GPwSI in Rheumatology: 
Autoantibody testing in rheumatic disease

MY Karim

Abstract
Immunology and autoimmune testing have the reputation for being complex and difficult to understand. However most of the tests in current practice are relatively straightforward and in this article I try and dispel some of the myths, and attempt to clarify the interpretation of the more common autoantibody tests used in clinical immunology.

Keywords
Antinuclear antibody, DNA, antiphospholipid, rheumatoid, vasculitis.

Anti-nuclear antibody
The anti-nuclear antibody (ANA) is assessed most commonly by testing patient serum on HEp-2 cells (human epithelial cell line), though some laboratories use an enzyme linked immunosorbent assay (ELISA). Patient serum is added to HEp-2 cells on a microscope slide. A fluorescinated antibody is added and the slide read using a fluorescence microscope. The ANA results are typically given as either positive with a titre and a pattern, or negative. The titre represents the dilution at which the serum remains positive. A weak ANA is often seen in the elderly and may represent a non specific-positive, rarely associated with connective tissue disease. Positive ANA can be found in 5-10% of patients without evidence of connective tissue disease, and can also occur in relatives of patients with rheumatic disease.

The ANA pattern may indicate which additional tests might be relevant. For example speckled ANA (figure 1) is associated with the presence of antibodies to extractable nuclear antigens (ENA) which include Ro and La amongst others. In contrast, homogeneous ANA (figure 2) is associated with antibodies to DNA, or more rarely to histones. However the association between ANA pattern and these antibodies is not absolute.

Anti-centromere antibodies are associated with limited cutaneous scleroderma, including the subgroup previously known as CREST syndrome (calcinosis cutis, Raynaud’s, oesophageal dysmotility, sclerodactyly, telangiectasia). The nucleolar pattern of ANA is associated with scleroderma, though this, like many autoantibodies, can also be found in healthy persons.

In the past it was said that 5% of lupus patients were ANA negative. This was largely because the previously used rodent and mouse tissue used for ANA testing was not particularly rich in Ro. With the advent of HEp-2 cells, richer in Ro, the occurrence of ANA negative lupus is much less common.

For monitoring of connective tissue disease, I would not advocate repeat testing of ANA once the diagnosis has been reached, unless of course the patient’s clinical features alter markedly to suggest a change in diagnosis.

Figure 1. Speckled immunofluorescence pattern on HEp-2 cells

Figure 2. Homogeneous pattern on Hep-2 cells.
Anti-double stranded DNA antibodies

Anti-double stranded DNA (anti-dsDNA) antibodies are strongly associated with systemic lupus erythematosus (SLE), though they occur in only 60% of patients. To some extent the anti-dsDNA titre correlates with disease activity, and these antibodies are associated with renal involvement. Very rarely anti-dsDNA antibodies can be found in other rheumatic conditions, and they can also be found in autoimmune lupoid hepatitis. Testing can be done either by ELISA, immunofluorescence (Crichidia luciliae), or radioimmunoassay (Farr assay). The newer technologies involved in testing do impact on the positivity rate. The older Farr assay is a very specific but less sensitive assay than the ELISA method which may generate more false positives than previously found.

ENA

ENA refers to extractable nuclear antigens which can be extracted using saline from the nucleus. There are a number of different types including Sm, RNP, Ro (SSA), La (SSB), Scl-70 and Jo-1. The antibodies Sm, RNP, Ro and La produce a speckled pattern on ANA testing. Anti-Sm antibodies are very tightly associated with SLE and are part of the American College of Rheumatology classification criteria for SLE.4 They are however only found in 10-30% of SLE and more frequently in Afro-Caribbean patients. Anti-RNP antibody is often found together with anti-Sm. However when it is found alone it is associated with an overlap syndrome known as mixed connective tissue disease, which may consist of features from lupus, scleroderma and polymyositis.

Anti-Ro antibodies are associated with Sjögren’s syndrome and SLE. They are also associated with neonatal lupus syndrome and congenital heart block, the latter resulting from maternal antibodies crossing the placenta and causing damage to the foetal cardiac conducting system. Approximately 80% of mothers of babies born with congenital heart block, have anti-Ro or anti-La antibodies, sometimes without actually having a connective tissue disease. Conversely the development of congenital heart block in children born to mothers with anti-Ro or anti La is rare, less than 1%. Anti-La is associated with Sjögren’s syndrome, and SLE.

Scl-70 antibodies are found in 20-40% of patients with diffuse cutaneous scleroderma. Jo-1 antibodies produce a cytoplasmic pattern on ANA testing (with negative nuclear staining), and are found in 25% of patients with polymyositis.

Antiphospholipid antibodies

Antiphospholipid antibodies (aPL) are associated with recurrent thrombosis, and/or pregnancy morbidity, known as the antiphospholipid syndrome (APS).1 This can be primary, or secondary to underlying connective tissue disease, typically SLE. Patients with recurrent miscarriage, or recurrent arterial or venous thrombosis should be tested for aPL, which includes anticoagulant antibodies by ELISA (immunology), and lupus anticoagulant (haematology).3 The latter shows prolongation of phospholipid-dependent clotting times in vitro, while in vivo conversely is associated with thrombosis. aPL can also be found in healthy controls, and in some infections, and at least 2 positive aPL measurements, of medium- or high titre, are generally advised before reaching a diagnosis. Table 1 summarises whom to test for aPL.

Rheumatoid factor

Rheumatoid factor is an antibody directed against the Fc portion of the IgG molecule. It is present in patients with rheumatoid arthritis (RA) but also can be found in other connective tissue diseases, and normal controls at an increasing rate with age. Therefore positive rheumatoid factor is not diagnostic of rheumatoid arthritis. In early disease only about 50% of patients are positive and around 15% patients never develop positive rheumatoid factor. The test is best used in patients with a high index of suspicion of RA.

Anti-CCP antibodies have a similar sensitivity as rheumatoid factor but higher specificity (90-95%) for RA. Anti-CCP antibodies may also be useful in early disease diagnosis. Furthermore, anti-CCP positive early RA may develop a more erosive course than anti-CCP negative. Anti-CCP antibodies are not currently widely available, and we are just starting to consider testing in our laboratory.

Vasculitis and anti-neutrophil cytoplasmic antibody testing

Vasculitis simply means inflammation of the blood vessels and it can be localised as in cutaneous vasculitis or generalised as in a primary systemic vasculitis. It can be classified (table 2) on the basis of vessel size, on the basis of ANCA positivity, or according to aetiology e.g. drug-related, post-infection etc. It can occur secondary to underlying connective tissue disease such as SLE.

In terms of laboratory testing the major step forward in the last 20 years has been the advent of the anti-

| Arterial thrombosis under age 45 yrs |
| Recurrent vascular thrombosis |
| History of venous and arterial thrombosis |
| Thrombosis after trivial provocation |
| Thrombosis in an unusual site |
| Underlying connective tissue disease, particularly SLE |
| Recurrent foetal loss |
| Association of foetal loss and thrombosis |
| Recurrent pregnancy morbidity e.g. PET, IUGR |
| Warfarin-induced skin necrosis |

PET = pre-eclampsia
IUGR = intra-uterine growth retardation
SLE = systemic lupus erythematosus

Table 1. Whom to test for antiphospholipid antibodies2
neutrophil cytoplasmic antibody (ANCA) test. This test, it should be stressed, is not positive in all patients with vasculitis. In addition some patients without vasculitis, for example with infection or inflammatory conditions such as ulcerative colitis and rheumatoid arthritis, can also have positive ANCA. Therefore as with other immunological tests it is best to restrict the requesting of this test to where there is a high degree of suspicion of vasculitis.

The test is performed by immunofluorescence microscopy of human neutrophils. There are two main immunofluorescence patterns, cytoplasmic (C-ANCA) and peri-nuclear (P-ANCA) (figure 3). If the ANCA immunofluorescence is positive then more specific neutrophil antigens are examined by ELISA testing, namely proteinase 3 (PR3) and myeloperoxidase (MPO). C-ANCA is associated with PR3 antibodies and clinically associated with Wegener’s granulomatosis and microscopic polyarteritis. P-ANCA is associated with MPO antibodies and can be associated with microscopic polyarteritis, Wegener’s granulomatosis or Churg–Strauss Syndrome (asthma, eosinophilia, and neuropathy). However P-ANCA is less specific, as it can also be found in inflammatory bowel disease, sclerosing cholangitis, RA, and SLE. In the latter diseases the association is not with vasculitis.

Patients who have positive ANCA immunofluorescence but negative specific tests for MPO and PR3 are less likely to have vasculitis. Rarely patients may be positive only by ELISA, with negative immunofluorescence. Polyarteritis nodosa affects medium-sized vessels, and is rarely ANCA-positive.

Tissue biopsy remains the gold standard for diagnosis of vasculitis, and ANCA testing provides useful supporting evidence. In occasional cases where biopsy might not be advised, ANCA testing may obviate its performance. Further details on immunological testing can be found in more extensive reviews of the subject, such as by Sheldon.

Table 2. Classification of vasculitis

<table>
<thead>
<tr>
<th>Vessel size</th>
<th>ANCA-positive</th>
<th>ANCA-negative</th>
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<tbody>
<tr>
<td>Large</td>
<td>Giant cell arteritis</td>
<td>Takayasu’s arteritis</td>
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<tr>
<td>Medium</td>
<td>Polyarteritis nodosa</td>
<td>Kawasaki’s disease</td>
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<tr>
<td>Small</td>
<td>Wegener’s granulomatosis</td>
<td>Henoch–Schönlein purpura</td>
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<td></td>
<td>Microscopic polyarteritis</td>
<td>Hypersensitivity vasculitis</td>
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<tr>
<td></td>
<td>Churg–Strauss syndrome</td>
<td>Cryoglobulinaemic vasculitis</td>
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References
1. Cross LS, Adam A, Misbah SA. Antinuclear antibody-negative lupus as a distinct diagnostic entity—does it no longer exist? QJM 2004; 97: 303-308