

EDITORIAL

Issue Seventeen, October 2004

Welcome to the Spring 2004 Edition of InfoLink. The feature article in this edition has been written by Dr Rodger Laurent, Head of Department, PaLMS Rheumatology Laboratory. The article explores Anti-neutrophil cytoplasmic antibodies (ANCA) and the role ANCA plays in the diagnosis and ongoing treatments of a small subgroup of vasculitides, which have been designated the ANCA associated vasculitides.

It is with pride that we announce that PaLMS Director, Professor Leslie Burnett, has been honoured by the American Association of Clinical Chemistry with the Management Sciences Division Award. This award is in recognition of his “pioneering work to make pathology and laboratory testing more accurate”. Prof Burnett is the first non-American to claim a top scientific award from this association.

For some years now PaLMS has used Telstra X400 for electronic delivery of reports. PaLMS has commenced delivery of electronic pathology reports via HealthLink and are phasing out the use of the Telstra X400. HealthLink is an established provider of delivery services for health related data throughout New Zealand and East Coast Australia.

If there is one area that seems to exasperate requesting practitioners, it is the effort one often has to go to meet the obligations under the Health Insurance Act 1973. This is particularly true in relation to valid requests for pathology services. This issue contains a flyer regarding our reciprocal obligations under the act and the processes PaLMS has put in place to assist us to meet our mutual obligations.

The Royal College of Pathologists of Australasia has launched a new magazine PathWay. A copy of the new and informative publication accompanies this issue of InfoLink. If you would like additional copies of PathWay for your patients, staff or colleagues please contact PaLMS Administration on 9926 8086. PaLMS are pleased to be able to support RCPA with this new endeavour.

If there is a topic you wish covered in future issues or you have any feedback relating to this issue, please contact a member of the editorial team whose details appear below.

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Anti-Neutrophil Cytoplasmic Antibodies - ANCA

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What are anti-neutrophil cytoplasmic antibodies?

Anti-neutrophil cytoplasmic antibodies (ANCA) are antibodies directed against several cytoplasmic antigens in neutrophils and monocytes. ANCA are associated only with a small subgroup of vasculitides, which predominantly involve small sized vessels. This subgroup have been designated the ANCA associated vasculitides.

Most of the antigens are in the primary granules and the majority have antibacterial properties. They can also be expressed on the surface of stimulated or apoptotic neutrophils but not on resting neutrophils.

There are several known target antigens of ANCA. These are proteinase 3, myeloperoxidase, elastase, cathepsin G, azurocidin, lactoferrin, lysozyme and bacterial permeability-increasing protein. At present only proteinase-3 (PR3) and myeloperoxidase (MPO) are clinically relevant and routinely measured.

ANCA probably have a pathogenetic role although its mechanism of action is unknown.

What vasculitides are associated with ANCA?

ANCA is associated with:

- Wegener's granulomatosis
- Microscopic polyangiitis
- Pauci-immune crescentic glomerulonephritis
- Churg-Strauss syndrome

The common pathology in these diseases is predominant involvement of capillaries and venules as well as arterioles. There is early invasion of the vessel wall with neutrophils which may be the cause of vessel wall damage and necrosis.

There is no association of ANCA with other vasculitides. These include polyarteritis, leukocytoclastic vasculitis, giant cell arteritis, Kawasaki disease, Takayasu's arteritis and Behcet's syndrome. ANCA may be present in a small percentage of these vasculitides but the association is too weak to be of any clinical relevance.

Wegener's granulomatosis:

Wegener's granulomatosis is characterised by inflammatory destructive lesions that predominantly affect the sinuses, ears, trachea, bronchi and lungs. Renal disease manifests as a glomerulonephritis. It may also affect the eyes and the joints.

The strongest association between ANCA and a disease is between C-ANCA with Wegener's granulomatosis. ANCA is present in about 90% of those with active disease and the target antigen is usually PR3. In 10% it can be a P-ANCA with the target antigen being MPO.

Microscopic Polyangiitis

Microscopic polyangiitis involves predominantly the lungs and kidney. There may also be secondary pulmonary haemorrhage. Renal involvement consists of a glomerulonephritis. Mononeuritis multiplex can also occur but is less common than in other vasculitides.

ANCA is present in 40-80% of patients with microscopic polyangiitis. It is usually the P-ANCA pattern and the target antigen is MPO.

Pauci-Immune Crescentic Glomerulonephritis

This is probably part of the Wegener's granulomatosis/microscopic polyangiitis spectrum. It is a vasculitis in which there is minimal inflammatory cell infiltrate in the kidney.

About 75-80% have a P-ANCA pattern with antibodies against MPO.

Churg-Strauss Syndrome

Churg-Strauss vasculitis predominantly affects the respiratory tract. It is associated with asthma and allergic rhinitis. It produces fever, weight loss, lung involvement and mononeuritis multiplex. There is often a peripheral blood eosinophilia and eosinophils deposited in the vasculitic lesions.

ANCA is present in about 60% of patients with Churg-Strauss syndrome. The target antigen can be either PR3 in 30% or MPO in 30% of cases.

How is ANCA measured?

Measurement of ANCA is by indirect immunofluorescence (IIF) and an enzyme linked immunoassay (ELISA).

Indirect immunofluorescence:

- Indirect immunofluorescence on neutrophils is used as the initial screen for ANCA.
- There are two main staining patterns C-ANCA and P-ANCA. The C-ANCA pattern is where the cytoplasm is diffusely stained and the P-ANCA pattern is where the staining is predominantly around the nucleus.
- The C-ANCA pattern is usually associated with antibodies to the serine protease, proteinase 3 (PR3). This pattern can occasionally be associated with antibodies to MPO.
- The P-ANCA pattern is usually associated with antibodies to myeloperoxidase (MPO).
- The P-ANCA pattern can occur in the absence of antibodies to MPO. This is because the P-ANCA pattern can be associated with other antigens, for example, elastase and lactoferrin.
- A pattern which is different to the C-ANCA or P-ANCA patterns is called atypical ANCA. These sera have antibodies to other neutrophil antigens which include nuclear, cytoplasmic and granular components.
- Antinuclear antibodies (ANA) can react with neutrophils in the indirect immunofluorescence assay making it difficult to interpret. In this situation it is essential to measure antibodies to PR3 and MPO.

Enzyme linked immunoassay:

- The ELISA assay is used to determine the specificity of the target antigen. The specific antigens currently tested are PR3 and MPO.
- If there is a positive ANCA with a particular staining pattern then it is important to test for the specific target antigens.
- A recently described capture enzyme immunoassay may be more sensitive and have a better correlation with disease activity.

When should I request an ANCA

- There is no value in using ANCA as a general screening test for vasculitis in patients with a vague symptom complex.
- It is only going to be of diagnostic value in patients with any of the following clinical abnormalities:
 - Proteinuria and haematuria
 - Acute or chronic renal failure
 - Haemoptysis
 - Abnormal chest x ray
 - Cutaneous vasculitis, particularly if there are associated systemic features
 - Mononeuritis multiplex or peripheral neuropathy
 - Chronic upper airways disease, particularly chronic sinusitis
 - Chronic iritis or retro-orbital swelling
- An ANCA should not be regarded as a diagnostic test. It is a useful aid to diagnosis and monitoring of therapy.
- Vasculitis should always be diagnosed on the histopathological findings in a biopsy of the involved structure.
- A biopsy is essential so that non-vasculitic disorders in which ANCA may occur are not inappropriately treated. These include connective tissue disorders, inflammatory bowel disease, infections, tuberculosis and bacterial endocarditis, malignancy and medications.

Why should I only order an ANCA if there are specific clinical features?

- The ANCA associated vasculitides are rare so the pretest probability of disease is very low. This results in a large number of false positives with a poor diagnostic yield for ANCA testing.
- The performance of the test can be improved by increasing the pretest probability. This is done by only ordering ANCA in patients with a high likelihood of the disease. They have the clinical features previously listed in the preceding section.
- Using the test in the appropriate clinical context reduces the number of false positives without missing any cases of ANCA associated vasculitis.

How does ANCA help with the diagnosis of vasculitis?

- Both the IIF and corresponding ELISA must be positive to support the diagnosis of an ANCA associated vasculitis.
- The clinical value of either test alone being positive is limited, providing little help with the diagnosis.
- Therefore, a positive result from the IIF method should always be confirmed with an antigen specific ELISA for PR3 and MPO.
- The combination of IIF and ELISA assays for PR3 and MPO improve the specificity and sensitivity of the test.
- If these criteria are met there is a specificity of 99% for an ANCA associated vasculitis, with a

sensitivity of 73% for Wegener’s granulomatosis and 67% for microscopic polyangiitis.

- It is important to remember that a negative ANCA does not rule out a diagnosis of vasculitis.

What does the ANCA report tell me?

The following is an example of the format of a laboratory report for ANCA:

Anti-neutrophil cyto. Ab	POSITIVE.
C-ANCA	160 titre
PR3	60 H Enz Units (<10)
MPO	< 10 Enz Units (<10)

This report indicates that there are antibodies against neutrophil cytoplasmic components. They produced the C-ANCA pattern on immunofluorescence with a titre of 1:160. This is due to antibodies against PR3, which are significantly elevated. There are no antibodies to MPO.

This pattern is associated with Wegener’s granulomatosis.

If the anti-neutrophil cytoplasmic antibodies are positive with either pattern but MPO and PR3 antibodies are negative (< 10 Enz Units), then the antibodies are probably against another target antigen, and are of no known clinical significance.

If an antinuclear antibody is present there will be an appropriate comment in the report.

Does ANCA have a role in the management of vasculitis

Is ANCA a risk factor for a relapse of the vasculitis?

Relapse of vasculitis is frequent, and over a 5 year period, occurs in about 50% of Wegener’s granulomatosis and 30% of microscopic polyangiitis. Relapse is more common in patients who have a persistence of ANCA after clinical remission. This has been clearly demonstrated for PR3-ANCA, but not for MPO-ANCA.

Vasculitis with PR3-ANCA is associated with more frequent relapses than MPO-ANCA irrespective of the diagnosis. This means that PR3-ANCA positive Wegener’s granulomatosis is more likely to relapse than MPO-ANCA positive Wegener’s granulomatosis.

Persistence of ANCA after remission of the vasculitis is also associated with a worse outcome. Patients with MPO-ANCA associated glomerulonephritis with persistent elevation of MPO-ANCA are more likely to have end stage renal disease.

Patients who initially are negative during remission and subsequently become positive are also at increased risk of a relapse.

Patients with a consistently negative ANCA after remission have a low probability of relapse. Therefore, patients with persistence of ANCA, or the reappearance of ANCA should be more carefully monitored for a flare in their disease.

How often should ANCA be measured?

Serial measurement of ANCA has been advocated as a means of predicting a disease relapse. However, there is a poor correlation between titres and disease activity. Rising titres of PR3-ANCA may predict relapses but there is no clear consensus as to what is a significant rise. At present the best criteria are a 75 % increase in PR3-ANCA levels or a fourfold increase in IIF titre of C-ANCA.

The frequency of testing has not been determined but it is considered that 6-8 weeks is reasonable. However, the frequency of subsequent testing is likely to be determined by the severity of the vasculitis at onset.

Should the ANCA result be used to change treatment?

The role of pre-emptive treatment based on an increase in ANCA to prevent a clinical relapse is still a controversial area. Two prospective studies have suggested that pretreatment with either azathioprine or cyclophosphamide reduces relapses. There was a relapse rate of about 25% in those given pre-emptive treatment compared to about a 60% relapse rate in those not given pre-emptive treatment.

However, 25% to 40% of those given pre-emptive treatment would have received unnecessary treatment. The benefits from pre-emptive treatment should be related to the number of patients that will have complications from an unnecessary increase in treatment. The persistence of ANCA suggests that the vasculitis is not well controlled, and careful monitoring is required so that treatment can be quickly altered if there are clinical features of a relapse.

What other diseases have a positive ANCA?

ANCA is present in numerous diseases other than systemic vasculitides. ANCA does not have any diagnostic value in these situations, but their presence can cause uncertainty and result in an incorrect diagnosis. It appears as though some of these ANCAs found on IIF are not true ANCAs and will probably be reclassified.

The following are some of these diseases associated with ANCA:

Gastrointestinal Diseases

Several gastrointestinal diseases are associated with a positive ANCA, usually only on IIF. These include ulcerative colitis where ANCA may be present in 60-80%, primary sclerosing cholangitis with a positive ANCA in 60-80% and Crohn's disease with a positive ANCA in 10-40%. The pattern is usually a P-ANCA or atypical ANCA directed against a variety of target antigens. These include cathepsin G, elastase, lysozyme and lactoferrin and MPO. However, the most important antigens are located in the chromatin and nuclear membrane and are not true ANCA.

ANCA and Rheumatoid Arthritis and Systemic Lupus Erythematosus

ANCA is also found in a small percentage of patients with rheumatoid arthritis and systemic lupus erythematosus. There is no association with the development of vasculitis in these diseases.

ANCA and Chronic Bacterial Infections

Patients with chronic recurrent infections, particularly cystic fibrosis and bronchiectasis may develop ANCA. ANCA is present in about 90% of patients with cystic fibrosis. This is usually a P-ANCA pattern, with the target antigen being bacterial permeability-increasing protein.

ANCA and Drug Induced Diseases

Drug induced vasculitis or lupus erythematosus like syndrome may be associated with a positive ANCA. The drugs most commonly producing ANCA are hydralazine, minocycline and propylthiouracil.

The commonest staining pattern is P-ANCA which is directed against several neutrophil derived antigens. These include MPO, elastase, PR3 and cathepsin G. Antibodies to both PR3 and MPO may develop together in drug induced ANCA. The combination of antibodies to both PR3 and MPO does not occur in the ANCA associated vasculitides. When the drug is discontinued the clinical features and ANCA usually resolve.

Summary:

- ANCA is a useful test only if there are clinical features suggestive of a vasculitis.
- ANCA has no value as a general screening test for vasculitis.
- Both the IIF and ELISA must be positive to support a diagnosis of vasculitis

- ANCA identifies only a small number of vasculitides, the ANCA associated vasculitides.
- ANCA may be present in other disorders but does not have any diagnostic or clinical relevance.
- ANCA can be drug induced.
- ANCA does not replace a biopsy of the involved tissue to make a diagnosis of vasculitis.
- A rapid increase or reappearance of antibodies to PR3 or MPO, in patients with an ANCA associated vasculitis should alert the physician to the possibility of a flare in the vasculitis.

Further reading

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Wiik A. Rational use of ANCA in the diagnosis of vasculitis (Editorial). *Rheumatology* 2002;41:481-483.

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