Guidelines for the Laboratory Use of Autoantibody Tests in the Diagnosis and Monitoring of Autoimmune Rheumatic Diseases

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Key Words: Antinuclear antibodies; Autoimmune rheumatic disease; Guidelines; Dermatopolymyositis; Systemic sclerosis; Systemic lupus erythematosus; Sjögren syndrome

Abstract

The Italian Society of Laboratory Medicine Study Group on the Diagnosis of Autoimmune Diseases has generated a series of guidelines for the laboratory diagnosis and monitoring of systemic autoimmune rheumatic diseases intended for the use of clinical pathologists and laboratory physicians. These guidelines are based on a systematic review of published works and expert panel discussion and consist of 13 recommendations for antinuclear antibodies, anti–double-stranded native DNA, and antinuclear specific antibodies. To improve analytic performances and help select the most appropriate test for specific autoantibodies, as well as provide education and guidance in the use of these tests, special emphasis is placed on laboratory methods.

Guidelines in the clinical laboratory recommend the most appropriate modalities for conducting a diagnostic procedure, with the aims of improving the efficiency and efficacy of the diagnosis and monitoring the disease. As guidelines are the result of a methodologic approach in which data based on scientific observation are screened by expert opinion and discussion and then coordinated, they thus represent a working tool and should be considered temporary steps in a continuous process of updating and formation. The practice of evidence-based medicine has resulted in an important upsurge of interest in guidelines; international experiences have confirmed that scientific-medical societies have a central role in their elaboration and in defining indicators of efficiency in the provision of diagnostic services. This issue is particularly important in the field of laboratory diagnosis of autoimmune diseases, because methods for the assay of organ-specific and non–organ-specific autoantibodies have evolved considerably since the lupus erythematosus (LE) cell phenomenon was reported more than 50 years ago.

Requests for these tests have risen remarkably, mainly owing to a growing understanding of the nature of autoantibodies, as well as the molecular characterization of the main target autoantigens and the recent insights into the diagnostic and prognostic significance of the presence and concentration of some autoantibodies in the serum of patients with autoimmune diseases.

On the other hand, the parallel, almost chaotic proliferation of new methods and analytic systems in clinical immunology has involved a constantly increasing expenditure of economic resources for the assay of autoantibodies, estimated in hundreds of millions of dollars throughout the world; this phenomenon thus demands the adoption of measures of...
standardization and verification of the quality of the methods, as well as the introduction and widespread use of guidelines for the diagnosis and monitoring of autoimmune diseases.

Autoantibodies directed against intracellular antigens (eg, antinuclear antibodies [ANAs], anti-DNA, antinuclear specific antibodies) and autoimmune rheumatic diseases constitute a major interest of physicians working in the field of clinical immunology (eg, rheumatologists, immunologists, dermatologists) and in the laboratory.

For about 10 years, progressive review of the cardinal criteria for the diagnosis of the principal autoimmune rheumatic diseases has increasingly centralized the role of the diagnostic laboratory since the presence of diagnostic criteria in the serum autoantibody profile of the patient represents an important prerequisite for the clinical diagnosis. Indeed, positive results for ANA and the presence of anti–double-stranded native DNA (anti-dsDNA) or anti-Sm antibodies constitute 2 of the 11 criteria for the diagnosis of systemic lupus erythematosus (SLE)\(^7,8\); positivity for ANA in high titer or the presence of anti-Ro/SSA or anti-La/SSB antibodies are diagnostic criteria for Sjögren syndrome\(^6\); the presence of anti–Jo-1 antibodies is a criterion for the diagnosis of dermatomyositis\(^10\); the presence of anticientromere or anti–topoisomerase I (anti-Scl70) antibodies is the criterion for classifying subtypes of cutaneous systemic sclerosis as limited or diffuse\(^11\); and a high titer of anti–uridin-rich 1 ribonucleoprotein (U1 RNP) antibodies is the main diagnostic criterion for mixed connective tissue disease (MCTD).\(^12\)

### Guidelines for the Laboratory Diagnosis of Inflammatory Connective Tissue Diseases

Many literature reports describe the standardization of methods and analytic procedures for the detection of autoantibodies,\(^13,14\) with particular attention to the preparation of reference materials,\(^15\) and a recent article gives guidelines for the clinical use of autoantibody testing\(^16\); however, none treats the production of guidelines for the use of laboratory methods to diagnose autoimmune rheumatic diseases. The Italian Society of Laboratory Medicine Study Group on the Diagnosis of Autoimmune Diseases has generated a series of guidelines intended for the use of clinical pathologists and laboratory physicians \(\text{Table I.}\) These guidelines are based on systematic review of published works (articles published from 1966 through 2000 were identified by searching the National Library of Medicine MEDLINE and by examining the bibliographies of retrieved original articles and review articles), as well as expert panel discussion, and consist of 13 recommendations subdivided for ANA, anti-dsDNA, and antinuclear specific antibodies.

### Table I

<table>
<thead>
<tr>
<th>Summary of Guidelines for the Laboratory Use of Autoantibody Tests</th>
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<tbody>
<tr>
<td>• Test for antinuclear and anticytoplasmic antibodies (ANA) only in patients with symptoms of autoimmune rheumatic disease (ARD), because weak ANA reactivity may be present in many nonrheumatic patients and even in “healthy” control subjects.</td>
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<tr>
<td>• To diagnose ARD, screen for ANA using indirect immunofluorescence (IIF) on HEp-2 cells, and specify immunohistochemical pattern (nuclear, cytoplasmic, mitotic) and quantity (titer, concentration).</td>
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<td>• Consider ANA titer (or concentration) of 1:40 (5 IU/mL) and 1:160 (20 IU/mL) as decision-making levels: negative if &lt;1:40, low positive from 1:40 to 1:80, and positive if 1:160 or more.</td>
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<td>• Do not use ANA titer or concentration to monitor ARD.</td>
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<td>• Use enzyme-linked immunosorbent assay (ELISA)-ANA screening test only when your procedure has shown good clinical and analytic correlation with the IIF method.</td>
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<td>• Test for anti–double-stranded native DNA (anti-dsDNA) antibody only in ANA-positive patients in whom systemic lupus erythematosus (SLE) is suspected clinically, using the IIF on \textit{Crithidia luciliae} or ELISA methods. Positive ELISA findings must be confirmed by IIF or the Farr technique.</td>
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<tr>
<td>• Use the ELISA or the Farr technique to monitor anti-dsDNA–positive SLE patients.</td>
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<tr>
<td>• Test for antinuclear specific antibody only in IIF-ANA–positive patients or IIF-ANA–negative patients who have clear symptoms of ARD (especially Sjögren syndrome and polymyositis).</td>
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<tr>
<td>• Use counterimmunoelectrophoresis, ELISA, line-immunoblot, or immunodiffusion to detect Ro/SSA, La/SSB, Sm, uridin-rich 1 ribonucleoprotein, Scl70, Jo-1, CENP-B, rRNP, and chromatin antibodies; reserve Western blot for diagnostic workups.</td>
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<tr>
<td>• In the case of isolated positivity for U1 RNP, report quantitative results because a high antibody titer is diagnostic for mixed connective tissue disease.</td>
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### Recommendations for ANA and Anticytoplasmic Antibodies

The prevalence of autoimmune rheumatic diseases in the population is not high; with the exception of rheumatoid arthritis, which affects about 1% of adults and for which at present there is no specific serologic autoantibody marker, the other rheumatic diseases on the whole do not affect more than 0.5% of the population.\(^17,18\) Most of the affected subjects initially have modest, nonspecific clinical signs, generally with an indistinct and gradual course; included among these is the possibility of a casual finding of abnormal laboratory test results in asymptomatic subjects.

Autoantibodies in low titer without clinical significance in healthy subjects or individuals with diseases other than rheumatic diseases are a relatively frequent finding in the population. On average, it is possible that 1 individual in 4 will show positivity for autoantibodies with common laboratory tests, and this finding does not necessarily indicate the existence of an autoimmune rheumatic disease\(^19,20\); for these reasons:

\textbf{We recommend that tests to detect autoantibodies are performed only when a consistent clinical suspicion of autoimmune rheumatic disease is present. ANA determination should not be used to screen subjects without specific symptoms,}
because weak ANA reactivity is present in many nonrheumatic patients and even in “healthy” control subjects.

Systemic autoimmune diseases are characterized by the presence of high titers of serum autoantibodies to intracellular proteins and nucleic acids. These autoantibodies are generally referred to as antinuclear antibodies owing to their predominant reactivity with nuclear antigens, but they also include anticytoplasmic antibodies. The test for ANAs has a very high diagnostic sensitivity, such that it is considered the best test for the screening for autoimmune diseases has been demonstrated. At present, of the approximately 40 types of fluoroscopic patterns identified, only 19 are reasonably associated with clinical pictures; moreover, for many autoantibodies, the target autoantigen has been identified with certainty.

Among the nuclear patterns, the most frequent consist of homogeneous/peripheral fluorescence (DNA, deoxyribo-nucleoprotein, histones) and speckled fluorescence (RNP, Sm, Ro/SSA, La/SSB); the relatively frequent patterns include the centromeric (CENP-A, CENP-B, CENP-C), nucleolar (PM/Scl, nucleolin, fibrillarin, RNA polymerase I, human upstream binding factor), and the diffuse grainy (topoisomerase I or Sc170) patterns, while the rarer pictures include the proliferating cell nuclear antigen (PCNA) or cyclin, nuclear dots (coilin, Sp-100), and nuclear membrane (lamin A, B, and C; glycoprotein 210) patterns.

Cytoplasmic patterns are relatively less frequent and consist of speckled fluorescence (tRNA synthetase), mitochondrial fluorescence (proteins of the pyruvate-dehydrogenase complex), ribosomal fluorescence (ribosomal ribonucleoprotein), cytoskeletal filament fluorescence (eg, actin, vimentin), and fluorescence of the Golgi apparatus and lysosomes.

In reference to the rare mitotic patterns, these include fluorescence of the spindle (tubulin), centrosomes (enolase), poles or NuMa (nuclear matrix protein), midbody, and the chromosomal protein CENP-F (autoimmunity to which is associated mainly with cancer and hepatitis B or C and not with systemic autoimmune disease). For this reason:

When performing IIF-ANA, use the epithelial cell line HEp-2 (American Type Culture Collection CCL 23) from human laryngeal carcinoma, provided that the expression and integrity of the clinically significant antigens is preserved.

Specify the immunohistochemical patterns as follows, and add a short interpretation of the significance:

1. Nuclear patterns: homogeneous/peripheral; speckled; centromeric; nucleolar; PCNA; nuclear dots; nuclear membrane; diffuse grainy
2. Cytoplasmic patterns: speckled; mitochondrial-like; ribosomal-like; Golgi apparatus; lysosomal-like; cytoskeletal filaments (actin, vimentin, cytokeratin)
3. Mitotic patterns: mitotic spindle; centrosomes; NuMA; midbody; CENP-F

The fluorescence intensity observed in IIF is expressed in different ways in clinical laboratories. Expression with a qualitative scale of values from + to ++++ has the advantage of saving time and resources because titration of serum samples is not requested; however, the intensity of fluorescence in the working dilution is not always proportional to the antibody concentration. This is particularly true for centromeric and nucleolar patterns in which the reduced amount of antigen makes the definition of fluorescence intensity highly subjective and, thus, poorly accurate.

In the majority of laboratories, antibody concentration is expressed with a quantitative scale of values, using titers (reciprocal of the last dilution of the reactive serum); this method better differentiates low- from high-positive titers.
and enables the establishment of different cutoff levels, each one with its own characteristics of sensitivity and specificity; however, this method also is bound to a subjective definition of the end point and, thus, is poorly reproducible, as evidenced by the wide scattering of the titers obtained in numerous collaborative studies.20,31,32

The analytic imprecision or inaccuracy related to the use of dilutions makes it preferable to use calibrators adjusted against an international reference standard (World Health Organization [WHO] International Reference Preparation [IRP] 66/233, homogeneous pattern) and to express the results in international units of concentration (IU/mL).23,33 Available since 1982 are a number of reference sera for the standardization and verification of the quality of the various commercial preparations of HEP-2 cells34 and the different types of nuclear fluorescence in the ANA test.31,35 However, the WHO standard is not easy to obtain on a regular basis, and secondary or tertiary standards should be prepared and eventually used.31 Consequently:

We recommend expressing ANA quantity in titer (reciprocal of the last dilution of the reactive serum). If a reference standard is available, expression in antibody concentration (IU/mL, WHO-IRP 66/233) is preferable.

When ANA is expressed as a titer, which is the most used method, the recommended initial dilution of the sample is 1:40 (corresponding approximately to 5 IU),36 and a titer equal to or greater than 1:160 (20 IU) should be considered positive.20

Low titers (1:40-1:80; 5-10 IU) of ANA may be present in healthy subjects (in particular, pregnant women, women older than 40 years, and elderly persons) and as a phenomenon associated with viral infections, paraneoplastic neurologic syndromes, liver disease, chronic fatigue syndrome, and cancers of various types.37 For the most part, these are natural autoantibodies with low avidity that in some cases may be directed against microbial antigens and environmental chemical haptons, with a repertoire of antigenic specificity similar to that of autoantibodies present in autoimmune rheumatic diseases.38

The autoantibodies detected in serum samples of patients with autoimmune disease are most likely a heterogeneous mixture of polyreactive, low-affinity natural autoantibodies of IgM isotypes and monoreactive, high-avidity pathogenic autoantibodies of IgG and IgA isotypes.39 The methods commonly used in the clinical laboratory are not able to distinguish these two types of autoantibodies, so that the differential diagnosis must necessarily be conducted on the basis of the patient’s clinical history and symptoms and the autoantibody levels present in serum.

Low autoantibody titers (1:40) are rarely present in autoimmune patients, but are observed in about 32% of healthy subjects20; this threshold is highly sensitive and poorly specific. However, it could have diagnostic value, since it would classify virtually all patients with SLE, systemic sclerosis, and Sjögren syndrome as positive for ANA.20

High autoantibody titers (>1:160) are present in sick subjects, but in only 5% of healthy people; this threshold is a little less sensitive but more specific.20 Indeed, at an end point titer less than 1:160, the follow-up secondary tests will yield positive results in fewer than 5% of the cases.36,40 Intermediate titers may be present in about 20% of the healthy population and in a small percentage of sick subjects. For these reasons:

- Titers of 1:40 and 1:160 (or concentrations of 5 and 20 IU, respectively) are considered decision-making levels that require different operative algorithms:
  1. Titers less than 1:40 (5 IU) should be considered as negative.
  2. Titers equal to or more than 1:40 (5 IU) and less than 1:160 (20 IU) should be considered positive at low titer (in the absence of specific symptoms, further diagnostic study is not advised, but the patient should be clinically monitored).
  3. Titers equal to or higher than 1:160 (20 IU) should be considered positive, and patients should undergo further diagnostic study because they are probably affected by an autoimmune disease.

Each laboratory should verify the consistency of these cutoff levels based on the characteristics of its own case material and possibly set cutoff levels at a dilution that gives high diagnostic specificity as determined against inflammatory diseases and healthy control subjects.

The variation in ANA titer in IIF seems to have a poor correlation with the course of the disease, with the sole exception of the anti-PCNA and antichromatin antibodies (homogeneous nuclear pattern) for which a titer decrease or disappearance is associated with the efficacy of therapy.41 This may be related to the fact that most assay systems are not rigorously quantitative, and the use of variations in antibody levels as a reflector of disease activity will need to await the introduction of more quantitative assays; therefore:

In general, the use of variations in the ANA titer in IIF to monitor the course and therapy of autoimmune rheumatic disease is not advised.

Despite recent advances in the standardization of the IIF method (automation of the analytic procedure and recognition of the immunohistochemical pattern by way of computerized systems), the technique still has some methodologic and interpretive limitations. A negative finding for ANA on IIF may occur in connective tissue diseases owing to the effective absence of antinuclear autoantibodies,42 the presence of antibodies to very soluble antigens (such as Ro/SSA), or the presence of autoantibodies directed against scarce cytoplasmic antigens (such as Jo-1 and Ro/SSA).

To simplify and standardize the ANA test, solid phase immunoenzymatic methods (EIA-ANA) have been promoted in recent years as alternatives to the immunofluorescence test
for both screening and detection of total ANAs. While such EIA-ANA tests are less labor intensive and can be used to screen and detect a panel of common autoantibodies usually observed in systemic rheumatic diseases, they are not 100% sensitive when compared with IIF-ANA; in addition, the EIA-ANA test may fail to detect certain ANAs with atypical or rare immunofluorescent patterns.

Moreover, there are remarkable qualitative differences between the different reagents; some kits contain only a narrow assortment of antigens, whereas other include whole cell extracts and others whole cell extracts spiked with scarcely represented antigens. Furthermore, some manufacturers use IgG-specific conjugates, some use conjugates directed to the whole immunoglobulin molecule, and some to all immunoglobulin classes. Therefore, to validate every single reagent or kit, it is necessary to study a sufficient number of patients with well-established diagnoses, as well as a large sample number of IIF-positive serum samples having all the different staining patterns and fully representative of the healthy population from the area (excluding hospital and laboratory personnel), before substituting IIF with the enzyme-linked immunosorbent assay (ELISA). In addition, in reporting negative EIA-ANA screening test results, it should be stated that autoantibodies to specific antigens in this test (eg, Ro/SSA, La/SSB, RNP, Sm, Scl70-topoisomerase I, Jo-1, and dsDNA) were not detected. This clarification will alert clinicians that a negative EIA-ANA test result is not the equivalent of a negative finding for all nuclear antigens. Furthermore, a positive EIA-ANA screen should always be confirmed by IIF-ANA on HEp-2 cells, reporting titer and pattern. For these reasons:

At the present state of scientific knowledge and technology, do not screen for ANAs with immunoenzymatic methods, unless the following conditions are present:

1. The method used must have at least 90% concordance with IIF, and prognostically important ANAs, such as those directed to nucleoli or the nuclear membrane, must be recognized as positive on the ELISA.
2. Positive results must be subsequently confirmed by IIF, specifying pattern and titer or reactivity.
3. Discordant results (ELISA-positive, IIF-negative) should be considered false-positive unless anti-Ro/SSA or anti-Jo-1 antibodies are found. If it is strongly suspected that the patient has an autoimmune rheumatic disease, the patient must be monitored over time.
4. Negative results should specify which autoantibodies were studied and found negative, and patients with a clinical picture that raises suspicion of autoimmune rheumatic disease must be evaluated successively over time.

**Recommendations for Anti-DNA Antibodies**

Since the 1950s it has been known that anti-DNA autoantibodies may exist in patients with SLE. It soon became evident that the DNA molecule presented several epitopes and, therefore, that the anti-DNA antibodies included a heterogeneous group of immunoglobulins with different specificities. The most commonly identified antibodies are those directed against single-stranded DNA (ssDNA), whose antigenic determinants seem to be localized in the basic purine and pyrimidine sequences; the antibodies directed against dsDNA instead recognize epitopes localized along the deoxyribose-phosphate backbone.

Compared with the lack of specificity of anti-ssDNA antibodies, anti-dsDNA antibodies are highly specific for SLE and are present in affected subjects with a prevalence varying from 40% to 80%, such that they constitute the 10th of the diagnostic criteria for SLE as defined by the American College of Rheumatology. Moreover, the presence of anti-dsDNA antibodies in an asymptomatic patient is highly suggestive of subclinical SLE, as these antibodies are negative in SLE induced by drugs and positive in fewer than 2% of the cases with other autoimmune diseases.

Anti-dsDNA and antichromatin antibodies are the main cause of the homogeneous/peripheral nuclear staining pattern in IIF (although they also may be present less frequently with other staining patterns). Patients with SLE and other autoimmune diseases may be initially recognized by means of this test, which constitutes the 11th American College of Rheumatology diagnostic criterion for SLE. Therefore:

**Determine anti-dsDNA autoantibodies only when clinical symptoms raise the suspicion of SLE and when ANA is positive by IIF.**

The diagnostic and prognostic usefulness of the titer of anti-dsDNA antibodies has led to the development of numerous quantification techniques. Among these, the most commonly used are the techniques of radiobinding (among which is the original method of Farr), IIF on Crithidia luciliae, and ELISA.

The technique of Farr prevalently detects high-avidity anti-dsDNA antibodies and provides greater specificity for diagnosing SLE, as well as greater usefulness for monitoring the clinical course. Nevertheless, the need for radioactive isotopes limits its application in many clinical laboratories.

IIF using an anti-IgG conjugate is highly specific, has a good sensitivity and a relative accuracy, reveals antibodies with high and intermediate avidity, and enables the identification of the various antibody classes and their ability to fix complement. This assay is easy to perform, very practical, less technically demanding than the Farr technique, and the most widely used test for anti-dsDNA antibody detection, but it does not provide an accurate quantitative determination.

The ELISA technique can be automated, it reveals different immunoglobulin classes, provides quantitative findings, and is more sensitive than the Farr and IIF techniques.
however, it may also detect antibodies with low avidity having uncertain clinical significance.\textsuperscript{55} Therefore, positive results require subsequent confirmation by IIF, which has a higher specificity.

Quantification of anti-dsDNA antibodies is useful for the clinical management of the patient with SLE. Several studies have documented a close relationship between antibody titer and disease activity, particularly lupus nephritis;\textsuperscript{56} an increase in the antibody concentration may precede flares of disease by a few weeks.\textsuperscript{57,58} In general, for patients with relatively active disease, anti-dsDNA can be tested every 6 to 12 weeks, whereas for those with less active disease, a frequency of every 6 to 12 months may suffice.\textsuperscript{16} Both the Farr and the ELISA technique will give an accurate quantitative result, but the Farr is not as cost-effective as ELISA. For these reasons:

In the diagnostic phase, use the highly specific radioimmunologic method (Farr technique) or the IIF assay on Crithidia luciliae at the initial serum dilution of 1:10 to determine anti-dsDNA antibodies. ELISA also may be used, but positive results must be subsequently confirmed by IIF.

In monitoring the clinical course of SLE, perform a quantitative determination of anti-dsDNA antibodies every 6 to 12 weeks, using ELISA or Farr assay, and express results in IU/mL (WHO/ISP Wo/80).

Recommendations for Antinuclear Specific Antibodies

In the initial diagnostic phase in patients with clinical symptoms that raise suspicion for autoimmune rheumatic disease, the first test is the detection of ANAs by IIF; the pattern of nuclear or cytoplasmic fluorescence determines the subsequent test, represented by the search for autoantibodies directed against one or more specific intracellular autoantigens.\textsuperscript{40} In some patients affected by rheumatic diseases (in particular, Sjögren syndrome and dermatomyositis), the IIF-ANA test may be negative;\textsuperscript{59} therefore, in patients in whom these diseases are suspected clinically, antinuclear specific antibodies should be sought even if the ANA screening test is negative.\textsuperscript{40} For these reasons:

In general, determine antinuclear specific antibodies only when ANA screening is positive. In the case of a negative ANA or positive cytoplasmic antibody finding, antinuclear specific antibody testing is indicated only if the patient has clear signs of an autoimmune rheumatic disease (especially Sjögren syndrome or polymyositis).

The variety of target autoantigens of the antinuclear autoantibodies is extremely wide; from a correct cost-benefit viewpoint, it is not reasonable to try to identify the specific autoantibody in all patients with a known fluoroscopic pattern, but it is wise to limit the study to the autoantibodies for which importance in the clinical diagnosis or as criteria of classification has been demonstrated. At present, the autoantibodies with these characteristics are anti-Ro/SSA, anti-La/SSB, anti-Sm, anti-U1 RNP, anti-Scl70, anti-Jo-1, anti-CENP-B, anti-rRNP, and antinucleosome (chromatin); therefore:

In the diagnostic phase, extend the detection of antinuclear specific autoantibodies to the following autoantigens: Ro/SSA, La/SSB, Sm, U1 RNP, Scl70 (topoisomerase I), Jo-1 (histidyl-tRNA synthetase), CENP-B, rRNP, and nucleosome (chromatin).

Serum antinuclear specific antibodies can be detected with several techniques currently used in the laboratory: double immunodiffusion, counterimmunoelectrophoresis, ELISA, line-immunoblot, immunodot, and Western blot. The strategy for choosing the techniques and their sequence of use depends on the clinical situation, the cost of the reagents, and the time required for a result, as well as the organization of the laboratory and the experience of its personnel.

The ideal method should fulfill criteria of clinical sensitivity and high specificity,\textsuperscript{60} precision and accuracy, ease of execution, limited use of technology, quick availability, and contained costs. At present, no method exists that fulfills all these requirements. Indeed, double immunodiffusion and counterimmunoelectrophoresis are not reliable for wide routine application, as they are remarkably affected by the method of preparation and execution, have a satisfactory sensitivity only for some antinuclear specific antibodies (anti-Ro/SSA),\textsuperscript{61,62} and are qualitative methods. ELISA or other immunoenzymatic methods have excellent sensitivity and are quantitative, but they do not give reliable results for the entire antinuclear specific pattern; if screening methods are not used, tests for each antigen are required.\textsuperscript{53} Western blot is a qualitative method that furnishes a complete pattern of the antinuclear specific antibodies and indicates the composition and molecular weight of the different antigenic systems,\textsuperscript{64} but the proteins used as antigens are modified in their conformation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.\textsuperscript{65} This method is used mostly to confirm autoantibody specificity and to attempt recognizing antigens different from those listed in the preceding guideline. Line-immunoblot is a qualitative method that furnishes limited information about the selected antigens and has the same technical limits as Western blot analysis.\textsuperscript{64} Immunodot has reached the market and is a qualitative method that uses native proteins; it is easy to use and interpret, but at the moment offers a limited panel of autoantigens.\textsuperscript{64}

Recent experiences of external quality assessment\textsuperscript{13,14,32,66-69} have shown significant variability in terms of analytic sensitivity among the methods and reagents commonly used in clinical laboratories for the detection of antinuclear specific antibodies, none of which was found to be completely reliable. Therefore, a correct diagnostic
strategy for the search for antinuclear specific antibodies must use several methodologic approaches. For these reasons:

In the diagnostic phase, determine antinuclear specific antibodies with counterimmunoelectrophoresis, immunoenzymatic, immunoblot, or immunodot methods.

The use of one of the aforementioned methods suffices to identify anti-Ro/SSA, anti-La/SSB, anti-U1 RNP, anti-Sm, anti-Jo-1, anti-Scl-70, anti-rRNP, anti—CENP-B, and antinucleosome (chromatin) antibodies; however, the finding of a low or negative antibody concentration in a subject for whom clinical suspicion of autoimmune rheumatic disease is strong imposes the use of 2 of the above methods. Use Western blot analysis for confirmation of autoantibody specificity and possible identification of antibodies that recognize antigens other than those listed.

In all of the rheumatic diseases, detection of the concentration of the antinuclear specific autoantibodies does not seem to provide additional information for the study of the disease course and prognosis or the efficacy of therapy, with the single exception of MCTD, in which the finding of a high anti-U1 RNP autoantibody titer represents the main diagnostic criterion; therefore:

Report quantitative results (ELISA method) only in the case of an isolated positivity for U1 RNP, because a high antibody concentration is a diagnostic criterion for MCTD.

Conclusions

These recommendations are subject to further modifications and additions as new scientific acquisitions become available; they are offered to the attention of all operators in the field, both in the clinic and the laboratory, as a working tool; they do not represent definitive documents, but temporary steps of a continuous process of updating and formation.

References


