

Antinuclear Antibody Profiles

The Lab's Role in Diagnosing Systemic Rheumatic Disease

BY CAROL L. PEEBLES, MS, MT (ASCP)

Clinicians can often diagnose patients with systemic rheumatic disease (SRD) through clinical observation alone. But some patients are difficult to diagnose, particularly those patients who are in the early stages of the disease or those who present with overlapping symptoms. In these cases, the diagnostic process can be long and tedious, and pinpointing the patient's specific type of SRD—such as systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), both limited and diffuse systemic sclerosis (SSc), rheumatoid arthritis (RA), polymyositis (PM), or Sjögren's syndrome (SjS)—is difficult. To arrive at a diagnosis, patients with suspected SRD first undergo a thorough physical exam, and the clinician obtains a complete medical history. Based on the patient's symptoms, the clinician may then request one or more diagnostic studies—including laboratory tests for autoantibodies—to establish the specific diagnosis.

A diagnostic hallmark of SRDs, autoantibodies are present in all types of rheumatic diseases and can be used, individually or in combination, to characterize a patient's specific disease. Because of the wide and often confusing reactivity of the major rheumatic autoantibodies—double-stranded DNA (dsDNA), chromatin, Sm, RNP, SS-A/Ro, SS-B/La, Scl-70, centromere, Jo-1, and cyclic citrillated peptide (CCP)—interpretation of the test results by the laboratory plays an important role in the specific diagnosis of SRDs. This article focuses on the tests most commonly used in the assessment of patients with systemic rheumatic complaints

due to the fixation method; in particular, fixation of cells with alcohol diminishes or destroys the SS-A/Ro speckled ANA pattern leading to a negative ANA. For this reason, it is important to always include a control for antibodies to SS-A/Ro.

The major patterns observed on HEp-2 slides include homogeneous, speckled, nucleolar, and centromere (Figure 1). Antibodies to dsDNA and chromatin give rise to a homogeneous pattern, while antibodies to Sm, RNP, SS-A/Ro, and SS-B/La produce a speckled pattern. The nucleolar pattern observed in Figure 1 is due to antibodies to fibrillarin detected in patients with SSc,

ribosomes, and various filaments—and many patients demonstrate mixed patterns.

The Key to Diagnosis: Specific Autoantibodies

Thirty years ago, researchers observed that each of the SRDs could be characterized by the presence of antibodies specific for that disease. The first description of antinuclear antibody profiles in SRD was in 1975 by Notman, Kurata, and Tan (1). They tested patients with SLE, RA, SjS, SSc, PM, and MCTD for antibodies to four different antigens: double-stranded DNA (dsDNA) and soluble nucleoprotein (now designated chromatin) were determined by radioimmunoassay, and Sm and RNP were determined by immunodiffusion and passive hemagglutination. The results demonstrated that antibodies to chromatin and dsDNA were present primarily in SLE and that antibodies to Sm were a "marker" for SLE. Antibodies to RNP, on the other hand, were present in most of the SRDs, but in MCTD, along with being present in high titer, they were the only antibody that was observed. Between 1969 and 1980, anti-SS-A/Ro and SS-B/La were associated with SjS, anti-Scl-70 and anti-centromere with SSc, and anti-Jo-1 with PM. At the same time, however, researchers failed to find a specific antibody marker for RA, other than rheumatoid factor (RF), which is detected in many other conditions. That situation changed in 2000 when Shellikens et al. (2) reported the diagnostic properties of antibodies to CCP in RA.

Consequently, while patients with SRD can have the multiple autoantibodies, the specificity and quantity of each antibody provides valuable diagnostic information for clinicians. Figure 2 shows a profile of each of the 10 major autoantibodies for eight rheumatic disorders. Each disease has a distinct ANA profile, allowing the clinician to make the differential diagnosis.

The Most Common SRDs

SRDs are complex disorders, and patients display a wide and highly variable number of symptoms. Presented below are the more common SRDs, along with their characteristic autoantibody patterns.

Systemic Lupus Erythematosus. SLE is a multi-system disease affecting all of the major organs, making the initial presentation highly variable. Patients with SLE are characterized by the presence of antibodies to multiple antigens including Sm, RNP, dsDNA, chromatin, and SS-A/Ro. There are

and discusses which tests are used for diagnosis of the major SRDs.

The Primary Screening Test: IIF

Indirect immunofluorescence (IIF) on a HEp-2 cell substrate is the primary screening test for diagnosis of SRD. A negative IIF result virtually rules out a diagnosis of SLE, and the patterns observed on HEp-2 slides can provide a key to the diagnosis of other SRDs. An important point to remember when performing IIF is that, while the major ANAs are detected on all HEp-2 slides, the detection of antibodies to SS-A/Ro var-

which is termed "clumpy." Other nucleolar reactivity associated with SSc may appear speckled or homogeneous. The centromere pattern is observed when anti-centromere antibodies react with proteins associated with the kinetochore region of the chromosomes; since characteristic changes in the location of the centromeres occur during the mitotic stages of the cell cycle, HEp-2 slides must contain actively dividing cells to confirm antibodies to the centromere. Other patterns may also be observed, including antibodies to cell cycle antigens and various cytoplasmic constituents—mitochondria,



Immunofluorescent Patterns of Nuclear Staining in IIF-ANA Tests Using HEp-2 Cell Substrate

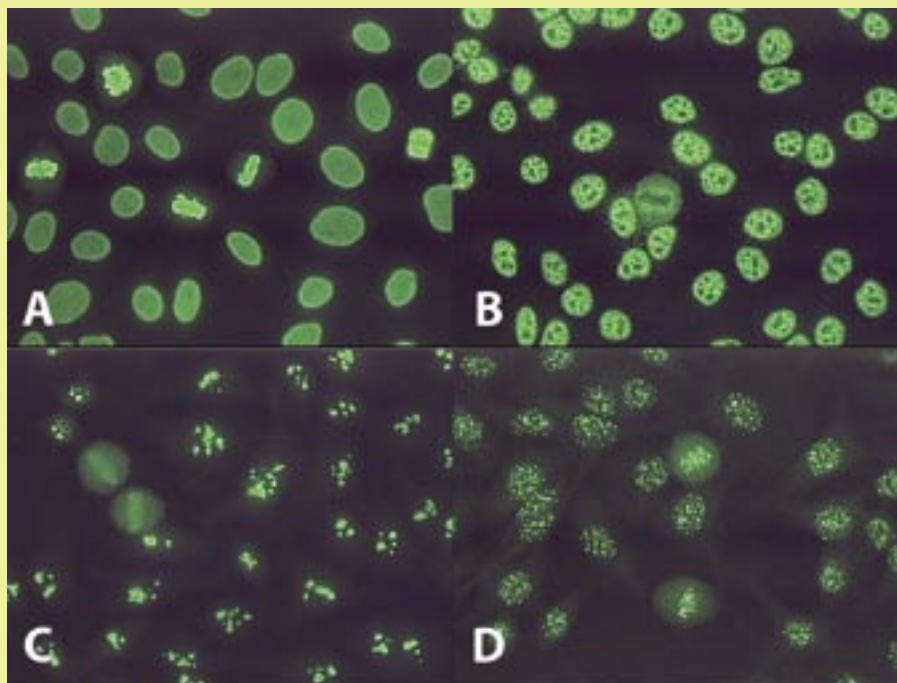


Figure 1—A. homogeneous, B. speckled, C. nucleolar, D. centromere

11 criteria for the diagnosis of SLE, and in order for a definitive diagnosis to be made, patients must meet at least four of these criteria. Two of the criteria are a positive ANA and the detection of antibodies to Sm, dsDNA, or cardiolipin. Antibodies to Sm are detected in 20%–30% of SLE patients, and antibodies to dsDNA may occur in up to 60% of patients, depending on the study.

Antibodies to Sm and RNP typically occur together, since they react with different proteins associated in an RNP particle termed a “spliceosome.” ELISA tests for these antibodies usually have one well that is coated with the whole Sm/RNP particle and a second well coated only with Sm. When the patient’s serum reacts only with the Sm/RNP particle but not the Sm, the patient is considered positive for antibodies to RNP. This test result is seen in a number of SRDs, usually in low to moderate titer. A positive reaction in the Sm well denotes the presence of additional antibodies to Sm and a high probability of SLE. In patients with MCTD—a disease with features that overlap those of SLE, SSc, PM, and RA—antibodies to RNP are the only antibodies; however, they are present in high titer.

The presence of antibodies to dsDNA is also one of the criteria for the diagnosis of SLE, and these antibodies are associated with active disease. A major concern in patients with SLE is the formation and deposition of immune complexes in various organ systems, and for this reason, the presence of these antibodies is monitored along with complement activation to predict exacerbations of the disease. Changes in the antibody titers are also used to monitor treatment. Laboratorians should be aware, however, that antibodies to dsDNA have recently been reported in RA patients being treated with the new TNF α inhibitors.

In general, patients with SLE have antibodies to chromatin more often than antibodies to dsDNA. Antibodies to chromatin are also associated with glomerulonephritis (3) and have been identified, along with dsDNA antibodies, in immune complexes eluted from patients’ kidneys. This is to be expected as the chromatin particle is composed of the core histone particle—containing H2A, H2B, H3, and H4—wrapped with 2.5 turns of dsDNA. Anti-

chromatin antibodies are the antibodies responsible for the LE cell phenomenon in which nuclei are phagocytosed by mature polymorphonuclear leucocytes and digested.

Lupus-like symptoms also occur in patients taking certain medications, one of which is procainamide. Patients with drug-induced lupus (DLE) develop antibodies to chromatin and in some instances to the histone component of chromatin, but not to dsDNA. Of the two antibodies, antibodies to chromatin are better for the diagnosis of DLE. While antibodies to both chromatin and histone may occur in these patients, antibodies to chromatin—but not histone—discriminate between symptomatic and asymptomatic patients. Also, antibodies to chromatin are detected in patients taking other drugs—such as quinidine, penicillamine, methyl dopa, and acebutalol—while histone antibodies are not.

Systemic Sclerosis. The SSc associated antibodies, Scl-70 and centromere, tend to differentiate between SSc patients with systemic disease that is considered to be diffuse versus that which is limited. Patients with the limited form of SSc have skin changes limited to the extremities, while those with the diffuse form have skin changes affecting the trunk as well as the extremities. Patients with diffuse SSc also have more severe disease with greater internal organ involvement.

Antibodies to Scl-70, identified as topoisomerase I and considered to be a marker for SSc, have been reported to occur in 20%–75% of patients with diffuse SSc, depending on the study. Other antibodies associated with the more severe form of SSc include antibodies to the nucleolar antigens fibrillarin and RNA polymerase I. When observed by IIF, these antibodies give clumpy and speckled nucleolar ANA patterns, respectively.

Patients with the limited form of SSc usually have antibodies that react with the centromere. These patients form a subset of SSc called CREST, characterized by calcinosis, Raynaud’s phenomenon, esophageal hypomotility, sclerodactyly, and telangiectasia (4). **Sjögren’s Syndrome.** Clinically characterized by dry eyes and mouth, SjS patients also present with specific autoimmune symptoms. Patients are classified as having either primary SjS if they do not show symptoms

ANA Profile of SRDs

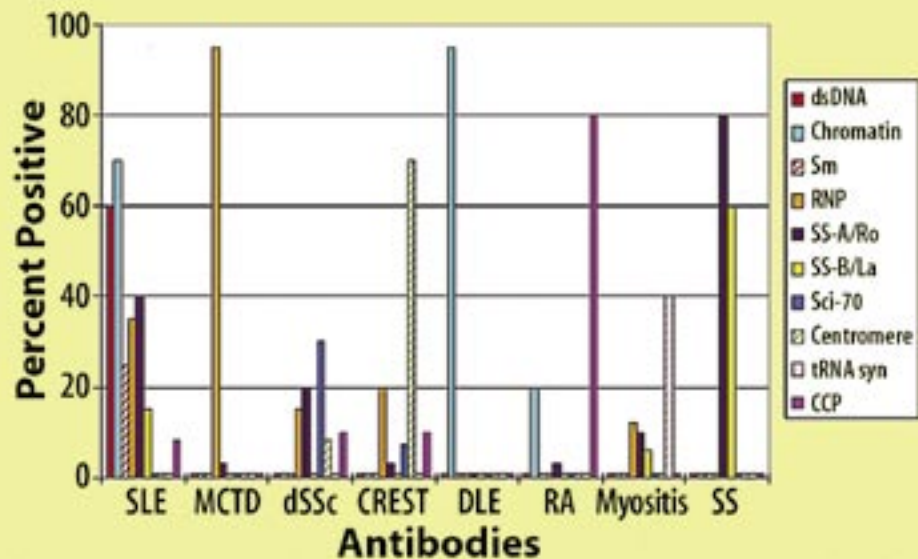


Figure 2—The chart shows the percent of patients who are positive for a specific antibody in each of the major SRDs. These include SLE, MCTD, dSSc (diffuse scleroderma), CREST (limited scleroderma), DLE (drug induced lupus, RA, myositis, and SS (Sjögren’s Syndrome).

of other SRDs, or secondary SjS if their symptoms are associated with RA, SSc, SLE, PM, or other SRDs. More than 30 years ago, researchers reported antibodies in patients with SLE and SjS, designated Ro and La, that reacted with cytoplasmic antigens. At about the same time, another team of researchers reported antibodies, which were designated SS-A and SS-B, that reacted with nuclear antigens. When the research groups exchanged sera, the antibodies to SS-A and Ro were determined to be identical, as were antibodies to SS-B and La (5).

While antibodies to SS-A/Ro occur alone in SLE, as well as in SjS, they are characteristically associated with the SS-B/La antibodies in patients with primary SjS. The detection of SS-A/Ro and SS-B/La antibodies together helps to differentiate primary and secondary SjS. Up to 70% of patients diagnosed with primary SjS have antibodies reacting with either SS-A/Ro only or with SS-A/Ro and SS-B/La. While patients with primary SjS have the SS-A/Ro and SS-B/La antibodies, those with secondary SjS associated with RA, SSc, SLE, and PM generally do not. The presence of SS-A/Ro and SS-B/La antibodies also aids in excluding a diagnosis of SjS among patients with conditions such as sarcoidosis, AIDS, hepatitis, and other causes of keratitis sicca and salivary gland enlargement.

Polymyositis. In 1990, a team of researchers observed the presence of two polypeptides on SDS-PAGE gels, which they designated SS-A/Ro (52 kD) and SS-A/Ro (60 kD), in the sera of patients with SLE and SjS who were considered to be monospecific for antibodies to SS-A by immunodiffusion. (6) While the antibodies to both proteins

were detected most often, in some patients antibodies could be detected to just one or the other of the components. Although the researchers assumed both antibodies were part of a particle, this assumption was never confirmed by cross-linking experiments.

As anti-SS-A 52 kD and anti-SS-A 60 kD reactivities were studied further, however, researchers discovered that they are indeed two separate antibodies that react with distinct antigens. This was most clearly demonstrated in a study of patients with PM in which it was shown that anti-SS-A 52 kD was common but anti-SS-A 60 kD was not. (7). At INOVA Diagnostics, Inc., a study confirmed these results and found that antibodies to SS-A/Ro 52 kD were present in about 45% of patients with PM/DM or a myositis overlap disease, but that percentage increased to 76% when limited to patients with antibodies to tRNA synthetases (Table 1). These results suggest that measuring antibodies to both SS-A 52 kD and SS-A 60 kD could be clinically useful.

Approximately 25%–30% of PM patients are diagnosed with a subset of PM termed “anti-synthetase syndrome,” which is characterized by myositis, Raynaud’s phenomenon, fever, myalgias, and interstitial lung disease that may be severe or even fatal. The sera from these patients contain antibodies that react with tRNA synthetase proteins. Each amino acid has its own tRNA synthetase, the function of which is to catalyze the binding of an amino acid to its specific t-RNA during protein synthesis, and antibodies to different synthetases have been reported. As protein synthesis occurs in the cytoplasm, staining for tRNA synthetase antibodies on the HEp-

Distribution of Antibodies in SLE, SjS, SSc, and PM

Disease (# patients)	SS-A/Ro 60 kD and 52 kD	SS-A/Ro 60 kD only	SS-A/Ro 52 kD only
SLE (161)	28%	9%	7%
SjS (72)	74%	2%	12%
SSc (122)	7%	2%	19%
PM/DM/OL (199)	12%	2%	45%
tRNA synthetase + (82)	12%	1%	76%
Others (84)	1%	1%	6%

The data shows the percent of patients who are positive for the two SS-A antibodies in the total patients tested for each disease.

2 cells is cytoplasmic and not nuclear, but IIF results are inconsistent. The most commonly detected tRNA synthetase antibody, Jo-1, occurs in about two thirds of the patients with synthetase syndrome and is specific for histidyl tRNA synthetase. Antibodies to other tRNA synthetases are rare. Anti-SS-A/Ro 52 kD is also detected in about 75% of patients with synthetase syndrome, including people with antibodies to tRNA synthetases other than Jo-1. Therefore, if a clinician suspects synthetase syndrome, the laboratory should run tests for specific antibodies.

Rheumatoid Arthritis. RA is characterized by bilaterally symmetrical polyarthritis involving three or more joints and morning stiffness lasting more than one hour. Approximately 80% of patients with RA have antibodies to RF. While some patients with RA have a mild disease, a majority of patients diagnosed with RA progress to an erosive arthritis.

The traditional markers associated with a poor prognosis in RA include high titers of RF, increased sedimentation rates, presence of C-reactive protein, and the development of erosions as determined by X-ray. The presence of antibodies to CCP is a new addition to this list. Recent studies in patients diagnosed with early RA indicate that the presence of antibodies to CCP is associated with progression to more erosive disease; in some patients, antibodies to CCP may be detected before symptoms of RA occur and before RF appears. Since patients vary in their presentation and development of symptoms, the ability to identify and predict those patients who will progress to more erosive disease means that treatment can be started earlier in the disease, thereby preventing the development of severe erosions.


Treatment for RA varies depending on the patient. Aiming both to alleviate pain and prevent progression of the disease, tradi-

tional treatment strategies consisted of starting out conservatively with the use of aspirin and non-steroidal anti-inflammatory drugs, and then progressing to corticosteroids and second-line agents including methotrexate in the more severe stages of the disease. Today this treatment regime has changed with the development and use of the new TNF inhibitors. The current trend in rheumatology is to identify those patients who may develop more severe RA early in the course of the disease so that more aggressive interventions involving the use of both methotrexate and TNF α inhibitors can be started. On the other hand, because TNF α inhibitors are expensive and may have serious side effects, clinicians carefully evaluate which patients should take the drug and often order lab tests for antibodies to CCP to aid in the selection.

A Final Word

For people with autoimmune diseases, get-

ting a specific diagnosis can be one of the most difficult challenges they face. Often these patients don't display a clear pattern of symptoms, making diagnosis difficult. In addition, the diagnostic process can be long and tedious. Although no one lab test defines a specific diagnosis, ANA profiles help clinicians distinguish between the many types of SRDs and make a more definitive diagnosis.

As discussed here, certain autoantibodies, individually or in combination, are characteristic of specific rheumatic diseases. Through the judicious use of IIF on a HEp-2 cell substrate and specific antibody testing, the laboratory can significantly aid clinicians in making the correct SRD diagnosis. 

SUGGESTED READING

Krapf AR, et al. *Atlas of Immunofluorescent Autoantibodies*. Baltimore, Md.: Urban & Schwarzenberg, 1996.

Peter JB, Shoenfeld Y, eds. *Autoantibodies*. New York, N.Y.: Elsevier, 1996.

Von Mühlen CA, Tan EM. Autoantibodies in the Diagnosis of Systemic Rheumatic Diseases. *Seminars in Arthritis and Rheumatism* 1995; 24:323-358.

REFERENCES

1. Notman DD, Kurata N, Tan EM. Profiles of antinuclear antibodies in systemic rheumatic disease. *Ann. Intern. Med.* 1975; 83:464-469.

2. Shellikens GA, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrillated peptide. *Arthritis Rheum.* 2000; 43:155-163.

3. Burlingame RW, et al. The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus erythematosus. *J. Clin. Invest.* 1994; 94:184-192.

4. Tan EM, et al. Diversity of antinuclear antibodies in progressive systemic sclerosis. Anti-centromere antibody and its relationship to CREST syndrome. *Arthritis Rheum.* 1980; 23:617-625.

5. Ben-Chetrit E, Fox RI, Tan EM. Dissociation of the immune responses to the SS-A (Ro) 52-kd and 60 kd polypeptides in systemic lupus erythematosus and Sjögren's syndrome. *Arthritis Rheum.* 1990; 33:349-355.

6. Alspaugh MA, Madison P. Resolution of the identity of certain antigen-antibody systems in systemic lupus erythematosus and Sjögren's syndrome: An interlaboratory collaboration. *Arthritis Rheum.* 1979; 22:796-798.

7. Frank MB, et al. The association of anti-Ro 52 autoantibodies with myositis and scleroderma autoantibodies. *J. Autoimmun.* 1999; 12:137-142.



Carol L. Peebles, MS, MT (ASCP), is currently a scientist at INOVA Diagnostics, Inc., in San Diego, Calif. From 1982 to 2001, she was a senior research assistant in the Autoimmune Disease Center at The Scripps Research Institute, La Jolla, Calif.

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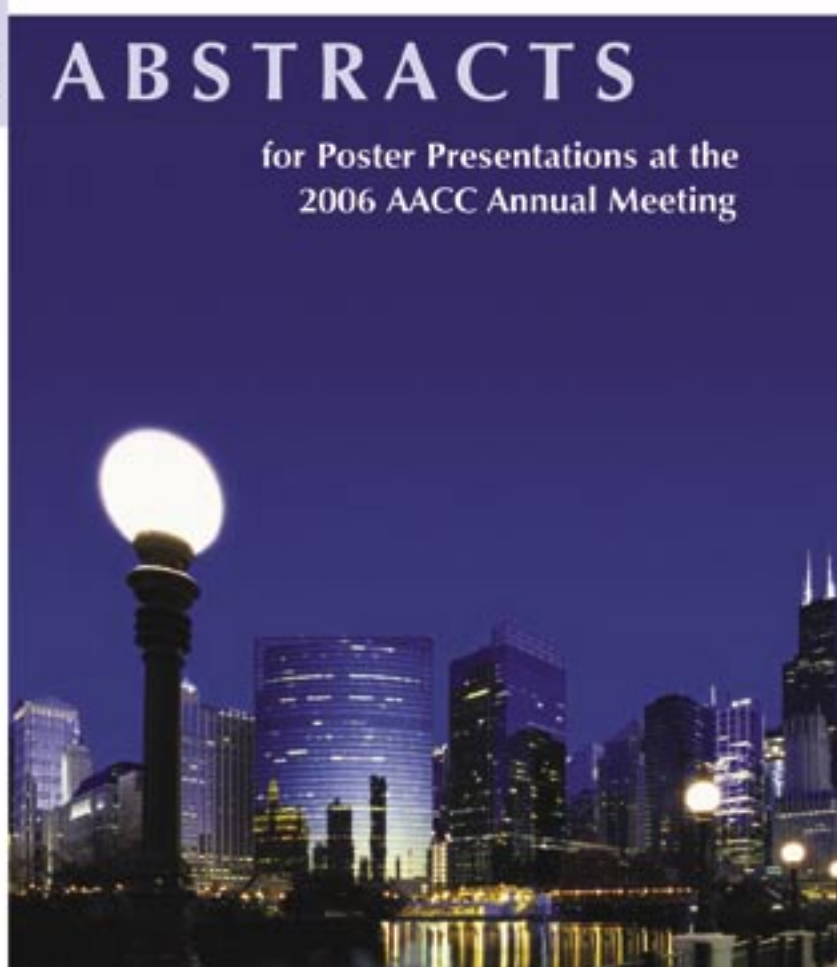
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