



SAN PATHOLOGY UPDATE

THE ANA TEST IN THE DIAGNOSIS OF IMMUNE DISEASE

by Dr Bevan Hokin

INTRODUCTION

The immune system is vital for our survival - but must be finely balanced. In immunodeficiency states, humans become more susceptible to infections and tumours. A hyperactive immune system can cause fatal conditions such as anaphylaxis in response to a bee sting. A further derangement of the immune system is noted when the body loses its ability to distinguish 'self' from 'non-self', resulting in the development of immunity against one's own tissue(s). In this condition called autoimmunity, antibodies develop and attack one's own cells.

Antinuclear antibodies (ANA) are unusual antibodies that are found in the blood that have the capacity to bind to structures within the cells' nuclei. They are auto antibodies found in patients that result in inflammation and damage to their own tissues.

The test for ANA is used to detect the presence of autoimmunity and provide an indication of possible autoimmune disease. The most common method of testing for ANA is the immunofluorescence method.

AUTOIMMUNE DISEASES

ANA are found in patients with a number of autoimmune diseases, including Systemic lupus erythematosus (SLE), drug induced LE, Sjogren's syndrome, Rheumatoid arthritis, inflammatory myopathies, scleroderma and pulmonary fibrosis. ANA are sometimes seen in non autoimmune conditions including chronic infections, haemolytic anaemia, psoriasis, pemphigus and some cancers. See Table 1.

The immunofluorescence test for ANA is positive in virtually every patient with SLE i.e. very sensitive. But the test is not specific for SLE as patients with other autoimmune diseases also test positive. Of assistance is a positive antibody test to double stranded DNA – the Sm antigen – where a positive result is virtually diagnostic of SLE. See Table 1.

DRUG INDUCED PRODUCTION OF ANA

Some medications can stimulate the production of ANA. Drug-induced ANA are false positives. Implicated drugs include hydralazine, isoniazid, procainamide and several anticonvulsants including dilantin.

About 5% of the normal population without disease may also test positive for ANA, usually at low levels (titres below 1:80). The incidence of false positive ANA results increases with age with up to one third of healthy individuals over age 65 years testing positive.

TEST METHOD AND RESULTS

The sample of choice is serum – 5 mL of blood is collected in a plain tube.

The ANA test is performed by adding patient serum to commercially prepared cells fixed to a microscope slide. If anti-nuclear antibodies are present they bind to the nuclei in the cells on the slide. A second antibody that is labelled with a fluorescent dye is added. This second antibody binds to the first antibody – and as it carries a fluorescent 'tag' the now labelled cells can be visualised. Areas with antinuclear antibodies fluoresce when the slide is viewed under an ultraviolet microscope.

If fluorescent cells are seen, the test is positive. When positive, the serum is diluted, or titred, and the test done again. These steps are repeated until the serum is so diluted it no longer gives a positive result. The last dilution that shows fluorescence is the titre reported. It is important to note that there is some subjectivity in interpreting the degree of fluorescence at these low levels, so any titre reported should be understood to be a semi-quantitative result.

The pattern of fluorescence within the cells gives some clue as to what the disease might be. The test result will be reported as "negative" or if "positive" will include the titre

and the pattern observed. E.g. ANA Positive at a dilution of 1:400 with a speckled pattern.

INTERPRETING ANA PATTERNS

Different reported patterns may be associated with different autoimmune diseases:

<i>Nucleolar pattern</i>	Associated with scleroderma and polymyositis
<i>Homogenous or diffuse pattern</i>	Associated with SLE and mixed connective tissue diseases
<i>Speckled pattern</i>	Associated with scleroderma, polymyositis, SLE, mixed connective tissue diseases, Sjogren's syndrome and rheumatoid arthritis
<i>Peripheral outline pattern</i>	Associated with SLE

SUPPLEMENTARY TESTING OF POSITIVE ANA PATIENTS: THE ENA TEST

In cases of positive ANA results, additional testing (Extractable nuclear antigen antibodies (ENA)) may be useful to categorise possible disease states. The ENA test may also be useful where there is high suspicion of connective tissue disease and the ANA test is negative (antibodies to Ro (SS-A) may not be detected by the ANA test).

The presence of antibodies against extractable antigens is highly suggestive of systemic rheumatic disease. Specific associations include:

<i>RNP</i>	Mixed connective tissue disease
<i>Sm</i>	Highly specific for SLE
<i>Ro (SS-A)</i>	subacute cutaneous lupus; associated with recurrent abortion, congenital heart block; Sjogren's syndrome
<i>La (SS-B)</i>	Sjogren's syndrome
<i>Scl 70</i>	scleroderma



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CONTINUED FROM PAGE 1

TABLE 1.

Anti-nuclear antibodies in various autoimmune diseases

Red-coloured entries indicate high correlation

Disease % positive							
NATURE OF ANTIGEN	ANTIBODY SYSTEM	SLE	DRUG-INDUCED SLE	SYSTEMIC SCLEROSIS-DIFFUSE	LIMITED SCLERODERMA-CREST	SJOGREN'S SYNDROME	INFLAMMATORY MYOPATHIES
Many nuclear antigens to DNA, RNA, proteins	ANA	>95	>95	70-90	70-90	50-80	40-60
Native DNA	Anti-double-stranded DNA	40-60	<5	<5	<5	<5	<5
Histones	Antihistone	50-70	>95	<5	<5	<5	<5
Core proteins of small nuclear ribonucleo-protein particles	Anti-Sm	20-30	<5	<5	<5	<5	<5
Ribonuclear protein	Nuclear RNP	30-40	<5	15	10	<5	<5
RNP	SS-A(Ro) SS-B(La)	30-50 10-15	<5 <5	<5 <5	<5 <5	70-95 60-90	10 <5
DNA topoisom-erase	Scl-70	<5	<5	28-70	10-18	<5	<5
Histidyl-RNA Synthetase	Jo-1	<5	<5	<5	<5	<5	25

CONCLUSION

While the ANA test is not 100% specific, it is a useful semi-quantitative test to identify several of the important autoimmune diseases.

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