

Review

Wegener's Granulomatosis – Autoimmunity to Neutrophil Proteinase 3

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Abstract. Wegener's granulomatosis (WG) is a small-vessel vasculitis associated with various clinical manifestations, among which the most common are respiratory tract disease and glomerulonephritis leading to renal failure. The pathogenesis of vascular injury in WG is ascribed to antineutrophil cytoplasmic antibodies (ANCA) directed mainly against proteinase 3 (PR3), an enzyme from neutrophil granules. The reasons for the breakdown of self tolerance to PR3 are unknown, and together with the molecular mechanisms underlying this immunoinflammation, are the subject of research. Standard treatment of WG consists of cyclophosphamide and corticosteroids. In patients resistant to this therapy or with refractory disease, some alternative strategies involving tumor necrosis factor blockade, polyclonal antithymocyte globulin or monoclonal anti-T cell antibodies are applied.

Key words: Wegener's granulomatosis; ANCA; proteinase 3.

Introduction

Wegener's granulomatosis (WG) is a necrotizing, granulomatous vasculitis with multiorgan involvement and predilection to the respiratory tract and kidneys⁴⁰. The first case of WG was reported by KLINGER in 1931⁵⁶. Friedrich Wegener was the one, who in 1936, distinguished the disease as a clinicopathologic entity¹⁰⁷. He established that the disease preferentially exhibits granulomatous lesions in the respiratory tract in addition to vasculitis. In 1954, GODMAN and CHURG²⁸ proposed diagnostic criteria for WG, known as Wegener's triad: 1) necrotizing granulomatous inflammation of the upper and/or lower respiratory tract, 2) sys-

temic or focal necrotizing vasculitis involving arteries and veins, and 3) focal, segmental necrotizing crescentic glomerulonephritis.

The pathogenesis of the disease remained unknown until the discovery of antineutrophil cytoplasmic antibodies (ANCA), described in a few patients with necrotizing glomerulonephritis by Davies et al.¹⁰ in 1982. In 1985, VAN DER WOUDE et al.¹⁰³ established a close association between active WG and ANCA. These antibodies suggested an autoimmune pathogenesis for WG. In 1990 the target antigen for ANCA in WG was identified as proteinase 3 (PR3), a constituent of neutrophilic granules, actively participating in vasculitic inflammation⁶⁵. Some other specificities of these antibodies are

Abbreviations used: ANA – antinuclear antibody, ANCA – antineutrophil cytoplasmic antibodies, ASV – ANCA-associated small-vessel vasculitis, CSS – Churg-Strauss syndrome, ECs – endothelial cells, ELISA – enzyme-linked immunosorbent assay, IIF – indirect immunofluorescence, MPA – microscopic polyangiitis, MPO – myeloperoxidase, PR3 – proteinase 3, RPGN – rapidly progressing necrotizing crescentic glomerulonephritis, WG – Wegener's granulomatosis.

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myeloperoxidase (MPO), lysozyme, lactoferrin and cathepsin G, all derived from the neutrophils⁸⁴. ANCA are not specific to WG^{30, 31}, as they also appear in microscopic polyangiitis (MPA), rapidly progressing necrotizing crescentic glomerulonephritis (RPGN), which seems to be a renal-limited form of MPA, Churg-Strauss syndrome (CSS), and other conditions^{5, 71, 84}. The presence of ANCA as well as the diameter of the vessels involved in the inflammation (small to medium arteries, capillaries and venules) allowed distinguishing the above-mentioned diseases from a large group of systemic vasculitides under the name ANCA-associated small-vessel systemic vasculitides (ASV). Another common feature of these disorders is the lack or paucity of immune complexes deposited at the sites of inflammation.

Epidemiology of WG

WG occurs in 3 out of 100,000 persons and is equally distributed between men and women⁸. Although 80–97% of WG patients are white, correlation between HLA genotype and disease occurrence seems to be insignificant. Familial cases are very rare⁸. The mean age for the onset of the disease (ranging from 9 to 78) is 41 years⁴⁰. WG is rare in the very young, 15% of patients are less than 19 years old⁴⁰. In children and adolescents the features resemble those of the adult disease⁸⁰. About 50% of patients are over 60 years old. The older population presents the same features as younger adults, but the outcome is worse due to treatment-associated infections, which are a common cause of death⁵⁷.

Environmental exposure to particulate material and gaseous substances appears to be unimportant in triggering disease onset¹⁸. Whether a seasonal variation in the onset can be observed is a matter of controversy. According to one of the pathogenic theories, the autoimmune process may be triggered by infection, suggesting a prevalence of autumn and winter in the onset, which is not confirmed by others¹⁸. Some authors report slight differences in epidemiology and clinical manifestation among patients with ANCA of various specificity. Their studies reveal a preponderance of males in patients with ANCA against PR3 and of females in patients with ANCA against MPO. Patients with anti-MPO are also older than those with anti-PR3²⁷.

Pathogenesis of WG

The pathogenesis of WG and other small-vessel vasculitides has not been yet elucidated. According to

the current ANCA-cytokine-sequence theory, the vascular inflammation derives from an autoimmune reaction between ANCA and neutrophil granule proteins. The reason for the breakdown of self tolerance to granule proteins is unknown and seems to be an acquired feature however, some undiscovered genetically mediated susceptibility cannot be dismissed. The characterization of PR3 Wegener's autoantigen is shown in Table 1. Reaction between ANCA and target antigens raises the net of proinflammatory cytokine interactions and activates leukocytes and endothelial cells (ECs), ending in vascular damage⁷². There are three hypotheses explaining the accessibility of intracellular neutrophilic proteins to circulating ANCA. According to one report, PR3 is expressed on the cell surface of a subset of circulating neutrophils and the proportion of neutrophils presenting PR3 is genetically controlled and highly stable. The phenotype of increased PR3 surface expression is significantly high in patients with ASV¹⁰⁹. The second possible cause of PR3 accessibility may be the increased level of apoptosis of neutrophils in vasculitic patients³⁴. Apoptosis is associated with a higher concentration of surface PR3. Whether the apoptosis occurs irrespective of or is secondary to ANCA transducing an apoptotic signal remains unknown. The greatest role in evoking the interaction between PR3 and ANCA is ascribed to the infection⁵². Unidentified infectious factors stimulate the synthesis of proinflammatory cytokines such as IL-1, IFN- γ and TNF- α , which results in priming the neutrophils. The priming is associated with the translocation of PR3 from neutrophil granules to the cell membrane, where it can be detected by flow cytometry²² and is accessible for ANCA. Despite the fact that conventional microbiological culture studies or histological staining methods have failed to detect any causative microorganism, the role of infection in the development of WG is supported by several considerations. Most WG patients in the active phase or reactivation of the disease present with neutrophilic alveolitis in the examination of their bronchoalveolar lavage⁴². Infection often precedes a rise in ANCA le-

Table 1. Characterization of proteinase 3

Biochemical features	• serine protease from chymotrypsin superfamily • cationic protein • physiological inhibitor: α1-antitrypsin
Functional characteristics	• enzymatic activity: proteolysis of elastin, fibronectin, laminin, vitronectin, collagen type IV • nonproteolytic antimicrobial activity • regulation of myeloid differentiation

vels and clinical activation of WG⁷⁷. Chronic nasal carriage of *Staphylococcus aureus* is a significant risk factor for the relapse of the disease⁹³. Beneficial effects of antibiotic therapy with sulphamethoxazole-trimethoprim in WG patients with the disease limited to the upper airways have been observed⁹¹. Several viruses, such as HBV, HCV, EBV and HIV, are known to be associated with vasculitis⁵⁹.

The role of ANCA in enhancing immunoinflammation has been supported by the vast evidence from *in vitro* studies. Binding of ANCA to primed neutrophils displaying PR3 on the cell surface results in neutrophil activation followed by a release of reactive oxygen species, degranulation of proteins and secretion of proinflammatory cytokines³⁵. The activating process takes place on the surface of EC. ANCA, as well as proinflammatory cytokines, increase the expressions of adhesion molecules (E-selectin, VCAM-1 and ICAM-1) on EC⁶⁹, thus localizing the inflammation on the intravascular side of the vessel wall. Upregulation of adhesion molecules leads to the increase of their soluble isoforms, which has been shown to correlate with disease activity⁹². PR3 released from the activated neutrophils adds to the vascular injury by forming immunocomplexes with circulating ANCA and binding via charge interactions to EC. Under cytokine stimulation, EC were shown to synthesise PR3 and express it on the surface⁶⁸. PR3-ANCA complexes are able to activate neutrophils via the Fc fragment of the IgG molecule. Blocking the FcγRIIa neutrophilic receptor by nonactivating monoclonal antibodies inhibits the activation of neutrophils by the PR3-ANCA complex⁷⁴. Fc receptors of the neutrophils express a variable affinity to certain immunoglobulin subclasses according to the amino-acidic sequence of the receptor molecule. Receptors with histidine at position 131 display a higher affinity for immunoglobulin G3 (IgG3) subclass than those with arginine. Relapses of WG were shown to be associated with increases in the IgG3 subclass of ANCA^{6, 101}.

Binding positively charged PR3 to the negatively charged EC has several pathological effects. EC with PR3 on their surface become the target for ANCA and are damaged due to the antibody-dependent cell cytotoxicity mechanism⁸². EC injury is also a consequence of the proteolytic properties of PR3. The enzyme degrades vessel-wall proteins such as elastin, fibronectin, laminin and collagen type IV, enabling neutrophil extravasation and penetration of inflamed tissue¹⁰⁶. Proteolysis of the basal membrane with the subsequent detachment of ECs leads to their apoptosis¹¹⁰.

The interaction between PR3 displayed on EC and ANCA has been shown to be responsible for increased

EC production of the tissue factor, the initiator of the coagulation cascade and a strong chemoattractant agent, such as IL-8⁷⁰. High intravascular concentrations of IL-8 impede neutrophil transmigration by trapping them in the microvasculature and cause reorganization of the cytoskeletal architecture of neutrophils, leading to their decreased deformability¹⁰⁸. Retention of neutrophils in the capillaries and the tissue-specific endothelium features may be responsible for the specific organ involvement in WG with the predilection of vascular lesions to pulmonary and renal microvasculature.

PR3 is naturally inhibited by α1anti-trypsin (α1AT) protease inhibitor, synthesized in the liver. It was suggested that disturbances in the PR3/α1AT balance might be implicated in WG pathogenesis. Several studies report that anti-PR3 ANCA-positive patients have an α1AT deficiency⁸³. The gene of α1AT is highly polymorphic: more than 70 alleles are known. Alleles responsible for subnormal α1AT concentrations have been shown to appear more often in vasculitic patients and are associated with greater organ involvement and poor prognosis⁸⁶. Under inflammatory conditions, α1AT can be degraded and inactivated by a number of proteases and reactive oxygen species released from the activated neutrophils², increasing the local activity of PR3. Furthermore, in WG patients anti-PR3 ANCA can interfere with the inhibition of PR3 by α1AT¹⁷. PR3-ANCA complexes interfere with PR3, clearance acting as its reservoir. PR3 released from such complexes in patients with α1AT deficiency may cause local tissue destruction²⁰.

Vascular lesions resulting from neutrophil activation belong to the early phase of WG. The subsequent phase is granuloma formation, which suggests T cell involvement. Renal biopsies reveal a predominance of CD4⁺ cells³. T cell activation is indicated by increased serum levels of IL-1, IL-6 and TNF-α in the acute phase of WG¹¹. An elevated level of soluble IL-2 receptors has been detected in WG patient sera and it seems to correlate with the disease activity³². *In vitro* studies report a stimulating effect of PR3 on T cell proliferation in WG patients¹⁰². The lymphocytes as memory cells may be responsible for the refractory nature of the disease.

Despite intensive research, the definitive etiology and pathogenesis of WG still remains unclear. Animal models and *in vitro* studies are imperfect in reflecting the unique human intravascular environment. Recent research aimed at revealing the molecular aspects of PR3-ANCA interactions is hampered by the fact that ANCA recognize conformational PR3 epitopes, which are difficult to obtain *in vitro*¹⁰².

Clinical Features of WG

WG may affect any organ system, although, the most common clinical manifestation is the respiratory tract disease¹³. The classic triad of WG is often not present. The onset is usually associated with non-specific features such as fatigue, fever and weight loss. In the upper respiratory tract, the inflammatory granulomatous process might be localized in the nose, ears and sinuses, and may present as recurring epistaxis due to ulceration and friability of mucous tissue. Nasal cartilage might be deformed. Inflammation and narrowing of the subglottic trachea appears in 20% of patients and is a life-threatening condition. This may develop in the absence of other manifestations. Such symptoms as dyspnea and hoarseness should be noted. Subglottic stenosis demands a specific therapeutic approach, because it is unresponsive to standard immunosuppressive treatment due to rapid cicatrization and scarring. Under these circumstances, tracheotomy or another surgical technique combining mechanical dilatation of the trachea with intratracheal injection of corticosteroid should be performed⁶⁰. In the lower respiratory tract, the pulmonary parenchyma, bronchi and, rarely, pleura might be affected. Common complaints that may suggest this disease localization are cough, dyspnea, chest pain, hemoptysis and wheezing. Diffuse alveolar hemorrhage is a rare, but serious manifestation. Bronchitis is poorly responsive to treatment due to scarring. Symptoms in the upper respiratory tract appear at presentation in 73% of patients, compared with symptoms from the lower part detected in 48% of patients. In the course of the disease, such symptoms appear in 92 and 85% of patients, respectively⁵⁸.

Glomerulonephritis is one of the manifestations of greatest concern because it may progress to end-stage renal failure. It is usually asymptomatic, detected by abnormal laboratory findings such as increased serum creatinine, decreased creatinine clearance and proteinuria³⁷. Although it occurs at presentation in 20% of patients, it develops in 80% during the disease course. According to a National Institutes of Health questionnaire, glomerulonephritis appears within 2 years of disease onset⁴⁰. Musculoskeletal symptoms (i.e. myalgias and arthralgias) appear in 67% of patients, and 52% of patients have ocular disease, which may threaten vision. There is a variety of ocular involvement, including conjunctivitis, scleritis, uveitis, retinal vasculitis, optic neuritis and retinal arteriae occlusion. Retroorbital disease presents with ophthalmoplegia, exophthalmus and compression of the optic nerve. Any complaints of diplopia or field cuts should be assessed ophthalmologically⁴⁰.

Several clinical types of WG can be recognized. Classic (generalized) WG with a wide organ involvement including the upper and lower respiratory tract and kidneys, accounts for 25% of disease cases¹². Limited pulmonary WG, associated with better prognosis, may develop into generalized WG⁶². Eighty percentage of patients with limited WG will develop renal involvement¹⁹. The protracted, superficial variant is characterized by chronic inflammation and ulceration involving nasal and sinus mucosa. Patients may also develop symptoms in the larynx, trachea and bronchi and may eventually progress to the classic form of WG²⁵. Another distinguished clinical type is diffuse alveolar hemorrhage, which is unusual but associated with a fulminant course and poor prognosis. Pulmonary hemorrhage caused by necrotizing alveolar capillaries may be accompanied by RPGN, leading to renal failure within a few days or weeks. The condition called pulmonary-renal syndrome appears mainly in WG and MPA⁷⁵. Isolated glomerulonephritis is another rare variant of WG⁵⁸. Other unusual manifestations include facial palsy, granulomatous mastitis and subarachnoid hemorrhage^{9, 66}.

The Diagnosis of WG

The diagnosis of WG is based on histopathological data supported by laboratory findings and on the appropriate clinical features. Detection of ANCA and their close association with WG and other small-vessel vasculitides added substantially to constructing the diagnosis however, the presence of ANCA is recognized by neither The American College of Rheumatology nor the Chapel Hill Consensus Conference criteria^{49, 61}. Moreover, the methods of ANCA testing await standardization.

The main histopathologic characteristics of WG include vasculitis, granulomas and necrosis¹⁰⁵. Vasculitis might be necrotizing or granulomatous and involves small- to medium-sized arteries, arterioles, capillaries and venules⁴⁹. Some authors suggest that diagnostic confirmatory findings from nonrenal biopsy should show an inflammatory exudate dominated by the granulocytes with at least one of the following: 1) necrotizing vasculitis, 2) epithelioid granulomas, or 3) giant cells¹⁵. The characteristic glomerular lesion is a focal segmental necrotizing glomerulonephritis, usually with crescents, but sometimes with a disruption of the Bowman's capsule. Immunofluorescent microscopy of the glomeruli and of other vessels demonstrates few immunocomplex deposits⁵⁰. This distinguishes WG from the

immunocomplex-associated glomerulonephritis with granular deposits and from the linear staining in anti-glomerular basement membrane disease³⁶. The vascular lesions, regardless of their localization (kidneys, lungs, and muscles), are characterized by focal distribution.

The likelihood of obtaining a positive biopsy is influenced by the amount of the tissue won and by the organ examined. Upper airway biopsies, which are low invasive, demonstrate diagnostic findings in 16–21% of the samples⁴⁰. Renal biopsies are less diagnostic than pulmonary ones. Pulmonary parenchyma obtained by open lung biopsy has the highest positive yield, with diagnostic findings in 91% of the specimens. Although less invasive, transbronchial biopsy is diagnostic in only 7% of cases^{99, 100}.

Conventional chest radiographs are useful when disclosing pulmonary involvement. In 34% of WG patients abnormalities in chest radiographs appear asymptotically⁴⁰. The pulmonary radiologic features of WG are highly variable and may show single nodules, multiple nodular infiltrates, diffuse, hazy interstitial patterns usually associated with alveolar haemorrhage, and cavitary lesions⁵⁵. Computed tomography scans as well as bronchoscopy may be useful while assessing tracheal or bronchial stenosis. Orbital and paranasal sinuses involvement is best detected by computer tomography⁷⁶.

Anemia is present in up to 50% of WG patients, but is usually mild, except in patients with alveolar hemorrhage. Leukocytosis rarely exceeds 18,000/ml, and is neutrophilic. The erythrocyte sedimentation rate may be normal or very elevated, especially in patients with active, generalized disease. Laboratory findings of renal function impairment are as follows: proteinuria, an active urine sediment with microscopic hematuria and red-cell casts, increased serum creatinine and decreased creatinine clearance¹⁴.

ANCA – Clinical Utility and Methods of Detection

The first method applied to the detection of ANCA was indirect immunofluorescence (IIF). Two main fluorescent staining patterns of ANCA, which reflect their antigen specificity, have been described⁴³. The C-ANCA staining pattern is characterized by diffuse cytoplasmic staining, which spares the nucleus of the neutrophil, often accentuated in the center of the cytoplasm (C stands for central or classical). The P-ANCA pattern (perinuclear) results from translocation of the cytoplasmic target antigen to the perinuclear region during etha-

nol fixation of the neutrophils (one of the steps of the method) and, as such, is a preparatory artefact⁹⁸. Translocation appears to be due to the migration of cationic proteins to the negatively charged nucleus. This translocation does not occur when paraformaldehyde is used as a cross-linking fixative. ANCA producing the P-ANCA pattern on ethanol-fixed neutrophils switch to the C-ANCA pattern on paraformaldehyde fixed cells. This allows one to distinguish the P-ANCA pattern from the pattern of antinuclear antibodies (ANA), characteristic for systemic lupus erythematosus and scleroderma. Some authors consider formalin fixation as unreliable and irreproducible⁹⁰. Other immunofluorescent patterns different from those mentioned above are termed atypical (A-ANCA or X-ANCA). The IIF method does not provide information about the antigenic specificity of ANCA. To determine the target antigens of ANCA, the enzyme-linked immunosorbent assay (ELISA) has been introduced. ANCA presenting the C-ANCA fluorescent staining pattern appear to be reactive against PR3⁹⁸. The P-ANCA pattern is associated with reactivity against a wider spectrum of antigens. The principal target antigen for P-ANCA is MPO, but reactivity against other neutrophil proteins, such as elastase, cathepsin G, azurocidine, lactoferrin, lysozyme and bacterial permeability-increasing protein, have been encountered⁸⁴. The A-ANCA fluorescence pattern is induced by various antigens, most of which have not been determined⁸⁵. P-ANCA is less specific for MPO than C-ANCA for PR3⁹⁸.

The strongest ANCA association has been reported between anti-PR3 ANCA and WG. Anti-PR3 ANCA appear in 70–90% of patients with WG, but they cannot be defined as specific for WG as they appear in 50% of MPA patients²⁷. Although most patients with active, generalized WG have C-ANCA by IIF and PR3-specific ANCA by ELISA, up to 25% of them have P-ANCA with MPO specificity⁵¹. Ten percentage of WG patients do not have ANCA by IIF or ELISA, and the majority of them are likely to have the limited form of the disease⁸⁹. P-ANCA with reactivity to MPO reveals diverse disease associations. Some of these are of clinical importance, for example in MPA, RPGN and CSS²¹. Anti-MPO ANCA are found in 80% of patients with RPGN, in 50% of patients with MPA²⁶, and in 70–80% of patients with CSS⁴.

Apart from the appearance of ANCA in small-vessel vasculitides, these antibodies can be found in other vasculitic syndromes, chronic inflammatory disease of various origin, and neoplastic processes, all of which constitute a group of differential diagnosis for WG. Anti-PR3 ANCA can be found in giant cell arteritis,

cutaneous leukocytoclastic angiitis, Kawasaki syndrome, polyarteritis nodosa, Henoch-Schonlein purpura and cryoglobulinemic vasculitis³⁰. They appear also in the inflammatory conditions confined to a single organ system, such as recurrent conjunctivitis, relapsing chondritis, cranial nerve paralysis, refractory otitis media and sensorineural deafness³⁰. ANCA are also occasionally reported in chronic endocarditis, pneumonia, fungal infections, tuberculosis, sepsis, amebiasis with liver abscess and HIV infection³⁹. The diversity of ANCA associations is shown in Table 2.

Certain drugs, such as propylthiouracil, methamizole, hydralazine, penicillamine, clozapine and phenytoine, may induce ANCA (predominantly P-ANCA with reactivity to MPO), sometimes associated with such vasculitic signs as glomerulonephritis or cutaneous symptoms⁷³. ANCA associated with such propylthiouracil therapy appear in 20% of patients at any time during treatment¹⁶, while hydralazine-associated ANCA are reported after the years of the therapy⁸⁷.

The strong association between WG and anti-PR3 C-ANCA suggests a potential clinical utility of ANCA testing. A positive ANCA test may serve as a non-in-

vasive diagnostic tool, and monitoring of the level of ANCA in the course of the disease may reflect the activity of the vasculitis and support therapeutic decisions. The specificity of positive ANCA tests in WG ranges between 85–95%. Combining two methods of testing (IIF and ELISA) enhances the specificity to approximately 99%³³. The sensitivity of ANCA testing is variable and depends on the activity and clinical type of WG. In active, generalized WG, the sensitivity of a positive anti-PR3 ANCA test result is 90–98%. In the limited disease and remission, the sensitivity is 65–70% and less than 35%, respectively⁵⁵. The assessment of a positive ANCA test should be taken with caution, because ANCA appear in a large group of the diseases with differential diagnosis of WG. Taking into account the high toxicity of WG therapy, there is no room for diagnostic mistakes. Therefore, the diagnosis should not be solely based on positive ANCA testing results and should be complemented with biopsy.

ANCA levels correlate with disease activity in 2/3 of patients⁵⁴; however there are patients in remission in whom high C-ANCA levels persist for years after treatment⁵⁵. Therefore, basing therapeutic decisions only on rising ANCA levels is not justified⁵⁴. In the majority of WG patients, C-ANCA levels decline during the treatment and rise in 50% prior to relapse⁷. WG patients who are persistently ANCA-positive at clinical remission tend to have a higher relapse rate than those who have been cleared of ANCA⁴⁷. Relapse can be expected when the rise in C-ANCA measured by IIF (greater than 4-fold⁷) is accompanied by an at least 50% increase of the IgG3 subclass of anti-PR3 concentration measured by ELISA⁶. The average time from ANCA increase to relapse is 7 weeks⁴⁷. Monitoring of ANCA level may also be a valuable aid in distinguishing between the progression of vasculitis and an infectious complication resulting from an aggressive immunosuppressive treatment⁶⁷.

Currently, both IIF and ELISA are used in ANCA detection. IIF is considered the screening method. It is based on the microscopic examination of various cytoplasmic fluorescence patterns found when patient serum is applied to normal, human neutrophils fixed in ethanol on glass slides (Fig. 1). Although IIF is regarded as the more sensitive^{63, 95}, it is limited by subjectivity in the interpretation of the fluorescence pattern³⁹. After the determination of ANCA target antigens, ELISA methods became commonly used. There are two ELISA methods, direct (standard) and capture (sandwich) ELISA³⁹. Capture ELISA seems to be more sensitive and allows one to avoid the conformational changes of the antigenic epitopes, that may

Table 2. Diversity of ANCA associations*

Disease	IIF pattern	Antigen specificity by ELISA
WG	C-ANCA 80–90%	PR3
MPA	P-ANCA 50–70%	MPO
CSS	P-ANCA, C-ANCA 40–60%	MPO, PR3
RA	P-ANCA, A-ANCA 15–30%	MPO, LF, LZ
SLE	P-ANCA 15–30%	MPO, CG, LF
RPGN	P-ANCA 80%	MPO
Anti-GBM disease	P-ANCA 20–40%	MPO
IBD	A-ANCA, PANCA 40–80% in ulcerative colitis 10–20% in Crohn disease	CG, BPI
Liver diseases	A-ANCA, P-ANCA 65–85% in PSC 65–95% in AIH I	CG, BPI

* Modified from¹⁰⁴.

WG – Wegener's granulomatosis, MPA – microscopic polyangiitis, CSS – Churg-Strauss syndrome, RA – rheumatoid arthritis, SLE – systemic lupus erythematosus, RPGN – rapidly progressing glomerulonephritis, anti-GBM disease – antiglomerular basement membrane disease, IBD – inflammatory bowel disease, PSC – primary sclerosing cholangitis, AIH I – autoimmune hepatitis type I, PR3 – proteinase 3, MPO – myeloperoxidase, LF – lactoferrin, LZ – lysozyme, CG – cathepsin G, BPI – bacterial permeability increasing protein.

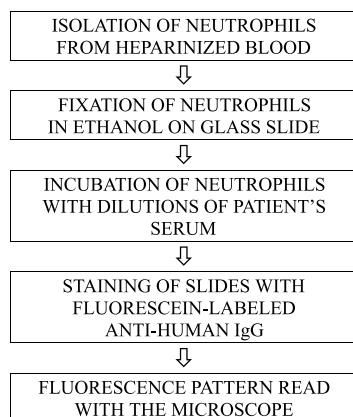


Fig. 1. Detection steps of ANCA by the indirect immunofluorescence method

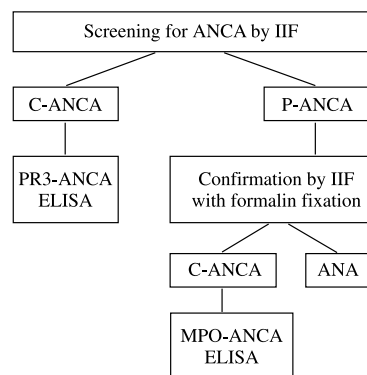


Fig. 2. Current algorithm of testing for ANCA (modified from ⁸¹)

occur during antigen purification from neutrophils and applying them to the plastic plate⁹⁷. Some authors, however, report that there is no advantage to the capture method over the direct ELISA⁸¹. The use of ELISA has improved ANCA detection specificity, made it more quantitative and circumvented the subjectivity of the fluorescent pattern interpretation. The current recommendation for ANCA testing is to combine both methods for maximal diagnostic accuracy⁸⁵ (Fig. 2). IIF should be followed by ELISA for anti-PR3 and anti-MPO ANCA^{33, 85}. Negative IIF results are recommended not to be reported as long as ELISA has not been performed⁸⁵. Where the ELISA test is positive, ELISA units rather than IIF titer should be reported. Where interfering antibodies such as ANA are present, ELISA in place of IIF should be performed.

Treatment and Outcome

WG is a potentially fatal disease; without treatment 80% of patients will die within one year and median

survival time is 5 months¹⁰⁵. Corticosteroids, which were the first agents applied in the treatment, have extended the median survival time to 12 months⁴⁵. Great progress was made after the development of a therapeutic approach combining corticosteroids and cyclophosphamide²⁴. The strongly immunosuppressive treatment strategy resulted in marked improvement in 90% and remission in 75% of patients⁴⁰. Despite these results, the administration of the treatment should be histopathologically confirmed because of the high toxicity of the agents used, especially cyclophosphamide. Morbidity associated with the treatment often exceeds the complications from the disease or relapse alone³⁹ therefore, minor clinical features should not be treated aggressively⁴⁸. Generally, the therapeutic strategy consists of inducing remission with an aggressive treatment followed by maintaining the achieved remission with a milder treatment. The agents used in the therapy and the period of their administration depends on the activity, clinical type of WG, age, renal function and bone marrow reserve of the patients. The standard induction regimen for patients with generalized WG consists of daily oral administration of corticosteroid with cyclophosphamide. After remission, cyclophosphamide is maintained for one year, then tapered and discontinued. Corticosteroids are tapered after one month according to the clinical symptoms and discontinued after 6–12 months. This regimen, although the most effective, is found to be highly toxic⁴⁰. The cumulative toxicity of cyclophosphamide results in myelosuppression, leukemia, infertility, hemorrhage cystitis and increased incidence of bladder cancer⁵⁹. Therefore, less harmful solutions are being sought. Intermittent intravenous cyclophosphamide administration resulted in lower cumulative dose and lower toxicity but fewer remissions, low recovery from dialysis, and more relapses and deaths⁴¹. To reduce the exposure to cyclophosphamide new trials have been developed⁴⁸. After a 3- to 6-month induction phase, cyclophosphamide can be replaced with azathioprine²³. Another agent acceptable in sustaining remission is methotrexate⁸⁸. This drug in combination with corticosteroids can also be used in the induction phase, but rather in patients with a limited form of the disease, without renal involvement, or in those who are intolerant to cyclophosphamide⁹⁶. Severe renal involvement is an indication for plasma exchange or pulse methylprednisolone in addition to the standard regimen. In patients with RPGN, pulse methylprednisolone results in the recovery of renal function even in those who are dialysis-dependent⁹⁴. Both methods of treatment are reported to have comparable effects, but the response to the plasma exchange is usually short lived⁷⁸. In pa-

tients who fail to respond to the optimal combination therapy or with the refractory disease, certain novel treatments more specifically interfering with the immune system are performed. Administration of intravenous (i.v.) Ig seems to be beneficial and the dosages of corticosteroids and cyclophosphamide can be decreased⁴⁶. Other studies are less optimistic, indicating that after i.v. Ig administration, complete remission is rare, renal function does not improve, and the relapse rate is high⁷⁹. Another approach aimed at interfering with T cell response involves such agents as antithymocyte globulin, humanized monoclonal anti-CD4 and anti-CD52 antibodies, tacrolimus, and mycophenolate mofetil⁶⁴.

One of the recent promising therapies is aimed at TNF blockade. Elimination of that proinflammatory cytokines by etanercept, a recombinant human antibody with TNF receptors attached in place of the antigen-binding site, or with infliximab, a chimeric monoclonal antibody, would interfere with the initial phase of vasculitis. TNF-blocking antibodies reduce interactions between neutrophils, EC and ANCA by inhibiting neutrophil priming and EC activation. Clinical studies have revealed that inclusion of etanercept to standard therapy with cyclophosphamide was associated with improvement and a decrease in vasculitis activity scores in the majority of patients. Additionally, daily prednisolone doses could be reduced⁵³. For cyclophosphamide-induced severe neutropenia, short-term treatment with low doses of granulocyte colony-stimulating factor (GCSF) can be introduced³⁸. Despite the proinflammatory potential of GCSF, exacerbation of the vasculitis does not occur due to the subnormal granulocyte levels.

The outcome in WG results from the effects of the disease and the hazards of the therapy. The most important factor in determining outcome is the presence of renal disease. Renal survival is inversely correlated with serum creatinine at presentation⁴⁴. Certain features of renal biopsy at diagnosis may also be indicative: the percentage of sclerosed glomeruli, the degree of tubular atrophy, and the extent of arterial sclerosis¹. About of 50% of the patients will experience at least one relapse in 4–5 years after the initial treatment²⁹. Relapses appear more commonly after rapid reduction of the immunosuppressants and after the exclusion of cyclophosphamide from the induction treatment⁴⁰.

Variations in the clinical presentation of WG and the high toxicity of the applied regimens indicate that treatment should be tailored to the individual patient and guided on a basis of discerning follow-up.

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