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Systemic Sclerosis:

Laboratory Support of Diagnosis and Management

CLINICAL BACKGROUND

Systemic sclerosis (SSc) is a chronic, multisystem, heterogeneous autoimmune disease. Individuals with SSc have a mortality rate 250% greater than that of the general population.[1,2] The disease is characterized by widespread microvascular dysfunction and progressive fibrosis of the skin and internal organs.[2,3] SSc is frequently referred to as scleroderma; however, scleroderma includes SSc and localized forms of scleroderma (morphea and linear scleroderma) that affect only the skin.[3,4]

SSc typically presents in people aged 20 to 30 years and is about 4 times more common in females than males. The incidence is approximately 3 to 20 cases per million persons overall but varies with ethnicity and the population studied.[5] While the disease is not genetically inherited, relatives of an affected individual have a higher risk of developing SSc than the general population.[6] Environmental factors may also play a role.[6,7]

There are 2 main types of SSc, which 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.[2-4] Multiple organs, including the heart, lungs, gastrointestinal tract, and kidneys, can be affected in both forms, though organ involvement is less severe in lcSSc.[2-4] Sine scleroderma is a rare form of SSc that affects internal organs but spares the skin.[4] Overlap syndromes, in which another autoimmune disease (eg, polymyositis, primary biliary cholangitis) is present along with SSc, have also been described.[2,8] Of patients with SSc, approximately 55% have lcSSc, 35% have dcSSc, and 10% have overlap or sine scleroderma.[9,10] CREST syndrome (calcinosis cutis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) is considered a variant of lcSSC.[4]

Secondary Raynaud phenomenon is a hallmark of SSc and may precede skin changes and organ disease by several years, especially in patients with lcSSc.[2-4] In patients with dcSSc, Raynaud phenomenon typically begins shortly after or is coincident with the onset of skin changes. Thus, the only presenting sign in dcSSc may be puffy fingers. Other nonspecific symptoms include general malaise, fatigue, arthralgia, myalgia, gastrointestinal symptoms such as reflux, and dyspnea.[2-4]

The highest risk for developing internal organ involvement, tendon friction rubs, joint contractures, and myopathy among SSc patients is during the first 3 to 5 years after disease manifestation.[2-4] Individuals with increasing skin thickness are generally considered to be at increased risk of internal organ involvement as well. Renal hypertensive crisis is an important cause of morbidity, but not mortality, as administration of angiotensin-converting enzyme inhibitors can minimize renal damage when diagnosed early.[2,3] Mortality is most often due to interstitial lung disease (ILD) or pulmonary artery hypertension (PAH), with PAH typically occurring more than 5 years after disease manifestation.[2,3,11]

Early treatment of SSc can improve outcomes, so prompt diagnosis is important. Although diagnosis can sometimes be made on the basis of characteristic skin changes alone, most SSc patients present with nonspecific symptoms. In addition, patients with other autoimmune disorders may present with symptoms suggestive of SSc. Both of these factors can contribute to a delay in diagnosis.

This Clinical Focus discusses the role of autoantibody testing in the diagnosis and categorization of SSc, as well as its use for assessment of prognosis and disease management.

INDIVIDUALS SUITABLE FOR TESTING

- People suspected of having SSc (ie, those with capillary nailfold changes; Raynaud phenomenon; skin changes; or unexplained, but suggestive, musculoskeletal, gastrointestinal, cardiac, or pulmonary symptoms)

TEST AVAILABILITY

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

TEST SELECTION AND INTERPRETATION

Diagnosis

Early diagnosis of SSc is important in order to delay, mitigate, or avoid irreversible end-organ damage.[2-4] The diagnosis is based primarily upon the presence of characteristic clinical findings and autoantibodies (**Table 2**).[4,10,15,17,18,20,22,25,29]

Table 1. Autoantibody Testing Used in the Diagnosis and Management of Systemic Sclerosis[4,12-31]

Test Code	CPT Code(s)[a]	Test Name	Clinical Use
249	86038	ANA Screen, IFA, with Reflex to Titer and Pattern	Diagnose autoimmune disease
16814[b]	86038	ANA Screen, IFA, with Reflex to Titer and Pattern and Reflex to Multiplex 11 Antibody Cascade Antibody cascade includes 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
90073[b]	86038	ANA Screen, IFA, with Reflex to Titer and Pattern (Systemic Sclerosis Panel 1) Includes ANA screen (IFA) with reflexes to titer and pattern; also includes centromere B (16088), RNA polymerase III (19899), and Scl-70 (4942) antibodies.	Diagnose SSc
16088	86235	Centromere B Antibody	Diagnose SSc; determine SSc type, organ involvement, and prognosis
91292	86235	Fibrillarin (U3 RNP)	Diagnose SSc; determine prognosis
18855	83516	Ku Autoantibodies	Predict muscle and joint involvement and digital vasculopathy-related complications
19899	83520	RNA Polymerase III Antibody	Diagnose SSc; determine prognosis
94646	86235	Anti-PM/Scl-100 Antibody, EIA	Determine prognosis
4942[c]	86235	Scleroderma Antibody (Scl-70)	Diagnose SSc; determine prognosis
38567[c,d]	86235	Sm/RNP Antibody	Determine prognosis
94685[e]	86235 (x10), 84182 (x2)	Systemic Sclerosis 12 Antibodies Panel 2 Includes centromere protein (CENP)-A, CENP-B, fibrillarin (U3-snRNP), PM/Scl-75, PM/Scl-100, RP11 (RNA polymerase III), RP155 (RNA polymerase III), Scl-70, Th/To, U1-snRNP RNP-70k, U1-snRNP RNP A, and U1-snRNP RNP C antibodies.	Diagnose SSc; determine SSc type, organ involvement, and prognosis

ANA, antinuclear antibodies; IFA, immunofluorescence assay; SSc, systemic sclerosis.
[a] The CPT codes provided are based on AMA guidelines and are for informational purposes only. CPT coding is the sole responsibility of the billing party. Please direct any questions regarding coding to the payer being billed.
[b] Reflex tests are performed at an additional charge and are associated with an additional CPT code.
[c] Test codes 4942 and 38567 are performed by immunoassay.
[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.

Autoantibody positivity may be present early in the course of the disease and assist in diagnosis in 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.[4,15,17,20] This is important for determining prognosis and optimal treatment.

The following sections describe the autoantibodies associated with SSc and their relevance for diagnosis and management. A summary of the autoantibodies, their prevalences, and their clinical associations are provided in the **Appendices**, and a simplified algorithm showing the use of antibody testing for SSc diagnosis is presented in the **Figure**.

Table 2. ACR-EULAR Systemic Scleroderma Classification Criteria[10,a]

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[b]	4
Lesions on fingertips (only count highest score)	
Ulcers on tips 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[c]:	3
Centromere B antibody	
Scl-70 antibody	
RNA polymerase III antibody	

ACR-EULAR, American College of Rheumatology-European League Against Rheumatism.
[a] Classify a patient as having systemic sclerosis if sum of points is ≥9.
[b] Distal to metacarpophalangeal joints but proximal to proximal interphalangeal joints.
[c] Maximum score is 3.

Antinuclear Antibody (ANA)

ANA immunofluorescence assay (IFA) is the initial test for a patient presenting with nonspecific symptoms, including Raynaud phenomenon. ANA is present in 85% to 97% of patients with SSc; however, ANA is also present in many other autoimmune disorders.[12,26] 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.[12,26] A negative ANA IFA test decreases the likelihood of SSc, but does not rule it out. The presence of characteristic skin changes, Raynaud phenomenon, and a positive ANA IFA test is diagnostic for SSc. However, in the rare case that Raynaud phenomenon is not present and ANA IFA testing is negative, skin changes alone are sufficient for a diagnosis (**Table 2**).[10]

Centromere Antibodies

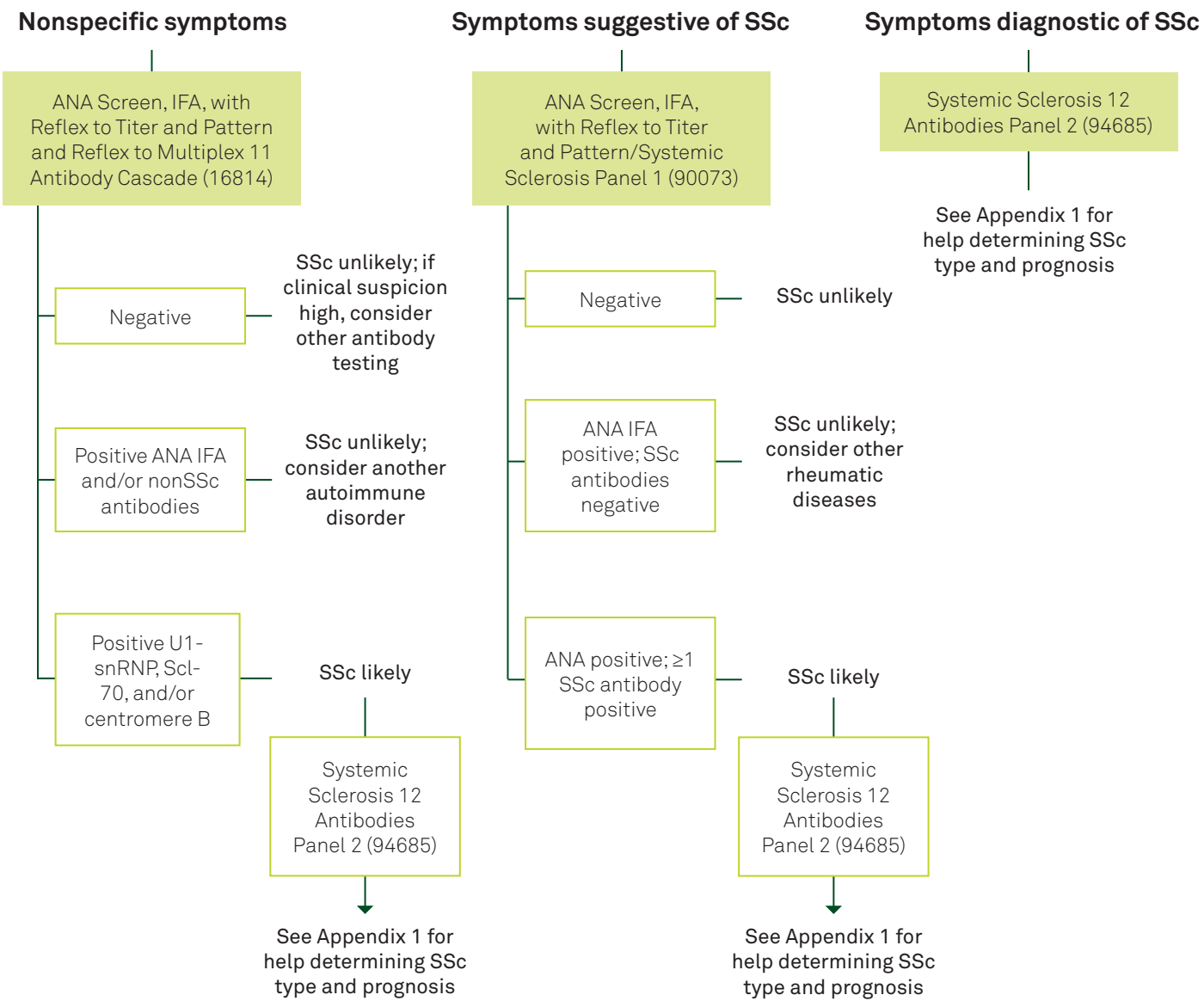
Centromere antibodies can be directed against a number of centromere proteins (CENP), including CENP-A, -B, -C.[17,24,25] Centromere B antibody is found in 20% to 40% of patients with SSc and up to 90% of patients with lcSSc.[17] Presence of centromere B antibody has a diagnostic sensitivity of 36% and specificity of >97% for SSc.[13] This antibody is 1 of 3 autoantibodies included in the 2013 American College of Rheumatology-European League Against Rheumatism (ACR-EULAR) classification criteria.[10] However, centromere B antibodies are also seen in other connective tissue diseases, including systemic lupus erythematosus (SLE), primary biliary cirrhosis, and Sjögren syndrome.

Presence of centromere antibodies is considered predictive of SSc development in patients with Raynaud phenomenon.[17] The antibodies are negatively associated with risk of cardiac and renal involvement but positively associated with risk of PAH. Although centromere antibodies are associated with a better prognosis overall than are other autoantibodies, their presence indicates a poorer prognosis if PAH is present.[17,20]

Fibrillarin (U3-snRNP or U3-RNP) Antibody

U3-snRNP (also called U3-RNP) antibodies target a 34 kDa protein of the U3 small nucleolar ribonucleoprotein (U3-RNP) complex called fibrillarin; hence the terms fibrillarin and U3-snRNP antibodies are used interchangeably.[17,29] These antibodies are found in 4% to 10% of patients with

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



Test codes are included in parentheses. SSc antibodies include U1-snRNP, Scl-70, and centromere B antibodies; nonSSc antibodies include all other antibodies in the 11-antibody cascade. ANA, antinuclear antibodies; IFA, immunofluorescence assay; SSc, systemic sclerosis.

This figure was developed by Quest Diagnostics based on references 4, 12, 17, 18, 20, and 26. 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.

SSc, depending on disease type and duration, and are rarely found in other autoimmune disorders.[17,20] Thus, the presence of U3-snRNP antibodies is considered diagnostic for SSc.[17] The antibodies tend to be associated with organ involvement, especially the heart and kidneys, and, in African American patients, with severe pulmonary and small bowel disease.[17] Overall, their presence indicates a poorer prognosis.

Ku Antibody

The Ku protein is involved in repair of DNA damage.[19] Ku antibodies are uncommon and are detected in only about 2% of patients with SSc.[21] The prevalence, however, varies with ethnicity. For example, Ku antibodies are found in about 4% of African Americans with SSc but are not found in Caucasians with SSc.[19,28] Presence is not specific for SSc, as the antibodies are also seen in patients with undifferentiated connective tissue disease, overlap syndrome, polymyositis/dermatomyositis, and systemic lupus erythematosus (SLE). Ku antibody testing may be more useful prognostically, as presence is strongly associated with muscle and joint involvement and ILD; presence also appears to be protective against severe digital vasculopathy-related complications.[16,21]

PM/Scl Antibody

PM/Scl antibodies target the PM/Scl human exosome complex, and the majority of reactivity is against 2 proteins, PM/Scl-75 and PM/Scl-100. They are present in 4% to 11% of SSc patients and are seen in other autoimmune diseases as well.[17,20] PM/Scl-75 antibodies tend to be associated with dcSSc, overlap syndrome, and ILD. Reactivity to both antigens, or to PM/Scl-100 alone, is associated with myositis and overlap syndrome in up to 55% of patients.[14,31] Though patients with PM/Scl antibodies have an increased risk of ILD and digital ulcers, their risk of PAH and lower gastrointestinal symptoms is lower, and their prognosis is generally good.[17,20,32]

RNA Polymerase III Antibody

RNA polymerase III antibodies are found in 5% to 30% of patients with SSc and are included in the 2013 ACR-EULAR classification criteria.[10,17,20,22,33] The antibodies target RNA polymerase epitopes 11 and 155 and are thus defined as anti-RP11 and anti-RP155. RP11 antibodies are found in about 10% of SSc patients, while RP155 is observed in about 8%.[23,30] They are diagnostic for SSc, as they are rarely found in other autoimmune diseases. Their presence is predictive of dcSSc, synovitis, myositis and joint contractures, renal crisis, and increased mortality.[17,22] It has also been noted that patients with RNA polymerase antibodies have an increased risk of malignancy within a 5-year timeframe before or after onset of SSc skin changes.[17,20,22]

Scl-70 (Topoisomerase I Antibody)

Topoisomerase I antibodies were initially named Scl-70 based on immunoblot testing, but later research indicated that the 70 kDa protein identified was a breakdown product of the full-length 100 kDa protein.[17] Scl-70 antibodies are found in 14% to 42% of patients with SSc, and are strongly associated with dcSSc (occurring in 40% of patients[15,17,20]), higher risk of pulmonary and cardiac involvement, and a poor prognosis.[17] The antibodies are rarely found in healthy individuals, patients with other connective tissue diseases, or primary Raynaud phenomenon and thus are considered diagnostic for SSc.[17,20] Scl-70 antibodies are included in the 2013 ACR-EULAR classification criteria. Scl-70 and centromere B antibodies are almost always mutually exclusive in SSc patients: only 0.5% test positive for both.[34] Patients with isolated Raynaud phenomenon who are Scl-70 positive have a 25-fold greater risk of developing SSc than Raynaud patients who are Scl-70-negative.[10]

Th/To Antibody

Th/To antibodies are found in 1% to 13% of SSc patients and are primarily associated with lcSSc.[17,20] The antibodies are rarely found in other autoimmune diseases and thus are considered diagnostic for SSc.[3] Patients who test positive for Th/To antibodies have a high incidence of pulmonary fibrosis and renal crisis and thus a poorer prognosis.[17,20]

U1-snRNP (Sm/RNP) Antibody

U1-snRNP antibodies, also referred to as U1-RNP and Smith (Sm)/RNP, are found in 2% to 14% of SSc patients; they are more frequent in lcSSc than in dcSSc.[17,20] The antibodies are also found in approximately 90% of patients with mixed connective tissue disease and thus are not diagnostic for SSc. 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.[17,20] Overall, the presence of U1-snRNP antibodies is associated with a good response to corticosteroids and a favorable prognosis.[17,20] Three major components of U1-snRNP that can be tested are U1-snRNP RNP A, U1-snRNP RNP C, and U1-snRNP RNP-70kd.

Management

Once a diagnosis of SSc is established, and clinical findings and autoantibody testing have established the type, further testing should be directed toward determining the degree of

end-organ involvement and treatment response.[2,3] Testing may include, but not be limited to, examination of liver and kidney function, complete blood count, pulmonary function testing, radiograph or high-resolution computed tomography (HRCT) of the chest, electrocardiography, and Doppler echocardiography.[2,3] Various tools have been developed to classify disease activity and assist in guiding treatment.[35-38] Though autoantibody testing is important for establishing a diagnosis of SSc and determining type and prognosis, serial monitoring of antibody levels has not proven useful for predicting treatment response or prognosis.[17,20]

Appendix 1. Clinical Characteristics Associated with Autoantibodies Used in the Diagnosis and Classification of Systemic Sclerosis (SSc)[a,4,12-31]

Antibody	Prevalence in SSc, %	Presence in Other Autoimmune Diseases	SSc Type Most Likely	Clinical Associations	Relative SSc Prognosis
ANA	85-97	Common	Does not help differentiate type	Most common in rheumatic diseases	Varies
Centromere A or B	20-40[b]	Uncommon	lcSSc	PAH, CREST syndrome, digital ischemia, esophageal involvement	Better
Fibrillarin (U3-snRNP or U3-RNP)	4-10[b] (depends on disease type and duration)	Rare	dcSSc (rare in lcSSc)	Renal and cardiac involvement, pulmonary disease	Poorer
Ku	0-4 (varies with ethnicity)	Common	dcSSc or lcSSc	Muscle and joint involvement, protective against digital vasculopathy-related complications	Unclear
PM/Scl-75	4-11	Common	dcSSc	Raynaud phenomenon, arthritis, pulmonary involvement, calcinosis, digital ulceration, overlap syndromes, younger patients	Better
PM/Scl-100	4-11	Common	lcSSc	Raynaud phenomenon, arthritis, pulmonary involvement, calcinosis, digital ulceration, overlap syndrome, less GI involvement	Better
RNA Polymerase III	5-30[b]	Rare	dcSSc	Renal crisis, hypertension, tendon friction rubs, synovitis, myositis, joint contractures, malignancy	Poorer
RNA Polymerase III, subunit RP11	10	Rare	dcSSc	Renal crisis, hypertension, tendon friction rubs, synovitis, myositis, joint contractures, malignancy	Poorer

(continued)

Appendix 1 *(continued)*. Clinical Characteristics Associated with Autoantibodies Used in the Diagnosis and Classification of Systemic Sclerosis (SSc)[a,4,12-31]

Antibody	Prevalence in SSc, %	Presence in Other Autoimmune Diseases	SSc Type Most Likely	Clinical Associations	Relative SSc Prognosis
RNA Polymerase III, subunit RP155	8	Rare	dcSSc	Renal crisis, hypertension, tendon friction rubs, synovitis, myositis, joint contractures, malignancy	Poorer
Scl-70	14-42[b]	Rare	dcSSc	Severe pulmonary fibrosis, cardiac and joint involvement, tendon friction rubs, digital ulcers	Poorer
Th/To	1-13[b]	Rare	lcSSc	Pulmonary involvement, renal crisis	Poorer
U1-snRNP[c]	2-14[b]	Common (90% in patients with MCTD)	lcSSc	MCTD, overlap syndrome	Better

ANA, antinuclear antibodies; lcSSc, limited cutaneous SSc; PAH, pulmonary arterial hypertension; CREST, calcinosis cutis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia; dcSSc, diffuse cutaneous SSc; GI, gastrointestinal; MCTD, mixed connective tissue disease.

[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.[39]

[b] In North America, prevalence has been shown to vary among Caucasians and African Americans (Appendix 2).

[c] U1-snRNP is also called Smith (Sm)/RNP and U1-RNP.

Appendix 2. Antibody Prevalence Among North Americans With Systemic Sclerosis[4]

Antibody	Caucasians	African Americans
Centromere	21%	11%
Fibrillarin (U3 snRNP)	4%	37%
PM/Scl	2%	0%
RNA Polymerase III	24%	11%
Scl-70	20%	11%
Th/To	5%	0%
U1 snRNP	14%	26%

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