Kidney International, Vol. 57 (2000), pp. 846–862

PERSPECTIVES IN RENAL MEDICINE

Antineutrophil cytoplasmic antibodies and associated diseases: A review of the clinical and laboratory features

JUDY SAVIGE, DAVID DAVIES, RONALD J. FALK, J. CHARLES JENNETTE, and ALLAN WIIK

Department of Medicine, University of Melbourne, Austin and Repatriation Medical Center, Heidelberg, Victoria, and South Western Area Pathology Service, Liverpool Hospital, Liverpool, New South Wales, Australia; Departments of Pathology and Laboratory Medicine, and Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; and the Department of Autoimmunology, Statens Serum Institut, Copenhagen, Denmark

Antineutrophil cytoplasmic antibodies and associated diseases: A review of the clinical and laboratory features. There have been a number of recent advances in this field. First, the "International Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies (ANCA)" has been developed to optimize ANCA testing. It requires that all sera are tested by indirect immunofluorescent (IIF) examination of normal peripheral blood neutrophils and, where there is positive fluorescence, in enzyme-linked immunosorbent assays (ELISAs) for antibodies against both proteinase 3 (PR3) and myeloperoxidase (MPO). Testing will be further improved when international standards and common ELISA units are available. Second, new diagnostic criteria for the small vessel vasculitides that take into account ANCA-positivity and target antigen specificity as well as histologic features are currently being produced. Third, we understand that the complications associated with treatment of the ANCA-associated vasculitides are often more hazardous than the underlying disease, and regimens that use effective but less toxic agents are being evaluated. The factors associated with increased risk of relapse, however, remain incompletely understood. Finally, ANCA with specificities other than PR3 and MPO are present in many nonvasculitic autoimmune diseases. Their clinical significance is still largely unclear, and some of the target antigens are present in other cells as well as neutrophils and thus are not strictly "ANCA."

The association of antineutrophil cytoplasmic antibodies (ANCA) with the small vessel vasculitides has proved helpful in the diagnosis and management of Wegener's granulomatosis, microscopic polyangiitis, and Churg–Strauss syndrome, but the lack of technical guidelines for antibody testing had meant that it was often difficult to compare results from different laboratories. The "International Consensus Statement on Testing and Reporting of ANCA" has been developed to optimize the usefulness of ANCA testing and to ensure more uniformity in the laboratory results that are issued. It requires that all sera should at least be tested by indirect immunofluorescent (IIF) examination of normal peripheral blood neutrophils and, where there is positive fluorescence, in enzyme-linked immunosorbent assays (ELISAs) for antibodies against proteinase 3 (PR3) and myeloperoxidase (MPO). Testing could be further improved by the development of international standards and the adoption of common ELISA units. Current diagnostic criteria for the small vessel vasculitides do not take account of ANCA positivity or target antigen specificity, despite correlations with patterns of organ involvement and with the tendency to relapse. New criteria that include these features are now being developed by the Chapel Hill group. "Overlap syndromes" in which there is medium, as well as small vessel involvement, are recognized with increasing frequency. We have come to understand that the ANCA-associated vasculitides are not invariably fatal when treated appropriately and that the complications associated with relapses, especially in the older population, can be less hazardous than the therapy. Thus, regimens that use effective but less toxic agents are being actively sought. The risk factors for relapses in the ANCA-associated vasculitides and for the development of generalized disease in Wegener's granulomatosis are incompletely understood at the present time. ANCA with specificities other than PR3 and MPO are frequently demonstrated in patients with inflammatory bowel disease, autoimmune liver disease, rheumatoid arthritis, and some drug-induced vasculitides. In these conditions, ANCA often have multiple specificities, and antibody levels are usually low. Some of these antigens are present in cells other than neutrophils and are thus not strictly "ANCA." The clinical significance of many of these antibodies is still unclear. There have been several

Key words: ANCA, crescentic glomerulonephritis, microscopic polyangiitis, pauciimmune segmental necrotizing glomerulonephritis, small vessel vasculitis, Wegener's granulomatosis.

Received for publication October 17, 1997
and in revised form July 13, 1999
Accepted for publication July 21, 1999

© 2000 by the International Society of Nephrology

excellent reviews of the clinical and pathologic aspects of ANCA-associated vasculitides [1–6], but this article emphasizes recent developments from the ANCA Workshops.

DIAGNOSIS OF SYSTEMIC SMALL VESSEL VASCULITIDES

The ANCA-associated small vessel vasculitides include Wegener's granulomatosis, microscopic polyangiitis, renal-limited microscopic polyangiitis, Churg–Strauss syndrome, and some drug-induced vasculitides [7–12]. The term "polyangiitis" is preferred to "polyarteritis" because vessels of various sizes are affected and arteries are often not involved. In patients with primary pauciimmune crescentic glomerulonephritis, the presence of a necrotizing glomerular lesion, the demonstration of ANCA, and the response to immunosuppressive agents suggested that this was a renal-limited form of microscopic polyangiitis, even though patients often have constitutional symptoms [13].

Wegener's granulomatosis, microscopic polyangiitis, and Churg–Strauss syndrome are relatively uncommon conditions but are being diagnosed with increasing frequency, in part because of the widespread use of ANCA testing [14]. However, there are currently no satisfactory definitions for these diseases. The American College of Rheumatology (ACR) criteria do not recognize the diagnosis of microscopic polyangiitis, and classify such patients as having Wegener's granulomatosis, Henoch-Schönlein purpura, or hypersensitivity angiitis [15, 16]. The Chapel Hill definitions require histologic evidence or the presence of clinical features that indicate the underlying pathology to make a diagnosis [17]. Thus, with the Chapel Hill criteria, a patient has Wegener's granulomatosis when there is necrotizing granulomatous inflammation, often of the respiratory tract, but no history of asthma, and microscopic polyangiitis when there is a pauciimmune small vessel vasculitis in the absence of granulomatous inflammation and asthma. When the ACR and Chapel Hill definitions are applied to a cohort of patients with vasculitis, they identify different but overlapping groups (abstract; Bruce et al, *Sarcoidosis Vasc Diffuse Lung Dis* 13:270, 1996) [18]. Neither the ACR nor current Chapel Hill criteria takes into account ANCA positivity and target antigen specificity, even though these are probably important clues to the nature of the underlying vasculitis and to subsequent disease behavior. For example, the demonstration of ANCA will indicate an ANCA-associated vasculitis when there is only glomerular sclerosis or tubulointerstitial disease, and when the histologic appearance cannot be distinguished from that seen with Henoch Schönlein purpura, cryoglobulinemia, and serum sickness. The subsequent demonstration of specificity for proteinase 3 (PR3-ANCA) or MPO has further implications for organ involvement, histopathology, and the likelihood of relapse [19, 20]. The Chapel Hill criteria are currently being revised to allow for ANCA positivity and antigen specificity.

Table 1. Summary of the International Consensus Statement on Testing and Reporting of ANCA

Testing

Minimum requirements

- IIF is performed on all sera, since about 10% of ANCA-positive sera are detected only by IIF
- Sera containing ANCA, any other cytoplasmic fluorescence, or an ANA that results in homogeneous or peripheral nuclear fluorescence should be tested promptly in ELISAs for both PR3- and MPO-ANCA
- Sera from patients that were previously ANCA-positive by IIF alone may be tested subsequently only by IIF
- Sera that were positive for either PR3- or MPO-ANCA may be tested subsequently only in the relevant ELISA (although ANCA sometimes change antigen specificity)

Optimal recommendations

- IIF titration should be performed for sera positive only by IIF, or if other cytoplasmic fluorescence, or an ANA is present
- ELISAs should be performed on all sera since about 5% of ANCA-positive sera are positive only by ELISA
- The inclusion of the most recent positive serum in the IIF or ELISA studies may be useful in demonstrating a change in antibody level

Reporting

Nomenclature

- Reports should use the terms "C-ANCA" for cytoplasmic fluorescence with interlobular accentuation; "C-ANCA (atypical)" for other types of cytoplasmic fluorescence; "P-ANCA" for perinuclear or granulocyte-specific nuclear fluorescence; and "atypical ANCA" for other, less common patterns, such as mixed cytoplasmic and perinuclear fluorescence
- Antigen specificities demonstrated by ELISA are described as "PR3-" and "MPO-ANCA"

Reports

- Any report of positive neutrophil fluorescence issued before the ELISA results are available should indicate that positive fluorescence alone is not specific for the diagnosis of Wegener's granulomatosis or microscopic polyangiitis
- Reports should indicate that decisions about treatment should not be based solely on the ANCA results

Modified from [23] with permission from the *American Journal of Clinical Pathology.* Abbreviations are in the **Appendix.**

TESTING FOR ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES

The most common reason to request a test for ANCA is to diagnose or exclude Wegener's granulomatosis or microscopic polyangiitis and to monitor inflammatory activity in these diseases. Recent retrospective and prospective studies have suggested that the results of ANCA testing have a low sensitivity and specificity for at least Wegener's granulomatosis [21, 22], but these analyses have used the ACR criteria for diagnosis and have demonstrated ANCA by neutrophil IIF alone.

The "International Consensus Statement on Testing and Reporting of ANCA" (Table 1) has been developed

Table 2. Clinical indications for ANCA testing

- Glomerulonephritis, especially rapidly progressive glomerulonephritis
- Pulmonary hemorrhage, especially pulmonary–renal syndrome
- Cutaneous vasculitis, especially with systemic features
- Multiple lung nodules
- Chronic destructive disease of the upper airways
- Long-standing sinusitis or otitis
- Subglottic tracheal stenosis
- Mononeuritis multiplex or peripheral neuropathy
- Retro-orbital mass

The presence of any of these features in the absence of another obvious cause indicates that ANCA testing is warranted. Modified from [**24**] with permission from *Nephrology.*

to optimize the diagnostic usefulness of ANCA testing in patients suspected of having vasculitis, by the adoption of standardized testing and reporting procedures [23]. Table 2 shows the clinical manifestations that suggest the diagnoses of Wegener's granulomatosis and microscopic polyangiitis [24], and indicate that a test for ANCA should be performed. In such patients, ANCA positivity is more likely to indicate a vasculitis than in other hospital patients. The International Consensus Statement has adopted the recommendation of the European standardization trials that ANCA testing for the diagnosis of vasculitis requires both IIF examination of normal peripheral blood neutrophils and ELISAs for PR3- and MPO-ANCA [25]. When the IIF assay is positive and one ELISA is performed depending on the IIF pattern, this approach produces sensitivities of 73 and 67% for Wegener's granulomatosis and microscopic polyangiitis, respectively, and a diagnostic specificity of 99% [26]. A similar approach yields positive and negative predictive values of 95 and 85% for Wegener's granulomatosis and microscopic polyangiitis, respectively, when there is kidney involvement [27]. These values should be increased by adhering to the minimum requirements of the International Consensus Statement, where both ELISAs are performed when the IIF is positive, or the optimal recommendations that all sera should be tested by IIF and in both ELISAs.

Four patterns are demonstrated with IIF (Fig. 1). Probably 90% of patients with active generalized Wegener's granulomatosis have granular cytoplasmic neutrophil fluorescence with central interlobular accentuation ("C-ANCA") that usually corresponds to PR3 specificity [28]. About 80% of patients with microscopic polyangiitis or Churg–Strauss syndrome are ANCA positive, and most with microscopic polyangiitis and about half with Churg–Strauss syndrome have perinuclear neutrophil staining, often with nuclear extension ("P-ANCA") and MPO specificity [10], while the rest have C-ANCA.

However, most neutrophil fluorescence detected in a routine immunodiagnostic laboratory is not seen in patients with Wegener's granulomatosis or microscopic polyangiitis [29, 30]. "C-ANCA (atypical)" with "flat" neutrophil cytoplasmic fluorescence and without central accentuation accounts for half of all cytoplasmic fluorescence in some laboratories [29]. These sera rarely have PR3 specificity and recognize such antigens as bactericidal/permeability-increasing protein (BPI), MPO, and so forth [31]. Cytoplasmic fluorescence is also seen with heat-treated sera, with antimitochondrial and antiribosomal antibodies, and with some alloantibodies [31–33]. Perinuclear fluorescence without the nuclear extension characteristic of MPO specificity [32] can still occur with MPO-ANCA, but is common in inflammatory bowel disease and rheumatoid arthritis [34, 35]. Granulocyte-specific ANA represent a form of P-ANCA, and the International Consensus Statement recommends that they are described as such. The corresponding antigen specificities are unclear. P-ANCA should be distinguished from the fluorescence seen with anti-dsDNA, anti-Ro, anti-lamin, and anti-Golgi antibodies [31]. "Atypical" ANCA patterns are uncommon and often comprise a mix of cytoplasmic and perinuclear fluorescence with multiple antigen specificities [23]. They occur with propylthiouracil and other drugs, even if there is no vasculitis, and are also observed in inflammatory bowel disease and rheumatoid arthritis.

In contrast to IIF, ELISAs for PR3- and MPO-ANCA produce an immediate estimate of antibody levels, which are usually high at the time of presentation with generalized Wegener's granulomatosis or microscopic polyangiitis and which fall with treatment [36]. The inclusion of a recent serum in the IIF assay or ELISA may demonstrate a change in antibody level that helps with patient management. The sensitivity of ANCA ELISAs is increased with the "capture" methodology [37], where the target antigens are bound to specific monoclonal antibodies adherent to the plastic ELISA plates, thus overcoming the effect of protein denaturation from extraction or coating procedures. The results with capture ELISAs probably correlate better with disease activity and predict relapses more accurately than conventional assays. Rapid-screening ELISAs, in which results are available in less than 60 minutes, and "near patient" testing assays that use whole blood are also available.

Most laboratories use commercial kits for both IIF and ELISAs, and there is good concordance between IIF positivity using different types of neutrophil preparations, although the fluorescent patterns may differ. However, sera with C-ANCA produced a concordance rate of 56% with seven different PR3-ANCA kits and sera with P-ANCA, a rate of only 30% using eight different MPO-ANCA assays [38]. Additionally, while PR3-ANCA levels correlated with C-ANCA intensity, there was no such correlation between MPO-ANCA and P-ANCA levels. Further obstacles to the direct comparison of ELISA results from different assays are the lack of international serum standards and the absence of units for PR3- and MPO-ANCA.

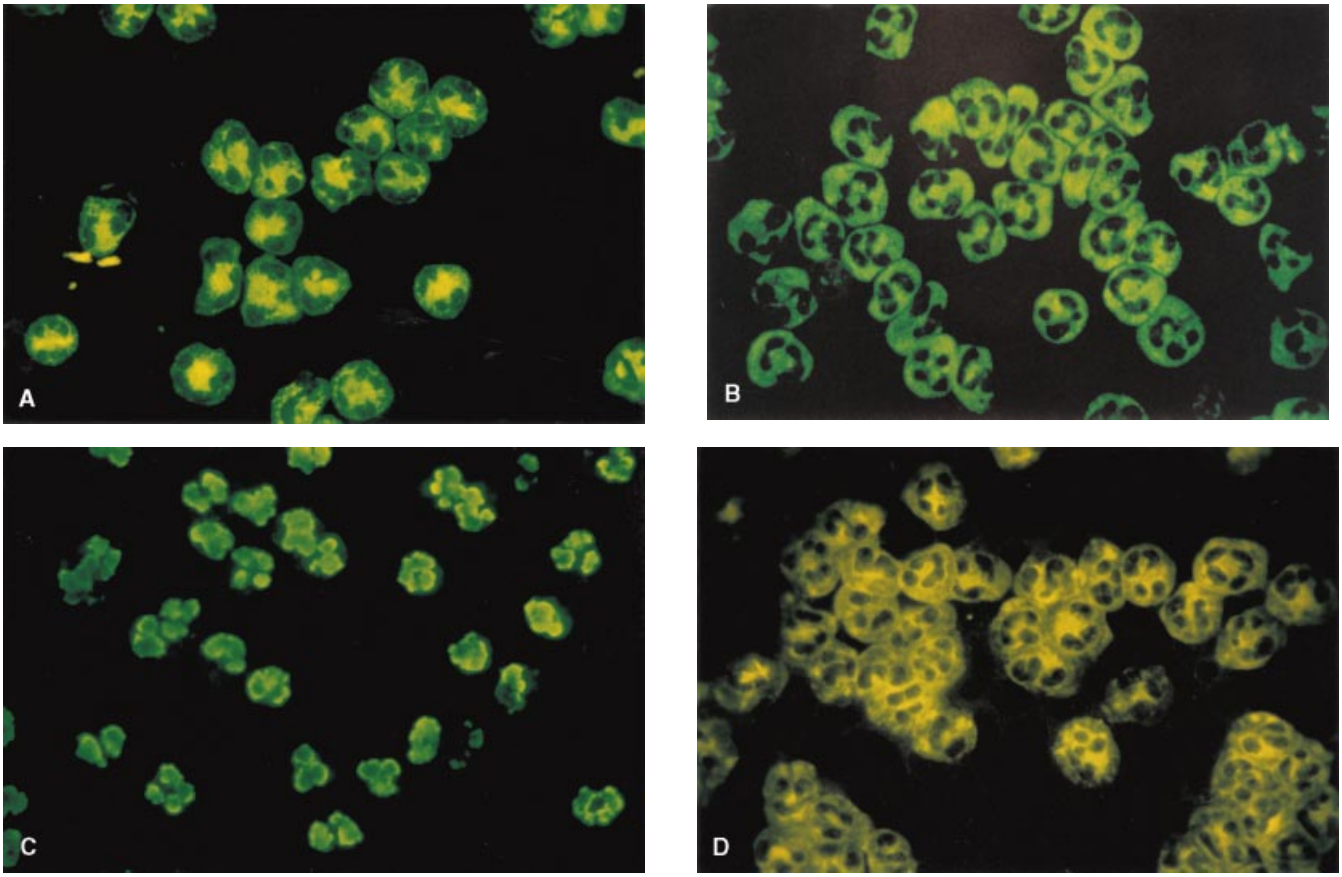

Fig. 1. Immunofluorescence patterns. (*A*) C-ANCA with cytoplasmic staining, central accentuation and PR3 specificity. (*B*) C-ANCA (atypical) with flat cytoplasmic fluorescence and where the antigen was not PR3. (*C*) P-ANCA with perinuclear fluorescence and nuclear extension, where the antigen was MPO. (*D*) Atypical ANCA, with both cytoplasmic and perinuclear fluorescence, and where both PR3 and MPO were recognized.

Other autoantibodies are common in patients with Wegener's granulomatosis and especially microscopic polyangiitis (abstract; Geffriaud et al, *Clin Exp Immunol* 93:41, 1993) [39, 40], but are not necessarily present contemporaneously with ANCA [40]. An ANA occurs in up to 30% of patients, and these IIF patterns vary and may mask a P-ANCA, which is why the International Consensus Statement recommends that sera containing an ANA should be tested for both PR3- and MPO-ANCA. Rheumatoid factor is also common [39]. Antiglomerular basement membrane (GBM) antibodies occur together with ANCA in about 5% of patients with vasculitis (abstract; Coulthart et al, *Clin Exp Immunol* 101:60, 1995). These are usually P-ANCA, but sometimes C-ANCA [41]. Anti-GBM disease can resemble Wegener's granulomatosis and microscopic polyangiitis clinically, and some laboratories routinely test all sera at presentation for anti-GBM antibodies as well as ANCA [42]. Anticardiolipin antibodies [40] and a lupus anticoagulant [43] occur in possibly 20% of patients and are associated with an increased risk of thrombosis (abstract; Mistry et al, *Sarcoidosis Vasc Diffuse Lung Dis* 13:270, 1996).

TARGET ANTIGENS

Many of the target antigens of ANCA are located in the primary granules of neutrophils and have antibacterial properties (Table 3). PR3 and MPO are recognized in most ANCA-positive small vessel vasculitides. PR3 is a 29 kD serine protease that breaks down tissue to allow the passage of neutrophils into an inflammatory focus [44] and is also involved in neutrophil maturation [45]. MPO helps generate hypochlorite molecules and reactive oxygen species that are bactericidal [46]. In patients with vasculitis affecting the kidney, ANCA specific for human lysosomal membrane glycoprotein (h-lamp2) have been described together with PR3- or MPO-ANCA, and may result from cross-reactivity with a renal endothelial surface protein [47]. Their significance, however, is unclear.

PR3- and MPO-ANCA occur also in a number of other nonvasculitic autoimmune diseases. Their immunoglobulin class, subclass, and epitope specificity are thought to differ from vasculitis-associated ANCA [48]. Additional ANCA antigens are recognized in inflammatory bowel disease, autoimmune liver disease, and rheuma-

Table 3. Disease associations of ANCA defined by immunofluorescence patterns and antigen specificities

IIF pattern	Antigens	Disease associations
C-ANCA	PR3 alone	Wegener's granulomatosis (80–90%) Microscopic polyangiitis (20–40%) Primary pauciimmune crescentic glomerulonephritis (20–40%) Churg–Strauss syndrome (35%)
C-ANCA (atypical)	BPI alone BPI, MPO, CG, etc., often multiple	Cystic fibrosis (80%) Inflammatory bowel disease Primary sclerosing cholangitis Rheumatoid arthritis
P-ANCA	MPO alone	Microscopic polyangiitis (50%) Primary pauciimmune crescentic glomerulonephritis (50%) Churg–Strauss syndrome (35%)
	Multiple specificities including: • HMG1/2 • catalase • αenolase • actin also, • lactoferrin • lysozyme • elastase • cathepsin G • defensin	Inflammatory bowel disease Rheumatoid arthritis Drug-induced vasculitis Autoimmune liver disease Drug-induced syndromes Some parasitic infestations
Atypical ANCA	Multiple specificities see above	Drug-induced systemic vasculitis Inflammatory bowel disease Rheumatoid arthritis

Granulocyte-specific ANA is a form of P-ANCA; many laboratories do not distinguish between P-ANCA and atypical ANCA, and for this reason the frequencies of atypical ANCA are not given. Data are from references given in the text.

toid arthritis and include catalase, α enolase, high mobility group nonhistone chromosomal proteins (HMG1 and HMG2), actin, BPI, cathepsin G, elastase, lactoferrin, and lysozyme (abstract; Flesch et al, *Am J Kidney Dis* 18:201, 1991) [49–56]. ANCA specific for these antigens individually result in P-ANCA, except for BPI-ANCA, which sometimes produces C-ANCA (atypical) [57]. However, ANCA in inflammatory bowel disease, autoimmune liver disease, and rheumatoid arthritis often have multiple specificities, resulting in an "atypical" fluorescence pattern. Interestingly h-lamp2, catalase, α enolase, HMG1 and HMG2, and actin are all found in cells other than neutrophils, and while currently described as "ANCA," may be more accurately named in the future. Further antigens include defensins [58] that have been recognized in some parasitic infections, and azurocidin [59], which is of uncertain significance.

PATHOLOGY

The characteristic histologic lesion of the ANCA-associated small vessel vasculitides is focal fibrinoid necrosis of the capillaries and venules. However, involvement of arterioles and small arteries is common [17], and "overlap" with medium or large vessel disease occurs in nearly half of all patients with microscopic polyangiitis [60]. By convention, when medium or large vessel involvement occurs, the disease is still called microscopic polyangiitis, Wegener's granulomatosis, or Churg–Strauss syndrome.

The characteristic glomerular lesion is a focal segmental necrotizing glomerulonephritis usually with crescents, and sometimes with disruption of Bowman's capsule and periglomerular tubulointerstitial inflammation from the spillage of inflammatory mediators. There is less endocapillary hypercellularity and more disruption of Bowman's capsule than occurs in immune complex crescentic glomerulonephritis. Uninvolved glomerular segments are often almost normal, and tubulointerstitial disease sometimes occurs without any obvious glomerulonephritis.

Immunofluorescence microscopy of the glomeruli and other vessels characteristically demonstrates few immunoglobulin deposits [61], which distinguishes the lesions from immune complex glomerulonephritis where there are granular deposits, and from the linear staining of anti-GBM disease. However, there is some overlap between the amount of immunoglobulin seen in pauciimmune and immune-complex–mediated glomerulonephritis, and thus, the frequency of ANCA positivity in immune complex glomerulonephritis depends on the definition of "pauciimmune" [62].

Lung disease is common in the ANCA-associated small vessel vasculitides (Tables 4 and 5). Capillaritis is the most frequently seen vascular lesion [63], and airways and interstitial disease are more common when C-ANCA is present, but otherwise, there is no histologic lesion that differentiates between C-ANCA and P-ANCA–

Table 4. Approximate frequency of organ involvement in ANCA-associated vasculitides

Organ	Wegener's granulomatosis	Microscopic polyangiitis	Churg–Strauss syndrome
Skin	40%	40%	60%
Kidney	80%	90%	45%
Lungs	90%	50%	70%
Ear, nose, throat	90%	35%	50%
Musculoskeletal	60%	60%	50%
CNS	50%	30%	70%
Gut	50%	50%	50%

Modified from [5] with permission from the *New England Journal of Medicine.*

Table 5. Pulmonary lesions in ANCA-associated vasculitides

	P-ANCA (N = 14)	C-ANCA (N = 13)	Total (N = 27)
Alveolar hemorrhage	11 (79%)	8 (62%)	19 (70%)
Vascular lesions	11 (79%)	10 (77%)	21 (78%)
Capillaritis	9 (64%)	8 (62%)	17 (41%)
Airway lesions	3 (21%)	8 (62%)	11 (41%)
Interstitial lesions	8 (57%)	13 (100%)	21 (78%)
Diffuse alveolar damage	9 (64%)	7 (54%)	16 (59%)
Pleural lesions	2 (14%)	5 (38%)	7 (26%)

Modified from [63] with permission from the *American Journal of Clinical Pathology.*

associated disease. The corresponding radiographic abnormalities are nodules, bilateral fluffy opacities, and less often lobar consolidation and honeycomb lung [64]. Open lung biopsy of the pulmonary parenchyma has the highest diagnostic yield, with characteristic abnormalities in more than 90% of specimens [65]. Transbronchial biopsies of tracheobronchial lesions, and even renal biopsies, are usually more helpful than transbronchial biopsies of alveolar tissue [66]. Within a week of starting treatment, the histologic appearance of these lung abnormalities begins to improve, but interstitial fibrosis commonly results [67].

CLINICAL SYNDROMES OF ANTINEUTROPHIL CYTOPLASMIC ANTIBODY-ASSOCIATED VASCULITIDES

The pattern of organ involvement is similar in Wegener's granulomatosis and microscopic polyangiitis (Table 4). Together these diseases account for 60% of all patients with rapidly progressive glomerulonephritis [68] and are the most common cause of the pulmonary–renal syndrome in adults [69]. These observations contrast with Churg–Strauss syndrome, in which renal failure and pulmonary hemorrhage are less common.

"Limited" Wegener's granulomatosis

Patients with "limited" Wegener's granulomatosis have disease affecting the eyes, ears, nose, or lungs, but not the kidneys, and about 60% are ANCA positive [70]. "Limited" Wegener's granulomatosis is recognized with increasing frequency, but probably 80% of patients go on to develop renal involvement [1], indicating that "limited" and "generalized" disease are part of a continuum and that long-term follow-up is essential.

"Generalized" Wegener's granulomatosis

Patients with "generalized" Wegener's granulomatosis have disease affecting the kidneys as well as other organs (Table 4). Involvement of the nose, ear, and eye is often overlooked, and subglottic stenosis occurs in up to 20% of patients, often at a time when other features have responded to treatment [71]. Central nervous system disease is recognized increasingly with the use of magnetic resonance imaging. Skin lesions usually parallel the activity in other organs.

Wegener's granulomatosis is rare in the very young. However, in children over the age of 7 and in adolescents, the features resemble adult disease, except that subglottic stenosis and nasal deformity are more common, and fewer cyclophosphamide-related malignancies ensue [72]. As in adults, early recognition and treatment are important to improve the renal outcome [73]. Pregnancy is uncommon in the age group affected by Wegener's granulomatosis, but may trigger the onset of disease and relapse [74]. Cyclophosphamide is teratogenic in the first trimester of pregnancy.

About half of all patients with Wegener's granulomatosis are aged over 60 years. In the older population, presenting features are similar to those in younger individuals, but the outcome is often worse, with uncontrolled pulmonary vasculitis and treatment-associated infections being common causes of death [75, 76]. ANCA do not occur incidentally in the normal older population, unlike ANA and rheumatoid factor [77].

Genetic risk factors for the development of Wegener's granulomatosis

Patients with Wegener's granulomatosis or PR3-ANCA are more likely to have abnormal α_1-antitrypsin (α_1AT) phenotypes than normal individuals [78–80]. Furthermore, patients with the Z phenotype have more organs involved, more progressive disease, and a higher mortality rate [79, 80]. α_1AT is the major inhibitor of PR3 (abstract; van der Wiel et al, *Am J Kidney Dis* 18:206, 1991) and competes with ANCA for binding to the PR3 catalytic site [81]. Thus, abnormal α_1AT phenotypes result in high circulating concentrations of unbound uninhibited PR3 that can lead to autoantibody production in an immunologically active environment. In addition, α_1AT inhibits other neutrophil proteolytic enzymes as well as PR3, and defective or deficient α_1AT probably results in uninhibited enzymes and increased tissue damage. Interestingly, however, patients with ab-

normal phenotypes often have normal serum levels of α_1AT at presentation because the protein is an acute phase reactant. α_1AT phenotyping has been recommended in patients with Wegener's granulomatosis or PR3-ANCA–associated vasculitis to identify those at risk of worse disease. Other genes that may predispose to the development of systemic vasculitis or increased tissue damage in vasculitis include those corresponding to the human leukocyte antigens and the neutrophil FcγRIIa receptor (abstract; Tse et al, *Sarcoidosis Vasc Diffuse Lung Dis* 13:269, 1996).

Microscopic polyangiitis

Most patients with microscopic polyangiitis have glomerulonephritis at presentation, sometimes as their only clinical manifestation [82]. Pulmonary hemorrhage occurs in up to 40% (abstract; Mistry et al, *Sarcoidosis Vasc Diffuse Lung Dis* 13:269, 1996), but an association with IgM ANCA [83] has not been confirmed. These patients, though, have an increased mortality rate and about 15% subsequently develop diffuse interstitial fibrosis (abstract; Mistry et al, *Sarcoidosis Vasc Diffuse Lung Dis* 13:269, 1996).

At least half of all patients with microscopic polyangiitis have arterial involvement and an "overlap syndrome" with features of both microscopic polyangiitis and polyarteritis nodosa [60]. Thus, in addition to glomerulonephritis, these patients may have bowel or renal ischemia and a peripheral neuropathy from medium vessel involvement. Arterial disease is indicated by an arcuate or interlobular arteritis in the renal biopsy and the demonstration of aneurysms at surgery or angiographically. This "overlap syndrome" differs from polyarteritis nodosa in that ANCA are present [60], the disease does not respond to corticosteroids alone, and relapses occur.

Differences between proteinase 3- and myeloperoxidase-antineutrophil cytoplasmic antibody–associated vasculitides

Patients with PR3-ANCA–associated disease have eye, ear, nose, and upper respiratory tract involvement more often, as well as tissue granulomata and an increased relapse rate [20, 84]. Patients with MPO-ANCA–associated vasculitis are often older, usually have glomerulonephritis, and have other autoantibodies too [19, 84]. While renal lesions are more active and kidney function deteriorates more rapidly in patients with PR3-ANCA–associated vasculitis [20], overall, the outcome for patients and for their renal function appears to be the same for both PR3-ANCA– and MPO-ANCA–associated disease [19].

Antineutrophil cytoplasmic antibody-negative Wegener's granulomatosis and microscopic polyangiitis

About 10% of patients with Wegener's granulomatosis or microscopic polyangiitis do not have ANCA that can be demonstrated by IIF or in antigen-specific ELISAs. Patients with Wegener's granulomatosis who are ANCA negative are likely to have local disease [70]. However, in one study in which only patients with generalized Wegener's granulomatosis were examined, the 14 ANCA-negative patients were younger, more likely to be female, and had less lung and kidney involvement, a lower relapse rate, and a better outcome overall than 14 ANCA-positive patients (abstract; Reinhold-Keller et al, *Sarcoidosis Vasc Diffuse Lung Dis* 13:267, 1996). In contrast, in microscopic polyangiitis, there was no difference in the clinical or laboratory features or prognosis between 22 ANCA-negative and 37 ANCA-positive patients, suggesting that ANCA-negative and ANCA-positive microscopic polyangiitis are the same disease (abstract; Adu et al, *Clin Exp Immunol* 101:62, 1995). One small study has suggested that patients with ANCA-negative microscopic polyangiitis have fewer organs affected [36].

Churg–Strauss syndrome

A review of more than 150 patients has indicated that the diagnosis of Churg–Strauss syndrome can be made when there is asthma, a peak peripheral blood eosinophilia $> 1.5 \times 10^9$/L, and a systemic vasculitis affecting two or more extrapulmonary organs [85]. These features occur sequentially over a period of years, and the clinical diagnosis should be confirmed histologically wherever possible. Myocardial vasculitis is not uncommon and is a major cause of morbidity. Renal involvement is less common than in the other small vessel vasculitides.

TREATMENT

Treatment of generalized Wegener's granulomatosis and microscopic polyangiitis

Histologic confirmation of the diagnosis of Wegener's granulomatosis or microscopic polyangiitis is almost always required before treatment is instituted because of the associated risks. Similar regimens are used for both Wegener's granulomatosis and microscopic polyangiitis, although there have been no prospective controlled trials in microscopic polyangiitis. The responses to treatment can be measured using "disease activity" [Birmingham vasculitis activity score (BVAS)] [86], "disease remission," "treatment resistance," and "relapse," as defined in Table 6 [87]. Treatment-associated morbidity often exceeds the complications from the disease or relapse [88] so that minor clinical features should probably be tolerated rather than treated aggressively, and less toxic regimens are being actively sought (Table 7) [89, 90].

Most induction regimens still use oral prednisolone 1 mg/kg and cyclophosphamide 2 to 3 mg/kg depending on age, renal function, and bone marrow reserve [91]. This results in 75% of patients with Wegener's granulo-

Table 6. Criteria for treatment response in ANCA-associated vasculitides

Remission
Stabilization or improvement of renal function (serum creatinine concentration), resolution of hematuria, and resolution of extrarenal manifestations of systemic vasculitis. Persistent proteinuria does not indicate disease activity.

Treatment resistance
(*A*) Progressive decline in renal function with the persistence of an active urinary sediment; or (*B*) persistence or new appearance of any extrarenal manifestations of vasculitis despite immunosuppressive therapy.

Relapse
Occurrence of at least one of the following: (*A*) rapid rise in serum creatinine concentration accompanied by an active urinary sediment; (*B*) a renal biopsy demonstrating active necrosis or crescent formation; (*C*) hemoptysis, pulmonary hemorrhage or new and expanding nodules without evidence for infection; (*D*) active vasculitis of the respiratory or gastrointestinal tract as demonstrated by endoscopy with biopsy; (*E*) iritis or uveitis; (*F*) new neuropathy; or (*G*) necrotizing vasculitis identified by biopsy in any tissue.

Modified from [87] with permission from the *Journal of the American Society of Nephrology.*

Table 7. Randomized treatment trials in the ANCA-associated vasculitides

Early systemic disease in WG and MPA (NORAM)
Any organ involvement except renal or imminent vital organ failure
- Oral corticosteroids and oral cyclophosphamide
- Or oral corticosteroids plus weekly methotrexate

Generalized WG, MPA and renal-limited vasculitis (CYCAZAREM)
Renal disease with serum creatinine <500 mmol/L and/or imminent vital organ failure
- Oral corticosteroids and cyclophosphamide for 3 months, and continued cyclophosphamide
- Or oral corticosteroids and cyclophosphamide for 3 months, and then azathioprine

Severe renal involvement in WG, MPA and renal-limited vasculitis (MEPEX)
Renal disease with serum creatinine >500 mmol/L
- Oral corticosteroids and cyclophosphamide and IV methylprednisolone
- Oral corticosteroids and cyclophosphamide and plasma exchange

Refractory disease in WG, MPA (SOLUTION)
Frequently relapsing or progressive disease, life-threatening, standard treatment of no use
- ATG daily for 10 subsequent days
  methylprednisolone and azathioprine as necessary adjuvants

Generalized or severe renal disease in WG, MPA, renal-limited vasculitis (CYCLOPS)
New disease, serum creatinine >150 mmol/L
- Oral corticosteroids and cyclophosphamide and switch to azathioprine
- Oral corticosteroids and continue cyclophosphamide
- Oral corticosteroids and intermittent pulse cyclophosphamide

Early systemic or generalized WG (MUPIBAC)
GFR >50 mL/min and in remission 18 months after start of another clinical trial
- No treatment
- Or mupirocin nasal ointment one week a month

Generalized or severe renal WG, MPA or renal-limited vasculitis (REMAIN)
GFR <50 mL/min for WG only, and in remission after 18 months from start of clinical trial
- Withdraw oral corticosteroids and azathioprine between 18 and 24 months
- Or continue low dose oral corticosteroids and azathioprine until 48 months

Abbreviations are: WG, Wegener's granulomatosis; MPA, microscopic polyangiitis. Modified from [90] with permission from *Clinical and Experimental Immunology.*

matosis achieving remission and 91% improving significantly. Prednisolone alone is ineffective [91]. In patients with rapidly progressive glomerulonephritis, pulse methylprednisolone of 7 to 15 mg/kg daily for three days results in the recovery of renal function even in those who are dialysis dependent [91–95], and while the response to plasma exchange is equivalent to this dose, it is usually short lived [95–98]. Both pulse prednisolone and plasma exchange appear, however, to be effective in the treatment of pulmonary hemorrhage [95]. Pulse cyclophosphamide probably has no advantage over continuous oral administration in the induction phase of aggressive disease.

Most patients respond immediately to treatment. Within a week, symptoms and signs improve, although deafness and neuropathy respond more slowly and sometimes incompletely. Urinary red blood cell counts and C-reactive protein (CRP) plateau in the first week and then fall, but take up to two months to become normal. Serum creatinine usually plateaus in the first week and then falls, and the maximal improvement in creatinine clearance occurs within two months. Lung hemorrhage often clears radiographically within a week, but nodules take a month or more. Although ANCA are reported to disappear within three months [99], in our experience, it is often longer.

Maintaining remission

Prednisolone is usually tapered to 20 mg at three months and is then reduced further. Cyclophosphamide is continued for at least a year after remission with the NIH regimen [91], but is replaced with azathioprine after three to six months with the Hammersmith protocol [96]. Relapses are more common with the shorter course of cyclophosphamide, but the overall loss of function and treatment-related morbidity may be acceptable.

In patients with Wegener's granulomatosis, the addition of one double-strength tablet of trimethoprim/sulfamethoxazole twice daily in addition to immunosuppressive medication reduces the frequency of upper respiratory tract relapses [100], possibly by eradicating local *S. aureus.* However, it is poorly tolerated, and mupiromycin may be preferable, although its efficacy in preventing relapses is still unproved.

Monthly pulse cyclophosphamide results in a lower cumulative dose and lower toxicity compared with daily administration, but there are fewer disease remissions, a lower rate of recovery from dialysis, and more relapses and deaths [101]. Patients who fail to respond to monthly

pulse cyclophosphamide can still respond to daily oral treatment [102]. Pulse cyclophosphamide may be effective when given more frequently or in combination with pulse prednisolone [103], but weekly low-dose cyclophosphamide is also associated with a high relapse rate [104].

Refractory disease

Fewer than 10% patients cannot tolerate cyclophosphamide or fail to respond to treatment. In such cases, pulse prednisolone, plasma exchange, intravenous immunoglobulin (IVIg), antithymocyte globulin (ATG), and humanized monoclonal anti-CD4 and anti-CD52 antibodies have been used [105–110].

Initial uncontrolled reports of treatment with IVIg and immunosuppressives were promising, but subsequent studies from the same group have shown that complete remission is rare, that renal function does not improve, and that the relapse rate is high [105–107]. In addition, the treatment is expensive, and the response may be batch dependent.

Anti–T-cell treatment has a role in occasional patients [108–110]. A single course of anti–T-cell therapy in which immunosuppressives were continued resulted in an improvement in four of five patients with a high cumulative dose of cyclophosphamide or refractory disease [108], and partial or complete remission was maintained for the 5 to 12 months of review. Side effects were transient, but repeated dosing was limited by an antiglobulin response. In a subsequent study, all six patients with unresponsive disease who were treated with anti-CD52 +/− anti-CD4 antibodies [110] and in whom immunosuppressives were withdrawn improved, and while four subsequently relapsed after 1.5 to 18 months, these responded to further treatment. Treatment was, however, associated with a prolonged reduction in circulating CD4 counts, and some patients relapsed when counts became normal but responded to further doses. There was no increased rate of opportunistic infections or lymphoma. Treatment with anti-CD52 antibodies, nevertheless, should be reserved for patients with resistant and life-threatening disease.

Limited Wegener's granulomatosis

In patients with limited Wegener's granulomatosis, corticosteroids alone are still ineffective [111], but agents less toxic than cyclophosphamide such as methotrexate [abstract; Handrock et al, *Arthritis Rheum* 37:353, 1994] [112–115], trimethoprim/sulfamethoxazole [116–118], and IVIg have been used.

Data support the use of weekly methotrexate in the induction phase and to maintain remission in patients with limited Wegener's granulomatosis. In one study, more than 40 patients were treated with corticosteroids and 20 to 25 mg methotrexate weekly, and were followed for a median of two years. Seventy-one percent achieved remission within a median of four months, and there was symptomatic improvement in an additional 12%. However, 36% of the patients who achieved remission relapsed after a median of 29 months, when the methotrexate dose was reduced to less than 15 mg/week. Furthermore, some patients with limited disease who were treated with methotrexate developed renal disease [115].

There are case reports in which patients with limited Wegener's granulomatosis have responded to treatment with trimethoprim/sulfamethoxazole [116], but none of eight patients treated with one double-strength tablet twice daily in addition to an unchanged dose of immunosuppressives achieved remission, while 3 (37.5%) improved for 4, 17, or 24 months, and 5 (62.5%) progressed [91, 111]. Any clinical improvement may have resulted from the continued use of immunosuppressives or treatment of intercurrent infections. In a further study [117], trimethoprim/sulfamethoxazole alone induced a complete or partial remission lasting a median of 43 months in 11 out of 19 patients (58%) with early or limited Wegener's granulomatosis, but three of the nonresponders (16%) developed severe generalized disease. Thus, trimethoprim/sulfamethoxazole alone cannot be used to induce remission even in patients with limited disease.

Future treatments

Novel treatments have been identified from our increased understanding of the pathogenetic mechanisms underlying the ANCA-associated vasculitides [118]. These variously reduce circulating ANCA levels, deplete neutrophils, inhibit the cytokines tumor necrosis factor-α and interleukin-1 (such as thalidomide, oxypentifylline, and soluble tumor necrosis factor receptors), inhibit the adhesion molecules that mediate neutrophil–endothelium interaction (abstract; Elliott et al, *Clin Exp Immunol* 112:57, 1998), or interfere with T-cell responses (cyclosporine A [119, 120], FK506, tacrolimus, mycophenolate mofetil [121], serolimus, and deoxyspergualin). Some of these agents have already proved effective in individual case reports or small prospective studies. Cyclosporine (5 mg/kg/day) together with prednisolone can induce remission even in patients with renal disease and may prevent relapses. Mycophenolate mofetil causes ANCA levels to fall and may maintain remission. Patients with a life-threatening disease may respond to immune ablation and peripheral blood stem cell rescue (abstract; Bacon et al, *Clin Exp Immunol* 112:57, 1998). However, in general, controlled prospective studies are lacking, and clinical efficacy has been unproved.

Treatment of Churg–Strauss syndrome

Churg–Strauss syndrome is rare, and its treatment has usually been studied in series that include patients with polyarteritis nodosa. In such reports, glucocorticoids

alone and glucocorticoids together with cyclophosphamide have been efficacious [122]. However, any patient with organ- or life-threatening disease should probably receive both glucocorticoids and a cytotoxic agent as the initial treatment [122]. In contrast to the other small vessel vasculitides, pulse cyclophosphamide together with steroids may be effective in the Churg–Strauss syndrome. High-dose interferon α also maintains remission in patients who have responded incompletely to cyclophosphamide (abstract; Tatsis et al, *Clin Exp Immunol* 112:56, 1998).

OUTCOME

Morbidity in patients with Wegener's granulomatosis or microscopic polyangiitis results from the effects of both the underlying disease and its treatment. It can be quantitated using the vasculitis damage index (VDI) and the short form 36 (SF36) [123], which measures quality of life, but these tools are more useful in evaluating treatment protocols than in assessing individual patients. Overall, the single most important factor in determining the outcome for a patient is the presence of renal disease, and the strongest predictor of renal outcome is the serum creatinine at presentation [124]. The predictive value of a renal biopsy at presentation is limited, but high ANCA levels or persistent circulating ANCA may be associated with worse disease [125, 126]. Mortality is increased in patients who present late, who have pulmonary hemorrhage or C-ANCA–associated disease, or who are treated with corticosteroids alone [90, 124].

Disease-related morbidity occurs in possibly 90% of patients and arises from delays in instituting treatment, progression of subclinical disease, and the tendency to relapse. Complications include sinus dysfunction (in 47%), renal impairment (42%), hearing loss (35%), and moderate to severe respiratory disease (17%) [91].

The greatest risks of treatment of Wegener's granulomatosis with the NIH cyclophosphamide regimen are infections and bladder cancer. In one series, infections required hospitalization in nearly half the patients, and transitional cell bladder cancer was 30 times more common than in the general population [127]. Half the patients had nonglomerular hematuria after a median of 8.5 years, 70% of these had cyclophosphamide-induced cystitis at cystoscopy, and 16% were estimated to develop bladder cancer at 15 years. This group recommended that all patients treated with cyclophosphamide should have urine microscopy every three to six months for life. If nonglomerular hematuria was present, patients should have a cystoscopy, and if hemorrhagic cystitis was demonstrated, cyclophosphamide should be ceased, except in life-threatening circumstances. Individuals with cyclophosphamide-associated cystitis should have urinary cytology every six months and cystoscopy and random biopsies every one to two years. The risk of bladder cancer is 11 times greater than for the rest of the population after just one year of cyclophosphamide treatment in microscopic polyangiitis [125].

Relapses

The frequency of relapses depends on how they are defined, different treatment regimens, and the duration of follow-up. With the Hammersmith regimen, 42% of 45 patients with Wegener's granulomatosis and 27% of 15 with microscopic polyangiitis relapsed within a year of presentation [36]. Relapses usually occur when the immunosuppressive dose is reduced or ceased, especially within the first two years of treatment. Relapses are more common in patients with C-ANCA and PR3 specificity, in patients with Wegener's granulomatosis with persistent nasal *S. aureus* [128], and when induction treatment does not include cyclophosphamide [91]. Relapses are equally likely in patients with limited and generalized disease [36].

Antineutrophil cytoplasmic antibodies recur or persist in at least half the patients who relapse, and there is usually a fourfold or greater increase in IIF titer [99], and at least a doubling of IgG3 subclass ANCA [129]. However, relapses (and disease progression) can occur in the absence of ANCA positivity, and only about half of all patients in whom ANCA recur or who are persistently positive will relapse.

The average time from ANCA increase to relapse is reported to be seven weeks [36]. Most relapses involve the same organs that were affected initially, but renal involvement can occur for the first time at relapse. Lung relapses usually occur if the lung was involved initially, but often at new sites. Lung relapses and infections may be difficult to distinguish clinically, but relapses are rare in the early phase of treatment when high doses of treatment are used. If confusion persists, a histologic diagnosis should be obtained as quickly as possible. Occasionally, relapses affect patients on dialysis or after transplantation [36].

Minor relapses are treated with an increase in dose of corticosteroids and immunosuppressives. Major relapses are treated with reinstitution of the induction regimen, after which doses can be tapered more quickly to just above the levels at which relapse occurred. Relapses usually respond quickly to treatment and may be prevented by longer initial immunosuppressive courses, monthly ANCA monitoring [36], and long-term trimethoprim/sulfamethoxazole in patients with Wegener's granulomatosis [100]. In one study, none of nine patients who were treated on the basis of an increase in ANCA subsequently relapsed, while 9 of 11 (82%) who were not treated did [130]. However, most clinicians would not reinstitute or increase immunosuppressive treatment on the basis of an increased ANCA level alone, but

Table 8. ANCA in different types of glomerulonephritis

	ANCA
Primary pauciimmune crescentic glomerulonephritis	80–90%
AntiGBM disease	20–25%
Immune complex crescentic glomerulonephritis	15–20%
Immune complex glomerulonephritis without crescents	5–10%
Other renal diseases	<5%

Modified from [141] with permission from the *American Journal of Kidney Diseases.*

might observe the patient more closely and reduce drug doses more cautiously. Many of these observations have been made in Wegener's granulomatosis, but the principles probably apply to microscopic polyangiitis as well [131].

Renal transplantation

Renal survival post-transplantation in patients with Wegener's granulomatosis or microscopic polyangiitis is the same as for other causes of end-stage kidney disease [132]. ANCA levels often fall progressively after transplantation [133]. Relapses following transplantation are uncommon [134] and occur less often than in dialyzed patients [132]. The risk of relapse is minimized if cyclophosphamide has been used in the induction regimen by waiting six months after presentation or the most recent relapse, and preferably until ANCA are undetectable, and by including azathioprine and possibly cyclosporine in the antirejection regimen [134–136]. Unfortunately, an increase in ANCA level does not necessarily precede a relapse in patients with transplants (abstract; Schmitt et al, *Clin Exp Immunol* 93:43, 1993), but those who relapse usually respond to treatment with cyclophosphamide [137].

ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES AND NONVASCULITIC DISEASES

Antineutrophil cytoplasmic antibodies occur in a number of other vasculitic and nonvasculitic diseases, and the frequency of these conditions means that ANCA positivity in a routine immunodiagnostic laboratory does not necessarily indicate the diagnosis of Wegener's granulomatosis or microscopic polyangiitis.

Other glomerulonephritides

Antineutrophil cytoplasmic antibodies occur occasionally in IgA, poststreptococcal, and other forms of glomerulonephritis [138–140], especially when crescents are present (Table 8) [141], so an association with ANCA usually implies worse disease [139] [abstract; Bommer et al, *Nephrology* 3:S794, 1997]. It is not clear whether ANCA contribute to or simply reflect glomerular damage.

Antineutrophil cytoplasmic antibodies are present in about 30% of patients with anti-GBM disease and can be demonstrated at presentation or subsequently (abstract; O'Donoghue et al, *Am J Kidney Dis* 18:208, 1991) [142–144]. These patients have more vasculitic features, can recover renal function even if initially dialysis dependent [144], and are more likely to relapse than patients with anti-GBM disease alone [142, 143]. Nevertheless, the prognosis is generally better than uncomplicated anti-GBM disease, possibly because patients present earlier with the constitutional symptoms typical of vasculitis [144]. They usually have P-ANCA with specificity for MPO (abstract; O'Donoghue et al, *Am J Kidney Dis* 18:208, 1991), and anti-GBM antibody levels are often lower than in uncomplicated anti-GBM disease [144]. There is no cross-reactivity between the GBM and MPO antigens (abstract; O'Donoghue et al, *Am J Kidney Dis* 18:208, 1991), and although ANCA are more common in all forms of crescentic glomerulonephritis, anti-GBM antibodies may occur secondary to ANCA-induced glomerular damage at least in some patients.

Drug-induced systemic vasculitis

The most common drugs that induce ANCA and an associated vasculitis are propylthiouracil and related drugs, and hydralazine [12, 145]. ANCA can be demonstrated at some time in about 20% of all patients treated with propylthiouracil (abstract; Cohen Tervaert et al, *Sarcoidosis Vasc Diffuse Lung Dis* 13:280, 1996), and antigen specificities are usually multiple and include MPO and elastase. However, only a few patients develop evidence of a vasculitis, and this may appear at any time after treatment has begun. The vasculitis may take the form of purpura, arthralgia, or crescentic glomerulonephritis. When propylthiouracil is stopped, the vasculitis usually resolves quickly and ANCA levels fall, but some patients have been treated aggressively for the glomerular lesion [146].

Hydralazine-induced ANCA and the associated vasculitis often occur after years of treatment [145]. Clinical features and ANCA characteristics are the same as for propylthiouracil-induced ANCA, but this syndrome differs from hydralazine-associated lupus in that antids DNA antibodies may be present, and there is no association with acetylator status [145]. ANCA also occur after treatment with other agents that cause a drug-induced lupus syndrome, namely penicillamine, phenytoin, and procainamide [145].

Inflammatory bowel disease and autoimmune liver disease

Antineutrophil cytoplasmic antibodies occur in 50 to 70% of patients with ulcerative colitis and 20 to 40% of those with Crohn's disease [34, 49, 51, 147–151]. Frequencies vary because of different testing methodologies and patient groups. IIF patterns are usually P-ANCA

or atypical ANCA. Antigens are multiple and include catalase, α enolase, HMG1/2, BPI, and less often cathepsin G, lactoferrin, lysozyme, PR3, MPO and elastase, and antibody levels are often low.

The demonstration of ANCA by IIF or antigen-specific ELISA does not correlate with disease activity in ulcerative colitis or Crohn's disease [34, 149]. In patients with ulcerative colitis, ANCA are independent of disease extent, persist after colectomy, and do not predict the development of pouchitis after surgery [150]. Furthermore, antibody status changes with time in individual patients [151]. In Crohn's disease, studies that suggested P-ANCA occur more often when the left side of the colon was affected [152], thus resembling the distribution in ulcerative colitis, have not been reproduced [153]. IgA ANCA occur in both ulcerative colitis and Crohn's disease, but their significance is uncertain. Interestingly, ANCA have been described in patients with infective enteritis [149].

Antineutrophil cytoplasmic antibodies occur in more than 70% of patients with primary sclerosing cholangitis or chronic active hepatitis and in about 30% of patients with primary biliary cirrhosis [154]. Again, these are P-ANCA or atypical ANCA, and the specificities include actin [53], HMG1/2 (abstract; Ozaki et al, *Clin Exp Immunol* 112:120, 1998), and the antigens recognized in inflammatory bowel disease. In these diseases, ANCA may correlate with the degree of cirrhosis. ANCA are uncommon in nonautoimmune liver disease [154].

Arthritis

Low levels of P-ANCA (including granulocyte-specific nuclear fluorescence) or atypical ANCA have been demonstrated in 20 to 70% patients with rheumatoid arthritis (abstract; Braun et al, *Clin Exp Immunol* 93:33, 1993) [155–160]. Multiple specificities, including HMG1/2 [53], PR3, MPO, BPI, cathepsin G, lactoferrin, and lysozyme are common, as well as unidentified nuclear antigens of 25 to 35 kD. Several small studies have suggested that ANCA correlate with a rheumatoid vasculitis, but associations with disease severity, nephropathy, nodules, lung and other extra-articular disease, and disease duration are unconfirmed (abstract; Braun et al, *Clin Exp Immunol* 93:33, 1993) [156–160]. ANCA are common in Felty's syndrome [155] and occur occasionally in juvenile chronic arthritis [161] and in reactive arthritis [162].

Antineutrophil cytoplasmic antibodies are described in about 20% of patients with systemic lupus erythematosus (SLE) and have similar patterns and specificities to those seen in rheumatoid arthritis [55, 163–168]. These ANCA probably do not correlate with particular patterns of organ involvement [164], the presence of vasculitis [165], or disease activity [166]. Individual studies have suggested that certain antigen specificities may be clinically significant, but these are inconsistent [167, 168].

Lung disease

Antineutrophil cytoplasmic antibodies occur in at least 5% of patients with interstitial lung disease, which may represent the end-result of vasculitis (abstract; Gaskin et al, *Clin Exp Immunol* 93:33, 1993). C-ANCA (atypical) with specificity for BPI have been described in cystic fibrosis [169], and antibody levels are higher in patients with an associated secondary vasculitis, or pseudomonas colonization. C-ANCA (atypical) occur in suppurative lung disease [170].

Infections

In addition to the associations with infections described earlier in this article, ANCA have been described in isolated cases of subacute bacterial endocarditis [171], and in malaria [172], invasive amoebiasis [173], blastomycosis [174], leptospirosis [175], and onchocerciasis [58]. The demonstration of ANCA in HIV [176] probably results from nonspecific serum stickiness after heat treatment to denature the virus, and the presence of ANCA in some of the other infections may be related in part to the associated hypergammaglobulinemia [30].

CONCLUSIONS

The demonstration of ANCA has proved enormously helpful in the diagnosis of the small vessel vasculitides. While it is still not clear that ANCA actually contribute to the pathogenesis of these diseases, there is increasing evidence, especially from models of vascular damage, that this is so. However, the clinical and pathogenetic significance of ANCA in the nonvasculitic diseases remains poorly understood.

ACKNOWLEDGMENTS

The figures were provided by Roger Silvestrini, Department of Immunopathology, Westmead Hospital, NSW.

Reprint requests to Dr. Judy Savige, University Department of Medicine, Austin and Repatriation Medical Center, Heidelberg, Victoria 3084, Australia.
E-mail: jsavige@austin.unimelb.edu.au

APPENDIX

Abbreviations used in this article are: α_1AT, α_1-antitrypsin; ACR, American College of Rheumatology; ANA, antineutrophil autoantibody; ANCA, antineutrophil cytoplasmic antibodies; ATG, antithymocyte globulin; BPI, bactericidal/permeability-increasing protein; BVAS, Birmingham vasculitis activity score; C-ANCA, cytoplasmic ANCA; CRP, C-reactive protein; GBM, glomerular basement membrane; IIF, indirect immunofluorescence; IL-1, interleukin-1; IVIg, intravenous immunoglobulin; MPO, myeloperoxidase; P-ANCA, perinuclear ANCA; PR3, proteinase 3; RBC, red blood cell; SF36, short form 36 index to measure quality of life; SLE, systemic lupus erythematosus; TNF-α, tumor necrosis factor-α; VDI, vasculitis damage index.

REFERENCES

1. Duna GF, Galperin C, Hoffman GS: Wegener's granulomatosis. *Rheum Dis Clin North Am* 21:949–986, 1995
2. Lhote F, Guillevin L: Polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome. *Rheum Dis Clin North Am* 21:911–947, 1995
3. Savage COS, Harper L, Adu D: Primary systemic vasculitis. *Lancet* 349:553–557, 1997
4. Bajema IM, Hagen EC, van der Woude FJ, Bruijn JA: Wegener's granulomatosis: Meta-analysis of 349 literary case reports. *J Lab Clin Med* 129:17–22, 1997
5. Jennette JC, Falk RJ: Small vessel vasculitis. *N Engl J Med* 1997:337:1512–23
6. Heeringa P, Brouwer E, Cohen Tervaert JW, Weening JJ, Kallenberg CGM: Animal models of antineutrophil cytoplasmic antibody associated vasculitis. *Kidney Int* 53:253–263, 1998
7. Davies DJ, Moran JE, Niall JF, Ryan GB: Segmental necrotising glomerulonephritis with antineutrophil antibody: Possible arbovirus aetiology? *Br Med J* 285:606, 1982
8. van der Woude FJ, Rasmussen N, Lobatto S, Wiik A, Permin H, van Es LA, van der Giessen M, van der Hem GK, The TH: Autoantibodies against neutrophils and monocytes: Tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* 1:425–429, 1985
9. Savage CO, Winearls CG, Jones S, Marshall PD, Lockwood CM: Prospective study of radioimmunoassay for antibodies against neutrophil cytoplasm in diagnosis of systemic vasculitis. *Lancet* 1:1389–1393, 1987
10. Falk RJ, Jennette JC: Antineutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotising and crescentic glomerulonephritis. *N Engl J Med* 318:1651–1657, 1988
11. Cohen Tervaert JW, von Goldschmeding R, dem Borne AEGK, Kallenberg CGM: Antimyeloperoxidase antibodies in the Churg Strauss syndrome. *Thorax* 46:70–71, 1991
12. Dolman KM, Gans RO, Vervaat TJ, Zevenbergen G, Maingay D, Nikkels RE, von Donker AJ, dem Borne AE, Goldschmeding R: Vasculitis and antineutrophil cytoplasmic autoantibodies associated with propylthiouracil therapy. *Lancet* 342:651–652, 1993
13. Ferrario F, Tadros MT, Napodano P, Sinico RA, Fellin G, D'Amico G: Critical re-evaluation of 41 cases of "idiopathic" crescentic glomerulonephritis. *Clin Nephrol* 41:1–9, 1994
14. Andrews M, Edmunds M, Campbell A, Walls J, Feehally J: Systemic vasculitis in the 1980s: Is there an increasing incidence of Wegener's granulomatosis and microscopic polyarteritis? *J R Coll Phys Lond* 24:284–288, 1990
15. Leavitt RY, Fauci AS, Bloch DA, Michel BA, Hunder GG, Arend WP, Calabrese LH, Fries JF, Lie JT, Lightfoot RW, Masi AT, McShane DJ, Mills JA, Stevens MB, Walace SL, Zvaifler NJ: The American College of Rheumatology 1990 criteria for the classification of Wegener's granulomatosis. *Arthritis Rheum* 33:1101–1107, 1990
16. Lightfoot RW, Michel BA, Bloch DA, Hunder GG, Zvaifler NJ, McShane CJ, Arend WP, Calabrese LH, Leavitt RY, Lie JT, Masi AT, Mills JA, Stevens MB, Wallace SL: The American College of Rheumatology 1990 criteria for the classification of polyarteritis nodosa. *Arthritis Rheum* 33:1088–1093, 1990
17. Jennette JG, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL, Hagen C, Hoffman GS, Hunder GG, Kallenberg CGM, McCluskey RT, Sinico RA, Rees AJ, van Es LA, Waldherr R, Wiik A: Nomenclature of systemic vasculitides: Proposal of an international consensus conference. *Arthritis Rheum* 37:187–192, 1994
18. Watts RA, Jolliffe VA, Carruthers DM, Lockwood M, Scott DGI: Effect of classification on the incidence of polyarteritis nodosa and microscopic polyangiitis. *Arthritis Rheum* 39:1208–1212, 1996
19. Franssen CFM, Gans ROB, Arends B, Hageluken C, ter Wee PM, Gerlag PGG, Hoorntje SJ: Differences between anti-myeloperoxidase- and anti-proteinase 3- associated renal disease. *Kidney Int* 47:193–199, 1995
20. Franssen C, Gans R, Kallenberg C, Hageluken C, Hoorntje S: Disease spectrum of patients with antineutrophil cytoplasmic autoantibodies of defined specificity: Distinct differences between patients with antiproteinase 3 and antimyeloperoxidase autoantibodies. *J Intern Med* 244:209–216, 1998
21. Rao JK, Weinberger M, Oddone EZ, Allen NB, Landsman P, Feussner JR: The role of antineutrophil cytoplasmic antibody (c-ANCA) testing in the diagnosis of Wegener's granulomatosis. *Ann Intern Med* 123:925–932, 1995
22. Rao JK, Allen NB, Feussner JR, Weinberger M: A prospective study of antineutrophil cytoplasmic antibody (c-ANCA) and clinical criteria in diagnosing Wegener's granulomatosis. *Lancet* 346:926–931, 1995
23. Savige J, Gillis D, Benson E, Davies D, Esnault V, Falk R, Hagen EC, Jayne D, Jennette JC, Paspaliaris B, Pollock W, Pusey C, Savage COS, Silvestrini R, van der Woude F, Wieslander J, Wiik A: International consensus statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA). *Am J Clin Pathol* 111:507–513, 1999
24. Deremee RA: Antineutrophil cytoplasmic autoantibody-associated diseases: A pulmonologist's perspective. *Am J Kidney Dis* 18:181–183, 1991
25. Hagen EC: Standardisation of solid phase assays for ANCA determination. *Nephrology* 3(Suppl):S764–S765, 1997
26. Hagen EC, Daha MR, Hermans J, Andrassy K, Csernok E, Gaskin G, Lesavre P, Ludemann J, Rasmussen N, Sinico RA, Wiik A, van der Woude FJ: Diagnostic value of standardized assays for antineutrophil cytoplasmic antibodies in idiopathic systemic vasculitis: EC/BCR Project for ANCA assay standardization. *Kidney Int* 53:743–753, 1998
27. Jennette JC, Wilkman AS, Falk RJ: Diagnostic predictive value of ANCA serology. *Kidney Int* 53:796–798, 1998
28. Ludemann J, Utecht B, Gross WL: Antineutrophil cytoplasm antibodies in Wegener's granulomatosis recognise an elastinolytic enzyme. *J Exp Med* 171:357–362, 1990
29. Wong RCW, Silvestrini RA, Savige JA, Fulcher D, Benson E: Cytoplasmic fluorescence in a routine immunopathology laboratory. *J Clin Pathol* 52:124–128, 1999
30. Blockmans D, Stevens E, Marien G, Bobbaers H: Clinical spectrum associated with positive ANCA titres in 94 consecutive patients: is there a relation with PR-3 negative c-ANCA and hypergammaglobulinaemia. *Ann Rheum Dis* 57:141–145, 1998
31. Savige JA, Paspaliaris B, Silvestrini R, Davies DJ, Sturgess A, Neil J, Pollock W, Dunster K, Hendle M: Immunofluorescent patterns associated with antineutrophil cytoplasmic antibodies (ANCA) and their differentiation from other auto- and alloantibodies. *J Clin Pathol* 51:568–575, 1998
32. Lock RJ: Detection of autoantibodies to neutrophil cytoplasmic antigens. *J Clin Pathol* 47:4–8, 1994
33. Stroncek DF, Egging MS, Eiber GA, Caly ME: Neutrophil alloantibodies react with cytoplasmic antigens as possible cause of false-positive indirect immunofluorescence assays for antibodies to neutrophil cytoplasmic antigens. *Am J Kidney Dis* 21:368–373, 1993
34. Oudkerk-Pool M, Ellerbroek PM, Ridwan BU, Goldschmeding R, von Blomberg BME, Pena AS, Dolman KM, Bril H, Dekker W, Nauta JJ, Gans ROB, Breed H, Meuwissen SGM: Serum antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease are mainly associated with ulcerative colitis: Correlation between perinuclear antineutrophil cytoplasmic autoantibodies and clinical parameters, medical and surgical treatment. *Gut* 34:46–50, 1993
35. Brimnes J, Halberg P, Wiik A, Heegaard NHH: Specificities of antineutrophil autoantibodies in patients with rheumatoid arthritis (RA). *Clin Exp Immunol* 110:250–256, 1997
36. Jayne DRW, Gaskin G, Pusey CD, Lockwood CM: ANCA and predicting relapse in systemic vasculitis. *Q J Med* 88:127–133, 1995
37. Westman KWA, Selga D, Bygren P, Segelmark M, Baslund B, Wiik A, Wieslander J: Clinical evaluation of a capture ELISA for detection of proteinase 3 antineutrophil cytoplasmic antibody. *Kidney Int* 53:1230–1236, 1998
38. Wang G, Csernok E, de Groot K, Gross WL: Comparison of

eight commercial kits for quantitation of antineutrophil cytoplasmic antibodies (ANCA). *J Immunol Methods* 208:203–211, 1997
39. Gross WL, Schmitt WH, Csernok E: Antineutrophil cytoplasmic autoantibody-associated diseases: A rheumatologist's perspective. *Am J Kidney Dis* 18:175–179, 1991
40. Savige JA, Chang L, Wilson D, Buchanan R: Autoantibodies in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides. *Rheumatol Int* 16:109–114, 1996
41. Kalluri R, Meyers K, Mogyorosi A, Madaio MP, Neilson EG: Goodpasture syndrome involving overlap with Wegener's granulomatosis and antiglomerular basement membrane disease. *J Am Soc Nephrol* 8:1795–1800, 1997
42. Wieslander J: A strategy to assay for ANCA. *Nephrology* 3:S766, 1997
43. Cohney S, Stewart MR, Savige JA: Lupus anticoagulant in antineutrophil cytoplasmic antibody-associated polyarteritis: A case report. *Am J Nephrol* 15:157–160, 1995
44. Kao RC, Wehner NG, Skubitz KM, Gray BH, Hoidal JR: Proteinase 3: A distinct human polymorphonuclear leukocyte proteinase that produces emphysema in hamsters. *J Clin Invest* 82:1963–1973, 1988
45. Bories D, Raynal MC, Solomon DH, Darzynkiewicz Z, Cayre Y: Down-regulation of a serine protease, myeloblastin, causes growth arrest and differentiation of promyelocytic leukemia cells. *Cell* 59:959–968, 1989
46. Weiss J, Victor M, Stendhal O, Elsbach P: Killing of gram-negative bacteria by polymorphonuclear leukocytes: Role of an O_2-independent bactericidal system. *J Clin Invest* 69:959–970, 1982
47. Kain R, Matsui K, Exner M, Binder S, Schaffner G, Sommer EM, Kerjaschki D: A novel class of antineutrophil cytoplasmic antibodies in necrotising and crescentic glomerulonephritis: The lysosomal membrane glycoprotein h-lamp-2 in neutrophil granulocytes and related membrane proteins in glomerular endothelial cells. *J Exp Med* 181:585–597, 1995
48. Locke IC, Leaker B, Cambridge G: A comparison of the characteristics of circulating antimyeloperoxidase autoantibodies in vasculitis with those in non-vasculitic conditions. *Clin Exp Immunol* 115:369–376, 1999
49. Roozendaal C, Zhao MH, Horst G, Lockwood CM, Kleibeuker JH, Limburg PC, Nelis GF, Kallenberg CGM: Catalase and α enolase: Two novel granulocyte autoantigens in inflammatory bowel disease (IBD). *Clin Exp Immunol* 112:10–16, 1998
50. Moodie FDL, Leaker B, Cambridge G, Totty NF, Segal AW: Alpha enolase: A novel cytosolic autoantigen in ANCA positive vasculitis. *Kidney Int* 43:675–681, 1993
51. Sobajima J, Ozaki S, Osakada F, Uesugi H, Shirakawa H, Yoshida M, Nakao K: Novel autoantigens of perinuclear antineutrophil cytoplasmic antibodies (P-ANCA) in ulcerative colitis: novel non-histone chromosomal proteins, HMG1 and HMG2. *Clin Exp Immunol* 107:135–140, 1997
52. Uesugi H, Ozaki S, Sobajima J, Osakada F, Shirakawa H, Yoshida M, Nakao K: Prevalence and characterisation of novel pANCA, antibodies to the high mobility group non-histone chromosomal proteins HMG1 and HMG2, in systemic rheumatic diseases. *J Rheumatol* 25:703–709, 1998
53. Orth T, Gerken G, Kellner R, Buschenfelde KH, Mayet W-J: Actin is a target antigen of antineutrophil cytoplasmic antibodies (ANCA) in autoimmune hepatitis type 1. *J Hepatol* 26:37–47, 1997
54. Stoffel MP, Csernok E, Herzberg C, Johnston T, Carroll SF, Gross WL: Antineutrophil cytoplasmic antibodies (ANCA) directed against bactericidal/permeability-increasing protein (BPI): A new seromarker for inflammatory bowel disease and associated disorders. *Clin Exp Immunol* 104:54–59, 1996
55. Nassberger L, Jonsson H, Sjoholm AG, Sturfelt G, Heubner A: Circulating antielastase in systemic erythematosus. *Lancet* 1:509, 1989
56. Schmitt WH, Csernok E, Flesch BK, Hauschild S, Gross WL: Autoantibodies directed against lysozyme: A new target of antineutrophil cytoplasmic antibodies (ANCA). *Adv Exp Med Biol* 336:267–272, 1993
57. Yang JJ, Tuttle R, Falk RJ, Jennette JC: Frequency of antibactericidal/permeability increasing protein (BPI) and anti-azurocidin in patients with renal disease. *Clin Exp Immunol* 105:125–131, 1996
58. Gallin MY, Jacobi AB, Buttner DW, Schonberger O, Marti T, Eritmann KD: Human autoantibody to defensin: Disease association with hyperreactive onchocerciasis (SOWDA). *J Exp Med* 182:41–47, 1995
59. Zhao MH, Lockwood CM: Azurocidin is a novel antigen for antineutrophil cytoplasmic autoantibodies (ANCA) in systemic vasculitis. *Clin Exp Immunol* 103:397–402, 1996
60. Kirkland GS, Savige JA, Wilson D, Heale W, Sinclair RS, Hope RN: Classical polyarteritis nodosa, and microscopic polyarteritis with medium vessel involvement: A comparison of the clinical and laboratory features. *Clin Nephrol* 47:176–180, 1997
61. Jennette JC, Wilkman AS, Falk RJ: Antineutrophil cytoplasmic autoantibody-associated glomerulonephritis and vasculitis. *Am J Pathol* 135:921–930, 1989
62. Harris AA, Falk RJ, Jennette JC: Crescentic glomerulonephritis with a paucity of glomerular immunoglobulin localisation. *Am J Kidney Dis* 32:179–184, 1998
63. Gaudin PB, Askin FB, Falk RJ, Jennette CJ: The pathologic spectrum of pulmonary lesions in patients with antineutrophil cytoplasmic autoantibodies specific for antiproteinase 3 and antimyeloperoxidase. *Am J Clin Pathol* 104:7–16, 1995
64. Shin MS, Young KR, Ho K-J: Wegener's granulomatosis upper respiratory tract and pulmonary radiographic manifestations in 30 cases with pathogenetic consideration. *Clin Imaging* 22:99–104, 1998
65. Travis WD, Hoffman GS, Leavitt RY, Pass HI, Fauci AS: Surgical pathology of the lung in Wegener's granulomatosis: Review of 87 open lung biopsies from 67 patients. *Am J Surg Pathol* 15:315–333, 1991
66. Schnabel A, Holl-Ulrich K, Dlahoff K, Reuter M, Gross WL: Efficacy of transbronchial biopsy in pulmonary vasculitides. *Eur Respir J* 10:2738–2743, 1997
67. Mark EJ, Flieder DB, Matsubara O: Treated Wegener's granulomatosis: Distinctive pathological findings in the lungs of 20 patients and what they tell us about the natural history of the disease. *Hum Pathol* 28:450–458, 1997
68. Andrassy K, Kuster S, Waldherr R, Ritz E: Rapidly progressive glomerulonephritis: Analysis of prevalence and clinical course. *Nephron* 59:206–212, 1991
69. Niles JL, Bottinger EP, Saurina GR, Kelly KJ, Pan G, Collins ABX, McCluskey RT: The syndrome of lung haemorrhage and nephritis is usually an ANCA-associated condition. *Arch Intern Med* 156:440–445, 1996
70. Specks U, Wheatley CL, McDonald TJ, Rohrbach MS, Deremee RA: Anticytoplasmic autoantibodies in the diagnosis and follow-up of Wegener's granulomatosis. *Mayo Clin Proc* 64:28–36, 1989
71. Langford CA, Sneller MC, Hallahan CW, Hoffman GS, Kammerer WA, Talar-Williams C, Fauci AS, Lebovics RS: Clinical features and therapeutic management of subglottic stenosis in patients with Wegener's granulomatosis. *Arthritis Rheum* 39: 1754–1760, 1996
72. Rotterm M, Fauci ASS, Hallahan CW, Kerr GS, Lebovics R, Leavitt RY, Hoffman GS: Wegener granulomatosis in children and adolescents: Clinical presentation and outcome. *J Pediatr* 122:26–31, 1993
73. Valentini RP, Smoyer WE, Sedman AB, Kershaw DB, Gregory MJ, Bunchman TE: Outcome of antineutrophil cytoplasmic autoantibodies-positive glomerulonephritis and vasculitis in children: A single centre experience. *J Paediatr* 132:325–328, 1998
74. Dayoan ES, Dimen LL, Boylen CT: Successful treatment of Wegener's granulomatosis during pregnancy. *Chest* 113:836–838, 1998
75. Krafcik SS, Covin RB, Lynch JP, Sitrin RG: Wegener's granulomatosis in the elderly. *Chest* 109:430–437, 1996
76. Vassallo M, Shepherd RJ, Iqbal P, Feehally J: Age-related variations in presentation and outcome in Wegener's granulomatosis. *J Roy Coll Phys Lond* 31:396–400, 1997
77. Maillefert JF, Pfitzenmeyer P, Thenet M, Olsson NO, Piroth C, Behin A, Tavernier C, Justrabo E: Prevalence of ANCA in

a hospitalised elderly French population. *Clin Exp Rheumatol* 15:603–607, 1997
78. Esnault VLM, Testa A, Audrain M, Roge C, Hamidou M, Barrier JH, Sesboue R, Martin J-P, Lesavre P: α1 Antitrypsin genetic polymorphism in ANCA-positive systemic vasculitis. *Kidney Int* 43:1329–1332, 1993
79. Segelmark M, Elzouki AN, Wieslander J, Eriksson S: The PiZ gene of α1 antitrypsin as a determinant of outcome in PR3-ANCA-positive vasculitis. *Kidney Int* 48:844–850, 1995
80. Mazodier P, Elzouki ANY, Segelmark M, Eriksson S: Systemic necrotising vasculitides in severe α1 antitrypsin deficiency. *Q J Med* 89:599–611, 1996
81. Dolman KM, Stegeman CA, van der Wiel BA, Hack CE, von dem Borne AE, Kallenberg CG, Goldchmeding R: Relevance of classic antineutrophil cytoplasmic autoantibody (cANCA)-mediated inhibition of proteinase 3-α1-antitrypsin complexation to disease activity in Wegener's granulomatosis. *Clin Exp Immunol* 93:405–410, 1993
82. Savage COS, Winearls CG, Evans DJ, Rees AJ, Lockwood CM: Microscopic polyarteritis: Presentation, pathology and prognosis. *Q J Med* 56:467–483, 1985
83. Jayne DRW, Jones SJ, Severn A, Shaunak S, Murphy J, Lockwood CM: Severe pulmonary haemorrhage and systemic vasculitis in association with circulating antineutrophil cytoplasm antibodies of IgM class only. *Clin Nephrol* 32:101–106, 1989
84. Geffriaud-Ricouard C, Noel LH, Chaveau D, Houhou S, Grunfeld JP, Lesavre P: Clinical spectrum associated with ANCA of defined antigen specificities in 98 selected patients. *Clin Nephrol* 39:125–136, 1993
85. Lanham JG, Elkon KB, Pusey CD, Hughes GR: Systemic vasculitis with asthma and eosinophilia: A clinical approach to the Churg-Strauss syndrome. *Medicine (Baltimore)* 63:65–81, 1984
86. Luqmani RA, Bacon PA, Moots RJ, Janssen BA, Pall A, Emery P, Savage C, Adu D: Birmingham Vasculitis Activity Score (BVAS) in systemic necrotising vasculitis. *Q J Med* 87:671–678, 1994
87. Nachman PH, Hogan SL, Jennette JC, Falk RJ: Treatment, response and relapse in antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *J Am Soc Nephrol* 7:33–39, 1996
88. Hoffman GS: Treatment of Wegener's granulomatosis: Time to change the standard of care? *Arthritis Rheum* 40:2099–2104, 1998
89. Jayne DRW, Rasmussen N: Treatment of antineutrophil-cytoplasm autoantibody-associated systemic vasculitis: Initiatives of the European Community Systemic Vasculitis Clinical Trials Study Group. *Mayo Clin Proc* 72:737–747, 1997
90. Rasmussen N, Jayne DRW: Review of the activities of the European Vasculitis Study Group (EUVAS). *Clin Exp Immunol* 112(Suppl 1):13–15, 1998
91. Hoffman GS, Kerr GS, Leavitt RY, Hallahan CK, Lebovics RS, Travis WD, Rottern M, Fauci AS: Wegener granulomatosis: An analysis of 158 patients. *Ann Intern Med* 116:488–498, 1992
92. Couser WG: Idiopathic rapidly progressive glomerulonephritis. *Am J Nephrol* 2:57–69, 1982
93. Bolton WK, Sturgill BC: Methylprednisolone therapy for acute crescentic rapidly progressive glomerulonephritis. *Am J Nephrol* 9:368–375, 1989
94. Stevens ME, McConnell M, Bone JM: Aggressive treatment with pulse methylprednisolone or plasma exchange is justified in rapidly progressive glomerulonephritis. *Proc Eur Dial Transplant Assoc* 19:724–731, 1982
95. Levy JB, Winearls CG: Rapidly progressive glomerulonephritis: What should be first-line therapy? *Nephron* 67:402–407, 1994
96. Pusey CD, Rees AJ, Evans DJ, Peters DK, Lockwood CM: Plasma exchange in focal necrotising glomerulonephritis without antiGBM antibodies. *Kidney Int* 40:757–763, 1991
97. Cole E, Cattran D, Magil A, Greenwood C, Churchill D, Sutton D, Clark W, Morrin P, Posen G, Bernstein K, Dyck R, the Canadian Apheresis Study Group: A prospective randomised trial of plasma exchange as additive therapy in idiopathic crescentic glomerulonephritis. *Am J Kidney Dis* 20:261–269, 1992
98. Rees AJ: Vasculitis and the kidney. *Curr Opin Nephrol Hypertens* 5:273–281, 1996
99. Cohen Tervaert JW, van der Woude FJ, Fauci AS, Ambrus JL, Velosa J, Keane WF, Meijer S, van der Giessen M, The TH, van der Hem GK, Kallenberg CGM: Association between active Wegener's granulomatosis and anticytoplasmic antibodies. *Arch Intern Med* 149:2461–2465, 1989
100. Stegeman CA, Cohen Tervaert JW, de Jong PE, Kallenberg CGM, the Dutch Cotrimoxazole Wegener Study Group: Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. *N Engl J Med* 335:16–20, 1996
101. Hoffman GS, Leavitt RY, Fleischer TA, Minor JR, Fauci AS: Treatment of Wegener's granulomatosis with intermittent high-dose intravenous cyclophosphamide. *Am J Med* 89:403–410, 1990
102. Genereau T, Lortholary O, Leclerc P, Grenet D, Tubery M, Sicard D, Caubarrere I, Guillevin L: Treatment of systemic vasculitis with cyclophosphamide and steroids: Daily oral low dose cyclophosphamide administration after failure of a pulse intravenous high-dose regimen in four patients. *Br J Rheumatol* 33:959–962, 1994
103. Steppat D, Gross WL: Stage-adapted treatment of Wegener's granulomatosis: First results of a prospective study. *Klin Wochenschr* 67:666–671, 1989
104. Martin-Suarez I, D'Cruz D, Mansoor M, Fernandes AP, Khamashta MA, Hughes GRV: Immunosuppressive treatment in severe connective tissue diseases: Effects of low dose intravenous cyclophophamide. *Ann Rheum Dis* 56:481–487, 1997
105. Jayne DRW, Davies MJ, Fox CJV, Black CM, Lockwood CM: Treatment of systemic vasculitis with pooled intravenous immunoglobulin. *Lancet* 337:1137–1139, 1991
106. Jayne DRW, Esnault VLM, Lockwood CM: ANCA-antiidiotype antibodies and the treatment of systemic vasculitis with intravenous immunoglobulin. *J Autoimmun* 6:207–219, 1993
107. Richter C, Schnabel A, Csernok E, De Groot K, Reinhold-Keller E, Gross WL: Treatment of antineutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis with high-dose intravenous immunoglobulin. *Clin Exp Immunol* 101:2–7, 1995
108. Hagen EC, de Keizer RJW, Andrassy K, van Boven WPL, Bruijn JA, van Es LA, van der Woude FJ: Compassionate treatment of Wegener's granulomatosis with rabbit antithymocyte globulin. *Clin Nephrol* 43:351–359, 1995
109. Lockwood CM, Thiru S, Isaacs JD, Hale G, Waldmann H: Humanised monoclonal antibody therapy for intractable systemic vasculitis. *Lancet* 341:1620–1622, 1993
110. Lockwood CM, Thiru S, Stewart S, Hale G, Isaacs J, Wraight P, Elliott J, Waldmann H: Treatment of refractory Wegener's granulomatosis with humanised monoclonal antibodies. *Q J Med* 89:903–912, 1996
111. Hoffman GS: Immunosuppressive therapy is always required for the treatment of limited Wegener's granulomatosis. *Sarcoidosis Vasc Diffuse Lung Dis* 13:249–252, 1996
112. Hoffman GS, Leavitt RY, Kerr GS, Fauci AS: The treatment of Wegener's granulomatosis with glucocorticosteroid and methotrexate. *Arthritis Rheum* 35:6112–6118, 1992
113. Sneller MC, Hoffman GS, Talar-Williams C, Kerr GS, Hallahan CW, Fauci AS: An analysis of 42 Wegener's granulomatosis patients treated with methotrexate and prednisone. *Arthritis Rheum* 38:608–613, 1995
114. Deleted in proof.
115. de Groot K, Muhler M, Reinhold-Keller E, Paulsen J, Gross WL: Induction of remission in Wegener's granulomatosis with low dose methotrexate. *J Rheumatol* 25:492–495, 1998
116. De Remee RA, McDonald TJ, Weiland LH: Wegener's granulomatosis: Observations on treatment with antimicrobial agents. *Mayo Clin Proc* 60:27–32, 1985
117. Reinhold-Keller E, de Groot K, Rudert H, Nolle B, Heller M, Gross WL: Response to trimethoprim/sulfamethoxazole in Wegener's granulomatosis depends on the phase of disease. *Q J Med* 89:15–23, 1996
118. Jayne D: Current trends in therapy for primary systemic vasculitis. *Nephrology* 3:S785–S787, 1997
119. Georganas C, Ioakimidis D, Iatrou C, Vidalaki B, Iliadou K, Athanassiou P, Kontomerkos T: Relapsing Wegener's granulo-

matosis: Successful treatment with cyclosporin A. *Clin Rheumatol* 15:189–192, 1996
120. Haubitz M, Koch KM, Brunkhorst R: Cyclosporin for the prevention of disease reactivation in relapsing ANCA-associated vasculitis. *Nephrol Dial Transplant* 13:2074–2076, 1998
121. Nowack R, Birck R, van der Woude FJ: Mycophenolate mofetil for systemic vasculitis and IgA nephropathy. *Lancet* 349:774, 1997
122. Langford CA, Sneller MC: New developments in the treatment of Wegener's granulomatosis, polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome. *Curr Opin Rheumatol* 9:26–30, 1997
123. Bacon PA, Moots RJ, Exley A, Luqmani R, Rasmussen N: VITAL assessment of vasculitis. *Clin Exp Rheumatol* 13:275–278, 1995
124. Hogan S, Nachman PH, Wilkman AS, Jennette JC, Falk RJ, the Glomerular Diseases Collaborative Network: Prognostic markers in patients with antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *J Am Soc Nephrol* 7:23–32, 1996
125. Westman KWA, Bygren PG, Olsson H, Ranstan J, Wieslander J: Relapse rate, renal survival and cancer morbidity in patients with Wegener's granulomatosis or microscopic polyangiitis with renal involvement. *J Am Soc Nephrol* 7:842–852, 1998
126. Franssen CFM, Stegemen CA, Oost-Kort WW, Kallenberg CGM, Limburg PC, Tiebosch A, de Jong PE, Cohen Tervaert JW: Determinants of renal outcome in antimyeloperoxidase-associated necrotizing crescentic glomerulonephritis. *J Am Soc Nephrol* 9:1915–1923, 1998
127. Talar-Williams C, Hijazi YM, Walther MM, Linehan WM, Hallahan CW, Lubensky I, Kerr GS, Hoffman GS, Fauci AS, Sneller MC: Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegener's granulomatosis. *Ann Intern Med* 124:477–484, 1996
128. Stegeman CA, Cohen Tervaert JW, Sluiter WJ, Manson WL, de Jong PE, Kallenberg CGM: Association of chronic nasal carriage of *Staphylococcus aureus* and higher relapse rates in Wegener's granulomatosis. *Ann Intern Med* 120:12–17, 1994
129. Mulder AHL, Stegmen CA, Kallenberg CGM: Activation of granulocytes by antineutrophil cytoplasmic antibodies (ANCA) in Wegener's granulomatosis: A predominant role for the IgG3 subclass of ANCA. *Clin Exp Immunol* 101:227–232, 1995
130. Cohen Tervaert JW, Huitema MG, Hene RJ, Sluiter WJ, The TH, van der Hem GK, Kallenberg CG: Prevention of relapses in Wegener's granulomatosis by treatment based on antineutrophil cytoplasmic antibody titre. *Lancet* 336:709–711, 1990
131. De Oliveira J, Gaskin G, Dash A, Rees AJ, Pusey CD: Relationship between disease activity and antineutrophil cytoplasmic antibody concentration in long-term management of systemic vasculitis. *Am J Kidney Dis* 25:380–389, 1995
132. Kliem V, Haubitz M, Ehlerding G, Nashan B, Schlitt HJ, Oldhafer KJ, Pichlmayr R, Koch KM, Brunkhorst R: Outcome of kidney transplantation in patients with systemic autoimmune diseases. *Transplant Proc* 29:957–958, 1997
133. Frasca GM, Neri L, Martello M, Sestigiani E, Borgnino LC, Bonomini V: Renal transplantation in patients with microscopic polyarteritis and antimyeloperoxidase antibodies: Report of 3 cases. *Nephron* 72:82–85, 1996
134. Rostaing L, Modesto A, Oksman F, Cisterne J-M, Le Mao G, Durand D: Outcome of patients with antineutrophil cytoplasmic autoantibody-associated vasculitis following cadaveric kidney transplantation. *Am J Kidney Dis* 29:96–102, 1997
135. Le Mao G, Rostaing L, Modesto A, Oksman F, Cisterne JM, Durand D: Recurrence of ANCA-associated microscopic polyangiitis after cadaveric renal transplant. *Transplantat Proc* 28:2803–2804, 1996
136. Clarke AE, Bitton A, Eappen R, Danoff DS, Esdaile JM: Treatment of Wegener's granulomatosis after renal transplantation: Is cyclosporine the preferred treatment? *Transplantation* 50:1047–1051, 1990
137. Grotz W, Wanner C, Rother E, Schollmeyer P: Clinical course of patients with antineutrophil cytoplasm antibody positive vasculitis after kidney transplantation. *Nephron* 69:234–236, 1995
138. Ronda N, Esnault VLM, Layward L, Sepe V, Allen A, Feehally HJ, Lockwood CM: Antineutrophil cytoplasmic antibodies (ANCA) of IgA isotype in adult Henoch-Schönlein purpura. *Clin Exp Immunol* 95:49–55, 1994
139. Ardiles LG, Valderrama G, Moya P, Mezzano SA: Incidence and studies on antigenic specificities of antineutrophil-cytoplasmic autoantibodies (ANCA) in post-streptococcal glomerulonephritis. *Clin Nephrol* 47:1–5, 1997
140. Deleted in proof.
141. Jennette JC: Antineutrophil cytoplasmic autoantibody-associated diseases: A pathologist's perspective. *Am J Kidney Dis* 18:164–170, 1991
142. Wahls TL, Bonsib SM, Schuster VL: Coexistent Wegener's granulomatosis and antiglomerular basement membrane disease. *Hum Pathol* 18:202–205, 1987
143. Jayne DRW, Marshall PD, Jones SJ, Lockwood CM: Autoantibodies to GBM and neutrophil cytoplasm in rapidly progressive glomerulonephritis. *Kidney Int* 37:965–970, 1990
144. Bosch X, Mirapeix E, Font J, Borrelas X, Rodriguez RX, Lopez-Soto A, Ingelmo M, Revert L: Prognostic implication of antineutrophil cytoplasmic autoantibodies with myeloperoxidase specificity in antiglomerular basement membrane disease. *Clin Nephrol* 36:107–113, 1991
145. Short AK, Lockwood CM: Antigen specificity in hydralazine associated ANCA positive systemic vasculitis. *Q J Med* 88:775–783, 1995
146. Yuasa S, Hashimoto M, Yura T, Sumikura T, Takahashi N, Shoji T, Uchida K, Fujioka H, Kihara M, Matsuo H: Antineutrophil cytoplasmic antibodies (ANCA)-associated crescentic glomerulonephritis and propylthiouracil therapy. *Nephron* 73:701–703, 1996
147. Saxon A, Shanahan F, Landers C, Ganz T, Targan S: A distinct subset of antineutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. *J Allergy Clin Immunol* 86:202–210, 1990
148. Cambridge G, Ramptom DS, Stevens TRJ, McCarthy DA, Kamm M, Leaker B: Antineutrophil antibodies in inflammatory bowel disease: Prevalence and diagnostic role. *Gut* 33:668–674, 1992
149. Walmsley RS, Zhao M, Hamilton MI, Brownlee A, Chapman A, Pounder RE, Wakefield AJ, Lockwood CM: Antineutrophil cytoplasm autoantibodies against bactericidal/permeability-increasing protein in inflammatory bowel disease. *Gut* 40:105–109, 1997
150. Esteve M, Mallolas J, Klaassen J, Abad-Lacruz A, Gonzalez-Huix F, Cabre E, Fernandes-Banares F, Bertran X, Condom E, Marti-Rague J, Gassull MA: Antineutrophil cytoplasmic antibodies in sera from colectomised ulcerative colitis patients and its relation to the presence of pouchitis. *Gut* 38:894–898, 1996
151. Vecchi M, Bianchi MB, Calabresi C, Meucci G, Tatarella M, de Franchis R: Long term observation of the perinuclear antineutrophil cytoplasmic antibody status in ulcerative colitis patients. *Scand J Gastroenterol* 33:170–173, 1998
152. Vasiliauskas EA, Plevy SE, Anders CJ, Binder SW, Ferguson DM, Yang H, Rotter JI, Vidrich A, Targan SR: Perinuclear antineutrophil cytoplasmic antibodies in patients with Crohn's disease define a clinical subgroup. *Gastroenterology* 110:1810–1819, 1996
153. Jamar-Leclerc N, Reumaux D, Duthilleul P, Colombel JF: Do pANCA define a clinical subgroup in patients with Crohn's disease? *Gastroenterology* 112:316–317, 1997
154. Mulder AHL, Horst G, Haagsma EB, Limburg PC, Kleibeuker JH, Kallenberg CGM: Prevalence and characterisation of neutrophil cytoplasmic antibodies in autoimmune liver diseases. *Hepatology* 17:411–417, 1993
155. Wiik A: Granulocyte-specific antinuclear antibodies: Possible significance for the pathogenesis, clinical features and diagnosis of rheumatoid arthritis. *Allergy* 35:263–289, 1980
156. Savige JA, Gallichio MC, Stockman A, Cunningham TJ, Rowley MJ, Georgiou T, Davies D: Antineutrophil cytoplasm antibodies in rheumatoid arthritis. *Clin Exp Immunol* 86:92–98, 1991
157. Coremans IEM, Hagen EC, Daha MR, van der Woude FJ, van der Vort EAM, Kleijburg-van der Keur C, Breedweld FC:

Antilactoferrin antibodies in patients with rheumatoid arthritis are associated with vasculitis. *Arthritis Rheum* 35:1466–1467, 1992
158. Mulder AH, Horst G, van Leeuwen MA, Limburg PC, Kallenberg CG: Antineutrophil cytoplasmic antibodies (ANCA) in rheumatoid arthritis: Characterisation and clinical correlations. *Arthritis Rheum* 36:1054–1060, 1993
159. Cambridge G, Williams M, Leaker B, Corbett M, Smith CR: Antimyeloperoxidase antibodies in patients with rheumatoid arthritis: Prevalence, clinical correlates and IgG subclass. *Ann Rheum Dis* 53:24–29, 1994
160. Mustila A, Korpela M, Mustonen J, Helin H, Huhtala H, Soppi E, Pasternack A, Miettinen A: Perinuclear antineutrophil cytoplasmic antibody in rheumatoid arthritis: A marker of severe disease with associated nephropathy. *Arthritis Rheum* 40:710–717, 1997
161. Mulder L, van Rossum M, Horst G, Limburg P, de Graeff-Meeder ER, Kuis W, Kallenberg C: Antineutrophil cytoplasmic antibodies in juvenile chronic arthritis. *J Rheumatol* 24:568–575, 1997
162. Locht H, Peen E, Skogh T: Antineutrophil cytoplasmic antibodies in reactive arthritis. *J Rheumatol* 22:2304–2306, 1995
163. Nassberger L, Sjoholm AG, Jonsson H, Sturfelt G, Akesson A: Autoantibodies against neutrophil cytoplasm components in systemic lupus erythematosus and in hydralazine-induced lupus. *Clin Exp Immunol* 81:380–383, 1990
164. Schnabel A, Csernok E, Isenberg DA, Mrowka C, Gross WL: Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus: Prevalence, specificities and clinical significance. *Arthritis Rheum* 38:633–637, 1995
165. Pauzner R, Urowitz M, Gladman D, Gough J: Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. *J Rheumatol* 21:1670–1673, 1994
166. Nishiya K, Chizawa H, Nishimura S, Hisakawa N, Hashimoto K: Antineutrophil cytoplasmic antibody in patients with systemic lupus erythematosus is unrelated to clinical features. *Clin Rheumatol* 16:70–75, 1997
167. Nassberger L: Distribution of antineutrophil cytoplasmic autoantibodies in SLE patients with and without renal involvement. *Am J Nephrol* 16:548–549, 1996
168. Galeazzi M, Morozzi G, Sebastiani GD, Bellisai F, Marcolongo R, Cervera R, De Ramon Garrido E, Fernandez-Nebro A, Houssiau F, Jedryka-Goral A, Mathieu A, Papasteriades C, Piette JC, Scorza R, Smolen J: Antineutrophil cytoplasmic antibodies in 566 European patients with systemic lupus erythematosus: Prevalence, clinical associations and correlation with other autoantibodies. European concerted action on the immunogenetics of SLE. *Clin Exp Rheumatol* 16:541–546, 1998
169. Zhao MH, Jayne DRW, Ardiles LG, Culley F, Hodson ME, Lockwood CM: Autoantibodies against bactericidal/permeability increasing protein in patients with cystic fibrosis. *Q J Med* 89:259–265, 1996
170. Nolan P, Singh B: Specificity of cANCA for Wegener's granulomatosis. *Aust NZ J Med* 26:720–721, 1996
171. Subra JF, Michelet C, Laporte J, Carrere F, Reboul P, Cartier F, Saint-Andre JP, Chevailler A: The presence of cytoplasmic antineutrophil cytoplasmic antibodies (C-ANCA) in the course of subacute bacterial endocarditis with glomerular involvement, coincidence or association? *Clin Nephrol* 49:15–18, 1998
172. Yahya TM, Benedict S, Shalabi A, Bayoumi R: Antineutrophil cytoplasmic antibody (ANCA) in malaria is directed against cathepsin G. *Clin Exp Immunol* 110:41–44, 1997
173. Pudifin DJ, Duursma J, Gathiram V, Jackson TFHG: Invasive amoebiasis is associated with the development of antineutrophil cytoplasmic antibody. *Clin Exp Immunol* 97:48–51, 1994
174. Stappaerts I, Bogers J, Ebo D, Vanden Broecke E, Stevens WJ, Vermeire P: C-ANCA positivity in a Belgian patient with pulmonary paracoccidiodomycosis. *Eur Respir J* 10:2419–2422, 1997
175. Constantin A, Marin F, Oksman F, Bouteiller G: Antineutrophil cytoplasmic antibodies in leptospirosis. *J Rheumatol* 23:411, 1996
176. Koderisch J, Andrassy K, Rasmussen N, Hartmann M, Tilgen W: "False-positive" antineutrophil cytoplasmic antibodies in HIV infection. *Lancet* 335:1227–1228, 1990