Systemic Sclerosis

Laboratory Markers for Diagnosis and Prognosis

CLINICAL BACKGROUND

Systemic sclerosis (SSc) is a chronic, multisystem, heterogeneous autoimmune disease. Individuals with SSc have a mortality rate approximately 2.8 times that of the general population.[1] In the United States, the incidence is approximately 15 cases per 100,000 person-years.[2] The disease is characterized by inflammation, vasculopathy, and progressive fibrosis of the skin and internal organs.[3] SSc is frequently referred to as scleroderma; however, scleroderma includes SSc and localized forms of scleroderma that affect only the skin.[4]

The 2 main types of SSc are defined according to the pattern of skin involvement: limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc). In lcSSc, skin thickening is present distal to the elbows and knees, and facial skin thickening may or may not be present. In contrast, dcSSc is characterized by thickening of the skin of the whole extremity, as well as that of the anterior chest, abdomen, and back, with or without facial skin involvement.[4,5] Multiple organs, including the heart, lungs, gastrointestinal tract, and kidneys, can be affected in both forms, though organ involvement is generally less severe in lcSSc.[4,5] CREST syndrome (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) occurs frequently in lcSSC but can also occur in longstanding dcSSc.[4]

Of patients with SSc, approximately 55% have lcSSc and 35% have dcSSc.[4] The remaining 10% of patients with SSc have sine scleroderma or an overlap syndrome.[4] Sine scleroderma is a form of SSc that has characteristic clinical features but spares the skin.[5] Overlap syndromes occur when features of another autoimmune disease are present along with SSc.[5]

Early treatment of SSc can improve outcomes, so prompt diagnosis is important. However, diagnosis can be challenging because many patients present with nonspecific symptoms such as Raynaud phenomenon, gastroesophageal reflux, puffy fingers, and fatigue.[5] In addition, patients with other autoimmune disorders may present with symptoms suggestive of SSc. Testing for autoantibodies that are associated with SSc assists diagnosis and can help predict organ involvement and severity of disease.

This Clinical Focus discusses the role of autoantibody testing in the diagnosis and categorization of SSc, as well as in the assessment of prognosis. The material is provided for educational purposes only and is not intended as medical advice. Test selection and interpretation, diagnosis, and patient management decisions should be based on the physician's education, specialization, clinical expertise, and clinical and laboratory assessment of the patient.

INDIVIDUALS SUITABLE FOR TESTING

- Individuals with suspected SSc (ie, those with capillary nailfold changes, Raynaud phenomenon, and/or characteristic skin changes)
- Individuals with unexplained, but suggestive, musculoskeletal, gastrointestinal, cardiac, or pulmonary symptoms

TEST AVAILABILITY

Tests available for the diagnosis of SSc and assessment of prognosis are shown in **Table 1.**

TEST SELECTION AND INTERPRETATION

Diagnosis and Prognosis

Early diagnosis of SSc is important to delay, mitigate, or avoid irreversible end-organ damage. The diagnosis is based primarily on the presence of characteristic clinical findings and autoantibodies. Classification criteria are available from the American College of Rheumatology-European League Against Rheumatism (ACR-EULAR) and have 91% sensitivity and 92% specificity for SSc (**Table 2**).[6] Autoantibody positivity may be present early in the course of the disease and assist in diagnosis of patients with nonspecific signs and symptoms. Autoantibody patterns can also help distinguish the various types of SSc (ie, lcSSc, dcSSc, sine scleroderma, overlap syndrome) and the organ(s) likely to be involved, which is important for determining prognosis and optimal treatment.[7,8]

The following sections describe the autoantibodies associated with SSc and their relevance for diagnosis and prognosis. A summary of the autoantibodies, their prevalences, and their clinical associations is provided in **Table 3**. An algorithm showing the use of antibody testing for SSc diagnosis is presented in the **Figure**.

Antinuclear Antibody

Antinuclear antibody (ANA) immunofluorescence assay (IFA) is the initial test for detecting autoantibodies in a patient presenting with nonspecific symptoms, including Raynaud phenomenon. ANA is present in 85% to 95% of patients with SSc; however, ANA is also present in many other autoimmune

Table 1. Autoantibody Testing Used in the Diagnosis and Prognosis of Systemic Sclerosis

Test code	Test name[a]	Clinical use
249[b]	ANA Screen, IFA, with Reflex to Titer and Pattern	Diagnose autoimmune disease
16814[b]	ANA Screen, IFA, with Reflex to Titer and Pattern and Reflex to Multiplex 11 Antibody Cascade Includes ANA screen, IFA, with Reflex to Titer and Pattern (249) and Reflex to dsDNA (255), Sm/RNP (38567), RNP (19887), Sm (37923), and chromatin antibodies (34088); if all 5 antibodies are negative, reflex to SS-A (38568), SS-B (38569), Scl-70 (4942), and Jo-1 antibodies (5810); if all 4 of these antibodies are negative, reflex to ribosomal P (34283) and centromere B antibodies (16088).	Diagnose autoimmune disease
36378[b]	ANAlyzeR™ ANA, IFA with Reflex Titer/Pattern, Systemic Autoimmune Panel 1 Includes 14-3-3η protein (91455); ANA screen, IFA, with Reflex to Titer and Pattern (249); beta-2-glycoprotein I antibodies (IgG, IgA, IgM) (30340); cardiolipin antibodies (IgA, IgG, IgM) (7352); CCP antibody (IgG) (11173); centromere B antibody (16088); chromatin (nucleosomal) antibody (34088); complement component C3c and C4c (5704); DNA (ds) antibody, Crithidia IFA with Reflex to Titer (37092); Jo-1 antibody (5810); scleroderma antibody (Scl-70) (4942); Sjögren antibodies (SS-A and SS-B) (7832), Sm antibody (37923); Sm/RNP antibody (38567); RF (IgG, IgA, IgM) (19705); thyroid peroxidase antibodies (TPO) (5081).	Diagnose autoimmune disease
94954[b,c]	ANA Screen, IFA, Reflex Titer/Pattern, Reflex Mplx 11 Ab Cascade with IdentRA® Includes ANA screen, IFA, with Reflex to Titer and Pattern and Reflex to Multiplex 11 Antibody Cascade (16814); RF (4418); CCP antibody (IgG) (11173); 14-3-3η protein (91455).	Diagnose autoimmune disease
90073[b]	ANA Screen, IFA, with Reflex to Titer and Pattern/Systemic Sclerosis Panel 1 Includes ANA screen, IFA, with Reflex to Titer and Pattern (249) and Reflex to centromere B antibody (16088); RNA polymerase III (19899); scleroderma antibody (Scl-70) (4942).	Diagnose SSc
16088	Centromere B Antibody	Diagnose SSc; determine prognosis
18855	Ku Autoantibodies	Determine prognosis of SSc
19899	RNA Polymerase III Antibody	Diagnose SSc; determine prognosis
4942	Scleroderma Antibody (Scl-70)	Diagnose SSc; determine prognosis
38567[d]	Sm/RNP Antibody	Determine prognosis of SSc
94685[e]	Systemic Sclerosis (Scleroderma) 12 Antibodies Panel 2 Includes centromere protein (CENP)-A, CENP-B, fibrillarin (U3-RNP), PM/Scl-75, PM/Scl-100, RP11 (RNA polymerase III), RP155 (RNA polymerase III), Scl-70, Th/To, U1-snRNP RNP-70kd, U1-snRNP RNP A, and U1-snRNP RNP C antibodies.	Diagnose SSc; determine prognosis

ANA, antinuclear antibodies; IFA, immunofluorescence assay; SSc, systemic sclerosis.
[a] Numbers in parentheses represent test codes for individual components.
[b] Reflex tests are performed at an additional charge and are associated with an additional CPT code.
[c] This test was developed and its analytical performance characteristics have been determined by Quest Diagnostics. It has not been cleared or approved by the US Food and Drug Administration. This assay has been validated pursuant to the CLIA regulations and is used for clinical purposes.
[d] Sm/RNP is also called U1-snRNP and U1-RNP.
[e] This panel is run as a single line blot; thus, not all components are available as individual tests.

disorders.[9,19] Thus, a positive ANA IFA test should be followed with testing for more specific antibodies such as centromere B, Scl-70 (topoisomerase I), and RNA polymerase III antibodies.[20] A negative ANA IFA test decreases the likelihood of SSc but does not rule it out.

Centromere Antibodies

Centromere antibodies can be directed against a number of centromere proteins (CENP), including CENP-A, -B, and -C, though CENP-B is thought to be the main target.[7,21] These antibodies are found in 20% to 40% of patients with SSc

Table 2. ACR-EULAR Systemic Sclerosis Classification Criteria[6]

Classify a patient as having systemic sclerosis if the sum of points for criteria below is ≥9.	
Criterion	**Points**
Skin thickened on fingers of both hands, extending proximal to the metacarpophalangeal joints	9
Skin on fingers thickened (only count highest score)	
Puffy fingers	2
Sclerodactyly[a]	4
Lesions on fingertips (only count highest score)	
Ulcers on tip of digits	2
Pitting scars on fingertips	3
Telangiectasia	2
Abnormal nailfold capillaries	2
Pulmonary arterial hypertension and/or interstitial lung disease (max score is 2)	
Pulmonary arterial hypertension	2
Interstitial lung disease	2
Raynaud phenomenon	3
Presence of 1 or more of the following[b]:	3
Centromere antibody	
Scl-70 antibody	
RNA polymerase III antibody	

ACR-EULAR, American College of Rheumatology-European League Against Rheumatism.
[a] Distal to metacarpophalangeal joints but proximal to proximal interphalangeal joints.
[b] Maximum score is 3.

and are associated with the lcSSc subtype.[7] Furthermore, centromere antibodies are included in the 2013 ACR-EULAR classification criteria.[6] However, these antibodies can occur in other connective tissue diseases, including systemic lupus erythematosus (SLE), primary biliary cholangitis, rheumatoid arthritis, and Sjögren syndrome.[7]

Presence of centromere antibodies is considered predictive of SSc development in patients with Raynaud phenomenon.[21] The antibodies are associated with lower risk of cardiac and renal involvement but higher risk of PAH.[7,21] For patients without PAH, centromere antibodies are associated with a better prognosis overall than are other autoantibodies.[21]

Ku Antibody

The Ku protein is involved in repair of DNA damage and regulation of nuclear proteins. Ku antibodies are uncommon and are detected in about 1.5% to 5% of patients with SSc.[7] These antibodies are associated with overlap syndromes and the lcSSc subtype.[7,10] Presence is not specific for SSc, as the antibodies are also seen in patients with other autoimmune disorders, including undifferentiated connective tissue disease, polymyositis/dermatomyositis, and SLE.[10]

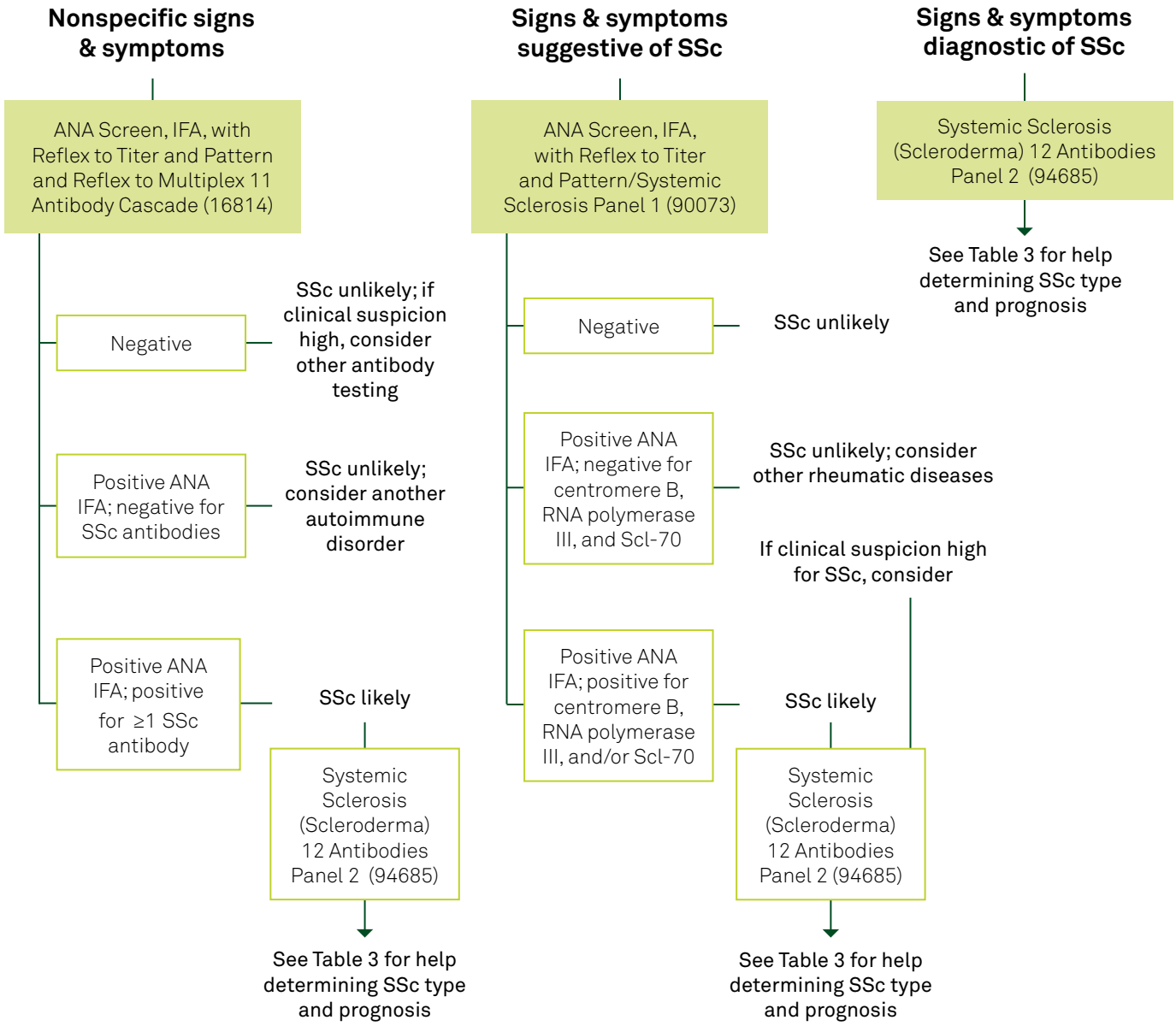
Ku antibody testing may be more useful prognostically, as presence is strongly associated with muscle and joint involvement; antibody presence also appears to be protective against vascular complications such as digital ulcers.[7,8] Internal organ involvement is generally mild and manifests most often as ILD or PAH.[7]

PM/Scl Antibody

PM/Scl antibodies target the PM/Scl exosome complex, and most reactivity is against 2 proteins, PM/Scl-75 and PM/Scl-100. They are present in 2% to 11% of SSc patients and are associated with SSc-myositis overlap syndrome and lcSSc.[7,8] PM/Scl antibodies also occur in other autoimmune diseases such as polymyositis and dermatomyositis.

The presence of either PM/Scl-75 or PM/Scl-100 antibody is associated with calcinosis, and the co-occurrence of both antibodies is associated with inflammatory myositis.[11,12] However, each antibody may be associated with a distinct clinical phenotype; gastrointestinal symptoms and ILD are common in patients with PM/Scl-75 antibodies but less so in those with PM/Scl-100 antibodies.[11,12] Internal organ involvement generally remains mild in patients with any

Figure. Autoantibody Testing for Systemic Sclerosis Diagnosis and Determination of Prognosis



ANA, antinuclear antibodies; IFA, immunofluorescence assay; SSc, systemic sclerosis.

Test codes are included in parentheses. SSc antibodies in the 11-antibody cascade include U1-snRNP, Scl-70, and centromere B antibodies.

This figure was developed by Quest Diagnostics based in on references 4, 16, and 20. It is provided for informational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient management decisions should be based on his/her education, clinical expertise, and assessment of the patient.

Table 3. Clinical Characteristics Associated With Autoantibodies Used in the Diagnosis and Classification of Systemic Sclerosis[a]

Antibody	Prevalence in SSc, %	Presence in other autoimmune diseases	SSc type most likely	Clinical associations	Relative SSc prognosis
ANA[9]	85-95	Common	Does not help differentiate type	Most common in rheumatic diseases	Varies
Centromere A or B[7,8]	20-40[b]	Uncommon	lcSSc	PAH, calcinosis, digital ischemia, intestinal involvement	Better
Ku[7,10]	1.5-5	Common	Overlap syndromes or lcSSc	Muscle and joint involvement	Unclear
PM/Scl-75 or PM/Scl-100[7,8,11,12]	2-11	Common	SSc-myositis overlap syndrome or lcSSc	Muscle and joint involvement, calcinosis; GI and lung involvement for those with PM/Scl-75	Better
RNA Polymerase III[7,8,13,14]	7-41	Rare	dcSSc	Renal crisis, progressive skin thickening, GAVE, malignancy	Poorer
Scl-70[7,8,15]	9.4-71[b]	Rare	dcSSc	Severe pulmonary fibrosis, cardiac involvement, renal crisis, digital ulcers	Poorer
Th/To[7,8]	2-5	Rare	lcSSc	Pulmonary involvement	Poorer
U1-snRNP[7,16]	2-14	Common (90% in patients with MCTD)	lcSSc	PAH, arthritis, esophageal dysfunction	Better
U3-RNP (Fibrillarin)[7,8]	4-10[b]	Rare	dcSSc	Multiorgan involvement, PAH	Poorer

ANA, antinuclear antibodies; dcSSc, diffuse cutaneous systemic sclerosis; GAVE, gastric antral vascular ectasia; GI, gastrointestinal; lcSSc, limited cutaneous systemic sclerosis; MCTD, mixed connective tissue disease; PAH, pulmonary arterial hypertension; SSc, systemic sclerosis.
[a] Data presented in this table have been compiled from the cited references. It is important to note that autoantibody frequency in SSc as well as other autoimmune diseases varies with sex, ethnicity, and population studied.[17]
[b] In the United States, prevalence has been shown to vary across ethnicities.[18]

PM/Scl antibodies, and the presence of these antibodies is associated with an overall favorable prognosis.[7]

RNA Polymerase III Antibodies

RNA polymerase III antibodies target RNA polymerase epitopes 11 and 155 and are thus also known as anti-RP11 and anti-RP155. These antibodies are found in 7% to 41% of patients with SSc and occur most often in dcSSc.[8,14] They are diagnostic for SSc, as they are rarely found in other autoimmune diseases, and are included in the 2013 ACR-EULAR classification criteria.[6]

The presence of RNA polymerase III antibodies is associated with progressive skin thickening, gastric antral vascular ectasia (GAVE), and renal crisis.[7,8] In addition, these antibodies are associated with onset of cancer within a 2-year timeframe before or after onset of SSc skin changes.[13] Historically, RNA polymerase III antibodies indicated a poor prognosis, but mortality rates improved after the introduction of ACE inhibitors to treat renal crisis; the prognosis for patients with RNA polymerase III antibodies is now better than for those with Scl-70 or U3-RNP antibodies.[8]

Scl-70 (Topoisomerase I Antibody)

Topoisomerase I antibodies were initially named Scl-70 based on immunoblot detection of a 70-kDa protein.[21] The prevalence of Scl-70 antibodies in SSc varies widely across

geographies and ethnicities, ranging from 9% to 71%.[7] These antibodies are strongly associated with dcSSc but also occur in lcSSc.[7,8] Furthermore, they are highly specific to SSc and are included in the 2013 ACR-EULAR classification criteria.[6,7]

The presence of Scl-70 antibodies is associated with pulmonary and cardiac involvement as well as renal crisis and vascular complications.[7,8] Therefore, these antibodies indicate a poor prognosis.[15] The increased mortality associated with Scl-70 antibodies results primarily from the higher risk for severe ILD.[7,8]

Th/To Antibody

Th/To antibodies target RNase P and mitochondrial RNase ribonucleoprotein complexes. These antibodies are found in 2% to 5% of SSc patients and are primarily associated with lcSSc.[7,8] Th/To antibodies are rarely found in other autoimmune diseases but can occur in patients with localized scleroderma.[8]

Patients with Th/To antibodies often develop both ILD and PAH and thus have a poorer prognosis than other patients with lcSSc.[7,8]

U1-snRNP (Sm/RNP) Antibody

U1-snRNP antibodies, also referred to as U1-RNP and Smith (Sm)/RNP, target 3 components of the U1 small nuclear ribonucleoprotein complex: U1-snRNP RNP A, U1-snRNP RNP C, and U1-snRNP RNP-70kd. These antibodies, found in 2% to 14% of SSc patients, are more frequent in lcSSc than in dcSSc.[16] The antibodies are also found in patients with other autoimmune diseases, including approximately 90% of patients with mixed connective tissue disease.[7,16]

Patients with U1-snRNP antibodies tend to have less prominent skin thickness and less renal involvement, but have increased risk of PAH, arthritis, and esophageal dysfunction.[8,16] Overall, the presence of U1-snRNP antibodies is associated with a good response to corticosteroids and a favorable prognosis.[16]

U3-RNP (Fibrillarin) Antibody

U3-RNP antibodies target the U3 small nucleolar ribonucleoprotein (U3-RNP) complex, which consists of the protein fibrillarin and U3 RNA.[7] These antibodies are found in about 4% to 10% of patients with SSc, and are especially common in African American SSc patients (approximately 30%).[7,8] U3-RNP antibodies are rarely found in patients with other autoimmune disorders; thus, the presence of these antibodies supports a SSc diagnosis.[8,21] These antibodies occur most often in dcSSc, but they can also occur in lcSSc.[7,8] U3-RNP antibodies are associated with multiorgan involvement, including the heart, kidneys, muscle, lungs, and gastrointestinal system. Their presence is an independent risk factor for the development of PAH, and PAH is the most common cause of death in U3-RNP–positive patients.[7,8] Therefore, their presence indicates a poorer prognosis.

Supportive Testing

Once a diagnosis of SSc is established, and clinical findings and autoantibody testing have established the type, further laboratory testing is performed to support evaluation of end-organ function and overall health. Testing may include examination of liver and kidney function, complete blood count, and inflammation assessment.[22] Since SSc is a chronic disease, follow-up assessment at least annually is recommended.[22]

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