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Laboratory Evaluation of Connective Tissue Disease

The diagnosis of systemic autoimmune disease (e.g. systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), scleroderma, Sjögren's syndrome, polymyositis or mixed connective tissue disease (MCTD)) can be challenging. The presenting signs and symptoms (fever, fatigue, arthralgias, weight loss, skin rash or lesions, serositis, Raynaud's phenomenon, dysphagia or mucosal ulcers) of these "connective tissue diseases" (CTD) are often nonspecific. Yet, significant organ damage may occur with some rheumatologic diseases if not diagnosed and managed appropriately. Serologic tests for autoimmune diseases are frequently ordered to "rule in" or "rule out" various rheumatologic disorders. However, the results of such testing can be confusing, as many people will have positive test results without having the suspected disease. In the absence of appropriate clinical correlation, overdiagnosis may result in unnecessary testing and inappropriate, if not dangerous, therapy.

ANA - A logical place to begin

One of the earliest tests associated with CTD was the "LE cell prep". While this aesthetically pleasing light microscopic test had its day (in the 50's and the 60's), it was labor-intensive, lacked sensitivity, specificity, and reproducibility (remember Tart cells?).[1,2] Recognition that the antinuclear antibodies (ANA) were responsible for the formation of LE cells led to more sensitive serologic tests to detect their presence. The earliest ANA tests were based upon indirect immunofluorescence (IIF). Initially kidney or liver tissue from mice or rats was used as substrate, but over time most laboratories migrated to a human tumor cell line (HEp-2). The different substrates had differing sensitivities, specificities and staining patterns, which led to some early confusion about the predictive value of positive and negative results. While some patients with SLE were reported to have (-) ANA when tested with some rodent substrates, this phenomenon has largely vanished with the use of HEp-2. However the improved HEp-2 sensitivity comes at the price of reduced specificity, even with the near-universal practice of requiring a titer of 1:40 for a "positive" result (Table 1).[3] (The Outreach charge for ANA is $34)

Table 1
(+) ANA by HEp-2 IIF

Disease/Condition	Frequency of (+) ANA, %
SLE	95-100
Scleroderma	60-80
Sjögren's syndrome	40-70
Dermatomyositis/polymyositis	30-80
Juvenile oligoarticular arthritis w/ uveitis	20-50
Raynaud's phenomenon	20-60
Drug-induced SLE*	~100
Autoimmune hepatitis (some types)*	~100
Mixed connective tissue disease*	~100
Rheumatoid arthritis	30-50
Multiple sclerosis	25
Idiopathic thrombocytopenic purpura	10-30
Thyroid disease	30-50
Discoid lupus	5-25
Infections	Variable
Malignancy	Variable
Silicone breast implants	15-25
Fibromyalgia	15-25
Relatives of patients w/ SLE or scleroderma	5-25
Normal persons (titer)**	
≥ 1:40	20-30
≥ 1:80	10-12
≥ 1:160	5
≥ 1:320	3

Modified from Kavanaugh et al [3]
* (+) ANA considered part of definition of condition by Kavanaugh et al
** Female sex and increasing age more commonly associated with (+) ANA in healthy individuals

The combination of the poor specificity of the IIF-ANA coupled with the relative rarity of SLE create an abysmal positive predictive value (from 0-10%) when physicians attempt to use ANA as a screening test for CTD.[3-6] As with many laboratory tests, careful evaluation of the patient's history and physical findings to determine the pre-test probability of disease will enhance the significance of a positive result. In patients with few criteria for SLE, a (+) ANA does little to increase the likelihood that the

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patient has the disease and may lead to erroneous diagnosis or inappropriate therapy. [3] The American College of Rheumatology developed classification criteria for SLE which, while originally designed for use in research studies, provide a useful clinical backdrop to determine if ANA testing is indicated (at least two organ systems involved as noted in Table 2). [6]

While appropriate patient selection is the most important factor in evaluating a (+) ANA result, other factors can enhance the specificity. The higher the titer of the ANA result, the greater the potential significance. ANA-IIF results < 1:160 have minimal clinical significance, may not be related to the patient's symptoms and are unlikely to yield positive second-order test results (see below). [4] However, the titer is not related to disease activity, and should not be used to "follow" the patient. Ordering an ANA on a patient with known SLE, who is admitted to the hospital for an acute condition offers little meaningful information. [4] The pattern of ANA-IIF staining may also be helpful in assessing a (+) ANA. There is overlap in staining patterns among different diseases, and more specific second-order tests have resulted in decreased reliance on this information. Nonetheless, the patterns are still reported by laboratories performing the ANA-IIF, and may suggest appropriate follow-up testing to confirm a specific disease. (Table 3).

Table 3
Putative Significance of (+) ANA-IIF Stain Patterns

Pattern	Disease Association	2nd/3rd Order Test(s)
Homogeneous	SLE (high titer) Nonspecific (low titer)	anti-DNA/ds-DNA
Speckled	SLE Scleroderma Mixed connective tissue disease Sjögren's syndrome	anti-Sm (Smith) anti-Scl-70 anti-RNP anti-SS-B
Nucleolar	Scleroderma Sjögren's syndrome	anti-Scl-70 anti-SS-B
Centromere	Scleroderma (CREST variant)	anti-Scl-70
SS-A (Ro)	Sjögren's syndrome SLE Subacute cutaneous lupus	anti-SSA anti-DNA/ds-DNA

modified from Golightly et al [7]

In recent years, some laboratories (including Mayo Medical Laboratories) have begun using enzyme immunoassays (EIA) for ANA testing (ANA-EIA). Advantages over the ANA-IIF include lower cost, less labor, and increased precision (less observer variability). While EIA methods have not been universally adopted and are considered "unproven" by some, their use is increasing and I suspect this is the way of the future. [3]

Table 2
Classification Criteria for SLE
The diagnosis of SLE requires at least 4 of the following 11 criteria, serially or simultaneously. Laboratory work-up recommended if 2 organ systems involved by conditions below.

Malar rash	Fixed erythema, flat or raised, sparing nasolabial folds
Discoid rash	Erythematous raised patches w/ keratotic scale, plugging +/- scar
Photosensitivity	By history or MD observation
Oral ulcers	Painless oral or nasopharyngeal ulcers observed by MD
Arthritis	Nonerosive, involving 2 or more peripheral joints
Serositis	Pleuritis or pericarditis
Renal disease	Proteinuria (> 500 mg/day) or cellular casts (RBC, WBC, epithelial)
CNS disease	Seizures or psychosis in absence of known drug, metabolic cause
Hematologic disease	Hemolytic anemia w/ reticulocytosis; WBCs < 4,000/uL or Lymphocytes < 1500/uL (2 or more occasions) or platelets < 100K/uL
(+) ANA	By IIF or "equivalent assay", in absence of drug-induced (+) ANA
2nd order tests	(+) anti-ds-DNA, anti-SM, anti-phospholipid, lupus anticoagulant or confirmed "false (+)" syphilis serology

modified from Gill et al [6]

Second-Order Tests

Given the lack of specificity for a (+) ANA, second- (and third-) order tests are helpful for determining the significance of this result and aid in classifying the type of disease (if any) present. Again, these tests perform better if used in patients with appropriate clinical manifestations of the suspected disease (e.g. "rule in" rather than "rule out"). Finally, although the results are often more specific than the ANA, a (+) lab result is insufficient to diagnose a specific disease. **Clinical correlation is essential.**

Anti-DNA (anti-ds-DNA)

A (+) anti-DNA/ds-DNA is highly specific for SLE, particularly when present in high titer. It is not as sensitive for SLE as the ANA, being present in 50-82% of cases. [2,8] Elevated levels may also serve as a marker for disease activity, but clinical correlation is necessary. Stated another way, the physician must determine if anti-DNA/ds-DNA activity serves as a marker for SLE flares in the individual patient. *(Outreach charge $34)*

Anti-ENA (Extractable Nuclear Antigen)

Anti-ENAs are a heterogeneous group of antibodies directed against a variety of nuclear enzymes and

ribonucleoproteins. A (+) ENA in the appropriate clinical setting supports the presence of a CTD and helps validate a (+) ANA. It does not confer any additional specificity. *(Outreach charge $36)*

Third-Order Tests

Anti-Sm (Smith)

Highly specific for SLE, but present in only 30% of patients. [2,8] Not related to disease activity. *(Outreach charge $29)*

Anti-U(1)RNP

May occur in either SLE (35%) or MCTD (71-100%). [2,8] A (+) anti-U1RNP in a patient with a (+) anti-DNA/ds-DNA suggests SLE, while a positive anti-U1RNP alone (without other (+) antibodies to specific ENAs) suggests MCTD. *(Outreach charge $29)*

Anti-SS-A/Ro

May occur in SLE (30-60%), Sjögren's syndrome (60-70%), or rheumatoid arthritis. [2,3,8] When present in isolation or in association with anti-SS-B/La, Sjögren's syndrome is implicated. In patients with SLE, a (+) anti-SS-A/Ro may be associated with photosensitivity, sicca symptoms, thrombocytopenia, and subacute cutaneous lupus. [3] It may also be associated with congenital heart block in neonatal SLE. [3] For this reason obstetrical patients with **known SLE** may benefit from screening for this antibody, although the incidence of this complication in (+) anti-SSA/Ro pregnancies is low. *(Outreach charge $29)*

Anti-SS-B/La

May occur in SLE (20%) and Sjögren's syndrome (60-70%). [2,8] Often observed in association with (+) anti-SS-A/Ro. *(Outreach charge $29)*

Anti-Scl 70

Highly specific for scleroderma, including the CREST variant - but present in only 20-40% of such patients. [2,8] Associated with an increased risk of pulmonary fibrosis. *(Outreach charge $27)*

Anti-Centromere

May occur in scleroderma, including the CREST variant (30-80%), Raynaud's phenomenon (25%), SLE, RA, and primary biliary cirrhosis. [2,4] *(Outreach charge $60)*

Anti-Jo 1

Highly specific for polymyositis, but present in only 20-30% of patients. [2,8] Associated with increased risk of pulmonary fibrosis. *(Outreach charge $27)*

CTD Tests At/Through Rex

The Rex Microbiology Laboratory performs ANA and anti-DNA testing by IIF. All of the other tests discussed in this article are forwarded to Mayo Medical Laboratories (MML) for evaluation by EIA. For the initial evaluation of patients with suspected CTD, two options are available. Physicians may wish to follow up (+) ANA-IIF results by selection of appropriate second-order and third-order tests based upon the clinical characteristics of the patient in question (and perhaps the staining pattern as reviewed in Table 3). A proposed testing algorithm to facilitate this "al a carte" approach is presented on one side of the bulletin insert (Work-Up of (+) ANA).

Alternatively, Mayo Medical Laboratories offers a connective tissue disease cascade, in which appropriate tests are selected based on the results of first-order testing for ANA-EIA and cyclic citrullinated peptide antibodies. The latter test has been added to facilitate separation of RA from other CTD (see article on page four). The details of this "prise fixe" approach to CTD diagnosis are presented on the other side of the insert (Connective Tissue Diseases Cascade). [8] (There is a baseline charge for the first and second order tests (Outreach charge $43), each third order test represents an additional charge.) While the cut-off levels employed by the cascade are intended to maximize diagnostic efficacy, the same caveats regarding the importance of patient selection discussed *ad nauseam* above still apply. The cascade is not intended for monitoring disease activity or response to treatment. (In general, except as discussed above, laboratory tests aren't helpful in "following" patients with CTD.)

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Cyclic citrullinated peptide antibodies (CCP)

Cyclic citrullinated peptide antibodies (CCP) is a relatively new test, which has been found to be a more specific and perhaps more sensitive than rheumatoid factor (RF) for the diagnosis of rheumatoid arthritis RA). It may be elevated early in the course of the disease, and may precede the development of recognizable RA changes. Often the test is ordered along with RF. If both tests are positive, it is likely the patient has (or will develop) RA. The same is true if the patient is CCP(+) and RF(-). For patients who are negative for both or CCP(-), RF(+) the clinical picture is critical for making the diagnosis. Like most CTD, RA is a clinical diagnosis and laboratory findings play, at best, a supporting role.

CCP is not entirely specific for RA (surprised?), as weakly (+) results may be observed in other CTD, including SLE. The low titer of CCP (coupled with (+) results for other laboratory markers as discussed elsewhere) helps distinguish RA from other CTD by laboratory methods. Mayo Medical Laboratories (MML) includes CCP in their Connective Tissue Disease Cascade to help split out RA patients from other CTD patients early in the cascade. It is also available as a separate test. *(RF is performed by nephelometry at Rex with an outreach charge of $16, while all CCP assays are referred to MML with an outreach charge of $30.)*

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1. *Lab Tests Online. http://www.labtestsonline.org/understanding /analytes/ccp/test.html*
2. *Mayo Medical Laboratories. Optimized laboratory testing for connective tissue diseases in primary care: The Mayo Clinic connective tissue diseases cascade. Mayo Communiqué 31(8):1-7 (June) 2006.*

Catrina Reading, MD, joins Rex Pathology Associates

We are pleased to announce the association of F. Catrina Reading, MD (Ree-ding) with the Rex pathologists beginning September 1. Catrina graduated *cum laude* with an A.B. degree in molecular and cell biology from the University of California at Berkeley in 1994. She completed medical school at the University of Texas Health Science Center in San Antonio in 2000, followed by a residency in anatomic and clinical pathology at the University of North Carolina at Chapel Hill in 2004. After a fellowship in surgical pathology at UNC, Catrina completed a second fellowship in cytopathology at M.D. Anderson Cancer Center in Houston Texas in July 2006. In addition to cytopathology, her interests within anatomic pathology include gynecologic and lymph node pathology.

She is married to Jeremy Reading, MD who practices pediatric anesthesiology at Wake Medical Center with Critical Health Systems. The Readings have two boys, 2 ½ years and 8 months old, and live in Raleigh. Her hobbies (when there is time) include arts and crafts, books and enjoying the outdoors. We welcome her to our group.

Stephen V. Chiavetta, MD

Inspection update

On August 29, 2006 a team of 9 inspectors from the College of American Pathologists (CAP) came to the Rex Hospital Laboratory for an unannounced inspection. CAP Laboratory inspections have "deemed" status by the Centers for Medicare and Medicaid Services and the Joint Commission on Accreditation of Healthcare Organizations. Our laboratory has been inspected numerous times by the CAP, but this is the first time the inspection had not been announced prior to arrival of the inspection team.[1] In response to Congressional hearings on laboratory problems in a Maryland hospital, the CAP agreed to convert to unannounced inspections.[2,3] In addition, the inspections now place more emphasis on observing all phases of the testing process, including specimen collection and processing, analysis, reporting and point of care testing. Using 12 checklists with over 2000 standards, the inspection found one deficiency which was corrected immediately. A successful inspection such as this reflects the collective efforts of the laboratory, medical, and hospital staff to provide quality laboratory data for Rex patients.

John D. Benson, MD

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3. *McDowell J. The status of lab quality: GAO report casts doubt on CLIA oversight. Clin Lab News 32(8). August 2006. (www.aacc.org)*

Rex Healthcare Laboratory (919) 784-3040. Telephone extensions are: Pathologists' Direct Line (3063), Robin Ivosic (Customer Service and Outreach Manager 3053), Elaine Patterson (Core Lab, Blood Bank and Microbiology Manager 3054), Diane Young (Anatomic Pathology Manager 3888).
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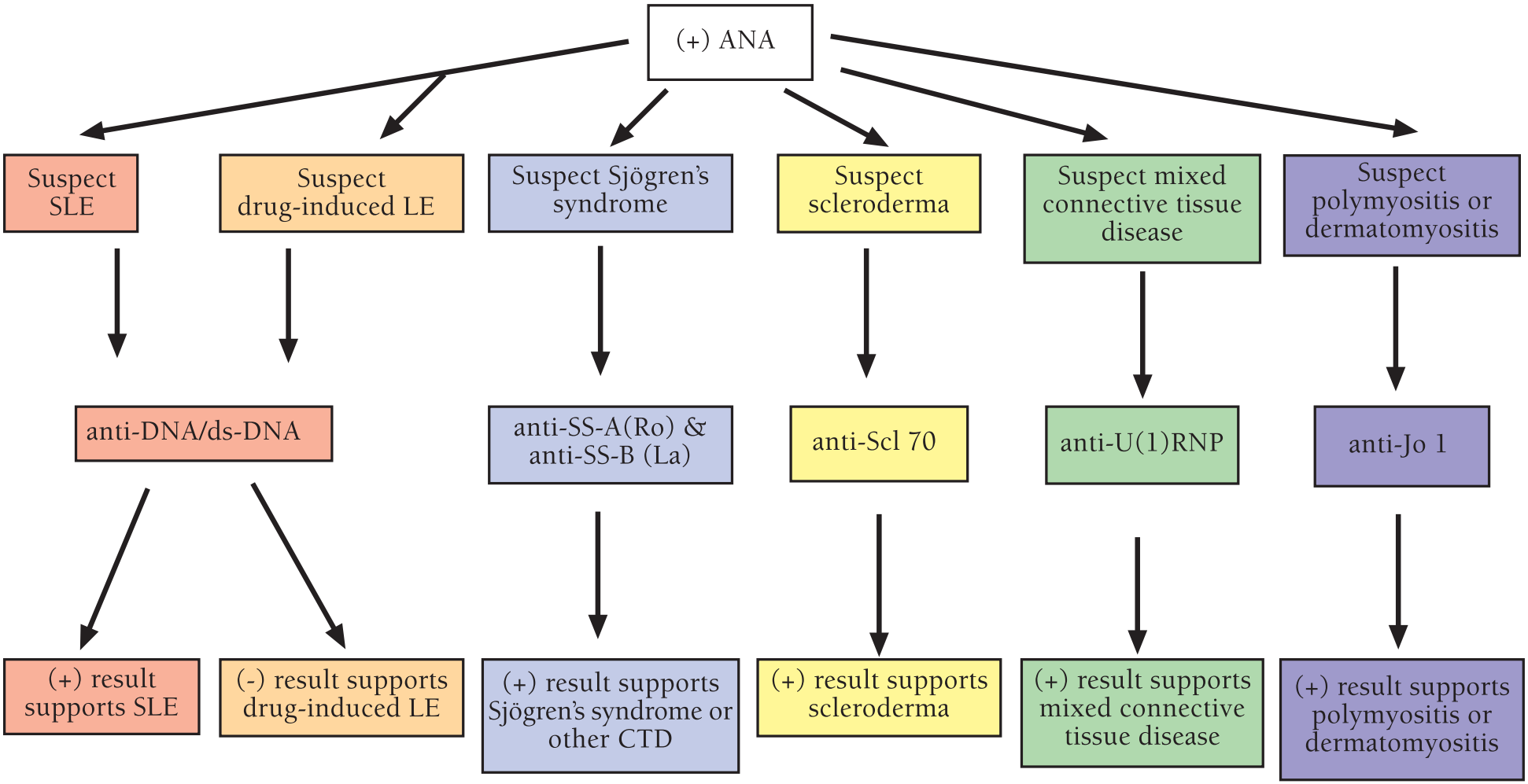
Work-Up of (+) ANA



Laboratory findings alone are insufficient to justify a conclusive diagnosis of connective tissue disease. Clinical correlation is essential.

JD Benson, MD
(06/28/06)

Mayo Medical Laboratory Connective Tissue Cascade (#83631)

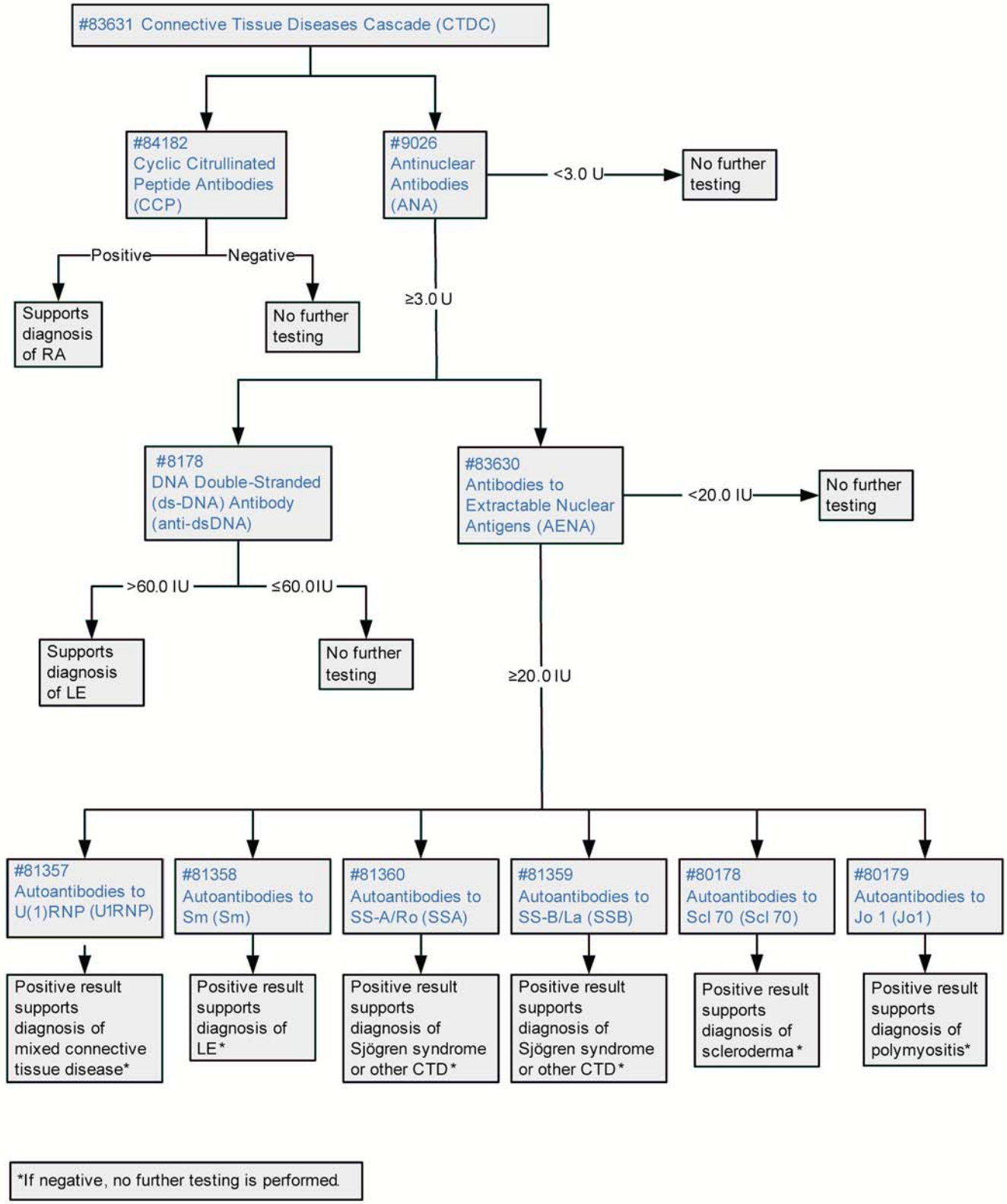


Figure 3. *Connective tissue disease cascade test-ordering algorithm.*