

# Chapter 146

## Approach to ANCA Interpretation

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### INTRODUCTION

ANCA testing is a routine in suspected cases of systemic necrotizing vasculitis, i.e. Wegner's granulomatosis, (WG), microscopic polyangiitis (MPO), Churg-Strauss syndrome (CSS) and polyarteritis nodosa (PAN). This article discusses the diagnostic significance and pitfalls of ANCA testing.

ANCA constitute a family of auto-antibodies directed against the constituents of neutrophil (PMN) and monocyte granules, such as proteinase3 (PR3), myeloperoxidase (MPO), azurocidin, bacterial permeability increasing protein (BPIP), cathepsin G, elastase, lactoferrin, lysozyme, etc. Of these only antibodies to PR3 and MPO are markers of systemic necrotizing vasculitis (SNV). Antibodies to other antigens are associated with a variety of non SNV diseases<sup>1-3</sup>.

### ANCA PATTERNS, AND THEIR INTERPRETATION

ANCA are detected by indirect immunofluorescence (IIF) staining using ethanol fixed neutrophils as the substrate. A variety of ANCA patterns can be appreciated with IIF staining in Table 1.

### ANCA ANTIGENS

Antigen specificity of ANCA is determined by ELISA using commercially available kits. In a given patient ANCA are most of the time directed against a single antigen. Occasionally, ANCA may have multiple antigen specificities which may or may not include PR3 and MPO. The diagnostic significance of antigens is shown in Table 2.

**Table 1:** ANCA immunofluorescence patterns

<i>Pattern</i>	<i>Description</i>	<i>Main disease association</i>	<i>Others</i>
Typical C - ANCA	Cytoplasmic staining with interlobular accentuation	Wegner's granulomatosis (*sens. 80% sp. 95%)	MPO CSS PAN
Typical P – ANCA**	Perinuclear staining with nuclear extension	Microscopic polyangiitis, Churg-Strauss syndrome (sens. 60% sp. 75%)	WG PAN
Atypical ANCA pattern(s)		Inflammatory bowel disease	
Atypical p-ANCA	Perinuclear pattern without nuclear extension	SLE and other rheumatic diseases, Infections (esp. chronic),	
Atypical c – ANCA	Diffuse cytoplasmic pattern without interlobar accentuation	Malignancies Drug induced vasculitis	
Other patterns			

\* Sens – sensitivity, Sp – specificity. \*\* P-ANCA pattern is ethanol fixation artefact. Under the effect of ethanol MPO migrates to perinuclear area giving a P-ANCA pattern. P-ANCA pattern is not seen with formalin fixed preparations. Sensitivity drops to 50% in limited forms of WG.

**Table 2:** ANCA antigens and their disease associations

Antigen	WG	MPA/CSS	Non-SNV diseases
PR3			
Sensitivity	85-90%	—	—
Specificity	95-100%		
MPO			
Sensitivity	10%	80-90%	—
Specificity		60-75%	
Other antigen ± MPO/PR3			
Sensitivity	—	—	70-80%
Specificity			85-90%

**Advantages of Antigen Determination**

- i. Antigens carry a better sensitivity and especially specificity for the diagnosis of SNV.
- ii. Antigen testing overcomes the problem of missing atypical P ANCA pattern.
- iii. When PR3 or MPO antigen specificity is present along with other antigen specificities, the disease association is with non SNV disorders especially drug induced vasculitis and not SNV<sup>11,12</sup>.
- iv. In patients with SLE a peripheral ANA pattern might be (mis) interpreted as P-ANCA. Antigen determination obviates the need to repeat ANCA testing using a formalin fixed PMN preparation<sup>11</sup>.
- v. A +ve ANCA along with PR3 or MPO antigen specificity has a 99-100% specificity for SNV. sensitivity for WG is 73% and for MPA 67%.

**CLINICAL APPLICATION OF ANCA DETERMINATION**

ANCA associated SNV are rare diseases. As is true for most antibody tests, when applied to patients with a low pretest disease probability, the positive predictive value of ANCA is poor. Indications for ANCA testing (if other causes are excluded) are:

Glomerulonephritis, RPGN, Pulmonary hemorrhage, cutaneous vasculitis with systemic features, lung nodules, chronic upper airways disease especially with destruction, peripheral neuropathy especially mononeuritis multiplex, unexplained fever, weight loss,

ANCA can be used to monitor response to therapy.

It is worth noting that ANCA are not a part of SNV diagnostic criteria. The accompanying figure provides an algorithmic approach to ANCA testing.

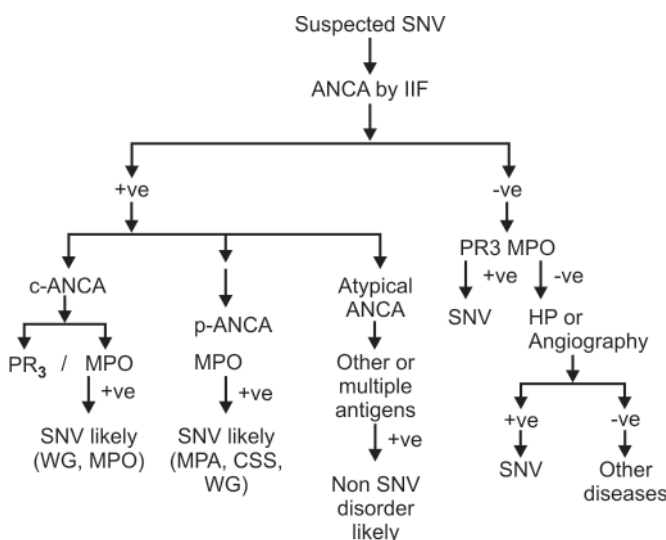
Ideally whenever possible a biopsy should be done to confirm the diagnosis of SNV. ANCA test is highly supportive but not diagnostic.

**CAUTIONS**

- i. Most laboratories use commercial kits which often fail to detect all the ANCA patterns. To be able to identify the various ANCA patterns a good quality PMN preparation is essential<sup>4,5</sup> or antigen specificity determination should be part of ANCA testing protocol.
- ii. There is lack of standardisation of commercial ELISA kits leading to inconsistent results.

**CONCLUSIONS**

ANCA are helpful to diagnose WG, MPA, CSS, and their limited forms such as pauciimmune necrotizing



crescentic glomerulonephritis and pulmonary capillaritis. ANCA can also be useful in monitoring disease activity. For proper application of ANCA following need to be kept in mind.

- i. It is good to have a PMN preparation which is able to display typical and atypical ANCA patterns.
- ii. Detection of ANCA by IIF is not enough. A positive result ANCA should always be followed with PR3, MPO determination
- iii. If possible a MPO/PR3 positivity should be followed by checking for other antigen as well, in order to exclude non SNV disorders.
- iv. Around 5% of patients with SNV may show a negative ANCA result. Testing for PR3 and MPO is useful in this situation<sup>13</sup>.

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