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Scleroderma (D Khanna, Section Editor)

Autoantibodies and Their Role in Scleroderma Clinical Care

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Opinion statement

The importance of autoantibodies in systemic sclerosis (SSc) diagnosis and prognosis has been increasingly recognized. This review discusses the current knowledge of autoantibodies in systemic sclerosis (SSc) with respect to clinical care. We focus primarily on the SSc-associated serum autoantibodies and their recognized SSc clinical phenotypes or characteristics. Geographic regional differences in autoantibody prevalence are discussed. Potential pitfalls in commercially available autoantibody testing are considered. Finally, contemporary literature in regards to antibodies to functional molecules is reviewed.

Introduction

For many decades, experienced clinicians have recognized that systemic sclerosis (SSc or scleroderma) is a highly heterogeneous disease in its clinical presentation, organ system involvement, natural history, and prognosis. In 1988, an international consensus statement recognized that two major disease subsets are present based on the extent of skin thickening [1]. This publication described the major features of diffuse cutaneous and limited cutaneous (previously termed CREST syndrome) SSc and noted the association of serum anti-topoisomerase I antibody (Scl-70) with diffuse SSc and anti-centromere antibody with limited SSc.

Since that time, there have been additional refinements. First, more precise, mutually exclusive definitions have been proposed for diffuse and limited SSc. Diffuse SSc patients have skin thickness proximal to the elbows and knees (upper arms, thighs, anterior chest, or abdomen) at some time during the disease, whereas in limited SSc patients, skin thickening remains restricted to the distal extremities (distal to the elbows and knees). Second, it is clear that SSc patients with no skin thickening (SSc sine scleroderma or ssSSc) are part of the limited SSc spectrum [2] and approximately 4 % of the total SSc population [3]. Finally, both diffuse and limited SSc can coexist with another connective tissue disease (CTD) as an overlap syndrome, most frequently one of the inflammatory myopathies, but also systemic lupus erythematosus (SLE) or inflammatory arthritis.

In addition, new SSc-related serum autoantibodies have been described, along with their clinical associations. Today, over 95 % of SSc patients have a positive antinuclear antibody (ANA) test, and over 85 % have

one or more autoantibodies either restricted to (specific for) or most commonly detected in (strongly associated) with SSc. All of these antibodies are directed against nuclear antigens and are thus antinuclear antibodies (ANAs). Detection of these autoantibodies aids in diagnosis and prognosis [4]. The importance of SSc-associated autoantibodies has been recently recognized in the new combined American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) clinical classification criteria for SSc [5, 6••], which incorporate the presence of three autoantibodies (anti-Scl-70, anti-RNA polymerase III (RNAP), and anti-centromere) as supporting the classification of SSc.

The combination of clinical classification by extent of skin thickening, augmented by serologic status by autoantibody type, has allowed a clinical-serologic classification of SSc (Fig. 1) which has proved to be very helpful for managing physicians, clinical and laboratory investigators, and patients. In this chapter, we will use this combined clinical-serologic classification system to focus on the role of the currently recognized ten SSc-associated serum autoantibodies in patient care and management. We will highlight recent publications.

In addition, a new group of autoantibodies reactive with functional proteins, such as cell surface receptors and extracellular matrix proteins, has been identified in SSc patients. They may contribute to disease pathogenesis by activating pathways involved in damaging vascular endothelial cells or promoting fibrosis. These will be reviewed under the section header, "Antibodies to Functional Molecules."

SSc-associated ANAs

As of this writing, ten ANA specificities associated with SSc have been reported and characterized, the most newly recognized autoantibody published in 2014. The original methods of antibody detection have included ANA by indirect immunofluorescence (IIF), double immunodiffusion (DID), and immuno-precipitation (IP). Today, ELISA and bead-based testing are more commonly used but have specific and significant limitations in SSc patients (see subheading "Important Clinical Limitations of Commercial Antibody Testing in SSc Patients" below).
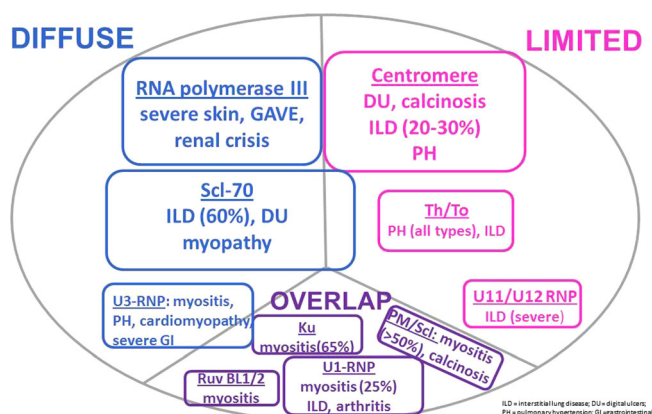

Fig. 1. Proportional representation of autoantibody frequencies, and their associations with cutaneous subtypes and internal organ manifestations.

These antibodies are rarely found in patients with other connective tissue diseases (CTDs) unless SSc features are also present, and thus, they are important diagnostic markers. The antibodies are nearly always present when first tested, and their presence likely precedes clinical findings by months or years, as has been confirmed in RA and SLE [7, 8]. SSc antibodies typically remain detectable throughout the course of disease, independent of treatment. SSc patients seldom (2–3 %) have more than one of these SSc-associated antibodies, suggesting that they occur in a mutually exclusive fashion. This statement does not include coexistence with SSA or Ro antibodies, which can occur in some SSc patients, and supports the possibility of coexistent Sjogren syndrome. In the Pittsburgh experience, anti-U1RNP is the autoantibody seen most frequently in combination with other SSc-associated antibodies.

Of interest, there is geographical variation in autoantibody frequencies as depicted in Table 1. These differences in regional autoantibody prevalence likely determine the phenotypic differences and reported predictors of prognosis and mortality reported in regional cohort studies.

Table 1. Worldwide frequency of SSc-related autoantibodies

	Europe	North America	Asia	Australia
Anti-topoisomerase	37 % EUSTAR 24 % Belgium 30 % Germany 20 % UK	18 % Pittsburgh 16 % Canada 28 % Mexico	10 % Malaysia	18 %
Anti-RNA polymerase III	2 % EUSTAR 6–9 % France 6 % Belgium 4–7 % Germany 17 % UK	22 % Pittsburgh 14 % Canada 1 % Mexico	6–10 % Japan	13–15 %
Anti-U3RNP	3 % UK	3 % Pittsburgh 1 % Canada	10 % Malaysia	
Anti-centromere	32 % EUSTAR 41 % Belgium 36 % Germany 32 % UK	19 % Pittsburgh 30 % Mexico 39 % Canada	10 % Malaysia	41 %
Anti-Th/To		8 % Pittsburgh		
Anti-U1RNP	5 % Germany 5 % Belgium	5 % Pittsburgh		
Anti-PM/Scl	5 % Germany 5 % UK	4 % Pittsburgh 3 % Canada 9 % Mexico		
Anti-Ku	1 % Germany	1 % Pittsburgh 1 % Canada 10 % Mexico		
Anti-U11/U12 RNP	–	2 % Pittsburgh	2 % Japan	–
Anti-RuvBL1/2	–	1 % Pittsburgh	2 % Japan	–

The Pittsburgh frequencies are unpublished data from the Pittsburgh SSc cohort as of December 31, 2014. Canadian frequencies are unpublished data provided by the Canadian Scleroderma Research Group

Anti-centromere antibody

Anti-centromere antibody (ACA) is directed against centromere proteins, and the gold-standard method of detection is by ANA using indirect immunofluorescence, and is widely available. Centromere proteins are necessary in the assembly of the kinetochore during cell division and are essential to mitosis. Recent work has confirmed that there are two centromere epitopes recognized, centromere-A and centromere-B. SSc patient antibodies can react to both centromere antigens. Centromere-A has greater specificity in SSc patients [9], but centromere-B antigen is the antigen included in most commercially available ELISA kits and multiplex assays. Anti-centromere-A antibodies are not part of naturally occurring human immunoglobulins [10]. Two novel motifs within the immunodominant centromere-A epitope have been identified, one of which is Ap17-30. Interestingly, this motif appears to be within the primary sequence of FOXE-3, which is a transcription factor known to be involved in the development of lens fiber cells. New work has demonstrated that FOXE-3 is expressed in monocytes, and that SSc patients with distinct seropositivity responded differently to cytokine stimulation [11].

ACA occurs in 20–25 % of most SSc populations and is strongly associated with limited SSc (Fig. 1) and scleroderma sine scleroderma. The prototypical ACA-positive SSc patient has long-standing (years) Raynaud phenomenon followed by swollen fingers (sclerodactyly) and then by thickening of the skin of the fingers which evolves over a number of years or even decades. For patients with Raynaud symptoms and no other clinical clues to an underlying CTD, the presence of ACA and/or nailfold capillary abnormalities is strongly predictive of the subsequent development of SSc [12]. Digital ischemia with digital tip ulcerations is disproportionately frequent in ACA-positive patients [13••]. Esophageal involvement is common (80 %), but severe interstitial lung disease, cardiomyopathy, and scleroderma renal crisis (SRC) are rare [14••, 15]. The most serious long-term complications of ACA-positive SSc are the late occurrence of pulmonary arterial hypertension (15–20 %) or small intestinal involvement with malabsorption [14••, 15].

Anti-topoisomerase I (Anti-Scl-70) antibody

This antibody is found in 20–30 % of SSc patients in many ethnic groups, but in Europe, the proportion is higher (40–60 %) [16••]. Three-fourths of patients with this antibody have dcSSc (Fig. 1). There is a high (60 %) risk of interstitial lung disease (ILD) regardless of the extent of skin thickening (diffuse or limited) [17].

Anti-Scl-70 is associated with myocardial disease and scleroderma renal crisis (SRC) in patients with rapidly progressive diffuse skin involvement [17]. Peripheral vascular complications such as digital ischemia with ulcerations are particularly common. In the multicenter digital ulcer outcome (DUO) registry, nearly half the patients were anti-Scl70 antibody positive [13••]. This association was confirmed in a recent EUSTAR multivariate analysis [18] where anti-

Scl-70 was an independent predictor of developing subsequent digital ulcers. Anti-Scl-70 is a marker for poor prognosis, primarily because of its association with ILD and its sequelae including secondary pulmonary hypertension.

The most reliable method for detecting anti-Scl 70, double immunodiffusion, has now been replaced in most commercial laboratories by ELISA or bead-based assays. Unfortunately, these methods result in a number of "low positives" which are, in fact, false positive tests in persons who do not have clinical evidence of SSc and often have a negative ANA by IIF. Clinicians should be cognizant of this when evaluating patients with a positive Scl-70 antibody test.

Anti-RNA polymerase III antibody

Anti-RNA polymerase III (RNAP III) is highly specific to SSc. The gold-standard method of detection is by immunoprecipitation, but reliable ELISA-based tests are commercially available. There is a higher frequency in North America Caucasian and UK patients (20–25 %) in comparison with mainland European or Japanese patients (5 %) [16••, 19]. Almost all patients with RNA P III have rapidly progressive diffuse skin thickening (Fig. 1). The peak skin thickness score is higher in RNAP III than in Scl 70 patients (Pittsburgh unpublished data),[20], and recent EUSTAR work has demonstrated that a higher rate of RNAP III-positive patients develop diffuse SSc within 3 years of the onset of Raynaud phenomenon than Scl-70-positive patients [18]. In many patients, skin thickness regresses during follow-up, even without treatment.

SSc patients with this antibody have the greatest risk for developing SRC (25 %) [21], typically during the early period when skin thickness progression is accelerated, and if tendon friction rubs are appreciated. Once SRC has occurred, survival is better than in Scl-70 positive SRC patients, perhaps because concomitant severe ILD and cardiomyopathy are infrequent in RNAP III patients.

In the last few years, two new associations with RNAP have come to light. The first is the occurrence of gastric antral vascular ectasia (GAVE or "watermelon stomach"), confirmed in two cohort studies [22, 23]. The second is a close temporal relationship between the onset of SSc in RNAP III-positive patients and the diagnosis of cancer, suggesting that the SSc may be a paraneoplastic process in this patient subset [24••, 25–27].

Anti-U3RNP antibody [28]

This antibody reacts with 34-KD fibrillarin complexed with U3RNA and produces bright nucleolar staining on ANA IIF testing. A reliable ELISA test is commercially available for detection of this antibody, and a recent publication suggested that a line immunoblot assay may be an attractive alternative [29]. Some commercial laboratories do have available immunoprecipitation testing, which is the gold standard.

Anti-U3RNP is found in 4–10 % of SSc patients and is more frequent in African Americans [28, 30]. It has been linked to a specific HLA-DRB1*08-04 allele [31] in African Americans. More than half of patients have diffuse SSc. A non-inflammatory skeletal myopathy is a distinctive feature, and early severe internal organ involvement including cardiomyopathy, PAH, and small bowel involvement with malabsorption and

episodes of pseudoobstruction may occur [19]. Prognosis in this subset of SSc patients is poor [30], similar to those with anti-Scl-70 antibody.

Anti-Th/To antibody

These antibodies are directed against subunits of mitochondrial RNA processing and ribonuclease P RNP complexes. Most patients with anti-Th/To antibody have nucleolar staining on ANA by IIF. One US-based company offers a commercially available immunoprecipitation assay for this test. Th/To antibody occurs in less than 5 % of SSc patients, almost all of whom have limited SSc (Fig. 1). Patients with this antibody can develop either ILD (45 %) or PAH (25 %), which are most often independent of one another [15]. These pulmonary complications result in a reduced survival compared to lcSSc patients with other SSc-associated antibodies. Regular screening for PAH in this patient population is prudent.

Anti-U11/U12RNP antibody

This is a rare antibody found in 1–3 % of SSc patients, equally divided between limited and diffuse subtypes (Fig. 1). A distinctive clinical feature is a very high frequency of ILD (80 %) which is often severe and rapidly progressive. As of this writing, there has only been one publication examining the internal organ associations with this autoantibody [32]. To the author's knowledge, testing for this antibody is not commercially available at this time.

Anti-PM/Scl antibody

PM/Scl antibodies target the human exosome complex, of which there are up to 16 proteins. The major B cell targets of the PM/Scl complex have been identified as PM100 (also called PM1-alpha) and PM75. SSc patients can have antibodies to one or both of these antigens. PM/Scl is one of three SSc-associated autoantibodies that have predominantly nucleolar staining on ANA by IIF, often with a moderately high ANA titer level. The gold-standard detection method for PM/Scl has been immunodiffusion and can be obtained commercially. The PM100 is available commercially using semi-quantitative immunblot testing.

Two to 4 % of SSc patients have anti-PM/Scl [33–35]. Patients with anti-PM/Scl antibody most often have an overlap with features of limited SSc and polymyositis (Fig. 1), but some have myositis only [36]. The myositis is usually mild and corticosteroid-responsive. Although there is a relatively high percentage of patients with lung involvement (40–50 %), serious internal organ involvement is rare, and patients have an excellent prognosis [33, 34]. Calcinosis has been a frequent reported clinical association [37••]. An interesting recent article combined Canadian, Australian, and US samples to examine the clinical associations with antibodies to each or both of the PM/Scl

complexes [34]. The presence of autoantibodies to both complexes was associated with an increased rate of inflammatory myositis.

Anti-Ku antibody

The Ku antigen is a heterodimer of 70 kDa (p70) and 80 kDa (p80) subunits. Ku is a multifunctional protein involved in DNA repair, immunoglobulin gene recombination, and transcription regulation [38]. Commercial testing for this antibody is available using immunoprecipitation through specific myositis testing panels. Anti-Ku patients represent less than 2 % of SSc populations, and anti-Ku has most commonly been detected in limited SSc patients with overlap disorders (myositis or lupus) (Fig. 1) [39, 40••, 41]. In the Pittsburgh experience, 60 % developed diffuse SSc skin changes. A more recent publication has linked it to a relatively higher rate of ILD [39] and several with arthritis [39, 41]. Although there are several case series of anti-Ku-positive SSc patients, overall numbers reported remain small, and thus, an estimate regarding prognosis cannot be made. The associated myositis is typically corticosteroid-responsive [42].

Anti-U1RNP antibody

This antibody was initially identified by Sharp et al. in the 1970s as being associated with features of SSc, PM and SLE and was thus considered to describe "mixed connective tissue disease" or MCTD [43]. Various combinations of these CTDs have been reported, and some patients have few or no scleroderma features. When we encounter anti-U1RNP-positive patients who have predominantly scleroderma features, we classify them as having SSc rather than using the term MCTD. High-titer speckled nuclear staining is most frequently found on ANA by IIF. Disease onset tends to be at a relatively young age, and limited SSc is far more common than diffuse SSc. The presence of arthritis and myositis is included in all proposed criteria for MCTD [44–47]. Five to 10 % of SSc patients have anti-U1RNP, which is particularly common in African American and Asian patients [28]. Serious complications are relatively uncommon. In our experience, the PAH that occasionally develops in anti-U1RNP-positive SSc patients is responsive to corticosteroids and immunosuppressive agents, which is not typically the case in PAH associated with other SSc antibodies.

Anti-RuvBL1/2 antibody

This newly identified antibody is directed against a double hexamer containing the proteins pontin and reptin, located in the nucleoplasm (Fig. 1). Detection is by immunoprecipitation less than 1 % of SSc patients have this antibody, which is more common in males with diffuse skin thickening and coexistent myositis. Only one publication, combining single cohorts from Japan and the US have reported [48••]. Information on internal organ and prognosis is limited at this time. To our knowledge, this antibody cannot be identified in a commercial setting at present.

Other SSc-associated ANAs

Anti-nucleolar organizing region (NOR) antibodies [49], anti-B23 [50], anti-SSA/R052/TRIMZ1 [51] antibodies to interferon-inducible genes designated HIN-200 [52], and antibodies to cytoplasmic mitochondrial proteins [53] have been reported in SSc patients. Anti-B23 was noted to be associated with PAH, but often coexisted with anti-U3RNP and anti-U1RNP; thus, its independent association with PAH is uncertain [51]. Anti-R052TRIM is strongly associated with SSc overlap syndromes and ILD [52].

ANA-negative SSc

Fewer than 5 % of patients with documented SSc on clinical grounds are ANA negative. A large North American SSc registry reported 6 % ANA-negative patients who were mostly males with less vasculopathy and an increase in lower gastrointestinal involvement [54].

Important clinical limitations of commercial antibody testing in SSc patients

In addition to the problem of false positive anti-Scl-70 antibody results, there are other issues with newer commercially available immunoassays that have been revealed recently. The majority of these assays require the availability of highly purified autoantigens, which contain the major epitopes recognized by virtually all sera positive for given SSc-related ANAs. However, since most autoantigens recognized by SSc ANAs are multiprotein complexes, determining the antigenic subunit most commonly recognized by autoantibodies is a major challenge.

Bead-based multiplex assay testing, which combines multibead arrays and flow cytometry, is capable of detecting multiple SSc-associated nuclear antibodies simultaneously. Unfortunately, in the commercially available multiplex ANA assays, many of the SSc-associated antibodies are not present. Although the common antibodies of centromere, Scl-70 and U1RNP antigens are included, the other seven SSc-associated antigens are not included. Thus, when this type of assay was used for ANA screening, up to 50 % of SSc patients were reported as a negative ANA in the US population [55] despite the fact that >90 % would have had a positive ANA test by immunofluorescence. Use of bead-based ANA testing for screening holds a potential danger for missing SSc patients, particularly at the primary care level.

More recently, a EUROLINE tests has become available which can detect multiple SSc-associated antibodies (PM/Scl, Ku, Scl-70, centromere-B, RNAP III, U3-RNP, Th/To as well as additional specificities). This immunoblot assay produces semi-quantitative results. Although not

the primary goal of the publication, recent work from the Australian Scleroderma Cohort Study using the EUROLINE test demonstrated that the rate of multiple antibody positivity was far higher than in cohorts that use the gold-standard testing, including immunodiffusion, suggesting high rates of false positivity using the EUROLINE test. In their principal component analysis, they demonstrated that the dominant autoantibody (if Scl-70, RNAP, or ACA) accurately reflects disease associations with this testing. We recommend caution in interpreting other results with this assay.

In the SSc literature, authors have commonly grouped and analyzed together patients presenting with a nucleolar pattern on ANA testing by immunofluorescence. The nucleolar pattern generally corresponds to one of three SSc-associated antibodies: anti-PM/Scl, anti-U3RNP, or anti-Th/To. Since the clinical and laboratory associations, internal organ involvement, and natural history of disease differ considerably in these three antibody subsets (see above sections), it is important to identify each if possible, both for patient care and research. Until very recently, the latter two of these antibodies could not be performed commercially and thus, have not usually been identified in cohort studies and clinical trials. This is an area in which the SSc community should work together in the future to identify and support resource laboratories capable of performing these tests in a reliable and validated manner.

Usefulness of SSc-related ANAs in SSc diagnosis

These antibodies are useful in supporting the diagnosis of SSc primarily because of their high specificity for the disease. As a consequence, the presence of ACA, anti-Scl-70, and anti-RNAP III are included in the serologic domain of the 2013 classification criteria for SSc jointly proposed by the American College of Rheumatology (ACR) and the European League against Rheumatism (EULAR). Their high specificity and the availability of reliable assay kits worldwide accounts for their inclusion in the criteria set.

Usefulness of SSc-related ANAs regarding disease activity

It has generally been believed that serial measurement of serum ANA titers is not useful in assessing SSc disease activity. However, few such studies have been attempted, and in the past, quantitation of antibody levels was not possible. Changing levels of anti-Scl 70 by ELISA were found to be associated with progression of skin thickness [63], and patients who lost anti-Scl 70 antibody reactivity over time had a more favorable outcome [55, 64]. Anti-RNAP III antibody levels correlated positively with the modified Rodnan total skin thickness score and with

the onset of scleroderma renal crisis (SRC). Now that semi-quantitative methods are available, additional studies are likely to be performed to clarify the usefulness of serial autoantibody testing in predicting future events in clinical practice and clinical trials.

Antibodies to functional molecules

These autoantibodies are potentially relevant to SSc pathogenesis by modulating disease-related pathways such as platelet-derived growth factor [56], transforming growth factor beta [57], angiotensin II type 1 receptor [58], or endothelin-1 type a receptor [59]. They have been reviewed in a recent textbook chapter [57]. Overall, the potential clinical utility of these autoantibodies remains uncertain. We will briefly discuss focused recent work examining anti-endothelin 1 type A receptors (ETAR) and anti-angiotensin II type I receptor (AT1R) in SSc which is relevant to patient care.

Serum AT1R and ETAR antibodies were reported to be elevated in autoimmune diseases [59] and in pre-eclampsia [60]. These antibodies have been found in human microvascular endothelial cells, but also are expressed in fibroblasts, epithelial, and immune cells. These antibodies have been shown to be biologically active and potentially mediate pathogenic effects of SSc [61]. Riemekasten et al. showed in a cross-sectional cohort study that there was an association between these antibodies and SSc mortality, pulmonary hypertension, renal crisis, and digital ulcers [59]. In 2015, Avouac et al. examined a small ($n = 90$), prospective SSc cohort and showed anti-ETAR antibodies to be an independent predictor of the occurrence of new ischemic DU over 48 months, but not anti-AT1R. A history of DU was also important in their models [62]. This work suggests a potential promising role of anti-ETAR and perhaps AT1R in future clinical management or risk stratification in study design of digital ulcers.

Conclusions

Systemic sclerosis-associated serum autoantibodies support the diagnosis of SSc, and because of their strong clinical associations they compliment the cutaneous-based subgrouping of SSc patients. A combined clinical-serologic classification system provides an essential framework for predicting patterns and frequency of organ system involvement in SSc patients. This is helpful for patient management and informing the design and conduct of future clinical and laboratory research and clinical trials.

Compliance with Ethical Standards

Human and Animal Rights and Informed Consent

With regard to the authors' research cited in this paper, all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. In addition, all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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