Antinuclear antibody test

Michael O'Sullivan
Andrew McLean-Tooke
Richard Loh

This article forms part of our 'Tests and results' series for 2013, which aims to provide information about common tests that general practitioners order regularly.

Keywords
antinuclear antibodies; immune system diseases; autoimmune disease

The antinuclear antibody (ANA) test is widely used as a serological marker of autoimmune disease. Antinuclear antibodies are immunoglobulins or antibodies that bind to one or more antigens expressed within the nucleus of human cells. Used selectively, the ANA test can be a useful laboratory tool to help confirm or exclude the diagnosis of systemic rheumatic disease. However, the relatively high prevalence of ANAs in other inflammatory conditions, as well as healthy individuals, can make a positive result difficult to interpret.

How is the test performed?

Although many methods are available for ANA detection, the indirect immunofluorescence antinuclear antibody test (IF-ANA) and enzyme immunoassay (EIA)/enzyme linked immunosorbent assay (ELISA) are commonly used.

Indirect IF-ANA involves incubating patient serum on a slide covered with a monolayer of cells from a malignant human epithelial cell line. These malignant cells are ideal for the ANA test as they have large nuclei in different stages of the cell cycle. The slides are washed and any remaining antibodies bound to cell nuclei are then visualised using a detection antibody binding human immunoglobulin which has been conjugated to a fluorescent tag. The detection antibody will then be visible using a fluorescence microscope if there is immunoglobulin from the patient serum that has bound to the cells on the slide.

In ELISAs wells are coated with antigens from cell nuclei. Serum is incubated in the wells and antibodies binding to the antigens are then detected using a detection antibody which has been conjugated with an enzyme tag. Antibody levels may be quantified by the amount of colour change of a substrate by the enzyme tag.

How is the test reported?

The results of ANA testing are reported in two components: the quantity of ANA in the serum (intensity) and, when the ANA is positive, the pattern of antibody binding to the nucleus (staining pattern).

The quantitation of an ANA is most commonly reported as a titre, reflecting the final step in a series of two-fold dilutions at which the ANA remains positive (eg. 1:1 280 for a strongly positive ANA, or 1:160 for a weaker, borderline positive ANA). An alternative method involves reporting the intensity of fluorescence, in international units per millilitre (IU/mL), at a pre-determined dilution. With this method, a result of >7 IU/mL is generally considered positive.

The different staining patterns provide clues to the significance of the ANA and type of rheumatic disease (*Table 1*).

When to request an ANA

While ANAs are associated with a number of autoimmune conditions, they are most useful in the diagnosis and classification of rheumatic diseases (*Table 1*). Therefore, it is in patients presenting with clinical symptoms or other laboratory results suggesting a high pre-test probability of rheumatic disease that the ANA test should be considered. These features include arthritis, photosensitive or discoid rash, alopecia, xerophthalmia and xerostomia, mouth ulcers, sclerodactyly or Raynaud's phenomenon, or laboratory findings of haemolytic anaemia, thrombocytopaenia,

lymphopaenia, hypergammaglobulinaemia, haematuria or proteinuria.

Changes in the ANA level are not associated with autoimmune disease activity and therefore repeat testing should not be performed unless there has been a significant change in the clinical picture.

What are the limitations?

Low-intensity ANAs are present in up to 40% of healthy individuals.[1] To address this issue, most laboratories will set a cut-off for reporting a positive ANA that excludes the majority of these low-intensity and clinically insignificant results. However, at least 5% of the healthy population have a moderate titre ANA that is considered positive; there are relatively higher rates in women and the elderly. If a randomly selected population were to be screened with ANA testing, 50 or more healthy people with a positive ANA would be identified for every one patient with systemic lupus erythematosis (SLE). A pre-existing clinical suspicion of systemic rheumatic disease is critical to enhance the clinical utility of a positive ANA result.[2] In the absence of clinical or laboratory markers supporting a diagnosis of rheumatic disease, a positive ANA is seldom useful.

A positive ANA will be seen in a range of conditions where it is not diagnostically helpful. These include non-autoimmune conditions such as chronic infection, viral hepatitis and malignancy, and also some autoimmune conditions such as multiple sclerosis or thyroid disease where the presence or absence of ANA does not play a significant role in diagnosis or prognosis.[3]

Many patients with autoimmune disease will not have a positive ANA. While the ANA test is highly sensitive for certain rheumatic diseases such as SLE and systemic sclerosis/scleroderma, a negative result doesn't exclude a wide range of other conditions including rheumatoid arthritis, spondyloarthropathies, idiopathic inflammatory myopathies and vasculitides.

Where does ANA testing fit with other investigations?

The ANA test is rarely interpreted in isolation, as there is likely to be a broad differential for the clinical presentation of a patient in whom autoimmune rheumatic disease is suspected. As a sensitive, but non-specific, marker of some systemic rheumatic diseases, the ANA test can be performed early in the diagnostic evaluation of patients with suggestive clinical symptoms to better direct further investigations.

In the majority of patients, the ANA test result alone is not sufficient to confirm the diagnosis of and characterise a systemic rheumatic disease. If ANA is positive, more specific tests may be performed based on clinical findings and ANA staining patterns. Antibodies directed against particular antigens within the cell nucleus are more strongly associated with particular rheumatic diseases. Therefore, testing for antibodies to these extractable nuclear antigens (ENA) is a useful follow-up test in patients with a positive speckled/peripheral ANA. Antibodies that can be characterised by the ENA test include anti-Sm (highly specific for SLE but only found in less than one-third of patients[4]), and anti-SSA and anti-SSB (also know as Ro and La respectively, seen in patients with Sjögren's syndrome and cutaneous lupus, and associated with a 1–2% risk of congenital heart block in foetuses of antibody-positive mothers).[5] Additionally, in patients with a homogenous pattern ANA, antibodies against double-stranded DNA can be quantitated by a separate test (anti-dsDNA antibody test). These antibodies are specific for SLE and the level of antibody may fluctuate with disease activity.

The ANA results are often interpreted in conjunction with other investigations selected according to the clinical scenario. The anti-cyclic citrullinated peptide antibody (anti-CCP) test (which has a high specificity for rheumatoid arthritis), full blood count (cytopaenias are a feature of SLE), urinalysis (haematuria and/or proteinuria may be due to renal manifestations of autoimmune disease), serum complement proteins C3 and C4 (low complement can reflect consumption in immune complex-mediated diseases such as SLE) and immunoglobulins (increased due to chronic inflammation, often markedly so with Sjögren's syndrome) are all potentially useful companion tests when interpreting a positive ANA.

Table 1. Common ANA patterns and their associated systemic rheumatic diseases

ANA pattern	Associated rheumatic disease
Homogeneous	• Systemic lupus erythematosus • Mixed connective tissue disease • Drug induced lupus • Juvenile idiopathic arthritis
Speckled	• Systemic lupus erythematosus • Sjögren's syndrome • Polymyositis/dermatomyositis • Systemic sclerosis/scleroderma
Nucleolar	• Diffuse systemic sclerosis/scleroderma • Polymyositis
Centromere	• Limited systemic sclerosis/scleroderma
Peripheral (rim)	• Systemic lupus erythematosus • Systemic sclerosis/scleroderma

What should a patient be told about the test?

Given the low specificity of ANA for systemic autoimmune disease, it is important to counsel the patient about the limitations of a positive test result, particularly if it is requested with low pre-test probability. A positive ANA is not diagnostic of lupus (or any other autoimmune disease) and most individuals with positive ANA will not develop an autoimmune disease in the subsequent three years if they don't have any suggestive symptoms at the time of initial testing.[2]

The ANA test is performed on serum and does not require any special patient preparation or collection. Due to the stability of immunoglobulins, stored serum samples will often be suitable. In patients with positive ANA, stored serum may be available to

perform additional testing for ENA and dsDNA antibodies to potentially provide more specific diagnostic information. While Medicare benefits are payable for each of these tests (ANA, ENA and dsDNA), they represent three separate item numbers and therefore episode coning rules may apply.

What do the results mean?

A positive test

The response to a positive test result will depend on the clinical scenario. Most commonly, the ANA test will be requested when there are features of systemic rheumatic disease. Therefore, follow-up testing for ENA antibodies is generally helpful as this might provide some more specific diagnostic information. If SLE is suspected, dsDNA antibody measurement is indicated.

The diagnosis of systemic rheumatic disease is usually based on a number of clinical and laboratory criteria, so a positive ANA could prompt a more targeted review of the clinical history and examination findings, and additional laboratory testing directed at those potential manifestations (see above).

Most positive ANA tests are not associated with systemic rheumatic disease, particularly at low titre. In subspecialty rheumatology clinics it is reported that <10% of patients referred with low to moderate titre positive ANA are ultimately diagnosed with an ANA-associated rheumatic disease.[6]

There is no absolute cut-off at which a positive ANA is clinically significant, however the higher the ANA titre the more likely it is to be associated with systemic rheumatic disease.[6] Therefore, in patients with high titre ANAs (the definition of which may change between laboratories but often >1:1 280) periodic assessment for development of new symptoms is reasonable. While many of these patients will never develop autoimmune disease, a positive ANA may appear several years before clinical symptoms in patients ultimately diagnosed with lupus.[7]

Table 2. Sensitivity of ANA for rheumatic diseases[3,8]

Disease	Sensitivity (%)
Systemic lupus erythematosus	93
Systemic sclerosis/ scleroderma	85
Polymyositis/ dermatomyositis	61
Juvenile idiopathic arthritis	57
Juvenile idiopathic arthritis with uveitis	80
Sjögren's syndrome	48–73
Rheumatoid arthritis	41

A negative test

A negative ANA test effectively excludes a diagnosis of ANA associated rheumatic disease such as drug-induced lupus and, in the majority of cases, SLE, systemic sclerosis/scleroderma and mixed connective tissue disease, particularly where there is a low pre-test probability of these conditions. However, while many other autoimmune diseases are associated with a positive ANA, it is a relatively insensitive marker for most of those conditions and therefore they can't be discounted based purely on negative ANA results (*Table 2*). These conditions include Sjögren's syndrome, rheumatoid arthritis, discoid lupus, juvenile idiopathic arthritis (without uveitis) and various forms of vasculitis including giant cell arteritis, polyarteritis nodosa, granulomatosis with polyangiitis (formerly Wegener's granulomatosis) and eosinophilic granulomatosis with polyangiitis (Churg Strauss syndrome).

An illustrative case

A 60-year-old gentleman presents with sore arms, upper abdominal pain and low grade fever. On examination he has tenderness on palpation of the abdomen without guarding, and over the wrists and metacarpophalangeal joints. Full blood picture revealed anaemia with Hb 90 g/L (normal 115–165), lymphopaenia of 0.5 x 10^9/L (normal 1.0–4.0) and thrombocytopaenia with platelets of 92 x 10^9/L (normal 150–450). Creatinine was slightly elevated at 128 mmol/L (normal 40–85). Liver function tests were normal other than an elevated total protein of 83 g/L (normal 60–82) despite slightly low albumin of 33 g/L (normal 35–50). ESR was increased to 48mm/hr (normal <20), with a normal CRP of <1 mg/dL.

An ANA test was performed, with a strongly positive (30 IU/mL or >1:1 280) homogeneous pattern. Dipstick urinalysis showed moderate blood and ++ protein. Complement protein C3 was low at 0.21 g/L (normal 0.95–1.4), as was C4 at 0.03 g/L (normal 0.16–0.32).

A diagnosis of systemic lupus erythematosus was made with clinical manifestations of arthritis, abdominal serositis and nephritis, supported by the laboratory findings of cytopaenias, low complement, haematuria and proteinuria, and a positive ANA. Subsequent tests confirmed positive anti-SSA antibodies (on the ENA test) and a high concentration of dsDNA antibodies, while the elevated total protein was due to a polyclonal hypergammaglobulinaemia.

Key points

- The ANA test is a useful tool for the evaluation of patients with symptoms of systemic rheumatic disease.
- ANA testing in patients with a low probability of rheumatic disease is likely to result in high numbers of false positive results.
- A positive ANA test is not diagnostic of autoimmune disease and is seen in many non-rheumatic conditions as well as healthy individuals.
- High titre ANAs are associated with a higher likelihood of rheumatic disease, but interpretation of their significance requires correlation with clinical symptoms and other investigations.

Authors

Michael O'Sullivan MBBS, FRACP, FRCPA, is a Consultant Immunologist, Fremantle Hospital and Princess Margaret Hospital for Children, WA. Michael.O'Sullivan@health.wa.gov.au

Andrew McLean-Tooke MBChB, MD, MRCP, FRCPath, FRCPA, FRACP, is Head of Immunology, Pathwest, QEII Perth, Western Australia

Richard Loh MBBS, FRACP, FAAAAI, FACAAI, is Head, Immunology Department, Princess Margaret Hospital for Children, Subiaco, Western Australia and President, Australasian Society of Clinical Immunology and Allergy

Competing interests: None.

Provenance and peer review: Commissioned; externally peer reviewed.

References

1. Marin GG, Cardiel MH, Cornejo H,Viveros ME. Prevalence of Antinuclear Antibodies in 3 Groups of Healthy Individuals. J Clin Rheumatol 2009;15:325–29.
2. Dinser R, Braun A, Jendro MC, Engel A. Increased titres of anti-nuclear antibodies do not predict the development of associated disease in the absence

of initial suggestive signs and symptoms. Scand J Rehabil Med 2007;36:448–51.
3. Solomon DH, Kavanaugh AJ, Schur PH; American College of Rheumatology Ad Hoc Committee on Immunologic Testing Guidelines. Evidence-based guidelines for the use of immunologic tests: antinuclear antibody testing. Arthritis Rheum 2002;47:434–44.
4. Migliorini P, Baldini C, Rocchi V, Bombardieri S. Anti-Sm and anti-RNP antibodies. Autoimmunity 2005;38:47–54.
5. Mevorach D, Elchalal U, Rein AJ. Prevention of complete heart block in children of mothers with anti-SSA/Ro and anti-SSB/La autoantibodies: detection and treatment of first-degree atrioventricular block. Curr Opin Rheumatol 2009;21:478–82.
6. Abeles AM, Abeles M. The clinical utility of a positive antinuclear antibody test result. Am J Med 2013;126:342–48.
7. Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med 2003;349:1526–33.
8. Shiboski SC, Shiboski CH, Criswell L, et al. American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance cohort. Arthritis Care Res 2012;64:475–87.

correspondence **afp@racgp.org.au**