Guidelines for Clinical Use of the Antinuclear Antibody Test and Tests for Specific Autoantibodies to Nuclear Antigens

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• The following guideline presents a series of recommendations based on published medical literature for use of the antinuclear antibody (ANA) test and tests for specific autoantibodies to nuclear antigens in the diagnostic evaluation, prognostic assessment, and monitoring of patients with systemic rheumatic diseases. The guideline emphasizes the need for clinical evaluation to improve the usefulness of test results in patient management. Consideration is given to appropriate use of the generic ANA test in the initial evaluation of patients with signs and symptoms of a systemic rheumatic disease, the evaluation of patients suspected of having lupus erythematosus, use in clinical situations in which the ANA test is required to establish a disease diagnosis, and identification of clinical situations in which the ANA test has little value. Sections are also devoted to recommendations aimed at improving the analytic methods used to detect and measure ANA and specific autoantibodies to nuclear antigens and to the appropriate use of tests for specific autoantibodies in several disease situations that commonly occur in patients with suspected or documented systemic rheumatic diseases. Emphasis is placed on the use of these tests only in situations in which the test results can be expected to provide information necessary for clinical decision making. Those tests of limited medical usefulness and situations in which test results are likely to be misleading are also identified.

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Results of serologic tests for autoantibodies, including tests for antinuclear antibodies (ANAs) and antibodies to specific nuclear antigens such as double-stranded DNA (dsDNA), play an important role in the diagnosis of systemic rheumatic diseases. Although the results are often useful, they can be misleading. Few tests yield results that are pathognomonic for particular diseases. For these reasons, test results for autoantibodies alone are insufficient to establish the diagnosis of a systemic rheumatic disease; they must always be interpreted in the clinical context. Positive results for tests such as the ANA test are seen quite commonly in patients with nonrheumatic diseases and even among normal, healthy persons. Moreover, technical considerations are critical to the accurate interpretation of results of autoantibody tests. Improper use of immunologic tests can result in misdiagnosis, inappropriate therapy, and wasted health care resources.

In an effort to help define the optimal clinical use of the ANA and related tests for specific autoantibodies (anti-dsDNA, anti–Ro [SS-A], anti–La [SS-B], anti-Sm, anti-nRNP, antihistone, and anti–Scl-70 antibodies), the College of American Pathologists (CAP) assembled a panel of representatives from the CAP (Drs Homburger and Tomar), the American College of Rheumatology (Drs Kavanaugh and Reveille), and the Clinical Immunology Society and Clinical Center of the National Institutes of Health (Thomas Fleisher, MD). The guidelines presented herein represent the work of members of the panel and other invited contributors (Robert Lahita, MD; Peter Schur, MD; Yvonne Sherrer, MD; and Dr Solomon).

Development of these recommendations began with a thorough search of the relevant medical literature. More than 800 published articles were retrieved and critiqued using criteria published by the Evidence-Based Medicine Working Group.[1,2] Individual recommendations in the guideline are based on the best relevant literature.

This guideline is intended to serve as an aid to the clinical use and interpretation of tests for autoantibodies. These recommendations are not designed to be a substitute for clinical judgment. In atypical cases, clinical judgment may require deviation from the recommendations. Also, many of the rheumatologic diseases discussed herein follow a variable or evolving course, and changes in clinical status may require reevaluation and repeat testing. Finally, the recommendations in this guideline may evolve over time, as newer analytic methods and additional clinical research yield important results.

The recommendations contained herein are presented in several parts. The first section reviews the history of development of tests for autoantibodies to nuclear antigens. The second section discusses technical considerations important to the performance of these laboratory tests. The third section presents guidelines for the use of autoanti-

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body tests to nuclear antigens in the evaluation of patients suspected of having a systemic rheumatic disease or for prognostic assessment of the disease. The final 2 sections are devoted to frequently asked questions about the clinical application of autoantibody tests and additional information about individual tests for autoantibodies.

HISTORY

Observation of the ''LE cell'' by Hargraves et al[3] in 1948 led to the first laboratory test for ANA. This was an important discovery, as it provided the clinician with a test that could be used to support the diagnosis of systemic lupus erythematosus (SLE). Previously, the diagnosis of SLE often could not be established until tissue specimens were obtained. Even though the lupus erythematosus (LE) cell preparation was recognized as a useful laboratory test, it soon became apparent that it was neither absolutely sensitive nor specific for the diagnosis of SLE. In an effort to further explain and refine this test, work conducted in a number of laboratories led to the recognition that the factors responsible for the LE cell phenomenon were a family of antibodies to various nuclear constituents.[4] By the late 1950s, tests were developed to detect these ANAs using immunofluorescence (IF) techniques on animal tissue substrates. An assortment of tissue types were used to detect ANA, and many of the early studies assessing clinical utility of the ANA test in SLE and other diseases used kidney or liver sections from rats or mice as substrates. Compared with the LE cell preparation, the immunofluorescent ANA test on rodent tissues was more sensitive for the diagnosis of SLE. However, this increased sensitivity was associated with reduced specificity, and substantial numbers of patients with other diseases and even healthy persons were found to have a positive ANA test result.[5] In an effort to reduce the numbers of false-positive or clinically irrelevant results, laboratories began to report titers; that is, the highest dilution of test serum that would still show immunofluorescent nuclear staining. In general, patients with SLE had higher titers of ANA than normal persons or persons with other diseases. Although testing often began at 1:10 or 1:20 dilutions, a positive test result typically was reported only when immunofluorescent staining persisted at dilutions of 1:40 or higher. While this practice reduced the problem of spurious results, large numbers of patients had positive ANA test results but did not have SLE, and occasional SLE patients had negative ANA test results. Despite these limitations, a positive ANA finding was incorporated as a diagnostic criterion for SLE (Table 1).[6,7]

Analysis of the results of ANA tests on animal tissue substrates revealed different appearances or patterns of immunofluorescent staining. It became standard practice for laboratories to report the patterns observed (eg, speckled, homogeneous/diffuse, rim/peripheral, nucleolar, or centromere) in addition to titers for positive ANA test results. In some cases, there appeared to be some clinical correlation with particular staining patterns. However, the relevance of these associations has been largely supplanted by the ability to categorize positive ANA test results on the basis of the antigen specificities of the autoantibodies. Using laboratory techniques such as immunodiffusion, immunoprecipitation, radioimmunoassay (RIA), hemagglutination, and enzyme immunoassay (EIA), it has been shown that ANA-positive sera react with several different nuclear antigens, including dsDNA; small nuclear ribonucleoproteins, including Ro (SS-A), La (SS-B), nRNP, and Sm; enzymes such as topoisomerase-1 (Scl-70); and histone proteins. Reactivity with these antigens is more disease specific than the above-mentioned patterns and may also provide clinically useful prognostic information (see below).

Table 1. American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus*

Malar rash: fixed malar erythema, flat or raised
Discoid rash: erythematous raised patches with keratotic scaling and follicular plugging; possible atrophic scarring
Photosensitivity: rash as an unusual reaction to sunlight
Oral ulcers: oral or nasal ulcers, usually painless
Arthritis: inflammatory arthritis of 2 or more peripheral joints
Serositis: documented pleuritis or pericarditis
Renal disease: persistent proteinuria (>0.5 g/d or >3+) or cellular casts
Neurologic disorder: unexplained seizures or psychosis
Hematologic disorder: hemolytic anemia; or leukopenia (white blood cell count $<4.0 \times 10^9$/L) or lymphopenia (white blood cell count $< 1.5 \times 10^9$/L) on 2 occassions
Immunologic disorder: anti-dsDNA antibodies; or anti-Sm antibodies; or antiphospholipid antibodies
Antinuclear antibody: positive antinuclear antibody test result

* Additional signs and symptoms suggestive of systemic lupus erythematosus include, Raynaud phenomenon, excessive hair loss, unexplained fever, unexplained lymphadenopathy or splenomegaly, and unexplained thromboembolic phenomena.

Over the past decade, most laboratories worldwide have come to use a human tumor cell line substrate (the HEp-2 cell line) for routine ANA testing.[5] The HEp-2 substrate has largely replaced rodent tissues and has become the standard substrate for performing the ANA test. Most relevant literature in recent years is based on results obtained with HEp-2 cells. There are several important differences between these 2 methods of ANA testing. The human cell line is more sensitive than the rodent line for the detection of ANAs. As a result, virtually all SLE patients have a positive ANA finding with use of the HEp-2 substrate. Increased sensitivity results from the expression of more relevant nuclear antigens in the human tumor cells. For example, rodents do not express Ro (SS-A) antigen. Also, centromeres, nucleoli, and other cellular organelles are more readily seen in transformed tumor cells like HEp-2. A sizable number of patients, for whom the term ''ANA-negative lupus'' was coined, have reactivity predominantly with Ro (SS-A).[8] Others have nucleolar reactivity that is not readily detected on rodent tissue substrates. While such patients may have had negative ANA test results on rodent tissue substrates, they are almost always positive when the HEp-2 substrate was used.[9]

The increased sensitivity of the HEp-2 ANA test compared with the ANA test performed on rodent tissue is associated with a lower specificity. Thus, more patients with diseases other than SLE as well as normal healthy persons have positive ANA test results. Some laboratories have attempted to adjust for this by using a higher titer of ANA as a cutoff for a positive result. For example, on rodent tissues, ANA titers of 1:20 or 1:40 or higher have been called positive, whereas on the HEp-2 substrate, titers of 1:80 or higher are usually called positive.

Another difference between the types of ANA tests is that the staining patterns previously described with rodent tissue are not the same on HEp-2 cells. For example,

a rim pattern is rarely seen on HEp-2 cells. Because of this and because the more relevant tests for autoantibodies to specific nuclear antigens are now widely available, interpretation of patterns has become much less important clinically.

More recently, EIA techniques have been used for ANA testing. In a typical EIA, nuclear antigens from cell preparations or mixtures of purified or recombinant antigens are adsorbed to microtiter plates. The EIA has several advantages over IF, including ease of performance and lower cost of the test. The EIA is now widely used to test for specific autoantibodies to nuclear antigens such as ds-DNA, Ro (SS-A), and La (SS-B). Nevertheless, at present, use of the EIA for the generic ANA test has not been subject to widespread population testing. Until comparative studies are available that contrast the operational characteristics of the EIA with those of the established immunofluorescent methods, it is difficult to make recommendations concerning the utility of the EIA technique for determining ANA.

Table 2. Regulatory Requirements for IF-ANA and EIA-ANA Test Methods*

Personnel: federal regulations define the minimum qualifications required for laboratory directors (doctoral degree), technical supervisors (doctoral degree, master's degree, or bachelor's degree plus experience), clinical consultants, general supervisors, and testing personnel (associate degree) engaged in ''high-complexity'' testing
Competency assessment: required yearly for individuals that perform tests and includes direct observation of testing and reporting of results
Quality control: laboratories must have an ''ongoing mechanism'' to identify problems and produce corrective actions
Proficiency testing: ANA is a ''regulated analyte''; acceptable performance on proficiency testing is defined by a result equal to the target value ± 2 dilutions; acceptable results must be obtained on 4 of 5 challenges in each mailing; specific ANAs are not ''regulated analytes'' and acceptable performance is defined by the proficiency provider

* IF indicates immunofluorescent; ANA, antinuclear antibody; and EIA, enzyme immunoassay.

TECHNICAL ISSUES AND QUALITY ASSURANCE

Quality assurance activities in clinical laboratory medicine include practices and procedures used to ensure the accuracy and reproducibility of test results (quality control) and efforts by laboratory scientists and clinicians to develop and communicate information about test results so that testing is performed in clinical situations where the results are most meaningful. Poorly controlled analytic methods are of little value to clinicians because they cannot rely on the accuracy or precision of test results. Reliable tests performed in inappropriate clinical situations are of little value to patients even though the results are accurate. These facts are appreciated by clinicians and laboratory scientists.

The paragraphs that follow summarize existing consensus recommendations for performing tests for ANA by indirect IF and EIA methods. Some of these recommendations are regulations that must be complied with by laboratories performing this group of tests and test methods; other recommendations are voluntary standards. Situations in which more information is needed to improve the usefulness of ANA testing are identified.

Testing for ANAs by Indirect IF

The following recommendations are adapted from an Approved Guideline published in December 1996 by the National Committee for Clinical Laboratory Standards (NCCLS).[10] The NCCLS Approved Guidelines are documents developed through a consensus process. Approved Guidelines are voluntary standards that have achieved consensus within the clinical laboratory testing community. This consensus document addresses general operating practices for the IF-ANA test. Certain of the recommendations are also appropriate to testing for ANA by the EIA method (EIA-ANA, see below).

Practices Designed to Ensure Appropriate Interpretation of Test Results.—It is important to ensure that IF-ANA results are presented to clinicians in the appropriate context. This requires consideration of consistent nomenclature and reporting format, appropriate reference intervals, and adequate representation of the limitations of the test.

Nomenclature and Report Format.—Results of the IF-ANA test should include a statement that describes whether the test result is negative (no discernible pattern of nuclear fluorescence) or positive at the cutoff dilution (nuclei exhibit specific pattern[s] of fluorescence), and if positive, a description of the fluorescence pattern(s) observed, eg, homogeneous or speckled, including cytoplasmic fluorescence. The report may also include a description of the intensity of fluorescent staining, and the end-point titer (or dilution) at which a discernible pattern of fluorescence is observed.

Reference Ranges.—Weakly positive IF-ANA results occur in variable percentages of healthy adults, and results in patients should be interpreted with knowledge of this fact. Several sources, including the above-mentioned NCCLS document, suggest that each laboratory should establish its own reference intervals and consider reporting these with laboratory results.[10,11]

Practices Designed to Ensure Accurate and Reliable Test Results.—Current federal regulations (and accepted laboratory inspection and accreditation criteria based on these regulations) define the levels of training required of supervisors and technicians who perform the IF-ANA test, ongoing competency assessment requirements, and requirements for proficiency testing and performance on proficiency specimens required to achieve ''acceptable'' laboratory performance.[12] These regulations are summarized in Table 2. Additional widely accepted recommendations for performing the IF-ANA test adapted from the NCCLS Approved Guideline are summarized below.

Specimen Collection and Storage.—Tests should be performed on serum. Specimens may be stored at 4°C for up to 72 hours or at −20°C or colder (without freezing and thawing) indefinitely.

Substrate Slides.—Acetone-fixed substrate slides are recommended; ethanol and methanol fixation is discouraged as these fixations may remove Ro (SS-A) antigen. HEp-2 cells are preferable to mouse or rat tissues since the latter do not contain detectable amounts of Ro (SS-A) antigen and do not reveal antibodies to a number of organelles, most importantly, antibodies to the centromere.

Anti-Ig Conjugate.—Anti-Ig conjugates used as detection proteins in the IF-ANA test must be evaluated and chosen with consideration of the following characteristics: isotype

Table 3. Recommended Characteristics of Anti-Ig Conjugate Reagents for IF-ANA Testing*
Isotype specificity: IgG specific; polyvalent conjugates may also be used but may detect increased percentages of clinically insignificant antibodies
FITC to protein ratio: approximately 3.0; higher FITC protein ratios may cause increased nonspecific staining
Antibody to protein ratio: ≥ 0.1
Specific antibody content: 30–60 μg/mL
Working dilution: determined by titration using serial dilutions of positive control sera with known patterns and endpoint titers

* IF indicates immunofluorescent; ANA, antinuclear antibody; and FITC, fluorescein isothiocyanate.

specificity (polyvalent vs IgG specific), fluorescein isothiocyanate to protein ratio, antibody to protein ratio, specific antibody content, and working dilution. Recommended characteristics of anti-Ig conjugates are summarized in Table 3.

Use of Reference Sera.—Reference sera of defined ANA content and specificity are available from the World Health Organization (WHO ANA International Reference Preparation 66/233) and from the AF/CDC ANA Reference Laboratory at the Centers for Disease Control and Prevention (AF/CDC 1–10). Reference sera for autoantibodies to dsDNA, Ro (SS-A), La (SS-B), nRNP, Sm, Scl-70, Jo-1, nucleolar, homogeneous, centromere, and speckled patterns are also available from the AF/CDC ANA Reference Laboratory. These sera are available in limited amounts to manufacturers of test reagents and individual laboratories, who should identify in-house reference sera closely comparable to the WHO/CDC reference sera for daily use.

Unresolved Issues.—The following issues are unresolved at this time.

Intermethod Standardization of IF-ANA Substrates and Anti-Ig Conjugates.—While proficiency testing data indicate there is reasonably good intermethod agreement of results of IF-ANA challenges (qualitative and quantitative), more complete information is needed about the performance characteristics (eg, reactivity with reference sera) of commercially available IF-ANA test systems.

Establishment of Reference Intervals and Use of IF-ANA in Test Algorithms.—No standard protocol has established the frequencies of weakly positive ANA results in healthy persons and in databases used in algorithms that employ the IF-ANA test. More complete information is also needed about the diagnostic sensitivity, specificity, and efficiency of testing algorithms in which the IF-ANA test is used as a screening test to identify patients with systemic rheumatic diseases or in which the test is used as a screening test prior to performing follow-up tests for specific autoantibodies, eg, antibodies to dsDNA and extractable nuclear antigens.

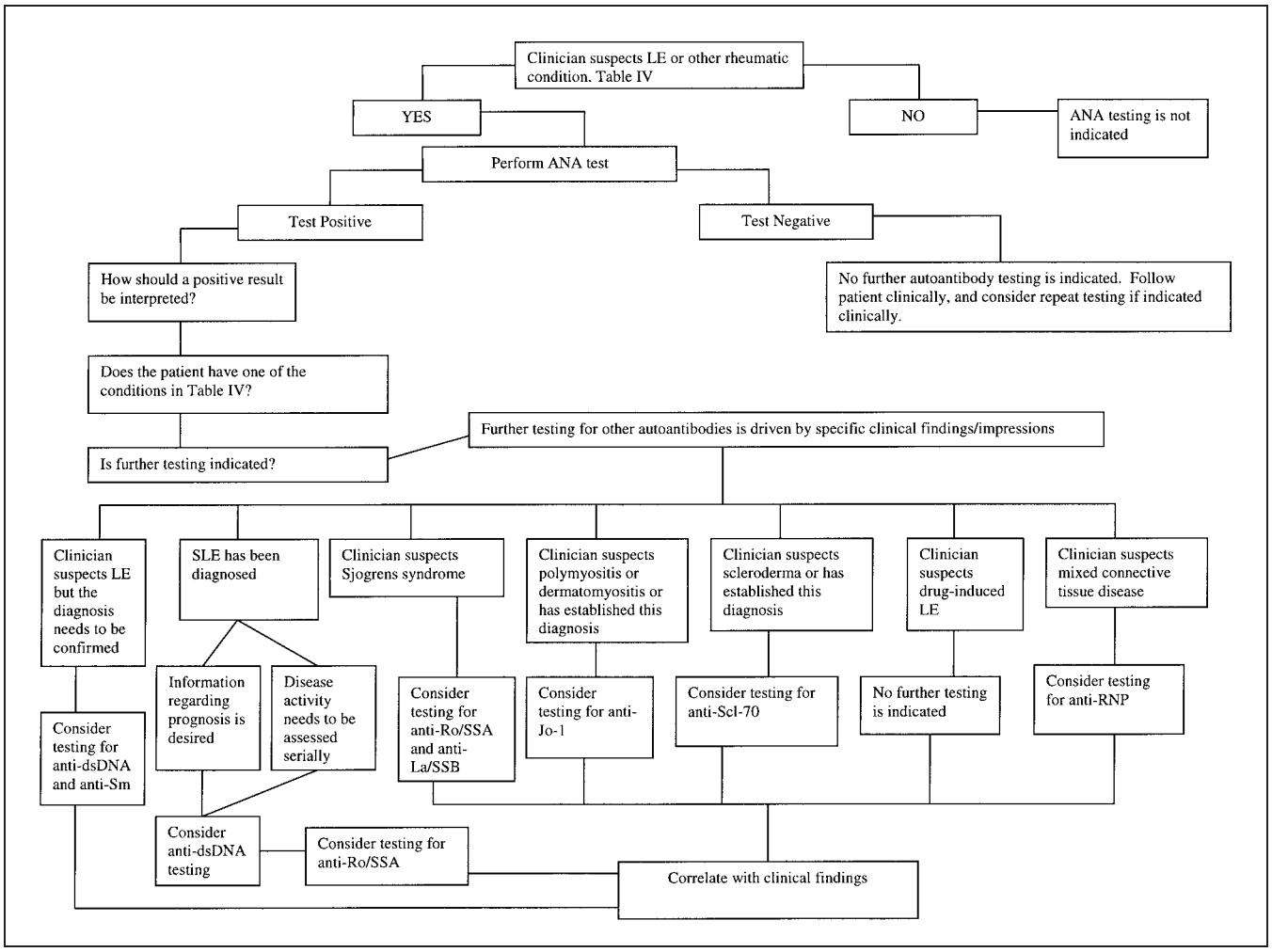
Testing for ANAs by EIA

Two types of EIA-ANA methods are commercially available for use in the clinical laboratory: assays that test for ANA of broad specificity, so-called generic ANA tests, and antigen-specific assays that detect ANA and react with a single autoantigen, eg, dsDNA or Ro (SS-A). The former type of assay gives results similar to the IF-ANA test without information about the pattern of fluorescence. Results are semiquantitative and are often expressed in arbitrary units by comparison with a standard curve or reference calibrator. This type of assay is becoming increasingly popular as an alternative to the IF-ANA test and as an initial test to identify sera to be tested for specific autoantibodies. The EIA-ANA methods have been available for only a few years, and there are no consensus guidelines for performing these assays. Few published studies support the efficacy of these methods for detecting ANA of different specificities or the clinical usefulness of test results of generic EIA-ANA tests or of testing algorithms in clinical practice.[13]

Practices Designed to Ensure Appropriate Interpretation of Test Results.—Since EIA-ANA assays are newer than IF-ANA methods, most clinicians are less familiar with the results of these tests. It is important in this context to provide information to clinicians about test specificity (the range of ANA detected), test sensitivity (lowest concentration or titer of ANA detected), the relationship of levels of reactivity expressed in units appropriate to the assay to IF-ANA titers or the concentrations of specific autoantibodies, and the frequencies of weakly positive results found in healthy individuals and in patients without systemic rheumatic diseases. This information is particularly important if EIA-ANA methods are used in testing algorithms. The levels of screening EIA-ANA results that are used as cutoffs to indicate further testing should be defined in different practice settings, eg, patients seen in primary care settings vs patients referred for specialty evaluation. The diagnostic specificity of results for specific EIA-ANA tests, eg, EIA-ANA to extractable nuclear antigens must also be defined in patients with systemic rheumatic diseases since detection of certain autoantibodies is often considered by clinicians to be disease specific, eg, anti-Sm in SLE and anti–Scl-70 in systemic sclerosis.[14,15] These relationships need to be reconfirmed for specific EIA-ANA test results.

Practices Designed to Ensure Accurate and Reliable Test Results.—Federal regulations currently in force for the IF-ANA test that address training requirements for supervisors and technicians, competency assessment of technicians and technologists, and proficiency testing also apply to EIA-ANA methods. However, these regulations do not recommend standard quality control measures required for optimum day-to-day performance of EIA-ANA tests. There is need for a consensus document analogous to the document for the IF-ANA test that sets forth recommended quality control practices for performing screening EIA-ANA tests and EIA-ANA tests for specific autoantibodies.

The EIA-ANA test methods are fundamentally different from IF-ANA methods. Preparation of EIA immunosorbents differs from kit to kit among manufacturers, and users cannot assume that kits may be used interchangeably and yield similar results in clinical testing situations. Kits may also vary in analytic sensitivity and specificity, and laboratories need to define these parameters for the assays they choose. In 1 recent study, significant discrepancies were demonstrated between commercial IF and EIA kits for detecting ANA.[16] Manufacturers can assist in this process by providing their customers with the results of tests performed on reference sera of known antibody specificity and content. Since testing with EIA-ANA assay kits can be performed manually or with semiautomated equipment, the consensus document should address the performance of EIA-ANA assays by both methods.



Flow chart for clinical antinuclear antibody testing.

TESTING GUIDELINES

Use of the ANA Test for Disease Diagnosis

The following recommendations are organized to simulate the thought processes a clinician might go through when ordering and interpreting an ANA test for disease diagnosis (Figure). No tests for autoantibodies should be performed without a clinical evaluation that leads to a presumptive diagnosis.

Systemic Lupus Erythematosus

An ANA test should be ordered if the clinician feels there is a reasonable clinical suspicion of SLE based on historical information (eg, symptoms suggestive of SLE), physical findings, and results of other laboratory tests (Table 1). The ANA test is sensitive for diagnosis of SLE (95%–100% of SLE patients have a positive ANA test result), but the test should not be used for random screening of patients for SLE. A large number of diverse conditions in addition to SLE are associated with a positive ANA test result (Table 4), and a substantial number of normal healthy persons have a positive ANA test result. The prevalence of positive ANA results among healthy individuals depends on sex and age: older persons, particularly women older than 65 years, more commonly have positive results.[17,18] Titer is also important. With use of the HEp-2 substrate, approximately 20% of normal persons have an ANA titer of 1:40 or higher, and approximately 5% have an ANA titer of 1:160 or higher. Due to the low prevalence of SLE (40–50 cases per 100 000 persons) in the general population, the majority of persons randomly discovered to have a positive ANA result do not have SLE. While the use of higher titers of ANA to define a positive test result may lead to better specificity for the diagnosis of SLE, this practice will decrease diagnostic sensitivity of the ANA test.

Ideally, clinicians use information obtained from the history, physical examination, and results of laboratory tests to more accurately gauge the pretest probability of disease. Patients who have few signs or symptoms suggestive of SLE have a low pretest probability of having the disease. In these patients, a positive ANA test result does little to increase the probability that the patient has SLE. In fact, positive ANA results in cases such as this can be misleading and may precipitate further unnecessary testing, erroneous diagnosis, or even inappropriate therapy.[19] However, if a patient has signs and symptoms suggestive of SLE (for example, arthritis, a malar rash, and autoimmune thrombocytopenia), then the pretest probability that this patient has SLE is greater. In cases such as this, a positive ANA result can be useful to support the diagnosis.

Table 4. Conditions Associated With Positive IF-ANA Test Results*

Disease	Frequency of Positive ANA Result, %
Diseases for which an ANA test is very useful for diagnosis	
SLE	95–100
Systemic sclerosis (scleroderma)	60–80
Diseases for which an ANA test is somewhat useful for diagnosis	
Sjögren syndrome	40–70
Idiopathic inflammatory myositis (dermatomyositis or polymyositis)	30–80
Diseases for which an ANA test is useful for monitoring or prognosis	
Juvenile chronic oligoarticular arthritis with uveitis	20–50
Raynaud phenomenon	20–60
Conditions in which a positive ANA test result is an intrinsic part of the diagnostic criteria	
Drug-induced SLE	~100
Autoimmune hepatic disease	~100
MCTD	~100
Diseases for which an ANA test is not useful in diagnosis	
Rheumatoid arthritis	30–50
Multiple sclerosis	25
Idiopathic thrombocytopenic purpura	10–30
Thyroid disease	30–50
Discoid lupus	5–25
Infectious diseases	Varies widely
Malignancies	Varies widely
Patients with silicone breast implants	15–25
Fibromyalgia	15–25
Relatives of patients with autoimmune diseases (SLE or scleroderma)	5–25
Normal persons†	
≥1:40	20–30
≥1:80	10–12
≥1:160	5
≥1:320	3

* IF indicates immunofluorescent; ANA, antinuclear antibody; SLE, systemic lupus erythematosus; and MCTD, mixed connective tissue disease.

† Values are titers. Prevalence of positive ANA test result varies with titer. Female sex and increasing age tend to be more commonly associated with positive ANA.

Scleroderma (Systemic Sclerosis)

Patients with scleroderma (systemic sclerosis) usually present with a distinct set of clinical signs and symptoms, and a positive ANA result is not required for diagnosis. However, 60% to 90% of patients with scleroderma have been reported to have a positive ANA finding.[20–22] Thus, an ANA result can support the diagnosis of scleroderma. The presence of a negative ANA test result might lead the clinician to consider other fibrosing illnesses that can resemble scleroderma, such as linear or local scleroderma, eosinophilic fasciitis, and scleredema.

Sjögren Syndrome

Approximately 40% to 70% of patients with Sjögren syndrome have a positive ANA test result. While this finding supports the diagnosis, it is not a requirement for the diagnosis of Sjögren syndrome.[23,24] Testing for ANA is indicated in patients with a reasonable suspicion of this condition, for example, in a patient with persistent sicca symptoms or in a woman who has given birth to a child with congenital heart block.

Idiopathic Inflammatory Muscle Disease

Included within this category are the clinically similar conditions polymyositis and dermatomyositis. The ANA test result is positive in 40% to 70% of patients and may be helpful in supporting a diagnosis.[22,25] However, the clinician should recognize that a negative ANA finding does not effectively rule out the diagnosis of dermatomyositis or polymyositis, and further investigation may still be indicated, depending on the clinical context.

Drug-Induced LE, Autoimmune Hepatitis, and Mixed Connective Tissue Disease

In each of these diseases, a positive ANA result is an integral component of the diagnosis. Either by consensus or by the use of published diagnostic criteria, patients must have a positive ANA result before these diagnoses can be made. For example, all studies of drug-induced LE have included a positive ANA result in the definition of the syndrome. Similarly, criteria for the diagnosis of certain types of autoimmune hepatitis and mixed connective tissue disease (MCTD) dictate that the ANA test result be positive for diagnosis. Given these constraints, it is impossible to assess the sensitivity or specificity of the ANA in these conditions. When the clinician is faced with a patient whose clinical course suggests 1 of these diseases, it is customary to obtain an ANA to support the diagnosis.

A number of other diseases are associated with a positive ANA test result at a prevalence higher than the general population (Table 4). The fact that the ANA result can be positive in substantial numbers of patients with various autoimmune diseases also illustrates why the ANA test is a poor screening tool. For example, if a clinician has a patient with arthritis, a positive ANA test result by itself does not help clarify whether the patient has SLE, rheumatoid arthritis, scleroderma, or other diseases that cause arthritis and can be associated with a positive ANA finding.

In addition to providing information that may support the diagnosis of SLE or other diseases, the ANA test may also be used to provide prognostic information (Table 4).

Raynaud Phenomenon

Raynaud phenomenon is diagnosed either by physical examination or by eliciting a specific clinical history. The prevalence of positive ANA results in patients with Raynaud phenomenon varies substantially depending on the population assessed. Although an ANA test does not help establish the diagnosis of Raynaud phenomenon, the test may provide information concerning prognosis.[26,27] Raynaud phenomenon may be associated with several rheumatic diseases, including SLE, rheumatoid arthritis, and scleroderma. However, Raynaud phenomenon is also common among the general population, and the vast majority (81%) of patients with Raynaud phenomenon never develop a systemic rheumatic disease. A positive ANA test result in a patient with Raynaud phenomenon increases the likelihood of development of a systemic rheumatic disease from approximately 19% to 30%, while a negative ANA test result decreases the likelihood to about 7%. The information from a negative ANA test result may help

reassure patients with Raynaud phenomenon that they are likely to have a favorable prognosis.

Juvenile Chronic Arthritis

The ANA test is not useful for diagnosing juvenile chronic arthritis (JCA). However, in children known to have JCA, the presence of a positive ANA test result may predict the development of uveitis, a serious complication.[28] Among patients with pauciarticular (≤3 joints involved) or polyarticular JCA and a positive ANA finding, 20% to 40% develop uveitis. Patients with JCA should be tested for ANA, and those with a positive result should be screened for uveitis.

Antiphospholipid Antibody Syndrome

In patients with an appropriate clinical presentation, antiphospholipid antibody syndrome (APS) is diagnosed by assays for antiphospholipid antibodies and by tests for lupus anticoagulant activity. An ANA test is not necessary for diagnosis. However, approximately 40% to 50% of patients with APS will have a positive ANA finding.[29] The presence of an ANA in a patient with APS increases the likelihood that APS is secondary to SLE.

Except for the 3 diseases mentioned above, ANA testing does not provide useful information about prognosis. This is true even for those diseases listed above in which the ANA test is useful for diagnosis. Since the ANA test does not provide useful information about prognosis or disease activity, there is no indication for sequentially monitoring the ANA level in patients with SLE or other rheumatic diseases. Indeed, there are few clinical indications to repeat a positive ANA test.

FREQUENTLY ASKED QUESTIONS ABOUT THE ANA TEST

If the ANA Test Result Is Negative, Should the Test Be Repeated or Should Other Tests Be Done?

Other than in exceptional cases in which an error in testing is strongly suspected, immediately repeating a negative ANA test is not necessary. However, because the clinical progression of systemic rheumatic disease is often dynamic and evolves over time, it may be worthwhile to repeat the ANA test at a future time, particularly if the clinical course changes. Using HEp-2 cells as a substrate has virtually eliminated false-negative ANA results. Aside from rare cases, further autoantibody testing, if a patient has a negative ANA finding, is not indicated (see below).

What Other Testing Should Be Done Following a Positive ANA Test Result?

In addition to the ANA test, several tests for autoantibodies to specific nuclear antigens are widely available, including dsDNA, Sm, nRNP, Ro (SS-A), La (SS-B), Scl-70 and Jo-1. Patients with SLE often have positive test results for multiple autoantibodies in addition to the generic ANA test.[30] Testing for these autoantibodies should be driven by the particular clinical circumstances and the suspicion of specific diseases.

One practice that has become more common recently is reflex or cascade testing. In such paradigms, panels of tests for various other autoantibodies are performed when an ANA test result is positive. Such additional testing is performed without regard to the clinical characteristics that initially may have prompted ANA testing. Although this approach may seem attractive theoretically, there is little empirical evidence that such reflex testing is actually an effective approach. Potential problems with reflex testing include increased costs and erroneous diagnoses. Additional testing should be guided by specific clinical indications.

As noted earlier, tests for specific autoantibodies are virtually never positive in patients who do not have a positive ANA result. Thus, with rare exceptions, these tests should not be ordered in patients with negative ANA test results. Also, with the exception of antibodies to dsDNA, variation in the concentrations or titers of these antibodies has not been shown to provide useful clinical information. Therefore, repeating tests (other than the anti-dsDNA, see below), if the results are positive, is not indicated. Because the diseases associated with these tests tend to be dynamic over time, negative findings might be rechecked if the clinical circumstances change considerably.

If the Clinician Suspects SLE, What Tests May Help Confirm the Diagnosis?

In the evaluation of patients suspected of having SLE, the initial test to obtain is the generic ANA test (see above). In some cases, for example, when the clinical picture is characteristic of SLE, tests other than the ANA to establish the diagnosis may not be needed. However, as noted above, the ANA test result may be positive in a number of other diseases, some of which have clinical features similar to SLE. In such cases, 2 additional tests can help establish the diagnosis of SLE: tests for autoantibodies to dsDNA (anti-dsDNA) and autoantibodies to the Smith antigen (anti-Sm).

Anti-dsDNA antibodies are assumed to be quite specific for SLE.[31–33] Thus, they are seen uncommonly in patients with other diseases or in normal persons and have a specificity for SLE of nearly 100%. Higher titers or higher concentrations of anti-dsDNA antibodies are more specific for SLE than are results just above the normal range. Weakly positive anti-dsDNA results may occur in patients who do not have SLE. This is particularly true for anti-dsDNA analysis performed by the EIA method. The percentage of SLE patients who have anti-dsDNA antibodies (ie, the sensitivity of the test) varies in published series from 25% up to 85%. In summary, in the appropriate clinical setting, the finding of anti-dsDNA antibodies supports the diagnosis of SLE, while the absence of anti-dsDNA antibodies does not rule out SLE.

Sm and related nuclear ribonucleoproteins (nRNPs) are constituents of subcellular particles (spliceosomes) composed of polypeptide-containing small nuclear RNAs.[30] Although anti-Sm antibodies are present in only 15% to 30% of patients with SLE, they are uncommonly found in patients with other diseases or in normal persons.[34] Thus, anti-Sm autoantibodies are relatively specific for SLE and can be used as an aid in diagnosis. Higher titers of anti-Sm are more specific for SLE. As with anti-dsDNA antibodies, the absence of anti-Sm does not exclude the diagnosis of SLE. Anti-nRNP antibodies, which are commonly tested for in conjunction with anti-Sm, are present in 30% to 40% of SLE patients. However, anti-nRNP antibodies are not specific for SLE and are not useful for establishing the diagnosis of SLE.

After the Diagnosis of SLE Is Made, What Tests Provide Information About Prognosis?

In patients with SLE, the clinician frequently wants to obtain more information relevant to the prognosis of dis-

ease. For example, the clinician may want to know if the disease is active, or which organs might be involved. Tests for autoantibodies can be used to assist in determining the prognosis of this disease.

Anti-dsDNA antibodies correlate with certain aspects of prognosis in SLE patients, including overall disease activity, the presence of lupus nephritis, and the activity of lupus nephritis.[35,36] However, the correlation between these clinical features and the presence of anti-dsDNA autoantibodies is not as strong as it is for diagnosis. Therefore, the finding of anti-dsDNA antibodies by itself does not establish that the patient has active SLE or active lupus nephritis. The anti-dsDNA must always be interpreted in the context of the complete clinical picture. For example, in SLE patients with some indication that they may have renal involvement (eg, proteinuria), the demonstration of a positive anti-dsDNA test result would make lupus nephritis more likely and should prompt the clinician to investigate renal function further and perhaps to initiate treatment. Similarly, if a patient known to have SLE is found to have a positive anti-dsDNA result and the patient also has symptoms that could be consistent with active SLE, the clinician should suspect a flare of disease and proceed with appropriate treatment. On the other hand, if an asymptomatic SLE patient is found to have anti-dsDNA, this does not necessarily indicate active disease or a need for therapy. Watchful waiting may be the best option in such a case. It is worth noting that higher concentrations of anti-dsDNA autoantibodies are more closely associated with measures of disease activity and prognosis than are lower titers. In difficult or confusing cases, consultation with a specialist may be appropriate to help determine the activity of SLE and the optimal approach to therapy.

Antibodies to the ribonucleoprotein Ro (SS-A) are detected in approximately 35% to 60% of SLE patients, depending on the technique used for measurement. There are a number of clinical associations in SLE patients with anti–Ro (SS-A) autoantibodies, including photosensitivity, sicca symptoms, thrombocytopenia, and subacute cutaneous LE rash.[30,37] One important clinical correlation with anti–Ro (SS-A) is neonatal lupus. In this setting, maternal IgG antibodies cross the placenta, causing disease in the neonate. Symptoms in the neonate may include rashes and other manifestations of SLE, but the most feared complication is complete congenital heart block. Although an uncommon manifestation even in SLE patients with anti–Ro (SS-A), women with SLE considering pregnancy may be screened for anti–Ro (SS-A) so that pregnancies can be closely monitored.

After the Diagnosis of SLE Is Made, What Tests Are Useful to Monitor Disease Activity Over Time?

A number of studies suggest that increases in the titers of anti-dsDNA antibodies accompany or precede flares of disease activity in patients with SLE.[38–40] Based on this association, many clinicians follow titers of anti-dsDNA antibodies serially in patients with SLE. Nevertheless, this association does not hold for all patients with SLE. Some patients experience flares of disease activity without changes in anti-dsDNA levels, and some patients have increases in titers without flares of disease. Thus, changes in titers of anti-dsDNA need to be interpreted in the context of the complete clinical picture. Although not thoroughly studied, 1 means to assess the utility of following anti-dsDNA levels may be to determine how closely changes in the levels of these autoantibodies correlate with disease activity in individual patients.[39] The optimal frequency for determining anti-dsDNA levels has not been clearly established. In general, for patients with relatively active disease, anti-dsDNA can be checked every 1 to 3 months, whereas for those with less active disease, a frequency of every 6 to 12 months may suffice. Consultation with a specialist may assist in determining the most appropriate approach to longitudinal follow-up of SLE patients. It should be noted that if the anti-dsDNA determination is repeated over time to ascertain disease activity, technical considerations regarding the analytic method used to measure this autoantibody are critically important. Ideally, the same analytic method should be used to make sequential measurements.

When the Clinician Suspects Sjögren Syndrome, What Additional Tests May Aid in Establishing the Diagnosis?

Sjögren syndrome is characterized by lymphocytic infiltration of exocrine glands, particularly the salivary and lacrimal glands, and by involvement of other organs. The most common clinical presentation is with sicca symptoms, xerophthalmia and xerostomia. The autoantibodies most closely associated with Sjögren syndrome are antibodies directed against the ribonucleoproteins Ro (SS-A) and La (SS-B). The frequency of antibodies to Ro (SS-A) in patients with Sjögren syndrome is 40% to 60% if the test is done by immunodiffusion and approaches 90% or greater if the test is done by EIA.[30,37] Antibodies to La (SS-B), which virtually never occur except in patients who have anti–Ro (SS-A), are found slightly less commonly. The presence of anti–Ro (SS-A) and anti–La (SS-B) antibodies can be used to support the diagnosis of Sjögren syndrome. However, these antibodies are also found in patients with SLE and other diseases, so their presence must be interpreted in the clinical context. In addition to diagnosis, these autoantibodies offer some information about prognosis in Sjögren syndrome. Patients with these autoantibodies more commonly have extraglandular disease, including vasculitis, purpura, lymphadenopathy, hematologic manifestations (eg, leukopenia and thrombocytopenia), hyperglobulinemia, and the presence of rheumatoid factor.

What Tests Provide Prognostic Information in a Patient With Idiopathic Inflammatory Myositis

In addition to inflammatory infiltration and destruction of muscle, patients with dermatomyositis or polymyositis may have involvement of other organ systems. Antibodies to aminoacyl-tRNA synthetases, including anti–Jo-1, have been associated with pulmonary involvement and, in some reports, arthritis.[41]

What Tests Provide Prognostic Information in a Patient With Scleroderma?

Patients with scleroderma are categorized primarily into 2 types of disease: limited and diffuse. Patients with limited disease (also known as CREST syndrome for calcinosis, Raynaud phenomenon, esophageeal dysmotility, sclerodactyly, telangiestasia) tend to have a better prognosis than those with diffuse disease. Autoantibodies may be useful in differentiating the 2 types of disease. Limited disease is most closely associated with the anticentromere pattern of ANA staining.[42,43] Centromere staining is deter-

mined readily from the IF-ANA test, and no additional tests are necessary. Diffuse disease is associated with autoantibodies to the enzyme DNA topoisomerase-1 (anti–Scl-70) or to components of nucleoli. Scleroderma patients with anti–Scl-70 autoantibodies tend to have more severe internal organ involvement, especially pulmonary fibrosis.[44] Neither anticentromere nor anti–Scl-70 autoantibodies are absolutely diagnostic of these different subsets of scleroderma, and the test results must be interpreted in the context of the clinical presentation.

Are Tests for Autoantibodies Useful in Patients Suspected of Having Drug-Induced LE?

A number of medications have been associated with the development of ANAs and clinical signs and symptoms suggestive of SLE. Common manifestations of drug-induced LE include arthritis, serositis, and rashes. Although many medications have been reported to cause drug-induced LE, those most closely associated with this syndrome include hydralazine, isoniazid, procainamide, and several anticonvulsants. Interestingly, studies of patients exposed to these medications reveal that many patients develop a positive ANA finding in the absence of any lupuslike symptoms. While there are no standard diagnostic criteria for drug-induced LE, all studies of this condition require a positive ANA result for the diagnosis.

Drug-induced LE is also associated with the development of autoantibodies to histones. Histones are a group of basic proteins that comprise the largest protein component of the eukaryotic cell nucleus. Antihistone autoantibodies are present in 90% to 100% of patients with drug-induced LE, particularly antibodies to the H2A-H2B histone proteins. Antihistone autoantibodies are also found in approximately 80% of patients with idiopathic SLE as well as a variety of other conditions, including rheumatoid arthritis, JCA, scleroderma, vasculitis, and autoimmune hepatic diseases.[45,46] Technical issues related to the available antihistone assays are also important, with different methods yielding discrepant results. Thus, outside a research setting, there are insufficient data to support the use of testing for antihistone autoantibodies for the diagnosis of drug-induced LE. If this condition is suspected, the clinician should base diagnostic and therapeutic decisions on the medical history and the generic ANA test.

If the Clinician Suspects MCTD, What Tests for Specific Autoantibodies Are Useful to Establish This Diagnosis?

Many patients present with clinical signs and symptoms that are compatible with more than 1 systemic rheumatic disease. One particular constellation of such overlapping features has been MCTD. Patients with MCTD have signs and symptoms characteristic of SLE, scleroderma, and myositis.[47]. In addition, these patients characteristically have high levels of anti-nRNP autoantibodies. While there is some controversy as to whether MCTD represents a unique entity or a scleroderma-SLE overlap syndrome, the test for anti-nRNP autoantibodies has been used to categorize such patients.

ADDITIONAL INFORMATION ABOUT TESTS FOR SPECIFIC AUTOANTIBODIES

Several factors affect the interpretation of tests for specific autoantibodies. Additional information about the following autoantibodies is presented below, including anti-dsDNA, antihistone, anti–Ro (SS-A) and anti–La (SS-B), anti-Sm, anti-nRNP, anti–Scl-70, anticentromere, and antinucleolar autoantibodies. A term that clinicians may encounter in reference to tests for certain autoantibodies is ''extractable nuclear antigen'' (ENA), which refers to the observation that some autoantigens can be extracted from nuclei with saline. In common usage, ENA often refers to Ro (SS-A) and La (SS-B), along with Sm and nRNP. An ENA test panel typically includes tests for autoantibodies to these 4 antigens.

Anti-dsDNA Autoantibodies

In common usage, the term ''anti-DNA antibody'' refers to antibodies that bind specifically to dsDNA. Tests for antibodies to single-stranded DNA (ssDNA) are available; however, their performance characteristics and clinical associations are quite distinct from anti-dsDNA antibodies. Anti-ssDNA antibodies are of no clinical utility and should not be ordered outside research settings.

Anti-dsDNA antibodies are detected by various methods. The initial techniques used to detect anti-dsDNA antibodies (immunodiffusion, complement fixation, and hemagglutination) were supplanted by RIA, also known as the Farr assay. Still later, immunofluorescent assays using a substrate of *Crithidia luciliae*, which contains a kinetoplast with circular dsDNA, and EIA tests were developed. Although results obtained by the 3 methods correlate, the Farr assay (which is not widely performed) and *Crithidia* assays, which detect high-affinity antibodies, are considered to be more specific for the diagnosis of SLE than the EIA. There is greater potential for false-positive results with the EIA, possibly related to low-affinity antibodies or contamination with ssDNA. Thus, caution should be used, particularly in interpreting weakly positive EIA results. For all methods, higher levels of anti-dsDNA autoantibodies are more specific for SLE and are more likely to be associated with active disease than are lower titers or concentrations.

Antihistone Autoantibodies

Histones are a group of highly conserved basic proteins found in eukaryotic cell nuclei. Five major classes (H1, H2A, H2B, H3, H4) and a number of subtypes of histones have been described.[45] Histones bind to native cellular DNA. Autoantibodies that bind to histones have been demonstrated since the 1950s. Over the years, a variety of assays have been used to detect antihistone antibodies. Initially, IF or complement fixation methods were used; more recently, RIA, immunoblotting, and EIA methods have been developed. Different analytic methods have yielded discrepant results.

Anti–Ro (SS-A) and Anti–La (SS-B) Autoantibodies

Ro (SS-A) and La (SS-B) are ribonucleoproteins that have been implicated in protein transcription and translation. Autoantibodies to these antigens were first described in the 1960s and have been found in a variety of systemic rheumatic diseases, particularly SLE and Sjögren syndrome.

Originally, Ouchterlony double immunodiffusion was the technique used for determination of anti–Ro (SS-A) and anti–La (SS-B) antibodies, and by this method the clinical utility of measurements of these autoantibodies was established. More recently, counterimmunoelectrophoresis and EIA techniques have been developed. The

EIA now has become the most widely used method to detect anti–Ro (SS-A) and anti–La (SS-B) autoantibodies. The EIA is more sensitive and detects lesser concentrations of autoantibodies. With increased sensitivity has come decreased specificity, and consideration of the technique used is important to the interpretation of test results. Assays for anti–Ro (SS-A) and anti–La (SS-B) are commonly performed together.

Anti-Sm and Anti-nRNP Autoantibodies

The Sm and RNP antigens are targets for autoantibodies in SLE. The antigens are parts of subcellular particles (spliceosome) composed of peptide-containing small RNAs. The Sm particle is complex, consisting of several different proteins associated with the small nuclear RNAs (U1, 2, 4, 5, 6). RNP contains a 70-kd protein and A and C antigens. Tests for anti-Sm and anti-nRNP antibodies are commonly performed together.

Immunodiffusion has been the standard technique used for determination of anti-Sm and anti-nRNP antibodies; most clinical studies have used this method. More recently, counterimmunoelectrophoresis and EIA techniques have become widely available to test for these autoantibodies. Immunodiffusion is less sensitive but more disease specific than EIA for detection of anti-Sm and anti-nRNP antibodies.

Anti–Scl-70 Autoantibodies

This autoantibody system was first described in the late 1970s and is widely used as a diagnostic aid in scleroderma.[48]. Anti–Scl-70 antibodies give a very fine speckled pattern on indirect IF, and the chromosomes may be stained as well. Anti–Scl-70 antibodies have classically been determined by immunodiffusion techniques, including counterimmunoelectrophoresis, although immunoblotting and EIA have also been used. Despite the suggestion that the EIA was more sensitive than other techniques in detecting anti–Scl-70 antibodies, 2 recent studies found no impact of the various methods used in determination of anti–Scl-70 autoantibodies on the sensitivity and specificity for scleroderma.[49,50]

One recent meta-analysis of 16 articles examining 1074 scleroderma patients in whom anti–Scl-70 antibodies were measured found an overall sensitivity for the diagnosis of scleroderma of 34%.[44] The sensitivity rose to 40% when only patients with diffuse cutaneous involvement were considered. These sensitivities have been shown to be similar in most ethnic groups, although some have reported slightly lower frequencies in caucasians and higher frequencies in African Americans.[42] Among 238 nondiseased controls, only 1 was found to have anti–Scl-70 antibodies (specificity, 99.6%). Among 1429 patients with other rheumatic diseases, the specificity of anti–Scl-70 was 98%. In patients with scleroderma, the presence of anti–Scl-70 has been most specifically and consistently found to correlate with the presence of diffuse cutaneous involvement. Some studies have also found predictive value of anti–Scl-70 either for the development of interstitial pulmonary fibrosis *per se* or for the severity thereof. Some studies have suggested that patients with anti–Scl-70 antibodies have a worse outcome, although other studies have not confirmed this.[51,52]

Anticentromere Autoantibodies

Anticentromere autoantibodies (ACAs) are most typically determined by their characteristic staining pattern on HEp-2 cells. Up to 5 different centromeric autoantigens have been recognized by immunoblotting, designated-CENP A-E. These autoantibody distinctions have not been shown as yet to have proven clinical relevance.

A recent meta-analysis of 19 articles reported the frequency of ACAs in scleroderma patients: 441 of 1379 patients were found to have ACA (overall sensitivity, 32%).[44] The sensitivity increased to 57% when only patients with limited cutaneous disease were studied. Studies examining ethnic differences in the frequency of scleroderma-associated autoantibodies have found ACA to occur strikingly more frequently in caucasians than in African Americans, Hispanics, or Asians. The presence of ACA has been associated with limited scleroderma, previously called the CREST variant of systemic sclerosis[51,52] (sensitivity, 60%, specificity, 98%) as well as with a lower frequency of interstitial pulmonary fibrosis. The specificity of ACA for scleroderma was 95% when 2115 nonscleroderma patients were tested. ACA and anti–Scl-70 autoantibodies rarely coexist in the same individual.

Antinucleolar Autoantibodies

The antinucleolar antibody system comprises a heterogeneous group of autoantibodies that produce nucleolar staining by IF on cells from a variety of species. The most widely recognized of these include anti–PM-Scl, anti–RNA polymerase I-III, anti–U3-RNP (antifibrillarin), and anti–Th (To RNP).[53] While of scientific interest, typing for these specific nucleolar autoantibodies has not found its way into clinical practice.

Antinucleolar antibodies are routinely determined by their characteristic staining pattern on IF. Indirect IF and immunoprecipitation are used for the determination of the various types of antinucleolar antibodies. The determination of subtypes of antinucleolar autoantibodies is used as a research tool and is not widely available commercially, and standardization between different laboratories has not been performed.

The overall sensitivity for scleroderma is low (8%–40%). Although family members of scleroderma patients have been reported to have antinucleolar antibodies, the presence of autoantibodies directed against specific nucleolar constituents is highly specific for scleroderma. Anti–PM-Scl has been most consistently associated with inflammatory myopathy in the setting of scleroderma.[53] Anti–U3-RNP (antifibrillarin), present in 4% to 8% of sera from scleroderma patients, is more frequent in African Americans and is associated with more severe diffuse disease. Likewise, anti–RNA polymerase I has also been associated with rapidly progressive diffuse scleroderma, with a high prevalence of internal organ involvement. Anti–RNA polymerase III has been more commonly described in patients with diffuse cutaneous disease, although no association with internal organ involvement has been described. Anti-Th To antibodies, on the other hand, are associated with limited skin disease. Individual scleroderma patients have been found to express only 1 specificity of antinucleolar autoantibody. Antinucleolar autoantibodies may also be found in patients with SLE, Sjögren syndrome, rheumatoid arthritis, and Raynaud phenomenon.

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