

Effective Use of Autoantibody Tests in the Diagnosis of Systemic Autoimmune Disease

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ABSTRACT: Screening for disease-specific autoantibodies may be useful in asymptomatic ANA-positive individuals as a means of evaluating the risk of developing a systemic autoimmune disease such as systemic lupus erythematosus (SLE), polymyositis/dermatomyositis (PM/DM), scleroderma (SSc), Sjögren's syndrome (SS), rheumatoid arthritis (RA), or primary biliary cirrhosis (PBC) in the future. In patients with known or suspected systemic autoimmune disease, a panel of disease-specific markers may help to establish a diagnosis and to assess the prognosis. The great strides in autoantibody testing over the last 20 years make it feasible to use specific autoantibody markers to improve diagnostic accuracy in systemic autoimmune disease. New technology enabling screening for multiple autoantibodies may further enhance the clinical usefulness of autoantibody testing, making it possible to diagnose autoimmune disease in its earliest stages and to intervene before serious end organ damage occurs.

KEYWORDS: antinuclear antibodies (ANA); asymptomatic; autoantibodies; scleroderma; Sjögren's syndrome; SLE; systemic autoimmune disease; test

INTRODUCTION

Disease-Specific Autoantibodies

Systemic autoimmune diseases are generally characterized by the production of autoantibodies that recognize a diverse array of cytoplasmic and nuclear antigens. It is important to distinguish between the terms "autoimmunity" and "autoimmune disease". Autoimmunity is an adaptive immune response (T- or B-cell mediated) against self-antigens either with or without concomitant clinical manifestations, whereas autoimmune disease implies the existence of clinical manifestations (e.g., kidney disease, arthritis, rashes, pleuritis) arising as a consequence of a T- or B-cell-mediated response to self. Thus, the production of antinuclear antibodies (ANA) in the absence of clinical manifestations constitutes autoimmunity, whereas the same antibodies accompanied by arthritis or glomerulonephritis would constitute an autoimmune disease.

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TABLE 1. Autoantibody associations with systemic autoimmune disease

Disease	Autoantibody to:	Sensitivity ^a		Specificity ^b		Onset prior to disease?
		London	Florida	London	Florida	
SLE	dsDNA	N/A		N/A		{10272, 12621} ^{7,8}
	Sm	7	10	100	100	{12621} ⁷
	Ribosomal P	3	2	100	100	N/A
	PCNA	3	0.3	100	100	N/A
PM/DM	Jo-1 (tRNA ^{his})	25	24	100	100	{12659} ⁵³
	PL-7 (tRNA ^{thr})	3	N/A	100	N/A	N/A
	PL-12 (tRNA ^{ala})	N/A	6	N/A	100	N/A
	EJ (tRNA ^{gly})	N/A	N/A	N/A	N/A	{4788} ¹⁰
	OJ (tRNA ^{ile})	N/A	3	N/A	100	N/A
SSc	Scl-70	16	N/A	100	N/A	{3803} ⁹
	Fibrillarin	N/A	2	N/A	100	N/A
	RNAP I/III	N/A	21	N/A	100	N/A
	Th (7-2 RNP)	N/A	N/A	N/A	N/A	{1444} ⁵²
SS	Ro (SSA)	75	54	87	82	{12621} ⁷
	La (SSB)	42	26	96	94	{12621} ⁷
RA	CCP ^c	N/A	65	N/A	N/A	{12657, 12658} ^{13,14}
PBC	Pyruvate dehydrogenase	88	N/A	100	N/A	{7367, 7370} ^{3,4}

^aPrevalence of the autoantibody in patients with the associated disease (number positive/number with disease × 100%) from reference 54 {2684} and our own data.

^bEstimated specificity for the disease.

^cCCP: Cyclic citrullinated peptide.

Autoantibodies can be used as adjuncts to diagnose autoimmune disease, to monitor disease activity and severity, and to predict the outcome of autoimmune disease. The fluorescent ANA assay using HEp-2 cells is a good initial screening test, but is not specific for a particular diagnosis. It provides information on the presence of serum autoantibodies as well as the subcellular localization(s) of the antigens they recognize.¹ In one population-based study of ANA-positive Caucasians, 18.8% had systemic lupus erythematosus (SLE), 10.9% had drug-induced lupus, 21.7% had other systemic autoimmune diseases (e.g., Sjögren's syndrome, myositis, scleroderma), 10.1% had autoimmune thyroiditis, 5.8% had other organ-specific autoimmune diseases, 8.3% had infections, 2.9% had neoplasms, and 24.3% had other conditions or "idiopathic" autoantibodies.² In view of this lack of specificity, attention has focused on tests for disease-specific autoantibodies that can be used to assess diagnosis or prognosis (TABLE 1).

AUTOANTIBODIES APPEAR YEARS BEFORE THE ONSET OF AUTOIMMUNE DISEASE

Autoantibody production can be the harbinger of autoimmune disease, especially in the case of disease-specific autoantibodies, which may appear months, years, or even decades before the onset of clinical symptoms. Detection of disease-specific autoantibodies in asymptomatic individuals may permit earlier diagnosis and preventative treatment. A striking example is antimitochondrial antibodies in primary biliary cirrhosis (PBC).

Antibodies against the mitochondrial antigen dihydrolipoamide acetyltransferase (E2), a component of pyruvate dehydrogenase, can appear in asymptomatic individuals decades prior to the onset of PBC.^{5,4} In one study of 29 asymptomatic individ-

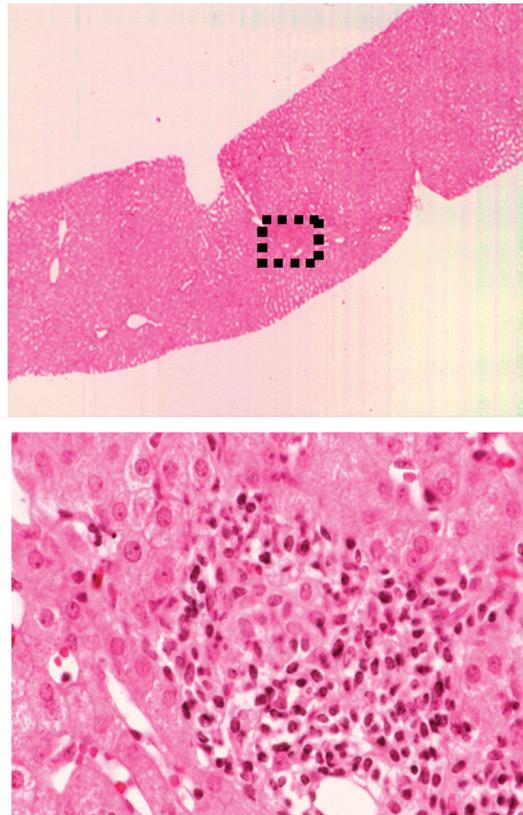


FIGURE 1. Liver biopsy from an SLE patient who developed new onset antimitochondrial antibodies. **(Top)** Low-power view of hematoxylin and eosin staining. An area of inflammatory cell infiltration is apparent within the box. **(Bottom)** High-power view of the area within the box showing mild periportal lymphocytic infiltration.

uals with antimitochondrial antibodies, 22 (76%) developed symptoms of PBC over an 11- to 24-year follow-up period.³ Antimitochondrial antibodies also may appear before the onset of PBC in the context of another systemic autoimmune disease. For example, we evaluated a 32-year-old woman with SLE with photosensitive malar rash, polyarthritis, Raynaud's phenomenon, a positive ANA, and anti-dsDNA antibodies by *Crithidia luciliae* kinetoplast staining who subsequently developed anti-cytoplasmic autoantibodies. She complained of mild right upper quadrant pain and had a mildly elevated alkaline phosphatase 187, normal AST and ALT, and an elevated serum IgM level of 323 mg/dL (normal range 25–210 mg/dL). IgG and IgA values were normal. Antimitochondrial antibodies were positive at a titer of 1:80. A liver biopsy (FIG. 1) revealed periportal lymphocytic infiltrates, suggesting that she had asymptomatic early PBC. She was treated with ursodeoxycholate, and her alkaline phosphatase and serum IgM levels normalized.

A variety of autoantibodies have been reported to precede the onset of SLE.^{5,6} ANA, anti-Ro, anti-La, and antiphospholipid antibodies may be present for extended periods before the onset of autoimmune disease, whereas anti-Sm and anti-nRNP are thought to appear much closer to the onset of disease.⁷ Anti-dsDNA antibodies are intermediate. In one study using stored serum samples from military recruits, 55% of individuals who subsequently developed SLE had positive anti-dsDNA antibodies. Anti-dsDNA antibodies were detected as long as 9.3 years before diagnosis, with a mean of 2.7 years.⁸ In the same cohort, at least one lupus autoantibody was present before diagnosis in 88% of patients, and ANA were present in 78%.⁷ Thus, many or most cases of lupus are preceded by serological abnormalities. Much less is known, however, regarding the likelihood that asymptomatic individuals with lupus autoantibodies will ultimately develop SLE.

Scleroderma-associated autoantibodies also predate disease onset. Anticentromere antibodies, a marker for limited scleroderma, can develop years before the onset of scleroderma or CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysfunction, sclerodactyly, telangiectasias), and their presence in individuals with Raynaud's phenomenon is associated with the development of telangiectasias over a period of 1–11 years.⁹ In the same study, anti-Scl70 (topoisomerase I) autoantibodies, a marker for diffuse scleroderma, were strongly associated with the subsequent development of skin tightening. Patients who had either of these autoantibodies were 63-fold more likely to develop signs of connective tissue disease by the end of the 11-year observation period.⁹

The production of autoantibodies against tRNA synthetases also may be seen years before the onset of myositis¹⁰ or may shift with alterations in disease manifestations. Autoantibodies also frequently precede the onset of rheumatoid arthritis (RA). Rheumatoid factor (RF) has been detected in RA patients months to years before the onset of clinical symptoms of RA,^{11,12} and the presence of RF is associated with a 20- to 40-fold greater risk of developing RA. Although the risk is relatively low (~10–15%),¹² it is highest in those with high RF titers.¹¹ Autoantibodies to citrulline-modified peptides precede the development of RA by several years.¹³ In one study, 93% of patients with these antibodies who were diagnosed with undifferentiated arthritis developed RA within 3 years.¹⁴

As in the case of systemic autoimmune disease, the onset of organ-specific autoimmune diseases, such as type I diabetes and autoimmune thyroiditis, is frequently preceded by the appearance of specific autoantibodies. Type I diabetes is associated

with autoantibodies against insulin, glutamate decarboxylase, and islet cells, which appear before the onset of clinical manifestations. The numbers of autoantibodies against these three antigens, not their specificities, best predict the risk of developing type I diabetes.¹⁵ Among first-degree relatives of patients with type 1 diabetes, the 5-year risk of developing diabetes is 0% if no antibody is present, 15% if 1 antibody is present, 44% if 2 antibodies are present, and 100% if all 3 antibodies are present.¹⁵ About 30–60% of family members of patients with type I diabetes with one of the diabetes-related antibodies develop the disease within 5–10 years.^{15–18} Likewise, the presence of thyroid peroxidase antibodies is predictive of the development of elevated TSH or hypothyroidism.^{19,20}

These data indicate that disease-specific autoantibodies are useful predictors of the future development of autoimmune disease. However, information about their frequency in at-risk subsets or in the general population is incomplete, and the risk factors determining whether an individual who produces one of these autoantibodies will remain asymptomatic or evolve an autoimmune disease have not been defined.

EVALUATION OF AN ASYMPTOMATIC POSITIVE ANA TEST

A positive ANA test in an asymptomatic individual prompts many referrals to autoimmune disease specialists.²¹ In many cases, this is not a cause for concern because some healthy individuals have low-titer ANA.^{22,23} The prevalence of a positive ANA is 3–5% in randomly selected healthy Caucasians,²³ but prevalence is strongly age dependent. It is estimated that 10–15% of healthy people over the age of 65 years are ANA positive, although the titers are usually $\leq 1:160$.²² Approximately 3% of normal individuals are ANA positive at a 1:320 serum dilution, and 32% are ANA positive at 1:40 serum dilution.^{24,25} Nevertheless, in view of the evidence summarized here that disease-specific autoantibodies are highly predictive of the future development of systemic autoimmune disease, an algorithm such as the one shown in FIGURE 2 may be useful for the differential diagnosis of an asymptomatic positive ANA test. This algorithm is based on the immunofluorescence pattern: nucleoplasmic, nucleolar, or cytoplasmic. Nucleoplasmic fluorescence is further categorized as homogeneous, speckled, peripheral, or centromere.

A diverse group of autoantibodies produce homogeneous-, speckled-, peripheral-, or centromere-specific nucleoplasmic staining. Because of the common occurrence of more than one pattern, it is best to consider all nucleoplasmic staining, with the exception of anticentromere staining, under a single differential diagnosis.

Homogeneous Nucleoplasmic Pattern

The differential diagnosis of homogeneous nucleoplasmic staining includes anti-dsDNA, antichromatin, antihistone, and anti-Sc170 (topoisomerase) antibodies. Two of these specificities are disease specific: anti-dsDNA for SLE and anti-Sc170 (topoisomerase I) for scleroderma (see below). Antichromatin and antihistone antibodies are found in a variety of autoimmune disorders and may be helpful for evaluating drug-induced lupus. Like anti-dsDNA, they frequently increase with lupus activity and decrease in remission, but they are poor predictors of disease outcome.

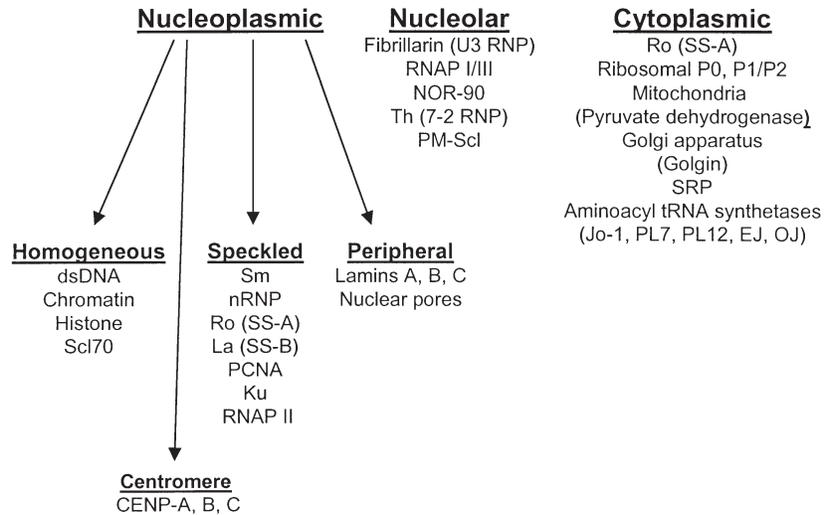


FIGURE 2. Algorithm for the assessment of a positive fluorescent “ANA” test in asymptomatic individuals. Immunofluorescence staining is classified as nucleoplasmic (homogeneous, speckled, peripheral, or centromere), nucleolar, or cytoplasmic, and the specificities of major types of autoantibodies producing these patterns are indicated.

Speckled Nucleoplasmic Pattern

The differential diagnosis of speckled nucleoplasmic staining includes anti-Sm and anti-nRNP (autoantibodies recognizing the U1, U2, U4–6, and U5 small nuclear ribonucleoproteins), anti-Ro60 (autoantibodies recognizing the Y1–5 small ribonucleoproteins), and anti-La (autoantibodies recognizing a 45 kDa protein associated with small RNAs synthesized by RNA polymerase III). The Ro60 antigen is mainly cytoplasmic, although speckled nucleoplasmic staining has been described in some cases. Autoantibodies against proliferating cell nuclear antigen (PCNA), Ku (p70/p80) antigen, and RNA polymerase II (RNAP II) also produce speckled nucleoplasmic staining. Anti-Sm antibodies are pathognomonic of SLE,¹ and when detected in asymptomatic individuals, the onset of SLE generally follows within a year.⁷ Anti-PCNA autoantibodies are uncommon but relatively specific for SLE. Their predictive value in asymptomatic individuals is not known. Anti-Ro60 and anti-La are frequently, but not invariably, associated with the development of sicca manifestations regardless of the underlying autoimmune disease. Autoantibodies to RNAP II and Ku are strongly associated with systemic autoimmune disease, but are not specific for a particular subset. There is some evidence that anti-Ku antibodies identify a clinical subset at risk for myositis. Anti-RNAP II autoantibodies are seen in both lupus and scleroderma and may have prognostic significance in the latter (see below).

Peripheral Nucleoplasmic Pattern

Peripheral nucleoplasmic staining results from autoantibodies against components of the nuclear envelope: nuclear lamins A, B, and C and nuclear pore complexes. Autoantibodies to the lamins are associated with SLE, antiphospholipid antibodies, and autoimmune hepatitis,^{26–28} whereas autoantibodies to nuclear pore complexes are seen in PBC.²⁹

Centromere Pattern

Centromere staining is associated with scleroderma or CREST. The fluorescence pattern consists of discrete nucleoplasmic dots in interphase cells that remain associated with the condensed chromosomes of mitotic cells, making this a distinctive pattern that does not usually require further evaluation.¹

Nucleolar Pattern

Nucleolar staining is associated with scleroderma.³⁰ Autoantibodies associated with a nucleolar staining pattern include antifibrillarin (U3 RNP), anti-RNA polymerase I/III (RNAP I/III), anti-NOR-90, and anti-Th (7–2 RNP). With the exception of anti-NOR-90, which may be less disease specific than once thought,^{31,32} all of these autoantibodies are highly specific for scleroderma³⁰ and have both diagnostic and prognostic significance (see below). Autoantibodies against the PM-Scl antigen, which are associated with polymyositis-scleroderma overlap syndrome, also give nucleolar staining. Although most frequent in the overlap syndrome, they are seen in patients with either polymyositis or scleroderma alone but have been reported in patients with neither myositis nor scleroderma, as well.³³

Cytoplasmic Pattern

The differential diagnosis of cytoplasmic staining includes anti-Ro (SS-A); the ribosomal P0, P1, and P2 antigens; signal recognition peptide (SRP); antimitochondrial antibodies (generally specific for pyruvate dehydrogenase); and an assortment of myositis-associated autoantibodies specific for various aminoacyl tRNA synthetases, including the enzymes specific for tRNA^{his} (Jo-1), tRNA^{thr} (PL-7), tRNA^{ala} (PL-12), tRNA^{gly} (EJ), tRNA^{ile} (OJ), and others.³⁴ Antiribosomal P autoantibodies, which recognize the P0, P1, and P2 antigens, are highly specific for SLE, and antimitochondrial autoantibodies are a diagnostic marker for PBC (TABLE 1).

USE OF AUTOANTIBODIES FOR DIAGNOSIS AND PROGNOSIS

For individuals with known or suspected systemic autoimmune disease, the detection of specific autoantibodies may be valuable both for confirming the clinical diagnosis and for assessing the prognosis.

SLE

Although the sensitivity of a positive fluorescent ANA test for lupus ranges from 90% to 95% or more,^{35,36} the specificity is low^{2,37} and the positive predictive value

is only 11–13%.^{36,38} By contrast, autoantibodies against Sm, dsDNA, the ribosomal P antigens (P0, P1, and P2), and PCNA are highly specific for SLE (TABLE 1). Increasing anti-dsDNA antibody levels may herald exacerbations of lupus nephritis or other organ involvement, and it has been suggested that corticosteroid therapy may be warranted to prevent flares in patients with an increasing anti-dsDNA antibody titer, even in the absence of other clinical evidence.³⁹

Anti-Sm antibodies are virtually pathognomonic for SLE and are detected in approximately 7–25% of lupus patients, depending on ethnic origin.¹ Unlike anti-dsDNA, the levels of anti-Sm antibodies do not correlate with disease activity. Anti-nRNP antibodies are associated with anti-Sm (virtually all anti-Sm sera are anti-nRNP positive), but are not disease specific (TABLE 1). Their prevalence in SLE is 20–40%. Antiribosomal P antibodies are reportedly associated with neuropsychiatric manifestations of lupus,⁴⁰ although this is somewhat controversial.⁴¹ They are, however, highly specific for the diagnosis of SLE (TABLE 1).

Sjögren's Syndrome

Anti-Ro (SS-A) and La (SS-B) autoantibodies are seen in Sjögren's syndrome and other systemic autoimmune diseases, such as SLE, myositis, and scleroderma, when they are accompanied by sicca symptoms. Anti-Ro60 (SS-A) antibodies are found in 10–50% of SLE and 60–80% of primary Sjögren's syndrome sera.⁴² Approximately 10–20% of SLE patients and a somewhat higher percentage of Sjögren's syndrome patients are anti-La (SS-B) positive. Anti-La is virtually always associated with anti-Ro, whereas anti-Ro60 antibodies frequently are detected without anti-La. The 52-kDa Ro52 antigen is recognized by autoantibodies in many sera from patients with Sjögren's syndrome and is associated with anti-Ro60. Anti-Ro52 is seen in the absence of anti-Ro60 in patients with polymyositis^{43,44} and less frequently in other disorders. In addition to being associated with sicca syndrome, autoantibodies to Ro52 and La (SS-B) are associated with cardiac conduction abnormalities in neonates.⁴⁵ Pregnant women with systemic autoimmune disease and asymptomatic mothers of children with congenital cardiac conduction abnormalities should be screened for these antibodies.

Polymyositis and Dermatomyositis

Polymyositis (PM) and dermatomyositis (DM) are associated with autoantibodies against a group of aminoacyl tRNA synthetases, the most common of which is anti-Jo-1 (histidyl tRNA synthetase), which is produced by approximately 20–25% of adult myositis patients. Other autoantibodies in this group are found in 1–4% of myositis patients. However, because only one antisynthetase autoantibody is usually detected in an individual patient, they are, in aggregate, relatively common. All are highly specific for myositis (TABLE 1) and are associated with a constellation of symptoms (myositis \pm skin involvement, interstitial lung disease, Raynaud's phenomenon, inflammatory arthritis, fever, and mechanic's hands) known as antisynthetase autoantibody syndrome.⁴⁶ Anti-SRP autoantibodies also are highly specific for polymyositis and are associated with severe disease but not with antisynthetase autoantibody syndrome. Anti-Mi-2 autoantibodies are a dermatomyositis marker generally associated with a relatively more favorable long-term prognosis.

Scleroderma

Anti-Scl70 antibodies are virtually pathognomonic of scleroderma and predict internal organ involvement, proximal scleroderma, and a poor outcome.^{30,47,48} Patients who develop both anti-Scl70 and anti-RNA polymerase II autoantibodies have an even worse prognosis.⁴⁹ Interestingly, anti-Scl70 autoantibodies are lost by a subset of patients, portending a more favorable outcome.⁵⁰ Like anti-Scl-70, anti-RNAP I/III autoantibodies are associated with severe disease and poor outcome.⁵¹ This is the most common disease-specific marker for scleroderma, with a sensitivity of 21% and 100% specificity (TABLE 1).

Antifibrillar autoantibodies, specific for the nucleolar U3 small ribonucleoprotein, are nearly 100% specific for scleroderma and are found in 2–8% of scleroderma sera (see ref. 30 and TABLE 1). The frequency of anti-Th (7–2 ribonucleoprotein) antibodies, another disease-specific marker for scleroderma, is approximately 4%.⁵² Interestingly, 3 of 244 controls were positive for anti-Th, all of whom had primary Raynaud's phenomenon of less than 2 years duration, raising the possibility that these individuals may go on to develop additional manifestations of scleroderma in the future. The PM-Scl antigen is a nucleolar/cytoplasmic complex of 11 proteins reported to be recognized by autoantibodies in approximately 3% of scleroderma, 8% of polymyositis, and 50% of polymyositis-scleroderma overlap syndrome sera.³⁰

Patients with limited symptoms and positive centromere staining have a high likelihood of developing additional manifestations of CREST syndrome. The centromere autoantigens recognized most commonly by these sera are CENP-A, -B, and -C.³⁰ Of these, CENP-B is the most important for predicting the subsequent onset of additional manifestations of CREST syndrome, especially telangiectasias.⁹

CONCLUSIONS

Screening for disease-specific autoantibodies may be useful in asymptomatic ANA-positive individuals as a means of evaluating the risk of developing a systemic autoimmune disease such as SLE, PM/DM, scleroderma, Sjögren's syndrome, RA, or PBC in the future. In this situation, a diagnostic algorithm such as that illustrated in FIGURE 2 may be employed. In patients with known or suspected systemic autoimmune disease, a panel of disease-specific markers may help to establish a diagnosis and to assess the prognosis. A panel for SLE should include assays for anti-dsDNA, anti-Sm, anti-nRNP, anti-ribosomal P, and anti-PCNA. A Sjögren's syndrome panel might include anti-Ro60 (SS-A), anti-Ro52, and anti-La (SS-B). A scleroderma panel would include anti-Scl70, anti-RNA polymerase I/III, antifibrillar, anti-Th (7–2) ribonucleoprotein, and anticentromere. A myositis panel would include anti-Jo-1, anti-PL-7, anti-PL-12, anti-EJ, anti-OJ, and anti-SRP as well as possibly anti-Mi-2 (specific for dermatomyositis) and anti-Ro52. The great strides in autoantibody testing over the last 20 years make it feasible to use specific autoantibody markers to improve diagnostic accuracy in systemic autoimmune disease. New technology enabling screening for multiple autoantibodies may further enhance the clinical usefulness of autoantibody testing, making it possible to diagnose autoimmune disease in its earliest stages and to intervene before serious end organ damage occurs.

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