Cayuga MEDICAL CENTER
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Laboratory Technical Bulletin

Date: March 25, 2016 Effective Date: March 28, 2016

Title: Connective Tissue Disease Cascade (CTDC) – New Algorithm

Tests: Antinuclear Antibodies (ANA) and Cyclic Citrullinated Peptide Antibodies (CCP)

Explanation of change: The CTD Cascade was adopted specifically to test patients with signs and symptoms compatible with a connective tissue disease. It also provides immediate disease-specific follow-up tests in those patients with presumptive serologic evidence of disease. The CTDC begins with 2 tests which are applied in all cases: ANA and CCP. Second order tests include Double-Stranded DNA Antibody (ds-DNA) and Extractable Nuclear Antigen Antibodies (AENA).

Attachments: Mayo Connective Tissue Disease Cascade, Mayo Connective Tissue Disease Communique

	Old	**New**
Test Name	ANA (Anti-Nuclear AB) Screen, Anti Double Stranded DNA, ENA Screen	Connective Tissue Panel
Test Code	ANA, ADNA, ENA	CONNECT
Perform Site	CMC	MAYO
Method	ELISA/FANA/Kit Tests	ELISA
Reference Range	Negative	Negative
Specimen	SST	SST
Volume Required	6 mL	6 mL
Turnaround time	2-5 days	3-4 days

Date 03-25-16
Dr. Elizabeth Plocharczyk, Laboratory Assistant Medical Director

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MAYO

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IMPROVING PATIENT CARE THROUGH ESOTERIC LABORATORY TESTING

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Optimized Laboratory Testing for Connective Tissue Diseases in Primary Care: The Mayo Connective Tissue Diseases Cascade

Autoimmunity is common in the general population, but systemic autoimmune diseases, including the so-called connective tissue diseases, are relatively rare. Many patients show some serologic evidence of autoimmunity, but few have the constellation of characteristic signs and symptoms and disease-specific autoantibodies required to establish the diagnosis of a connective tissue disease (Figure 1, see page 2). These statements describe the challenging environment in which primary care physicians must evaluate and treat patients suspected of having a connective tissue disease.

Signs and symptoms of systemic inflammation including fatigue, arthralgias, fever and weight loss are common manifestations of autoimmunity, and occur in connective tissue diseases. This group of diseases includes rheumatoid arthritis, lupus erythematosus (LE), scleroderma, Sjögren syndrome, polymyositis and mixed connective tissue disease. Nevertheless, the signs and symptoms often associated with connective tissue disease are not specific for autoimmune disease and also occur in diseases of other etiologies (Figure 2, see page 3). In addition, several connective tissue diseases have the potential to produce inflammation of vital organs that can lead to serious end organ damage including renal failure, irreversible pulmonary fibrosis, and even cardiac or central nervous system disease. Optimum medical management of patients with connective tissue diseases requires early and accurate diagnosis. Overdiagnosis can result in inappropriate treatment with potentially dangerous medications and unnecessary referrals to specialist physicians; whereas failure to diagnose a connective tissue disease can lead to a delay in instituting appropriate treatment and development of serious complications.

Laboratory testing is important in the diagnosis of connective tissue diseases and relies on the detection of autoantibodies. Certain autoantibody tests such as the test for antinuclear antibodies (ANA) are quite sensitive for disease diagnosis, while other tests detect autoantibodies that are less sensitive for diagnosis but have high specificity for a particular connective tissue disease. The diagnostic efficacy of tests for autoantibodies is determined not only by inherent properties of the tests such as sensitivity and specificity, but by the decision to perform the various tests in particular patients. To detect a connective tissue disease accurately in the primary care setting, sensitive tests must be applied early in the evaluation with the

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objective of identifying presumptive serologic evidence of autoimmunity. Follow-up tests for disease-specific autoantibodies are reserved for those instances in which autoimmune disease is likely. Screening tests are useful to increase the prevalence of autoimmune disease in patients who are tested for disease-specific autoantibodies. This strategy affords the highest likelihood of identifying patients with disease while avoiding the pitfall of incorrectly labeling patients with benign autoimmunity. The Mayo Medical Laboratories' #83631 Connective Tissue Diseases (CTD) Cascade, Serum is an established algorithm of tests based on this screening strategy.[1] It is designed to be used in the primary care setting of low disease prevalence.

Test Logic and Components

The CTD Cascade was developed specifically to test patients with signs and symptoms compatible with a connective tissue disease. It also provides immediate disease-specific, follow-up tests in those patients with presumptive serologic evidence of disease (Figure 3, see page 5). The test sequence is based on empirical data that define the likelihood of detecting a disease-specific autoantibody based on the level of reactivity in the screening ANA test. We believe this algorithmic approach is more efficient than a panel of tests. Panels, when applied indiscriminately, result in unnecessary testing for specific autoantibodies in ANA-negative samples and carry an increased potential to overdiagnose patients with benign autoimmunity. The CTD Cascade includes all necessary tests for autoantibodies performed on a single blood specimen, while eliminating unnecessary tests. A maximum of 10 different autoantibody tests may be performed depending upon the results of the initial screening tests and second-order follow-up tests.

First-Order Tests

The CTD Cascade begins with 2 tests which are applied in all cases:

#84182 Cyclic Citrullinated Peptide Antibodies, Serum Autoantibodies to cyclic citrullinated peptide (CCP) are quite specific for rheumatoid arthritis (RA), the most common connective tissue disease. Unlike the test for rheumatoid factor, which has poor specificity for RA, the test for CCP antibodies has proven to be reliable for differentiating RA from other connective tissue diseases. Nevertheless, this test is not an ideal screening test since it is positive in fewer than 80% of patients with RA and negative test results do not conclusively exclude RA.[2] The decision to include this test in the CTD Cascade was based on the need to have a test for RA and on the recognition that a strongly positive test for CCP antibodies has a very high positive predictive value for this disease. While weakly positive results for CCP antibodies

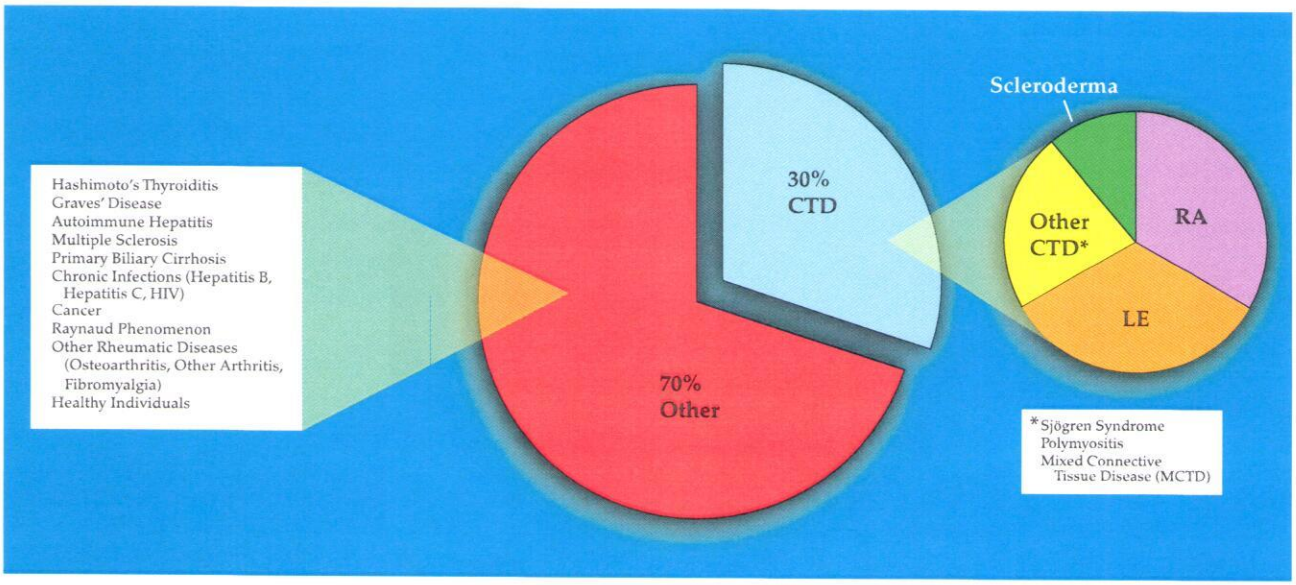

Figure 1. *Relative distribution of disease status for positive ANA tests.*

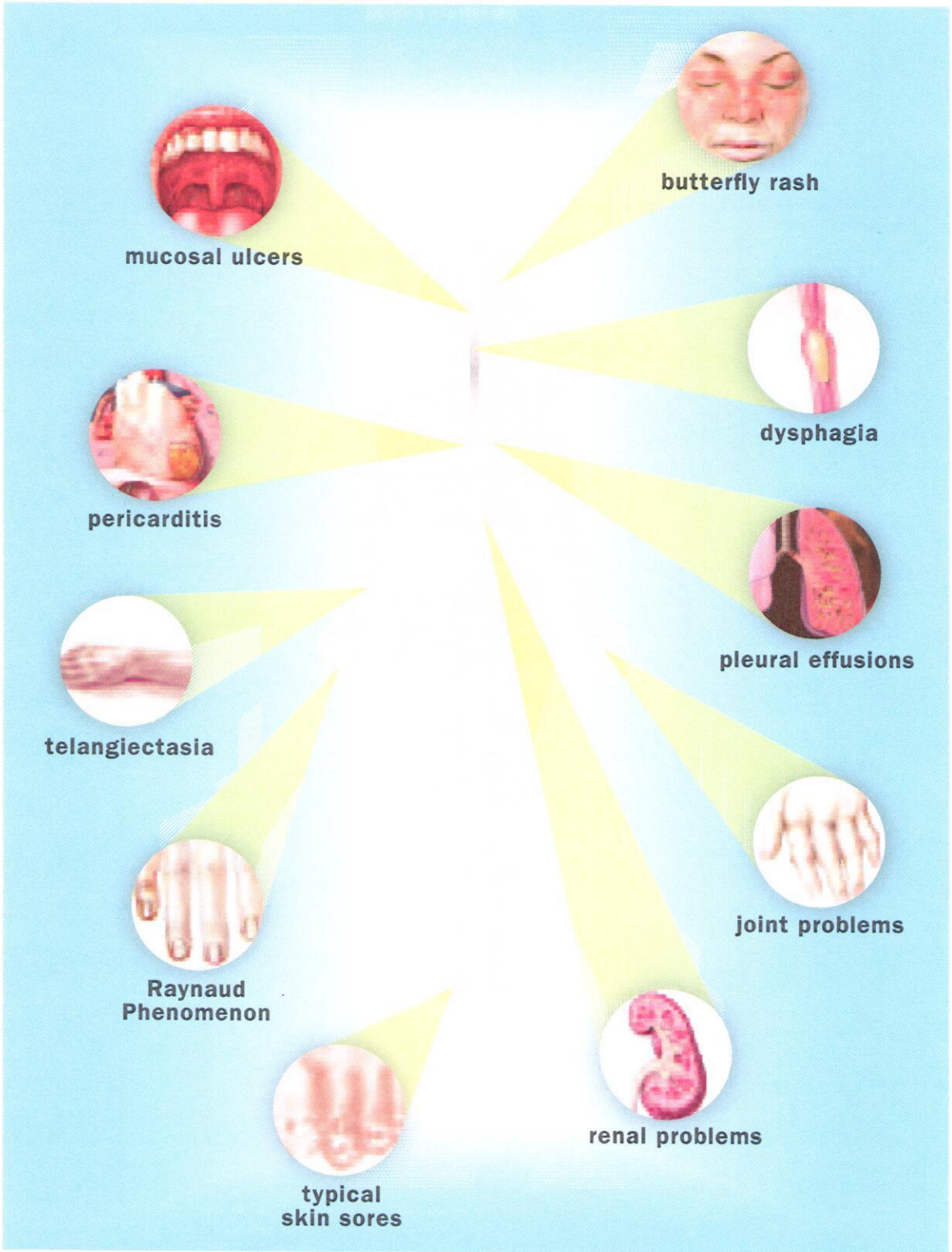

Figure 2. *Connective tissue disease-associated symptoms.*

may occur in some patients with other connective tissue diseases (eg, LE), the levels of reactivity are typically much lower than in RA, and other autoantibodies are often present that allow these diseases to be distinguished from RA.

#9026 Antinuclear Antibodies (ANA), Serum

Consensus guidelines suggest that patients suspected of having a connective tissue disease should be screened with the generic ANA test.[3-8] Data presented in these guidelines indicate that a negative ANA test result is useful (as defined by a negative likelihood ratio <0.5) for excluding both lupus erythematosus and scleroderma, the 2 most commonly encountered connective tissue diseases other than RA. Results of the ANA test are often positive in connective tissue diseases, but cannot be relied upon to establish the diagnosis of a particular connective tissue disease. For this purpose, further testing for disease-specific autoantibodies is required. In the CTD Cascade, the decision to test for additional autoantibodies is determined by the level of positivity of the ANA test. Although the ANA test is not strictly quantitative, higher concentrations of antibodies are accompanied by more strongly positive test results and the likelihood of finding a disease-specific autoantibody varies directly with the level of positivity of the ANA result. In addition, data from the Mayo Immunology Antibody Laboratory clearly indicate that disease-specific autoantibodies rarely occur in sera that test negative for ANA or have low levels of reactivity. The CTD Cascade uses empirical data from the Mayo Clinic practice to define a cutoff level of ANA reactivity that simultaneously maximizes the number of positive results on follow-up testing and minimizes the total number of negative results. Using a cutoff level of 3.0 U in our enzyme immunoassay for ANA permits detection of >90% of sera with an identifiable specific autoantibody on follow-up testing.[8,9] Since positive ANA test results may occur in patients with nonrheumatologic, autoimmune diseases and in healthy individuals, the importance of being selective in performing second-order tests cannot be overemphasized. When second-order tests are performed in patients with low levels of ANA reactivity who do not have connective tissue diseases, the results are rarely positive (<3% of cases) and are seldom helpful in diagnosis.[9]

In the CTD Cascade, if the results of screening tests for CCP antibodies and ANA are negative, no further reflex testing is required. If the screening test for CCP antibodies is positive and the ANA is negative or <3.0 U, no further reflex testing is required; a diagnosis of RA is likely if supported by characteristic clinical features of this disease. If the screening test for ANA is ≥3.0 U, second-order testing is performed for additional disease-specific autoantibodies in all cases.

Second-Order Tests

As noted above, a positive ANA result >3.0 U greatly increases the likelihood of finding 1 or more disease-specific autoantibodies. Second-order tests are designed to identify these antibodies with maximum efficiency. The second level of testing includes the following 2 components:

#8178 DNA Double-Stranded (ds-DNA) Antibody, IgG, Serum (anti-dsDNA)

A positive test result for anti-dsDNA antibodies is found in up to 82% of patients with active LE.[10] Anti-dsDNA antibodies are also highly specific for LE, thus making this test very useful for confirming the diagnosis. Some caution must be used when interpreting the result of the test for anti-dsDNA antibodies because low levels of positivity may represent low avidity antibodies that account for false-positive reactivity in some patients. For this reason, the results of the CTD Cascade must always be interpreted in the complete clinical context even when a disease-specific autoantibody such as anti-dsDNA is detected. The levels of anti-dsDNA also have been found to correlate with disease activity in patients with LE[11]; however, it is important to point out that the CTD Cascade is not designed to be used to monitor disease activity. For this purpose, it is more appropriate to order the test for anti-dsDNA as a stand-alone test.

#83630 Antibodies to Extractable Nuclear Antigens, Reflex, Serum (AENA)

The antibodies to the AENA group are comprised of 6 autoantibodies directed against small nuclear ribonucleoproteins (snRNPs) and enzymes. Autoantibodies to these antigens are important serological markers of particular connective tissue diseases. Their name derives from the fact that several

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Connective Tissue Disease Cascade (CTDC)

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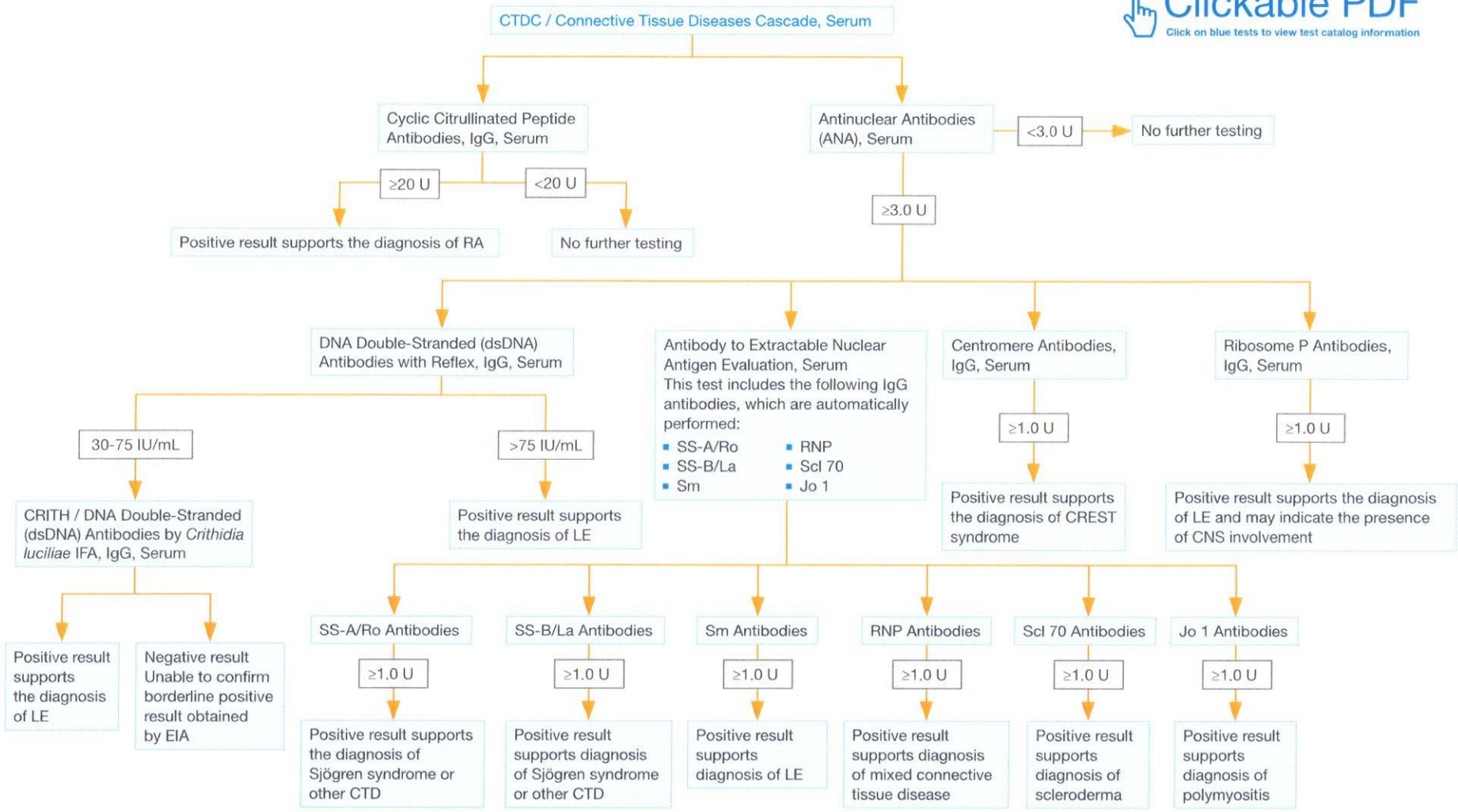

CNS=central nervous system
CREST=calcinosis, Raynaud disease, esophageal motility disorder, sclerodactyly, and telangiectasia.
CTD=connective tissue disease
SLE=systemic lupus erythematosus

NOTE: Positive results not diagnostic for any CTD and should be interpreted within the clinical context of the patient.

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of the antigens are soluble in physiologic saline solution. Each of these autoantibodies and the diseases with which they are associated are described below. The AENA screen is an enzyme immunoassay test that detects the presence of 1 or more extractable nuclear antigen antibodies simultaneously in the same test. In this way it is possible to screen for the presence of all 6 autoantibodies, and perform additional testing on only those sera that give presumptive positive results on the screening test.

If the results of these second-order tests are negative, no further reflex testing is required. If the test result for anti-dsDNA is >60 IU, the diagnosis of LE is likely, provided other characteristic clinical signs and symptoms are also present. If the test result for AENA is ≥20 IU, third-order testing is performed to identify individual autoantibodies to extractable nuclear antigens.

Third-Order Tests

The third level of tests in the CTD Cascade includes the following 6 tests for antibodies to individual extractable nuclear antigens (ENAs).

#81357 Autoantibodies to U(1) RNP, Serum (U1RNP)
Antibodies to U1RNP occur in several different connective tissue diseases including mixed connective tissue disease and LE. The diagnostic specificity of U1RNP antibodies depends largely upon finding these antibodies in isolation. The finding of U1RNP antibodies in the absence of anti-dsDNA antibodies and antibodies to other ENAs is consistent with the diagnosis of mixed connective tissue disease. U1RNP antibodies have been reported in 71% to 100% of patients with mixed connective tissue disease; and the specificity of positive results ranges from 84% to 100%.[12]

#81358 Autoantibodies to Sm, Serum (Sm)
Sm antibodies are reported to be highly specific for LE.[12] Fewer than 5% of positive results occur in other connective tissue diseases. The test for Sm antibodies lacks sensitivity; and this autoantibody is detectable in only approximately 30% of patients with documented LE.[12]

#81360 Autoantibodies to SS-A/Ro, Serum (SSA)
SSA antibodies occur with variable frequencies in several connective tissue diseases including Sjögren syndrome, LE and RA. When present in isolation or with SSB antibodies, the finding of this autoantibody is consistent with Sjögren syndrome. SSA antibodies are found in approximately 60% of patients with Sjögren syndrome and 35% of patients with LE.[13]

#81359 Autoantibodies to SS-B/La, Serum (SSB)
SSB antibodies rarely occur in isolation and are most often encountered in sera that contain SSA antibodies. Positive results for SSB antibodies are observed in up to 60% of patients with Sjögren syndrome and may also occur in patients with LE.

#80178 Autoantibodies to Scl 70, Serum (Scl 70)
Scl 70 antibodies react with the enzyme DNA topoisomerase 1 and are highly specific for scleroderma. Like other antibodies to ENAs, Scl 70 antibodies are not sensitive indicators of a disease but are considered diagnostic markers because of their specificity. Scl 70 antibodies have been reported in approximately 40% of patients with scleroderma when tests are performed by enzyme immunoassay.[14] The presence of Scl 70 antibodies is consistent with the diagnosis of scleroderma and indicates an increased risk for systemic involvement including pulmonary fibrosis.

#80179 Autoantibodies to Jo 1, Serum (Jo1)
Jo1 antibodies react with the enzyme histidyl tRNA synthetase and are highly specific for polymyositis. Jo1 antibodies are a marker for this disease, but occur in only approximately 20% of polymyositis patients when tests are performed by enzyme immunoassay. The finding of Jo1 antibodies is consistent with the diagnosis of polymyositis and indicates an increased risk for severe disease with pulmonary involvement and fibrosis.

Interpreting Results of the Mayo Connective Tissue Diseases Cascade

A typical test report for the CTD Cascade includes reference values for each antibody test performed, test results, and interpretive comments appropriate for the results obtained. Interpretive comments include information about the association of particular patterns of results with each connective tissue disease mentioned above.

Diagnostic Limitations of Serologic Testing

The Diagnosis of Connective Tissue Diseases Must Emphasize Clinical Criteria

Although serological testing is useful for assessing patients suspected of having a connective tissue disease, none of the individual autoantibody tests is specific enough to provide a definitive diagnosis of a particular connective tissue disease in the absence of clinical information. For example, some patients with classic clinical features of connective tissue disease and a positive ANA test result, will not test positive for disease-specific autoantibodies. Patients with nonrheumatic diseases may also have a positive ANA test result and not test positive for specific autoantibodies. Conversely, even highly specific autoantibodies such as CCP and anti-dsDNA may occur in patients who do not have sufficient clinical evidence to justify a diagnosis of RA or LE.[3]

Monitoring Disease Activity and Responses to Treatment in Patients with Connective Tissue Diseases

The CTD Cascade is not recommended for monitoring disease activity or responses to treatment in patients with known connective tissue diseases. Mayo Medical Laboratories recommends monitoring disease activity and responses to treatment be performed by ordering only those specific tests known to have efficacy for these applications (eg, testing for anti-dsDNA antibodies in patients with LE). Current practice guidelines do not, in general, recommend the use of serologic tests to monitor disease activity or responses to treatment in patients with connective tissue diseases.

Summary

Accurate diagnosis combined with appropriate therapy can delay or prevent morbidity in many patients with connective tissue diseases. However, recent studies indicate that up to 50% of patients with a positive ANA test result are incorrectly diagnosed with LE and are sometimes treated with unnecessary and potentially toxic medications.[15] Such errors are likely when large numbers of tests are applied to poorly selected patients in situations of low disease prevalence. The Mayo Medical Laboratories' #83631 Connective Tissue Diseases Cascade, Serum has been developed based on empirical data to provide physicians with a cost-effective and efficient algorithm of tests with proven efficacy for detecting connective tissue diseases in a low prevalence setting of primary care.

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