Antinuclear Antibody Testing for the Diagnosis of Systemic Lupus Erythematosus

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KEYWORDS

• Antinuclear antibody (ANA) • SLE • Sensitivity • Specificity • Predictive value
• Diagnosis

KEY POINTS

- Systemic lupus erythematosus (SLE) is a systemic autoimmune inflammatory condition that may involve multiple organ systems.
- Although the antinuclear antibody (ANA) test is positive in nearly every case of SLE, it is not specific for this disease and, therefore, must be interpreted in the appropriate clinical context.
- Key features that may warrant ANA testing include unexplained multisystem inflammatory disease, symmetric joint pain with inflammatory features, photosensitive rash, and cytopenias.
- In select cases, ANA staining patterns and more specific autoantibody testing may be helpful in suggesting a diagnosis of suspected SLE or another ANA-associated disease.
- For patients with nonspecific symptoms, such as malaise and fatigue (who have a low likelihood of SLE or a related disease), ANA testing is of limited value.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune inflammatory condition that may cause inflammation and damage in multiple organ systems and is associated with significant morbidity and premature death. It affects people of all ethnicities and geographic locations. Estimates suggest a female prevalence that is 10 times higher than among men and, within the United States, groups at elevated risk include African American and Hispanics/Latinx communities.[1] In addition to its direct impact on multiple organ systems, SLE is associated with morbidity related

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to cardiovascular disease and malignancy,[2] presumably due to a chronically heightened inflammatory state.

SLE may be difficult to diagnose due to the diversity of presenting symptoms and signs and because its symptoms are common and nonspecific. Although symptoms, such as arthritis and the classic malar rash, may be readily recognized, other presentations, such as hemolytic anemia, seizures, or psychosis, alone or even in combination, may be attributed to other causes or conditions. Furthermore, the challenge of diagnosis is compounded by the lack of a single gold standard test. Although a positive antinuclear antibody (ANA) test is a clue, it must be considered within the context of other clinical features, including details of the history, physical examination, and other test results.

The pathophysiology of SLE is incompletely understood. Several factors have been linked to its development and expression, including genetic, hormonal, and environmental influences.[3] Studies examining monozygotic twins have found SLE concordance of 24% to 57%.[4,5] But genetics alone do not fully explain disease manifestations. Other important pathogenetic factors may include exposure to hormones, such as estrogen (which is believed to be pathogenic in murine models of SLE) and exposure to ultraviolet light. Impaired clearance of apoptotic cellular debris and a dysregulated immune response to self-antigens may be important in triggering SLE or flares of the disease.[6] These processes may act in synergy to impair the host's self-tolerance and immune regulation, leading to the development of autoantibodies and immune complex deposition, the primary drivers of organ damage and clinical manifestations.

THE CLINICAL USEFULNESS OF ANTINUCLEAR ANTIBODY TESTING

ANAs are a collection of autoantibodies that target proteins within the nucleus of the cell. A positive result can signify breakdown of self-tolerance and herald the onset of autoimmune disease, such as SLE. A patient's ANA may be positive, however, in a variety of other settings and even may be present in healthy individuals. As a result, the usefulness of any individual's ANA test result is highly dependent on that individual's specific clinical presentation and pretest probability of SLE or other ANA-associated disease.

The ANA typically is reported as a titer, a quantitative measure of the amount of antibody, expressed as the number of dilutions a sample can undergo and still demonstrate detectable antibody. An ANA of 1:40, for example, is a low titer found in many people without autoimmune disease and, in some laboratories, is considered negative. The most recent classification criteria from for SLE from the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR)[7] require a titer of at least 1:80 for consideration of the diagnosis. In general, the higher the ANA titer, the more likely the result is an indication of autoimmunity,[8] but the ANA titer is not an accurate reflection of disease activity.

A range of sensitivities and specificities for ANA testing have been reported; this is not surprising because the types of patients tested and methodologies used for testing vary. Indirect immunofluorescence using human epithelial type 2 (HEp-2) cell lines and solid-phase testing techniques (including enzyme immunoassays and multiplex bead assays) currently are the most common ways to assess the presence of an ANA; the former is the most widely used and is recommended by the ACR[9] as the gold standard methodology. Solid-phase immunoassays may be less expensive to perform, easier to standardize, more efficient, and less expensive to perform; however, lower sensitivity has been a limiting factor.

According to a recent meta-regression analysis of ANA tests (96% of which used indirect immunofluorescence with HEp-2 cell substrate),[10] a positive ANA at a titer of 1:80 had sensitivity of 98% and specificity of 75%. Given the high sensitivity of the ANA for SLE, a negative ANA is a powerful argument against the diagnosis. The modest specificity means, however, that unless testing is highly selective (ie, limited to settings of high pretest probability), false-positive results are common; this represents a significant limitation of the test.[11]

As a diagnostic test, the ANA test can be useful in suggesting the diagnosis of SLE but other diseases, including nonrheumatologic conditions, also are associated with a positive ANA. In addition to SLE, rheumatic diseases in which a positive ANA often is found include

- Systemic sclerosis (scleroderma)
- Sjögren syndrome
- Rheumatoid arthritis
- Dermatomyositis and polymyositis
- Drug-induced lupus

Nonrheumatologic diseases associated with a positive ANA include autoimmune thyroid disease (such as Hashimoto thyroiditis), autoimmune hepatitis, and primary biliary cholangitis. In addition, many infections and malignancies are associated with immune dysregulation and autoantibody formation that includes ANAs that may be present in high titers.[12,13]

As indicated by its limited specificity, ANAs often are positive in people without a clinically relevant disease; 25% or more of the population are ANA-positive even without a rheumatic disease or other ANA-associated condition. The prevalence of positive ANAs in the population may be rising in the United States,[14] although the clinical importance of this is unclear. A higher incidence of false-positive results may be found in individuals who are aged[15] or have a family member with a positive ANA or autoimmune disease.

For these reasons, a single ANA result does not have a single interpretation: a low-titer ANA in a patient without a strong clinical suggestion of SLE or other ANA-associated disease usually is of little clinical significance; however, that same result in a patient with a malar rash, inflammatory arthralgia, and hematuria may reflect the presence of a systemic rheumatic disease, such as SLE. The value of detecting a positive ANA in a patient with nonspecific symptoms (such as malaise, fatigue, and generalized pain) is low, particularly if more specific symptoms are absent and other laboratory studies, such as the complete blood cell count, renal function, and urinalysis, are normal.

ANTINUCLEAR ANTIBODY STAINING PATTERNS

ANAs typically are reported with a pattern of fluorescent staining, which can be a clue regarding the underlying antigen specificity:

- A homogenous staining pattern may reflect antibodies directed against histone proteins and DNA
- A speckled staining pattern may be due to antibodies against U1-ribonucleoprotein (RNP), Smith (Sm), and Ro and La antigens
- A nucleolar pattern often signifies antibodies against RNA polymerase
- A peripheral, or rim, staining pattern suggests the presence of anti–double-stranded (anti-ds) DNA antibodies

- A centromeric pattern typically is found in patients with CREST syndrome (discussed later)

Because certain antigen specificities are highly associated with particular diseases (eg, anti-Sm and anti-dsDNA antibodies are highly specific for SLE), these staining patterns can be clinically useful.[16] The interpretation and reporting of ANA patterns, however, may be operator-dependent and subject to interobserver variability. In addition, the correlation between staining pattern and specific diseases is not particularly strong.

TESTING FOR ANTIBODIES DIRECTED AGAINST SPECIFIC AUTOANTIGENS

ANAs may be positive due to antibodies directed against several autoantigens (often called extractable nuclear antibodies). Among the most common and clinically useful include

- Anti-dsDNA and anti-Sm antibodies—these are highly specific for SLE. In addition, anti-dsDNA antibodies may correlate with SLE disease activity, especially among those with nephritis.
- Anti-RNP antibodies are associated with mixed connective tissue disease and SLE. A positive anti-RNP antibody is not enough, however, to establish a diagnosis of either disease.
- Anti-Ro (anti-SSA) and anti-La (anti-SSB) antibodies are associated with primary Sjögren syndrome and SLE; in addition, anti-Ro antibodies are strongly linked to the development of neonatal lupus.
- Antihistone antibodies are present in approximately half of patients with SLE but nearly always are present in drug-induced lupus
- Anti–Scl-70 (anti-topoisomerase 3) and anti-RNA polymerase antibodies are highly specific for scleroderma.
- Anticentromere antibodies are strongly associated with the limited form of systemic sclerosis, or CREST syndrome (manifest by calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia); they occasionally are present in patients with SLE.

As diagnostic tests, these antibodies are variably sensitive but their clinical value lies in their high specificity (**Table 1**). Testing for these more specific autoantibodies is not recommended routinely for patients who are ANA-negative or whose ANA status is unknown, especially in the setting of low pretest probability of disease.[17]

Table 1
Specific antinuclear antibodies, associated diseases, sensitivity, and specificity

Specific Antinuclear Antibody	Associated Disease	Sensitivity (%)	Specificity (%)
Anti-Sm	SLE	40[24]	98.6[24]
Anti-dsDNA	SLE	90[25]	96[25]
Anti-SSA (anti-Ro)	Primary Sjögren syndrome	49[26]	87.5[26]
Anti-SSB (anti-Lo)	Primary Sjögren syndrome	29[26]	95[26]
Anti-RNP	Mixed connective tissue disease	100[27]	84–100[28]
Anti–Scl-70	Systemic sclerosis (scleroderma)	28[29]	100[29]
Anticentromere	Limited scleroderma	33[30]	99.9[30]

HOW IS SYSTEMIC LUPUS ERYTHEMATOSUS DIAGNOSED?

A diagnosis of SLE requires a compelling combination of symptoms, physical examination findings, and laboratory and/or pathologic studies. Ultimately, clinical acumen and judgment are required to integrate the various features of the illness: asking the right questions, eliciting compatible physical findings, and interpreting the results of selected tests are essential. A young woman with diffuse aching and morning stiffness with a low-titer positive ANA could have fibromyalgia, SLE, a self-limited viral syndrome, or several other conditions. But, if she also has a family history of SLE, unexplained fever, and a photosensitive rash, the suspicion of SLE should rise much higher. And if these same symptoms were present in someone taking infliximab or procainamide, the possibility of drug-induced lupus would be appropriate. Thus, interpreting an ANA result requires an assessment of pretest probability, a recognition of its sensitivity and specificity, and an actual or estimated calculation of positive and negative predictive values.

The various iterations and revisions of lupus classification criteria can provide useful guidance for the evaluation of possible SLE. They include manifestations that are relatively common and, in cases of the 2019 EULAR/ACR classification criteria for SLE,[7] they provide a sense of how various manifestations of disease should be weighed (**Table 2**). For example, a renal biopsy demonstrating pathologic evidence of lupus nephritis is far more suggestive of the diagnosis than the (much less specific) finding of fever.

But, there is a reason the classification criteria are not called diagnostic criteria—a patient can have SLE without meeting these criteria or not have SLE even though meeting the criteria. These criteria are intended to standardize studies of the disease, not to be a tool to allow a clinician to establish or rule out the diagnosis in an individual patient.

Some aspects of the 2019 classification criteria for SLE[7] deserve particular emphasis:

- As discussed previously, a patient must have an ANA of at least 1:80 using a HEp-2 immunofluorescence assay (or an equivalent positive test).
- Criteria include 10 clinical or immunologic domains with 1 or more manifestations in each; only the highest scoring item within a domain can be counted (so a patient with discoid lupus and oral ulcers only gets credit for discoid lupus); each criterion has specific definitions (eg, fever must be >38.3°C and leukopenia is <4000 cells/mm^3).
- The 7 clinical domains are constitutional, hematologic, neuropsychiatric, mucocutaneous, serosal, musculoskeletal, and renal.
- The 3 immunology domains are antiphospholipid antibodies, complement proteins, and SLE-specific antibodies (anti-dsDNA or anti-Sm).
- A criterion should not be counted if there is a more likely explanation for it than SLE,
- Even with a positive ANA of at least 1:80 and at least 10 points, 1 or more clinical criteria must be met.
- Criteria do not have to be present at the same time,

Because a diagnosis of SLE has immediate as well as long-term implications with potential for a poor prognosis, a strong suspicion of SLE should prompt timely referral to a rheumatologist. The ANA is an important part of the evaluation but it is only 1 part of the diagnostic process. For nonrheumatologists, it probably is more important to decide whether to refer the patient based on clinical grounds to a rheumatologist and to determine how urgent that consultation should be than it is to decide whether an ANA should be requested.

Table 2
European League against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus

Clinical Domains and Criteria	Weight (Points)
Constitutional	
Fever	2
Hematologic	
Leukopenia	3
Thrombocytopenia	4
Autoimmune hemolysis	4
Neuropsychiatric	
Delirium	2
Psychosis	3
Seizure	5
Mucocutaneous	
Nonscarring alopecia	2
Oral ulcers	2
Subacute cutaneous or discoid lupus	4
Acute cutaneous lupus	6
Serosal	
Pleural or pericardial effusion	5
Acute pericarditis	6
Musculoskeletal	
Joint involvement	6
Renal	
Proteinuria >0.5 g per 24 h	4
Renal biopsy class II or V lupus nephritis	8
Renal biopsy class III or IV lupus nephritis	10
Immunologic Domains and Criteria	**Weight (Points)**
Antiphospholipid antibodies	
Anticardiolipin antibodies OR anti–beta-2 glycoprotein 1 antibodies OR lupus anticoagulant	2
Complement proteins	
Low C3 OR low C4	3
Low C3 AND low C4	4
SLE-specific antibodies	
Anti-dsDNA OR anti-Sm antibodies	6

See text for details.

Adapted from Aringer M, Costenbader K, Daikh D, et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. Arthritis & Rheumatology 2019;71(9):1409; with permission.

WHEN IS IT APPROPRIATE TO ORDER AN ANTINUCLEAR ANTIBODY TEST?

Several studies have appropriately decried the problem of overtesting, over-reliance on test results, and/or the costs they incur.[18–20] There is little consensus, however, on the specific clinical scenarios for which it is appropriate to order an ANA. This is

understandable: clinicians want to avoid delaying a diagnosis as important as SLE, the disease has protean manifestations, so testing may seem easy to justify, and the nonspecific nature of the presentation (eg, fatigue and anemia) makes casting a wide net a tempting approach. For patients with multiple, nonspecific symptoms, it can be a challenge to decide whether or not to request ANA testing.

In 2002, the ACR Ad Hoc Committee on Immunologic Testing Guidelines published a review of conditions in which ANA testing might be particularly useful.[21] It recommended ANA testing for a limited number of conditions, including a suspicion of SLE, systemic sclerosis, mixed connective tissue disease, or drug-induced lupus and stratifying risk of uveitis in patients with juvenile idiopathic arthritis. These guidelines did not specify which symptoms, signs, or other test results should make a clinician suspect SLE or other condition that would prompt ANA testing.

Although the presentation of SLE can vary widely, some clinical manifestations are more suggestive than others (as reflected by their weighting in the latest classification criteria[7]). The clinical setting and demographics of the patient matter: other (non-SLE) explanations must be considered and suggestive signs or symptoms are more likely to reflect SLE if they occur in a woman of childbearing age.

Here are some specific presentations that individually or in combination may warrant a high suspicion of SLE:

- Inflammatory polyarthralgia (including prolonged morning stiffness) or polyarthritis in a rheumatoid distribution (including metacarpophalangeal, proximal interphalangeal, and wrist joints)
- Persistent photosensitive rash (including a malar rash that spares the nasolabial fold), discoid lupus, and subacute or acute cutaneous lupus. These should be distinguished from more evanescent sun or heat triggered flushing.
- Hemolytic anemia or idiopathic thrombocytopenic purpura
- Unexplained and recurrent seizures or serositis
- Unexplained nephrotic syndrome or glomerulonephritis
- Multisystem inflammatory disease that otherwise is unexplained and includes features of the classification criteria[7]

Although the list of clinical presentations of SLE is long, the list of presentations in which SLE is unlikely is even longer. For example, individuals whose dominant symptoms are chronic low back pain, noninflammatory knee pain, or distal interphalangeal bony enlargement are unlikely to derive benefit from ANA testing. In such settings, a negative result is unlikely to add useful diagnostic information whereas a positive result likely is difficult to interpret and may lead to additional and unnecessary testing, referral, and treatment. Similarly, repeat ANA testing commonly is performed but rarely helpful.[20]

A major limitation of algorithms designed to guide clinicians through a rational sequence of diagnostic evaluation for suspected SLE is that the entry criterion is "suspicion of SLE."[22,23] Without providing details of why that diagnosis would be in play, it is unclear how useful such approaches may be.

SUMMARY

ANA testing clearly is an important part of the evaluation of a patient with possible SLE. It is important, however, to understand the strengths and weaknesses of the test and to interpret the results in the context of the specific clinical scenario that lead to testing in the first place. ANAs are present in nearly everyone with SLE but also may be present in other rheumatic disease, autoimmune thyroid disease, and liver disease and in

many healthy individuals. Ideally, an ANA should be ordered only when there is at least a moderate clinical suspicion of SLE (or other ANA-associated disease) and when the results are likely to advance diagnostic confidence. Considering the protean manifestations of SLE and the innumerable permutations of their presentation, it is unlikely that counting up criteria or running clinical algorithms can do more than provide general guidance—the final determination relies on the expertise of the evaluating clinician.

CLINICS CARE POINTS

- Although ANA testing is an important part of the evaluation of a patient with possible SLE, a positive ANA is not specific for SLE because it may be associated with a variety of rheumatic and nonrheumatic diseases.
- Because of its high sensitivity in SLE, a negative ANA is a strong argument against the diagnosis.
- To optimize clinical utility, the ANA should be ordered when there is a significant clinical suspicion of an ANA-associated disease and the results help rule in or rule out that condition.
- Recent classification criteria for SLE require a positive ANA at a titer of at least 1:80, emphasizing both the importance of a positive ANA in the diagnosis and the uncertain relevance of a minimally positive result.
- Repeated ANA testing rarely is helpful.
- If there is enough concern about possible SLE (or other ANA-associated rheumatic disease) to request ANA testing, it generally is advisable also to order a complete blood cell count with differential, serum creatinine, and urinalysis.

DISCLOSURE

The authors have no financial conflicts to disclose.

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