with CYC, this trial has clearly established the superiority of AZA over CYC in preventing relapse after initial induction of remission in AAV. The results support the concept of aggressive treatment of active disease and lower-intensity therapy for the maintenance of remission. The possible need for further CYC treatment for late relapse adds to the importance of minimizing the initial level of exposure [68].

**Mycophenolate:** Encouraged by a preliminary experience with mycophenolate (MMF) in smaller studies [69], the EUVAS has recently launched the IMPROVE trial comparing MMF and AZA as a maintenance therapy in patients in remission. MMF could have a place not only in the treatment of patients in remission, but also in patients with chronic active disease unresponsive to CYC, or in whom further courses of CYC would be inappropriate.

**Methotrexate:** MTX has been given for maintenance treatment of AAV with good success, but no controlled studies comparing MTX and AZA are available, nor under way. In one controlled study, MTX has been shown superior to trimethoprim-sulfamethoxazole in maintaining remission [70, 71].

**Trimetoprim-sulfamethoxazole (co-trimoxazole):** It has been shown in a double blind, placebo-controlled, multicentric trial, that treatment with co-trimoxazole (in a dose of 800 mg of sulfamethoxazole and 160 mg of trimethoprim given twice daily for 24 months) reduces the incidence of relapses in patients with AAV in remission (especially in the upper airways). In addition, fewer respiratory and non-respiratory tract infections were found in the treated group. These findings suggest that the drug exerts its protective effect by preventing infections. Given the reported association between nasal carriage of *Staphylococcus aureus* and an increased risk of relapse of WG, it is tempting to postulate that co-trimoxazole reduces the frequency of relapses by eliminating or reducing *S. aureus* in the upper airways. On the other hand, co-trimoxazole, through its antagonism of folic acid metabolism or other yet unknown mechanisms, may have immunosuppressive properties [72].

**Cyclosporin A:** Little data is available on the use of cyclosporin A. Haubitz *et al.* treated 7 patients for one year after inducing remission and none of the patients suffered a relapse during the year of follow-up. This is a very small and short-term study, but supports data showing low rates of relapse in patients with vasculitis receiving cyclosporin A after renal transplantation [73].

**Recommendations for maintenance therapy:** Once complete remission is achieved, CYC is discontinued and either MTX (which is an option only in those with a serum creatinine  $< 177$  mmol/L) or AZA is initiated. Maintenance therapy is



usually continued for 12 to 24 months. Slow tapering of CS is initiated once there is a significant response, which usually occurs after one month. A low-dose (5–10 mg per day of prednisone), possibly in an alternate day regimen, is maintained for as long as immunosuppressive therapy is continued.

#### *Treatment of relapse*

Treatment of relapse is determined by severity and by whether or not the patient is still being treated with immunosuppressive therapy. Among those with relatively minor relapses determined clinically or by biopsy who are still receiving maintenance therapy, a trial of increasing the dose of CS and immunosuppressive agents can be considered. By comparison, reinstatement of the initial induction regimen is warranted in patients with more severe disease and in those who are no longer on immunosuppressive therapy [26]. Thus, treatment of recurrent vasculitis is largely similar to that of the primary disease. Given the increased drug exposure, greater attention must be paid to potential toxicity. In terms of maintenance therapy, the duration after reinduction is prolonged to two years after remission. In addition, if relapse occurred while on maintenance therapy, a different drug should be used (e.g. MMF rather than AZA in a patient with renal disease).

#### *Other therapeutic approaches*

**Intravenous immunoglobulin:** A number of smaller studies have reported beneficial effects of intravenous immunoglobulin (IVIg) in patients with chronic granulomatous vasculitis despite more conventional treatments or in patients with acute disease [74, 75].

**Deoxyspergualin:** The mechanism of action of deoxyspergualin (DSG) includes inhibition of IL-1 synthesis and anti-proliferative effects. It appears to be an effective and safe agent to treat patients with AAV refractory or with contraindications to standard immunosuppressants. The experience with this drug is very limited so far and further studies are warranted (and already under way) to investigate DSG as secondary or even primary agent in patients with AAV [76].

**Leflunomide and mizoribine:** Leflunomide inhibits pyrimidine nucleotide synthesis, inhibits proliferation of activated lymphocytes, and reduces IL-2, TGF $\alpha$  and antibody production. Unlike in rheumatoid arthritis, in AAV there is only a single report of the use of leflunomide in the maintenance phase with very good results [77]. A purine synthesis inhibitor mizoribine has been shown useful for preemptive treatment for patients with AAV at high risk for relapse.

**Entirely new approaches:** Lymphocyte depletion using monoclonal antibodies (CAMPATH 1H-anti-CD52 pan lymphocyte antigen, or anti-CD4) has been reported in a handful of patients with relapsing or persistent disease [78]. Anti-thymocyte globulin (ATG) has been used in at least 10 patients with refractory disease with limited success [79], and is the subject of another EUVAS trial. At

least 5 patients have been treated with an anti-CD18 monoclonal antibody with clinical improvement in four of them [80]. Rituximab (anti-CD20 chimeric monoclonal antibody) is another option. More specific approaches include co-stimulation blockade (with anti-CD40 ligand for example) to prevent antigen-driven immune responses, and anti-TNF antibody therapy. In view of the importance of ANCA in the pathogenesis of AAV, semispecific removal of these antibodies has been attempted using L-tryptophan immunoadsorption [81], and more specifically with MPO-bound immunosorbent columns to remove anti-MPO ANCA [82]. Finally, a few patients with severe disease have received immunoablation with autologous bone marrow stem cell transplant, with only short-term benefit [77].

#### *Supportive treatment*

Together with an aggressive immunosuppression, a prophylaxis against corticosteroid-induced gastritis, fungal infection, and *Pneumocystis carinii* pneumonia is strongly recommended. Patients > 50 years usually receive calcium and vitamin D tablets for bone protection. With high-dose CYC in i.v. pulses, uromitexan is used. Special attention is paid to those in fertile age before CYC is initiated.

#### *PR3-ANCA and MPO-ANCA disease – is there a difference?*

It has been noted that PR3-ANCA are predominantly found in patients with WG and MPO-ANCA in patients with MPA, its renal limited form, or CSS. These associations led to the question whether patients with PR3-ANCA differ from those with MPO-ANCA with respect to clinical presentation, histopathological findings and clinical outcome. Clinical features, pattern of pre-treatment renal function loss, renal morphology and outcome have been analyzed in a consecutive series of 46 patients with PR3-ANCA and 46 patients with MPO-ANCA [83]. Patients with MPO-ANCA had a higher median age than that of patients with PR3-ANCA (63 and 56 years, respectively). The prevalence of renal involvement did not significantly differ between PR3-ANCA and MPO-ANCA positive patients (83% and 67%, respectively), but, prior to treatment, renal function deteriorated significantly faster in PR3-ANCA. Moreover, kidney biopsies showed a higher activity index (cellular and fibrocellular crescents, necrosis, insudation, infiltration by inflammatory cells) and a lower chronicity index (glomerulosclerosis, interstitial fibrosis, and tubular atrophy) than biopsies from patients with MPO-ANCA. However, although PR3-ANCA positive patients showed a more active renal disease, kidney survival did not differ between PR3-ANCA positive patients (73%) compared to MPO-ANCA positive patients (61%). The authors suggest that the more acute clinical presentation of patients with PR3-ANCA results in the earlier institution of immunosuppressive treatment explaining the comparable or even better renal outcome. These data are in agreement with other studies [84–86].



Taken together, PR3-ANCA and MPO-ANCA are now believed to be markers of different disease entities within the spectrum of AAV.

#### *Factors involved in relapse of AAV*

AAV is a relapsing disease. The reported relapse rate differs from 16% in 18 months of follow-up [68] to up to 50% in long-term observations [27]. Several groups have noted that PR3-ANCA relapse more frequently than patients with MPO-ANCA associated vasculitis [29, 83, 87, 88]. The reason for this difference is not clear. In patients with PR3-ANCA persistence of PR3-ANCA after induction of remission is a risk factor for relapse. Longitudinal observations made by several groups [29, 89, 90] showed that relapses of WG were preceded by rises in ANCA titres. It has been even suggested that rising titres of ANCA may be used as a guideline for the institution of immunosuppressive therapy, but this has not been proven beneficial to the patient as the amount of cyclophosphamide required to prevent relapses may be harmful to the patient in the long term due to its toxicity [91]. A prospective study of preemptive therapy with AZA and prednisone or no preemptive therapy once a rise in ANCA titer had occurred, was recently performed [92] and proved, that early relapses could be prevented with preemptive treatment but that late relapses occurred in many cases after stopping preventive treatment. Thus, rising titers of ANCA are frequently followed by relapses, but the use of an elevation in ANCA titer as the sole parameter to justify immunosuppressive therapy cannot be endorsed; the patients with rising titers should be followed closely for signs of clinical activity.

Besides rising titers of ANCA, persistence of ANCA after induction of remission in WG has also been identified as a risk factor for an ensuing relapse [72, 90, 93], which suggests that long-term maintenance treatment should be instituted in patients who are persistently positive for ANCA after induction of remission. On the other hand, a persistently ANCA-negative status is not an absolute proof of remission. This was illustrated in a report, where 8% of patients were ANCA negative at the time of relapse [94].

A second factor relevant for relapse in WG is chronic nasal carriage of *Staphylococcus aureus*. In one study, 63% of patients with WG were chronic nasal carriers of *S. aureus* and relapses occurred almost exclusively in these patients. In agreement with these data, maintenance treatment with co-trimoxazole resulted in a reduction of relapses in WG, as it was already previously mentioned [72]. Several hypotheses of the mechanisms involved in relapse induction by nasal carriage of *S. aureus*, have been suggested – *S. aureus* derived superantigens may activate the immune system, *S. aureus* derived cationic proteins may adhere to (glomerular) basement membrane, induce a subclinical vasculitis/ glomerulonephritis which, in the presence of ANCA, develops into clinically overt disease [95, 96].

Genetic risk factors have been implicated as well in the occurrence of relapses. Patients with polymorphic forms of Fc-gamma receptors that exhibit low affinity for certain IgG-subclasses were more prone to relapses in WG in the first 5 years after diagnosis [97]; high membrane expression of PR3 on resting circulating neutrophils was associated with a significantly increased risk for relapse [98].

In conclusion, a frequent monitoring of ANCA levels, a closer follow-up of those with rising titers and an eradication of *S. aureus* are necessary. The increased relapse rate in PR3 disease should be probably taken into consideration in the length of maintenance treatment.

### **Outcome of patients with AAV**

#### *Morbidity and mortality*

AAV is a life-threatening disease that requires prompt recognition and therapy. Prognosis is an especially important tissue as the disease process is aggressive and the therapeutic options are inherently dangerous. Since the introduction of CYC and CS in the treatment of AAV, mortality has significantly decreased from 82% in 1 year to 59–95% in various patients groups during various follow-up periods, and remission rates have increased to up to 93% [29]. Different authors have reported following survival periods in their cohorts of patients: 85.5% at 1 year and 63% at 5 years, 88% at 2 years and 74% at 5 years [99], 73% at 5 years and 62% at 10 years [100], 84% at 1 year and 76% at 5 years [101]. In our group of 61 patients, the estimated patient survival at 5 and 10 years was 78.3 and 62.2%, respectively (submitted for publication). At the time of disease presentation, clinicians are faced with several factors that may influence the outcome of the patients. The treatment used in AAV is toxic and carries the potential risk of life-threatening infection. Additionally, cytotoxic agents are associated with cancerogenesis, mutagenesis, infertility, and interstitial cystitis. Significant prognostic factors for mortality were found to be the entry age [88, 99, 100, 101], the serum creatinine level and dialysis-dependence at presentation [88, 99, 100, 101] (both confirmed by our results as well), the developing dialysis-dependence during follow-up [100], the multi-system manifestation and the presence of pulmonary hemorrhage [102]; however, the latter was not confirmed by others [100]. Some authors reported higher mortality in C-ANCA disease and WG [102], whereas others did not confirm this finding [88, 99, 101]. The male gender was sometimes [100] associated with increased mortality, that was not confirmed by others [101]. Factors unrelated to vasculitis, such as functional status (as quantified by Karnofsky score) and non-vasculitic co-morbidity were, not-surprisingly, found to be potent predictors of poor outcome [99]. Some authors report low-intensity immunosuppression to be associated with a worse outcome [99, 102], whereas others stress the treatment-associated leukopenia and ensuing sepsis as an independent risk factor for death [101], which indicates the need for more

effective better targeted therapy. Overall, the morbidity and mortality results from several factors. In the early phase of the disease it is associated with irreversible organ dysfunction because of inflammatory injury (within days), further on with aggressive immunosuppressive therapy and its short-term adverse effects, namely infections (within months), and long-term sequels, such as secondary tumors, myelodysplastic syndrome, accelerated atherosclerosis etc. [88, 100].

#### *Renal outcome*

The reported renal survival in patients with AAV with renal involvement differs according to the severity of renal disease at presentation from 65% at 5 years and 51% at 10 years [100] to 44% at 48 months [102]. In our group of patients, the estimated renal survival time at 5 and 10 years was 69.2 and 55.8%, respectively. The strongest predictors of long-term renal outcome are the entry serum creatinine level and dialysis-dependence and the occurrence of renal relapses [88, 100, 101, 102]. Some authors found the older age to be associated with an increased renal death [101]; others reported a worse renal survival in patients with very high titers of PR3-ANCA and in those with low blood thrombocytes [88], or in those with proteinuria at diagnosis and during follow-up in MPO-ANCA disease [100]. The importance of the race with a worse renal outcome in African Americans and arterial sclerosis on renal biopsy has been stressed [102]. The predictive value of renal biopsy findings for renal outcome in ANCA-associated necrotizing glomerulonephritis has been evaluated [103]. The percentage of normal glomeruli in the biopsy was the best predictor for renal recovery and outcome. Reversibly, glomerular sclerosis, diffuse interstitial infiltrates, tubular necrosis and atrophy were each associated with a worse recovery and outcome. This study, also, shows that the extent of chronicity in the renal biopsy at presentation is the major factor for renal outcome and not the activity of the glomerular lesions. The latter, apparently, are largely reversible once adequate immunosuppressive therapy has been instituted.

#### **Summary**

AAV is a multi-factorial disease with increasing incidence. Although our knowledge of etiopathogenesis is increasing rapidly, the exact role of ANCA and so far poorly defined environmental and genetic factors possibly involved in the etiology remain to be elucidated. The last two decades, with the advent of cytotoxic therapy, have brought greatly increased survival probability, but significant risk of infective complications in particular and many other problems with the management of chronic grumbling and relapsing disease with accumulating morbidity and mortality. The vasculitides are relatively rare and are heterogeneous in their presentation; hence the importance of well conducted, multi-centre collaborative trials to identify promising new

therapies, and to maximize the benefit of existing treatment regimens. Outcome in AAV was found to be related to age and presenting creatinine level. Therefore, diagnostic delay may have a major influence on outcome. An increased awareness of AAV with subsequent rapid ANCA testing, recognition of the presence of organ involvement and quick referral of the patient to a specialist is therefore warranted. The study of AAV thus remains a challenge in all aspects.

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## Multiple extrarenal complications of Wegener's granulomatosis

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**key words:** Wegener's granulomatosis, ANCA, aortic valvulitis, subglottic stenosis

### SUMMARY

**Background:** We describe a case of Wegener's Granulomatosis (WG), a disease characterized by necrotizing granulomatous lesions and vasculitis of the upper and lower respiratory tract, necrotizing glomerulonephritis with crescent formation and systemic vasculitis.

**Case report:** The patient initially presented with a corneal perforation. In addition to the eye involvement, multiple other organ involvement was found during the course of the disease. The renal biopsy showed a pauciimmune necrotizing glomerulonephritis with occlusive fibrocellular crescents, the interstitium was focally fibrotic with extensive active inflammation. She had typical upper and lower airway and pulmonary involvement, as well as the skin vasculitis and arthralgias. Cardiac involvement included an acute myocardial infarction and aortic valve vegetation, most likely caused by WG. Another complication during the initial presentation was a massive intestinal bleeding. The diagnosis of WG was established by renal, conjunctival and upper airway biopsies and by positive c-ANCA. The clinical and laboratory remission of the disease was achieved by combined immunosuppressive therapy with cyclophosphamide and corticosteroids. Later in the course of the disease the patient developed a subglottic stenosis, most probably due to reparative changes.

**Conclusions:** In conclusion, this is a case of WG with multiple extrarenal complications, that had a grave clinical course.

### BACKGROUND

WG is an ANCA (antineutrophil cytoplasmic antibodies) positive systemic inflammatory disorder of unknown etiology. It is characterized by necrotizing granulomatous lesions and vasculitis of the upper and lower respiratory tract, necrotizing glomerulonephritis with crescent formation and systemic vasculitis involving small and, less frequently, medium-sized vessels [1]. The clinical presentation of WG depends on the organ(s) involvement. It is a rare disease, although its incidence has recently increased. This finding may be the result of an increased awareness, as well as easier and earlier diagnosis, especially due to the availability of routine ANCA testing. More than 90 percent of patients with active WG have serum ANCA. Most patients with WG have c-ANCA (with cytoplasmic type of fluorescence), characterized by autoantibodies

directed against proteinase-3 (PR-3) [2]. Only a minority has p-ANCA (with perinuclear type of fluorescence), directed against myeloperoxidase (MPO).

In 1990, the American College of Rheumatology established four major criteria for the diagnosis of WG:

1. Nasal or oral inflammation characterized by ulcers or purulent or bloody nasal discharge
2. Abnormal chest radiogram
3. Glomerular microscopic hematuria
4. Granulomatous inflammation on biopsy.

The presence of two or more of these four criteria yielded sensitivity of 88 percent and specificity of 92 percent [3]. The criteria had been established before the routine ANCA testing was introduced.

### CASE REPORT

A 34-year-old woman presented to her primary care physician with a two weeks' history of fever and ge-

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neralized arthralgias. She was treated with antibiotics without any improvement. Chest radiography revealed a right upper lobe infiltrate, which did not change after prolonged antibiotic treatment. She was admitted to hospital and empiric antituberculous chemotherapy was started. Sputum AFB (acid-fast bacilli) smear and AFB cultures were negative. During the hospital, stay she developed conjunctivitis that rapidly progressed into a corneal perforation despite treatment.

She was transferred to the Department of Ophthalmology at our hospital. The conjunctival biopsy revealed perivasculitis. The corneal perforation was later treated with lamellar keratoplasty. The ENT examination was performed because of dysphonia and nasal deformation. Atrophic mucosa with crust formation was found in both nasal cavities. Multiple mucosal defects covered by fibrin were seen in the pharynx. In the larynx, granulations affecting both vocal cords and the subglottic area were described. The biopsy from the nasal mucosa revealed intensive mixed inflammatory infiltration and focal fibrinoid necrosis of the connective tissue with macrophages in the surroundings. The finding was compatible with WG.

Because of fever and electrocardiographic signs of an acute myocardial infarction, the patient was transferred to the Department of Internal Medicine. She was found to have a generalized skin rash. The lesions were hemorrhagic and papular with ulcerations and necrosis. They were located predominantly over the elbows, hips and on the fingers. Similar lesions were found on the oral mucosa.

Laboratory examination revealed moderate anemia (hemoglobin 8.0 g/dl), leukocytosis with white blood cell count  $20 \times 10^9/l$  and thrombocytosis (platelet count  $606 \times 10^9/l$ ). The diagnosis of WG was based on the clinical presentation and positive c-ANCA with anti-proteinase 3 titer 42 U/ml on enzyme-linked immunosorbent assay, where the normal levels (N) are below 3.5 U/ml.

On admission, the patient had an acute myocardial infarction of the inferior and lateral walls and signs of congestive heart failure. The electrocardiogram showed a slow r wave progression on the anterior wall with the transition zone in V6 and an elevation of the ST segment in leads II, III and V<sup>4-6</sup>. Cardio-specific enzymes were positive – creatine kinase 3.96  $\mu\text{kat/l}$  (N below 2.9  $\mu\text{kat/l}$ ), creatine kinase-MB fraction 0.78  $\mu\text{kat/l}$  (N below 0.42  $\mu\text{kat/l}$ ) and troponin-T 1.0  $\mu\text{g/l}$  (N negative). Transthoracic echocardi-

graphy showed an apical aneurysm with slightly decreased left ventricular ejection fraction (45–50%). Transesophageal echocardiography showed  $7 \times 4 \times 12$  mm vegetation on the left cusp of the aortic valve (Figure 1). Infectious endocarditis and valvulitis due to WG were both considered in the differential diagnosis. From multiple sets of blood cultures only one grew *Staphylococcus haemolyticus*. The skin lesions were considered to be rather due to the vasculitis than septic embolisms. The clinical condition of the patient, as well as the elevation of the inflammatory markers (sedimentation rate, C-reactive protein) admitted both possibilities. A chest computed tomography showed the right upper lobe alveolar infiltrates and nodules. Bronchoscopy was not performed because of severe vocal cord involvement.

The patient developed transient oliguria due to dehydration and cardiac insufficiency with mild renal insufficiency (creatinine 1.47 mg/dl, on admission 1.16 mg/dl). Urinalysis revealed mild proteinuria (0.75 g/l) and erythrocyturia (10–15 erythrocytes per high-power field). Renal biopsy revealed 1–3 normal glomeruli and 3–6 glomeruli with capillary tufts and occlusive fibrocellular crescents formation. The interstitium was focally fibrotic, saturated with extensive active inflammation. The finding was described as florid pauciimmune necrotizing glomerulonephritis with crescent formation (Figure 2).

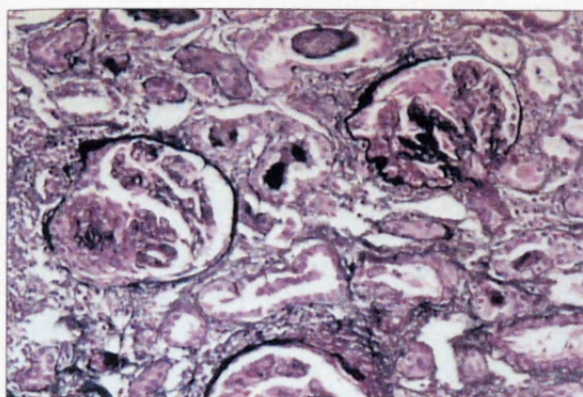
During the initial treatment, the patient developed massive intestinal bleeding requiring multiple blood transfusions. Colonoscopy revealed superficial suffusions of the rectal mucosa. Gastrosocopy was normal.

The diagnosis of WG was based on the ENT, pulmonary and eye involvement and on the positive c-ANCA findings. It was confirmed by histological findings, particularly by the renal biopsy with a pauciimmune necrotizing glomerulonephritis with crescent formation. The patient also fulfilled three out of four American College of Rheumatology criteria (see above). Immediately after the diagnosis had been established, the patient was started on a combined immunosuppressive therapy with cyclophosphamide and corticosteroids. A complete remission was achieved: c-ANCA became negative (anti-proteinase-3 titer = 0.79 U/ml). Echocardiography showed no pathologic formations on the aortic valve and coronary arteriography was normal (it was not performed on presentation because of the large aortic valve vegetation). The skin lesions resolved and renal functions, as well as the eye lesion stabilized. The patient showed no clinical signs of the upper or lower airway involvement.





**Figure 1.** Aortic valve in the short axis in the systole. Transesophageal echocardiography. Hyperechogenic oval pathologic formation sized 7x4x12 mm on the left coronal cusp next to the commissure with the right coronal cusp of the aortic valve.



**Figure 2.** Pauciimmune necrotizing glomerulonephritis. A segmental epithelial crescent with a partial collapse of the capillary tuft and with a small defect of the Bowmann's capsule on the left. A glomerule with a small segmental fibrin crescent on the right. Silver methenamine. Magnification 30x.

Six weeks after the discharge from hospital, she was readmitted because of rapidly progressive shortness of breath and stridor due to a very tight subglottic stenosis. At that time, no other clinical or laboratory signs of WG relapse were present. Electrocauterization and dilation were done during rigid bronchoscopy. Six months after the diagnosis of WG, she remains in clinical and laboratory remission. There were no signs of restenosis during control bronchoscopies.

## DISCUSSION

This is a case report of a patient with WG with an unusually high number of extrarenal complications. The patient had a two-year history of intermittent bloody nasal discharge; subsequently she developed nasal cartilage deformation (a 'saddle' nose). After

a febrile episode and arthralgias, the disease directly presented with a corneal perforation. This finding had led to the correct diagnosis, which was supported by positive c-ANCA. The conjunctival, ENT and renal involvement were verified histologically. However, such manifestation, as well as the pulmonary involvement is frequent in WG, the symptomatic cardiac involvement, is a rare condition. Pericarditis, coronary arteritis, myocarditis, cardiomyopathy, valvulitis and arrhythmia were described [4–7]. Some authors [6] suspect more frequent cardiac involvement, which is often asymptomatic. In our case, the patient had two cardiac findings (acute myocardial infarction and granulomatous valvulitis of the aortic valve). She suffered from acute myocardial ischemia most likely due to coronary vasculitis. A potential atherosclerotic disease, clinically very unlikely, was excluded by the normal coronarogram obtained during the remission of the disease. The embolism from the aortic valve vegetation was also considered as a reason for the myocardial infarction, though less likely because of the localization of the myocardial ischemia. Infectious endocarditis was considered as a reason for aortic valve vegetation. However, the patient did not fulfill the diagnostic criteria according to Durack [8]. One major criterion (echocardiographic evidence of the valve involvement) and one minor criterion (fever above 38°C) were present. The skin lesions were rather of immunologic than embolic origin. The only positive blood culture (*Staphylococcus haemolyticus*) was most likely caused by contamination during the sampling. The echocardiographic finding of the aortic valve was more suspicious than the granulomatous involvement due to the WG. Since infectious endocarditis involving the abnormal aortic valve could not be excluded, the patient was treated with IV antibiotics according to established guidelines.

Gastrointestinal tract (GIT) involvement in WG has been described only in sporadic cases. It may manifest by vomiting, diarrhea, abdominal pain, enterorrhagia and visceral perforation [9–11]. In our case, it was impossible to verify the GIT involvement especially due to the technical difficulties encountered during the colonoscopy. The scheduled examination of the small intestine was cancelled because of the poor condition of the patient and resolution of the bleeding after conservative treatment. However, bleeding from diffuse ulcerations is consistent with WG.

Another relatively infrequent complication was the development of a very tight subglottic stenosis during the remission of the disease. The biopsy obtained during bronchoscopy showed only inflammatory chan-

**Table 1.** Approximate frequency of individual organ involvement in WG.

Organ involved	Frequency
E.N.T. region	90%
Lungs	85-90%
Kidney	77-80%
Musculoskeletal signs	67%
Eye	52%
Skin	40-50%
Fever	50%
Nervous system	PNS 15%, CNS 8%
Heart	5-10%, in some sources [6] up to 44%
GI/T	Sporadic cases
Others parenchymal organs, endocrinal glands, urogenital tract...	Sporadic cases

ges. Since all markers of pathologic activity were normal, we consider the development of stenosis as a result of reparative changes. The development of the stenosis should have been anticipated because of the extensive airway involvement during the initial presentation.

The approximate frequency of organ involvement in WG according to the literature data is summarized in table 1.

## CONCLUSIONS

In conclusion, this is a case of WG with multiple extrarenal complications, which had a grave clinical course. This diagnosis should be considered primarily when a patient presents with E.N.T. and/or pulmonary signs and has an abnormal urinary sediment with or without renal insufficiency. Other possibili-

ties of organ involvement by this systemic vasculitis include the musculoskeletal system, eye, skin, nervous system, heart and GIT. A very precious help to establish the correct diagnosis is provided by the ANCA tests.

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## CLINICAL STUDY

# Silica and Asbestos Exposure in ANCA-Associated Vasculitis with Pulmonary Involvement

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Silica and asbestos exposure are thought to belong to the triggering factors of antineutrophil cytoplasm antibodies (ANCA)-associated vasculitis. We carried out a study to find out whether patients with pulmonary involvement attributable to ANCA-associated vasculitis (AAV) have been exposed to silicon-containing materials. Thirty-one patients (12 women, 19 men, median age 51 years) were interviewed using a structured questionnaire. Occupational exposure to silicon-containing chemicals was reported by 22.6% of the patients (12.9% to SiO<sub>2</sub>, 9.7% to asbestos), compared with 0% of control subjects ( $p < 0.05$ ). Our findings support the pathophysiologic role of silica in AAV.

**Keywords** ANCA, asbestos, etiology, silica, vasculitis

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## INTRODUCTION

The ANCA-associated vasculitides (AAV) are complex, immune-mediated disorders characterized by a necrotizing pauci-immune vasculitis affecting multiple organs, especially the respiratory tract and kidney. These AAV include three major categories: Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), and Churg-Strauss Syndrome (CSS). They are strongly associated with the presence of antineutrophil cytoplasmic antibodies (ANCA). The ANCA are most often directed to either proteinase 3 (PR3-ANCA) or to myeloperoxidase (MPO-ANCA). Both PR3 and MPO are located in the azurophilic granules of neutrophils and the peroxidase-positive lysosomes of monocytes.<sup>[1]</sup>

The tissue injury in AAV results from the interplay between an initiating inflammatory event and a highly specific pathogenic immune response to previously shielded epitopes of neutrophil granule proteins. This generates high-titer ANCA directed against antigens within the primary granules of neutrophils and monocytes. These antibodies produce tissue damage via interactions with primed neutrophils and endothelial cells. The exact events leading to the initiation of the disease



**Table 1**  
Detailed analysis of patients exposed to silica or asbestos

No	Sex	Age/yrs	ANCA	Dg.	Exposure	T1/yrs	T2/yrs	Smoker	Profession
1	Man	64	P	MPA	SiO <sub>2</sub>	5	30	Yes	Fireplace building
2	Man	67	C	WG	SiO <sub>2</sub>	8	8	Yes	Ceramics teacher
3	Woman	75	P	WG	SiO <sub>2</sub>	2.5	48	No	Stonework labourer
4	Man	22	C	WG	SiO <sub>2</sub>	2	2	Yes	Glass industry
5	Man	38	C	WG	Asbestos	7	17	Yes	Heating engineer
6	Man	36	P	MPA	Asbestos	1	1	Yes	Floorer (floor coverings)
7	Man	59	P	MPA	Asbestos	2	15	No	Traffic policeman

Abbreviations are: T1=the length of exposure, T2=the period between the start of exposure and the diagnosis.

are unclear. Infectious, genetic, and environmental risk factors and combinations of all three have been suggested.<sup>121</sup>

The first symptoms of WG very often occur in the respiratory tract. Exposure to infectious and noninfectious agents or toxins is, therefore, believed to be an inciting event. One of the possible candidates is silicon-containing dust. Exposure to silica dust has been repeatedly reported to be significantly higher in patients with ANCA and AAV than in healthy controls, lupus nephritis, or other conditions.<sup>13,41</sup> Recently, occupational exposure to asbestos, another silicon-containing mineral, even without typical signs of asbestosis such as interstitial lung fibrosis, has been reported to result in ANCA positivity.<sup>151</sup> We have, therefore, focused on occupational histories and established the silica and asbestos exposures in our patients with pulmonary AAV.

## PATIENTS AND METHODS

We included a total of 31 patients with AAV with pulmonary and renal involvement diagnosed in our center between the years 1993 and 2002.

The diagnosis of AAV was determined in all cases by a renal biopsy showing pauci-immune crescentic glomerulonephritis and ANCA positivity. Positive ANCA findings were defined as a C-ANCA or P-ANCA staining pattern for ethanol-fixed human neutrophils, as determined by indirect immunofluorescent microscopy. All P-ANCA were further characterized as MPO-specific and all C-ANCA as PR3-specific by enzyme-linked immunosorbent assay. Pulmonary involvement in patients with AAV was defined by the presence of hemoptysis, pulmonary hemorrhage, respiratory failure, and/or radiographic proof of infiltrates in the absence of evidence of any other cause, mainly infectious.

The patients were asked to complete questionnaires designed by the occupational health physicians to

evaluate their exposure to silica-containing chemicals and estimate their extent by the intensity  $\times$  frequency  $\times$  duration.

The control subjects provided by the Occupational Medicine Department were age, sex, and residence-matched healthy individuals, working as office employees. All but one with borderline positivity of MPO-ANCA, clinically insignificant, was ANCA-negative.

The statistical analysis was performed using a test of hypothesis of equality of two relative frequencies. *P* values  $<0.05$  were considered significant.

## RESULTS

The group of patients consisted of 12 women and 19 men. Their median age at diagnosis was 51 years (range 18–75 years). Their respective diagnoses according to the Chapel Hill Consensus Conference definitions<sup>161</sup> were as follows: 22 had WG, 8 had MPA, and one patient had CSS. All patients were ANCA positive, 21 of them had C-ANCA (anti-PR3), and 10 P-ANCA (anti-MPO).

All patients had pulmonary involvement diagnosed by clinical signs and/or abnormal findings on chest x-ray. Twenty-three of them had alveolar or diffuse hazy opacities, the remaining eight patients had cavitating nodules. Eleven patients presented with hemoptysis. Twenty-four patients had spirometry performed at some stage of the disease. Lung functions were normal in 10 patients, 11 had airflow obstruction, two had restrictive impairment, and one patient had a mixed obstructive and restrictive pattern. Fourteen patients had the transfer factor for carbon monoxide (TLCO) tested. It was normal in five cases, slightly reduced in another five, and more severely reduced in four cases. Exposure to smoking was comparable in the AAV and control groups (41.9% vs. 43.3% of smokers).

A total of seven AAV patients (22.6%) had a former exposure to silicon-containing chemicals (12.9% to SiO<sub>2</sub>,

9.7% to asbestos). The mean length of exposure was almost 4 years, in all cases the exposure was considered as low. We explored the possibility that the exposure was more likely to be associated with sex, smoking, and specific ANCA pattern or disease category. None of these was proven significant, although there was a tendency towards higher exposure in the MPO group. The detailed analysis including the occupational histories of the exposed patients is shown in Table 1. The results were compared to those of 30 age, sex, and residence-matched controls, where no patients were found to have any previous occupational exposure to silicon-containing materials. The difference was statistically significant ( $p < 0.05$ ).

## DISCUSSION

Environmental factors are thought to play a role in the development of autoimmune diseases. Since the beginning of the last century an increased prevalence of different autoimmune diseases (rheumatoid arthritis, scleroderma, systemic lupus erythematosus) in patients exposed to silica has been reported.<sup>[7-9]</sup> A combination of risk factors is involved in susceptibility to AAV. It is largely accepted that AAV is genetically based but environmentally triggered. Since 1960, several patients with silicosis developed pauci-immune necrotizing crescentic glomerulonephritis. Later on, it was reported that these patients had ANCA that was, in most cases, directed to myeloperoxidase. In one study, 27% of chronically silica-exposed individuals had anti-MPO antibodies.<sup>[10]</sup>

Furthermore, several case reports, case series, and case-control studies have demonstrated an association between AAV and exposure to silica-containing materials.<sup>[11-14]</sup> In summary, there is increasing evidence of a pathophysiologic role of silica in AAV, although the mechanisms by which silica may induce AAV are not well known.

Silica (silicon dioxide,  $\text{SiO}_2$ ) is the earth's most abundant mineral.<sup>[11]</sup>  $\text{SiO}_2$  occurs in a noncrystalline (amorphous) or a crystalline form. The crystalline forms are constituents of soil, rock, and sand. When these materials are processed and subsequently used, the workers can be exposed to respirable crystalline silica.<sup>[14]</sup> The mechanisms by which silica-containing compounds induce acute and chronic lung damage are well described and understood.<sup>[15]</sup> It has been shown that silica aspirated through the airway may activate alveolar macrophages and not only induce inflammation and activation of fibroblasts, but also stimulate lymphocytes through T-cell receptors and attract neutrophils, which are the source of MPO. Myeloperoxidase (MPO) secondarily taken up by

alveolar macrophages may be presented to immunocompetent cells to develop autoimmunity against MPO.<sup>[16,17]</sup> Silica is also considered to induce apoptosis of monocytes, macrophages, and possibly neutrophils. Surface expression of MPO during apoptosis of neutrophils in the absence of priming has been shown. The ANCA may bind to the antigen on apoptotic cells, resulting in an amplified release of cytokines, oxygen radicals, and lysosomal enzymes operative in vasculitis.<sup>[15]</sup> Silica, therefore, affects the immune response in many ways and the host's genetic susceptibility is probably the factor that decides whether or not the affected (exposed) individual will develop the autoimmune disease. Similarly to silica, the inhaled asbestos fibers persist in the respiratory systems for decades. A higher rate of ANCA positivity was found in asbestos-exposed patients who did not show any signs of asbestosis in the form of interstitial lung fibrosis, but had asbestos-induced pleural hyalinoses. Therefore, asbestos seems to have a more pronounced effect on the formation of ANCA than silica.<sup>[5]</sup>

We focused our attention on patients with AAV with pulmonary involvement. The pulmonary involvement in all patients was attributable to AAV; none of the patients was diagnosed with any kind of pneumoconiosis. Spirometry findings in patients with AAV have not been satisfactorily discussed in literature. Both restrictive and obstructive patterns can be found.<sup>[18]</sup> The majority of our patients had an obstructive disease, which is consistent with the literature.<sup>[18]</sup> Spirometry in our patients was performed irrespective of the stage of the disease, mostly in remission. The findings, therefore, reflect the consequences of pulmonary AAV attributable to vasculitis damage, and not the vasculitis activity. The reduced carbon monoxide transfer factor (TLCO) in pulmonary AAV is also a finding that confirms the available literary data. Unlike the lung function, which frequently improves following treatment, the diffusing capacity may not return to normal.<sup>[18]</sup> The impaired TLCO in our patients did not correspond to any ventilation abnormality. Four patients with reduced TLCO had normal ventilation parameters, three had a moderate airflow obstruction and two had a restrictive pattern on spirometry. Three patients with normal TLCO had a mild obstruction and two had normal ventilation. We have not detected any case of abnormally high TLCO, which comes under conditions of alveolar bleeding, again because the patients were not tested at the time of diagnosis.

We were able to show a significantly higher anamnestic exposure to silicon-containing compounds in our group of AAV patients compared to the control group. Case-control studies are known to frequently involve biases, especially with respect to the selection of control subjects and the recall of exposure history. We are certain

that none of the control subjects had AAV. In keeping with the published data,<sup>[11,14,15]</sup> we found a tendency to higher exposure in the P-ANCA subgroup, none of the other factors studied (sex, diagnosis, smoking) were significant.

## CONCLUSION

The results of this study indicate that activities and environments known to cause higher levels of exposure to silica dust are associated with AAV. Silica and asbestos dust exposure is, therefore, likely to be a factor that facilitates the pathogenesis of AAV in the Czech population. The exact mechanisms of causality must be further explored on a larger population of AAV patients.

## ACKNOWLEDGMENTS

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## Letter to the Editor (Case Report)

### Two familial cases of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis

SIR, Familial cases of ANCA-associated vasculitis (AAV) have not yet been described in the Czech Republic.

In 2000, a 74-yr-old man was referred to us with polyarthralgia and acute renal failure detected during a work-up for nasal polyposis surgery. The laboratory results showed cytoplasmic (c)-ANCA antiproteinase 3 (anti-PR3) positive (15.43 U/ml), the urinalysis protein 3+ and blood 4+. The chest X-ray, eye and ear, nose and throat (ENT) examinations were normal. The polyposis was thought to have receded thanks to local corticosteroids (CS). A renal biopsy (RB) showed a pauci-immune crescentic glomerulonephritis (GN). Wegener's granulomatosis (WG) with renal, musculoskeletal and ENT involvement was diagnosed. Treatment with CS and cyclophosphamide (CYC) induced remission, but the patient remained on dialysis.

This patient's daughter underwent maxillar sinus surgery in 1999 at the age of 44 yr. A carcinoma was diagnosed and treated with chemotherapy and radiotherapy. She has since suffered from epistaxis and hoarseness. She also had polyarthralgia and necrotizing skin lesions that were biopsied and showed a small vessel vasculitis. In 2003 her hoarseness progressed and dry cough appeared and persisted after antibiotics. The initial work-up revealed renal failure requiring dialysis, c-ANCA anti-PR3 positive (26 U/ml). An RB showed a pauci-immune crescentic GN. A second opinion was requested on the original maxillar mass; it contained a dense inflammatory infiltration, active 'fibrinoid' vasculitis and no morphological features of carcinoma. The induction treatment led to remission, creatinine dropped to 143  $\mu\text{mol/l}$ .

In June 2004, a 48-yr-old woman with polyarthralgia, cough, hoarseness, fever and purpura was admitted. In February 2004 she was treated with local CS for keratoconjunctivitis. Her father died at the age of 59 yr of pulmonary cavitation. Admission laboratory data showed creatinine 180  $\mu\text{mol/l}$ , perinuclear (p)-ANCA anti-myeloperoxidase (anti-MPO) positive (72.66 U/ml) and erythrocyturia. A chest X-ray showed diffuse interstitial thickening. An RB showed a pauci-immune crescentic GN. The patient was diagnosed with microscopic polyangiitis (MPA) with renal, lung, eye, skin and musculoskeletal involvement. Treatment with CS and CYC led to remission, creatinine decreased to 112  $\mu\text{mol/L}$ .

This patient's sister (56 yr old) had suffered from polyarthralgia, fever and anorexia for 2 months prior to admission in October 2004. Her erythrocyturia persisted after antibiotic treatment. The initial work-up revealed creatinine 160  $\mu\text{mol/l}$  and p-ANCA anti-MPO positive (41.72 U/ml). Chest X-ray was negative. An RB

confirmed the diagnosis of MPA. Induction therapy with CS and CYC led to remission, creatinine dropped to 81  $\mu\text{mol/l}$ .

This observation illustrates the diversity of the AAV determined by the sites and the activity/chronicity of organ involvement. Generalized 'flu-like' manifestation, ENT or respiratory symptoms lead mostly to antibiotic treatment. The detection of erythrocyturia often prompts another course of antibiotics and/or urological work-up. A temporary spontaneous remission of the symptoms further delays the diagnosis. As the disease persists, it may start to resemble a malignancy. Nevertheless, an erroneous diagnosis of carcinoma in the setting of a histological diagnosis of cutaneous vasculitis is a grave mistake. However varied the AAV may be, their clinical presentation in the two families described was in some aspects very similar. Both the father and daughter had WG, c-ANCA and a history of ENT involvement that preceded dialysis-requiring renal failure with some corresponding features in renal histology. The two sisters both had MPA, p-ANCA, non-specific constitutional symptoms and histological and laboratory evidence of a rather slower decline in the renal function. We were unable to obtain objective data on their father. Nevertheless, the daughters described a suggestive picture of a pulmonary involvement in AAV. The difference in the presentation in the two families shows that PR3-ANCA and MPO-ANCA are markers of different diseases within the spectrum of AAV with a more acute presentation of patients with PR3-ANCA [1–3]. Last, but not least, the presented case reports raise the question of a familial predisposition to AAV. A number of familial cases have been described. A shared environment with exposure to silica has been thought to explain the cluster occurrence in some [4], whereas others have stressed the genetic predisposition [5, 6]. No consistent HLA association has been identified. Our patients within the two families shared a similar genetic background (Table 1). As mutations in the gene encoding  $\alpha$ -1 antitrypsin (AAT) are more frequent in patients with AAV [7], we tested all our patients and found their AAT levels to be within the normal range. Our patients did not share the same environment. The fact that two members of the two families fell ill with the same disease and their similar HLA typing seem to favour the role of a genetic predisposition to AAV.

The authors have declared no conflicts of interest.

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TABLE 1. HLA tissue typing

Patient	A	B	Cw	DRB1
I/1	1/11	51/57	2/6	07/11
I/2	1/2	14/57	6	07/11
I/3	2/11	18/51		11
II/1	2/30	8/62	3	04
II/2	2/30	8/41		04/13
II/3	2/30	8/62	3	04
II/4	26/30	8/38		04/13

I/1, father; I/2, daughter; I/3, healthy sister.

II/1, younger sister; II/2, her healthy daughter; II/3, older sister; II/4, her healthy son.

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## Regulatory cytokines in ANCA-associated vasculitis

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### INTRODUCTION

ANCA-associated vasculitis (AAV) is a complex immune-mediated disorder in which tissue injury results from the interplay between an initiating inflammatory event and a highly specific pathogenic immune response to previously shielded epitopes of neutrophil granule proteins. This generates high titre of ANCA (anti-neutrophil cytoplasmic antibodies) directed against antigens within the primary granules of neutrophils and monocytes. These antibodies induce tissue damage via interactions with primed neutrophils and endothelial cells. Inflammatory cytokine production and adhesion molecule activation or upregulation are important determinants of the pathogenic inflammatory responses noted in vasculitis (1). The whole spectrum of immunocompetent cells and their cytokines play important roles in the pathogenesis of AAV.

T cell contribution is indicated by a T cell-dependent ANCA production combined with the presence of T cells in inflammatory infiltrates. The Th1/Th2 concept is useful for a better understanding of the disease process and development of novel immunotherapeutic strategies in AAV. In Wegner's granulomatosis (WG), a shift in T cell response, from a Th1 pattern in localized disease towards a Th0/Th2 pattern in generalized disease, appears to occur. Although less thoroughly studied, data in Churg-Strauss syn-

drome (CSS) and microscopic polyangiitis (MPA) indicate that these diseases are predominantly associated with Th2 patterns. Nevertheless, the exact role of T cells and the true nature of the Th1/Th2 responses remains to be elucidated (2).

### PATIENTS

We examined 48 patients (27 men, 21 women, mean age 55.5 years) with AAV. Their respective diagnoses were WG in 28 of them, MPA in 10 of them, renal limited vasculitis (RLV) in 10 of them. ANCA specificity was anti-PR3 in 29 of the patients and anti-MPO in 19 of them. Fifteen of them had active disease and 33 were in remission at the time of examination.

### METHODS

Between August and December 2004, we examined 48 peripheral blood samples of the patients with AAV and 21 peripheral blood samples of age and sex matched healthy controls.

Using flow cytometry, following markers were assessed: T lymphocytes activation markers (HLA-DR+, CD28+, chemokine receptors CXCR3 and CCR5), surface molecules CD4, CD8, CD3, CD19, costimulation molecule CD80 on B lymphocytes, and intracellular cytokines: interferon gamma (IFN $\gamma$ ), tumor necrosis factor alpha (TNF $\alpha$ ), interleukin 2 (IL-2), interleukin 4 (IL-4) in CD3+ T cells, and



interleukin 10 (IL-10) and interleukin 12 (IL-12) in monocytes.

Furthermore, peripheral blood cells were stimulated in incubator (37°C, i.e. 98.6 °F , +5% CO<sub>2</sub>, 4 hours) by mitogens - lipopolysaccharide (LPS) for monocytes and PMA + ionomycin for T lymphocytes - in the presence of transport inhibitor protein Brefeldin A. The cells were fixed, permeabilized and stained by monoclonal antibodies BD Bioscience (anti CD3 PerCP, anti TNFα FITC, anti IL2 PE, anti IFNγ FITC and anti IL-4 PE, anti IL-10 PE, anti IL-12 PE and anti CD14 FITC). For immunophenotyping of costimulatory molecule CD 80 PE on B cells we used CD 19 PerCP, for chemokine receptor CCR5 PE on T helper cells we used CD4 PerCP, and for activation marker HLA DR PE on T cells we used CD3 FITC.

Erythrocytes were lysed by lysing solution (BD Bioscience). The samples were washed and analyzed by flow cytometer FACSCalibur (BD) using three-colour imaging. T lymphocytes were gated using FSC and CD3 PerCP, and the production of TNFα, IL-2 and IFNγ was measured by the CellQuest

program. Monocytes were gated using SSC and CD14 FITC, and the same program measured the production of IL-10 and IL-12.

Statistical significance was calculated using non-parametrical Kruskal-Wallis and Mann-Whitney U tests.

## RESULTS AND DISCUSSION

Results are shown in table 1.

The higher IL-2 and activation markers on T lymphocytes (DR+) and B lymphocytes (CD80+) in the patients when compared with healthy subjects (p = 0.007, p = 0.02, resp.) implies a higher activation of the immune system in AAV patients, which may persist even during remission. On the other hand, the lower IL-10 and IL-12 (p = 0.0003, p = 0.0000001) in the patients is most probably the result of previous or ongoing immunosuppressive treatment. The IL-2 production was significantly lower in MPA when compared with WG (16% vs. 24% positive cells, p<0.05) suggesting less inflammation in MPA.

Patients with AAV had higher levels

Cytokine/surface molecule	All patients (N=48)	WG (N=28)	MPA (N=10)	RLV (N=10)	cANCA+ (N=29)	pANCA+ (N=19)	Active disease (N=15)	Remission (N=33)	Healthy controls (N=16)
IFN	29,09	27,3	35,07	28,81	27	32,17	22,19	32,83	20,29
IL4	2,11	2,18	1,93	2,06	2,36	1,73	2,19	2,06	3,76
TNF	32,41	34,41	28,81	30,28	33,52	30,85	31,34	32,91	25,92
IL2	24,39	28	15,28	23,9	27,29	20,53	30,03	21,87	15,46
IL10	12,73	12,7	13,33	12,05	12,75	12,69	12,46	12,84	8,71
IL12	17,89	16,52	25,72	14,25	15,71	21,03	17,92	17,87	4,82
CXCR3	38,65	37,4	43,06	38,06	36,5	42	32,35	41,5	43,6
CCR5	27,93	24,54	31,75	33	25,42	31,62	23,93	29,93	16,22
D80	8,32	8,25	7,55	9,08	8,52	8,01	9,21	7,87	6,03
CD3	68,75	68,96	68,7	68,2	70,49	65,63	67,8	69,18	70,75
CD3+HLADR+	7,45	6,43	11,93	6,8	6,28	9,33	4,64	8,64	4,1
CD4	39,52	39,89	35,8	42,2	41,17	37	46,2	36,48	47,15
CD8	29,85	29,82	33,5	26,3	30,21	29,32	23	32,97	22,95

Table 1 - Mean values of the main cytokines and surface molecules in AAV patients, in different subgroups according to the disease category and ANCA specificity, and in healthy controls.

of IFN $\gamma$  and CCR5 when compared with healthy controls ( $p = 0.01$ ,  $p = 0.0001$  resp.), which represents a significant shift towards Th1 population. The levels of IFN $\gamma$  in the patients were very high (41.7% positive cells) and correlated with increased numbers of CCR5+ and CXCR3+ cells.

The higher number of CD8 positive cells and lower number of CD4 positive cells indicates that cytotoxic T lymphocytes are involved in the pathogenesis of AAV. The number of DR positive activated T lymphocytes and TNF $\alpha$  production increased with advanced renal failure.

### CONCLUSION

In conclusion, our study supports the view that AAV is a Th1 mediated dis-

ease. The difference in the IL-2 production between WG and MPA patients suggests a different cytokine regulation of immune reaction in these patients. Confirmation of the results on larger series of patients in various disease stages are clearly needed.

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## Current treatment strategies in ANCA-positive renal vasculitis—lessons from European randomized trials

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### Abstract

Antineutrophil cytoplasmic antibody (ANCA)-positive renal vasculitis is the most common cause of rapidly progressive (crescentic) glomerulonephritis. Its life-threatening natural course may be modified substantially by current treatment modalities. The European Vasculitis Study Group (EUVAS) developed a subclassification of ANCA-positive vasculitides based on the disease severity at presentation, and have organized (so far) two waves of clinical trials. The first wave of randomized clinical trials had the aim of optimizing the existing therapeutic regimens; the second wave concentrated on testing some newer therapeutic approaches. Here, the design and available results of the first wave and the design of some second wave trials are reviewed briefly. The potential of the new targeted approaches (e.g. anti-tumour necrosis factor therapy) is also briefly mentioned.

**Keywords:** anti-neutrophil cytoplasmic antibodies; treatment; vasculitis

syndrome and isolated pauci-immune necrotizing/crescenting glomerulonephritis. Based on the subtype of ANCA, we can differentiate between anti-PR3 and anti-MPO diseases [1]. These classifications are clearly overlapping: patients with Wegener's granulomatosis usually have anti-PR3 antibodies, while anti-MPO antibodies are more common in microscopic polyangiitis (micro-PAN). Some renal features are typical of these different categories. Wegener's granulomatosis and anti-PR3 disease may present with more active renal disease (necrosis, epithelial crescents, acute or rapidly progressive renal failure) with a better chance of improving renal function, but with higher risk of relapses. Micro-PAN and anti-MPO disease may be characterized more frequently by a more indolent renal disease with less active and more chronic changes (fibrous crescents, glomerulosclerosis, interstitial fibrosis) in the renal biopsy at presentation and with a lower chance of improving renal function. Micro-PAN is usually associated with lower risk of relapses.

### Classification of ANCA-positive vasculitides based on disease severity

### Introduction

Pauci-immune rapidly progressive (crescentic) glomerulonephritides commonly are accompanied by the presence of antineutrophil cytoplasmic antibodies (ANCA) directed toward several epitopes of azurophilic neutrophil granules. The most common types of ANCA are antibodies directed against proteinase-3 (PR3) and myeloperoxidase (MPO).

ANCA-positive (or ANCA-associated) vasculitides can be subclassified based on different criteria: from a clinical point of view, we can define Wegener's granulomatosis, microscopic polyangiitis, Churg–Strauss

The European Vasculitis Study Group (EUVAS) was created gradually during the first half of the 1990s, starting with the investigation of the diagnostic role of ANCA, followed by the standardization of ANCA testing, histological assessment and the classification of ANCA-positive vasculitis. It was assumed that despite their different clinicopathological characteristics, ANCA-positive vasculitides could be studied together, and a new classification of ANCA-positive vasculitides, based on the severity of the disease, was introduced [2]. Pauci-immune small vessel vasculitides were subclassified as follows: (i) localized vasculitis (with serum creatinine < 120 µmol/l, no constitutional symptoms, no threat to any vital organ function and positive or negative ANCA); (ii) early systemic vasculitis (with serum creatinine < 120 µmol/l and constitutional symptoms, no threat to any vital organ

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function and positive or negative ANCA); (iii) generalized vasculitis (with serum creatinine < 500 µmol/l and constitutional symptoms, dysfunction of any vital organ and positive ANCA); (iv) severe renal vasculitis (with serum creatinine > 500 µmol/l, constitutional symptoms and positive ANCA); and (v) refractory vasculitis (any serum creatinine, constitutional symptoms, threatened function of any vital organ and positive or negative ANCA). Daily oral cyclophosphamide for 1 year in combination with a tapering dose of oral prednisolone was considered as a standard therapeutic regimen [3].

### Lessons from clinical trials organized by EUVAS

In the so-called first wave of randomized clinical trials aimed at optimizing existing therapeutic regimens, the most important studies compared the effect of methotrexate and cyclophosphamide on the remission rate in early systemic vasculitis (NORAM trial), the effect of short-term vs long-term treatment with cyclophosphamide (and early or later switch to azathioprine) on the relapse rate (CYCAZAREM trial) in generalized vasculitis, and finally, the effect of plasma exchange, or pulsed methylprednisolone as add-on therapy on renal survival in severe renal vasculitis (MEPEX trial).

In the CYCAZAREM trial, all patients were treated with oral cyclophosphamide for 3–6 months (until remission) and were then randomized either to switch to azathioprine or to undergo prolonged treatment with cyclophosphamide for 1 year and followed for 18 months. Remission rate (93%) and relapse rate (16% altogether, 19% in patients with Wegener's granulomatosis and 8% in patients with microscopic polyangiitis) were the same in both arms of the trial, with a non-significant trend to more frequent severe adverse events during remission in patients on prolonged treatment with cyclophosphamide [4]. Therefore, in patients with generalized vasculitis (with impaired renal function, but without overt renal failure), the early switch to azathioprine seems to be as safe and comparably effective as prolonged treatment with cyclophosphamide.

In the MEPEX trial, the patients were randomized to adjunctive therapy with either seven plasma exchange treatments (each 60 ml/kg) or three pulses of intravenous methylprednisolone (each 15 mg/kg). The 3 months follow-up data have been published recently [5]. Although the mortality in both trial arms was the same (16%), renal survival was much better in patients treated with plasma exchange. Only 14.8% of patients treated by plasma exchange remained dialysis dependent compared with 36.5% of patients treated by pulsed methylprednisolone. These data confirmed the meta-analysis of several smaller studies [4] and strongly suggest that plasma exchange should be used as an adjunctive treatment in patients with ANCA-positive renal vasculitis with acute renal failure.

The NORAM trial compared treatment either with oral cyclophosphamide (2 mg/kg/day) or with oral methotrexate (15–25 mg weekly) with a similarly tapering dose of prednisolone for 1 year and follow-up of 18 months. According to preliminary data [6], the remission rates were similar in both trial arms (at the end of 6 months, 83% vs 84% for methotrexate and cyclophosphamide, respectively), but the relapse rate was higher (69% vs 42%) in patients treated with methotrexate. The final evaluation should include the analysis of the adverse event rate which is not yet available. A high relapse rate in both arms suggests that even in patients with early systemic vasculitis, immunosuppression should be prolonged beyond 1 year.

Second wave randomized controlled trials were aimed at testing newer approaches. Pulsed cyclophosphamide was compared with oral continuous cyclophosphamide in patients with generalized vasculitis (the primary end-point is the disease-free period; the CYCLOPS trial), nasal mupirocin ointment is compared with placebo in patients with Wegener's granulomatosis in stable remission (the end-point is the relapse rate; the MUIBAC trial) and prolonged treatment with azathioprine and prednisolone (4 years) is compared with shorter treatment (2 years, the end-point is the relapse rate; the REMAIN trial).

Patient recruitment for the CYCLOPS trial is almost finished. Our centre recruited for this trial 28 patients with newly diagnosed, biopsy-proven, ANCA-associated renal vasculitis (generalized vasculitis with serum creatinine < 500 µmol/l). Currently, 18 patients have completed the trial. Seven of them were randomized to the pulse arm (10 pulses of cyclophosphamide 15 mg/kg i.v. during 6 months) and 11 patients were treated with oral cyclophosphamide (2 mg/kg/day for months 0–3 and 1.5 mg/kg/day for months 3–6). Non-fatal adverse events (mostly infections) were frequent in both arms of the trial (45%). The mortality was relatively high and tended to be higher in patients treated with oral cyclophosphamide (1/7 vs 4/11), mostly due to infectious complications. All other patients with the exception of one in the oral arm went into remission, and only one relapse was documented in the pulsed arm. These data are in keeping with the recently published meta-analysis [7] demonstrating a lower cumulative dose of cyclophosphamide (17 g vs 34 g), a higher remission rate (93% vs 77%), a lower infection rate (39% vs 58%), but also a higher relapse rate (42% vs 29%) in patients treated with pulsed (compared with oral continuous) cyclophosphamide. The final results of the CYCLOPS trial should help us to define newer recommendations concerning the role of cyclophosphamide treatment in generalized ANCA-positive vasculitis.

### Newer therapeutic approaches

Newer immunosuppressive drugs currently are being tested in patients with ANCA-positive vasculitis. Based

on a small pilot study with mycophenolate mofetil [8], EUVAS recently launched the IMPROVE trial comparing mycophenolate and azathioprine as a maintenance therapy in patients in remission. Anti-tumour necrosis factor (TNF) strategies (anti-TNF antibody infliximab and humanized soluble TNF receptor etanercept) hopefully may also improve further the outcome and the quality of life in patients with ANCA-positive vasculitis (Wegener's granulomatosis [9]).

Based on the experience from these European randomized trials, the treatment of ANCA-positive vasculitides should be tailored according to the disease severity at presentation. Current therapy with cyclophosphamide is quite effective, but has relatively high short- and long-term toxicity. The search for therapeutic modalities which are at least as effective but less toxic is therefore warranted.

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## Daily oral versus pulse intravenous cyclophosphamide in the therapy of ANCA-associated vasculitis – preliminary single center experience

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**Abstract:** The aim of the multicentric randomized trial CYCLOPS is to optimize the treatment of induction of remission in patients with generalized, but not immediately life-threatening ANCA (antineutrophil cytoplasmic antibodies) -associated vasculitis. This will be achieved by reducing the dose of cyclophosphamide by administering it as intermittent pulses. The lower cumulative dose will be very probably accompanied with lower toxicity, whereas the effectivity should be comparable. We have enrolled 28 patients to the study. At present, 18 of them are suitable for evaluation. Our preliminary results show that pulse intermittent administration of cyclophosphamide is safer from the point of morbidity and mortality due to infectious complications. In our hands, this treatment modality does not seem to be less effective than the conventional daily oral cyclophosphamide. However, unambiguous results and treatment recommendations will not be available until the final evaluation of all patients enrolled in the trial.

**Key words:** ANCA – Wegener's granulomatosis – Microscopic polyangiitis – Vasculitis – Cyclophosphamide

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### Introduction

Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) are systemic autoimmune diseases characterized by pauciimmune necrotizing vasculitis that involves mainly small vessels. They are strongly associated with ANCA (antineutrophil cytoplasmic antibodies). ANCAs are circulating autoantibodies directed against different target antigens of azurophilic granules of polymorphonuclear leucocytes. In WG they are usually directed against proteinase 3 (antiPR3) and have a cytoplasmic type of immunofluorescence (c-ANCA). In MPA the target antigen is myeloperoxidase (MPO) with the perinuclear type of immunofluorescence (p-ANCA). Renal-limited vasculitis (RLV), or idiopathic pauciimmune rapidly progressive glomerulonephritis is an early form that does not involve any other organ but kidney. It is more often antiMPO positive. All these three entities, together with less frequent Churg-Strauss syndrome (CSS), are ranked among ANCA-associated vasculitides. At present, it is unknown whether there is any difference between them in prognosis or response to therapy [1]. Their incidence is not as low as it was originally believed, most probably due to the higher awareness of the disease and the possibility of routine ANCA testing. ANCA-associated vasculitis represents the end-stage renal disease in about 5 % [2].

Untreated, generalized WG and MPA follow a progressive course with a fatal outcome due to vital organ failure. The average survival of untreated patients was 5 months. In the 1970s Fauci and Wolff introduced empirical therapeutic scheme of daily oral cyclophosphamide (CYC) and corticosteroids (prednisolone 1mg/kg/day on a tapered-off basis) for one year after remission achievement. This "Fauci-scheme" dramatically improved the prognosis of the patients. Remission was induced in 80–100 % of cases. However, the toxicity of the regimen caused considerable morbidity and mortality. Moreover, in a long-term follow-up it turned out that at least 50 % of the patients relapse even under continuing immunosuppression or when the therapy is tapered [3]. The aim of the European Vasculitis Study Group (EUVAS) is to improve the clinical, histological and serological diagnostics of vasculitides and to optimize their treatment based on the severity of the disease at presentation. Therefore, EUVAS has designed and conducted several randomized trials and continues to do so. Our center closely cooperates with EUVAS and repeatedly contributes by a substantial number of patients [4].

The aim of the international randomized trial CYCLOPS is to optimize the treatment of induction of remission in patients with generalized, but not immediately life-threatening ANCA-associated vasculitis. There is the intent to reduce the toxicity of induction therapy. This will be achieved by reducing the overall dose of CYC during the induction period by using it in an intermittent pulsed form. There is uniform international consensus that a regimen comprising

pulsed CYC will be associated with less toxicity compared to the current, gold standard, continuous oral CYC regimen. However, there are concerns that lower toxicity may be at the expense of reduced efficacy [5,6].

Consequently, the induction treatment consisted of either continuous oral or pulsed intravenous CYC together with corticosteroids (CS). The maintenance therapy, azathioprine for 12 months, was the same for both limbs. The primary end-point was the rate of remission at 9 months and relapse rate until the 18th month – the disease-free period. Secondary end-points were adverse events of the therapy, cumulative dose of CYC and CS and cumulative damage to the organs affected by vasculitis. Patients with newly diagnosed WG, MPA or RLV with renal involvement without life-threatening manifestation were included into the study. Renal involvement was defined as biopsy proven necrotizing glomerulonephritis and/or microhematuria and proteinuria with serum creatinin 150–500 mmol/L. The inclusion criteria further comprised ANCA positivity and the age limit was 18–80 years. Following patients were a priori excluded: those who had already received immunosuppressive treatment, anti-GBM positive, patients with other autoimmune diseases, pregnant women, women without adequate contraception, patients with known malignancy, HbsAg, antiHCV or HIV positive.

### Results

The CYCLOPS trial was designed for 160 patients. Between December 1999 and November 2001 our center included a total of 28 patients. Eighteen of them (12 women, 6 men) are to date evaluated. The mean age at presentation was 54.6 years (18–72). Seven patients (5 women, 2 men, mean age 57.9 years) were randomized to the pulse limb (CYC 15 mg/kg body weight in every 2 weeks the 1<sup>st</sup> month, every 3 weeks until the end of the 6th month). Eleven patients (7 women, 4 men, mean age 52.5 years) were randomized to the oral limb (CYC 2 mg/kg body weight/day month 0–3, 1,5 mg/kg body weight/day month 3–6). The proportion of diagnosis was as follows: in pulse limb: 4×WG, 2×MPA, 1×RLV, in oral limb: 4×WG, 4×MPA, 2×RLV, 1×CSS. All patients had biopsy proven renal involvement and all were ANCA positive. The cumulative dose of CYC in the pulse limb was approximately 150 mg/kg body weight/6 months. In the oral limb the cumulative dose of CYC was more than two-fold, 315 mg/kg body weight/6 months.

All patients in the pulse limb (100 %) achieved remission, in contrary to only 55 % of patients in the oral limb.

During the whole period studied, there was only one relapse in the pulse limb that occurred 5 months after the cessation of immunosuppressive treatment (i.e. 23 months after the enrollment). The patient was treated again with CYC and CS. After remission achievement she was put on mycophenolate mophetil, which is now gradually tapered.

The number of infectious complications of immunosuppressive treatment was comparable in both limbs (pulse 43 % vs. oral 45 %). However, there were only 14 % of serious events (i.e. those requiring hospitalization) in the pulse limb. One patient had leucopenia ( $1,7 \cdot 10^9/L$ ) caused by CMV infection and he was successfully treated with ganciclovir. In the oral limb, 27 % of infectious complications were severe and unfortunately resulted in death of the patients. All these three patients died during the induction treatment even though CYC was always stopped as soon as leucopenia and infection were ascertained. The first patient was a 56-year-old woman that died of septic shock despite intensive treatment with antibiotics, antiviral and antimycotic agents. Her leucocytes were constantly above  $1 \cdot 10^9/L$ . The necropsy revealed pulmonary aspergillosis. The second, 60-year-old patient died despite intensive care including growth factors because of transient leucopenia (nadir leucocytes  $0,6 \cdot 10^9/l$ ) of respiratory failure due to bilateral pneumonia. She had extensive lung interstitial disease due to WG. The third patient died of febrile neutropenia in another hospital.

The overall mortality was also higher in the oral limb (36 %) compared to the pulse limb (14 %). Apart from three patients from the oral limb that died of infectious complications there was one more death in this group that was not related to the diagnosis or therapy. This patient died in the local hospital due to bleeding caused by cumarine overdose. In the pulse limb, only one death was recorded. This patient died of pulmonary embolism while on maintenance dose of CS (prednisolone 10mg/day). We cannot confidentially exclude that the therapy did not contribute to the fatal complication.

### Conclusion

The final results of the multicentric randomized trial CYCLOPS should be published in the second half of 2004 and it will answer the question whether the intermittent pulse application of CYC in the treatment of generalized, but not immediately life-threatening ANCA-associated vasculitides is comparably effective as continuous oral administration. The cumulative dose of CYC in the oral limb of this study is more than two-fold compared to the pulse limb. Therefore, it is practically certain that the toxicity of the pulse regimen will be lower. This benefit might even outweigh possibly slightly lower efficacy of the pulse regimen.

Our preliminary results of a small group of patients confirm the higher toxicity of oral CYC that resulted in higher morbidity and mortality in this group. Surprisingly, in our hands, the efficacy of pulse CYC seemed to be better. This was certainly due to the small number of patients and the high mortality in the oral limb. There was no early relapse in the followed-up period (18 months after enrollment). In the pulse limb, one patient relapsed later on.

However, these are only preliminary findings. Unequivocal results and therapeutic guidelines will not be available until the complete evaluation of all patients enrolled to the CYCLOPS trial.

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# Long-Term Outcome of Patients with Antineutrophil Cytoplasmic Autoantibody-Associated Vasculitis with Renal Involvement

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## Key Words

Antineutrophil cytoplasmic autoantibody ·  
Microscopic polyangiitis · Vasculitis, outcome ·  
Wegener's granulomatosis

## Abstract

**Background:** Despite treatment, renal involvement in antineutrophil cytoplasmic autoantibody (ANCA)-positive vasculitis is still associated with significant long-term mortality and remains an important cause of end-stage renal failure. **Methods:** We retrospectively analyzed a series of 61 consecutive patients with newly diagnosed ANCA-associated renal vasculitis (54.1% Wegener's granulomatosis, 23% renal-limited vasculitis, 16.4% microscopic polyangiitis, 4.9% Churg-Strauss syndrome) diagnosed between 1986 and 1997. **Results:** The median creatinine level at diagnosis was 221.5 (63–762)  $\mu\text{mol/l}$ , i.e. 2.5 (0.7–8.6) mg/dl, 32.8% were dialysis-dependent. All patients were treated with cyclophosphamide. Remission was achieved in 87% of patients. Relapses occurred in 44.7%. The median renal disease-free interval was 62.5 (0–138) months. The estimated patient survival at 5 and 10 years was 78.3 and 62.2%, respectively. Mor-

tality was associated with age ( $p = 0.04$  when age limit 50 years) and advanced renal failure ( $p = 0.038$  when compared dialysis-dependent and independent patients). Estimated renal survival time at 5 and 10 years was 69.2 and 55.8%, respectively. At the end of follow-up, 50.8% of patients were in complete remission, 31% had died. The median serum creatinine level was 137.5 (77–469)  $\mu\text{mol/l}$ , i.e. 1.56 (0.87–5.3) mg/dl, 24.6% of patients were on regular dialysis treatment. **Conclusion:** Patient survival, relapse rate and mortality were comparable to similar reports. In view of the severity of the renal disease and the length of follow-up, renal survival was very good. Despite effective treatment, the long-term outcome of patients with ANCA-associated renal vasculitis remains unsatisfactory.

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## Introduction

Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) and Churg-Strauss syndrome (CSS) are small vessel vasculitides with frequent renal involvement characterized by necrotizing/crescentic pauci-immune glo-

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merulonephritis and a strong association with antineutrophil cytoplasmic autoantibodies (ANCA) directed against proteinase-3 (anti-PR3, C-ANCA) or myeloperoxidase (anti-MPO, P-ANCA). Isolated ANCA-positive necrotizing/crescentic pauci-immune glomerulonephritis, also called renal limited vasculitis (RLV) is considered a 'forme fruste' of either WG or MPA. It is more often anti-MPO-positive.

The annual incidence of renal vasculitis in Europe may exceed 20 per million per year and is probably different in the northern and southern parts of the continent. It represents the cause of renal failure in 5% of patients with end-stage renal disease [1]. Currently, it is not clear whether there is any difference between WG, MPA, RLV with regard to the response to treatment [2].

If untreated, ANCA-associated vasculitides have a poor outcome, e.g. the 1-year mortality of untreated WG was 80% [3]. The introduction of cyclophosphamide (CYC) into combined immunosuppression with corticosteroids (CSs) has dramatically improved the prognosis of these patients. Mortality has significantly decreased from 82% in 1 year to 59–95% in various patients groups during various follow-up periods, and remission rates have increased to up to 93% [4, 5]. Although effective treatment has substantially decreased 1-year mortality, it has also changed the course of the disease to that of a chronic relapsing disorder with cumulative morbidity and mortality related not only to disease scars due to the relatively high relapse rate, but also to treatment-related toxicity [5].

In this retrospective analysis, we studied the presenting features, response to therapy and the overall and renal survival in patients with ANCA-associated vasculitis (AAV) with renal involvement who were diagnosed and followed in our center between 1986 and 1997. Special attention was paid to the impact of age, diagnostic subgroups, level of renal function and type of ANCA on the outcome of the patients.

## Patients and Methods

Between January 1986 and December 1997, 61 patients were newly diagnosed with AAV with the evidence of renal involvement, determined mostly by renal biopsy showing necrotizing and/or crescentic pauci-immune glomerulonephritis (87%) and/or by deterioration of renal function with active urinary sediment. The patients were reclassified to 4 diagnostic subgroups according to the Chapel Hill disease definitions [6] with following results: 54.1% had WG, 23% RLV, 16.4% MPA and 4.9% CSS.

## Treatment

All patients received homogenous induction treatment according to the local treatment guidelines in the respective years. It consisted of oral continuous CYC and CS. The initial dose of CYC was 2 mg/kg body weight, the median dose of CYC was 15 (range 1–70) g and the median time of CYC administration 8.5 (range 1–25) months. A 25% reduction in CYC dose was made for those aged >65 years and for a glomerular filtration rate of <50 ml/min. The maximal oral prednisone dose was 60 mg/day, in most of the patients (92%) it was preceded by intravenous pulse methylprednisolone for 3 consecutive days. Plasma exchange was added to the treatment regimen in patients treated within the MEPEX trial. This multi-center randomized study compared adjunctive therapy with either 7 plasma exchange treatments or 3 pulses of intravenous methylprednisolone with regard to the overall and renal survival [7]. Except from patients recruited for the MEPEX trial, plasma exchange was used in all patients presenting with dialysis-dependent renal failure and most patients with life-threatening pulmonary involvement – alveolar hemorrhage (especially those with high titer of ANCA). In all, plasma exchange was used in 29.3% of patients. In 45.2% of patients CYC was switched to azathioprine (AZA) after reaching stable remission.

## Definitions

Remission was defined as the absence of clinical signs or symptoms or laboratory evidence of vasculitis activity and stabilization or improvement in renal function in combination with the absence of erythrocyturia. Relapse was defined as the reappearance of clinical signs or symptoms or laboratory evidence of disease activity sufficient to increase or introduce immunosuppressive treatment (intention to treat).

## Adverse Effects

Because adverse effects are likely to be underreported in a retrospective study, only the occurrence of infections requiring hospital admission, gastrointestinal bleeding, hepatopathy, and malignancy were recorded in this study.

## Statistical Analysis

Kaplan-Meier life survival analysis was used to assess patient survival, the log-rank test to compare survival for different groups of patients and the Cox proportional hazard model to test the impact of different variables on survival. To evaluate categorical data we used  $\chi^2$  test for independence in contingency tables and in the cases of repeated observation we used the McNemar test of symmetry in contingency tables. *p* values of <0.05 were considered significant. Data were analyzed on a personal computer using statistical package SPSS v10.

## Results

### Patient Classification

The median age was 54 (18–81) years, and 60.7% were men. Patients with RLV and MPA (median age 67 (27–81) and 65 (36–72) years, respectively) were significantly older than patients with WG (median age 49 (18–75)

**Table 1.** Organ involvement (%) in all patients and in the respective subgroups (WG, MPA, CSS) and in C-ANCA (PR3)- and P-ANCA (MPO)-associated disease

	Total	WG	MPA	CSS	PR3	MPO
Kidney	100	100	100	100	100	100
Lungs	45.9	63.6	50	66.7	48.6	26.9
Arthralgias	41	54.5	50	66.7	54.3	23
ENT	34.4	54.5	20	33.3	45.7	19.2
Skin	18.0	24.2	20	33.3	22.6	11.5
PNS	14.8	18.2	20	33.3	17.1	11.5
CNS	13.1	15.2	20	33.3	14.3	11.5
Eye	14.8	24.2	10	0	17.1	11.5
Heart	3.3	3	0	33.3	2.9	3.8
GIT	3.3	3	10	0	2.9	3.8

ENT = Ear, nose, throat; PNS = peripheral nervous system; CNS = central nervous system; GIT = gastrointestinal tract.

years;  $p = 0.008$  and  $0.085$ , respectively). No difference in age was found between the C-ANCA and P-ANCA subgroups. There were no differences in sex distribution between the diagnostic subgroups or C-ANCA/P-ANCA subgroups. The median follow-up was 90.5 (range 1–168) months.

#### Clinical Data

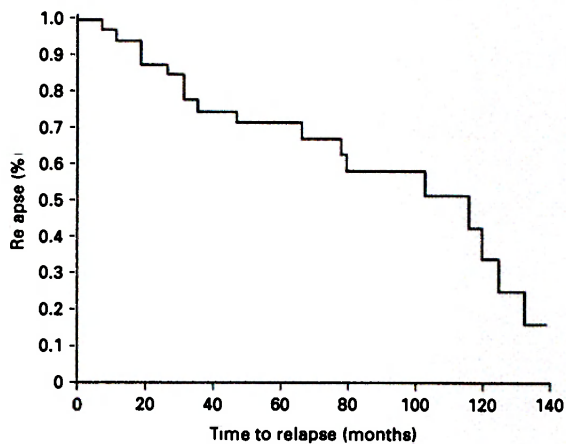
All patients were tested for ANCA by immunofluorescence (since 2000 both by immunofluorescence and ELISA). ANCA was detected in 85.2% (C-ANCA in 57.4%, P-ANCA in 42.6%). The median CRP was 30 (0–192) mg/l, the median hemoglobin 88.5 (57–164) g/l. Organ involvement at presentation is summarized in table 1. By definition, all patients had renal involvement, which was biopsy-proven in 87%. The median serum creatinine level of patients dialysis-independent at diagnosis was 221.5 (63–762)  $\mu\text{mol/l}$ , i.e. 2.5 (0.7–8.6) mg/dl, and 32.8% were dialysis-dependent. The median percentage of crescents in the renal biopsy was 50 (0–100) and the median percentage of sclerosed glomeruli was 20 (0–90). Microscopic hematuria was present in all patients, and the median proteinuria was 1.54 (0.15–10.92) g/24 h. Initial renal function did not differ according to sex, age or C-ANCA/P-ANCA. The highest percentage of initially dialyzed patients was in the RLV group (50%), whereas for WG it was 34.4% and for MPA only 10%. However, the differences did not reach statistical significance ( $p = 0.098$  for RLV versus MPA).

#### Remission

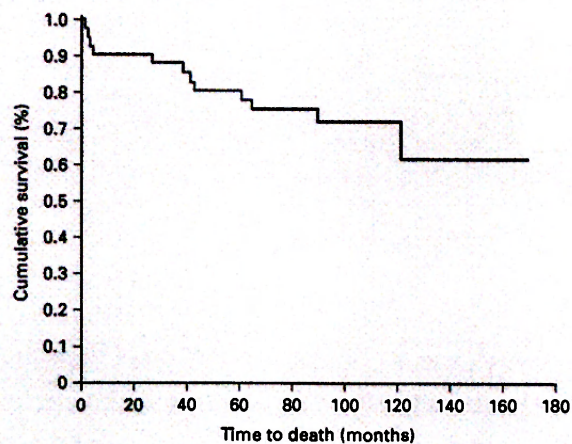
Remission was achieved in 87% of the patients, more often in patients with independent renal function at presentation (in 92.7%) than in the subgroup of patients initially dialyzed (75%, in 60% of them with independent renal function at the time of remission). All patients who did not achieve remission died. The median time to remission was 3 (1–10) months. The median serum creatinine at remission was 124.5 (76–400)  $\mu\text{mol/l}$ , i.e. 1.4 (0.86–4.52) mg/dl, 6.6% of patients in remission had to be dialyzed. The median CRP was 3 (0–52) mg/l, the median hemoglobin 104 (81–141) g/l, microscopic hematuria was not present, the median proteinuria was 0.8 (0–12.7) g/24 h. ANCA was detected in 3.3%.

#### Relapses

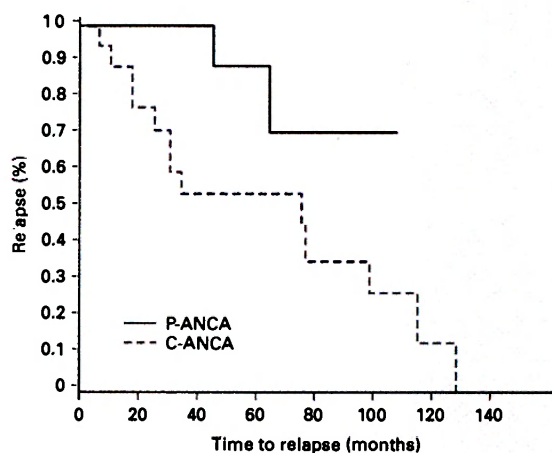
Relapses occurred in 44.7%. The median disease-free interval was 58 (0–138) months, the median renal disease-free interval was 62.5 (0–138) months (fig. 1). Relapses mostly occurred when the patients were no longer on immunosuppressive treatment (in 81.5%), in 4 patients on AZA (14.8%), and 1 patient on CYC (3.7%). Virtually all the relapses were associated with ANCA positivity. Only 2 patients remained ANCA-positive in remission, and they did not experience any relapse. The relapse rate was higher in C-ANCA-associated disease when compared to P-ANCA, although the difference was of borderline significance ( $p = 0.059$ ; fig. 2). No difference in relapse rate was seen between dialysis-dependent and independent patients at presentation.



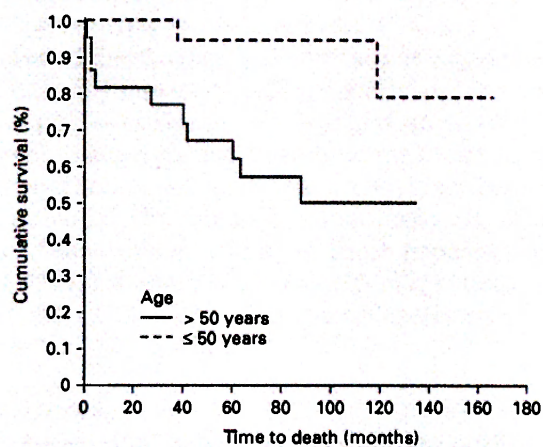
**Fig. 1.** Survival without relapse in patients with ANCA-associated vasculitis.



**Fig. 3.** Cumulative patient survival.



**Fig. 2.** Survival without relapse in patients with ANCA-associated vasculitis and the type of ANCA at presentation ( $p = 0.059$ ).



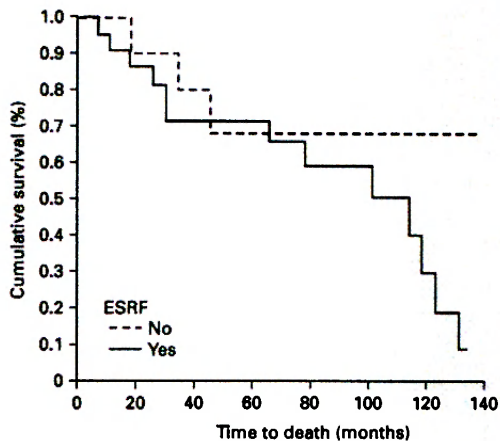
**Fig. 4.** Cumulative patient survival and age ( $p = 0.04$ ).

#### Overall Survival

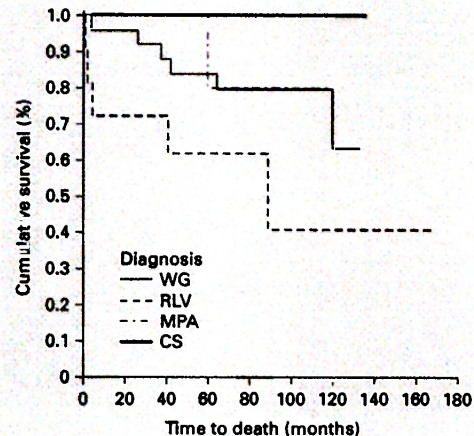
For the group of 61 patients, the estimated patient survival at 5 and 10 years was 78.3 and 62.2%, respectively (fig. 3). The estimated survival was significantly shorter in patients >60 years at diagnosis when compared to those <60 years ( $p = 0.029$ ). Similar results were shown when

the age limit was 50 years ( $p = 0.04$ ; fig. 4). The estimated survival was not dependent on sex, initial proteinuria, C-reactive protein or hemoglobin level. Patients who had to be initially hemodialyzed had significantly worse estimated survival compared to patients who were dialysis-independent ( $p = 0.038$ ; fig. 5). When we compared patients

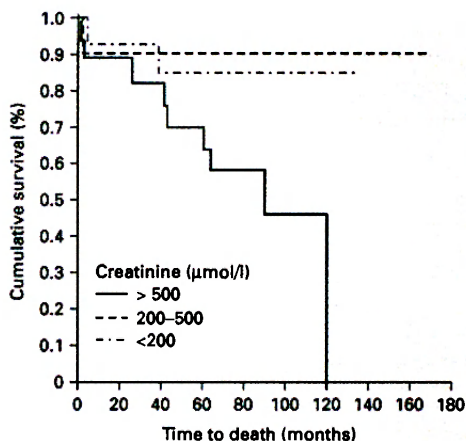




**Fig. 5.** Cumulative patient survival and dialysis dependence at presentation ( $p = 0.038$ ).



**Fig. 7.** Cumulative patient survival based on clinical diagnosis.



**Fig. 6.** Cumulative patient survival and presenting serum creatinine levels ( $p = 0.03$  and  $0.02$ , respectively). To convert  $\mu\text{mol/l}$  to  $\text{mg/dl}$ , divide by 88.4.

with serum creatinine levels of  $<200 \mu\text{mol/l}$  ( $2.26 \text{ mg/dl}$ ),  $200\text{--}500 \mu\text{mol/l}$  ( $2.26\text{--}5.66 \text{ mg/dl}$ ) and  $>500 \mu\text{mol/l}$  ( $5.66 \text{ mg/dl}$ ), we found a statistically significant difference only between the first 2 subgroups and the 3rd one ( $p = 0.03$ ,  $p = 0.02$  respectively; fig. 6). There was no

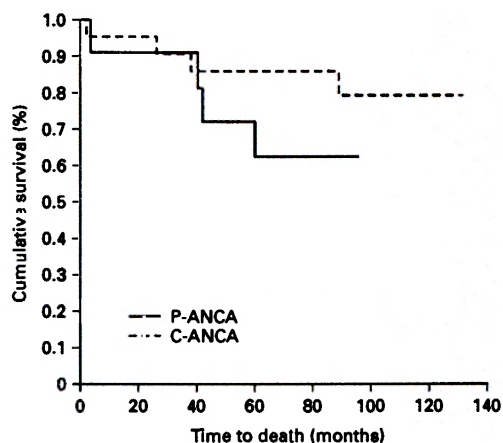
significant difference in survival between the 4 diagnostic subgroups (fig. 7) or between C-ANCA- and P-ANCA-associated disease (fig. 8). Pulmonary involvement did not have any impact on estimated survival, nor did the use of plasma exchange in the initial treatment (data not shown). Using data calculated from the Czech National Statistics Series, death rates were analyzed according to age (table 2). The standardized mortality ratio (SMR) was 2.6486 compared with the Czech population (95% CI 1.4575; 3.8396). The relative risk of age equals 8.038 (95% CI 1.614–40.031) and renal insufficiency 3.710 (95% CI 1.124–12.250).

#### Mortality

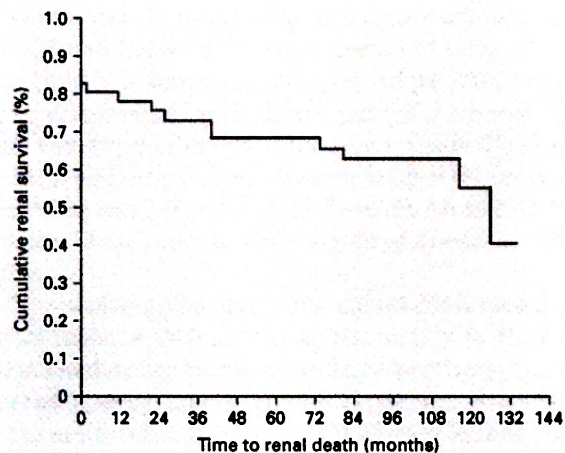
Nineteen of 61 patients (31%) died. The median time to death was 41.3 (1–120) months. Six patients died of infectious complications related to immunosuppressive treatment; 7 patients due to cardiovascular events; 2 of exsanguination from a gastric ulcer; 2 of cancer; 1 of multi-organ failure, and 1 cause of death is unknown (table 3). No patient died of active vasculitis. Nine patients presented with lung hemorrhage and none of these patients died of this condition.

#### Renal Survival

The estimated renal survival at 5 and 10 years was 69.2 and 55.8%, respectively (fig. 9). It did not differ according to sex, initial proteinuria, C-ANCA- or P-ANCA-associ-



**Fig. 8.** Cumulative patient survival and the type of ANCA.



**Fig. 9.** Cumulative renal survival.

**Table 2.** Relationship between age at presentation and death rate using the Czech national statistics series

Age group years	Annual death rate/1,000 population	Total patient years at risk	Patient deaths	Patient annual death rate/1,000	95% CI	Expected annual death rate/1,000	Ratio real/expected deaths
15-24	0.7235	10	0	0.0000	0; 308.5	0.0072	0.00
25-34	1.5023	44	0	0.0000	0; 80.4	0.0661	0.00
35-44	3.6090	44	1	22.7273	0.6; 120	0.1588	6.30
45-54	8.6104	99	2	20.2020	2.46; 71.1	0.8524	2.35
55-64	23.1390	58	5	86.2069	28.6; 189.8	1.3421	3.73
65-74	44.8954	80	5	62.5000	20.6; 139.9	3.5916	1.39
75-84	97.2186	8	5	625.0000	244.9; 914.8	0.7777	6.43

Calculated standardized mortality ratio 2.6486, 95% CI (1.4575; 3.8396).

ated disease or between the 4 diagnostic subgroups. However, the difference between patients <50 years and >50 years of age at presentation was of borderline significance ( $p = 0.097$ ). We did not find any statistically significant difference between the number of dialysis-dependent patients at diagnosis and the end of follow-up. Four of the 61 patients who were initially dialyzed now have independent renal function. On the other hand, 6 of those who had independent renal function at presentation are currently on chronic hemodialysis treatment.

**Table 3.** Causes of death (%)

Infection	31.57
Cardiovascular	36.84
Gastric ulcer	10.52
Multi-organ failure	5.26
Unknown	5.26
Active vasculitis	0.0



**Table 4.** Comparison of the laboratory data at diagnosis, at remission and at the end of follow-up

	At diagnosis	At remission	At the end of follow-up
ANCA positivity, %	85.2	3.3	31.1
Creatinine, $\mu\text{mol/l}$	221.5	124.5	137.5
Dialysis, %	32.8	6.6	24.6
Proteinuria, g/day	1.54	0.8	0.23
Microhematuria, %	100	0	4.9
CRP, mg/l	30	3	7
Hemoglobin, g/l	88.5	104	123.5

#### *Adverse Effects*

Twenty-five of 61 patients (41%) suffered from the adverse effects of therapy. Severe bacterial infection requiring hospital admission occurred in 16 patients (26%). Severe viral infection requiring hospital admission occurred in 5 patients (8%; 2 varicella zoster virus and 3 cytomegalovirus disease). Four patients experienced gastrointestinal bleeding, while in 1 patient CYC was believed to cause transient hepatopathy. Solid tumors occurred in 3 patients. However, only in 1 of them (lung cancer 5 years after the diagnosis of WG) was it possibly secondary to the immunosuppression. In the remaining 2 the diagnosis of malignancy was made either with or soon after the diagnosis of AAV.

#### *Clinical and Laboratory Data at the End of Follow-Up*

At the end of the follow-up, 50.8% of patients were in remission, 31% have died and 3.3% have active disease. The outcome of 14.9% is not known. The median serum creatinine is 137.5 (77–469)  $\mu\text{mol/l}$ , i.e. 1.56 (0.87–5.3) mg/dl, and 24.6% of patients are on dialysis. The median CRP is 7 (0–263) mg/l, median hemoglobin 123.5 (84–155) g/l, median proteinuria 0.23 (0–2) g/24 h, and 4.9% of patients have microscopic hematuria. ANCA is present in 31.1%. Laboratory data at presentation, at remission and at the end of follow-up are shown in table 4.

#### **Discussion**

During the last two decades many studies have reported outcome data on AAV. Our retrospective study is interesting from several points of view. It comprises a rela-

tively high number of patients from one center treated in principle with uniform induction treatment with CYC and CS and followed for a long period of time. The median time of follow-up is 7.5 years. All patients had relatively severe renal involvement, most of them were biopsied. One third of the patients were initially dialysis-dependent and the median creatinine level of the remaining two thirds was 221.5  $\mu\text{mol/l}$  (2.5 mg/dl). More than three quarters of the patients had generalized disease at presentation.

The demographic data in our cohort confirmed that at presentation with renal vasculitis, mainly in RLV and MPA (median age 67 and 65 years, respectively), patients were of older age compared to WG patients, possibly due to the more indolent course of the disease leading also to the higher serum creatinine level at presentation in the RLV group [5, 8–10]. In line with previous reports, there was no significant sex difference, although men slightly predominated. Organ involvement and ANCA analysis corresponded well to the findings of other investigators [5]. ANCA negativity (14.8%) was probably caused mostly by low sensitivity of immunofluorescence testing in the early 1990s, a fact supported by a detailed analysis showing that most of these patients were observed in the earliest studies. In some of these patients ANCA became positive during the follow-up.

AAV is a life-threatening disease that requires prompt recognition and therapy. Prognosis is an especially important issue as the disease process is aggressive and the therapeutic options are inherently dangerous. Different authors have reported the following survival periods in their cohorts of patients: Booth et al. [5] 84% at 1 year and 76% at 5 years; Slot et al. [11] 73% at 5 years and 62% at 10 years; Little et al. [12] 85.5% at 1 year and 63% at 5 years, and Aasarod et al. [13] 88% at 2 years and 74% at 5 years.

The cumulative 5- and 10-year patient survival in our cohort was 78.3 and 62.2%, respectively, which is comparable to the above-mentioned reports. Mortality was associated with age over 50 and advanced renal failure at presentation. No patient died of active vasculitis. The causes of early mortality were related to the adverse effects and toxicity of the treatment (infections, gastrointestinal bleeding). Infections remained the main cause of morbidity as well. Late mortality was mainly due to cardiovascular events. The entry age, the serum creatinine level and dialysis-dependence at presentation are well known risk factors [5, 9, 11, 12]. Some authors described the presence of pulmonary hemorrhage as a risk factor for death [10]; however, this was not confirmed in our study,

nor by others [11]. Some authors [10, 14] also reported higher mortality in C-ANCA-associated disease and WG, whereas we and many others did not confirm this finding [5, 9, 12]. Male gender was sometimes [11] associated with increased mortality, again not confirmed by our study or others [5]. Some authors report that low-intensity immunosuppression is associated with a worse outcome [10, 12], whereas others stress the treatment-associated leukopenia and ensuing sepsis as an independent risk factor for death [5], which indicates the need for more effective and better targeted therapy. Overall, the morbidity and mortality results from several factors. In the early phase of the disease it is associated with irreversible organ dysfunction due to inflammatory injury (within days), further on with aggressive immunosuppressive therapy and its short-term adverse effects, namely infections (within months), and long-term sequelae, such as secondary tumors, myelodysplastic syndrome, accelerated atherosclerosis, etc. [9, 11].

In view of the severity of the renal disease at presentation and the length of follow-up, the renal survival in our study (69.2% at 5 years and 55.8% at 10 years) was very satisfactory. At presentation, one third of the patients were dialysis-dependent, some of the patients have recovered renal function, so that at remission only 6.6% remained dialysis-dependent. At the end of follow-up three quarters of the living patients still have independent renal function. The reported renal survival in patients with AAV with renal involvement differs according to the severity of renal disease at presentation from 65% at 5 years and 51% at 10 years [11] to 44% at 48 months [10]. We found no difference in renal survival between C-ANCA- and P-ANCA-associated disease or between the 4 diagnostic subgroups, whereas other authors [14] have recent-

ly brought suggestive data about a more aggressive disease course in C-ANCA-associated disease. Large prospective studies will be needed to responsibly address this important issue.

AAV is a relapsing disease. In this study, a relapse rate of 44.7% was noted with the median renal disease-free interval of more than 5 years; the vast majority of patients were no longer on immunosuppressive treatment at the time of relapse. There was no difference in the occurrence of relapses between the patients treated with CYC only and those switched to AZA. Renal relapses probably have a major impact on the loss of independent renal function in the course of the disease [11, 15, 16]. However, some of our patients progressed to end-stage renal failure without experiencing any relapse. In these cases, the non-immunologic progression of renal disease was probably involved, which highlights the importance of optimal conservative care, in particular careful blood pressure control, preferentially using ACE inhibitors, or angiotensin-II antagonists. Consistent with previous reports [5, 8, 17–19] we have noticed an increased relapse rate in C-ANCA-associated disease, which should probably be taken into consideration, especially in the length of maintenance treatment.

In conclusion, our data confirm that despite effective induction treatment, the long-term outcome of patients with ANCA-associated renal vasculitis remains unsatisfactory with a relatively high overall mortality (with standardized mortality ratio compared to the common population of about 2.5) and dialysis dependence due to a high relapse rate and toxicity of current treatment. Search for newer, more effective and less toxic modes of treatment is therefore warranted.

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