# 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 ethiopathogenesis 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 cryglobulinemic 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 Concensus 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 immunofluorescencce from that caused by antinuclear antibodies (ANA).

Individuals with ANA frequently have false-positive results on ANCA testing by immunofluorescence. The use of both formol- 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 a-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,

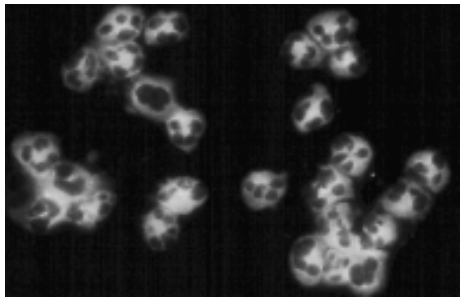

*Fig. 1 – C-ANCA pattern - cytoplasmic type of immunofluorescence.*

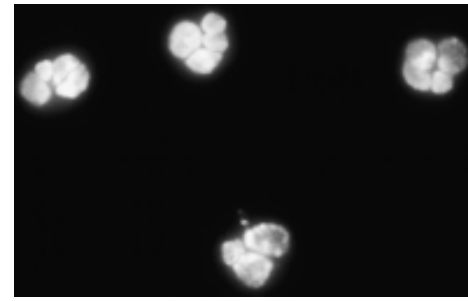

*Fig. 2 – P-ANCA pattern – perinuclear type of immunofluorescence.*

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 cholangiitis, 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%) then 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].

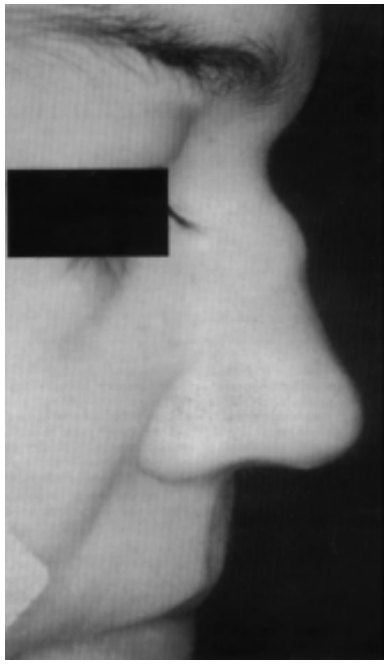

*Fig. 3 – A typical saddle nose deformity in a patient with Wegener's Granulomatosis.*

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

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

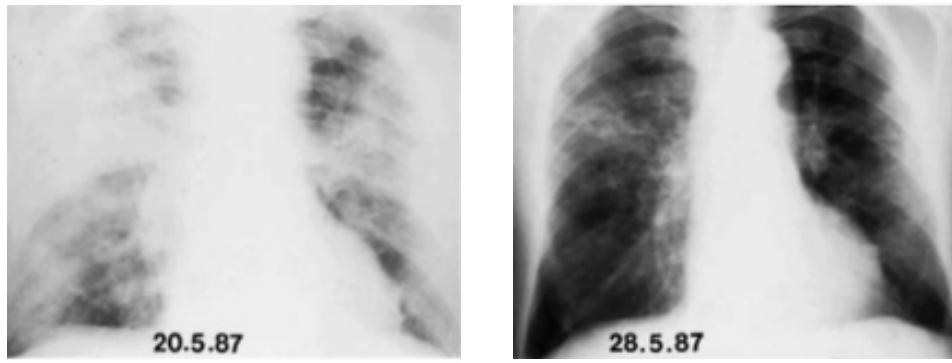


*Fig. 4 – Chest x-ray – alveolar shadowing caused by pulmonary hemorrhage in a patient with microscopic polyangiitis, improved after immunuosuppression and plasma exchange therapy.*

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

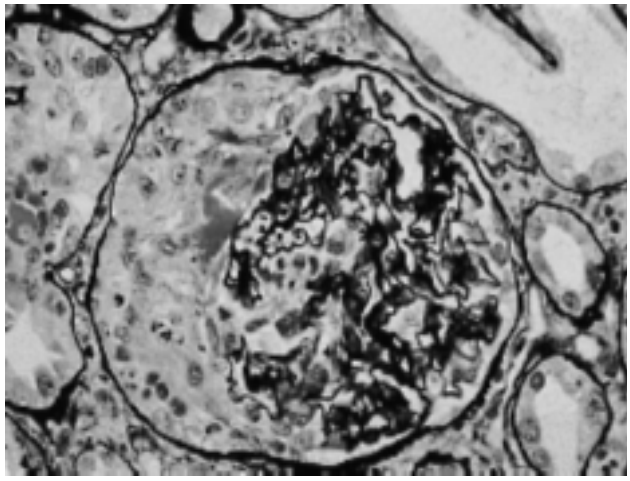

*Fig. 5 – Kidney biopsy – cellular crescent in a glomerulus from a patient with Wegener's Granulomatosis.*

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 intracellulary 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 FcgRII/FcgRIII [42]. Mutations in the gene encoding a-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 polymorhisms 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 TNFa 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-/-) [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 dates 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 methyprednisolone (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 causes 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 particular 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-sufamethoxazole 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, reinstitution 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 grumbling 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, TGFa 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 immunoadsorbtion [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 immunosupression, 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 ethiopathogenesis 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.

## References


1. HUNDER G. G., AREND W. P., BLOCH D. A., ET AL.: The American College of Rheumatology 1990 criteria for the classification of vasculitis. *Arthritis Rheum.* 33: p. 1065–1073, 1990.
2. JENNETTE J. C., FALK R. J., ANDRASSY K., ET AL.: Nomenclature of systemic vasculitides – Proposal of an international concensus conference. *Arthritis Rheum.* 37: p.187–192, 1994.
3. KALLENBERG C. G. M., COHEN TERVAERT J. W.: Renal manifestations. In: Ball GV, Bridges jr. L. [eds] Vasculitis. Oxford University Press, 2002, p. 139–149.
4. KALLENBERG C. G. M., NROUWER E., WEENING J. J., ET AL.: Anti-neutrophil cytoplasmic antibodies: current diagnostic and pathophysiological potential. *Kidney Int.* 46: p.1–15, 1994.
5. FALK R. J., JENNETTE J. C.: Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N. Engl. J. Med.* 318: p.1651–1657, 1988.
6. DAVIES D. J., MORAN J. E., NIALL J. F., ET AL.: Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *Br. Med. J.* [Clin. Res. Ed.] 285: p. 606, 1982.
7. VAN DER WOUDE F. J., RASMUSSEN N., LOBATTO S., ET AL.: Autoantibodies against neutrophils nad monocytes: Tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* 1: p. 425, 1985.
8. COHEN TERVEART J. W., GOLDSCHMEDING R., ELEMA J. D., ET AL.: Autoantibodies against myeloid lysosomal enzymes in crescentic glomerulonephritis. *Kidney Int.* 37: p. 799, 1990.
9. JENNETTE J. C., WILKMAN A. S., FALK R. J.: Diagnostic predictive value of ANCA serology. *Kidney Int.* 53: p. 796, 1998.
10. NILES J. L., PAN G., COLLINS A. B., ET AL.: Antigen-specific radioimmunoassays for anti-neutrophil cytoplasmic antibodies in the diagnosis of rapidly progressive glomerulonephritis. *J. Am. Soc. Nephrol.* 2: p. 27, 1991.
11. STONE J. H., TALOR M., STEBBING J., ET AL.: Test characterisitics of immunofluorescence and ELISA tests in 856 consecutive patients with possible ANCA-associated conditions. *Arthritis Care Res.* 13: p. 424, 2000.
12. BARTŮŇKOVÁ J., TESAŘ V., ŠEDIVÁ A.: Diagnostic and pathogenetic role of antineutrophil cytoplasmic autoantibodies. *Clinical Immunology* 106: p. 73–82, 2003.
13. HAGEN E. C., DAHA M. R., HERMANS J., ET AL.: Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. *Kidney Int.* 53: p. 743–753, 1998.
14. SUN J., FASS D. N., HUDSON J. A., ET AL.: Capture-ELISA based on recombinant PR3 is sensitive for PR3-ANCA testing and allows detection of PR3 and PR3-ANCA/PR3 immunecomplexes. *J. Immunol. Methods* 211: p.111–123, 1998.
15. CHOI H. K., MERKEL P. A., WALKER A. M., ET AL.: Drug-associated antineutrophil cytoplasmic antibody-positive vasculitis: prevalence among patients with high titres of antimyeloperoxidase antibodies. *Arthritis Rheum.* 43: p. 405, 2000.

16. LAM D. C., LINDSAY R. H.: Accumulation of 2-[14C]-propylthiouracil in human polymorphonuclear leukocytes. *Biochem. Pharmacol.* 28: p. 2289, 1979.
17. LEE H., HIROUCHI M., HOSOKAWA M., ET AL.: Inactivation of perixidase of rat bone marrow by repeated administration of propylphiouracil is accompanied by a change in the heme structure. *Biochem. Pharamcol.* 37: p. 2151, 1988.
18. SHORT A. K., LOCKWOOD C. M.: Antigen specificity in hydralazine associated ANCA positive systemic vasculitis. *Q. J. Med.* 88: p. 775, 1995.
19. ZHAO M. H., JAYNE D. R. W., ARDILES L., ET AL.: Autoantibodies against bactericidal/ permeability-increasing protein [BPI] in cystic fibrosis. *Clin. Exp. Immunol.* 101: p. 38, 1995.
20. ZHAO M. H., JONES S. J., LOCKWOOD C. M.: Bactericidal/permeability-increasing protein [BPI] is an important antigen for anti-neutrophil cytoplasmic antiboides [ANCA] in vasculitis. *Clin. Exp. Immunol.* 99: p. 49–56, 1995.
21. LOCHT H., SKOGH T., WIIK A.: Characterisation of autoantibodies to neutrophil granule constituents among patients with reactive arthritis, rheumatoid arthritis, and ulcerative colitis. *Ann. Rheum. Dis.* 59: p. 898–903, 2000.
22. CHOI H. K., LAMPRECHT P., NILES J. L., ET AL.: Subacute bacterial endocarditis with positive cytoplasmic antibodies and anti-proteinase 3 antibodies. *Arthritis Rheum.* 43: p. 226–231, 2000.
23. PUDIFIN D. J., DUURSMA J., GATHIRAM V., ET AL.: Invasive amoebiasis is associated with the development of anti-neutrophil cytoplasmic antibody. *Clin. Exp. Immunol.* 97: p. 48–51, 1994.
24. DIEHL V., SCHRAPPE M.: Seroprevalence and disease association of antineutrophil cytoplasmic autoantibodies and antigens in HIV infection. *Infection* 27: p. 92–96, 1999.
25. WEBER M. F., ANDRASSY K., PULLIG O., ET AL.: Antineutrophil-cytoplasmic antibodies and antiglomerular basement membrane antibodies in Goodpasture's syndrome and in Webener's granulomatosis. *J. Am. Soc. Nephrol.* 2: p. 1227, 1992.
26. SAVIGE J., DAVIES D., FALK R. J., ET AL.: Antineutrophil cytoplasmic antibodies and associated diseases: A review of the clinical and laboratory features. *Kidney Int.* 57: p. 846, 2000.
27. HOFFMAN G. S., KERR G. S., LEAVITT R. Y., ET AL.: Wegener's granulomatosis: An analysis of 158 patients. *Ann. Int. Med.* 116: p. 488, 1992.
28. JOHNSON R. J., FEEHALLY J.: Comprehensive clinical nephrology. Text book – ISBN 07234 31175, p. 1–13, 2000.
29. JAYNE D.R. W., GASKIN G., PUSEY C. D., ET AL.: ANCA and predicting relapse in systemic vasculitis. *Q. J. Med.* 88: p. 127–133, 1995.
30. HAUER H. A., BAJEMA I. M., VAN HOUWELINGEN H. C., ET AL.: Renal histology in ANCA-associated vasculitis: Differences between diagnostic and serologic subgroups. *Kidney Int.* 61: p. 80–89, 2002.
31. SCHNABEL A., HOLLULRICH K., DALHOFF K., ET AL.: Efficacy of transbronchial biopsy in pulmonary vasculitides. *Eur. Respir. J.* 10: p. 2738, 1997.
32. KOBOLD A. C., VAN DER GELD Y. M., LIMBURG P. C., ET AL.: Pathophysiology of ANCA-associated glomerulonephritis. *Nephrol. Dial. Transplant.* 14: p. 1366, 1999.
33. RAYNAULD J. P., BLOCH D. A., FRIES J. F.: Seasonal variation in the onset of Wegener's granulomatosis, polyarteritis nodosa and giant cell arteritis. *J. Rheumatol.* 20: p. 1524–1526, 1993.
34. BARRETT T. G., TAYLOR C. M., THOMASON P., ET AL.: Environmental trigger for anti-neutrophil cytoplasmic antibodies. *Lancet* 342: p. 369–370, 1993.
35. STEGEMAN C. A., TERVAERT J. W., MANSON W. L., ET AL.: Chronic nasal carriage of Staphylococcus aureus in Wegener granulomatosis identification of a subgroup more prone to relapses. *Ann. Intern. Med.* 120: p. 12–17, 1994.

36. HOFFMAN G. S., SECHLER J. M., GALLIN J. I., ET AL.: Bronchoalveolar lavage analysis in Wegener's granulomatosis: a method to study disease pathogenesis. *Am. Rev. Respir. Dis.* 143: p. 401–407, 1991.
37. LAWYER C., HENKLE J., BAKIR H.: Nasal carriage of staphylococcal infection in Wegener granulomatosis. *Ann. Intern. Med.* 121: p. 74–75, 1994.
38. PRESTON G. A., FALK R. J.: ANCA signaling: not just a matter of respiratory burst. *Kidney Int.* 59: p. 1981–1982, 2001.
39. TERVEART J. W. C., STEGEMAN C. A., KALLENBERG C. G. M.: Silicon exposure and vasculitis. *Curr. Opin. Rheumaol.* 10: p. 12–17, 1998.
40. BARTŮŇKOVÁ J., KOLÁŘOVÁ I., ŠEDIVÁ A., ET AL.: Does silica cause vasculitis and kidney damage? *Clin. Immunol.* 99: p. 107, 2001.
41. PELCLOVÁ D., FENCLOVÁ Z., LEBEDOVÁ J., ET AL.: Silicosis, asbestosis and ANCA positivity. *Eur. Respir. J.* 18: p. 437–438, 2001.
42. GENCIK M., BORGMANN S., ZAHN R.: Immunogenetic risk factors for anti-neutrophil cytoplasmic [ANCA]-associated systemic vasculitis. *Clin. Exp. Immunol.* 117: p. 412–417, 1999.
43. CALLEA F., GREGORINI G., SINICO A., ET AL.: Alpha 1-antitrypsine [AAT] deficiency and ANCA-positive systemic vasculitis: genetic and clinical implications. *Eur. J. Clin. Invest.* 27: p. 696–702, 1997.
44. AUDRAIN M., SESBOUE R., BARANGER T. A., ET AL.: Analysis of anti-neutrophil cytoplasmic antibodies [ANCA]: frequency and specificity in a sample of 191 homozygous [PiZZ] alpha1-antitrypsin-deficient subjects. *Nephrol. Dial. Transplant.* 16: p. 39–44, 2001.
45. MELLER S., JAGIELLO P., BORGMANN S., ET AL.: Novel SNPs in the CD18 gene validate the association with MPO-ANCA+ vasculitis. *Genes Immun.* 2: p. 269–272, 2001.
46. SPENCER S. J., BURNS A., GASKIN G., ET AL.: HLA class II specificities in vasculitis with antibodies to neutrophil cytoplasmic antigens. *Kidney Int.* 41: p. 1059–1063, 1992.
47. KOBOLD A. C., KALLENBERG C. G., TERVEART J. W.: Monocyte activation in patients with Wegener's granulomatosis. *Ann. Rheum Dis.* 58: p. 237, 1999.
48. MOINS-TEISSERENC H. T., GADOLA S. D., CELLA M., ET AL.: Association of a syndrome resembling Wegener's granulomatosis with low surface expression of HLA class-I molecules. *Lancet* 354: p. 1598, 1999.
49. MASUTANI K., TOKUMOTO M., NAKASHIMA H., ET AL.: Strong polarization toward Th1 immune response in ANCA-associated glomerulonephritis. *Clin. Nephrol.* 59: p. 395, 2003.
50. JENNETTE J. C., FALK R. J.: Pathogenic potential of anti-neutrophil cytoplasmic autoantiboides. *Lab. Invest.* 70: p. 135, 1994.
51. FALK R. J., TERRELL R. S., CHARLES L. A., ET AL.: Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proc. Natl. Acad. Sci. USA* 87: p. 4115, 1990.
52. TERVAERT J. W.: PROTEINASE 3: A cofactor for the binding of antineutrophil cytoplasm antibodies [ANCA] to endothelial cells? *Kidney. Int.* 57: p. 2171, 2000.
53. HONG XIAO H., HEERINGA P., HU P., ET AL.: Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J. Clin. Invest.* 110: p. 955–963, 2002.
54. MULLER KOBOLD A. C., KALLENBERG C. G., COHEN TERVAERT J. W.: Leucocyte membrane expression of proteinase 3 correlates with disease activity in patients with Wegener's granulomatosis. *Br. J. Rheumatol.* 37: p. 901–907, 1998.
55. SAVAGE C. O. S., POTTINGER B. E., GASKIN G. ET AL.: Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity towards cultured endothelial cells. *Am. J. Pathol.* 141: p. 335–342, 1992.

56. HABER M. A., TSO A., TAHERI S.: Wegener's granulomatosis in pregnancy: the terapeutic dilemma. *Nephrol. Dial. Transplant.* 14: p. 1789–1791, 1999.
57. HEERINGA P., BROUWER E., COHEN TERVAERT J. W., ET AL.: Animal models of anti-neutrophil cytoplasmic antibody associated vasculitis. *Kidney Int.* 53: p. 253–263, 1998.
58. KERR G. S., FLEISHER T. A., HALLAHAN C. W., ET AL.: Limited prognostic value of changes in antineutrophil cytoplasmic antibody titer in patients with Wegener's granulomatosis. *Arthritis Rheum.* 36: p. 365–371, 1993.
59. LANGFORD C. A.: Treatment of ANCA-associated vasculitis. *N. Engl. J. Med.* 349: p. 1, 2003.
60. TESAŘ V., ŘÍHOVÁ Z., JANČOVÁ E., ET AL.: Current treatment strategies in ANCA-positive renal vasculitis – lessons from European randomized trials. *Nephrol. Dial. Transplant.* 18: suppl. 5, p. V2–V4, 2003.
61. HAUBITZ M., SCHELLONG S., GOEBEL U., ET AL.: Intravenous pulse administration of cyclophosphamide versus daily oral treatment in patients with antineutrophil cytoplasmic antibody-associated vasculitis and renal involvement. *Arthiritis Rheum.* 41: p. 1835–1844, 1998.
62. DE GROOT K., ADU D., SAVAGE C. O. S. FOR EUVAS: The value of pulse cyclophosphamide in ANCA-associated vasculitis: meta-analysis and critical review. *Nephrol. Dial. Transplant.* 16: p. 2018–2027, 2001.
63. DE GROOT K., RASMUSSEN N., COHEN TERVAERT J. W., ET AL.: Randomized trial of cyclophosphamide versus methotrexate for induction of remission in "non-renal" ANCA-associated vasculitis. *Cleveland Clin. J. Med.* 69: suppl. 2, p. 116, 2002.
64. GASKIN G., JAYNE D.: Adjunctive plasma exchange is superior to methylprednisolone in acute renal failure due to ANCA-associated glomerulonephritis. *J. Am. Soc. Nephrol.* 13: suppl S, p. 2A–3A, 2002.
65. JAYNE D.: Conventional treatment and outcome of Wegener's granulomatosis and microscopic polyangiitis. *Cleveland Clin. J. Med.* 69: suppl 2, p.110–115, 2002.
66. KLEMMER P. J., CHALERMSKULRAT W., REIF M. S., ET AL.: Plasmapheresis Therapy for Diffuse Alveolar Hemorrhage in Patients with Small-Vessel Vasculitis. *Am. J. Kidney Dis.* 42: p. 1149–1153, 2003.
67. NACHMAN P. H., HOGAN S. L., JENNETTE J. C., ET AL.: Treatment response and relapse in antinutrophil cytoplasmic antibody-associated microscopic polyangiitis and glomerulonephritis. *J. Am. Soc. Nephrol.* 7: p. 33, 1996.
68. JAYNE D., RASMUSSEN N., ANDRASSY K.: A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N. Engl. J. Med.* 349: p. 36–44, 2003.
69. NOWACK R., GOEBEL U., KLOOKER P., ET AL.: Mycophenolate Mofetil for maintenance therapy of Wegener's granulomatosis and microscopic polyangiitis: A pilot study in 11 patients with renal involvement. *J. Am. Soc. Nephrol.* 10: p. 1965–1971, 1999.
70. LANGFORD C. A., TALAR-WILLIAMS C., BARRON K. S., ET AL.: Use of a cyclophosphamide-induction methotrexate-maintenance regime for the treatment of Wegener's granulomatosis: extended follow-up and rate of relapse. *Am. J. Med.* 114: p. 463–469, 2003.
71. DE GROOT K., REINHOLD-KELLER E., TATSIS E., ET AL.: Therapy for the maintenance of remission in sixty-five patients with generalized Wegener's granulomatosis. *Arthritis Rheum.* 39: p. 2052, 1996.
72. STEGEMAN C. A., COHEN TERVAERT J. W., DE JONG P. E., ET AL.: Trimethoprim-sulfamethoxazole [Co-trimoxazole] for the prevention of relapses of Wegener's granuloamtosis. *N. Engl. J. Med.* 335: p. 16–20, 1996.
73. HAUBITZ M., KOCH K. M., BRUNKHORST R.: Cyclosporin for the prevention of disesase reactivation in relapsing ANCA-associated vasculitis. *Nephrol. Dial. Transplant.* 13: p. 2074–2076, 1998.

74. JAYNE D. R., CHAPEL H., ADU D. ET AL.: Intravenous immunoglobulin for ANCA-associated systemic vasculitis with persistent disease activity. *Q. J. Med.* 93: p. 433–439, 2000.
75. SCHOENFELD Y., SHERER Y., LANGEVITZ P., ET AL.: Treatment of 13 patients with vasculitis and other autoimmune diseases with intravenous immunoglobulin. *Clin. Exp. Immunol.* 120: suppl. 1, p. 12–13, 2000.
76. BIRCK R., WARNATZ K., LORENZ H. M., ET AL.: 15-deoxyspergualin in patients with refractory ANCA-associated systemic vasculitis: a six-month open-label trial to evaluate safety and efficacy. *J. Am. Soc. Nephrol.* 14: p. 440–447, 2003.
77. LEVY J.: New aspects in the management of ANCA-positive vasculitis. *Nephrol. Dial. Transplant.* 16: p. 1314–1317, 2001.
78. LOCKWOOD C. M., THIRU S., STEWART S.: Treatment of refractory Wegener's granulomatosis with humanised monoclonal antibodies. *Q. J. Med.* 89: p. 903–912, 1996.
79. SCHMITT W. H., BIRCK R., NOWACK R., ET AL.: Anti-thymocyte globuline: A therapeutic option for wegener's granulomatosis untreatable with conventional therapies. *J. Am. Soc. Nephrol.* 11: p. 165A, 2000.
80. LOCKWOOD C. M., ELLIOTT J. D., BRETTMAN L., ET AL.: Anti-adhesion molecule therapy as an interventional strategy for autoimmune inflammation. *Clin. Immunol.* 93: p. 93–106, 1999.
81. ELLIOTT J. D., LOCKWOOD C. M., HALE G., ET AL.: Semi-spcific immuno-absorption and monoclonal antibody therapy in ANCA positive vasculitis: experience in four cases. *Autoimmunity* 28: p.163–171, 1998.
82. ALEXANDRE S., MOGUILEVSKY N., PAINDAVOINE P., ET AL.: Specific MPO immunoabsorption for the treatment of vasculitis. *Clin. Exp. Immunol.* 120: Suppl. 1, p. 73, 2000.
83. FRANSSEN C. F. M., GANS R. O. B., ARENDS B., ET AL.: Differences between anti-myeloperoxidase and anti-proteinase 3 associated renal disease. *Kidney Int.* 61: p. 80–89, 1995.
84. HAUER H. A., BAJEMA I. M., VAN HOUWELINGEN H. C., ET AL.: Renal histology in ANCA-associated vasculitis: differences between diagnostic and serologic subgroups. *Kidney Int.* 61: p. 80–89, 2002.
85. FRANSSEN C. F. M., HUITEMA M. G., MULLER KOBOLD A. C., ET AL.: In vitro neutrophil activation by antibodies to proteinase 3 and myeloperoxidase from patients with crescentic glomerulonephritis. *J. Am. Soc. Nephrol.* 10: p. 1506–1515, 1999.
86. FRANSSEN C. F. M., GANS R. O. B., KALLENBERG C. G. M., ET AL.: Disease spectrum of patients with antineutrophil cytoplasmic antibodies of defined specificty: distinct differences between patients with anti-proteinase 3 nad anti-myeloperoxidase autoantibodies. *J. Intern. Med.* 244: p. 109–216, 1998.
87. GEFFRIAUD-RICOUARD C., NOEL L. H., CHAUVEAU D., ET AL.: Clinical spectrum associated with ANCA of defined antigen specificities in 98 selected patients. *Clin. Nephrol.* 39: p. 125–136, 1993.
88. WESTMAN K. W., BYGREN P. G., OLSSON H., ET AL.: Relapse rate, renal survival, and cancer morbidity in patients with Wegener's granulomatosis or microscopic polyangiitis with renal involvement. *J. Am. Soc. Nephrol.* 9: p. 842–852, 1998.
89. COHEN TERVEART J. W., VAN DER WOUDE F. J., FAUCI A. S., ET AL.: Association between active Wegener's Granulomatosis nad anticytoplasmic antibodies. *Arch. Intern. Med.* 149: p. 2461–2465, 1989.
90. EGNER W., CHAPEL H. M.: Titration of antibodies against neutrophil cytoplasmic antigens is useful in monitoring diseease activity in systemic vasculitides. *Clin. Exp. Immunol.* 82: p. 244–249, 1990.
91. COHEN TERVEART J. W., HUITEMA M. G., HENE R. J., ET AL.: Prevention of relapses in

Wegener's granulomatosis by treatment based on antineutrophil cytoplasmic antibody titer. *Lancet* 336: p. 709–711, 1990.
92. BOOMSMA M. M., STEGEMAN C. A., HERMANS J., ET AL.: Prevention of relapses of vasculitis in ANCA-associated vasculitis by treatment based on autoantibody levels as detected by solid phase assay. In: Boomsma MM, Academic Thesis, Groningen, The Netherlands. Doctoral thesis– ISBN 90-367-1451-6, 2001.
93. DE'OLIVEIRA J., GASKIN G., DASH A., ET AL.: Relationship between disease activity and anti-neutrophil cytoplasmic antibody concentration in long-term management of systemic vasculitis. *Am. J. Kidney. Dis.* 25: p. 380–389, 1995.
94. BOOMSMA M. M., STEGEMAN C. A., VAN DER LEIJ M. J., ET AL.: Prediciton of relapses in Wegener's granulomatosis by measurement of antineutrophil cytoplasmic antibody levels: a prospective study. *Arthritis Rheum.* 43: p. 2025, 2000.
95. POPA E. R., STEGEMAN C. A., KALLENBERG C. G. M., ET AL.: Staphylococcus aureus and Wegener's granulomatosis. *Arthritis Res.* 4: p.77–79, 2002.
96. BRONS R. H., KALLENBERG C. G. M., COHEN TERVAERT J. W.: Are ANCA-associated vasculitides pauci-immune? *Rheum. Dis. Clin. North. Am.* 27: p. 833–848, 2001.
97. DUSTELBLOEM H. M., SCHEEPERS R. H. M., OOST W. W., ET AL.: Fc gamma receptor polymorhisms in Wegener's granulomatosis: risk factors for disease relapse. *Arthritis Rheum.* 42: p. 1823–1827, 1999.
98. ROROK A. A., STEGEMAN C. A., LIMBURG P. C., ET AL.: Neutrophil membrane expression of proteinase 3 [PR3] is related to relapse in PR3-ANCA-associated vasculitis. *J. Am. Soc. Nephrol.* 13: p. 2232–2238, 2002.
99. LITTLE M. A., NAZAR L., FARRINGTON K.: Outcome in glomerulonephritis due to systemic small vessel vasculitis: effect of functional status and non-vasculitic co-morbidity. *Nephrol. Dial. Trnasplant.* 19: p. 356–364, 2004.
100. SLOT M. C., COHEN TERVAERT J. W., FRANSSEN C. F. M., ET AL.: Renal survival and prognostic factors in patients with PR3-ANCA associated vasculitis with renal involvement. *Kidney Int.* 63: p. 670–677, 2003.
101. BOOTH A. D., ALMOND M. K., BURNS A., ET AL.: Outcome of ANCA-associated renal vasculitis: A 5-year retrospective study. *Am. J. Kidney Dis.* 41: p.776–784, 2003.
102. HOGAN S. L., NACHMAN P. H., WILKMAN A. S., ET AL.: Prognostic markers in patients with antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *J. Am. Soc. Nephrol.* 7: p. 23–32, 1996.
103. BAJEMA I. M., HAGEN E. C., HERMANS J., ET AL.: Kidney biopsy as a predictor for renal outcome in ANCA-associated necrotizing glomerulonephritis. *Kidney Int.* 56: p. 1751–1758, 1999.