Topics in Primary Care Medicine

Clinical Significance and Interpretation of Antinuclear Antibodies

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Primary care physicians often see patients with rheumatic complaints such as joint pain, muscle weakness and other symptoms that suggest a "connective tissue" disease. When this occurs, there is a tendency to order a panel of tests that often includes the erythrocyte sedimentation rate, a fluorescent antinuclear antibody (FANA) test and a test for rheumatoid factor. The FANA test, in particular, is usually done as a "screening" test for systemic lupus erythematosus or one of the closely related systemic rheumatic diseases. Unfortunately, most clinicians do not have a clear understanding of how to interpret the antinuclear antibody test result. Complicating this issue even further is the fact that many clinical laboratories are now offering tests for antibodies to a variety of more specific nuclear antigens, such as antibodies to native (double-stranded) DNA (nDNA). When should a FANA test be done, what does it mean and how should it be interpreted? Moreover, when should the antinuclear antibody tests that detect unique nuclear antigens, such as nDNA, be done? In this brief review we summarize what is known about the clinical utility of the various antinuclear antibody tests that are currently available and provide suggested guidelines for their use.

Biology of Antinuclear Antibodies

Antinuclear antibodies are a spectrum of autoantibodies that react with various nuclear molecules, including DNA, ribonucleic acid (RNA), histones, acidic nuclear proteins or complexes of these molecular elements. The presence of antinuclear antibodies in serum appears to be associated with various factors including genetic predisposition (such as the histocompatibility locus DR3), environmental agents (ultraviolet light, viruses, certain drugs and chemicals and intravenous drug use), estrogen-androgen balance, chronic infections, neoplasms and advancing age. Aging cannot be overemphasized as a factor associated with the presence of antinuclear antibodies. One recent study noted that 18% of otherwise healthy persons older than 65 years had a positive—that is, a titer of more than 1:10—FANA test. While clinicians generally associate the presence of antinuclear antibodies with a rheumatic disease, particularly systemic lupus erythematosus, these antibodies can also be detected in persons with other immunologically mediated diseases such as Hashimoto's thyroiditis and immunopathic chronic active hepatitis.

While there is some evidence that antibodies to nDNA and other antinuclear antibodies may play a direct role in the pathogenesis of nephritis in patients with lupus erythematosus, their pathogenic role in the rheumatic diseases is poorly understood. Although recent research characterizing the nature and disease associations of the nuclear antigens to which various antinuclear antibodies are directed is exciting and may eventually provide insight into the pathogenesis of various autoimmune diseases, this information is of little practical use to clinicians. Therefore, tests for antinuclear antibodies are most often used as aids in the diagnosis and management of disease.

General Principles of Test Interpretation

When considering the possibility of doing an antinuclear antibody test, clinicians should keep certain general principles of testing in mind. (For a more detailed discussion of these principles, see the article by Sox.) First, if the result of a test will have no effect on any patient-management decision, the test should not be done. In the case of antinuclear antibody testing, it should be stressed that the diagnosis and management of most systemic rheumatic diseases rest primarily on clinical manifestations and the results of more routine laboratory tests.

Second, if the sensitivity and specificity of a test are known, after estimating the chance (pretest probability) that a patient has a specific disease, such as systemic lupus erythematosus, it is possible to apply Bayes's theorem and calculate the effect of a positive (or negative) test result on the posttest probability (see Table 1 for definitions). Whereas few physicians think in such a quantitative fashion, two important generalizations can be easily remembered and applied:

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ABBREVIATIONS USED IN TEXT

FANA = fluorescent antinuclear antibody
nDNA = native DNA
nRNP = nuclear ribonucleoprotein
RNA = ribonucleic acid

- When a patient has a low but appreciable probability of having a particular disease, such as 5% to 40%, a test of high specificity, when positive, is helpful in establishing the diagnosis—that is, increasing the posttest probability.
- When a patient has a moderate to high probability of having a particular disease, a test of high sensitivity, when negative, is useful in excluding this disease—that is, decreasing the posttest probability.

As the sensitivity and specificity of a test diminish, the usefulness of the test also diminishes.

The third principle is that any test that falls short of being 100% specific should not be used indiscriminately to screen patients who have a low pretest probability of disease, as it is likely that a positive result will be "false-positive" rather than "true-positive." Stated another way, the ability of a positive test result, by itself, to accurately predict the presence of disease (positive predictive value) is critically dependent on the prevalence (pretest probability) of the disease in the population being tested. An example that illustrates this principle is the use of the FANA test to screen patients for lupus erythematosus. Several studies have shown that in a group of "healthy" persons between the ages of 20 and 40 years, about 1% of the population has a positive FANA test without apparent disease (the specificity for systemic lupus erythematosus = 99%), and it is known that 95% of patients with lupus erythematosus have a positive FANA test (sensitivity = 95%). Assuming that the prevalence of the disease in this group is about 1 in 700, a positive FANA test will accurately reflect the presence of lupus in only 13% of cases (seven false-positive results for every single true-positive result). Interestingly, if a FANA test is done on a more select group of persons, such as patients with polyarthritis, the predictive value of a positive FANA test in detecting lupus does not increase. This is because the specificity of a FANA test for the disease in this population is significantly lower (specificity = 50% to 70%) because patients with rheumatoid arthritis and other inflammatory arthritides frequently have a positive FANA test. Assuming that the prevalence of systemic lupus erythematosus in patients with polyarthritis is 1 in 20, the predictive value of a positive test is still less than 15%.

The fourth principle to remember is that the initial estimates of the specificity of a test for a particular disease usually overestimate the true specificity. This is because the patients selected for these studies usually have well-defined "classic" disease, whereas the "control" cases are usually healthy persons or patients in hospital in whom the prevalence of false-positive test results is low. A more ideal "control" group to study to define the specificity of a test would be a group of patients presenting with signs or symptoms suggesting the disease in question, such as joint pain, skin rashes and so forth. This is particularly the case for the rheumatic diseases.

The FANA Test

Most physicians are familiar with the general principles that underlie the indirect immunofluorescence technique, which is the most widely used method of detecting antinuclear antibodies. While rat and mouse liver and kidney were commonly used cell substrates in the past, human epithelial cell lines, particularly the KB and HEp-2 cell lines, are currently the most commonly used substrates. It is important to know that methodologic differences and particularly differences in the substrates used—such as HEp-2 cells versus mouse kidney cells—may lead to differences in FANA test results from different laboratories. A report of a FANA test generally includes the pattern of staining and the highest serum dilution (titer) that has detectable antibody.

FANA Titer

The titer of the FANA test that should be interpreted as "positive" varies from one laboratory to another and should be noted on the report slip. In patients with a well-defined rheumatic disease, the titer of the FANA test varies widely. For example, about a third of patients with lupus erythematosus will have a FANA titer of 1:40 or less, whereas a third will have a titer of 1:360 or more. The titer is not helpful in predicting the activity of any rheumatic disease. While most false-positive FANA tests are low titer and most high-titer tests are associated with clinically obvious disease, there may be no discernible disease in some patients who have a high-titer FANA test.

Antinuclear Antibody Patterns

The most common patterns of nuclear fluorescence reported and the specificity of each of these patterns are listed in Table 2. With the exception of the rim pattern, the pattern of nuclear staining lacks sufficient specificity to be diagnostically useful. There is often interobserver variation in pattern interpretation, and the pattern can vary as the test serum is

TABLE 1.—*Definition of Terms*

Sensitivity: Percentage of patients with a particular disease who have a positive test*
Specificity: Percentage of patients who do not have the disease who have a negative test†
Positive predictive value of a test for a disease: (number of true-positives)/(number of true positives) + (number of false-positives)
Bayes's theorem: A mathematical formula for calculating the effect of a positive (or negative) test result on the posttest probability of disease using the pretest probability of disease and the sensitivity and specificity of the test

*The true-positive rate.
†The true-negative rate = 1 − (the false-positive rate).

TABLE 2.—*Specificity of Commonly Reported Immunofluorescent Antinuclear Antibody Patterns**

Pattern	*Specificity*	*Seen in Patients With*
Homogeneous	Low	SLE, drug-induced SLE, RA, vasculitis, polymyositis
Speckled	Low	SLE, MCTD, Sjögren's syndrome, scleroderma, polymyositis, RA
Nucleolar	Low	polymyositis, scleroderma, vasculitis, SLE
Rim	High	SLE (98%), occasionally noted in patients with other rheumatic diseases

MCTD = mixed connective tissue disorder, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus

*Sensitivity of a positive fluorescent antinuclear antibody test, any pattern: SLE = > 95%, drug-induced SLE = 100%, RA = 30% to 50%, Sjögren's syndrome = 75% to 80%, MCTD = 100%, scleroderma = 55% to 70%, dermatomyositis-polymyositis = 30% to 40%, vasculitis = 30%, Raynaud's phenomenon = 30%.

diluted because antibodies directed to several different nuclear antigens may be present in the same serum specimen.

A rim pattern is strongly associated with antibodies to nDNA. Homogeneous fluorescence is associated with antibodies directed against nuclear histone or DNA-histone complexes. A speckled antinuclear antibody pattern is associated with antibodies to acidic nuclear proteins and RNA-protein complexes, such as nuclear ribonucleoprotein (nRNP). A large-speckled pattern may be reported in patients who have anticentromere antibodies. A nucleolar pattern is associated with antibodies to elements that make up this organelle. The sensitivity of a positive FANA test in various diseases is also listed in Table 2.

Interpreting the FANA Test

There are only three diseases that include or require a positive antinuclear antibody test in their diagnostic criteria. The first is systemic lupus erythematosus. It is generally accepted that greater than 95% of patients with this disease will have a positive antinuclear antibody test and that more than 85% will have a positive FANA test two dilutions above the lowest titer that is acceptable as being "positive." In addition, virtually 100% of patients with mixed connective tissue disease and drug-induced lupus erythematosus have a positive antinuclear antibody test. Because the sensitivity of the FANA test in detecting these diseases is high, if clinical and laboratory findings suggest the presence of lupus erythematosus, mixed connective tissue disease or drug-induced lupus, a FANA test should be done. A negative test essentially *excludes* any of these diseases. As outlined earlier, a positive FANA test by itself is not diagnostic. In patients who have signs or symptoms compatible with either of these three disorders, however, a positive FANA test does increase the posttest probability that they have the disease.

Tests for Antinuclear Antibodies to Individual Nuclear Antigens

A variety of methods is available for detecting antibodies to individual nuclear antigens. As the number of different methods used to detect specific nuclear antigens has increased, a wider spectrum of patients than previously suspected has been noted to have low or moderate titers of antibodies to one or more of these nuclear antigens. The best characterized antinuclear antibodies and the specificity and sensitivity for the most common rheumatic diseases are listed in Table 3.

Antibodies to nDNA

The 1982 revised American Rheumatism Association criteria for classifying patients with systemic lupus erythematosus include, in addition to a positive FANA test, "an immunological disorder," which is satisfied if there is an elevated titer of antibody to nDNA *or* a positive lupus erythematosus preparation *or* antibodies to the Smith (Sm) antigen *or* a biologic false-positive serology test for syphilis. In some patients, the level of antibodies to nDNA varies directly with

TABLE 3.—*Sensitivity and Specificity of Antibodies to Nuclear Antigens*

Antinuclear Antibody	Sensitivity	Specificity	Disease
Anti-nDNA	SLE 60% to 70%	High	SLE (>90%) if present in high titer
Antihistone	Drug-induced SLE 100%, RA 15% to 20%, SLE 30%	Low	
Anti-Sm	SLE 30% to 40%	High	SLE 98%
Anti-nRNP	MCTD 100%, SLE 30%, scleroderma 20% to 30%, rheumatoid arthritis 10%, discoid lupus 20% to 30%	Low	
Anti-Ro (anti-SSA)	SLE 30% to 40%, Sjögren's syndrome 60% to 70%, RA 10%	Low	
Anti-La (anti-SSB)	SLE 10% to 15%, Sjögren's syndrome 50% to 60%	Low	
Anticentromere	Scleroderma 10% to 15%, CREST 50% to 90%, Raynaud's disease 10% to 30%	High	Scleroderma or a closely related disease such as CREST or Raynaud's disease, >95%
Anti-Scl_{70}	Scleroderma 15% to 20%	High	Scleroderma >95%
Anti-PM-Scl	Polymyositis 10%	Unknown	? Polymyositis
Anti-Jo_1	Polymyositis 30%, dermatomyositis <10%	Unknown	? Myositis

CREST=calcinosis, Raynaud's phenomenon, esophageal dysfunction, sclerodactyly and telangiectasia; MCTD=mixed connective tissue disease; RA=rheumatoid arthritis; SLE=systemic lupus erythematosus

TABLE 4.—*Indications for Doing Tests for Antinuclear Antibodies*

Test	Indication	Usefulness
Fluorescent antinuclear antibody (FANA)	Patient with possible SLE, drug-induced lupus or MCTD	A negative test virtually excludes these diseases A positive antinuclear antibody test, while nonspecific, increases the posttest probability of disease
Anti-nDNA*	Patient with features of SLE and a positive FANA	A positive test (high titer) substantially increases the posttest probability that the patient has SLE
Anti-Sm	Patient with features of SLE and a positive speckled FANA test pattern	A positive test substantially increases the posttest probability that the patient has SLE
Anti-Ro (anti-SSA)	Woman of child-bearing age with a known connective tissue disease	Counseling, a positive test associated with a small but real risk of neonatal SLE and congenital heart block
	Patient with a negative FANA but a high pretest probability of SLE	A positive test increases the posttest probability that the patient has SLE
Anti-nRNP	Patient with possible MCTD	A negative test essentially excludes the diagnosis of MCTD A positive test in high titer, while nonspecific, increases the posttest probability that the patient has MCTD

MCTD=mixed connective tissue disease, SLE=systemic lupus erythematosus

*The titer of anti-nDNA is used by some specialists to monitor the activity of SLE; clinical features and other laboratory tests, however, are of equal or greater usefulness.

disease activity, making this a potentially useful index to monitor response to therapy. In a given patient, however, other clinical and laboratory features such as hematologic values, a urinalysis, serum complement levels and the like may be more helpful than the level of antibodies to nDNA.

Anti-Sm and Anti-nRNP Antibodies

The Sm (Smith) and nRNP antigens are closely related immunologically and are composed of protein and small RNA species. The presence of antibodies to Sm, like antibodies to nDNA, counts as a criterion for an "immunologic disorder" in the revised American Rheumatism Association criteria for lupus erythematosus. While the presence of antibodies to nRNP is not specific for patients with a mixed connective tissue disorder, the exclusive presence of a high titer of antibody to nRNP without other antinuclear antibodies appears to be unique to patients with this disorder. The absence of antibodies to nRNP virtually excludes the diagnosis of the disease.

Antibodies to Histone

The presence of antibodies to histone is associated with several rheumatic diseases including drug-induced lupus erythematosus, rheumatoid arthritis and systemic lupus. In a patient with suspected drug-induced lupus, measuring the level of antihistone antibodies adds little diagnostic value to a positive FANA test.

Antibodies to the Centromere Antigen

Antibodies to the centromere antigen can usually be detected only in rapidly dividing tissue culture cell lines that include numerous mitotic figures. Occasionally they can be detected using the HEp-2 substrate. Although early studies suggested that the presence of antibodies to the centromere was specific for the CREST (calcinosis, Raynaud's phenomenon, esophageal dysfunction, sclerodactyly and telangiectasia) variant of scleroderma, further studies have shown that the presence of anticentromere antibodies is not specific for this syndrome. The exact diagnostic and prognostic significance of anticentromere antibodies remains to be defined.

Antibodies to the Ro (SSA) Antigen

The Ro antigen (identical to SSA, which stands for Sjögren's syndrome A antigen) is a protein-RNA complex that can be found both in the nucleus and in the cytoplasm of HEp-2 cells. It cannot be detected on mouse, kidney or liver tissue sections. As noted in Table 3, the presence of antibodies to Ro is not specific for any disease. The real significance of anti-Ro antibodies rests with certain disease associations:

- Anti-Ro positivity in a woman with a connective tissue disease is associated with a small but real risk of neonatal systemic lupus erythematosus and congenital heart block if the patient becomes pregnant.
- Patients with "subacute cutaneous systemic lupus erythematosus," who frequently have a negative FANA test, usually have detectable anti-Ro antibodies.
- Patients with Sjögren's syndrome who have anti-Ro antibodies appear to have more aggressive disease, particularly vasculitis.
- Anti-Ro antibodies are commonly detected in patients who have the lupuslike syndrome associated with a deficiency of the second component of complement.

Thus, the presence of anti-Ro antibodies may be helpful diagnostically or prognostically in a select few patients.

Antibodies to Other Nuclear Antigens

Many different nuclear antigens including anti-La (identical to anti-SSB), anti-Scl_{70}, anti-PM-Scl and anti-Jo_1 have been well characterized. The clinical value of finding elevated levels of antibodies to one of these nuclear antigens in a given patient has not been adequately defined.

Conclusion

The indications for doing specific antinuclear antibody tests are summarized in Table 4. Patients possibly having systemic lupus, drug-induced lupus or a mixed connective tissue disorder (pretest probability of disease ranging between 10% and 90%) should be tested for the presence of antinuclear antibodies. A negative FANA test is very useful in excluding these possible diagnoses. With the possible exception of a rim pattern FANA test, a positive test is nonspecific. Tests for antibodies to nDNA and the Sm antigen may be diagnostically useful when there is a moderate probability of systemic lupus erythematosus. The absence of antibodies to nRNP is helpful in excluding the diagnosis of mixed connective tissue disorder. Antibodies to Ro may be useful in a number of rather rare clinical situations.

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