

PRACTICE

RATIONAL TESTING

When to order an antinuclear antibody test

This article describes the diagnostic steps and tests for someone with symptoms suggestive of an autoimmune rheumatic disease

Allan Binder *consultant rheumatologist*, Spencer Ellis *consultant rheumatologist*

Rheumatology, Lister Hospital, East and North Hertfordshire NHS Trust, Stevenage SG1 4AB, UK

This series of occasional articles provides an update on the best use of key diagnostic tests in the initial investigation of common or important clinical presentations. The series advisers are Steve Atkin, professor, head of department of academic endocrinology, diabetes, and metabolism, Hull York Medical School; and Eric Kilpatrick, honorary professor, department of clinical biochemistry, Hull Royal Infirmary, Hull York Medical School. To suggest a topic for this series, please email us at practice@bmj.com

A 23 year old woman presents to her general practitioner with a three month history of malaise, fatigue, and arthralgia which she attributes to a viral illness. She had also attended the emergency department with pleuritic chest pain at the start of symptoms. She has had two miscarriages in the past, but has otherwise been healthy. On examination, there are small, non-tender lymph nodes in her neck and mildly swollen, tender, proximal interphalangeal joints in her hands.

What are the next investigations?

The presentation of systemic ill health, pleuritic chest pain, lymphadenopathy, and joint pain could follow a viral infection in addition to several differential diagnoses, including rheumatoid arthritis. However, with this collection of symptoms, it is important to consider a connective tissue disease, particularly systemic lupus erythematosus (SLE). A detailed history and examination (box 1) can provide additional clues to connective tissue disease including overlap syndromes. The presence of frequent mouth ulcers, photosensitivity, alopecia, joint swelling, sicca symptoms, pleurisy, or Raynaud's phenomenon would support the need to test for an autoimmune rheumatic cause.¹ The most common symptoms of SLE are shown in box2.²

In this case the general practitioner should first request a full blood count, erythrocyte sedimentation rate, and tests for C reactive protein, antinuclear antibody, and antibodies to extractable nuclear antigens. As antinuclear antibody positivity can depend on the method used to detect it and is not universally present across the range of rheumatic autoimmune disorders, it is not an absolutely reliable screening test. Testing for antibodies

to extractable nuclear antigens should therefore also be requested when pre-test suspicion is high, to ensure these conditions are not missed. It is important to indicate the diseases being considered when requesting extractable nuclear antigens, rather than simply ordering an "autoantibody profile," as antibodies associated with autoimmune liver disease (smooth muscle and anti-mitochondrial antibodies) may otherwise be reported without necessarily being relevant.

Where SLE is suspected, assess electrolytes, renal function, liver function tests, and urine analysis to identify possible organ involvement, especially nephritis. Blood or protein on urine analysis may indicate nephritis if infection has been excluded. Other tests would be defined by the patient's medical history, and in this case should include antibodies for rheumatoid arthritis, and potentially serology for Epstein-Barr virus, cytomegalovirus, parvovirus, viral hepatitis, and HIV.

Initial test results in patient

The initial blood tests in our patient showed a mild normochromic normocytic anaemia, lymphopenia, erythrocyte sedimentation rate of 105 mm in first hour, and C reactive protein of <5 mg/L. Renal function, liver function tests, and electrolytes were normal. Dipstick testing of urine was negative for blood and protein. The test for antinuclear antibody was strongly positive to a titre of 1: 2540 but with negative extractable nuclear antigens antibodies.

Interpreting a positive antinuclear antibody test

Where the antinuclear antibody test is positive, findings such as normochromic normocytic anaemia, neutropenia, lymphopenia, thrombocytopenia, and a disproportionate rise in the erythrocyte sedimentation rate (or plasma viscosity) compared with C reactive protein are further clues to an autoimmune aetiology. In SLE, the erythrocyte sedimentation rate often reflects disease activity, whereas C reactive protein is usually normal or only minimally raised. A markedly elevated

Learning points

- Most patients with a positive test result for antinuclear antibody do not have systemic lupus erythematosus (SLE) or connective tissue disease, so antinuclear antibody testing should not be requested without a strong suspicion of autoimmune rheumatic disease
- Clues from the clinical assessment such as photosensitivity, alopecia, Raynaud's phenomenon, mouth ulcers, and arthritis should guide the need for testing for antinuclear antibody (and antibodies to extractable nuclear antigens)
- Normochromic normocytic anaemia, neutropenia, lymphopenia, and thrombocytopenia are common findings in autoimmune rheumatic diseases
- Although 95% of SLE patients are positive for antinuclear antibodies, the test specificity is only 57% for the disease
- Antibodies to extractable nuclear antigens have a high specificity for particular clinical autoimmune features or complications
- Urine analysis should always be undertaken when SLE is suspected
- Antinuclear antibody titres cannot be used to monitor SLE and do not need to be repeated once a diagnosis is established. The best tests to serially monitor SLE activity are erythrocyte sedimentation rate, titres of antibodies to double stranded DNA, and complement levels

Box 1: Features suggesting autoimmune rheumatic disease*History: review of systems*

- Joint pain or swelling
- Raynaud's phenomenon
- Photosensitivity, malar rash, hair loss
- Shortness of breath, pleuritic or pericarditic chest pain
- Sicca symptoms (primary or secondary Sjögren's syndrome)
- Thromboembolism, recurrent miscarriage (antiphospholipid syndrome)
- General symptoms (depression, fatigue, fever, weight loss, headache)

Physical examination

- Rashes (variable), alopecia, oral ulcers
- Peripheral oedema
- Lymphadenopathy, splenomegaly
- Pericardial or pleural effusions

Urine analysis

- Blood or protein

Haematology

- Anaemia (may be haemolytic)
- Neutropenia or lymphopenia
- Thrombocytopenia

Box 2: Main symptoms of systemic lupus erythematosus (SLE)

Prevalence of most common clinical manifestations affecting a series of 1000 European patients with SLE²

- Arthritis (84%)
- Malar rash (58%)
- Fever (52%)
- Photosensitivity (45%)
- Nephropathy (39%)
- Serositis (36%)
- Raynaud's phenomenon (34%)
- Neurologic involvement (27%)
- Oral ulcers (24%)
- Thrombocytopenia (22%)
- Sicca syndrome (16%)
- Livedo reticularis (14%)
- Thrombosis (14%)
- Lymphadenopathy (12%)

C reactive protein in SLE should raise a suspicion of an intercurrent bacterial infection,³ but it can also be increased in the presence of certain clinical features such as serositis or arthritis. In primary Sjögren's syndrome, a similar discrepancy between erythrocyte sedimentation rate and C reactive protein often exists but the high erythrocyte sedimentation rate usually

reflects polyclonal hypergammaglobulinaemia (which affects the way the erythrocyte sedimentation rate or plasma viscosity is measured) and not disease activity.

Antinuclear and extractable nuclear antibodies can be directed at a variety of nuclear and cytoplasmic antigens and are found in patients with many rheumatic diseases, including SLE,

scleroderma, Sjögren's syndrome, and rheumatoid arthritis. Antinuclear antibody can also be found in non-rheumatic diseases such as autoimmune hepatitis, primary biliary cirrhosis, Crohn's disease, chronic infectious diseases (such as infectious mononucleosis, subacute bacterial endocarditis, and tuberculosis), lymphoproliferative disorders, and in normal individuals. Antinuclear antibodies are also induced by a variety of drugs, including procainamide and hydralazine.⁴

Although higher titres of antinuclear antibody are more likely to be pathological, no titre is diagnostic of disease. One multicentre study reported that the percentage of normal individuals who were antinuclear antibody positive was as high as 32% at the 1:40 titre, but this decreased to 5% at the 1:160 dilution. At the 1:160 titre, 95% of patients with SLE, 87% of patients with scleroderma, 74% of those with Sjögren's syndrome, and 14% of those with rheumatoid arthritis were antinuclear antibody positive.⁵ Despite the high prevalence of antinuclear antibody in these diseases, specificity is only around 50% (57% for SLE), limiting the value of the test for screening for SLE.⁶ A positive antinuclear antibody may also be found with increasing age, particularly in women,⁷ and in relatives of patients with rheumatological disease.⁸ In the appropriate clinical setting, a positive antinuclear antibody result assists the diagnosis of connective tissue disease, but the antibody titre does not reflect disease activity and antinuclear antibody cannot be used serially to monitor the disease.

It is important to reserve testing for antinuclear antibody (and extractable nuclear antigens) to patients for whom there is a high pre-test suspicion of systemic autoimmune disease,^{4 9} and to avoid using it as a screening tool in patients complaining of fatigue and generalised musculoskeletal pain. A positive antinuclear antibody result in a patient with a low pre-test probability of autoimmune disease often leads to confusion or unnecessary referral to secondary care. The prevalence of SLE is low (40-50/100 000), so the vast majority of people with a positive antinuclear antibody result do not have SLE (positive predictive value 11%).⁸

Although the pattern of antinuclear antibody staining on indirect immunofluorescence is often reported by laboratories and gives some indication of the specificity of the autoantibodies in the serum, it is of limited diagnostic value, varying between laboratories and at different dilutions.¹⁰ More clinically helpful data can be found from the identification of the type of antibodies to extractable nuclear antigens present, given their high specificity for distinct autoimmune conditions and association with important complications that might influence outcome (see table 1). Both positive and negative results for antinuclear antibody should be accompanied by testing for extractable nuclear antigens where there is still a strong suspicion of autoimmune disease.

Referral and further testing

A high index of clinical suspicion should prompt referral even in the absence of positive tests for autoantibodies. Our patient's clinical and test findings warrant referral to a rheumatologist for further investigation. Within the specialist setting we would proceed to check for antibodies to double stranded DNA, complement, and, given her history of miscarriages, anti-cardiolipin antibody and lupus anticoagulant. However, these investigations are widely available in primary care and can be requested before referral depending on the confidence of the clinician in interpreting the initial test results.

Antibodies to double stranded DNA are 97% specific for the diagnosis of SLE (see table 1), and, unlike antinuclear antibody,

the titre generally reflects disease activity and can therefore also indicate response to treatment.¹¹ Complement activation is another common feature of active SLE, and a reduction in the C3 and C4 components of the complement pathway may correlate with disease activity, particularly with renal involvement.¹² A rise in erythrocyte sedimentation rate and titres of antibodies to double stranded DNA and a fall in complement levels can all indicate a worsening of SLE and are the best parameters to measure serially to assess disease control.³ Other antibodies such as anti-Sm (Smith) antibodies (not to be confused with anti-smooth muscle antibodies), although present in only a proportion of SLE patients (table 1), are highly specific for the disease¹³ and are included in the SLE classification criteria from the American College of Rheumatology.¹⁴

Because our patient had had miscarriages, anti-phospholipid antibodies and lupus anticoagulant should also be assessed. These antibodies are found in SLE, but also in other rheumatic diseases or without any rheumatic association. Recurrent venous or arterial thromboses and pregnancy morbidity suggest the anti-phospholipid syndrome, but, as these antibodies can develop transiently after acute infections, the test needs to show elevated titres on at least two occasions 12 weeks apart to confirm the presence of the anti-phospholipid syndrome.

Outcome

The patient's titre of antibodies to double stranded DNA was raised to three times the upper limit of normal. Anti-cardiolipin antibodies were also positive, with a mild reduction in C3 and C4 complement levels supporting a diagnosis of SLE.

The positive result for anti-cardiolipin antibodies was confirmed three months later in the rheumatology clinic and was consistent with a diagnosis of anti-phospholipid syndrome in association with SLE. In view of the patient's anaemia, a direct anti-globulin antibody test was also undertaken and was positive, indicating a degree of autoimmune haemolysis.

The patient was treated with a short course of low dose prednisolone and maintenance therapy of hydroxychloroquine and aspirin, with rapid improvement. The use of heparin during future pregnancies was discussed. Long term monitoring of disease activity and organ damage in a secondary care setting would be recommended.

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Table

Table 1 | Widely available autoantibody tests that assist in diagnosis of autoimmune rheumatic diseases (adapted from Satoh)¹⁰

Disease and autoantibody tests	Frequency in the disease	Specificity	Important clinical associations
Antibodies to extractable nuclear antigens			
Systemic lupus erythematosus:			
Sm	15%	High	Some association with neuro-lupus
Sjögren's syndrome:			
SS-A (Ro)	70%	Low	Also in 35% of patients with systemic lupus erythematosus, some of whom are negative for antinuclear antibodies Associated with congenital fetal heart block or neonatal rash
SS-B (La)	40%	Moderate	Also in 15% of patients with systemic lupus erythematosus
Mixed connective tissue disease:			
U1RNP	100%	Low	Also in 30% of patients with systemic lupus erythematosus
Scleroderma:			
Scl-70	15%	High	Associated with lung fibrosis
Centromere	25%	Moderate	Limited cutaneous scleroderma
Polymyositis or dermatomyositis:			
Jo-1	20%	High	Associated with lung fibrosis
Antibodies to double stranded DNA*			
Systemic lupus erythematosus:			
dsDNA	50–80%	High	High titres associated with nephritis

*Tests for antibodies to double stranded DNA do not come under the umbrella of antibodies to extractable nuclear antigens and need to be requested separately.