Antineutrophil cytoplasmic antibodies

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Lancet 2006; 368: 404–18

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Much like other autoantibodies (eg, anti-double stranded DNA in systemic lupus erythematosus or antiglomerular basement membrane antibodies in Goodpasture's syndrome), antineutrophil cytoplasmic antibodies (ANCA) have provided doctors with a useful serological test to assist in diagnosis of small-vessel vasculitides, including Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, and their localised forms (eg, pauci-immune necrotising and crescentic glomerulonephritis). 85–95% of patients with Wegener's granulomatosis, microscopic polyangiitis, and pauci-immune necrotising and crescentic glomerulonephritis have serum ANCA. ANCA directed to either proteinase 3 or myeloperoxidase are clinically relevant, yet the relevance of other ANCA remains unknown. Besides their diagnostic potential, ANCA might be valuable in disease monitoring. In addition, data seem to confirm the long-disputed pathogenic role of these antibodies. Present treatments for ANCA-associated vasculitis are not free from side-effects and as many as 50% of patients relapse within 5 years. Accurate understanding of the key pathogenic points of ANCA-associated vasculitis can undoubtedly provide a more rational therapeutic approach.

Antineutrophil cytoplasmic antibodies (ANCA) are predominantly IgG autoantibodies directed against constituents of primary granules of neutrophils and monocytes' lysosomes. Although several antigenic targets have been identified, those ANCA directed to proteinase 3 or myeloperoxidase are clinically relevant, whereas the importance of other ANCA remains unknown. Both are strongly associated with small-vessel vasculitides, the ANCA-associated vasculitides, which include Wegener's granulomatosis, microscopic polyangiitis, and Churg-Strauss syndrome, and the localised forms of these diseases (eg, pauci-immune necrotising and crescentic glomerulonephritis).[1]

ANCA were discovered by chance in 1982 when Davies and associates[2] were studying antinuclear antibodies in serum samples from patients with segmental necrotising glomerulonephritis. Using indirect immunofluorescence applied to neutrophils, a diffuse cytoplasmic—but not nuclear—staining pattern was observed. In 1985, van der Woude and colleagues[3] noticed that cytoplasmic ANCA mainly arose in patients with Wegener's granulomatosis and interest in these antibodies skyrocketed. In 1988, Falk and Jennette[4] reported a distinct perinuclear pattern in serum samples from patients with systemic vasculitis and idiopathic necrotising and crescentic glomerulonephritis. The researchers showed by ELISA that myeloperoxidase was the chief antigenic target of perinuclear ANCA. 2 years later, proteinase 3 was recognised as the major autoantigen accounting for the cytoplasmic ANCA pattern of Wegener's granulomatosis.[5,6]

Data from animals seem to confirm the long-disputed pathogenic role of ANCA, and as a result, research into these antibodies is gaining momentum once again. Understanding of their intimate pathogenic mechanisms will lead to development of new therapeutic strategies in the not-too-distant future.

Here, we aim to provide doctors with a comprehensive review about ANCA. We will pay special attention to their clinical usefulness and pathogenic role.

Search strategy and selection criteria

We searched MEDLINE and PubMed for published work with the keywords "ANCA", "antineutrophil cytoplasmic antibodies", "ANCA-associated vasculitis", "Wegener granulomatosis", "Wegener's granulomatosis", and "microscopic polyangiitis" from 1982 (when antineutrophil cytoplasmic antibodies were first reported) to March, 2005. We also searched the reference lists of articles identified by this strategy and selected those we judged relevant. Publications were selected for review on the basis of original research and evidence-based reviews.

Pathogenesis

Current treatments for ANCA-associated vasculitides (ie, glucocorticoids and immunosuppressants) are not free from side-effects and 25% of patients have severe adverse events.[7] Furthermore, up to 50% of individuals in remission will relapse within 5 years.[8] There is, thus, an urgent need for less toxic and more effective treatments than are currently available. Accurate understanding of the pathogenic key points of ANCA-associated vasculitides will provide a more rational therapeutic approach. Transfer of laboratory findings to state-of-the-art management is underway.

Are ANCA pathogenic?

On the basis of the association of ANCA with the aforementioned small-vessel vasculitis, a pathogenic role has always been suspected. Since 1990, when Falk and associates[9] showed that ANCA can stimulate neutrophils to undergo a respiratory burst and release primary granule constituents, many in-vitro studies have revealed that these antibodies might cause in-vivo vascular damage by inducing a wide range of neutrophil effector functions, such as cytokine and chemokine release, and increased adhesion to cultured endothelial cells with eventual lysis of them.[10] These 15 years of in-vitro work in human beings

are invaluable to understand the mechanisms by which ANCA induce vasculitis. Further, their pathogenic role has been reinforced after findings in animals showed that ANCA do cause human-like vasculitis. Xiao and colleagues[11] immunised myeloperoxidase-knockout mice with murine myeloperoxidase. When myeloperoxidase-immunised splenocytes were transferred to mice lacking B-functioning and T-functioning lymphocytes [Rag2 (–/–)] (figure 1), myeloperoxidase ANCA arose in a dose-dependent manner. Mice that were administered the highest amount

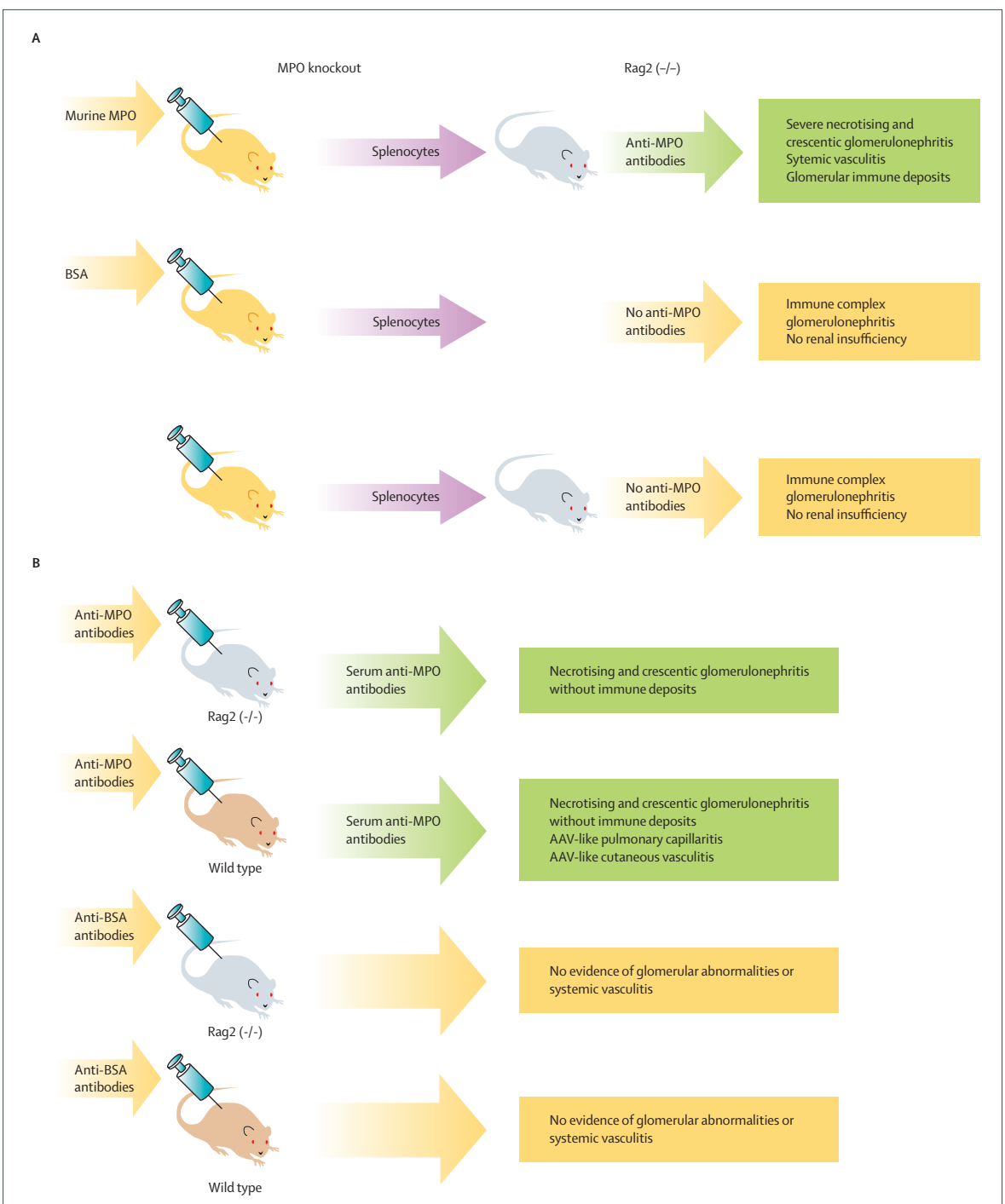

Figure 1: **Animal model of adoptive transfer of mouse anti-myeloperoxidase (MPO) splenocytes (A) and passive transfer of mouse anti-MPO IgG antibodies (purified from MPO knockout mice) (B)[11]**
Rag2 (–/–)=recombinase-activating gene 2 knockout mice. AAV=ANCA-associated vasculitis. BSA=bovine serum albumin.

of myeloperoxidase-immunised splenocytes developed severe necrotising and crescentic glomerulonephritis and systemic vasculitis, including pulmonary capillaritis. However, all mice receiving the highest splenocyte dose developed non-severe immune complex-mediated glomerulonephritis. In a second step, the researchers injected myeloperoxidase ANCA into Rag2 (–/–) and wild-type mice, which will trigger anti-idiotype antibodies, which will react with the original autoantigen. Both strains presented focal necrotising and crescentic glomerulonephritis without immune complexes. Xiao and colleagues thus concluded that myeloperoxidase ANCA possess the intrinsic ability to produce pauci-immune necrotising and crescentic glomerulonephritis. However, because renal lesions in Rag2 (–/–) mice receiving myeloperoxidase ANCA were not as widespread as those seen in Rag2 (–/–) mice that were given myeloperoxidase-immunised splenocytes, other factors (eg, T lymphocytes, low-level immune complex deposition) might enhance the inflammatory process. Although ANCA-associated vasculitides do not have, by definition, immune complexes, immune deposits were shown by electron microscopy in 50% of renal biopsy specimens from patients with ANCA-positive necrotising and crescentic glomerulonephritis, necrotising arteritis, or both.[12]

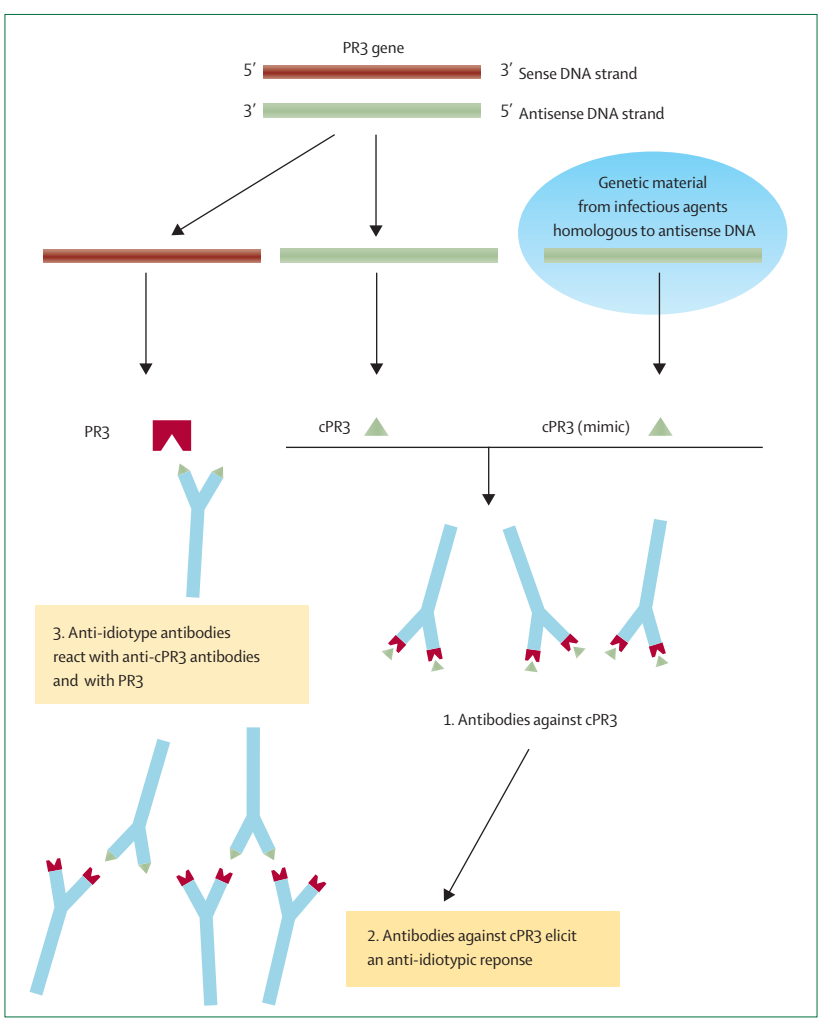

***Figure 2*: Generation of proteinase 3 ANCA according to the autoantigen complementarity theory[13]**
cPR3=complementary peptide of proteinase 3.

Why do ANCA appear?

Although nobody has a definitive answer to this pivotal question, two attractive theories have been postulated. Pendergraft and co-workers[13] suggested that generation of proteinase 3 ANCA could be attributable to so-called autoantigen complementarity. The theory relies on the assumption that a given peptide, encoded by the sense DNA strand, binds to the peptide encoded by the antisense strand (complementary peptide). Accordingly, autoantibodies in autoimmune diseases would not be elicited by an autoantigen or its mimic, but they would be by the complementary peptide or its mimic. This complementary peptide can originate from transcription of either endogenous antisense DNA or identical genetic material from an infectious agent. This peptide can lead to production of antibodies, which will trigger an anti-idiotype response, and the latter antibodies will also react with the original autoantigen (figure 2). With a recombinant complementary peptide corresponding to the proteinase 3 middle region (cPR3[105–201]), Pendergraft and colleagues showed that seven of 34 patients with vasculitis associated with proteinase 3 ANCA also harboured antibodies to cPR3(105–201). By contrast, only one of 89 controls had such antibodies. To test this finding, mice were immunised with cPR3(105–201) and they produced not only antibodies specific to cPR3(105–201) but also proteinase 3 ANCA. Both types of antibodies were distinct and did not cross-react, making an idiotypic pair. Furthermore, 50% of patients with proteinase 3-ANCA vasculitis showed cPR3(105–201) transcripts in circulating leucocytes by real-time PCR. For the exogenous source of complementary peptides, multiple proteinase 3-encoding gene complementary sequences were identified, including sequences from *Staphylococcus aureus* and *Entamoeba histolytica*, pathogens reportedly associated in proteinase 3 ANCA production in human beings.[14,15] These results lent support to the role of infectious agents as triggering factors of vasculitis associated with ANCA and might account for both the common influenza-like symptoms at the onset of ANCA-associated vasculitides and the association between chronic nasal carriage of *S aureus* and higher relapse rate in patients with Wegener's granulomatosis.[14]

Other researchers suggest that a dysfunction in neutrophil apoptosis might lead to ANCA generation. When neutrophils undergo apoptosis, primary granules constituents translocate to cell surface.[16,17] In two experiments,[18,19] injection of apoptotic neutrophils into rodents resulted in the presence of ANCA. Uptake of apoptotic cells is typically undertaken by macrophages.

Under certain conditions, dendritic cells might also take up apoptotic neutrophils, leading to cross-presentation of self-antigens, activation of specific T lymphocytes, and, ultimately, to autoimmunity.[20] Clayton and colleagues[21] showed that immature dendritic cells took up human apoptotic neutrophils, although a decrease in T lymphocyte proliferation took place. Nevertheless, addition of tumour necrosis factor α counteracted this suppressor effect. For ANCA to appear, apoptotic bleb aberrant capture by dendritic cells might not suffice and a second signal can be needed to achieve cross-presentation of self-antigens. Tumour necrosis factor α presence during the influenza-like symptoms heralding ANCA-associated vasculitides might be that signal.[20] However, if apoptotic neutrophils were the triggering factor of ANCA production, ANCA-associated vasculitides patients would be expected to have antibodies against a range of antigens (not just proteinase 3 ANCA or myeloperoxidase ANCA), because all granule constituents are available on apoptotic cells.

Where do ANCA bind?

In-vitro studies have shown that neutrophil priming with tumour necrosis factor α (perhaps taking place in vivo as a result of infection) allows myeloperoxidase and proteinase 3 to travel to the cell surface. Yang and associates[22] showed that myeloperoxidase and proteinase 3 genes (silenced in mature neutrophils) become expressed in patients with vasculitis associated with ANCA, with amounts of transcripts correlating with disease activity. These researchers suggested that such activation might increase antigen availability on the plasma membrane. ANCA bind to target antigens via their F(ab')2 portion and to neutrophil Fc-gamma receptors IIa and IIIb via the Fc portion.[20] Substantial evidence suggests that the main epitopes recognised by proteinase 3 ANCA locate near the enzyme's catalytic site.[23] Proteinase 3 ANCA can inhibit the action of this enzyme and prevent binding of its inhibitor α1 antitrypsin at the catalytic site, resulting in unregulated protease activity.[24,25] Variations in the affinity of proteinase 3 ANCA with their antigen were noted when comparing serum samples from patients with active disease with patients in remission, who showed more effective inhibition of proteinase 3 cleavage activity.[23,26] Such variations may influence the course of disease in a given patient. Epitope mapping studies have shown that myeloperoxidase ANCA recognise a restricted number of epitopes on the heavy chain of myeloperoxidase. Unlike proteinase 3 ANCA, myeloperoxidase ANCA do not impede myeloperoxidase enzymatic activity. Even though myeloperoxidase ANCA can interfere with caeruloplasmine-induced myeloperoxidase physiological inhibition, the clinical importance of this effect is unknown.[27]

How do ANCA act?

Binding of ANCA causes activation of neutrophils, with subsequent increased adhesion and migration to endothelium, release of proteolytic granule enzymes (including myeloperoxidase and proteinase 3) and proinflammatory cytokines, generation of respiratory burst, and, eventually, endothelial cell damage. Both ANCA F(ab')2 and Fc engagements are needed to allow for effective neutrophil activation. β2 integrin (CD11b/CD18) might cooperate with the Fc-gamma receptor to propagate the signal. ANCA-induced intracellular signal transduction pathways are different depending on whether the signal is initiated by the F(ab')2 or Fc portion of the autoantibody.[28] The ANCA IgG F(ab')2 fragment can activate inhibitory G proteins and RAS p21 protein activator but not tyrosine kinases (sarcoma virus kinases, SYK, PI 3 kinase, protein kinase B, and protein kinase C) whose activation probably relies on the Fc-gamma receptor binding.[29,30] Tyrosine kinase pathways are thought to induce respiratory burst through activation of NADH oxidase.[31] Inhibitory G protein and tyrosine kinase pathways can have a cooperative relation in oxidative burst generation because they converge on the GTPase RAS p21 protein activator.[29] Transient enhanced activity of RAS p21protein activator was reported to precede a rise in superoxide production.[30]

By using microarray technology, Yang and colleagues[32] noted that ANCA IgG F(ab')2 and ANCA IgG can activate a common subset of genes. The researchers also showed that different changes in gene expression happened depending on ANCA IgG F(ab')2 or ANCA IgG binding. In particular, the cyclooxygenase2 gene (*PTGS2*) was selectively induced by IgG F(ab')2, whereas differentiation-dependent gene-2 and interleukin 8 gene were activated by both ANCA IgG F(ab')2 and the whole antibody. This in-vitro activation mimicked changes in neutrophils from patients with vasculitis associated with ANCA.

Binding of ANCA allows neutrophils to adhere in a fixed, stationary way and migrate to previously cytokine-primed (tumour necrosis factor α) endothelium, which presents enhanced expression of adhesion molecules.[8] ANCA promote the interaction between neutrophils and endothelial cells depending on β2 integrins (CD11a/CD18 and CD11b/CD18) and GTP binding protein chemokine receptors.[33] Once adhered, noxious neutrophils' constituents—reactive oxygen species and proteolytic enzymes—are secreted and ready to damage vessel walls. Released myeloperoxidase and proteinase 3 can bind to endothelial cells and then interact with circulating ANCA, allowing for additional cytotoxic effects. Proinflammatory cytokines secreted by neutrophils as a result of ANCA binding include interleukin 1 β, tumour necrosis factor α, interleukin 6, interleukin 8, monocyte chemoattractant protein 1, and leukotriene B4. ANCA-mediated cytokine secretion will activate and recruit extra inflammatory cells, amplifying and perpetuating the inflammatory response, with monocytes and T cells participating later in the process.[1,8,20]

T cells might have a major role in ANCA-associated vasculitides. Supportive data include: (1) ANCA are high-affinity, class-switched antibodies and their generation

necessarily relies on T cells; (2) T cells accumulate in the kidney and their number correlates with renal impairment;[34,35] (3) T cells in patients with vasculitis associated with ANCA react to proteinase 3 and myeloperoxidase in proliferation assays;[20] and (4) T-cell reactivity markers (eg, cytotoxic T lymphocyte-associated antigen 4) are increased in active disease.[36]

Rituximab, an anti-CD20 monoclonal antibody against B cells, was proved effective in patients with refractory ANCA-associated vasculitides. Remission was achieved in all patients and maintained while circulating B lymphocytes were absent.[37] This finding, and the fact that disease-inducing ANCA must be produced by B cells, points to a fundamental intervention of them in the ANCA-associated vasculitides pathogenic puzzle.

ANCA-mediated neutrophil activation disrupts apoptosis by delaying expression of apoptotic neutrophil surface phosphatidylserines, which are necessary for macrophage-mediated cell removal. Because of delayed clearance, neutrophils undergo secondary necrosis with subsequent release of inflammatory mediators, hence amplifying the process.[38] Furthermore, ANCA-opsonised apoptotic neutrophils enhance both the phagocytotic activity of macrophages and their production of proinflammatory cytokines.[39]

By contrast, Abdel-Salam and colleagues[40] detected a low affinity of proteinase 3 ANCA for their antigen, challenging the activating role of ANCA. In this study, only high concentrations of ANCA permitted their binding. However, these results do not exclude the possibility that ANCA, at high amounts, could interact with primed neutrophils at the capillary lumen and thus exert their pathogenic effects.[41] ANCA can also induce polymerisation of actin cytoskeleton, thus strengthening neutrophil rigidity, which can contribute to their sequestration in capillaries. This might account for why ANCA-associated vasculitides have a predilection for small vessels.[42]

Who will develop ANCA-associated vasculitis?

Although evidence is still scarce, the above-mentioned pathogenic mechanisms might make ANCA-associated vasculitides easier to develop in individuals harbouring some genetic and environmental propensities.

Genetic factors

The baseline level of expression of proteinase 3 on the surface of resting neutrophils varies widely in individuals and remains steady over time. Many investigations have shown highest degrees of proteinase 3 surface expression in people with Wegener's granulomatosis. Such patients are more prone to clinical relapse.[43–45] The higher the surface expression of proteinase 3, the easier the proteinase 3 ANCA binding might be. High expression of proteinase 3 in the neutrophil membrane has also been associated with higher amounts of neutrophil degranulation and superoxide generation.[46] A polymorphism [A(–564)G] in the proteinase 3 promoter region, which can lead to overexpression of this enzyme, has been linked to Wegener's granulomatosis.[47] However, Pieters and colleagues[48] showed that this polymorphism does not cause the increased amounts of proteinase 3 on the surface of neutrophils that are seen in individuals with Wegener's granulomatosis.

Patients with Wegener's granulomatosis have a 100-fold increased carrier frequency of the defective *Z* allele of the SERPINA1 gene of α1 antitrypsin. By extrapolation, only about 5% of individuals with Wegener's granulomatosis have this allele.[49] Such patients have a more disseminated disease and a worse prognosis, probably accounted for by an imbalance between proteinase 3 and its inhibitor.[50]

Patients with Wegener's granulomatosis with certain genotypes of polymorphisms of Fc-gamma IIa (homozygous for the Arg131 form) and IIIa (homozygous for the Phe158 form) receptors are greater prone to clinical relapse. These polymorphisms are not associated with the highest degree of ANCA-mediated neutrophil activation but with decreased Fc-gamma-mediated bacterial clearance, which might be relevant to the chronic nasal carriage of *S aureus*.[51]

By investigating polymorphisms in adhesion molecules associated with the interaction between neutrophils and endothelial cells, Genzik and colleagues showed[52] that a restriction fragment-length polymorphism in exon 11 of the *CD18* gene was associated with myeloperoxidase-ANCA-associated vasculitis. The researchers suggested that a common variant of *CD18* (*AvaII+* allele) might predispose to the development of vasculitis by a CD18 quantitative regulation, by means of the highest proadhesive behaviour, or by both processes.

Despite the fact that evidence for association of complement in ANCA-associated vasculitides is scarce, Persson and colleagues[53] reported an amplified frequency of certain alleles of polymorphisms in *C3* (*C3F* allele) and *C4* (*C4A3* allele) genes in patients with ANCA-associated vasculitides.

Interleukin 10 has a pivotal role in the polarisation of T cells towards T-helper-2 and immunoglobulins production by B cells. A microsatellite CA repeat (interleukin 10.G)—located in the promoter region of interleukin 10 gene and associated with high antibody production—has been linked to Wegener's granulomatosis.[54] A rise in interferon-γ +874 T/T and tumour necrosis factor α-238 G/A genotypes, which are associated with high expressor cytokine phenotypes, has also been detected in individuals with Wegener's granulomatosis.[55]

While using an extended association screen with 202 microsatellite markers—representing apoptosis-related genes—in 150 patients with Wegener's granulomatosis, Jagiello and co-workers[56] found an association between one microsatellite allele pattern in the immediate vicinity of the retinoid X receptor β gene (*RXRB*) and the disease. The *RXRB* gene locates in the major histocompatibility complex region between major histocompatibility complex class II, DP beta 1 (HLA-

DPB1), and death-associated protein 6 (DAXX). Further analysis revealed a strong link between HLA-DPB1 0401 allele and Wegener's granulomatosis. Certain HLA-DPB1 alleles are implicated in chronic beryllium disease, another granulomatous lung disorder. Furthermore, the researchers identified an extended haplotype DPB1*0401/RXRB03, which showed an even stronger association with Wegener's granulomatosis.

CTL4 plays an important part in T-cell activation downregulation. The frequency of the shortest allele (86) of an (AT)n microsatellite polymorphism of the *CTLA4* gene is decreased in Wegener's granulomatosis patients.[57] This polymorphism might account for a hyperactive immune response.[49] Another polymorphism in the promoter region of the *CTLA 4* gene [C(–318)T] has been associated with Wegener's granulomatosis, although it is unlikely to have functional importance.[58]

Environmental factors

Many case-control studies have shown an association between silica exposure and ANCA detection, ANCA-associated vasculitides, or both.[59–62] In a series of 65 patients with ANCA-associated vasculitides, Hogan and associates[61] showed that the odds ratio of silica dust exposure was 4·4 times greater in patients compared with controls. Lane and colleagues[62] reported a substantial association between high occupational exposure to silica and Churg-Strauss syndrome (odds ratio 5·6) and ANCA positivity (4·9). Because silica particles are powerful stimulators of T cells and B cells, their inhalation by susceptible individuals might trigger production of autoantibodies including ANCA. Moreover, release of proteinase 3 or myeloperoxidase (and subsequent ANCA production) can be induced by activation of monocytes and macrophages by silica.[61] Data from in-vitro and murine studies have shown that silica accelerates apoptosis.[63,64] Silica might cause disturbances in normal apoptosis leading to cross-presentation of self-antigens by dendritic cells. In view of the growing evidence on the potential role of silica in ANCA-associated vasculitides pathogenesis, it seems reasonable to explicitly ask patients with these disorders about previous or current exposure to specific sources. Doctors can then consider whether such individuals who remain actively exposed to silica should be advised to move away from its source, which might improve their disease prognosis.

Other occupational factors associated with ANCA-associated vasculitides, ANCA positivity, or both (although to a lesser degree than silica) are farming (especially livestock), high occupational solvent exposure, allergy in general,[62] asbestos,[65] and pesticides.[66]

Cocaine-induced face midline destructive lesions have been linked to ANCA displaying double-specificity for human neutrophil elastase and proteinase 3. This association helps to distinguish midline lesions arising as a result of sniffing cocaine from those in Wegener's granulomatosis. In patients with this disorder, ANCA

	Reference
Infections	
Tuberculosis	Flores-Suarez[71]
HIV/AIDS	Klaassen[72]
Malaria	Yahya[73]
Hepatitis C	Wu[74]
Subacute endocarditis due to *S aureus* or streptococci	Hellmich;[75] Choi[76]
Parvovirus B19	Chou[77]
Leprosy	Medina[78]
Pseudomonas (cystic fibrosis)	Carlsson[79]
Aspergillosis	Cho[80]
Histoplasmosis	Mead[81]
Leptospirosis	Constantin[82]
Amoebiasis	Pudifin[15]
Pulmonary sporotrichosis	Byrd[83]
Digestive disorders	
Inflammatory bowel disease	Saxon[84]
Primary sclerosing cholangitis	Schwarze[85]
Autoimmune hepatitis	Schwarze[85]
Primary biliary cirrhosis	Sobajima[86]
Neoplasms	
Carcinoma	Vassilopoulos[87]
Lymphoma	Savige[88]
Liebow's disease	Savige[88]
Chronic myelocytic leukaemia	Chevailler[89]
Myelodysplasia	Savige[88]
Monoclonal gammopathies	Esnault[90]
Drugs	
Propylthiouracil	Slot[69]
Hydralazine	Short[91]
Methimazole	Guma[92]
Minocycline	Elkayam[93]
Carbimazole	Miller[94]
Allopurinol	Choi[95]
Cocaine	Wiesner[67]
D-penicillamine	ten Holder[70]
Phenytoin	ten Holder[70]
Levamisole	ten Holder[70]
Pimagedine	ten Holder[70]
Connective-tissue diseases	
Systemic lupus erythematosus	Schnabel[96]
Rheumatoid arthritis	Bosch[97]
Felty's syndrome	Juby[98]
Systemic sclerosis	Ruffatti[99]
Dermatomyositis	Merkel[100]
Sjögren's syndrome	Font[101]
Mixed connective tissue disease	Cooper[102]
Reactive arthritis	Locht[103]
Ankylosing spondylitis	Lotch[103]
Juvenile chronic arthritis	Mulder[104]
Relapsing polychondritis	Papo[105]
Eosinophilia myalgia syndrome	Schnabel[106]

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Systemic vasculitides other than ANCA-associated vasculitis	
Polyarteritis nodosa	Baranger[107]
Horton's arteritis	Bosch[108]
Takayasu's arteritis	Uppal[109]
Schönlein-Henoch purpura	Saulsbury[110]
Kawasaki disease	Rider[111]
Behçet's disease	Baleva[112]
Cryoglobulinemic vasculitis	Lampretch[113]
Renal diseases	
Poststreptococcal glomerulonephritis	Ardiles[114]
IgA nephropathy	van den Wall Bake[115]
Membranous nephropathy	Dwyer[116]
Antiglomerular basement membrane disease	Bosch[117]
Other disorders	
Silica exposure	Hogan[61]
Sarcoidosis	Forde[118]
Sweet syndrome	Burrows[119]
Idiopathic pulmonary haemosiderosis	Attia[120]
Retroperitoneal fibrosis	Sakemi[121]
Erythema elevatum diutinum	Ayoub[122]

Table 1: **Disorders other than ANCA-associated vasculitis, in which ANCA have been detected either by indirect immunofluorescence or ELISA**

will react with proteinase 3 but not with neutrophil elastase.[67] This double-reactivity suggests the existence of cocaine-induced polyclonal B-cell stimulation, an event noted also with other drugs (eg, allopurinol, D-penicillamine). The almost universal nasal carriage of *S aureus* in cocaine abusers might also account for the presence of ANCA in those with midline destructive lesions. Finally, bearing in mind the association between ANCA and infections, a persistent superinfected necrotic tissue in cocaine-induced lesions might account for the existence of ANCA in such cases.[68]

Among drug treatments, propylthiouracil, hydralazine, methimazole, carbimazole, D-penicillamine, and minocycline have mostly been implicated in the development of ANCA-associated vasculitides.[69,70] Other uncommon reported agents are listed in table 1. Cases of drug-induced ANCA-associated vasculitides are not as severe as the primary forms of disease and withdrawal of the agent has generally resulted in a decrease in ANCA titres and disease resolution.[123] The prevalence of ANCA in patients treated with propylthiouracil ranges between 33% and 64%. Yet clinical manifestations of vasculitis are uncommon.[28] Of note, Slot and associates[69] detected a higher frequency of ANCA, with or without vasculitis, in patients administered antithyroid drugs (compared with hyperthyroid patients who received other therapies) even after years of treatment. Most cases of drug-induced ANCA-associated vasculitides are associated not only with myeloperoxidase ANCA but also with elastase, proteinase 3, and lactoferrin. The detection of ANCA against two or more antigens might hint at drug-induced disease.[69,123] ANCA-associated vasculitides with drug-induced disease can show an atypical ANCA pattern along with a high concentration of myeloperoxidase ANCA;[124] the pathogenesis is not well understood. Propylthiouracil can accumulate in neutrophils and is oxidised to reactive intermediates that bind to self-peptides and provoke T-cell sensitisation, potentially leading to ANCA production.[28] After finding differences in epitope recognition, Ye and colleagues[125] suggested that the mechanism of generation of ANCA in propylthiouracil-induced myeloperoxidase ANCA-associated vasculitis is dissimilar to that of idiopathic ANCA-associated vasculitides.

Methods for detection of ANCA

Indirect immunofluorescence and ELISA remain the most widely used techniques for ANCA detection, their combination being the most suitable approach.[87,124,126–128] When positive results from indirect immunofluorescence and ELISA are combined, specificity for ANCA-associated vasculitides is 99% and sensitivity for Wegener's granulomatosis and microscopic polyangiitis is 73% and 67%, respectively.[126] Moreover, ANCA testing by both methods is highly useful to exclude pauci-immune necrotising and crescentic glomerulonephritis in patients with low pre-test likelihood (negative predictive value 99%); when there is high clinical suspicion, ANCA determination is most valuable to lend support to such a diagnosis (positive predictive value 95%).[129]

When searching for ANCA by indirect immunofluorescence, four immunostaining patterns can be seen. The cytoplasmic pattern suggests the presence of serum proteinase 3 ANCA. The perinuclear pattern is defined as any perinuclear fluorescence with or without nuclear extension. In a clinically suitable scenario, most perinuclear ANCA, usually with nuclear extension, will match myeloperoxidase ANCA. However, perinuclear ANCA, generally other than myeloperoxidase ANCA, can also be in disorders different from ANCA-associated vasculitides, such as inflammatory bowel disease and other autoimmune conditions. Atypical cytoplasmic ANCA (diffuse flat cytoplasmic staining without interlobular accentuation) has specificity for bactericidal/permeability-increasing protein and is mainly linked to chronic infections.[124] Any other ANCA staining makes up an atypical ANCA pattern, which is not typically associated with ANCA-associated vasculitides.

In clinical practice, ELISA will prove the presence of myeloperoxidase ANCA or proteinase 3 ANCA. Yet there might be sensitivity issues because of the quality of ELISA kits.[130] Other techniques are being developed to overcome ELISA limitations (eg, capture ELISA).[131]

An international group of experts has produced guidelines on ANCA testing and reporting to minimise technical difficulties and improve the homogeneity of results.[124,128] They propose to screen ANCA by indirect

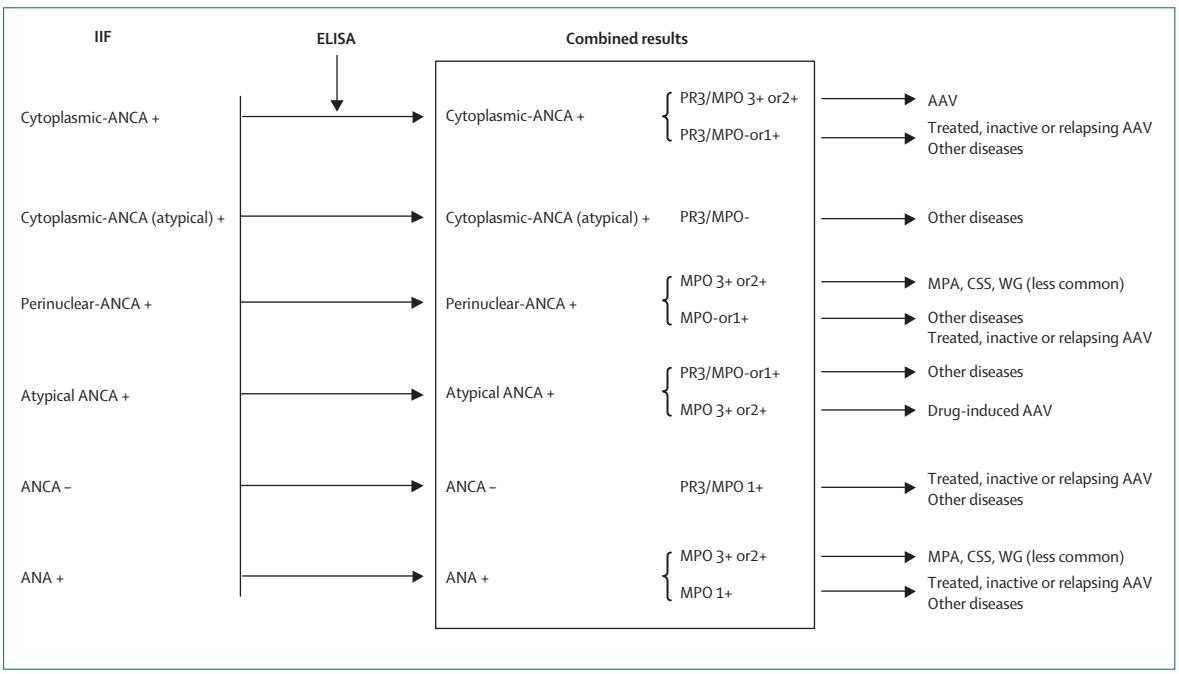

***Figure 3*: Scheme of ANCA determination and reporting based on the International Consensus Statement on testing and reporting of ANCA[124,128]**
AAV=ANCA-associated vasculitides. IIF=indirect immunofluorescence. MPO=myeloperoxidase. CSS=Churg-Strauss syndrome. MPA=microscopic polyangiitis. WG=Wegener's granulomatosis.

immunofluorescence and to confirm any positivity by both proteinase 3-ELISA and myeloperoxidase-ELISA. Negative results from indirect immunofluorescence should also be tested by ELISA because 5% of serum samples are positive by ELISA only. When results point to ANCA-associated vasculitides, histological confirmation is mandatory. Because ANCA concentrations might be useful for diagnosis and disease monitoring, the group recommends determination of proteinase 3 ANCA and myeloperoxidase ANCA concentrations. These should be reported semiquantitatively because no consensus on standardised measurement units has been reached so far (figure 3).

Most ANCA in disorders other than ANCA-associated vasculitides do not have specificity for myeloperoxidase and proteinase 3, and usually display a perinuclear-ANCA pattern (table 1). However, myeloperoxidase ANCA and proteinase 3 ANCA (usually at low concentrations) can rarely be detected in non-vasculitic conditions including inflammatory bowel disease, other autoimmune disorders, and infections such as tuberculosis.[71,74,99,124] The ANCA-associated vasculitides are very rare disorders. If ANCA detection methods were applied to the general population (low pre-test probability), we would be expect to record a large number of false-positives results, irrespective of a high test specificity. The same would happen if ANCA testing were indiscriminately done in a clinical setting. However, if tests are ordered in patients with high suspicion of ANCA-associated vasculitides (higher pre-test probability), the total number of false-positive results will be reduced.[132] Therefore, to avoid misdiagnosis, unnecessary and potentially harmful treatments, and medical examinations (and laboratory charges), we advise use of ANCA tests only when there exists a high suspicion of ANCA-associated vasculitides.

Clinical usefulness of ANCA

Much like other autoantibodies (eg, anti-double stranded DNA in systemic lupus erythematosus or antiglomerular basement membrane antibodies in Goodpasture's syndrome), ANCA have provided doctors with a serological test useful to assist in the diagnosis of Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, and their localised variants, such as pauci-immune necrotising, crescentic glomerulonephritis, and pulmonary capillaritis. Thus, if we consider Wegener's granulomatosis, microscopic polyangiitis, and necrotising and crescentic glomerulonephritis together, ANCA are detected in 85–95% of cases.[133] Besides their diagnostic potential, ANCA might be valuable in disease monitoring. However, ANCA are not currently considered a diagnostic criterion. The American College of Rheumatology published in 1990 a series of criteria for diagnosis of systemic vasculitis. The College reports, however, did not mention ANCA and, furthermore, microscopic polyangiitis was not thought of as a separate entity. Because the American College of Rheumatology does not require histological confirmation as mandatory diagnostic criteria, if we would adhere to the report's definitions today, microscopic polyangiitis would be misdiagnosed as Wegener's granulomatosis or as polyarteritis nodosa. The

Wegener's granulomatosis	Granulomatous inflammation affecting the respiratory tract, and necrotising vasculitis affecting small-sized to medium-sized vessels (ie, capillaries, venules, arterioles, and arteries). Necrotising glomerulonephritis is common.
Churg-Strauss syndrome	Eosinophil-rich granulomatous inflammation in the respiratory tract, and necrotising vasculitis affecting small-sized to medium-sized vessels, and associated with asthma and eosinophilia.
Microscopic polyangiitis	Necrotising vasculitis, with few or no immune deposits, affecting small vessels (ie, capillaries, venules, or arterioles). Necrotising arteritis involving small-sized and medium-sized arteries might be present. Necrotising glomerulonephritis is very common. Pulmonary capillaritis frequently arises.

Table 2: **Operative definitions of ANCA-associated vasculitis adopted by the Chapel Hill conference consensus on the nomenclature of systemic vasculitis[134]**

publication in 1994 of the Chapel Hill consensus conference on the nomenclature of systemic vasculitis[134] addressed some limitations of the American College of Rheumatology's criteria. For the first time, the necrotising vasculitides affecting medium-sized and small-sized (including arterioles, venules, and capillaries) vessels, commonly associated with ANCA, were classified as a separate group, termed ANCA small-vessel vasculitis (table 2). Of note, this group does not include polyarteritis nodosa. Although this disorder affects medium-sized and small-sized vessels, it respects arterioles, venules, and capillaries and ANCA are typically negative. Chapel Hill's operative definitions (but not diagnostic criteria) for each entity do not explicitly include ANCA positivity or the target antigen specificity. Nevertheless, even in absence of histopathological confirmation (eg, incipient renal damage), in a coherent clinical context, ANCA remain an important factor strongly suggestive of small-vessel vasculitis. Moreover, knowledge of the precise antigenic specificity might orient the doctor towards a specific disease in ANCA-associated vasculitides. Thus, to assist the early recognition—and prompt treatment—of Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome and their localised forms, ANCA might well be regarded, in future, a criterion (among others) for diagnosis of ANCA-associated vasculitides. However, because ANCA might be absent in some ANCA-associated vasculitides scenarios (and false-positive results can happen) they should never be regarded as an absolute essential diagnostic prerequisite (figure 4).

Wegener's granulomatosis

This disease affects predominantly the upper and lower respiratory tracts, and kidneys, where it can produce a rapidly progressive glomerulonephritis as a result of necrotising and crescentic glomerulonephritis. In the lungs, Wegener's granulomatosis can cause life-threatening diffuse alveolar haemorrhage as a result of (pauci-immune) alveolar necrotising capillaritis (figure 5). The eyes, heart, gut, skin, and the peripheral nervous system might also be affected in Wegener's granulomatosis as a result of necrotising vasculitis affecting small-sized to medium-sized vessels.[135] The localised form of the disease usually refers to the involvement of eyes, ears, nose, and lungs.[136] The histological hallmark of Wegener's granulomatosis is a granulomatous inflammation in the respiratory tract. In its generalised form, proteinase 3 ANCA are seen in 70–80% and myeloperoxidase ANCA in 10% of patients.[123] In limited Wegener's granulomatosis, ANCA are detected only in 60% of cases.

Microscopic polyangiitis

Microscopic polyangiitis is characterised by pauci-immune necrotising small-vessel vasculitis without granuloma formation, with or without involvement of medium-sized arteries. The clinical range is similar to Wegener's granulomatosis, although ear, nose, throat, and lung involvement is less common in microscopic polyangiitis. About 90% of patients have renal diseases in the form of pauci-immune necrotising and crescentic glomerulonephritis, which is indistinguishable from that seen in Wegener's granulomatosis.[135] Renal involvement might constitute the only manifestation of microscopic polyangiitis. About half of patients with microscopic polyangiitis develop necrotising alveolar capillaritis-induced pulmonary haemorrhage. Moreover, this disorder is the most common cause of pulmonary-renal syndrome.[135] About 60% of microscopic polyangiitis patients have myeloperoxidase ANCA and 30% have proteinase 3 ANCA.[123] People with microscopic polyangiitis have fewer relapses than do those with Wegener's granulomatosis.[137]

Churg-Strauss syndrome

Clinically characterised by asthma, hypereosinophilia, and transient pulmonary infiltrates, Churg-Strauss syndrome's typical histopathological features include extravascular granulomas and eosinophil-rich infiltrates in the respiratory tract, along with small-sized and medium-sized necrotising vasculitis. Rapidly progressive glomerulonephritis and pulmonary haemorrhage are less familiar in Churg-Strauss syndrome than in microscopic polyangiitis and Wegener's granulomatosis. However, life-threatening myocardial vasculitis is most likely in Churg-Strauss syndrome.[8] ANCA are detected in 60% of patients (30% myeloperoxidase ANCA and 30% proteinase 3 ANCA).[123]

ANCA in disease monitoring

Early evidence about the usefulness of ANCA as markers of clinical activity in Wegener's granulomatosis was reported in 1985 by van der Woude and colleagues,[3] who noted that disease remission was generally paralleled by ANCA negativity. Persistent or reappearing cytoplasmic-ANCA positivity within the first year in patients in remission is clinically significant associated with disease relapse in ANCA-associated vasculitides. On the contrary, the risk of relapse in patients who show persistently negative cytoplasmic ANCA or perinuclear ANCA seems to be very low.[138] Should we prophylactically treat a patient who has an elevation of ANCA concentrations while in

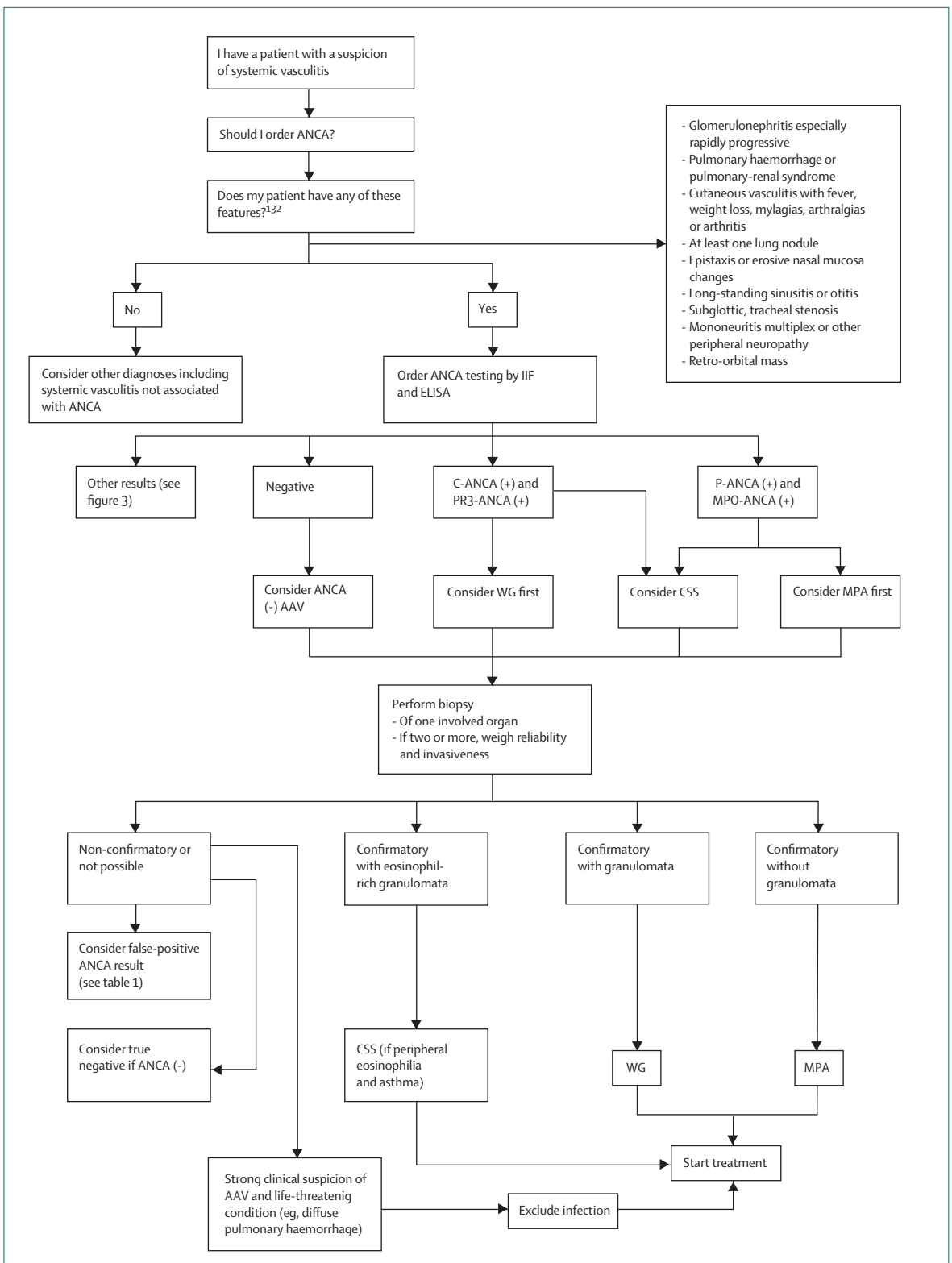

***Figure 4*: Value of ANCA in the clinical approach to ANCA-associated vasculitides**
IIF=indirect immunofluorescence. CSS=Churg-Strauss syndrome. WG=Wegener's granulomatosis. MPA= microscopic polyangiitis. ENT=ear, nose, and throat. AAV=ANCA-associated vasculitides.

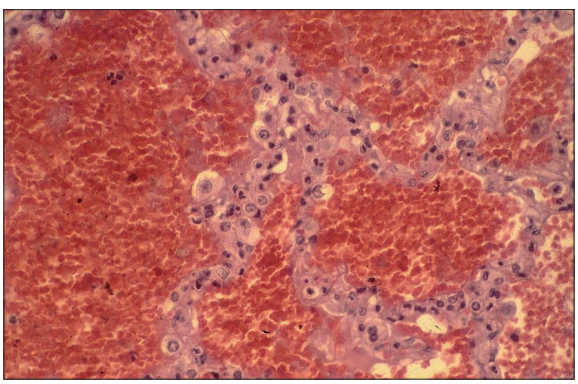

Figure 5: **Severe alveolar capillaritis with acute alveolar haemorrhage in a patient with Wegener's granulomatosis**
Haemoxylin and eosin stain, original magnification ×60.

remission, as previously suggested?[139,140] Several, mostly retrospective, studies have attempted to calculate the number of ANCA-associated vasculitides patients who develop a relapse after a rise in ANCA titres. After analysis of 15 of these reports, Schmitt and van der Woude[141] noted that 155 of 365 (42%) and 75 of 295 (25%) patients did not have a relapse after concentration of ANCA in serum rose when indirect immunofluorescence and ELISA, respectively, were done. Because statistically powerful trials assessing the benefit-risk balance of a pre-emptive treatment are absent, it does seem reasonable to argue that ANCA titres alone are not valuable for guiding treatment. Otherwise, patients would be unnecessarily left to the potential risks of immunosuppressive treatment.[141,142] In our opinion, any patient with ANCA-associated vasculitides in remission with persistent or reappearing ANCA positivity or rise in titre should be closely followed up, and diagnostic efforts intensified, to promptly detect—and treat—relapses.

Clinical value of ANCA in other diseases

Antiglomerular basement membrane disease

About a third of patients with antiglomerular basement membrane disease have ANCA, mainly myeloperoxidase ANCA.[117,143] Furthermore, up to 7·5% of ANCA-positive patients can harbour antiglomerular basement membrane antibodies.[144] Previous studies have noted that patients with such a double-positivity have a better clinical outcome (better response to immunosuppressants and renal recovery), even when presenting with severe renal failure, than do those with antiglomerular basement membrane antibodies alone.[117,145] However, Levy and co-workers[143] reported a series of patients with both antibodies who presented with severe disease and had a poor outlook. Unlike ANCA-associated vasculitides, these patients were unresponsive to immunosuppressive treatment and did not recover from dialysis, hence somewhat behaving like genuine antiglomerular basement membrane disease. It seems, therefore, advisable to check any patient with ANCA-related renal disease for antiglomerular basement membrane antibodies, and vice versa, because this association might confer distinct prognostic and therapeutic implications.

Inflammatory bowel disease and other autoimmune diseases

ANCA are recorded in 50–70% of patients with ulcerative colitis[84] and 10–30% of individuals with Crohn's disease.[124] A subgroup of patients with inflammatory bowel disease (10–15%) cannot be initially classified as having ulcerative colitis or Crohn's disease (indeterminate colitis). In this subset, ANCA might help to identify the precise disorder, when combined with anti-*Saccharomyces cerevisiae* antibody testing by ELISA. A positive perinuclear-ANCA but negative anti-*S cerevisiae* antibody result suggests ulcerative colitis, whereas a negative perinuclear-ANCA but positive anti-*S cerevisiae* antibody test hints at Crohn's disease. In inflammatory bowel disease, ANCA concentrations might correlate with disease activity.[146] ANCA determination can also assist in the diagnosis of certain autoimmune disorders. In particular, they have been noted in primary sclerosing cholangitis (up to 87%), autoimmune hepatitis type I (up to 96%), and Felty syndrome (90%).[85,124]

The predominant pattern in these disorders is a perinuclear-ANCA immunostaining with a broad rim-like fluorescence of the periphery of the nucleus without nuclear extension. Antigenic specificities are protean, such as nuclear and cytosolic antigens and other antigens contained in specific and primary granules.

Bactericidal/permeability-increasing protein ANCA have been detected in most of the above-mentioned non-ANCA-associated vasculitides disorders and in chronic airway infections (mainly in cystic fibrosis and bronchectases).[123] Bactericidal/permeability-increasing protein is a neutrophil-granule protein with significant antimicrobial activity against gram-negative bacteria that strongly neutralises the endotoxic activity of bacterial lipopolysaccharide. Reported data suggest that bactericidal/permeability-increasing protein ANCA mainly take place in disorders in which prolonged exposure to gram-negative bacteria and endotoxin happens, leading to widespread mobilisation of neutrophils, release of bactericidal/permeability-increasing protein, and formation of extracellular bactericidal/permeability-increasing-endotoxin complexes. Bactericidal/permeability-increasing protein lipopolysaccharide complexes (or other proteins from degraded neutrophils) might possibly be taken up and processed by monocytes. As a result, some bactericidal/permeability-increasing protein domains (and other neutrophil protein domains) can be presented on the monocyte surface, paving the way to generation of bactericidal/permeability-increasing protein ANCA and other ANCA.[147] Alternatively, on grounds of the apparent association with infections, ANCA in these disorders might represent cross-reactive antibodies induced by bacterial proteins.

Conflict of interest statement
We declare that we have no conflict of interest.

Acknowledgments
No funding was used for the preparation or submission of this manuscript.

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