

# CAT

## Critically Appraised Topic

<b>The use of antineutrophil cytoplasmic antibodies in the diagnosis and monitoring of small vessel vasculitis</b>
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### **CLINICAL BOTTOM LINE**

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*Antineutrophil cytoplasmic antibodies (ANCA) are useful markers in the diagnosis of small vessel vasculitis, such as Wegener's granulomatosis and microscopic polyangiitis. However, the diagnostic value of ANCA depends largely on the pretest probability of small vessel vasculitis. If a patient presents with little clinical evidence of a small vessel vasculitis, the diagnostic probability of ANCA-associated vasculitis remains low, despite a positive ANCA result. Guidelines are necessary to prevent overuse and hence raise the diagnostic performance of the test. Furthermore it may be useful to educate the requesting clinician about the clinical indications, limitations and interpretation of ANCA-testing. The use of ANCA-testing as a 'screen for vasculitis' should be discouraged and clinicians should provide evidence that a small vessel vasculitis is more than something that they hope a negative ANCA will exclude. Many studies show that ANCA-testing cannot confirm or exclude the diagnosis of small vessel vasculitis, and that it should only be used as a part of a carefully considered investigation. The use of ANCA for follow-up of small vessel vasculitis is still a point of controversy.*

### **CLINICAL/DIAGNOSTIC SCENARIO**

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*The pauci-immune necrotizing small vessel vasculitides, including microscopic polyangiitis, Wegener's granulomatosis, the Churg-Strauss syndrome, and pauci-immune necrotizing and crescentic glomerulonephritis are currently known as "ANCA-associated vasculitides" (AAV). These diseases are relatively rare, but the number of assays that are performed in the laboratory does not reflect this rarity. Therefore, we suspect an overuse of ANCA-testing as a diagnostic tool for AAV, and will attempt to uncover a feasible 'gating-policy'. In addition to the use of this test in a clinical setting with a low pretest probability, the ANCA-assay has other ongoing controversies regarding its application as a clinical tool: the lack of standardisation, the diversity of performance characteristics, and the use of ANCA-titers in the follow-up of AAV. These controversies have consequences not only for the use of ANCA in the diagnosis and monitoring of AAV, but also for the laboratory responsible for providing the requesting clinician with an appropriate answer.*

## QUESTIONS

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- 1) *In which patients is ANCA-testing useful as a diagnostic tool?*
- 2) *Can we develop a feasible 'gating-policy' to increase the pretest probability of the test and hence improve its diagnostic impact?*
- 3) *Is our current flowchart for ANCA-detection sufficiently reliable? If not, is there a way to improve test performances?*
- 4) *Can ANCA-testing be used in the follow-up of patients with ANCA-associated vasculitis?*

## SEARCH TERMS

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- 1) *MeSH Database (PubMed): "Antibodies, Antineutrophil Cytoplasmic"[MeSH] AND "Vasculitis"[MeSH]; (ANCA [MeSH Major Topic] OR (ANCA[TITLE WORD])) AND (1950:2005[DP] AND English[Language] AND (2005/02:2005/03[DP] OR Humans[MESH]) AND (1995[Publication Date] : 2005[Publication Date]) AND jsubsetaim[All Fields])*
- 2) *Pubmed (Medline; from 1966), SUMSearch (<http://sumsearch.uthscsa.edu/>): "ANCA"*
- 3) *UpToDate Online version 13.1 (2005): "ANCA" and "vasculitis"*
- 4) *Google advanced search on "ANCA" and "vasculitis"*

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## APPRAISAL

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After going through the medical literature, we find it more useful to discuss the current opinion on ANCA-testing first, before assessing ANCA-testing in our own laboratory. In the first part of this paper we will summarise the current situation in the field of ANCA-testing in general and attempt to provide the answers on the questions stated on page 2. For the sake of completeness, a second part is added in which we will review ANCA-testing in our laboratory. As the latter was not the primary goal of this critically appraised topic, the description will be very schematical.

### I. CURRENT INSIGHT

#### *Introduction*

ANCA stands for antineutrophil cytoplasmic antibodies. These autoantibodies are directed against certain proteins in the cytoplasm of neutrophils and monocytes. They were first described in 1982 in patients with necrotizing glomerulonephritis and were believed to be associated with Ross River virus infections [1]. Nowadays, ANCA have been clearly linked to small vessel vasculitis and seem to be an important tool in the diagnosis, pathogenesis, classification and follow-up of ANCA-associated vasculitides. The term ‘ANCA-associated vasculitides’ (AAV) describes the pauci-immune necrotizing small vessel vasculitides, including Wegener’s granulomatosis (WG), microscopic polyangiitis (MPA) and its renal limited variant (*ie* pauci-immune necrotizing and crescentic glomerulonephritis) and the Churg-Strauss syndrome (CSS).

In addition to their relationship with AAV, ANCA have also been reported in many other disorders (Attachment 1). This is due to the fact that ANCA recognize a multitude of antigens, such as proteinase 3 (PR3), myeloperoxidase (MPO), bactericidal/permeability-increasing protein (BPI), cathepsin-G, lactoferrin, elastase,  $\beta$ -glucuronidase, lysozyme,  $\alpha$ -enolase, azurocidine and others [2-4]. Of this spectrum of antigens only 2 have a (been) proven clinical relevance in the context of AAV, *ie* PR3 and MPO (proteinase 3 and myeloperoxidase). As far as all other ANCA are concerned, the literature suggests that these do not help to elucidate the diagnosis or prognosis of the disorders they have been associated with [4]. In addition, increasing evidence arises that ANCA may be of help, albeit limited, in categorising inflammatory bowel disease [5]. However, this item goes beyond the goal of this paper.

Two major categories of ANCA are described: c-ANCA and p-ANCA.

The terminology of **cytoplasmic (c-) ANCA** refers to the diffuse, granular cytoplasmic staining pattern (with central or interlobular accentuation) observed by immunofluorescence microscopy when serum antibodies are incubated with normal granulocytes.

The major c-ANCA antigen is PR3, one of four serine protease homologues (namely 29-kd neutral serine protease) in the azurophilic granules of neutrophils [6].

The terminology of **perinuclear (p-) ANCA** refers to the more perinuclear or nuclear staining pattern of the neutrophils observed by immunofluorescence microscopy after serum incubation with normal granulocytes. The major p-ANCA antigen is MPO, which constitutes nearly 5% of the total protein content of the neutrophil. MPO is found in the same granules as PR3.

A minority of immunofluorescence positive ANCA patterns differs from the typical c- or p-ANCA and are called ‘atypical’ ANCA. These patterns may result from interactions between serum autoantibodies and neutrophilic antigens others than MPO or PR3.

A specific ELISA can mark the presence of anti-MPO or anti-PR3 antibodies.

**Summary:**

Antineutrophil cytoplasmic antibodies are autoantibodies directed against antigens found in cytoplasmic granules of neutrophils. They are useful as a diagnostic tool in the context of small vessel vasculitis. On the basis of immunofluorescence pattern, two major types of ANCA are distinguished: c-ANCA and p-ANCA. ELISA can demonstrate the specific antigen. Only MPO and PR3 are associated with AAV.

*Performing an ANCA-test:*

The lack of standardisation has been an ongoing problem with ANCA-testing and has led to numerous standardisation efforts. A large international study by Hagen and colleagues [2] reported that the use of indirect immunofluorescence technique (IIF) or ELISA alone resulted in an unsatisfactory specificity. On the other hand, the combination of the two methods resulted in a 99% specificity for the diagnosis of AAV; the sensitivity for newly diagnosed WG and MPA was 73% and 67%, respectively. As a result of this report, consensus guidelines for ANCA-testing were published by an international group of ANCA researchers [3]. The IIF technique can give false-positive results due to the interaction of antinuclear antibodies (ANA) with the test-neutrophils. Therefore, the investigators stated that a positive IIF result should always be confirmed with antigen specific ELISA for PR3 and MPO. Further reason to support this approach is the existence of ANCA directed towards antigens other than PR3 and MPO, which are not specific for any disease.

To distinguish ANCA from ANA, control cells, such as lymphocytes, can be used. These cells do not bind ANCA, but they do bind ANA. Surprisingly, most commercially available slides for ANCA screening do not have these control cells, what may result in more false-positive p-ANCA patterns. In addition to false-positive IIF test, MPO-ANCA assays have also been reported with false-positive results, due to the formation of DNA/anti-DNA antibody complexes. [7]

Different IIF patterns for a single serum sample are also described and may be caused by differences in neutrophil substrates.[8] Commercially available ELISA-kits, measuring anti-PR3 antibodies in a direct binding assay, also demonstrated a wide variation in sensitivity varying from 22% to 70%, with a negative predictive value between 43% and 70% [9]. Substantial variation was also noted for MPO-ANCA ELISA. [9]

As we mentioned, current guidelines are based on the study of Hagen et al, and recommend dual testing by standard indirect immunofluorescence and target antigen-specific assays to maximize diagnostic utility [3]. However, it is important to realise that the lack of standardisation and the diversity of performances between commercial IIF and ELISA kits, make a formulation of worldwide guidelines almost impossible, and necessitate the assessment of local guidelines. For example, our laboratory uses IIF for screening and MPO- and PR3 ELISA for confirmation of AAV. At the Mayo Clinic however, the ANCA-testing algorithm is the exact opposite: they screen with PR3- and MPO-ELISA, and positive samples are confirmed by IIF on ethanol-fixed neutrophil slides. Other groups report the highest diagnostic accuracy in the reverse scenario.

We are currently studying the performance of both ELISA and IIF on a selection of samples from the department of internal medicine (all samples requested by (or under supervision of) Prof. Dr. Blockmans and Prof. Dr. Bobbaers), over a period of 18 months. Using statistical

analysis, we will assess the most appropriate flow-chart for detecting ANCA in the patient population of our hospital. It will also enable us to adjust, if necessary, the cut-off points for the ELISA-test and to investigate whether it would be better to perform both ELISA and IIF on all samples that are accepted after a first screening for warranted ANCA-requests. The deadline for this CAT renders it impossible for us to answer these questions at this point of time.

#### *Clinical indications for ANCA-testing.*

#### PREVALENCE AND INCIDENCE OF SMALL VESSEL VASCULITIS:

The ANCA-associated vasculitides are rare disorders. The estimated prevalence for Wegener's granulomatosis is 3 per 100 000 [14]. The Churg-Strauss syndrome has an estimated annual incidence of 1 to 3 per million [14]. It is difficult to establish an accurate incidence of microscopic polyangiitis due to its previous inclusion as part of polyarteritis nodosa (PAN). The incidence of MPA is thought to be approximately 1 per 100 000 [15]. There is abundant data indicating that the likelihood of false-positive results greatly increases if ANCA-testing is performed outside an appropriate clinical context. Several large studies showed that less than half of all ANCA detected in an unselected group of patients are associated with AAV. [10,16-18]

#### DISEASE ASSOCIATION OF ANCA:

The detection of ANCA is associated with many cases of primary small vessel vasculitis (*ie* WG, MPA and CSS). The target antigens of these ANCA are consistently either PR3 or MPO (almost never both). ANCA with specificity for other antigens may be detected in a variety of other disorders (Attachment 1).

#### **Wegener's granulomatosis:**

The combination of a c-ANCA pattern on IIF and a PR3-ANCA assay is very typical for patients with active, generalized WG. Nearly 90% of patients with active WG are ANCA-positive [19]. On the other hand, the absence of ANCA does not rule out the diagnosis of WG, and up to 40% of patients with limited WG (*ie* without renal involvement) may be ANCA-negative [19].

In a large multicentre study [2] 75% of the ANCA-positive samples in WG patients showed a c-ANCA pattern. In that same study 66% of patients with newly diagnosed WG showed PR3-ANCA and 24% showed MPO-ANCA. The sensitivity of c-ANCA for WG in new patients was 64%. The combination of the two patterns (c-ANCA or p-ANCA positive) raised sensitivity to 85%. There was no significant difference in sensitivity for ANCA-positivity between treated or untreated patients. The specificity for the IIF test (c-ANCA and p-ANCA) was 76% towards disease controls (various kinds of vasculitis, glomerulonephritis, rheumatologic diseases,...) and 94% towards healthy controls. Therapy did not change the sensitivity of PR3- or MPO-ANCA. The cut-off points for the ELISA were set to reach a specificity of approximately 90% towards disease control patients. Combining the IIF with ELISA increased specificity to 99% towards disease control patients, and 100% towards healthy controls. The sensitivity for the combination of c-ANCA with PR3-ANCA for new WG patients decreased to 57%.

WEGENER	Positive IIF	New patients: 97		Historical patients: 75	
		82 (85%)		62 (83%)	
		Sensitivity		Sensitivity	
	C-ANCA positive	62 (76%*)	64%	42 (68%*)	56%
	P-ANCA positive	20 (24%*)	21%	20 (32%*)	27%
	Positive ELISA	87 (90%)		66 (88%)	
		Sensitivity		Sensitivity	
	PR3-ANCA positive	64 (74%°)	66%	44 (67%°)	59%
	MPO-ANCA positive	23 (26%°)	24%	22 (33%°)	29%

(\*) percentage on all positive IIF samples

Kidney International, Vol. 53 (1998), pp 743-753

(°) percentage on all positive ELISA samples

### Microscopic polyangiitis:

Patients with MPA are ANCA positive in about 70%, and a p-ANCA pattern, which usually corresponds to the presence of MPO-ANCA, is more typical. In the study by Hagen and colleagues [2] approximately 78% of MPA patients with a positive IIF were p-ANCA positive. Almost 60% of new MPA patients had MPO-ANCA and 27% had PR3-ANCA. The sensitivity of IIF (p- or c-ANCA) in detecting MPA was 82%. There was no significant difference in sensitivity between treated or untreated patients. Combining the IIF with ELISA increased specificity to 99% towards disease control patients, and 100% towards healthy controls. The sensitivity for the combination of p-ANCA with MPO-ANCA for new MPA patients decreased to 49%.

MPA	Positive IIF	New patients: 44		Historical patients: 19	
		36 (82%)		13 (68%)	
		Sensitivity		Sensitivity	
	C-ANCA positive	10 (28%*)	23%	2 (15%*)	11%
	P-ANCA positive	26 (72%*)	59%	11 (85%*)	58%
	Positive ELISA	38 (86%)		17 (89%)	
		Sensitivity		Sensitivity	
	PR3-ANCA positive	12 (32%°)	27%	7 (41%°)	37%
	MPO-ANCA positive	26 (68%°)	59%	10 (59%°)	53%

(\*) percentage on all positive IIF samples

Kidney International, Vol. 53 (1998), pp 743-753

(°) percentage on all positive ELISA samples

### Churg-Strauss Syndrome:

Approximately 50% of the patients with CSS are ANCA positive. These ANCA can be both MPO or PR3 [3,19].

The following table summarizes the most important disease associations of ANCA defined by IIF patterns and antigen specificities.

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

IIF pattern	Antigens	Disease associations
<b>C-ANCA</b>	<b>PR3 alone</b>	<b>Wegener's granulomatosis (80-90%)</b> <b>Microscopic polyangiitis (20-40%)</b> <b>Primary pauciimmune crescentic glomerulonephritis (20-40%)</b> <b>Churg-Strauss syndrome (35%)</b>
C-ANCA (atypical)	BPI alone BPI, MPO, CG,... (often multiple)	Cystic fibrosis (80%) Inflammatory bowel disease Primary sclerosing cholangitis Rheumatoid arthritis
<b>P-ANCA</b>	<b>MPO alone</b>	<b>Microscopic polyangiitis (50%)</b> <b>Primary pauciimmune crescentic glomerulonephritis (50%)</b> <b>Churg-Strauss syndrome (35%)</b> <b>Wegener 's granulomatosis (10%)</b>
	Multiple specificities including: - HMG1/2 - catalase - $\alpha$ -enolase - 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

Many laboratories do not distinguish between P-ANCA and atypical ANCA, and for this reason the frequencies of atypical ANCA are not given.  
(Data from reference 18).

**Summary:**

WG patients are typically positive for c-ANCA and PR3. MPA patients typically show a p-ANCA pattern with MPO positivity. It is important to note that AAV are rare disorders and that a significant proportion of non-AAV patients will test positive for ANCA as well. On the other hand, a proportion of the patients with proven small vessel vasculitis do not have ANCA.

## CLINICAL INDICATIONS

Edgar et al. showed that a very high proportion of requests for ANCA-testing (73%) was demanded for patients with disorders other than AAV in a clinician led environment [21]. This is a strange observation, given the fact that there is a clear association only between ANCA and AAV.

There are no clear diagnostic criteria for AAV. The diagnosis is based on detailed history and clinical examination, supported by technical investigations, with tissue biopsy as the gold standard. ANCA-testing can be very useful to provide further diagnostic certainty when the diagnosis of AAV is suspected. However, the presence of ANCA is not diagnostic for AAV [22] and not all patients with AAV will have a positive ANCA test. The application of ANCA-testing must be well considered and should only be performed when history and

clinical signs suggest the diagnosis of an AAV. It should be bared in mind that AAV are rare disorders and the likelihood of false-positive results will be very high if the test is not performed in patients with a high pretest probability for the disease. The reason for this high frequency of false-positive results is two-fold: firstly antinuclear antibodies can react with neutrophils and give false positive results; and secondly ANCA can interact with a wide variety of antigens, but only ANCA directed against PR3 and MPO are associated with AAV. Raising the pretest probability will result in a better diagnostic performance of ANCA-testing. In 1999, an international consensus statement on testing and reporting of ANCA [3] listed the clinical manifestations that suggest the diagnosis of MG or MPA.

The following signs warrant an ANCA-testing when there is no other obvious cause for the clinical manifestation [3]:

- 1) Glomerulonephritis, especially rapidly progressive glomerulonephritis
- 2) Pulmonary hemorrhage, especially pulmonary renal syndrome
- 3) Cutaneous vasculitis with systemic features
- 4) Multiple lung nodules
- 5) Chronic destructive disease of the upper airways
- 6) Chronic active sinusitis or otitis
- 7) Subglottic tracheal stenosis
- 8) Mononeuritis multiplex or other peripheral neuropathy
- 9) Retro-orbital mass

The diagnostic usefulness of ANCA in the diagnosis of CSS is less well studied. CSS is suspected when asthma and eosinophilia are present in addition to vasculitis.

It is generally accepted that non-specific symptoms, such as fever, arthralgias, myalgias and fatigue do not justify ANCA-testing. However, if we take into consideration that the clinical picture of a small vessel vasculitis can vary widely and it can be especially difficult to diagnose in an early stage AAV, ANCA-testing can be justified for a clinical picture of serious, acute illness, particularly when lungs and/or kidneys are involved. In all other cases ANCA-testing should not be performed.

**Summary:**

ANCA-testing should be restricted to patients with a high pretest probability of having AAV. The use of ANCA-testing as a screening method for AAV must be discouraged because of a high percentage of false positive results.

REQUESTING BEHAVIOUR IN THE UNIVERSITY HOSPITALS OF LEUVEN:

Our study period was one and a half year (April 2003 – October 2005), and all ANCA-tests were identified by a computer search of the laboratory database.

During that period, there were 7781 ANCA-tests performed, of which 6391 (82,14%) were negative, 911 (11,7%) were positive and 479 (6,16%) were dubious on IIF.

597 clinicians (intra- and extra-muros) requested an ANCA-test in the studied period. The frequency of test application per clinician varied between 1 per 18 months and 21 request per month. One extra-muros clinician accounted for 307 requests, with an IIF-positivity of only 5%.

We performed further analysis on the requests of intra-muros clinicians and summarized the application behaviour of the hospital departments with more than one ANCA-request per month:

<i>Department</i>	<i># requests</i>	% IIF positive		% ELISA positive	
		<i>Positive</i>	<i>Positive and Dubious</i>	<i>IIF positive</i>	<i>IIF dubious</i>
IAL	1245	14,5	21,9	49 (56)	15 (22)
NEU	638	3,1	7,1	15 (16)	0 (12)
NEF	585	13,9	18,8	42 (56)	10 (28)
HEP	450	10,7	16,5	6 (19)	12 (15)
REU	374	24,6	32,9	16 (28)	3 (6)
PNE	345	9,6	16,3	27 (36)	4 (8)
GE	212	17	22,7	ND	ND
ALL	203	2,9	5,8	ND	ND
NKO	107	4,7	7,4	ND	ND
GER	63	6,3	14,2	ND	ND
PED	52	11,5	13,5	ND	ND
HEM	34	5,8	14,7	ND	ND

ND = not determined.

**Department:**

IAL = general internal medicine, NEU = neurology, NEF = nephrology, HEP = hepatology, REU = rheumatology, PNE = pneumology, GE = gastroenterology, ALL = allergology, NKO = otorhinolaryngology, GER = geriatric medicine, PED = pediatrics, HEM = haematology.

**# requests:**

Number of requests made in the studied period of one and a half year by the clinicians (of the mentioned department) who made more than one request per month.

**% IIF positive:**

Percentage of positive samples on all requested samples of the mentioned department (under 'positive') and percentage of positive samples plus dubious samples (under 'positive and dubious').

**% ELISA positive:**

Percentage of IIF positive samples (split up in 'IIF positive' and 'IIF dubious') that are also found ELISA positive. This percentage reflects the 'true positive' samples for AAV. Between brackets are the percentages when our MPO cut-off is used (see chapter II.). The numbers not placed between brackets are more meaningful.

This table suggests that the nephrology department and the department of general internal medicine have the best strategy regarding ANCA-testing, with a 'true' positivity rate of 52% (84%) and 64% (78%), respectively.

The rheumatology department has a high percentage of IIF positive samples. As the percentage of true positives is relatively low in these patients, this probably reflects the fact

that many rheumatologic patients express ANCA with other target-antigens than MPO or PR3. The neurology department is responsible for the second most number of requests, but with a very low rate of true positive results.

This wide variation in diagnostic yield between departments is likely due to differences in clinical practice and experience, and in patient population. The overuse of ANCA-testing in the neurology department is a common fact in many hospitals, as a 'vasculitis screening' is routinely performed in some hospitals in patients presenting with both explained and unexplained neurological symptoms and signs. In clinical practice, the most frequent neurological presentation in ANCA-associated disease is peripheral neuropathy, less commonly seen is cranial nerve palsy or cerebral involvement [15,16].

#### *Feasibility of a gating policy on ANCA-testing*

ANCA-testing is being widely applied despite its poor return. The high rates of false-positive results is a well known problem in the literature and many authors [10,16-18] report the need for guidelines for its more effective application. In contrast, we have only found two studies [18,23] that addressed the effect of guidelines for accepting or refusing an ANCA-test.

To understand the true value of ANCA-testing, clinicians need to be informed about the methods used for their detection and the associated problems and pitfalls. It also implies that the clinician is fully aware of the indications for the test. Informing the applicants about ANCA-testing can be a first step in the more accurate use of ANCA-testing.

In 2004, Sinclair et al. reported on the effects of a symptom related gating policy on ANCA requests [18]. They accepted or refused ANCA-testing on the clinical data provided by the clinician, by means of positive selection criteria similar to those reported by the international consensus statement [3]. Of all requests, 72,5% was accepted for ANCA-testing. The reason for this high percentage of acceptance was that their laboratory had promoted the selection criteria for more than 10 years, and subsequently the requesting clinicians were already 'trained' in the appropriate use of the test. Of the accepted requests 73% was negative, and almost 74% of the patients with positive results were found to have an AAV – indicating a low false positive rate. In the two years after the study was initiated, only one patient developed an AAV before an ANCA-testing was performed/accepted. However, the delay in performing the (positive) test was only two days, indicating that the potential delay in diagnosis of AAV when selection criteria for ANCA-testing are used is negligible. They concluded that selecting requests on the basis of a clinical symptom score has a positive impact on the usefulness of the test and renders the test more clinically relevant and cost effective.

In a study by Mandl et al. [17], guidelines (see attachment 2) were applied in a retrospective manner to the existing ANCA requests and they saw that ANCA-testing would have fallen by 23% if the guidelines were used when the samples were received. They also calculated that the false positivity rate for the detection of AAV would have fallen by 27%. The positive predictive values of IIF ANCA for AAV were still very low (55%), with negative predictive values of >90%. However, given the rarity of AAV in the population studied, a high negative predictive value may have little impact. In this study, the chances of having an AAV decreased from 3% to 1% if the ANCA-test was negative. More importantly, their study confirmed that no cases of AAV would have been missed by applying gating criteria. By the way, they showed that in the context of neurological disease the positive predictive value of IIF was 0%.

These two studies demonstrate that the implementation of investigative protocols based on published guidelines can provide a major impact on the efficiency of ANCA-testing. Moreover large savings could be made by implementing such guidelines into routine clinical practice.

Our laboratory, like most others, offers a testing service 'on demand'. This means an unrestricted testing of all samples arriving in the laboratory without any regard to the clinical background. As mentioned, this scenario leads to a large number of ANCA detected with IIF outside the context of small vessel vasculitis (*ie* false positive IIF results).

As an exercise we applied the criteria of Mandl and colleagues in a retrospective manner to all requests (over a period of 18 months) of two staff members of the department of internal medicine (Prof. Dr. Blockmans and Prof. Dr. Bobbaers). We determined the clinical diagnosis by review of the medical records in all patients tested for ANCA in the department most specialised in the treatment of patients with small vessel vasculitis. This method has limitations and depends on the clinician's ability to diagnose an AAV and to record a clear medical report which reflects the contact with the patient. Accordingly we also sought evidence for an AAV in histological, radiological or serological records. All the records of those departments were reviewed to establish if there were clinical indications for ANCA-testing.

We reviewed a total of 286 requests, 199 requests resulted in a negative IIF result, 56 requests had a positive IIF result and 31 requests had a dubious IIF test. If we looked at the 199 requests resulting in a negative IIF, we saw that only 78 requests (39%) would have passed the selection criteria. Of the accepted requests, 20 samples were from proven AAV-patients. No AAV-patients were found in the rejected patients. Concerning the requests with a positive IIF test, 32 of 56 requests would have been accepted (57%). No AAV-patients were found in the rejected samples. And for the dubious results, 16 of 31 requests (52%) would have been accepted, and here as well, no AAV-patients were found in the rejected population. (*Note the similarity in percentages of the 'positive IIF' and 'dubious IIF' group: the requests with a dubious IIF result act like the positive results, this may be a consequence of the fact that our screening dilution for the serum to be tested is 1/40, which is actually a first titration step if we would follow the manufacturer's guidelines (these state a screening with a 1/20 dilution - see part II.). In this context, we wonder if the split into 'positive' and 'dubious' for IIF in AAV-patients is meaningful.*) This means that on a total of 286 requests, 44% (or 126 requests) would be accepted if we applied the criteria of Mandl et al. With approximately 5500 ANCA requests per year in our laboratory, we could save a substantial amount of time and money. If we assume that 50% of the current ANCA requests are not warranted (which is no correct assumption, since we the selection criteria do not accept ANCA-testing for inflammatory bowel disease or auto-immune hepatitis), a total of €17 700 could be saved per year.

Selection criteria should be determined in close consideration with the clinical staff of our hospital and should be evidence based. The acceptance or refusal of the ANCA-test should be reviewed by a clinical biologist before the test is performed. A lack of clinical information should result in refusal of the test, on the other hand it should be possible for the clinician to overrule the criteria if they believe that the test is warranted for a peculiar clinical case despite the patient not meeting the selection criteria.

In addition, ANCA-testing for non-vasculitis cases (for example inflammatory bowel disease and auto-immune hepatitis) should also be justified by providing accurate clinical information. In these cases, the ANCA-testing should be restricted to the performance of IIF, since the performance of an MPO and PR3 ELISA is not meaningful in this clinical context.

Of course, implying a gating-policy on ANCA-testing would have several implications for the laboratory:

Firstly, personal experience suggests that request forms are often inadequately completed, many with no clinical details included at all. How do we respond to these requests?

Secondly, who is the gatekeeper? A senior member of staff, a resident, or a laboratory technician? That person would need to dedicate a considerable amount of time to this task: at 30 seconds to review each form, this would equate to 42 hours each year for 5000 requests, a full working week.

Thirdly, in case of electronic requesting, codes such as 'vasculitis screening' would effectively bypass the request form review.

Fourthly, necrotising vasculitis causes some additional problems. After all, the potential cost to the patient and to the service of a missed diagnosis of necrotising vasculitis could be extensive and exceed the savings made by rejecting 25% of samples dictated by the gating policy. However, it should be mentioned that ANCA-testing is only one point in the diagnosis of necrotising vasculitis and that the presence of an ANCA is neither essential (not currently included in disease definitions) nor sufficient to make a diagnosis of AAV [22]. This being the case, it is the responsibility of the clinician to interpret any given test result, not in isolation, but in the context of the patient's case history and other investigations.

A last remark is that the gating policy itself may lead to a more educated clinician base with clear understanding of the limitations of ANCA-testing, potentially leading to self limitation of use.

**Summary:**

It seems that workload can be reduced by introducing a gating policy (studies report 25% reduction in samples) and that laboratory costs can be saved. There is need for a prospective study based on a clear audit of current workload, case mix, and predictive values in a centre using an open door strategy (*ie* all request are performed), followed by the introduction of a policy based on selection criteria and subsequent re-audit.

*Role of ANCA in follow-up of AAV*

Despite treatment, relapses occur in half of the cases of AAV. Long-term therapy increases the risk of side-effects and treatment toxicity must be carefully monitored [24]. Because relapses of AAV are associated with significant morbidity and mortality due both to the disease and its treatment, their prevention is of single most importance. [25]. A correlation between ANCA and AAV activity would be very useful in reaching this goal.

The increasing evidence from *in vitro* data that ANCA in patients with AAV have a pathogenetic role [26], combined with the observation that ANCA often become undetectable after patients go into remission [27] raise hopes that ANCA may be closely related to disease activity and that results of ANCA-testing may have prognostic significance. In 1985 van der Woude et al [27] reported that ANCA were useful in both the diagnosis and monitoring of disease activity in WG. Patients with active WG tended to have higher titres than those in

remission. In another study the presence of ANCA has been associated with an increased incidence of relapse of systemic vasculitis [28].

However, there is still controversy about the relationship between ANCA and disease activity.

In patients with ANCA positive AAV, a relapse of AAV is in 80% to 100% associated with persistent or renewed positivity for ANCA (either measured by IIF or ELISA) [25,29-32]. On the other hand, a diagnosis of relapse in a patient with AAV must be seriously questioned if ANCA levels persist to be negative. These diagnoses should be histologically proven and other diagnoses have to be ruled out. Whether persistently positive or increasing ANCA levels are good indicators of increasing disease activity is more controversial.

Within the first few months of treatment, ANCA levels fall or become negative in 30% to > 80% of the patients [25]. Although persistent disease activity during treatment is associated with persistent ANCA positivity, most patients with persistent ANCA achieve remission [25,27,29]. If c-ANCA persist or reappear during the first year, there is a significant chance to subsequent relapse in both WG and other AAV [25,29,30]. For p-ANCA this is less clear [30]. In addition, the relapse risk for patients with persistent undetectable c- or p-ANCA is very low [25]. Because many relapses occur during reducing or ceasing of immunosuppressive therapy [29], it seems logical to question whether the response of ANCA levels during treatment should guide the dosage and duration of treatment. A clear relationship between ANCA-titers and disease activity appears to be very difficult to establish and the need for prospective trials continues.

Stegeman reviewed several studies on the relationship between rises in ANCA levels and disease activity [25]. He correctly argues that many of these studies are retrospective, involve small numbers of patients and relapses, and do not standardise the interval between sequential ANCA measurements or the interval between a rise in ANCA level and a clinical event to be associated. In addition he reported problems with the diagnoses of 'relapse', as some authors made the diagnosis on clinical basis, others on histological proof or on non validated disease activity scores. Another problem highlighted by Stegeman is the inter-assay variation. Kerr et al reported a change of one titer step in about one in five determinations of the same sample with IIF on ethanol-fixed neutrophils [31]. All these objections make it very difficult to assess the value of serial ANCA-testing in the follow-up of patients with AAV.

If Stegeman confined to the prospective studies with a reasonable number of patients and at last 2 years of follow-up, predefined criteria for relapse, a standardized interval for sequential ANCA measurements and elimination of the inter-assay variation by measuring two sequential samples in the same assay, the following results could be restrained: 4-fold rises in c-ANCA titre by IIF or > 75% increase in PR3-ANCA level by ELISA are in more than 50% of the cases associated with a relapse within 6 or 12 months [33-35]. Boomsma et al also indicate that PR3-ELISA may be superior to IIF for predicting relapse [33].

Another prospective study by Girard et al [32] showed again that ANCA positivity was associated with relapse of AAV, but that this correlation was weak. Relapses were associated with reappearance of ANCA in only 37% of the patients and persistence of ANCA in 31%. They also demonstrated that ANCA can be positive for several months before relapse. They concluded that WG patients with persistent or reappearing ANCA are potential candidates for relapse. However, they added that because of the weak correlation between relapse and ANCA, ANCA monitoring should not be used as a tool for treatment decisions or adaptations. According to these authors, ANCA should be seen as a warning signal for relapse, which means that the patient's monitoring should be intensified. However, a rise in ANCA levels

does not mean that the patient is relapsing, especially not if the clinical context does not suggest a relapse.

***Summary:***

To date, no markers have been clearly identified as useful predictors of AAV relapses. There appears to be a correlation between ANCA levels and activity of AAV. However, this correlation is weak and prudence is in order if we want to guide treatment on ANCA-levels in the individual patient. In the literature, lack of standardisation of the ANCA-assays is problematic if we want to compare results within and between centres.

## II. ANCA-TESTING IN THE UNIVERSITY HOSPITALS OF LEUVEN

### *Flow chart:*

Screening: indirect immunofluorescence with 1/40 dilution.

- If negative: reported as such.
- If positive:
  - o reporting of the pattern (c-ANCA or p-ANCA) and titration of serum sample (reporting of titre)
  - o MPO and PR3 ELISA (reporting of results)
- If 'dubious' (or uninterpretable):
  - o See 'If positive'

Note:

- 'Dubious' is said for samples of which only the 1/40 dilution is positive on IIF.
- Screening titres for IIF are 1/40, while instruction leaflet recommends 1/20.
- The manufacturer's cut-off value is followed for the PR3-ELISA, but not for the MPO-ELISA (The manufacturer proposes a cut-off of 20 U, while we use a cut-off of 5 U. This is based on a former in-house study. Unfortunately, this study was untraceable.). However, if we take a closer look at the cases with the combination 'IIF positive' and 'MPO positive' (considering our cut-off of 5U), we notice that there are not much pros for this approach. In the studied population (see part I.) we identified 50 samples meeting the combination 'IIF positive' and 'MPO positive', of these samples 18 were identified with a MPO value between our cut-off (5 U) and the manufacturer's cut-off (20 U). When we examined these 18 samples, we found that only 3 were associated with AAV. For the remaining 32 samples (with a MPO of > 20 U), 16 were associated with AAV and another 13 were associated with some kind of vasculitis (PTU-induced vasculitis, aortitis,...). There appears to be no profit by operating a cut-off value of 5 U:
  - o AAV detected with a cut-off of > 20 U: 16 on 32 samples (50%)
  - o AAV detected with a cut-off of > 5 U: 19 on 50 samples (38%)

The advantage of finding 3 additional AAV-patients is overshadowed by 15 additional false positive samples. If we do not consider the absolute negative samples (with MPO values < 5 U), we find a sensitivity of 53% and a specificity of 99% for a cut-off of 20 U compared with a sensitivity of 100% and a specificity of 0% for a cut-off of 5 U.

New cut-off values will be marked, but the deadline of this CAT renders it impossible to solve this problem at this moment.

### *The immunofluorescence test:*

#### 1) Analytical performance characteristics

##### *1.1 Preanalytical considerations*

##### Sample stability:

This test requires a serum specimen: samples should be (immediately) separated from cells to avoid hemolysis.

Serum can be stored at room temperature for no longer than 8 hours. For longer delays it should be stored at 2-8°C until tested. This means that samples from outside our hospital should be transported at 2-8°C. However, our guidelines do not require this refrigerated transport. For longer term of storage, it is recommended to store the samples on -20°C or lower. This according to NCCLS guidelines. Heat inactivated,

hemolysed, microbially contaminated or incompletely defibrinated samples may cause high background staining and make interpretation difficult and are therefore not accepted.

### 1.2 Analytical considerations

#### Reproducibility:

Immunofluorescence microscopy implies semiquantitative results. Determinations are thus dependent on the expertise of the technician, the variable quality of test reagents, and the equipment used. A recent study performed in our hospital [36] assessed the interassay and interobserver variability in the detection of ANCA in patients with ulcerative colitis and they showed large interassay and interobserver variability for the p-ANCA detection (which are associated with ulcerative colitis). For further details, see reference 36.

### 1.3 Turn around time (TAT)

The TAT for ANCA in our laboratory is < 10 days.

The IIF-test is performed four times a week (not on Wednesday).

## 2) Diagnostic performance

### 2.1 Sensitivity, specificity

The literature clearly shows that arguing about performance characteristics of IIF or ELISA alone is of no use because both are proven to be unsatisfactory.

Data provided by the manufacturer of the slides used in our hospital (INOVA):

#### **For Wegener's Granulomatosis:**

Sensitivity: 89% (all c-ANCA positive)

Specificity: 100% (toward normal controls)

Specificity: 96% (towards disease controls)

#### **For Microscopic polyangiitis:**

Sensitivity: 100% (all p-ANCA positive)

Specificity: 100% (towards normal controls)

Specificity: 96% (towards disease controls)

If we analyse the test performances in our laboratory (at random checks from samples from the department of general internal medicine) the following results are found for AAV:

Sensitivity: 68% and specificity: 80% (towards disease controls)

### 2.2 Likelihood ratio's (LR)

Data provided by the manufacturer (INOVA):

**For Wegener's granulomatosis:**

Likelihood ratio for a positive test: 22,25

Likelihood ratio for a negative test: 0,1

**For microscopic polyangiitis:**

Likelihood ratio for a positive test: 2500

Likelihood ratio for a negative test: 0

If we analyse the test performances in our laboratory (at random checks from samples from the department of general internal medicine) the following results are found for AAV:

Likelihood ratio for a positive test: 3,4

Likelihood ratio for a negative test: 0,4

*The ELISA:*1) Analytical performance characteristics*1.1 Preanalytical considerations*Sample stability:

See above.

*1.2 Analytical considerations*Reproducibility:

Data provided by the manufacturer.

## MPO ELISA:

Six replicates each of negative, weak positive and strong positive samples were run in six separate assays. The mean of the strong positive was 100.9, the weak positive was 25.5 and the negative was 19.3. The standard deviation and coefficient of variation for each sample are summarized below.

	Negative		Strong Positive		Weak Positive	
	SD	CV	SD	CV	SD	CV
Overall	1.14	5.9%	7.55	7.5%	1.40	5.5%
Within run	0.88	4.6%	2.11	2.1%	0.84	3.3%
Between run	1.05	5.4%	8.09	8.0%	1.37	5.4%

## PR3 ELISA:

Six replicates each of negative, weak positive and strong positive samples were run in six separate assays. The mean of the strong positive was 100.1, the weak positive was

25 and the negative was 18.75. The standard deviation and coefficient of variation for each sample are summarized below.

	Negative		Strong Positive		Weak Positive	
	SD	CV	SD	CV	SD	CV
Overall	0.81	4.3%	8.90	8.9%	0.80	3.2%
Within run	0.60	3.2%	2.01	2.0%	0.83	3.3%
Between run	0.79	4.2%	9.40	9.4%	0.57	2.3%

Becoming pressed for time, we did not calculate our 'in house' SD and CV so far. Considering these data and our own experience, running samples in duplicate appears to be unnecessary. This will prove (or not) in a more directed study on this matter.

### 1.3 Turn around time (TAT)

The TAT for ANCA in our laboratory is < 10 days.

The ELISA is performed twice a week (on Tuesday and Friday).

## 2) Diagnostic performance

### 2.1 Sensitivity, specificity

The literature clearly shows that arguing about performance characteristics of IIF or ELISA alone is of no use because both are proven to be unsatisfactory.

The article by Csernok et al [9] gives an overview of the performance characteristics of several commercial kits, it is obvious that sensitivity is a major problem, especially in the ELISA. Note that the artificial situation of the tested population (92 proven AAV-patients, 30 healthy controls, 30 disease controls) makes the sensitivity and specificity more rosy than in routine clinical testing. In addition, cut-off values for the ELISA (and the IIF interpretation) have been raised towards a specificity of > 90%.

Assay	WG					MPA				
	Sensitivity (%)	Specificity (%)	AUC	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	AUC	PPV (%)	NPV (%)
IIF	80	100	0.900	100	75	69	97	0.832	97	74
In-house direct ELISA	42	100	0.710	100	51	52	100	0.762	100	66
In-house capture ELISA	74	100	0.870	100	70	62	100	0.810	100	70
Commercial ELISA kits										
A	54	93	0.737	93	55	50	90	0.697	84	62
B	62	100	0.810	100	61	48	97	0.725	95	63
C	56	100	0.790	100	59	45	100	0.726	100	62
D	50	100	0.750	100	55	50	100	0.750	100	64
E	56	100	0.780	100	58	67	79	0.728	78	68
F	60	100	0.800	100	60	62	100	0.810	100	70
G	68	100	0.840	100	65	55	100	0.774	100	67
H	22	100	0.610	100	43	48	97	0.725	95	63
I	70	100	0.850	100	67	55	100	0.774	100	67
J	56	100	0.780	100	58	60	90	0.745	86	67
K	46	100	0.730	100	53	55	100	0.774	100	67

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AUC, area under the curve; ELISA, enzyme-linked immunosorbent assay; IIF, indirect immunofluorescence; MPA, microscopic polyangiitis; NPV, negative predictive value; PPV, positive predictive value; WG, Wegener granulomatosis.

In a routine clinical setting, the combination of IIF with a PR3 ELISA and MPO ELISA shows a 99% specificity for the diagnosis of AAV. Using this approach, the

sensitivity for newly diagnosed Wegener's granulomatosis and microscopic polyangiitis is 73% and 67%, respectively. [12]

Analysis of the ELISA performances in our laboratory could not be evaluated before the deadline of this CAT.

### 3) Clinical impact

- a. *Diagnostic aspect: see text.*
- b. *Treatment: see text.*
- c. *Health outcome: see text.*

### 4) Cost impact:

#### 1. *Actual cost*

#### **Immunofluorescence test:**

- Consumable cost: 4,34 euro.
- Task Unit and logistics: 1,73 euro.
- Support: 1,79 euro.
- Invest: 0 euro

Total cost: 7,86 euro

Each titration step costs an extra 7,86 euro. Knowing that titrations may be up to 1/1280 (this means five dilutions after the screening dilution), the maximum cost for the IIF may be as high as 47,16 euro.

#### **ELISA (MPO or PR3):**

- Consumable cost : 15,65 euro.
- Task Unit and logistics : 2,84 euro.
- Support: 2,80 euro.
- Invest: 0,12 euro.

Total cost : 21,47 euro x 2 (MPO and PR3) = 42,94 euro.

The manufacturer 'recommends' to run the samples in duplicate for both MPO and PR3. However, if we take a closer look at the results of samples run in duplicate, no big differences appear between the two measurements. The sense or nonsense of samples run in duplicate needs to be clarified in a more specific study. If the samples would not be run in duplicate, the cost would be reduced to approximately 26,52 euro.

#### 2. *Reimbursement*

#### **Immunofluorescence test:**

ANCA-detection: B300 = 8,4 euro.

Titration (if ANCA detected): B400 = 11,20 euro.  
Total reimbursement for a positive sample: B700 = 19,60 euro.

**ELISA:**

PR3 detection: B350 = 9,80 euro.  
MPO detection : B350 = 9,80 euro.  
Total: B700 = 19,60 euro.

*3. Cost impact per test*

**Negative IIF:**

Total cost: 7,86 euro.  
Total reimbursement: 8,40 euro.  
Net profit : 0,54 euro.

**Positive IIF:**

Total cost:  $7,86 + 7,86 + \dots + 42,94 = 58,66$  euro (minimum) or 90,10 euro (maximum).  
Total reimbursement: 39,20 euro.  
Net loss : 19,46 euro (minimum) or 50,90 euro (maximum).

5) Decision making

5.1 *Is ANCA-testing helpful in clinical decision making and patient management:  
see part I.*

5.2 *Overexploitation/underutilization of the ANCA-test:  
see part I.*

## **To DO/ACTIONS**

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- 1) To confer with the clinical staff on the gating policy and to determine selection criteria for accepting or refusing ANCA-testing.
- 2) To complete the statistical analysis on our ANCA-detection policy.
- 3) To adjust problems with our ANCA-detection policy once the results of our study are known. Following items should be cleared out:
  - a. The use of ANCA-testing for other disorders than AAV does not require an ELISA if a positive IIF is identified.
  - b. The sense or nonsense of running samples in duplicate on the ELISA.
  - c. Adjusting the cut-off values of the ELISA.
  - d. Appraisal of the flow-chart for ANCA-testing in our hospital.

## **TAKE-HOME MESSAGE**

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*“The problems of antineutrophil cytoplasmic antibody testing include the diversity of antineutrophil cytoplasmic antibody target antigens, assay standardisation and performance, the application of antineutrophil cytoplasmic antibody testing in a clinical setting with a low pretest probability, and, finally, the widespread assumption that antineutrophil cytoplasmic antibody titers alone may closely reflect disease activity and therefore may be used to guide therapy. Recent findings demonstrate that the combined use of indirect immunofluorescence tests and solid phase assays to detect antineutrophil cytoplasmic antibody directed against myeloperoxidase and proteinase 3 can minimize the occurrence of false-positive antineutrophil cytoplasmic antibody results. Furthermore, the yield of antineutrophil cytoplasmic antibody testing can be improved by the use of a well standardised test, adherence to published guidelines, and restricting the use of the test to clinical situations with a rather high pretest probability for antineutrophil cytoplasmic antibody-associated vasculitides. However, treatment decisions should be based on the clinical presentation of the patient and histological findings and not on the results of antineutrophil cytoplasmic antibody testing alone.”*

SCHMITT AND VAN DER WOUDE, 2004.  
(Reference 12)

## ATTACHMENTS

Attachment 1: Disorders different from the AAV for which positive results for ANCA by indirect immunofluorescence and/or solid phase assays have been described.

Disorder	Study
<b>Connective tissue disorders</b>	
Systemic lupus erythematosus	Schnabel, 1995; Chin, 2000; Manolova, 2002
Systemic sclerosis	Ruffatti <i>et al.</i> [34]
Mixed connective tissue disease	Cooper, 2000
Sjögren syndrome	Font, 1998; Nishiya, 1999
Dermatomyositis	Merkel, 1999
Antiphospholipid syndrome	Merkel, 1999
Rheumatoid arthritis	Mulder, 1993; Braun, 1993; Afeltra, 1996; Mustila, 1997
Felty syndrome	Juby, 1992
Juvenile chronic arthritis	Nässberger, 1991
Reactive arthritis	Locht, 1999
Ankylosing spondylitis	Locht, 1999
<b>Non-ANCA associated vasculitides</b>	
Takayasu vasculitis	Kerr <i>et al.</i> [53]
Giant cell arteritis	McHugh, 1990
Kawasaki disease	Savage, 1989
Polyarteritis nodosa	Guillevin, 1993; Hauschild, 1994
Schönlein-Henoch Purpura	Saulsbury, 1991; O'Donoghue, 1992; Shaw, 1992
Behcet disease	Burrows, 1996
Cryoglobulinemic vasculitis	Lamprecht, 1998
<b>Gastrointestinal disorders</b>	
Ulcerative colitis	Snook, 1989; Saxon, 1990
Crohn disease	Snook, 1989; Saxon, 1999
Autoimmune hepatitis	Hardarson, 1993; Roozental, 1999
Primary biliary cirrhosis	Roozental, 1999
Primary sclerosing cholangitis	Roozental, 1998; Schwarze, 2003
<b>Infectious disorders</b>	
Tuberculosis	De Clerk, 1989; Adebajo, 1993; Flores-Suárez, <i>et al.</i> [29•]
Leprosy	Medina 1998
<i>Pseudomonas</i> infection in cystic fibrosis	Zhao, 1996; Sediva, 1998; Schultz, 2000
Bacterial septicemia	Mege <i>et al.</i> , 1993
Subacute bacterial endocarditis	Choi, 2000
Leptospirosis	Constantin, 1996
<i>Aspergillus</i>	Cho 1995
Sporotrichosis	Byrd, 1998
Paracoccidioidomycosis, chromomycosis	Stappaerts, 1999, Galnerin <i>et al.</i> , 1996
Amoebiasis	Pudifin, 1994
Malaria	Adebajo, 1993
Influenza	Staud, Ramos, 2001
HIV	Davenport, 1991; Savige, 1994, Cornely, 1999
Hepatitis C	Wu <i>et al.</i> [27]
<b>Neoplasia</b>	
Lymphoid neoplasia	Hamidou, 2000
Lymphomatoid granulomatosis	Hamidou <i>et al.</i> , 2000
Monoclonal gammopathies	Savige, 1994
Myeloproliferative disorders	Hamidou, <i>et al.</i> , 2000
Carcinomas	Edgar, 1993; Norris, 2003; Vassilopoulos <i>et al.</i> [36•]
<b>Miscellaneous disorders</b>	
Sweet syndrome	Kemmett, 1991
Poststreptococcal glomerulonephritis	Ardiles, 1997
IgA nephropathy	van den Wall Bake, 1989; O'Donoghue, 1992
Sarcoidosis	Forde, 1996
Nonspecific interstitial pneumonia	Gal 2003
<b>Drugs</b>	
Thiamazole	Hori, 1996; Wada, 2002
Propylthiouracil, benzythiouracil	Wada, 2002; Yamashita, 2002; Kaaroud, 2002, Katayama, 2002
Methimazole	Kawachi, 1995; Thong, 2002; Guma, 2003
Minocycline	Schaffer 2001
Hydralazine	Nässberger, 1990; Short, 1995; Norris, 2003

ANCA, antineutrophil cytoplasmic antibody.

*Curr Opin Rheumatol* 2004 (reference 12)

Attachment 2: Clinical ordering guidelines for ANCA testing (by Mandl)**Table 1. Clinical Indications for ANCA Testing\***

Guidelines†	Clinical Definition
1. Glomerulonephritis, especially rapidly progressive	(A) Creatinine level >2.0 mg/dL (>176.8 µmol/L) (normal range, 0.7-1.3 mg/dL [61.9-114.9 µmol/L]) immediately prior to ANCA testing or (B) urinary red blood cell casts or hematuria with >5 red blood cells per high-powered microscopic field
2. Pulmonary hemorrhage, especially pulmonary renal syndrome	Hemoptysis or pulmonary hemorrhage
3. Cutaneous vasculitis with systemic features myalgias, arthralgias, or arthritis	Purpura, rash or livedo with concurrent fever, weight loss, myalgias, arthralgias, or arthritis
4. Multiple lung nodules	At least 1 nodule seen on any imaging study‡
5. Chronic destructive disease of the upper airways	Epistaxis or erosive changes seen on clinical examination or imaging studies not due to previous surgery
6. Long-standing sinusitis or otitis	(A) Hearing loss, blocked ears, or ear pain or (B) sinusitis or otitis specified as the reason for ANCA test ordering by the physician
7. Subglottic, tracheal stenosis	(A) Visualized on imaging studies or (B) tracheal stenosis specified as the reason for ANCA test ordering by the physician
8. Mononeuritis multiplex or other peripheral neuropathy	Sensory or motor changes, including cranial nerve palsies
9. Retro-orbital mass	Radiographic visualization of a mass lesion

\*ANCA indicates antineutrophil cytoplasmic antibody.

†Based on the article by Hagen et al.<sup>12</sup>

‡Not all patients had specialized imaging studies to detect multiple lesions, so a single nodule was accepted.

*Arch Intern Med 2002 (reference 17)*