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## Journal Article



N.B.: When citing this work, cite the original article.

Original Publication:

Maria Weiner and Mårten Segelmark, The clinical presentation and therapy of diseases related to anti-neutrophil cytoplasmic antibodies (ANCA), *Autoimmunity Reviews*, 2016. 15(10), pp.978-982.

<http://dx.doi.org/10.1016/j.autrev.2016.07.016>

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Postprint available at: Linköping University Electronic Press

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-132472>



**THE CLINICAL PRESENTATION AND THERAPY OF DISEASES RELATED TO  
ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA)**

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Short title: Diseases related to ANCA

## Abstract

Anti-neutrophil cytoplasmic antibodies (ANCA) are a family of autoantibodies that reacts with proteins predominantly expressed in cytoplasmic granules of polymorphonuclear neutrophil granulocytes (PMNs). ANCA was initially detected using indirect immunofluorescence, allowing for different patterns such as p-ANCA (perinuclear) and c-ANCA (cytoplasmic) to be distinguished. Today it is common to detect the antibodies by immunochemical assays such as ELISA using purified proteins as antigens. The strongest association with ANCA is found in the pauci-immune small vessel vasculitides granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA). There are compelling evidence that ANCA contribute to the pathogenesis in these conditions. ANCA also occur in 30-40 % of patients with eosinophilic granulomatosis with polyangiitis (EGPA) and anti-GBM disease, but is uncommon in other forms of vasculitis. ANCA with different specificities have been described with varying frequencies in diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, inflammatory bowel disease, endocarditis, chronic infections and hematopoietic malignancies. ANCA can also develop as an adverse event during pharmacological treatment. These entities are treated quite differently, with therapies ranging from immunosuppressive agents over antibiotics to simply removing the causative drug. A positive ANCA test thus requires a careful diagnostic work-up.

Key words: ANCA, vasculitis,

Take-home messages

- ANCA is a sensitive biomarker for certain forms of small vessel vasculitis but specificity is limited especially when detected with indirect immunofluorescence
- A careful diagnostic work-up is warranted as ANCA occurs in a wide range of conditions requiring very different actions.

## 1. Definition and nomenclature

Anti-neutrophil cytoplasmic antibodies (ANCA) are a family of autoantibodies that reacts with proteins predominantly expressed in cytoplasmic granules of polymorphonuclear neutrophil granulocytes (PMNs). ANCA was initially detected using indirect immunofluorescence (IIF), where positive staining of PMNs and negative staining of lymphocytes distinguish ANCA from other classes of autoantibodies such as ANA which stain all nucleated cells. IIF allows for different patterns such as p-ANCA (perinuclear) and c-ANCA (cytoplasmic) to be distinguished. Beside c- and p-ANCA other patterns have been recognized, and are often referred to as atypical-ANCA (a-ANCA). Today it is common to detect the antibodies by immunochemical assays such as enzyme linked immunosorbent assay (ELISA) using purified proteins as antigens[1]. Autoantibodies with specificity for myeloperoxidase are referred to as MPO-ANCA and those against proteinase-3 as PR3-ANCA. The c-ANCA pattern is in most cases caused by antibodies to PR3, but in some cases it can be traced back to reactivity with MPO or Bactericidal/permeability increasing protein (BPI). There is a variety of antigens that can be responsible for the p-ANCA pattern including MPO, elastase, lactoferrin, BPI and cathepsin G. Consequently the p-ANCA pattern is less specific regarding both autoantibody reactivity and disease association.

## 2. ANCA in vasculitis

The discovery of ANCA has totally changed diagnosis and classification of small vessel vasculitis. Today, ANCA associated vasculitis (AAV) is a collective term for three diseases: microscopic polyangiitis (MPA; formerly microscopic polyarteritis nodosa), granulomatosis with polyangiitis (GPA; formerly Wegener's granulomatosis) and eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss syndrome)[2]. There are

also compelling evidence that ANCA is a crucial mediator in the pathogenesis of these disorders.

## 2.1 GPA

Characteristic for GPA (formerly Wegener's granulomatosis) is granulomatous inflammation of the upper and lower respiratory tract in combination with necrotizing vasculitis affecting small vessels[2]. Typical symptoms are ear, nose and throat manifestations such as bloody nasal discharge, nasal crusting, sinusitis and otitis media. Destruction of the nasal cartilage can lead to the characteristic saddle nose deformity. Involvement of the lower respiratory tract with lung nodules and alveolar hemorrhage is also common, as is renal involvement.[3] Disease onset is most common at the age of 50-75 years. The incidence of GPA varies from around 2.5-10/million and there is a proposed north-south gradient with more GPA in the north of Europe[4]. Treatment of GPA is divided into induction treatment to achieve remission, and maintenance treatment to keep patients in remission and avoid relapses. Current induction therapies comprise high doses of glucocorticoids in combination with cyclophosphamide[5]. In case of localized disease, methotrexate can replace cyclophosphamide. Rituximab, a monoclonal anti-CD20-antibody that causes B-cell depletion, is now an alternative to cyclophosphamide[6]. Azathioprine is the first choice for maintenance of remission. Before the introduction of glucocorticoids and cyclophosphamide for the treatment of GPA, mortality was up to 90%. With the current treatment regimens, the disease has become more of a chronic relapsing-remitting disease. Remission is achieved in about 90%, but relapses occur in 50% or more during long-term follow-up. PR3-positivity and lung/ENT involvement is associated with increased relapse risk[7]. Relapses are often preceded by a rise in ANCA and very few patients are negative at time of relapse, but due to limited specificity serial measurements of ANCA cannot be used alone to guide treatment decisions.[8].

The type of ANCA usually associated with GPA is PR3-ANCA, but 10-20 % in Europe have MPO-ANCA (in Asia > 50%). Although called ANCA-associated vasculitis the diagnosis can be made in the absence of a positive ANCA-test. The fact that the great majority of patients with GPA are ANCA-positive and that titers often rise in active disease[9, 10] speaks in favor of a pathogenic role of ANCA, as do the genetic association between GPA and PRTN3 (the gene encoding PR3) and SERPINA1 (the gene encoding  $\alpha$ 1-antitrypsin, the inhibitor PR3)[11].

## 2.2 MPA

The hallmark of MPA is necrotizing vasculitis of small vessels and the most common manifestation is glomerulonephritis[12]. In contrast to GPA, granulomatous inflammation is not present[2]. Lung manifestations with pulmonary capillaritis occur, in the most severe cases in the form of massive lung bleedings. MPA is also associated with pulmonary fibrosis. Skin manifestations such as purpura, and involvement of peripheral nerves is also frequently seen. Sometimes glomerulonephritis is the only documented manifestation of the disease, and it is debated whether such cases should be called MPA or renal limited vasculitis. Patients with MPA are often older compared to patients with GPA, and the incidence for MPA varies between 3-15/million[13]. MPA is treated in a similar way as GPA, with induction of remission followed by maintenance therapy. Since relapses are more uncommon, especially in MPO-positive patients, the need for prolonged maintenance therapy is argued to be smaller than in GPA/PR3-vasculitis[14]. Mortality rates are higher in MPA[15], as the patients are older and more often have severe renal impairment. Early mortality is caused by infections and active vasculitis[16], whereas long-term risks include malignancies and increased cardiovascular risk. Most patients with MPA are MPO-ANCA positive at diagnosis, but 20-30 % have PR3-ANCA and the number of ANCA negative cases are small in most published series. Experimental mouse models have shown strong support for the pathogenic role of

MPO-ANCA in development of a clinical picture with glomerulonephritis and pulmonary capillaritis congruent with MPA in humans[17].

### **2.3 EGPA**

Eosinophilic granulomatosis with polyangiitis, formerly called Churg-Strauss syndrome is characterized by asthma, eosinophilia and granulomatous inflammation. Cardiac, skin and gastrointestinal engagement and involvement of the peripheral nervous system is common[18]. It is a rare disease with an incidence of around 1/million. EGPA is generally treated in a similar way as other AAVs, but in mild cases there is evidence that monotherapy with steroids may be sufficient[18]. EGPA is classified as an ANCA-associated vasculitis even though a majority of patients are ANCA-negative[2]. Between 30-40 % of EGPA patient test positive for MPO-ANCA, while PR3-ANCA is found only infrequently. There are reports that ANCA-positive EGPA differs from ANCA-negative EGPA. Renal involvement is more common among those testing positive for ANCA, while cardiac involvement show the opposite association[19]. Like in other forms of AAV, ANCA titres usually decrease in response to therapy and may rise again when the disease is reactivated.

### **2.4 Anti-GBM disease**

Anti-glomerular basement membrane disease, earlier called Goodpasture's syndrome or disease, is also a small vessel vasculitis[20]. Its characteristic hallmark is the linear deposits of IgG along the glomerular basement membrane. In most patients with active disease circulating autoantibodies specific for the non-collagenous domain of the alpha3 chain of type IV collagen can be detected by ELISA, and in addition about 30 % have ANCA. The vast majority of ANCA-positive anti-GBM patients have MPO-ANCA, but other specificities have been described. Patients with anti-GBM disease most often present with a very aggressive form of glomerulonephritis where most glomeruli are affected by crescentic lesions. A substantial proportion of the patients also have lung involvement. Hemoptysis is

the typical symptom, but less overt findings such as hypochromic anemia and diffuse pulmonary infiltrates are sometimes the only signs present. The therapy aims at curbing inflammation, removing toxic autoantibodies and stopping their production. These three goals are targeted by the simultaneous institution of high dose corticosteroids, plasma exchange and cyclophosphamide. Without therapy anti-GBM disease is usually lethal, but with aggressive therapy most patients survive and if detected early before all glomeruli have been affected by crescent formations there is also a good chance of renal recovery. Compared with ANCA negative patients, double-positive patients tend to be older, and some have features normally seen in GPA suggesting an overlap syndrome. It's debated whether double positive patients have a worse or better prognosis, but most authors agree that they have a greater tendency for relapse.

### **2.5 Other forms of vasculitis**

ANCA is usually absent in large vessel vasculitis such as Giant cell arteritis and Takayasu's syndrome. According to the definition in the Chapel Hill Consensus Conference nomenclature, classic polyarteritis nodosa (cPAN) is an ANCA-negative disease[2]. With older less stringent classifications there are reports of ANCA in a sizable proportion of PAN cases. Most of them would now probably be classified as MPA. In Kawasaki's disease, ANCA is infrequent[21]. In IgA-vasculitis (formerly known as Henoch-Schönlein purpura) there are reports of IgA-ANCA during episodes of active disease, but IgG-ANCA is rare[22].

### **3. ANCA in inflammatory bowel disease and liver diseases**

Next to vasculitis, ANCA has been most intensively studied in inflammatory bowel disease (IBD). ANCAs as well as several other autoantibodies have been shown to occur much more frequently among IBD patients as compared to healthy controls, but their usefulness as a biomarker and their possible role in the pathogenesis is debated.



### 3.1 Ulcerative colitis

Abdominal pain and frequent stools are the typical symptoms of ulcerative colitis (UC), a disease with inflammation confined to the colon. Steroids, topical or in severe cases systemically administered, are effective in bringing disease activity under control in most cases. To sustain remission, 5-aminosalicylic acid or sulfasalazine are used. In more severe cases purine analogues are recommended and in therapy failure TNF-alpha inhibitors or other biologicals. If necessary, colectomy is curative. A large percentage of UC patients have ANCA when detected by IIF[23-25]. The IF pattern is often referred to as atypical-ANCA (a-ANCA). Antigen specificity varies, only a few are PR3-ANCA or MPO-ANCA positive. In contrast to UC, Crohn's disease (CD) can affect the entire gastrointestinal tract. About half of CD patients have antibodies to the yeast *Saccharomyces cerevisiae* (ASCA), but ANCA is typically absent. It has been suggested that combining ANCA and ASCA is helpful in differentiating UC from CD[26].

### 3.2 Primary sclerosing colangiitis

Primary sclerosing colangiitis (PSC) is an insidious chronic liver disease with inflammation emanating from the bile ducts. Most cases of PSC occur as a complication to UC. Acute colangiitis and bile duct obstruction with jaundice and malabsorption can occur and ultimately liver cirrhosis develops. There is no effective treatment for PSC and the response to anti-inflammatory drugs is usually poor. Ursodeoxycholic acid is often tried. In advanced cases, liver transplantation is the only option. There are reports of ANCA positivity in 80 % or more in PSC[24]. ANCA does not seem to be associated with any specific features in PSC and there is no reported link to prognosis or response to treatment.

ANCA have been reported in a moderate to large percentage of most kinds of inflammatory liver disease. This includes conditions such as autoimmune hepatitis, primary biliary cirrhosis and chronic viral hepatitis[27]. Compared with PSC, titres tend to be lower. As with PSC and UC it is most often IIF positivity.

#### **4. ANCA in other systemic inflammatory conditions**

ANCA have been reported in huge variety of inflammatory conditions and in some diseases ANCA positivity has been shown to correlate with certain features. In some cases ANCA positivity heralds an overlap syndrome[28]. However, there is no proven benefit to search for ANCA in patients who are diagnosed with a non-vasculitic condition.

##### **4.1 Rheumatoid arthritis**

Rheumatoid arthritis (RA) is a common and severe chronic systemic inflammatory condition that primarily affects the synovial membrane of joints. It often starts with morning stiffness that slowly deteriorates into erosive arthritis. The course is often relapsing and remitting.

Early initiation of disease modifying drugs (DMARDs) such as methotrexate has been shown to significantly alter the course of the disease. When DMARDs are insufficient to control disease activity TNF-alpha inhibitors and other biologicals are employed. There are varying reports on the frequency of ANCA in RA[25]. The highest frequency is found among those with splenomegaly and neutropenia (Felty's syndrome) [29]. More patients are positive with IIF than with ELISA. Lactoferrin-ANCA seems to be the most common antigen.

##### **4.2 SLE**

Systemic lupus erythematosus (SLE) is a rheumatic disease primarily affecting young women. Inflammation and organ injury is driven by deposition of immune complexes along vessel walls. Typical features include skin rash, arthralgia and serositis, and in severe cases central nervous system engagement and renal involvement[30]. Steroids are useful to halt inflammation but in order to induce long-term remission a course with cyclophosphamide or mycophenolate mofetil is usually needed. Chloroquine, which blocks Toll-like receptor 9 signaling, is used in patients with moderate disease activity and during maintenance phase to prevent flares. There is a plethora of antibodies in SLE, some of them show ANCA specificity, a frequency of about 25 % have been reported[31]. ANCA in SLE often have low

affinity, and the frequency of positive results have been shown to go down considerably when increasing the stringency of ELISA testing by increasing the salt concentration in the buffers[32]. ANCA positivity in SLE has been shown to correlate to both disease activity and severity, being more common in patients with nephritis and CNS engagement.

#### **4.3 Other diseases**

ANCA detected by IIF has been reported in several other inflammatory diseases including Sjögren's Syndrome[33], systemic sclerosis, mixed connective tissue disease and reactive arthritis, usually with prevalence rates around 20 % [25].

### **5. ANCA in infective diseases**

ANCA (and other autoantibodies) are rarely analyzed in patients with infections even though infections of many are considered as the probable cause of AAV[34]. Most reports of ANCA in infections disease stems from situations where infections can mimic vasculitis or where patients with infections have been used as disease controls. For instance several features typical for tuberculosis (Tb) can also be found in GPA including cavitating pulmonary lesions and granulomatous inflammation. ANCA positivity by IIF has been shown in up to 40 %, but lower frequencies have also been reported [35]. Other autoantibodies are known to occur during or after infections. When diagnosing anti-phospholipid syndrome it is mandatory to re-analyze autoantibodies after 3 month before a diagnosis can be made[36].

#### **5.1 Endocarditis**

Bacterial colonization of the cardiac valves may lead to local meltdown with abrupt onset cardiac failure as well as systemic spread of infectious particles covered with antibodies and complement. The later may develop into a subacute inflammatory condition mimicking systemic vasculitis. The treatment is urgent administration of combinations of antibiotics; sometimes surgery is necessary to restore the integrity of the valves. There are several case

reports on both MPO-ANCA and PR3-ANCA in patients with infective endocarditis, and a larger study showing ANCA by IIF in 18 % and PR3- or MPO-ANCA in 8 % [37].

### **5.2 *Pseudomonas aeruginosa* infection in Cystic fibrosis**

Cystic fibrosis (CF) is a genetic disorder caused by mutations in an epithelial chloride channel, leading to mucus stagnation, chronic airway colonization and decreased lung function. Infection with *Pseudomonas aeruginosa* (Pa) is common. Exercise and physical therapy in order to mobilize stagnant mucus is most important, but early aggressive antibiotic treatment can prevent permanent colonization. In CF, 50 % or more have ANCA with specificity to BPI in ELISA, and this is strongly linked to Pa infection [38]. There is also a correlation between BPI-ANCA and disease severity and as well as with prognosis.

### **5.3 HIV**

The human immunodeficiency virus (HIV) selectively and progressively destroys CD4 positive T-helper cells leading to severe impairment of adaptive immunity. Similarly to genetic immunodeficiencies, HIV infections can be accompanied by hypergammaglobulinemia, autoantibody production and other autoimmune manifestations. ANCA have been described in a substantial proportion of sera from patients with HIV infections, varying from a few up to over 80 % of tested specimens [39]. There are also cases described where ANCA positive small vessel vasculitis develop [40].

### **5.4 Amoeba infection**

Invasive infections with *Entamoeba histolytica* result in liver abscesses and colitis. PMNs are known to participate in the defense against the invasion and their activation leads to release of proteolytic enzymes and collateral damage, including blood vessel injury. In one study 97 % of patients were ANCA positive, 75 % with PR3-ANCA specificity by ELISA [41]. Treatment leads to declining titers.

## **6. ANCA in malignancies**

Autoimmune manifestations sometimes occur as paramalignant phenomenon. This is especially common in different forms of hematological neoplasia's. There are reports of systemic vasculitic syndromes in conjunction with thymomas, lymphomas and myelodysplastic syndromes, some of which have typical PR3- or MPO-ANCA serology[42]. There are also several reports of ANCA without any vasculitic symptoms in for instance Hodgkin lymphoma and monoclonal gammopathy[43, 44].

## **7. ANCA in adverse drug reactions**

While isolated cutaneous vasculitis can occur as an adverse event during treatment with many drugs, systemic inflammatory conditions is much rarer and have been described only for a limited number of pharmaceutical agents. Such reaction can be lupus like or they can mimic systemic small vessel vasculitis[45].

### **7.1 Hydralazine**

Hydralazine is an old anti-hypertensive drug, rarely used today. Production of autoantibodies is common during hydralazine treatment and patients may develop either a lupus-like or an AAV-like condition. Patients with hydralazine-induced vasculitis typically have very high levels of MPO-ANCA along with ANCA of different specificities such as elastase-ANCA and lactoferrin-ANCA[45]. In mild cases it is sufficient to stop the drug treatment, but in severe cases immunosuppression is needed. There is no need for maintenance therapy.

### **7.2 Propylthiouracil**

Propylthiouracil (PTU), a drug used to treat hyperthyroidism, is strongly linked to ANCA. Studies have shown that MPO-ANCA can be found in around 20 % of patients taking the drug and among these around 20 % develop vasculitic symptoms[46]. In most cases there are

only mild skin lesions, but severe multi-organ failure may occur. A correlation between disease severity and affinity of MPO-ANCA has been reported.

### 7.3 Levamisole

There are many case reports and a few case series of AAV in cocaine abusers, and this is attributed to contamination with levamisole, a veterinary drug used for deworming. Multiple ANCA specificities such as MPO-ANCA and elastase-ANCA are seen, along with neutropenia and destructive nasal lesions.

## 8. Concluding remarks

Even if ANCA is a sensitive marker for pauci-immune small vessel vasculitis, ANCA especially detected IIF, is a rather unspecific phenomenon. A common characteristic of most of the conditions where ANCA develop is an increased turnover and/or impaired non-apoptotic clearance of PMNs resulting in an increased burden of neutrophil debris. Autoantibodies with ANCA specificity belong to the repertoire of natural antibodies appearing without specific immunization[47]. It has been suggested that ANCA aid in the clearance of PMN debris, such as brake-down fragments of neutrophil extracellular traps (NETs)[48, 49]. As ANCA also have been shown to induce NETs in vitro it is possible that ANCA reacting with antigens present on the surface of PMNs starts a vicious circle. Surface expression of ANCA antigens have only been described for PR3 and MPO, which might explain the close relationship with small vessel vasculitis and their better performance as biomarkers in these diseases.

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