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Serologic Testing in Connective Tissue Diseases

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Serological testing is primarily applied to assist in confirming a specific diagnosis, to formulate appropriate management strategies and, in some cases, to evaluate disease activity relative to connective tissue diseases (CTD). Based on a high index of clinical suspicion, physicians should have a compelling reason to order serologic autoantibody tests to diagnose CTD. This article is designed to serve as a guide for physicians to better understand the appropriate use and interpretation of rheumatologic tests.

It is important that clinicians evaluate the indications for sensitivity, specificity, and positive and negative predicative values of serologic tests since these parameters are important in order to understand and appropriately interpret the results. The term sensitivity when used in the field of rheumatology refers to the likelihood or probability that a patient suggestive of having a rheumatologic or CTD will have a positive serologic test result. A specific serologic test is more likely to identify patients with a particular disease and exclude those without the disease. A test with a high specificity rules in, but does not rule out, a disease. A positive test result provides additional supporting evidence that the disease in question is present.

Positive and negative predictive values of a test refer to the proportion of persons who test positive or negative and who have or do not have a disease, respectively. The results of these tests are highly dependent upon the prevalence of disease in the population being tested. In a population with a low prevalence of CTD, a positive test result is more likely to be a false positive.

Utility of the Antinuclear Antibody (ANA) Test in Diagnosis and Monitoring

The ANA test utilizes the indirect immunofluorescence technique to detect autoantibodies that bind to a variety of nuclear antigens and is often used to evaluate the possible presence of autoimmune disease. The ANA test is sensitive in that ANAs are detected in more than 95% of patients with systemic lupus erythematosus (SLE) and are also found in conjunction with CTDs. However, the ANA test lacks specificity and the presence of the antibody is not necessarily diagnostic for SLE. These antibodies may be found in patients with other autoimmune diseases (e.g., hepatitis C), may be medication induced and may even be present in otherwise healthy individuals (table 1). Thus, a negative ANA test is associated with a high negative predictive value. If this test is ordered in a population or in a person with a

Table 1. Sensitivity and specificity of antinuclear antibody (ANA) tests in certain connective tissue diseases.[1]

Disease	Sensitivity (%)	Specificity (%)
Polymyositis/dermatomyositis	61	63
Rheumatoid arthritis	41	56
Scleroderma	85	54
Secondary Raynaud	64	41
Sjögren's syndrome	48	52
Systemic lupus erythematosus	93	57

low probability of having a CTD, a positive result is more likely to be a false positive.

Compared to patients with SLE, the ANA test has a lower sensitivity and specificity in patients with other CTDs.[1] Therefore, an ANA test should not be routinely ordered in patients who present with joint pain to exclude or diagnose SLE. The result, if positive, is more likely to be a false positive, particularly in the elderly, given the low prevalence and predictive value of SLE in this patient population.

ANA immunofluorescence testing is associated with various staining patterns (homogenous, particulate, diffuse and localized to the centromere), but lacks specificity because of overlap between staining patterns and diseases. The advent of more specific autoantibody tests has diminished the use of nuclear staining patterns evaluation. ANA are reported in titers with values of at least 1:160 having possible clinical significance and warranting further diagnostic evaluation.[2]

ANA titers do not correlate with disease activity and the practice of ordering this test to monitor the course of SLE should be abandoned.[2] In fact, the history and clinical examination, supported in some cases by determination of an estimated sedimentation rate, anti-double stranded DNA (dsDNA) antibody titer and complement levels, better correlate with disease activity and serves to guide treatment decisions.

Tests with greater specificity that are more likely to support the diagnosis of SLE include the anti-dsDNA-antibody and anti-smooth-muscle (Sm) antigen tests (table 2). These tests are less sensitive relative to other CTDs, since they are not commonly found in those conditions. Anti-dsDNA has been shown in some SLE patients to correlate with greater severity of renal involvement and, as noted, is a marker of disease activity.[2-4] Elevated levels of anti-nucleosome antibodies have also been shown to be associated with renal involvement and are useful in assessing disease activity, particularly in anti-dsDNA negative patients.[5] Absence of these antibodies in a patient with a high probability of SLE does not rule out the disease (low negative predictive value). Thus, anti-dsDNA tests should be performed only in patients with a positive ANA test in whom SLE is clinically suspected or in ANA-negative patients whose symptoms are highly suggestive of SLE.

Another serological nuclear marker used in cases of drug-induced lupus is the anti-histone antibody test. Antihistone antibodies are sensitive but nonspecific for drug-induced lupus. They occur in over 95% of patients with drug-induced lupus but are also found in over 50% of patients with SLE.[1] In fact, most patients who develop these antibodies do not develop symptomatic disease. A negative test result makes the diagnosis of drug-induced lupus less likely; however, like all serologic tests, the results must be interpreted in the appropriate clinical content. Drug-induced lupus involves the ingestion of a drug (e.g., Procainamide) and is associated with high-titer positive ANA, absence of dsDNA antibody and other clinical features of lupus with the exception of nervous system and renal involvement.

Appropriate Use of Extractable Nuclear Antibodies

Extractable nuclear antibodies are directed against small ribonuclear proteins (RNA) and include anti-Sm, uracil-rich 1 ribonucleoprotein (U_1RNP), anti-SSA/Ro and anti-SSB/La. The ribonuclear proteins play an important role in mRNA splicing. The high specificity of these tests means that they should only be ordered in ANA positive patients with clinical features suggestive of a particular CTD and in ANA negative patients with known or suspected CTD (table 2).[2] These tests are intended for diagnostic confirmation but do not exclude a specific CTD.[1] These antibodies in general do not correlate with disease activity and, therefore, may be found in patients without active disease.

Anti-Sm antibodies are specific but lack sensitivity for SLE. They only occur in approximately 25% to 30% of patients with SLE.[1] Therefore, the absence of these antibodies does not exclude the disease despite their high specificity for SLE. Anti-Sm and anti-dsDNA antibodies may be particularly useful in confirming a diagnosis in ANA positive patients who have not fully met the American College of Rheumatology Classification criteria for SLE.

Antibodies directed against U_1RNP may be found in patients diagnosed with mixed CTDs. Patients with mixed CTDs have, by definition, a positive anti-U_1RNP antibody with overlapping symptoms of other CTDs. The absence of these antibodies in the presence of other clinical features of CTDs by convention is termed "overlapping syndrome." U_1RNP antibodies are specific but lack sensitivity for mixed CTDs. Furthermore, these antibodies are neither sensitive nor specific for SLE. The presence of a positive U_1RNP result and a negative dsDNA antibody in a patient with SLE is associated with a clinical course characterized by the absence of nephritis but more typical scleroderma-like features including sclerodactyly, esophageal hypomotility and Raynaud's phenomenon.

Table 2. Sensitivity and specificity of specific antinuclear antibody tests.[1]

Antigen	Associated Condition	Sensitivity (%)	Specificity (%)
Anticentromere	Limited cutaneous systemic sclerosis	65	99.9
Anti-dsDNA antibody	Systemic lupus erythematosus	57	97
Anti-SSB/La antibody	Sjögren's, subacute cutaneous lupus erythematosus, neonatal lupus syndrome	16-40	94
Anti-SSA/Ro antibody	Sjögren's, subacute cutaneous lupus erythematosus, neonatal lupus syndrome	8-70	87
Anti-Smooth muscle antibody	Systemic lupus erythematosus	25-30	High*
Anti-U3-RNP antibody	Scleroderma	12	96
Scl-70	Systemic sclerosis	20	100

* Precise data not available.

Anti-SSA/Ro and anti-SSB/La are specific but less sensitive for SLE. The presence of an anti-SSA/Ro antibody in a patient with SLE is often associated with subacute cutaneous lupus and increased risk of congenital heart block. Anti-SSB/La antibodies are less commonly found in both patients with SLE and Sjögren's syndrome compared to anti-SSA/Ro antibodies. Their presence is associated with a higher risk of late-onset SLE, secondary Sjögren's syndrome and neonatal lupus syndrome. They are commonly found in patients with Sjögren's syndrome and may occur in other CTDs. Thus, these tests are sensitive, but have lower specificity for Sjögren's syndrome relative to SLE.

Usefulness of the Rheumatoid Factor in Diagnosis

Until recently the traditional laboratory test used to support the clinical diagnosis of rheumatoid arthritis was the rheumatoid factor, an IgM antibody directed against the Fc portion of IgG. Rheumatoid factors have a wide range of sensitivity (50% to 85%) but have moderate specificity (80% to 95%) for diagnosing rheumatoid arthritis depending upon the age and health of the population studied.[1] The sensitivity of the rheumatoid factor in diagnosing rheumatoid arthritis depends upon the clinical suspicion and the prevalence of the disease in the population. Even if a physician's clinical suspicion is high, the rheumatoid factor may be absent (20% are seronegative), particularly early in the course of the disease (up to 40%).[6] Therefore, a negative test result with a high clinical suspicion of rheumatoid arthritis should not dissuade the physician from the diagnosis. Although rheumatoid factor may correlate with the severity of extraarticular manifestation of disease severity, this test is not useful for monitoring the course of disease.

IgG anti-cyclic citrullinated peptide is an antibody directed against the anti-keratin epitope that contains citrulline. Citrullinated extracellullar fibrin is found within the synovium of patients with rheumatoid arthritis. The development of an enzyme-linked immunosorbent assay based upon a synthetic modified amino acid, citrulline (anti-cyclic citrullinated peptide), has provided the clinician with a highly specific tool for the diagnosis of rheumatoid arthritis at an earlier stage of the disease. The presence of this antibody may be a marker for the development of more severe erosive disease and thus call for more aggressive treatment strategies compared to those without this antibody.

Compared to the rheumatoid factor, this antibody has a higher specificity (97.4%) with a sensitivity of 47.1% for the diagnosis of rheumatoid arthritis.[7] Additionally, this antibody is more likely to be present early in the course of the disease and may be helpful in confirming the serological diagnosis in patients with a negative or equivocal rheumatoid factor.[1,8] Other indications for anti-cyclic citrullinated peptide testing include confirming a presumed false positive rheumatoid factor and monitoring disease activity and prognosis. Currently, in all instances, the best evidence available when evaluating disease activity is the history and physical examination. Patients with classical symptoms of rheumatoid arthritis should not undergo testing for rheumatoid factor.

In conclusion, a positive ANA test result must be interpreted by the physician in the appropriate clinical context and confirmed by a more specific autoantibody test. The diagnosis of a CTD remains a clinical one based on the history and physical examination. Properly applied and interpreted, serological testing can be an important tool to support or confirm diagnoses and disease management strategies.

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