Current Perspectives on Rheumatic Laboratory Tests

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Clinicians face a considerable dilemma when deciding which tests to utilize for the diagnosis and management of rheumatic diseases. These authors describe some of the most commonly used rheumatic tests and offer their recommendations as to when they should be ordered.

Despite advances in rheumatic therapeutics over the past decade, older tests continue to be used for diagnostic purposes. Most rheumatic laboratory tests initially were described over 50 years ago and are based on the detection of autoantibodies. Although autoantibodies are associated with rheumatic diseases, their pathogenetic role remains unclear. In addition, they have been detected in individuals with unrelated disorders, such as infections and malignancies, in the absence of any systemic autoimmune disorder. So, while autoantibody tests provide useful information, they require careful interpretation in conjunction with detailed history taking and physical examination.

In this article, we briefly review the most commonly used rheumatic tests and the historical context in which they emerged. We discuss the clinical importance of each in the diagnosis and prognosis of various rheumatic disorders and make recommendations for their use in clinical practice.

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ANTINUCLEAR ANTIBODY (ANA) TESTING

The lupus erythematosus (LE) cell test—first described in 1948 by hematologists Malcolm Hargraves and Robert Morton at the Mayo Clinic—led to the discovery of ANAs.[1] The researchers observed that the LE cell phenomenon occurred only in the presence of what are now known as antihistone antibodies, which are part of the ANA family.

The ANA test used most commonly is indirect immunofluorescence (IIF) using human epithelial or rodent liver cells as substrates (with human epithelial cells being the more sensitive of the 2). With more than 30 nuclear antigens known to be associated with autoimmune disorders, traditional enzyme-linked immunosorbent assay (ELISA), which detects a single antigen epitope, is less efficient than IIF in testing for ANAs.

IIF-ANA tests are highly sensitive for diagnosing systemic lupus erythematosus (SLE) at the screening titer of 1:40 (sensitivity > 97%).[2] Specificity is sacrificed, however, because up to one-third of the general population may test positive for ANAs at that level, and positivity increases with age.[2,3] Although the IIF-ANA test is an excellent tool for detecting SLE when there is a high degree of clinical suspicion for the condition, it is not a useful screening tool and should not be used to rule out other rheumatic diseases because ANAs are present in a variety of conditions[2] (Table 1).

In identifying SLE, the negative predictive value of the IIF-ANA test is estimated to be > 95%, but its positive predictive value (PPV) is only 57%,[4] which is even lower when the test is ordered inappropriately. In 1 study, the PPV of IIF-ANA testing was only 29% for connective-tissue diseases because the test was being ordered inappropriately for large numbers of noninflammatory conditions, including fibromyalgia and localized soft-tissue rheumatism.[5]

Although there are case reports of "ANA-negative" SLE, it remains unclear whether these represent a subgroup of SLE or whether they are technical artifacts.[6] Defining positivity at a higher titer makes the IIF-ANA test more specific but less sensitive: At ANA titers of 1:80, 1:160, and 1:320, the proportion of normal patients testing positive is 13.3%, 5%, and 3.3%, respectively.[2]

The different types of ANAs are defined by their target antigen: double-stranded (ds) DNA; single-stranded DNA; nuclear histones; nucleoproteins; and such RNA-protein complexes as ribonucleoprotein (RNP), Smith, Scl-70, SSA, and SSB. In binding to nuclear antigens, ANAs produce different staining patterns on IIF, such as homogenous, speckled, nucleolar, and centromeric. These patterns, however, are not sufficiently

sensitive or specific to diagnose rheumatic disorders; for that, more specific tests are required.

ANTI-dsDNA ANTIBODY

Autoantibodies to DNA were associated with SLE in the mid 1960s[7] and with nephritis shortly thereafter.[8] The accurate measurement of this antibody, however, was impeded by the lack of a good substrate for IIF. In 1975, Aarden and colleagues reported that the dsDNA in the kinetoplast of *Crithidia luciliae* could be used as a substrate for determining anti-dsDNA antibodies.[9] The ELISA technique for detecting immunoglobulin (Ig) G antibodies to dsDNA is more sensitive but less specific than using *C luciliae* immunofluorescence (CLIF).[10]

The sensitivity and specificity of the CLIF test for anti-dsDNA in SLE range from 61% to 79% and 73% to 95%, respectively.[10-12] The PPV for SLE is 50% with ELISA and 94% with CLIF,[12] which is an important consideration when evaluating a patient's laboratory studies for SLE, especially if the patient belongs to a population with a low SLE prevalence.

Anti-dsDNA testing is a useful complement to positive IIF-ANA results in patients with suspected SLE, but, because of its lower sensitivity, the test should not be ordered for patients with negative IIF-ANA results. Anti-dsDNA tests correlate modestly with SLE disease activity and renal involvement. Increasing titers may precede an SLE flare, but studies addressing this observation are not conclusive.

ANTI-SMITH AND ANTI-RNP ANTIBODIES

The anti-Smith antibody is named after the patient with SLE in whom it was first described in 1966.[13] The extractable nuclear antigen it targeted was identified by immunodiffusion, using phosphate-buffered saline extract of calf thymus. The Smith antigen consists of small nuclear RNPs. Anti-RNP antibodies react against proteins involved in the splicing of heterogeneous nuclear RNA to messenger RNA. Anti-Smith and anti-RNP antibodies typically are grouped together because their antigen targets are found on the spliceosomes and often coexist. Patients with anti-RNP antibodies eventually develop anti-Smith antibodies, possibly as a result of epitope spreading.[14]

Anti-Smith antibodies have a low sensitivity but high specificity for SLE. Of patients with SLE, 25% to 47% have anti-RNP antibodies and 5% to 30% have anti-Smith antibodies,[15] though the method used for measurement affects both the sensitivity and specificity of these tests.[16] Many laboratories use ELISA because it can be performed quickly and easily, but the early reports of sensitivity and specificity were based on immunoprecipitation techniques, such as countercurrent immunoelectrophoresis (CIE) or double diffusion (Table 2).[16]

If these antibodies are present, SLE generally is diagnosed within a year.[17] In patients with clinical fea-

tures of SLE, whose IIF-ANA tests are positive, the anti-Smith antibody test is useful in confirming an SLE diagnosis, though a negative anti-Smith test does not exclude SLE, and the anti-Smith test is not recommended for distinguishing SLE from other autoimmune disorders. Several studies have shown that there is little correlation between anti-RNP antibodies and SLE disease activity,[18,19] and the anti-RNP test has proven unsuccessful in predicting organ damage in SLE.[20]

The anti-RNP test is, however, a diagnostic criterion for mixed connective-tissue diseases (MCTD) because affected patients have higher levels of anti-RNP than patients with SLE. For MCTD, anti-RNP's sensitivity ranges from 71% to 100%, and its specificity ranges from 84% to 100%. The Alarcon-Segovia criterion for MCTD specifies that the hemagglutination titer of anti-RNP must be at least 1:1600 to be diagnostic.[21]

In addition to MCTD, anti-RNP can be positive in patients with Raynaud phenomenon, systemic sclerosis (SSc), and SLE. Anti-RNP and anti-Smith also can be found in the absence of systemic autoimmune disorder, though it is relatively uncommon.[22]

Table 1. Conditions associated with positive antinuclear antibodies

Systemic autoimmune disorders	Organ-specific autoimmune disorders	Other Systemic Causes
• SLE • Scleroderma • Polymyositis/ dermatomyositis • MCTD • RA • Pauciarticular juvenile chronic arthritis • SS • Drug-induced lupus • Discoid lupus	• Hashimoto thyroiditis • Graves disease • Autoimmune hepatitis • Primary biliary cirrhosis • Primary autoimmune cholangitis	• Chronic infections: hepatitis C, HIV, tuberculosis, lepromatous leprosy, infectious mononucleosis, infective endocarditis • Malignancies: chronic lymphocytic leukemia, lymphoma • Drugs • Pregnancy

MCTD = mixed connective-tissue disease; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; SS = Sjögren's syndrome.

SJÖGREN'S SYNDROME (SS) ANTIBODIES

In the late 1950s, Jones discovered, in extracts from patients' lacrimal and salivary glands, a pair of antibodies (later known as anti-SjD and anti-SjT) that precipitated the development of SS.[23] In 1969, Clark and colleagues described Ro (SjD) and La (SjT) as cytoplasmic antigens.[24] In 1975, SSA and SSB were identified as nuclear antigens with properties similar to Ro and La, respectively.[25] It was not until 1979 that Tan and colleagues discovered that Ro and La were identical to SSA and SSB, respectively.[26]

Two closely related proteins constitute the SSA/Ro antigen: 60 kDa—which is associated with small human cytoplasmic RNAs, called hY RNAs—and 52 kDa. The SSB/La antigen is a 48 kDa protein that binds to vari-

Table 2. Sensitivity and specificity of anti-Smith and anti-RNP tests for SLE with different immunoprecipitation methods[16]

Test	Method	Sensitivity, %	Specificity, %	PPV, %
Anti-Smith	Double diffusion	17-35	99-100	89-100
	CIE	11-56	98-100	90-100
	ELISA	34-45	88-100	73-100
Anti–U1-RNP	Double diffusion	17-30	87-99	31-90
	CIE	8-35	83-99	37-57
	ELISA	39-64	84-97	70-95

CIE = countercurrent immunoelectrophoresis; ELISA = enzyme-linked immunosorbent assay; PPV = positive predictive value; RNP = ribonucleoprotein; SLE = systemic lupus erythematosus. Adapted with permission from Lock RJ, Unsworth DJ. Antibodies to extractable nuclear antigens. Has technological drift affected clinical interpretation? *J Clin Pathol.* 2001;54(3):187-190.

Table 3. Sensitivities and specificities of tests for antibodies to SSA/Ro and SSB/La in SS and SLE[34]

Test	Major clinical associations	CIE		ELISA	
		Sensitivity, %	Specificity, %	Sensitivity, %	Specificity, %
Anti-SSA/Ro	SS	85-95	50-60	90-97	45-50
	SLE	25-30	50-60	35-60	45-50
Anti-SSB/La	SS	70-80	60-70	75-85	50-60
	SLE	10-15	50-55	20-30	45-50

CIE = countercurrent immunoelectrophoresis; ELISA = enzyme-linked immunosorbent assay; SLE = systemic lupus erythematosus; SS = Sjögren's syndrome. Adapted with permission from Phan TG, Wong RC, Adelstein S. Autoantibodies to extractable nuclear antigens: Making detection and interpretation more meaningful. *Clin Diagn Lab Immunol*. 2002;9(1):1-7.

ous components of RNA polymerase III and plays an important role in its expression.[27] Although SSA/Ro and SSB/La initially were associated with SS, they also are associated with such other autoimmune disorders as SLE, subacute cutaneous LE, primary biliary cirrhosis, neonatal lupus, scleroderma, polymyositis, MCTD, and rheumatoid arthritis (RA).

The antibodies to SSA/Ro and SSB/La have low specificity for SS and SLE because they are present in other autoimmune disorders (Table 3).[28] It has been estimated that they are present in 3% to 15% of patients with RA,[29] in 3% to 11% of patients with SSc,[30-32] and in 5% to 15% of patients with polymyositis/dermatomyositis.[33-35] They also are found frequently in patients who test positive for cryoglobulin, rheumatoid factor (RF), or polyclonal hypergammaglobulinemia.[36]

CIE is the gold standard for detecting anti-SSA/Ro and anti-SSB/La in autoimmune disorders, with an overall sensitivity of 89% and a specificity of 100% for both antibodies.[37] Due to budgetary and time constraints,[28,38] however, most laboratories use ELISA to measure these antibodies. Therefore, it often is suggested that clinicians order CIE as a confirmatory test for patients who receive positive ELISA results.

Positivity to SSA/Ro or SSB/La is a criterion for SS diagnosis.[38] In pregnant women with suspected autoimmune disorder, anti-SSA/Ro should be checked early in the pregnancy because its presence indicates an increased risk of fetal heart block. In 1 Italian study, 2 of 112 women with anti-SSA had newborns with congenital complete heart block.[39]

ANTINUCLEOLAR ANTIBODIES

Both antiscleroderma (anti-Scl) antibodies and anticentromere antibodies (ACAs) belong to the family of antinucleolar antibodies. Tan and colleagues described a histone-associated basic 70 kDa protein antigen, isolated from the nuclei of rat liver, and demonstrated monospecific antibodies for this protein antigen, called Scl-70, in 2 of 5 patients with scleroderma.[40] Later, Scl-70 was identified as part of the DNA topoisomerase I.[41]

Initially, antibodies to Scl-70 were identified using immunodiffusion technique, which had a sensitivity and specificity of 20.2% and 100%, respectively.[42,43] Immunoblotting was more sensitive and just as specific at 41% and 99.4%, respectively.[42,43] Some studies suggest that ELISA may be even more sensitive than immunodiffusion or immunoblotting, with a sensitivity of 43% and a specificity of 100%,[44,45] though further evidence is required.

The high specificity of the antibody to Scl-70 only pertains to distinguishing diffuse SSc from normal individuals. However, it is not useful in distinguishing SSc from other rheumatic diseases because there are subsets of patients with limited SSc, who also are positive for this antibody. The EULAR Scleroderma Trial and Research database reported the prevalence of anti–Scl-70 antibody in diffuse SSc and limited SSc to be 60.8% and 23.4%, respectively ($P < .001$).[46]

A year after describing the association between anti–Scl-70 and SSc, Tan and colleagues, using IIF with human epithelial cells, discovered that ACAs are even more selective for patients with limited SSc.[47] Currently, there are at least 7 centromeric nuclear proteins (CENPs), A through F and O, known to be associated with SSc.[48,49] Although all ACA-positive serum on IIF showed antibodies to

CENP-B on ELISA, the use of ELISA in detecting SSc has not been studied as thoroughly as the use of IIF.[50]

The prevalence of ACAs in diffuse SSc and limited SSc is reported to be 6% and 47.6%, respectively ($P < .001$).[46] By contrast, the sensitivity and specificity of ACAs for SSc are 33% and 99%, respectively, when affected patients are compared with healthy controls, and the overall sensitivity and specificity of ACAs for differentiating SSc from other connective tissue diseases are 31% and 97.4%, respectively.[51] In a retrospective study of 120 patients with positive ACAs, 70% had SSc, and 30% had such other rheumatic diseases as RA, SLE, idiopathic inflammatory myopathy, primary biliary cirrhosis, hepatitis C, and overlap syndromes.[50]

While ACAs measured by IIF is highly specific for limited SSc, and useful in diagnosing the condition, it is unhelpful in distinguishing it from other rheumatic diseases, and the diagnosis is questionable when anti–CENP-B ELISA results occur in the presence of a negative centromeric ANA pattern. The presence of ACAs in patients without a definite diagnosis of SSc predicts an increased risk of progression to SSc.[48]

RF AND ANTICYCLIC CITRULLINATED PEPTIDE (ANTI-CCP)

Meyer first described the agglutination of sheep red blood cells with human serum in 1922.[52] In 1940, Waaler associated the phenomenon with RA, though he did not consider it to be diagnostic of RA because of its sporadic occurrence.[53] In 1948, Rose and colleagues observed the phenomenon in all patients with active RA, but in only 8 of 16 patients with inactive RA at titers ≥ 16.[54] He also observed that there were 2 false-positives out of 26 patients without RA, but their titers were < 16. In 1956, Singer and Plotz described the IgG-coated latex granules agglutination test as an alternative method of diagnosing RA, which is easier to perform than the sheep red blood cell agglutination tests.[55] The IgG-coated latex granules and the sheep red blood cell agglutination tests remain the principal bases of our current tests for RF today.

Table 4. Prevalence of positive RF in various rheumatic diseases[a]

Disease	RF-positive tests, %
RA[58-60]	65-80
Primary SS[61]	70-80
SSc[62]	20-40
Polymyositis/dermatomyositis[61]	10-20
MCTD[62]	40-50
SLE[61]	30-35

MCTD = mixed connective-tissue disease; RA = rheumatoid arthritis; RF = rheumatoid factor; SLE = systemic lupus erthematosus; SS = Sjögren's syndrome; SSc = systemic sclerosis.
[a]In addition to the rheumatic diseases listed here, there are many other conditions associated with positive RF—in particular, chronic infections and lymphoproliferative malignancies.

RF is an autoantibody that binds to the Fc component of IgG (particularly in the Cγ2 and Cγ3 regions). In 1967, Torrigiani and Roitt described the quantification of different Ig classes of RF.[56] IgG and IgA RFs are not measured by agglutination methods because they do not agglutinate as well as IgM. The precise physiologic function of RF is unknown, but it is recognized as an excellent scavenger of immune complexes.[57] As Rose observed in 1948, other rheumatic and nonrheumatic diseases, besides RA, also are associated with RF[54] (Table 4).[58-62] About 1% to 4% of healthy individuals are RF positive, and the presence of RF increases with age.

The anti-CCP antibody is directed against a synthetic peptide derived from the large protein filaggrin and expressed in the epidermis. It has been measured and verified by ELISA to be highly specific for RA.[63] Antiperinuclear factor was discovered to be associated with serum of patients with RA in the 1960s.[64] Although antiperinuclear factor was known to be specific for RA, it was not feasible to be used as a standardized laboratory test because of the need for buccal mucosa cell donors and IIF techniques. When the target antigen for antiperinuclear factor was discovered to be filaggrin, it was renamed antikeratin antibody.[65] Filaggrin extracted from the human epidermis, however, has heterogeneous amino acid sequences, and the variability of its charge and degree of deimination make it an unsuitable standardized substrate for ELISA.

Van Venrooij and colleagues discovered that the antikeratin antibody epitope on filaggrin contains citrulline residues formed by post-translational deimination of arginine residues.[66] They made synthetic peptides that included cystine residues, which resulted in a cyclic peptide that mimics the β-turn conformation antikeratin antibody epitope on the parent protein, resulting in high antibody affinity.[67] The sensitivity and

Table 5. Summary of recommendations

Test	When to order?	Test advantages	Test disadvantages
IIF-ANA	Symptoms suggest: • SLE • SS • SSc • MCTD • Idiopathic inflammatory myopathy • Pauciarticular juvenile chronic arthritis	High sensitivity	Low specificity
Anti-dsDNA	ANA positive and to confirm SLE	High specificity	Low sensitivity Most laboratories use ELISA, which is less specific than IIF
Anti-Smith	ANA positive and to confirm SLE	Higher specificity than anti-dsDNA	Lower sensitivity than anti-dsDNA
Anti-RNP	ANA positive and symptoms suggest MCTD	Specific for MCTD at high positive titers	Low positive titers are nonspecific and not useful to distinguish MCTD from other rheumatic diseases
Anti-SSA (anti-Ro)/ anti-SSB (anti-La)	ANA positive and • Symptoms suggest SS • Early in pregnancy for women with symptoms suggestive of autoimmune disorder	Sensitive for SS Positive anti-SSA (anti-Ro) antibody associated with increased risk for congenital heart block	Moderate specificity and not useful to distinguish SS from other rheumatic diseases
Anti–Scl-70	Symptoms suggest diffuse SSc	High specificity for diffuse SSc	Moderate sensitivity
Anticentromere	Symptoms suggest limited SSc (CREST)	High specificity for limited SSc	Moderate sensitivity
RF	Symptoms suggest RA	May reflect RA disease activity	Low specificity; many other diseases are associated with positive RF (such as hepatitis C infection)
Anti-CCP	Symptoms suggest RA	High specificity for RA Useful to distinugish RA from other conditions with positive RF	Moderate sensitivity May not reflect RA disease activity

ANA = antinuclear antibody; anti-CCP = anticyclic citrullinated peptide; anti-Scl = antiscleroderma; CREST = calcinosis, Raynaud phenomenon, esophageal disease, sclerodactyly, and telangiectasias; dsDNA = double-stranded DNA; ELISA = enzyme-linked immunosorbent assay; IIF = indirect immunofluorescence; MCTD = mixed connective-tissue diseases; RA = rheumatoid arthritis; RF = rheumatoid factor; RNP = ribonucleoprotein; SLE = systemic lupus erythematosus; SS = Sjögren's syndrome; SSc = systemic sclerosis.

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specificity of anti-CCP antibody for RA were found to be 66% to 85% and 90% to 98%, respectively.[58,59,63,68] As highly specific as it may be, anti-CCP also may be positive in nonrheumatic diseases. Unlike RF, for which titers change with treatment, anti-CCP titers typically do not.[60]

GENE EXPRESSION PROFILING

The collection of rheumatic laboratory tests discussed herein is not exhaustive, but there is some consensus on when each should be ordered (Table 5). Gene expression profiling has provided new information regarding disease pathogenesis in various immunologic disorders and has led to the development of novel treatment regimens. Describing the use and interpretation of gene expression profiling is beyond the scope of this article, but, in short, such tests are complicated and limited in terms of sensitivity, specificity, and reproducibility.

The field of personalized medicine currently is under active research. This is an exciting era in the field of rheumatology, in which, bedside-to-bench techniques are soon expected to gain popularity.

Acknowledgments

The authors thank Ms. Sonora Hudson for reviewing the manuscript. This work was supported in part by the Houston VA Health Services Research and Development Center of Excellence (HFP90-020).

Author disclosures

The authors report no actual or potential conflicts of interest with regard to this article.

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REFERENCES

1. Hargraves MM. Discovery of the LE cell and its morphology. *Mayo Clin Proc*. 1969;44(9):579-599.
2. Tan EM, Feltkamp TE, Smolen JS, et al. Range of antinuclear antibodies in "healthy" individuals. *Arthritis Rheum*. 1997;40(9):1601-1611.
3. Juby AG, Davis P. Prevalence and disease associations of certain autoantibodies in elderly patients. *Clin Invest Med*. 1998;21(1):4-11.
4. Wananukul S, Voramethkul W, Kaewopas Y, Hanvivatvong O. Prevalence of positive antinuclear antibodies in healthy children. *Asian Pac J Allergy Immunol*. 2005;23(2-3):153-157.
5. Suarez-Almazor ME, Gonzalez-Lopez L, Gamez-Nava JI, Belseck E, Kendall CJ, Davis P. Utilization and predictive value of laboratory tests in patients referred to rheumatologists by primary care physicians. *J Rheumatol*. 1998;25(10):1980-1985.
6. Bohan A. Seronegative systemic lupus erythematosus. *J Rheumatol*. 1979;6(5):534-540.
7. Casals SP, Friou GJ, Myers LL. Significance of antibody to DNA in systemic lupus erythematosus. *Arthritis Rheum*. 1964;7:379-390.
8. Hughes GR. Significance of anti-D.N.A. antibodies in systemic lupus erythematosus. *Lancet*. 1971;2(7729):861-863.
9. Aarden LA, de Groot ER, Feltkamp TE. Immunology of DNA. III. Crithidia luciliae, a simple substrate for the determination of anti-dsDNA with the immunofluorescence technique. *Ann N Y Acad Sci*. 1975;254:505-515.
10. Wong KH, Lawton JW, Cheng SK, Lee SS, Lau CS. Measurement of anti-dsDNA: A comparative study of two ELISA and the Crithidia assay. *Pathology*. 1998;30(1):57-61.
11. Janyapoon K, Jivakanont P, Choosang K, Surbrsing R, Charoenying V, Baithong S. Comparative study of anti-double stranded DNA detection by ELISA and Crithidia luciliae immunofluorescence. *Southeast Asian J Trop Med Public Health*. 2003;34(3):646-650.
12. Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: The role of analytical diversity. *Ann Rheum Dis*. 2004;63(4):386-394.
13. Tan EM, Kunkel HG. Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. *J Immunol*. 1966;96(3):464-471.
14. James JA, Harley JB. B-cell epitope spreading in autoimmunity. *Immunol Rev*. 1998;164:185-200.
15. Arnett FC, Hamilton RG, Roebber MG, Harley JB, Reichlin M. Increased frequencies of Sm and nRNP autoantibodies in American blacks compared to whites with systemic lupus erythematosus. *J Rheumatol*. 1988;15(12):1773-1776.
16. Lock RJ, Unsworth DJ. Antibodies to extractable nuclear antigens. Has technological drift affected clinical interpretation? *J Clin Pathol*. 2001;54(3):187-190.
17. Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med*. 2003;349(16):1526-1533.
18. Field M, Williams DG, Charles P, Maini RN. Specificity of anti-Sm antibodies by ELISA for systemic lupus erythematosus: Increased sensitivity of detection using purified peptide antigens. *Ann Rheum Dis*. 1988;47(10):820-825.
19. Yamamoto AM, Amoura Z, Johannet C, et al. Quantitative radioligand assays using de novo-synthesized recombinant autoantigens in connective tissue diseases: New tools to approach the pathogenic significance of anti-RNP antibodies in rheumatic diseases. *Arthritis Rheum*. 2000;43(3):689-698.
20. Yee CS, Hussein H, Skan J, Bowman S, Situnayake D, Gordon C. Association of damage with autoantibody profile, age, race, sex and disease duration in systemic lupus erythematosus. *Rheumatology (Oxford)*. 2003;42(2):276-279.
21. Alarcon-Segovia D, Cardiel MH. Comparison between 3 diagnostic criteria for mixed connective tissue disease. Study of 593 patients. *J Rheumatol*. 1989;16(3):328-334.
22. Azizah MR, Azila MN, Zulkifli MN, Norita TY. The prevalence of antinuclear, anti-dsDNA, anti-Sm and anti-RNP antibodies in a group of healthy blood donors. *Asian Pac J Allergy Immunol*. 1996;14(2):125-128.
23. Jones BR. Lacrimal and salivary precipitating antibodies in Sjogren's syndrome. *Lancet*. 1958; 2(7050):773-776.
24. Clark G, Reichlin M, Tomasi TB Jr. Characterization of a soluble cytoplasmic antigen reactive with sera from patients with systemic lupus erythmatosus. *J Immunol*. 1969;102(1):117-122.
25. Alspaugh MA, Tan EM. Antibodies to cellular antigens in Sjogren's syndrome. *J Clin Invest*. 1975;55(5):1067-1073.
26. Alspaugh M, Maddison P. Resolution of the identity of certain antigen-antibody systems in systemic lupus erythematosus and Sjogren's syndrome: An interlaboratory collaboration. *Arthritis Rheum*. 1979; 22(7):796-798.
27. van Venrooij WJ, Slobbe RL, Pruijn GJ. Structure and function of La and Ro RNPs. *Mol Biol Rep*. 1993;18(2):113-119.
28. Phan TG, Wong RC, Adelstein S. Autoantibodies to extractable nuclear antigens: Making detection and interpretation more meaningful. *Clin Diagn Lab Immunol*. 2002;9(1):1-7.
29. Skopouli FN, Andonopoulos AP, Moutsopoulos HM. Clinical implications of the presence of anti-Ro (SSA) antibodies in patients with rheumatoid arthritis. *J Autoimmun*. 1988;1(4):381-388.
30. Parodi A, Puiatti P, Rebora A. Serological profiles as prognostic clues for progressive systemic scleroderma: The Italian experience. *Dermatologica*. 1991;183(1):15-20.
31. Drosos AA, Andonopoulos AP, Costopoulos JS, Stavropoulos ED, Papadimitriou CS, Moutsopoulos HM. Sjogren's syndrome in progressive systemic sclerosis. *J Rheumatol*. 1988;15(6):965-968.
32. Fujimoto M, Shimozuma M, Yazawa N, et al. Prevalence and clinical relevance of 52-kDa and 60-kDa Ro/SS-A autoantibodies in Japanese patients with systemic sclerosis. *Ann Rheum Dis*. 1997;56(11):667-670.
33. Kubo M, Ihn H, Asano Y, Yamane K, Yazawa N, Tamaki K. Prevalence of 52-kd and 60-kd Ro/SS-A autoantibodies in Japanese patients with polymyositis/dermatomyositis. *J Am Acad Dermatol*. 2002;47(1):148-151.
34. Frank MB, McCubbin V, Trieu E, Wu Y, Isenberg DA, Targoff IN. The association of anti-Ro52 autoantibodies with myositis and scleroderma autoantibodies. *J Autoimmun*. 1999;12(2):137-142.
35. Rutjes SA, Vree Egberts WT, Jongen P, Van Den

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Hoogen F, Pruijn GJ, Van Venrooij WJ. Anti-Ro52 antibodies frequently co-occur with anti-Jo-1 antibodies in sera from patients with idiopathic inflammatory myopathy. *Clin Exp Immunol*. 1997;109(1):32-40.
36. Alexander EL, Provost TT. Cutaneous manifestations of primary Sjogren's syndrome: A reflection of vasculitis and association with anti-Ro(SSA) antibodies. *J Invest Dermatol*. 1983;80(5):386-391.
37. Manoussakis MN, Kistis KG, Liu X, Aidinis V, Guialis A, Moutsopoulos HM. Detection of anti-Ro(SSA) antibodies in autoimmune diseases: Comparison of five methods. *Br J Rheumatol*. 1993;32(6):449-455.
38. Franceschini F, Cavazzana I. Anti-Ro/SSA and La/SSB antibodies. *Autoimmunity*. 2005;38(1):55-63.
39. Brucato A, Frassi M, Franceschini F, et al. Risk of congenital complete heart block in newborns of mothers with anti-Ro/SSA antibodies detected by counterimmunoelectrophoresis: A prospective study of 100 women. *Arthritis Rheum*. 2001;44(8):1832-1835.
40. Douvas AS, Achten M, Tan EM. Identification of a nuclear protein (Scl-70) as a unique target of human antinuclear antibodies in scleroderma. *J Biol Chem*. 1979;254(20):10514-10522.
41. Guldner HH, Szostecki C, Vosberg HP, Lakomek HJ, Penner E, Bautz FA. Scl 70 autoantibodies from scleroderma patients recognize a 95 kDa protein identified as DNA topoisomerase I. *Chromosoma*. 1986;94(2):132-138.
42. Aeschlimann A, Meyer O, Bourgeois P, et al. Anti-Scl-70 antibodies detected by immunoblotting in progressive systemic sclerosis: Specificity and clinical correlations. *Ann Rheum Dis*. 1989;48(12):992-997.
43. Weiner ES, Earnshaw WC, Senécal JL, Bordwell B, Johnson P, Rothfield NF. Clinical associations of anticentromere antibodies and antibodies to topoisomerase I. A study of 355 patients. *Arthritis Rheum*. 1988;31(3):378-385.
44. Hildebrandt S, Weiner ES, Senecal JL, Noell GS, Earnshaw WC, Rothfield NF. Autoantibodies to topoisomerase I (Scl-70): Analysis by gel diffusion, immunoblot, and enzyme-linked immunosorbent assay. *Clin Immunol Immunopathol*. 1990;57(3):399-410.
45. Tsay GJ, Fann RH, Hwang J. Specificity of anti-Scl-70 antibodies in scleroderma: Increased sensitivity of detection using purified DNA topoisomerase I from calf thymus. *J Rheumatol*. 1990;17(10):1314-1319.
46. Walker UA, Tyndall A, Czirjak L, et al. Clinical risk assessment of organ manifestations in systemic sclerosis: A report from the EULAR Scleroderma Trials And Research group database. *Ann Rheum Dis*. 2007;66(6):754-763.
47. Tan EM, Rodnan GP, Garcia I, Moroi Y, Fritzler MJ, Peebles C. Diversity of antinuclear antibodies in progressive systemic sclerosis. Anti-centromere antibody and its relationship to CREST syndrome. *Arthritis Rheum*. 1980;23(6):617-625.
48. Rattner JB, Rees J, Arnett FC, Reveille JD, Goldstein R, Fritzler MJ. The centromere kinesin-like protein, CENP-E. An autoantigen in systemic sclerosis. *Arthritis Rheum*. 1996;39(8):1355-1361.
49. Saito A, Muro Y, Sugiura K, Ikeno M, Yoda K, Tomita Y. CENP-O, a protein localized at the centromere throughout the cell cycle, is a novel target antigen in systemic sclerosis. *J Rheumatol*. 2009;36(4):781-786.
50. Miyawaki S, Asanuma H, Nishiyama S, Yoshinaga Y. Clinical and serological heterogeneity in patients with anticentromere antibodies. *J Rheumatol*. 2005;32(8):1488-1494.
51. Reveille JD, Solomon DH. Evidence-based guidelines for the use of immunologic tests: Anticentromere, Scl-70, and nucleolar antibodies. *Arthritis Rheum*. 2003;49(3):399-412.
52. Meyer K. Ueber Hämagglutininvermehrung und Hämagglutination fordernde Wirkung bei menschlichen Seren. Ztschr. f. *Immunitätsforsch u exper Therap*. 1922;34:229-234.
53. Waaler E. On the occurrence of a factor in human serum activating the specific agglutination of sheep blood corpuscules. *Acta Pathol Microbiol Scand*. 1940;17:172-188.
54. Rose HM, Ragan C, Pearce E, Lipmann MO. Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis. *Proc Soc Exp Biol Med*. 1948;68(1):1-11.
55. Plotz CM, Singer JM. The latex fixation test. I. Application to the serologic diagnosis of rheumatoid arthritis. *Am J Med*. 1956;21(6):888-892.
56. Torrigiani G, Roitt IM. Antiglobulin factors in sera from patients with rheumatoid arthritis and normal subjects. Quantitative estimation in different immunoglobulin classes. *Ann Rheum Dis*. 1967;26(4):334-340.
57. Carson DA, Chen PP, Kipps TJ. New roles for rheumatoid factor. *J Clin Invest*. 1991;87(2):379-383.
58. Lee DM, Schur PH. Clinical utility of the anti-CCP assay in patients with rheumatic diseases. *Ann Rheum Dis*. 2003;62(9):870-874.
59. Sauerland U, Becker H, Seidel M, et al. Clinical utility of the anti-CCP assay: Experiences with 700 patients. *Ann N Y Acad Sci*. 2005;1050:314-318.
60. De Rycke L, Verhelst X, Kruithof E, et al. Rheumatoid factor, but not anti-cyclic citrullinated peptide antibodies, is modulated by infliximab treatment in rheumatoid arthritis. *Ann Rheum Dis*. 2005;64(2):299-302.
61. Dörner T, Egerer K, Feist E, Burmester GR. Rheumatoid factor revisited. *Curr Opin Rheumatol*. 2004;16(3):246-253.
62. Mimura Y, Ihn H, Jinnin M, Asano Y, Yamane K, Tamaki K. Rheumatoid factor isotypes in mixed connective tissue disease. *Clin Rheumatol*. 2006;25(4):572-574.
63. Schellekens GA, Visser H, de Jong BA, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum*. 2000;43(1):155-163.
64. Nienhuis RL, Mandema E. A new serum factor in patients with rheumatoid arthritis; the antiperinuclear factor. *Ann Rheum Dis*. 1964;23:302-305.
65. Simon M, Girbal E, Sebbag M, et al. The cytokeratin filament-aggregating protein filaggrin is the target of the so-called "antikeratin antibodies," autoantibodies specific for rheumatoid arthritis. *J Clin Invest*. 1993;92(3):1387-1393.
66. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Invest*. 1998;101(1):273-281.
67. Dorow DS, Shi PT, Carbone FR, Minasian E, Todd PE, Leach SJ. Two large immunogenic and antigenic myoglobin peptides and the effects of cyclisation. *Mol Immunol*. 1985;22(11):1255-1264.
68. Dubucquoi S, Solau-Gervais E, Lefranc D, et al. Evaluation of anti-citrullinated filaggrin antibodies as hallmarks for the diagnosis of rheumatic diseases. *Ann Rheum Dis*. 2004;63(4):415-419.