

## **AUTO-ANTIBODIES - Dr. N. Manolios. 2007**

Autoantibody testing can be of value in making the diagnosis in a number of connective tissue disorders such as systemic lupus erythematosus, mixed connective tissue disease, and polymyositis to name a few. Correct interpretation of the results is important and the test results must always be correlated with the patients symptoms and signs. *In general*, auto-antibodies, are of value in assisting with the diagnosis, but not as a marker of disease activity. The first screening test for a connective tissue disorder is an anti-nuclear antibody.

About 5 % of the normal population have a positive anti-nuclear antibody at a titre of greater than or equal to 1:160. This group usually has only a slight increase in titre with a speckled pattern. The percent with positive ANA increases with age and in people, 70 -80 years, up to 15% will have a positive low titre anti-nuclear antibody. In the over 70 age group, the ANA's are usually antibodies to histone proteins, and there is no increase in antibodies to the significant antigens, for example, DNA, RNP, Scl-70.

It is important to remember that a positive anti-nuclear antibody is not diagnostic of systemic lupus erythematosus and can occur with other illnesses, e.g. infection, both bacterial and viral, drug therapy and other inflammatory disorders.

### **ANTI-NUCLEAR ANTIBODIES**

The anti-nuclear antibody (ANA) is a useful screening test and may give information about other auto-antibodies that need to be specifically measured. It indicates whether the patient's serum has an antibody against a nuclear component. Interpretation of a positive anti-nuclear antibody is not always simple and it needs to be correlated with the clinical findings, the age of the patient and the antibody titre. The titre does not always correlate with activity, since it is the avidity of antibody binding that is important and not the amount.

The ANA is a good screening test for SLE but it is not specific for SLE. More than 95% of patients with SLE have a positive ANA. ANA negative SLE is very uncommon and may be due to the presence of anti-cytoplasmic antibodies, the commonest being SSA/Ro. Active SLE can have a low titre ANA.

Different staining patterns are reported, which may give clues as to the significance of the ANA, and any further tests required. The staining patterns are homogenous, rim, speckled, and nucleolar. There can be variation in reporting of staining patterns between laboratories. They are not diagnostic but are a useful guide as how to proceed with investigations.

If the clinical findings and the ANA result do not agree, believe the clinical findings.

## **ANA Staining Patterns**

The different staining patterns reflect the nature of the antigen and its distribution. Different patterns include:

*Homogenous Pattern.* This pattern is most common in SLE, drug induced lupus erythematosus and chronic active hepatitis. The antigen is usually DNA, histones or deoxyribonucleoprotein.

*Rim Pattern.* This pattern is also associated predominantly with SLE. It is thought that rim and homogenous are the same but appear different due to sectioning of cells.

*Speckled Pattern.* This is the most frequent staining pattern and the pattern that usually occurs in other illnesses and normal people with low titres of anti-nuclear antibody. A special speckled pattern is antibodies to anti-centromere which result in exactly 46 nuclear speckles. Anti-centromere antibodies are associated with the CREST syndrome, a limited variant of scleroderma. Anti-centromere antibody is uncommon in the more diffuse form of systemic sclerosis and is only rarely found in other connective tissue disorders

*Nucleolar Pattern.* This pattern is suggestive of systemic sclerosis (scleroderma), polymyositis.

## **Specific Antibodies:**

The clinical findings and ANA result will be a useful guide as to more specific antibody testing. The most important antibody is to double stranded DNA. There is also a group of specific autoantibodies that are usually associated with a particular autoimmune disease or group of diseases. They usually produce a speckled ANA. These include the extractable nuclear antigens, RNP, Sm, SSA/Ro, or SSB/La, Scl-70, Jo-1 and ribosomal-P. They are of diagnostic value and are reported as being present or absent. Levels are not a guide to disease activity.

### *Antibodies to dsDNA*

This is the antibody that is of diagnostic value for systemic lupus erythematosus.

Antibodies to ds-DNA are specific for systemic lupus erythematosus, being present in 60%-70% of patients. Elevated levels are usually associated with active disease, but patients can have elevated DNA antibodies and be clinically quiescent. Patients who are clinically well with high DNA antibody levels, require more frequent review, but there is no need to change treatment.

Rapid rises or falls in DNA antibody levels with doubling or halving time of four weeks or less, may precede flares in the disease. Levels may fall with successful treatment by corticosteroids and immunosuppressive drugs.

Serum complement levels of C3 and C4 are useful in monitoring disease activity in conjunction with DNA levels. A rapid rise or fall of DNA levels in association with a fall in complement C3 and/or C4, is more likely to be significant than when the complement levels remain normal.

There are two major types of assays used to measure antibodies to DNA, a radioimmunoassay or Farr assay and an enzyme linked immunoassay (ELISA). The Farr assay measures antibodies that are clinically relevant, and is more reliable. ELISA assays have a higher rate of false positive results, so that if there is doubt about the result it should be checked using a Farr assay.

## **Extractable Nuclear Antigens (ENA)**

The extractable nuclear antigens consist of a number of antigens which include RNP, Sm, SSA/Ro and SSB/La.

*Anti-RNP.* Antibodies to RNP are present in about one third of patients with SLE and are the only auto-antibody present in patients with mixed connective tissue disease.

*Anti-Sm.* Anti-Sm antibody is highly specific for SLE but is present in only about 10% of patients. Therefore, it is not particularly sensitive.

*Anti-SSA/Ro.* Anti-SSA/Ro is a relatively common antibody found in about 30% of people with SLE and 75% of patients with primary Sjogren's syndrome. People with SLE who have anti-Ro are more likely to have cutaneous involvement and women with this antibody when pregnant are more likely to have children with congenital heart block. This applies particularly if they also have antibody to SSB/La.

*Anti-SSB/La.* Antibody to SSB/La is less common and usually occurs in conjunction with antiSSA/Ro. It is present in only about 10% of people with SLE and in about 50% of primary Sjogren's syndrome. It may indicate a milder course of disease in SLE. The presence of both SSA/Ro and SSB/La is more commonly associated with primary Sjogren's syndrome.

*Antibodies to Scl-70/Topoisomerase.* Antibodies to Scl-70 are highly specific for diffuse scleroderma. They occur in about 50% of patients with diffuse scleroderma.

*Antibodies to Jo-1.* Antibodies to Jo-1 are present in 30% of people with polymyositis, particularly in those who also have pulmonary fibrosis.

*Antibodies to ribosomal-P* These antibodies are present in about 15% SLE, and may be associated with psychiatric manifestation of SLE. However, the association is strong enough to make them of diagnostic value.

Autoantibodies can be useful in helping with the diagnosis of connective tissue disorders. (Table 1). The clinical findings are the most important factors in determining what autoantibody to measure. Their interpretation should always be made in association with the clinical findings.

Table 1. Specific Autoantibodies and their Disease Association

ANTIBODY	DISEASE ASSOCIATION
dsDNA	SLE
Sm	SLE
SSA/Ro with SSB/La	Primary Sjogrens syndrome
SSA/Ro without SSB/La	SLE, Sjogrens syndrome
Jo-1	Polymyositis/dermatomyositis
RNP	Mixed connective tissue disease
Scl-70	Diffuse scleroderma
Anti-centromere	CREST syndrome