

Anti-Neutrophil Cytoplasmic Autoantibodies (ANCA) and Vasculitis

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Introduction

Vasculitis denotes inflammation in and around the vessel wall, often accompanied by necrosis. Vasculitic disorders may present with a multitude of signs and symptoms, many of which are common to inflammatory diseases, and there are often overlapping clinicopathologic features. An early diagnosis is important as dysfunction or irreversible damage to the organs involved in the vasculitic process develop easily, and certain forms, such as Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA), may rapidly take a fatal course unless aggressive immunosuppressive and anti-inflammatory therapy is instituted (1). The diagnosis of vasculitic disorders may be difficult because of the diverse clinical manifestations and the many different forms of presentation of these diseases and certain similarities to several systemic connective tissue diseases. Because of the paucity of pathognomonic clinical manifestations, a definitive diagnosis requires a constellation of clinical manifestations and confirmation by histopathology (2). Unfortunately, even biopsy findings may not be diagnostic for many reasons: too small or too few biopsies from selected areas, superinfection-induced tissue changes, and prior treatment.

A laboratory test that could strongly support the diagnosis of vasculitis was not available until the beginning of the 1980s. In 1982, Davies et al. described autoantibodies directed against neutrophil granulocyte cytoplasmic components (ANCA) in eight patients with renal vasculitis suspected to be caused by virus (3). In 1985,

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the connection between ANCA and WG was clearly established, showing their common presence in active disease (4), and this finding was later confirmed by others (5,6). Since then, our knowledge about these autoantibodies has grown remarkably. The use of ANCA in clinical practice is the main topic of this review.

ANCA Terminology

ANCA were first detected by indirect immunofluorescence (IIF), and this method is still the standard method for ANCA screening, though complementary tests are now available (7,8). The IIF technique is cheap and easy to perform, but both the methodological details and interpretation of results requires great experience. Two patterns can be distinguished: a finely granular cytoplasmic pattern with interlobular accentuation called classical ANCA (cANCA) (*see* Fig. 1A), and a perinuclear/nuclear pattern (pANCA) (*see* Fig. 1B). The major antigen recognized by cANCA is now known to be a serine protease (9) called by different names, such as proteinase 3 (PR3), myeloblastin, and azurophil granule protein 7 (AGP7) (10). The pANCA pattern is mainly caused by antibodies to myeloperoxidase (MPO), although it is now clear that the same or very similar pANCA pattern can be seen with antibodies to lactoferrin, cathepsin G, or elastase. These ANCA antigens derive from the granules of the neutrophilic granulocyte cytoplasm. The pANCA pattern is a fixation artifact. During fixation in ethanol, the granule membranes are disrupted and basic proteins (positively charged) are redistributed to the negatively charged nucleus (8,11).

Purified antigens are now commonly used in solid-phase systems to determine the antigenic specificity of ANCA, and such specified ANCA are designated PR3-ANCA, MPO-ANCA, and so on (12). Contrary to cANCA, pANCA can be found in a variety of vasculitic disorders, but also in other chronic inflammatory disease states, such as connective tissue diseases (13).

Clinical Associations

WG is typically characterized by granulomatous inflammation and necrotizing vasculitis involving the upper respiratory tract, the lungs, and the kidneys. WG can run a rapid clinical course leading to renal failure, respiratory failure, and death. To avoid this outcome, the diagnosis has to be made quickly so that suitable treatment can be initiated. The degree of granulomatous inflammation varies markedly from patient to patient. Some have extensive granulomatous inflammation, whereas others have minimal evidence of

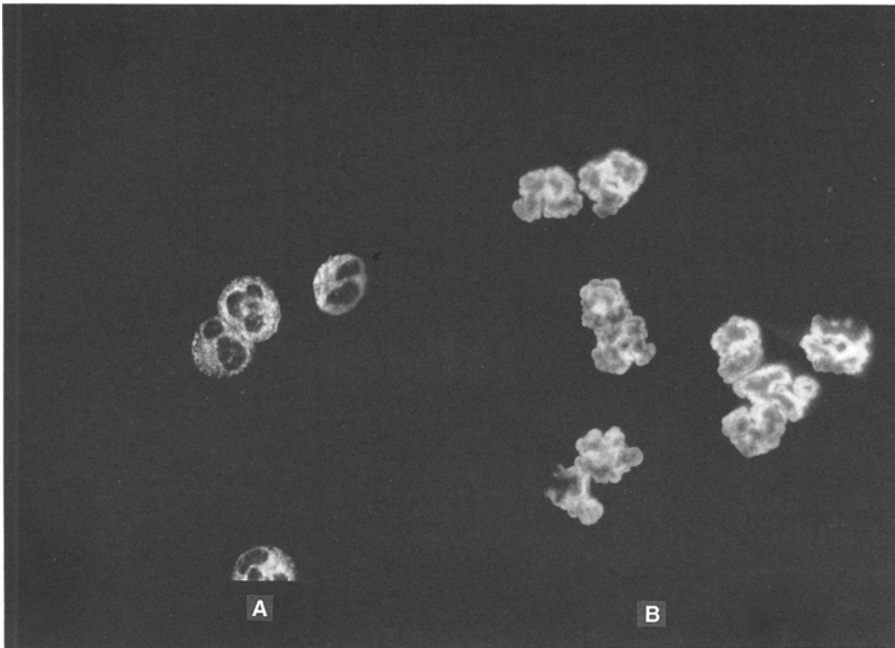


Fig. 1. Antineutrophil cytoplasmic autoantibodies as shown by indirect immunofluorescence using human leukocytes as antigen substrate. (A) Classical granular cytoplasmic pattern (cANCA). (B) Perinuclear pattern (pANCA).

it, making biopsy diagnosis extremely difficult. The diagnostic workup may be aided by finding a positive ANCA. cANCA has a rather high sensitivity in active WG: In systemic forms the sensitivity is above 95% and in the limited forms about 70% (6). A serum found positive for cANCA should be examined further in an enzyme-linked immunoabsorbent assay (ELISA) to establish whether it is PR3-ANCA or not. In patients with active WG, the percentage of patients having PR3-ANCA is slightly lower than those having cANCA (14). This discrepancy may be explained by loss of epitopes during protein purification or during coating onto ELISA plates. The discrepancy could also reflect a true difference in cANCA antigen specificity, as other antigens than PR3 may reside in the granules after the IIF procedure (15). Furthermore, titers of cANCA do not necessarily correlate to the ELISA values, although most positive cANCA are positive in the PR3-ANCA ELISA as well. Some patients with WG are cANCA negative at the beginning of the disease, but later on become positive. Once a patient has been found positive, cANCA may be used as a marker for disease activity, as titers seem to rise with disease activity, and relapses may be preceded by a rise

in ANCA titer by weeks to months (14,16). However, this is not true for all patients, and ANCA should not be used as the only marker for disease activity. In patients in complete remission, ANCA is most often not detectable, but some may continue to be positive. Patients in partial remission may have low titers of ANCA (5,16). It has been postulated that IgG class cANCA belonging to the IgG3 subclass is particularly found in active disease states (17,18), but not all studies confirm this (19). A summary of ANCA findings in vasculitis is found in Table 1.

MPA

This is a necrotizing vasculitis with few or no immune deposits and no granulomas (20). Crescentic necrotizing glomerulonephritis is very common, as is pulmonary vasculitis. Thus there are both clinical and pathological similarities as well as differences between WG and MPA. The ANCA pattern found in the latter is often pANCA, although cANCA is nearly as common, and, correspondingly, MPO-ANCA and PR3-ANCA are both common in these patients. An association to disease activity has also been described in MPA (13).

Churg-Strauss Syndrome (CSS)

This is characterized by eosinophil-rich granulomatous inflammation involving the respiratory tract and necrotizing vasculitis, associated with asthma and blood eosinophilia. This form of vasculitis is much more rare than WG and MPA, and examination of ANCA in a large group of patients is lacking. In small series, MPO-ANCA seem to be the most common (21).

Polyarteritis Nodosa (PAN)

Both pANCA and cANCA have been described in a few cases of classical PAN, but it is very difficult to see whether these patients in reality also had small vessel vasculitis, which, according to the Chapel Hill Conference consensus criteria, would categorize these patients as MPA patients (22).

Other Vasculitides

Although some authors report the presence of ANCA in other vasculitis syndromes, such as Kawasaki disease (23), IgA nephritis, and Henoch-Schönlein purpura, the frequency and titers are low and the antigenic specificity for neutrophil cytoplasm components are unknown.

Table 1
ANCA in Primary and Secondary Vasculitides^a

	cANCA	pANCA	PR3-ANCA	MPO-ANCA	Other ANCA
Wegener's granulomatosis	C	R	C	R	U
Microscopic polyangiitis	C	C	C	C	U
Churg-Strauss syndrome	U	C	U	C	U
Polyarteritis nodosa	R	R	R	R	U
Henoch-Schönlein purpura	U	U	U	U	U
Kawasaki disease					
Takayasu arteritis					
Secondary vasculitides ^b	R	R	R	R	U ^c

^aC = common, R = rare, and U = unknown.

^bIn connective tissue diseases.

^cSome authors find antilactoferrin in about one fourth of patients with rheumatoid vasculitis.

Vasculitis in Systemic Rheumatic Diseases

In vasculitis, complicating rheumatoid arthritis and systemic lupus erythematosus pANCA directed toward lactoferrin have been described (24,25), but such ANCA are not readily demonstrated by IIF because of the simultaneous presence of antinuclear antibodies, so specific ELISAs must be utilized. It should be emphasized that autoantibodies to neutrophils, which have been designated granulocyte-specific antinuclear antibodies (GS-ANA) (26), have been described in rheumatoid arthritis (25) and in ulcerative colitis (27,28). A substantial part of these antibodies are probably directed to cytoplasmic components of neutrophils (25), but further studies on these antibodies are needed before firm conclusions can be made.

It is well known that prolonged treatment with certain drugs may be complicated by vasculitis. Recently, Dolman et al. described PR3-ANCA and MPO-ANCA in patients with vasculitis associated with propylthiouracil therapy. In these cases, ANCA concentrations fell on withdrawal of the drug (29).

False-Positive ANCA

The use of neutrophils as antigen source for antibody detection may lead to some immunoglobulin binding because of the interaction of immune complexes with Fc-receptors on the cells and, hence, a false-positive ANCA result. Although there has only been few descriptions of this, these cases point out the need for a careful use of the IIF test (7,30). The nonspecific Fc-receptor reaction looks like an atypical pANCA, and since pANCA has been found in a variety of

diseases with and without vasculitis, this phenomenon should be kept in mind. It is difficult to talk about a false-positive test result, as the clinical spectrum in which this test should be used is not yet clarified. However, ANCA has been described in HIV-positive patients and in patients with tuberculosis of the lungs (31,32). The former may be complicated by vasculitis; the latter presents clinical difficulties, as the X-ray of the chest may mimic alterations characteristic of WG. Thus, a positive ANCA needs to be further examined by specific ELISA tests, and even if this is positive too, a positive test alone without sufficient clinical criteria for vasculitis should not lead to a vasculitic diagnosis or treatment.

Histopathology

There seem to be at least two features that are common to vasculitides accompanied by presence of ANCA: First, there is a predominance of neutrophils in and around the vessel wall; and, second, a characteristic fibrinoid necrosis involving arterioles, capillaries, and venules. In WG and CSS, granulomas are found in the vicinity of the vessels, and these are built up by macrophages and lymphocytes often with scattered giant cells. Whereas neutrophils predominate in the areas around the granulomas in WG, eosinophils predominate in CSS (1).

Pathogenicity

The close relationship between PR3-ANCA levels and WG has led to intensive research on a possible role for these autoantibodies in the pathogenesis of WG. Several possible mechanisms have been proposed: First, it has been shown that ANCA can further activate neutrophils that have been preactivated by cytokines (33,34); second, endothelial cells activated by cytokines may bring PR3 onto their surface after neosynthesis of PR3 (35), leading to antibody-dependent target cell destruction; and, third, ANCA may inhibit the neutralization of PR3 activity after lysosome component release from neutrophils (36). Tissue injury could then be a result of uncontrolled PR3 enzyme activity, since IgG can be degraded by PR3. These possible mechanisms are currently being explored.

Classification of Vasculitis

It is possible that the production of a given autoantibody reflects important aspects of a disease, such as genetic background, clinical manifestation spectrum, course, prognosis, and responsiveness to treatment (37). Whether presence of ANCA bears a similar relation-

ship to subpopulations of vasculitis patients is not yet known. The question arises, however, whether the various ANCA specificities should be included in a subclassification of primary vasculitides with the aim of collecting data from a large group of patients in a consecutive manner (22).

Conclusion

ANCA are now established to be of considerable value as a tool to support the diagnosis of certain vasculitides and for monitoring disease activity. Further research is needed to clarify whether ANCA have a pathogenetic role in these diseases, and it will be interesting to study both ANCA positive and negative patients with seemingly identical diseases to clarify whether ANCA may have prognostic significance and thus be useful for the classification of vasculitis.

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