Periodic Synopsis

The value of autoantibodies in autoimmune connective tissue disease

D A C Dissanayake[1], T D P Lokunarangoda[2], S Prasad W Kumarasinghe[3]

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Autoimmune connective tissue diseases are a group of disorders characterized by the presence of autoantibodies directed against an array of autoantigens which are all cellular components; the nucleus, the cytoplasm and the cell membrane[1] (Table 1). Detection of autoantibodies in serum is an integral part of the diagnostic process of many of both organ specific and non-organ specific connective tissue disorders. It may confirm the suspected clinical diagnosis, help to identify the subsets of the disease and aid prognostication[2]. Serial testing of some of these auto antibodies are being adopted as markers of the disease activity, as well as to predict the sustained response to a particular treatment modality. Some of these autoantibodies are non-specific; that is, they do occur in a number of connective tissue diseases and therefore their diagnostic value is limited. In contrast, the detection of certain autoantibodies in serum may confirm the clinical diagnosis in question. For example, although rheumatoid factor (RF) is positive in approximately 70% of patients with rheumatoid arthritis (RA), its presence is not diagnostic of the disease (Table 2) since it is also found in many other connective tissue diseases (CTD). On the other hand, detection of anti dsDNA and anti Sm antibodies in suspected systemic lupus erythematosus (SLE) is diagnostic of the disease, although the sensitivity is low. It has been proved that some autoantibodies are involved in the pathogenesis of the disease. For example, anti dsDNA antibodies in SLE are found to be directly pathogenic. In addition they help in the determination of the development and expression of the disease. The presence of antibodies to anti-nucleosome subset of dsDNA and histones are more frequent in patients with lupus nephritis. In addition it has been observed that there is a positive correlation with these antibodies and the disease activity. In patients with RA who possess HLA-DRB1, it is estimated that the risk of developing bony erosions at one year is 13 times higher in seropositive patients[3]. At present, it is not clear whether the other autoantibodies also do so, it is simply an association or generated as a consequence of the disease process. However, in a recent study on personnel of US armed forces, it has been observed that clinical SLE is preceded by an autoimmune response evolving through many years[4].

Table 1. Autoimmune connective tissue diseases

1 Lupus erythematosus
  Systemic lupus erythematosus
  Discoid lupus erythematosus
  Subacute cutaneous lupus erythematosus
  Overlap of two more lupus erythematosus subsets
  Overlap of lupus erythematosus with other CTD
  Neonatal lupus erythematosus

2 Systemic sclerosis (Scleroderma)
  (a) Localized sleroderma
  (b) Systemic scleroderma
    Limited cutaneous systemic sclerosis (acrosclerosis, CREST syndrome*)
    Diffuse cutaneous systemic sclerosis
    Systemic sclerosis sine scleroderma**

3 Sjogren's syndrome
  Primary
  Secondary

4 Mixed connective tissue disease

5 Dermatomyositis

6 Overlap and undifferentiated CTDs

* CREST - Calcinosis, Raynaud's phenomenon, Esophageal dysmotility, Sclerodactily, Telangiectasia.

** A form with little or no cutaneous sclerosis despite the presence of systemic features.

Table 2. Associations of rheumatoid factor

Disease	*Positivity*
Rheumatoid arthritis	70 - 80%
Felty syndrome	up to 100%
Sjogren's syndrome	up to 100%
Mixed cryoglobulunaemia	80 - 100%
MCTD	40 - 60%
Infective endocarditis	upto 50%
SLE	up to 40%

(Rheumatoid factor is also found in cryptogenic fibrosing alveolitis, viral infections including infectious mononucleosis, tuberculosis, leprosy and in 5 - 10% of normal controls.)

[1] *Registrar in Medicine,* [2] *Senior Registrar in Dermatology,* [3] *Consultant Dermatologist, North Colombo Teaching Hospital, Ragama.*

Diagnostic value of serology

There are several parameters by which the value of a given serologic test could be estimated. They include sensitivity, specificity, positive predictive value, negative predictive value and marginal benefit[1]. Sensitivity refers to the probability of a test to have a positive result in a patient with a disease whereas specificity refers to that of a negative result in a person without disease. Positive predictive value is indicative of the probability of patient with a positive test to have the disease; whereas negative predictive value refers to that of a person with a negative test to be free of disease. Therefore in a given test the higher the specificity, the higher the positive predictive value is. On the other hand, a higher sensitivity of a test will have a higher predictive value when the test is negative. However, of all the parameters the marginal benefit has the greatest practical value for the clinician who attempts to confirm or exclude a diagnosis in question with an index serological test. The marginal benefit of a given diagnostic serological test is maximal when the possibility of the disease is intermediate[1]. However the clinical usefulness of the results depends on the quality of the laboratory test used[3]. The increasing commercial availability of tests could result in problems of standardization. In difficult cases access to a reference laboratory may be indispensable; especially if the result of test of an ordinary laboratory does not support the clinical diagnosis.

Techniques of serologic testing

Immunologic techniques have been the method of choice for autoantibody testing although the method by which a given autoantibody is tested has changed over past few decades[1]. Radioimmunoassay and immunoelectrophoresis were the commonly used methods in the past. Both tests have recently been replaced by newer techniques such as immunofluorescence (IF) and enzyme linked immunosorbant assay (ELISA). The ELISA has several advantages over the former although the principle of both tests is similar; i.e. use HEp-2 as the substrate. It is cheaper, more sensitive, less labour intensive and reproducible. In addition it can be used to screen a large number of sera together. The ELISA is however less specific compared to IF and therefore needs to be interpreted with caution. Immunoblotting is the method of choice for the detection of U1RNP antibodies, the SM B/B proteins being the predominant antigen against which these antibodies are directed[5].

Important autoantibodies

Rheumatoid factor (RF)

This is primarily an IgM antibody (rarely an IgG) antibody directed against the Fc portion of the patient's own IgG molecules. RF is usually tested with latex agglutination test[4]. Although it is positive in 70-80% of patients with RA predicting more aggressive disease with extra-articular manifestations, these antibodies are not specific for the disease and also occurs in a wide range of autoimmune diseases, a variety of infections and in old age (Table 2).

Antinuclear antibodies (ANA)

Antinuclear antibodies are autoantibodies directed against cellular proteins or nucleic acids (Table 3). Screening for ANA plays a central role in the workup of a suspected connective tissue disease. The presence of ANA is characteristic of SLE and may be detected in more than 90% of untreated disease. A high titre ANA (>1:160 or above) is more likely to be clinically significant[6]. Indirect immunofluorescence (IIF) is the gold standard test although ELISA is increasingly being used. Of the two substrates used (fresh frozen sections of rodent liver and/or kidney and cultured human cell lines such as HEp-2 which are cultured human oesophageal carcinoma cells) the human substrate has a better sensitivity. It is very interesting to note that the pattern of immunofluorescence of ANA test is usually associated with the presence of specific antinuclear antibodies. For example, peripheral or ring pattern is associated with antibodies to dsDNA and thus correlates with a diagnosis of SLE. The homogenous pattern is associated with antibodies to dsDNA or histones. The nucleolar pattern is seen in patients with both SLE and systemic sclerosis (SSc) whereas centromere pattern can be observed in CREST (Calcinosis, Raynaud's phenomenon, Esophageal dismotility, Sclerodactily and Telangiectasia) syndrome. Mixed connective tissue disorder (MCTD), SLE, SSc, Sjogrens syndrome all show ANA of spekled pattern[1].

Table 3. Antinuclear antibody and disease

Disease	*Positivity*
SLE	90 - 95%
Juvenile idiopathic arthritis	76%
Chronic active hepatitis	75%
Sjogren's syndrome	68%
Progressive SSc	64%
Rheumatoid arthritis	32%

(Anti nuclear antibodies are also found in the elderly [20% at 70 years], pregnancy, medication with certain drugs, chronic infections, neoplasms, relatives with patients with CTDs and in other autoimmune diseases.)

Table 4. Antibodies to extractable nuclear antigens in CTD

Autoantibody	*Disease*	*Prevalence*	*Clinical associations*
U1RNP	MCTD	almost 100%	Raynaud's phenomenon, slerodactily, deforming arthritis myositis, oesopageal dysmotility, pulmonary dysfunction, low incidence of renal disease.
	SLE	30%	
Sm	SLE	15 - 40%	Vasculitis, CNS lupus
Anti Ro (SSA) Anti La (SSB)	SLE	Ro - 40% La - 15%	Subacute cutaneous lupus erythematosus photosenssitive dermatitis neonatal lupus congenital heart block.
	Sjogern's syndrome	Ro - 60% La - 25%	More aggressive extraglandular systemic disease, vasculitis, lymphoma.
Scl 70	Systemic sclerosis	25%	Diffuse cutaneous systemic sclerosis and systemic involvement, particularly cardiopulmonary.
Anti centromere	Systemic sclerosis	30%	limited cutaneous systemic sclerosis CREST syndrome, absence of lung disease.
Anti Jo - 1	Polymyositis	20%	Interstitial lung disease.

Low-titre ANA positivity occurs in the majority of cases of dermatomyositis. Those with associated connective tissue disease tend to have higher titre ANAs (>1:160)[7]. Although ANA testing has a high negative predictive value in numerous studies, proper selection of patients is the key to improve predictive value of a positive result. The American College of Rheumatology criteria are reviewed and recommended as apart of the patient selection process[8].

Anti dsDNA antibodies

Antibodies to dsDNA are highly specific for SLE, especially if the titre is higher than 2-3 standard deviations above the mean value. They are present only in 70% of patients with the disease and a negative test does not exclude the diagnosis. On the other hand, its presence predicts the future occurrence of lupus nephritis and generally indicates a poor prognosis. Serial measurements of the antibody titre are useful in monitoring the disease activity some patients. IIF test performed using (*Crithidia luciliae*, a hemoflagellate that possesses giant mitochondria containing a circular dsDNA, is the standard test used[1,3]. However ELISA using calf thymus extract is more sensitive than IIF. A low level of antibodies may be detected in RA, MCTD, SSc, Sjogren's syndrome, autoimmune liver disease and some other organ specific autoimmune diseases[1].

ssDNA antibodies

These autoantibodies, detected by ELISA, are found in a number of connective tissue diseases and infections. Therefore it has a poor disease specificity and rarely done in diagnostic work up of connective tissue diseases.

Antihistone antibodies

Antihistone antibodies are found in 75-90% of drug induced SLE[9]. But it may also be found in up to 50% patient with true SLE and therefore has poor disease specificity.

Antibodies to extractable nuclear antigen

Detection of antibodies to extractable nuclear antigen is useful in the diagnosis (e.g. mixed connective tissue disease), identification of subsets of a CTD (e.g. scleroderma) and prognostication (see Table 4) of several autoimmune connective tissue diseases. MCTD is characterized by the presence of U1RNP and it is found in almost all patients with the disease, it is associated with protean clinical features. The presence of anti Sm antibodies is closely related to occurrence of central nervous system lupus and vasculitis. The detection of anti Ro (SSA) and anti La (SSB) in patients with both SLE and Sjogren's syndrome has significant prognostic implications. Pregnancy in SLE may be complicated by neonatal lupus and congenital heart block, the latter of which may be permanent. It is not a prerequisite for the mother to have overt disease for the baby to be affected (though almost all have auto antibodies to SSA/Ro associated proteins). On the other hand only a minority of all babies, whose mothers are positive for anti Ro in their serum, will develop the complications. The prevalence of Scl 70 and anticentromere antibodies are very low although they are specific to each subset of SSc . The latter is found in approximately 90% of patients with CREST syndrome. The pathogenesis of the disease is complex, but the detection of these autoantibodies may be useful in respect of early diagnosis and to distinguish scleroderma / myositis overlap syndromes. The newly recognized autoantibodies for myositis such as anti-Mi-2, anti-PM-Scl and anti signal recognition particles may be useful in this respect[9].

Antiphospholipid antibodies (APAs)

The antiphospholipid syndrome is characterized by the presence of APAs and the syndrome of hypercoagulability. This is manifested by arterial, venous or small vessel thrombosis, occurring within any tissue or organs[10]. The criteria for diagnosis and the terminology used to describe the disease are still in the process of revision[10]. The most commonly detected APAs are lupus anticoagulant antibodies, anticardiolipin antibodies and anti B2 glycoprotein 1(anti B2 gp1) antibodies. The division into three subgroups are broadly based on the method of detection. Lupus anticoagulant antibodies are identified by coagulation assays (activated partial thomboplastin time and dilute Russel's viper venom time) whereas anticardiolipin and antiB2gpl antibodies are detected by immunoassays that measure immunogenic reaction to the respective antigen[10]. In general lupus anticoagulant antibodies are reported to be more specific for antiphospholipid syndrome.

However, in antiphospholipid syndrome, there are no specific clinical manifestations for a particular antibody. Multiple tests may need to be performed in the event of thrombosis as the patient may turn out to be positive for one test but negative for other tests. Although anti B2gpl antibodies are strongly associated with the clinical syndrome of antiphospholipid syndrome, it is currently not included in the diagnostic criteria for the disease. Livedo reticularis, purpura, skin necrosis, and ulcers are the usual presentations to the dermatologist and testing for APAs should be included in the routine diagnostic work up. In addition they are found in up to 50% of patients with SLE and this should be included in the routine work up of the disease. Use of certain drugs, and certain infections are also associated with the presence of APAs in serum[1]. The syndrome is labeled as primary if evidence of association of other connective tissue diseases cannot be elucidated.

Anti-Ro/SS-A and anti-La/SS-B antibodies

Nearly all infants with neonatal lupus erythematosus have anti-Ro/SSA and sometimes anti-La/SS-B antibodies. In almost all cases mothers have auto antibodies to SS-A associated proteins but may not have clinical disease[11].

Anti neutrophil cytoplasmic antibodies (ANCA)

This heterogenous group of autoantibodies encompasses cytoplasmic (cANCA) and perinuclear (pANCA) subsets. Their detection has been a useful tool for the diagnosis of small vessel vasculitides, especially Wegener's granulomatosis, microscopic polyangiitis and Churg-Strauss syndrome, since its discovery in mid-1980s. In recent studies, it has been shown that 15-20% of patients with systemic lupus erythematosus have detectable pANCA[12]. ANCA to myeloperoxidase are associated with drug induced lupus. Further studies are needed to confirm a link between pANCA level and disease activity.

Antibodies to cyclic citrullinated peptide antigens (Anti-CCP)

The detection of antibodies to cyclic citrullinated peptide antigen in serum by second generation ELISA has been shown to be useful in the diagnosis of rheumatoid arthritis. In one study conducted in Japan, the sensitivity and the specificity of the test for RA were 87.6% and 88.9% respectively. This showed a higher discriminative power for a positive diagnosis compared to rheumatoid factor[13]. The results were further supported by a recently conducted US study

that showed the sensitivity and specificity of 66% and 90.4% respectively[14]. Although the second study showed a lower sensitivity, the higher specificity of the test merits its usefulness in the diagnosis of rheumatoid arthritis, may be even more than the rheumatoid factor.

Autoantibodies and monitoring of disease activity

Rising levels of anti dsDNA may correlate with disease activity. However the autoantibody level may predate a relapse by many months. On the contrary, it has been observed that in some patients the levels decrease immediately before a relapse. Nevertheless an increase in the titre should arouse the clinician's suspicion of a relapse and should lead to an increase in the frequency of the monitoring rather than inadvertent increase in immunosupression[6]. In general serial measurement of autoantibodies has a limited role in disease monitoring in autoimmune connective tissue diseases.

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