Review

The clinical relevance of autoantibodies in scleroderma

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Abstract

Scleroderma (systemic sclerosis) is associated with several autoantibodies, each of which is useful in the diagnosis of affected patients and in determining their prognosis. Anti-centromere antibodies (ACA) and anti-Scl-70 antibodies are very useful in distinguishing patients with systemic sclerosis (SSc) from healthy controls, from patients with other connective tissue disease, and from unaffected family members. Whereas ACA often predict a limited skin involvement and the absence of pulmonary involvement, the presence of anti-Scl-70 antibodies increases the risk for diffuse skin involvement and scleroderma lung disease. Anti-fibrillarin autoantibodies (which share significant serologic overlap with anti-U3-ribonucleoprotein antibodies) and anti-RNA-polymerase autoantibodies occur less frequently and are also predictive of diffuse skin involvement and systemic disease. Anti-Th/To and PM-Scl, in contrast, are associated with limited skin disease, but anti-Th/To might be a marker for the development of pulmonary hypertension. Other autoantibodies against extractable nuclear antigens have less specificity for SSc, including anti-Ro, which is a risk factor for sicca symptoms in patients with SSc, and anti-U1-ribonucleoprotein, which in high titer is seen in patients with SSc/systemic lupus erythematosus/polymyositis overlap syndromes. Limited reports of other autoantibodies (anti-Ku, antiphospholipid) have not established them as being clinically useful in following patients with SSc.

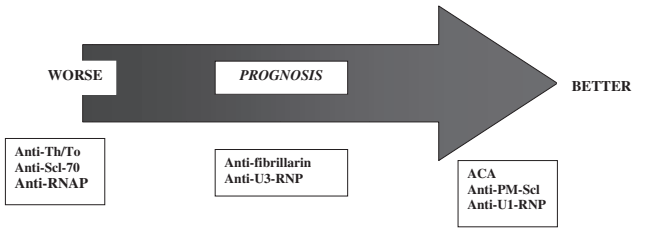
Keywords: anti-centromere, anti-Scl-70, autoantibodies, scleroderma, systemic sclerosis

Introduction

Systemic sclerosis (scleroderma or SSc) is a heterogeneous disorder characterized by autoantibody subsets, which in turn have their own clinical associations. Much controversy resides in whether these autoantibodies contribute directly to the pathology seen in SSc or whether they are merely epiphenomena of the underlying disease process. Nevertheless, various autoantibodies found in patients with SSc carry significant value in diagnosis and in predicting clinical outcomes (Fig. 1). The autoantibodies classically associated with SSc include anti-centromere antibodies (ACA) and anti-Scl-70 (otherwise known as anti-topoisomerase I or anti-topo I). In addition to these is the less commonly occurring anti-nucleolar antibody (ANoA) system, which comprises a mutually exclusive heterogeneous group of autoantibodies that produce nucleolar staining by indirect immunofluorescence (IIF) on cells from a variety of species [1]. The most widely recognized of these include anti-PM-Scl [2], antifibrillarin/anti-U3-ribonucleoprotein (AFA) [3], anti-Th/To [4], and the anti-RNA-polymerase family (anti-RNAP), including anti-RNAP I [5], II [6], and III [7] (although anti-RNAP frequently do not produce nucleolar staining on IIF). In addition to these disease-specific antibodies, anti-Ku, anti-Ro, antiphospholipid antibod-

ACA = anti-centromere antibodies; aCL = anticardiolipin antibodies; AFA = antifibrillarin/anti-U3-RNP; ANA = anti-nuclear antibodies; ANCA = anti-neutrophil cytoplasmic antibodies; ANoA = anti-nucleolar antibodies; anti-RNAP = anti-RNA-polymerase antibodies; anti-Sm = anti-Smith antibodies; aPL = antiphospholipid antibodies; $\beta_2$gp I = β_2 glycoprotein I antibodies; CENP = centromeric nucleoprotein; CIE = counterimmunoelectrophoresis; CREST = a variant of SSc defined by the presence of calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangectasia; CTD = connective tissue diseases; dcSSc = diffuse cutaneous systemic sclerosis; DL_{CO} = diffusion capacity for carbon monoxide; DM = dermatomyositis; ELISA = enzyme-linked immunosorbent assay; FVC = forced vital capacity; HLA = human leukocyte antigen; IB = immunoblotting; ID = immunodiffusion; IIF = indirect immunofluorescence; IP = immunoprecipitation; lcSSc = limited cutaneous systemic sclerosis; MCTD = mixed connective tissue disease; PFT = pulmonary function tests; PM = polymyositis; PM/SSc = myositis/scleroderma overlap; RLD = restrictive lung disease; RNP = ribonucleoprotein; SLE = systemic lupus erythematosus; snRNP = small nuclear RNP; SSc = systemic sclerosis.

Figure 1



Prognosis and systemic sclerosis-associated autoantibodies.

ies (aPL), anti-Smith (anti-Sm), anti-U1-ribonucleoprotein (anti-U1-RNP), and other autoantibodies are also found in SSc, each with a degree of clinical significance.

The present review details the various autoantibodies associated with SSc, their frequency (including in different ethnic groups), clinical correlates, pathophysiology, and genetic associations.

Anti-nuclear antibodies (ANA)

Since the early 1960s it has been known that ANA are common in the sera of patients with SSc [8,9], reported in as many as 95% and as few as 75% of patients with SSc with an overall diagnostic sensitivity of 85% and specificity of 54% when tested by IIF as published in a recent meta-analysis [10]. The presence of anti-Scl-70 and anti-U1-RNP antibodies in the sera yields a speckled appearance, whereas anti-Th/To, anti-AFA, and anti-PM-Scl give a nucleolar staining pattern. Anti-RNAP I antibodies yield a nucleolar staining, whereas those against RNAP II and III give a speckled appearance or no fluorescence [10]. The specificity and sensitivity of ANA vary depending on the antigen substrate used for the assay. The use of HEp2 cells yields a better sensitivity for the detection of nuclear antigens present during cell division (for example centromere antigen) than the use of tissue sections of murine liver or kidney [10]. ANA can also be measured by enzyme-linked immunosorbent assay (ELISA), a much less cumbersome technique now employed by many commercial laboratories. Although ANA by ELISA is appealing because the assay is automated, it often produces false positive results [10]. In addition, ANA by ELISA can yield false negative results, especially in patients with ANoA, and should not be used in the diagnosis of SSc without corroborative IIF [10].

ACA

ACA were initially described in 1980 [11] when HEp-2 cells were used as the substrate for the ANA. ACA had not been seen previously with the use of IIF on tissue substrates such as mouse liver, because the tissues in question undergo cell division much less commonly. ACA have been most typically determined by their characteristic staining pattern on immunofluorescence, giving rise to a speckled appearance on HEp-2 cells [11]. Subsequently was shown that SSc patients with ACA produce autoantibodies recognized by immunoblotting (IB), which react against six different centromeric proteins [12–20]. However, these distinctions have not been shown to have clinical relevance. So far, six centromeric nucleoproteins are known to be bound by sera from patients with SSc, designated CENP-A through CENP-F. Molecular analyses have shown that CENP-A is a 17 kDa centromere-specific histone H3-like protein [13]. CENP-B is an 80 kDa haploid DNA-binding protein [14–16]. CENP-C is a 140 kDa chromosomal component required for kinetochore assembly [16,17]. CENP-D is a centromere antigen of unknown function, with a molecular mass of 50 kDa [18]. CENP-E is a 312 kDa kinesin-like motor protein [19]. CENP-F is a nuclear matrix protein that accumulates in the nuclear matrix during S phase, assembling onto kinetochores at late G2 during mitosis [19,20].

All sera containing ACA react with CENP-B [21]. A solid-phase ELISA has been established by using a cloned fusion protein of CENP-B as antigen [21–24].

The frequency of ACA in patients with SSc has been reported to be 20–30% overall, but it varies depending on the ethnicity of the SSc patient. When determined by IIF, ACA are rather specific for the diagnosis of SSc. They are rarely found in healthy patients [25–27]. They are likewise seldom found to be positive in patients with other connective tissue diseases (CTD) [25,26,28,29] and are rarely found in unaffected relatives [30,31] (Table 1). When found in patients evaluated for Raynaud's phenomenon, ACA can predict the future development of SSc [32–36].

The presence of ACA has long been strongly associated with CREST, a variant of SSc, defined by the presence of calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangectasia [11]. Finding ACA can also distinguish CREST serologically from patients with other variants of SSc [34–37], from patients with other CTD [26–28,34], and from patients with primary Raynaud's phenomenon [34,38] (Table 2).

Although IIF remains the 'gold standard' in determining the presence of autoantibodies in SSc, many commercial laboratories have adopted ELISA testing to detect the presence of such autoantibodies. More recently, an ELISA using a cloned fusion protein of CENP-B as an antigen against which ACA are directed has shown no added sensitivity in the diagnosis of CREST compared with other patients with SSc, patients with primary Raynaud's phenomenon and patients with other CTD [22,23]. One must be very cautious of the specificity of this type of testing, although recent refinements have improved its performance [23,24].

Table 1

Overall sensitivity and specificity of anti-centromere antibodies by indirect immunofluorescence in diagnosis of SSc

SSc versus:	Sensitivity (%)	Specificity (%)
Normal controls	33*†	99.9*†
Other connective tissue diseases	31*	95†–97*
Primary Raynaud's phenomenon	24*	90*
Non-SSc relatives	19*	>99†*

*Reference [35]. †Reference [36].

Table 2

Overall sensitivity and specificity of anti-centromere antibodies by indirect immunofluorescence in diagnosis of CREST [40]

CREST versus:	Sensitivity (%)	Specificity (%)
Normal controls	65	99.9
Other connective tissue diseases	61	98
Primary Raynaud's phenomenon	60	83
SSc	61	84

The presence of ACA generally carries a better prognosis than many other SSc-associated autoantibodies. In addition, ACA are associated with certain cutaneous and cardiopulmonary manifestations.

ACA are most often seen in the presence of limited cutaneous involvement [39,40], and are also correlated with the presence of calcinosis [41] and ischemic digital loss in patients with SSc [42].

Pulmonary disease occurs in more than 70% of patients with SSc, second only to the esophagus in frequency of visceral involvement. The presence of ACA has been associated with a lower frequency of radiographic interstitial pulmonary fibrosis and a lesser severity thereof [37,39,40,43].

Lung involvement in SSc is defined by numerous measures, most commonly either by the presence of radiographic interstitial fibrosis, but also by abnormal forced vital capacity (FVC) or diffusion capacity for carbon monoxide (DL_{CO}) on pulmonary function tests (PFT). Although pulmonary involvement can also be defined by high-resolution computed tomography of the chest (HRCT) or by bronchoscopy with alveolar lavage, no studies have looked at the presence of autoantibodies in SSc-associated lung disease diagnosed by these means.

The lack of uniform criteria employed for the definition of restrictive lung disease (RLD) makes it difficult to compare studies of PFT. ACA have been found in association with a lower frequency of RLD in some studies [37,44] but not others [45,46]. It is noteworthy that ACA-positive patients are more likely to have an abnormal DL_{CO} but a normal chest radiograph and FVC [47], underscoring that pulmonary hypertension, in the absence of hypoxia from pulmonary fibrosis, is a more common feature of ACA-positive patients with SSc.

Studies on two recent large cohorts of 1321 patients have found there is a lower mortality in ACA-positive patients than in those with positive anti-Scl-70 autoantibodies or AnoA [41,48]. Within 10 years of diagnosis, patients who are positive for ACA and negative for anti-Scl-70 or negative for AnoA had a significantly better survival [41,48].

There seems to be no clinical utility in serially following ACA levels once the SSc patient has been found to be positive for ACA. ACA-positive patients remained positive in nearly all determinations, whether tested by IIF or IB [49–51], and no correlation with extent of disease involvement in any organ system has been established with ACA levels as determined by ELISA [51].

The frequency of ACA in patients with SSc varies depending on their ethnicity. It is highest in Caucasians, where they are found in approximately a third of those, compared with a significantly lower frequency in Hispanic, African American and Thai patients with SSc [52,53] (Table 3).

*HLA-DRB1*01*, *HLA-DRB1*04*, and *HLA-DQB1*05* are associated with the presence of ACA [53,54] and it seems likely that the generation of ACA is influenced by the presence of both human leukocyte antigen (HLA)-DRB1 and HLA-DQB1 alleles [55]. In Caucasian and Japanese patients with SSc, the presence of at least one HLA-DQB1 allele not coding for leucine at position 26 of the first domain was found to be necessary but not sufficient to generate ACA [31,55].

Anti-Scl-70 (anti-topoisomerase I) antibodies

In 1979, a basic, heat-labile, chromatin-associated, nonhistone 70 kDa protein against which autoantibodies from patients with SSc are detected was described; it was isolated from rat liver nuclei with a combination of biologic and immunologic methods. This was initially designated Scl-70 [56]. Subsequent analyses revealed this response to be directed against topoisomerase I [57].

Anti-Scl-70 antibodies have classically been determined by double immunodiffusion techniques against calf or rabbit thymus extract, including Ouchterlony and counter-immunoelectrophoresis (CIE) [49]. However, ascertainment of anti-Scl-70 antibodies by immunodiffusion (ID)

Table 3

Major histocompatibility complex class II associations with autoantibodies seen in patients with SSc

Autoantibodies	HLA association	Comments	References
ACA	HLA-DRB1		
	*DRB1*01*	In Hispanics and Caucasians	[53–55]
	*DRB1*04*		
	HLA-DQB1		
	*DQB1*05*		[53,55]
Anti-Scl-70	HLA-DRB1		
	*DRB1*1101*	In Caucasians and African Americans	[53–55]
	*DRB1*1104*	In Japanese	[53,55]
	*DRB1*1502*	In Hispanics and Caucasians	[55,136]
	HLA-DQB1		
	*DQB1*0301*		[71]
	*DQB1*0601*		
	HLA-DPB1		
	*DPB1*1301*	In Caucasians	[55]
	*DPB1*0901*	In Japanese	[136]
Anti-PM-Scl	*HLA-DQA1*0501*	Not seen in Japanese	[54,80]
	*HLA-DRB1*0301*		
Anti-Th/To	HLA-DRB1*11		[87]
Anti-RNAP	*HLA-DQB1*0201* ?		[91,93]
	No association		
Anti-U1-RNP	HLA-DR2	In Japanese and Caucasians	[117]
	HLA-DR4		
	HLA-DQw5	In African Americans	[118]
	*DQA1*0101*		
	*DQB1*0501*		
	HLA-DQw8		
	*DQB1*0302*		
Anti-U3-RNP/ antifibrillarin antibodies	*HLA-DQB1*0604* ?		[91,93]
	No association		

Question marks denote associations seen in one study but not confirmed elsewhere. ACA, anti-centromere antibodies; HLA, human leukocyte antigen; RNP, ribonucleoprotein.

usually requires 2–3 days and is difficult to automate. To circumvent this problem, IB and ELISA have been introduced more recently [24,57,58]. Topoisomerase I, initially purified from calf thymus glands, was used as antigen [59], although more recent studies have used recombinant topoisomerase I fusion proteins as the substrate for the ELISAs [60].

Anti-Scl-70 antibodies are found in 15–20% of patients with SSc by ID [35,36]. When determined by ID, anti-Scl-70 autoantibodies are virtually never seen in healthy controls [30,34–36,58] or in non-affected relatives of patients with SSc [30,31], nor in those patients with other CTD [28,29] or primary Raynaud's phenomenon [28,29,35,36] (Table 4). As with ACA, the presence of anti-Scl-70 antibodies in a patient initially evaluated for Raynaud's phenomenon can confer an increase in the future development of SSc [33]. The identification of anti-Scl-70 antibodies by IB or ELISA carries a similar specificity to that of ID, with overall higher sensitivity in earlier studies [34–36,52,58,59]. However, a recent article raises concern about the specificity of anti-Scl-70 ELISA assays for SSc, reporting positive results in sera of patients with systemic lupus erythematosus (SLE) that were correlated with disease activity, although this was not reproducible by ID [61].

ACA and anti-Scl-70 antibodies are virtually always mutually exclusive, being present in less than 0.5% of all patients with SSc simultaneously [35,36,41,48,62].

Anti-Scl-70 antibodies are found in about 40% of patients with diffuse cutaneous systemic sclerosis (dcSSc) and less than 10% of patients with limited cutaneous systemic sclerosis (lcSSc) [35,36].

The frequency of anti-Scl-70 antibodies in SSc with pulmonary fibrosis is about 45% [35]. Anti-Scl-70 antibodies have been associated with both the presence and severity of radiographic interstitial pulmonary fibrosis [39,47], whether determined by ID, IB, immunoprecipitation (IP), or ELISA [43]. Anti-Scl-70 antibodies have also been found

Table 4

Sensitivity and specificity of Anti-Scl-70 in diagnosis of SSc

SSc versus:	Assay used	Sensitivity (%)	Specificity (%)
Normal controls	ID	20*	100*
	IB	41*	99.4*
	ELISA	43*	100*
	Overall	34†	99.6†
Other connective tissue diseases	ID	26*	99.5*
	IB	40*	99*
	ELISA	43*	90*
	Overall		97.9†
Primary Raynaud's phenomenon	ID	28*	98*
Non-SSc relatives	ID	25.5*	100*†

*Reference [35]. †Reference [36]. ELISA, enzyme-linked immunosorbent assay; IB, immunoblotting; ID, immunodiffusion.

in correlation with RLD [63] and with a higher rate of decline in PFT [64], although this association is not universal [37,46].

Anti-Scl-70 antibodies carry an increased SSc-related mortality rate, owing to the higher rate of right heart failure in association with RLD and pulmonary fibrosis [65,66]. Although no convincing association has been established for anti-Scl-70 and scleroderma renal crisis in other studies, such an association has been shown in one study of Japanese patients with SSc [67].

Repeated testing for anti-Scl-70 antibodies is unlikely to be useful in clinical practice; although several recent studies have examined serial determinations of anti-Scl-70 antibodies in patients with SSc, a clear role for this in patient care has not been established. Patients who are initially positive tend to remain so over time [45,68], although in one recent study some patients with milder disease became anti-Scl-70-negative later in their disease course [69]. Three studies have shown variations in anti-Scl-70 levels (determined by ELISA) with extent of disease involvement and even seronegative conversion with disease remission [68–70], although this was not seen in others [51].

Unlike ACA, anti-Scl-70 antibody frequency has been shown not to vary depending on ethnic distribution. The presence of anti-Scl-70 antibodies is mediated by the presence of the genes for both HLA-DRB1 and DQB1, although primarily by the former in both Caucasian and Japanese patients with SSc [55,71–72] (Table 3). *HLA-DRB1*11* is associated with anti-Scl-70 antibodies in all ethnic groups, with *HLA-DRB1*1101* and *HLA-DRB1*1104* found in anti-Scl-70-positive Caucasians and African Americans and *HLA-DRB1*1104* found in anti-Scl-70-positive Hispanics [53,55]. HLA-DPB1 alleles have also been implicated in the anti-Scl-70 antibody response in patients with SSc, specifically *HLA-DPB1*1301* in Caucasians and *DPB1*0901* in Japanese [55].

ANoA

Since at least 1970 it has been recognized that the ANoA staining pattern of ANA was associated with SSc. ANoA actually comprises a group of mutually exclusive and heterogeneous autoantibodies that exhibit a typical nucleolar staining pattern of ANA by IIF on various cells (most often HEp2 cells) [1]. They include anti-PM-Scl, anti-Th/To, anti-U3-RNP, AFA, and anti-RNAP I, anti-RNAP II, and anti-RNAP III. Anti-RNAP II and anti-RNAP III do not always yield a nucleolar staining pattern by IIF.

ANoA have been reported in 15–40% of patients with SSc [39,73]. Unlike with ACA and anti-Scl-70, the number of published studies on frequency of ANoA is relatively small. Nevertheless, specific ANoA are rarely seen in healthy controls [1,74] nor in healthy non-affected relatives of patients with SSc [75]. ANoA are perhaps less specific for SSc than was previously thought, because they can be found in patients with other diseases such as SLE and Sjögren syndrome [76,77].

Anti-PM-Scl antibodies were the first of the AnoA to be characterized in 1977. Originally discovered in patients with myositis/scleroderma overlap syndrome (PM/SSc) with the use of Ouchterlony ID techniques [78], anti-PM-Scl are usually identified by IP techniques today [77]. Recently, the anti-PM-Scl autoantibodies have been shown to target six human exosome components that make up an RNA-processing complex, namely hRrp4p, hRrp40p, hRrp41p, hRrp42p, hRrp46p and hCs14p. hRrp4p and hRrp42p are most frequently targeted by the anti-PM-Scl antibody [79]. The frequency of anti-PM-Scl varies between different ethnic groups, ranging from about 3% of patients with SSc and 8% of patients with myositis in Caucasians [1,78], to being absent from a large series of 275 Japanese patients with SSc [43].

Anti-PM-Scl antibodies have been associated with the PM/SSc overlap syndrome [80,81]. As many as 80% of patients with anti-PM-Scl antibodies will have a PM/SSc overlap syndrome [81]. Anti-PM-Scl antibodies are found in as many as 50% of patients with PM/SSc overlap in comparison with less than 2% of patients with SSc in general [2,5]. The PM/SSc-associated overlap syndrome is associated with a more benign and chronic course of disease and responds to a low to moderate dose of corticosteroids [80]. Anti-PM-Scl antibodies predict limited

cutaneous involvement when they are present [43,75,82], although less reliably than ACA. This is likely to be secondary to the relative infrequency of anti-PM-Scl antibodies compared with ACA, reported in less than 15% patients with lcSSc [43,75,82]. Anti-PM-Scl antibodies are strongly linked to *HLA-DQA1*0501* and *HLA-DRB1*0301* [54] (Table 3).

After the discovery of anti-PM-Scl antibodies, the refinement of IP assays using [^{32}P]orthophosphate or [^{35}S]methionine-labeled cell extracts allowed the recognition of another ANoA, anti-Th/To, in 1983 [4,83]. The Th/To antigen has recently been identified. Anti-Th/To antibodies are directed against components of the ribonuclease MRP and ribonuclease P complexes, more frequently Rpp25 and hPop1. The Th40 autoantigen is identical to Rpp38 protein [84]. Anti-Th/To are present in about 2–5% of patients with SSc, being perhaps more common in the Japanese, and were previously virtually never seen in healthy control patients (less than 1%) [47]. This no longer seems to be so, because anti-Th/To antibodies have also been described in patients with SLE, PM and primary Raynaud's phenomenon [76,77]. Anti-Th/To antibodies are also almost never seen in the presence of ACA [76]. Like ACA, their presence most specifically predicts limited skin involvement [47,75,76,84], although routine testing is hardly useful as anti-Th/To autoantibodies are found so infrequently (Fig. 2).

Because of the low frequency of anti-Th/To antibodies, few studies have addressed their clinical significance. One report found that no particular clinical features were associated with anti-Th/To [47]. In another, anti-Th/To-positive patients with lcSSc carried a worse prognosis [85] with a smaller frequency of joint involvement but a greater frequency of puffy fingers, small bowel involvement, hypothyroidism, and a greater risk for reduced survival at 10 years [85], succumbing primarily to pulmonary arterial hypertension. In still another study, anti-Th/To antibodies were described in those patients with SSc who developed the combination of scleroderma renal crisis and pulmonary hypertension without interstitial lung disease [86]. In a study of sera from 172 patients with various CTD [77], anti-Th/To antibodies were increased in those patients with xerophthalmia, esophageal dysmotility and decreased DL_{CO}. The presence of anti-Th/To antibodies has been associated with *HLA-DRB1*11* [55,87] (Table 3).

Anti-RNAP I, anti-RNAP II, and anti-RNAP III were not discovered until 1987 and 1993 [7,88]. Determined by IP techniques, these specific autoantibodies are found in about 20% of patients with SSc [5,82,89] and, like other disease-specific autoantibodies, carry diagnostic and prognostic value. The specificity of anti-RNAP I and anti-RNAP III for SSc is similar and higher than that of anti-RNAP II, which can also be found in patients with

Figure 2

Skin involvement and autoantibody subset of systemic sclerosis.

SLE/SSc and overlap syndrome [90]. Anti-RNAP I and anti-RNAP III almost invariably coexist [5,82,89].

Anti-RNAP antibodies are associated with diffuse cutaneous involvement and have the highest likelihood of being associated with dcSSc than any other disease-specific autoantibodies apart from anti-Scl-70 [7,43,82,88,91,92]. They are found in about 40% of patients with dcSSc. The presence of anti-RNAP II antibodies has been found to independently predict lower lung function, even when ethnicity, age, smoking history, and disease duration were considered simultaneously [64], although this is not uniformly seen [7].

Anti-RNAP antibodies, like anti-Scl-70 antibodies, are correlated with a higher rate of SSc-related mortality, though not independently so. There exists a highly significant association between anti-RNAP antibodies and right heart failure unrelated to pulmonary fibrosis (probably related to pulmonary hypertension), which accounts for this increase [66].

Anti-RNAP I, anti-RNAP II, and anti-RNAP III were found to be associated with *HLA-DQB1*0201* in one study, and no HLA association was seen in another [91,93] (Table 3).

In 1985, anti-U3-RNP antibodies were isolated by IP techniques [94]. More recently it was shown that the mammalian U3 small nuclear RNP (snRNP) is one member of a family of nucleolar snRNPs that are immunoprecipitable by anti-fibrillarin autoantibodies [95]. AFA are present in about 4% of patients with SSc and are mutually exclusive with ACA, anti-Scl-70, and anti-RNAP [96]. AFA have also been described in patients with SLE, UCTD, and primary Raynaud's phenomenon [77]. The frequency of AFA is much higher in patients of African descent with SSc and is reported to be as high as 16–22% compared with only 4% in Caucasian patients with SSc [40,88,95]. AFA are highly specific for dcSSc [1,40,43,47,92,96] and when found in African American patients with SSc are virtually always associated with dcSSc [40,89,96]. Their presence in Caucasian patients with SSc is associated with diffuse skin involvement, but the correlation is not nearly as strong [96]. AFA-positivity in those patients with dcSSc also has

been associated with myositis, pulmonary hypertension, and renal disease. These autoantibodies also identify a younger subset of SSc patients with frequent internal organ involvement. However, in patients with lcSSc the presence of AFA did not predict pulmonary hypertension. Strangely, for its degree of internal organ involvement, AFA were not associated with a higher mortality rate, although those who died tended to succumb to pulmonary hypertension [96].

Although not seen in all studies [93], the autoantibody response to U3-RNP was associated in one study with *HLA-DQB1*0604* [40] (Table 3).

Other autoantibodies

Although the autoantibodies discussed in this section are much less specific to SSc than those already described, the following do carry valuable information.

Anti-Ku antibodies

Anti-Ku autoantibodies were originally thought to be relatively specific for SSc, although they have been reported more recently in sera from patients with SLE, SSc, and overlap syndrome [97,98]. By ELISA, IB, ID, or IP, a total of 159 anti-Ku-positive patients were identified: one-third had an overlap syndrome, 28% SLE, 4% dermatomyositis/-polymyositis (DM/PM), 14% SSc, and 20% other autoimmune diseases. Of those patients with overlap syndrome, nearly 65% had clinical features of scleroderma [98].

aPL

aPL, a group of autoantibodies composed of anticardiolipin antibodies (aCL), lupus anticoagulant antibody, and β_2 glycoprotein I antibodies ($\beta_2$gpI), are found in the antiphospholipid antibody syndrome but also in connection with various autoimmune, inflammatory, infectious, and neoplastic conditions. aPL are correlated with arterial and venous thromboses, livedo reticularis, recurrent fetal loss, thrombocytopenia, and cerebral and myocardial infarction. Although secondary antiphospholipid syndrome is rare in SSc (found in less than 1% of scleroderma patients [99,100]), the frequency of aPL in SSc is about 20–25% (ranging widely from 0% to 63%) [100–107]. Of note, though not widely recognized, aCL and ACA seem to be mutually exclusive [105,106].

There is a great deal of interlaboratory variability in testing aPL as measured by ELISA, which makes it difficult to compare and interpret the association of this antibody with various disease manifestations [104]. In addition, the role of aPL in pathogenesis and determining long-term outcomes in SSc is not clear at present.

The presence of aCL seems to be correlated with higher extent of disease involvement in SSc as defined by the presence of more skin and visceral involvement [100,105] in some studies, although not in others [101,106]. In two studies, aCL were also associated with myocardial ischemia or necrosis [100], although not with the presence of valvular lesions or diastolic dysfunction.

In one study, coexisting $\beta_2$gpI and aCL antibodies were found to be significantly associated with the presence of isolated pulmonary hypertension, and higher levels of these antibodies were correlated with higher mean pulmonary arterial pressure [104].

Although previously believed to have a potential role in the vasculitic phenomenon observed in SSc [100,105], the presence of aCL is not correlated with the presence of vascular lesions, ischemic cutaneous lesions, or digital ulcers [106,108]. aCL-positivity is less commonly present in SSc patients with proximal skin involvement, scarring ,or esophageal hypomotility and is more often associated with limited cutaneous involvement [106]. Thus the clinical utility of determining aCL in patients with SSc has not yet been established.

Antibodies against extractable nuclear antigens

Anti-Sm and anti-U1-RNP antibodies

Autoantibodies against saline-soluble extractable nuclear antigens, including those against Sm antigen and RNP, are found in many CTD. The presence of anti-Sm antibodies is considered to be highly specific for SLE [108] but occasionally occurs in patients with SSc [108]. In contrast, anti-U1-RNP antibodies bind to RNP, a ribonuclease-sensitive antigen involved in splicing heterogeneous nuclear RNA into mRNA. These antibodies are associated with a variety of CTD, including SLE, SSc, PM, and overlap syndrome previously designated 'mixed connective tissue disease' (MCTD) [108].

Anti-Sm and anti-U1-RNP antibodies can be identified by IP in agarose gel by using radial ID or CIE, ELISA, or hemagglutination [24,108]. Of these techniques, CIE is the most rapid; passive ID lacks sensitivity and is most time consuming; hemagglutination is complicated when both Sm and RNP are present; and although ELISA is the most sensitive it does not have the same specificity as ID techniques, particularly when anti-RNP antibodies are present in low levels [24].

Anti-Sm antibodies are rarely found in patients with SSc [108,109]. When found, they are most often present in SSc patients with SLE overlap and portend a poor prognosis with multiple serious organ involvement such as lupus nephritis, renal crisis, and pulmonary hypertension [109]. There is no evidence that the levels of anti-Sm antibodies coincide with SSc severity [110].

The frequency of anti-U1-RNP antibodies in SSc is about 8% (ranging from 2% to 14%) [47,53,104,108]. Anti-

U1-RNP antibodies in high titers are most often found in association with an overlap syndrome/MCTD with a frequency of more than 90% [108,111–114]. More recently, the diagnosis of MCTD as a distinct entity has been disputed [115], being thought of instead as a disease continuum overlap between SLE, SSc, and DM/PM. Clinically, the presence of anti-U1-RNP, whether seen in MCTD, SLE, DM/PM, or SSc, usually portends a favorable response to corticosteroids [108,111] and a more benign prognosis with less tendency for systemic disease characterized by less cutaneous [47,108,112], renal [108,112], and central nervous system disease [43]. Anti-U1-RNP antibodies in patients with CTD are associated with the presence of Raynaud's phenomenon [108,111], puffy hands [47,104,111], sicca [111], pulmonary disease [108,110,111,], arthritis/arthralgia [47,114], myositis [47,108,111], and esophageal disease [108], although this is not seen in all studies [116]. Septal hypertrophy and cor pulmonale secondary to pulmonary hypertension has also been linked to the presence of anti-U1-RNP antibodies [43].

More recently, anti-U1-RNP antibodies have been described to bind a snRNP known as p70 protein (70 kDa). These antibodies against p70, found in SSc and MCTD by IB, are not detected in SLE. Their presence correlates with pulmonary fibrosis, a decrease in FVC, and joint involvement [110].

HLA class II associations with anti-U1-RNP antibodies are less consistent. In some studies they have been associated with HLA-DR2 and DR4 [117]. In others, an increased frequency of HLA-DQw5-associated *DQA1*0101* and *DQB1*0501*, and the HLA-DQw8-associated allele *DQB1*0302*, was seen [118] (Table 3).

Anti-Ro antibodies

Anti-Ro antibodies do occur in patients with SSc, but at a lower frequency than in those with SLE or Sjögren syndrome (less than 35%) [119]. However, Sjögren syndrome has been described in up to 20% of all patients with SSc [120,121] with about one-third to one-half of those with anti-Ro antibodies. Sjögren syndrome is actually associated with about 35% of SSc patients positive for anti-Ro.

Less extensively studied autoantibodies in SSc

The association between more recently characterized autoantibodies and the clinical manifestations of SSc has been less well examined. One report described autoantibodies recognizing granzyme B-cleaved autoantigens as being specifically associated with ischemic digital loss in lcSSc [122].

Anti-neutrophil cytoplasmic antibodies (ANCA) have been reported at a low incidence in SSc (about 3%) without any significantly associated clinical features [123], although there are anecdotal reports of elevated anti-myeloperoxidase antibodies associated with microscopic polyangiitis in SSc [86]. A recent study identified two patients with a positive ANCA and diffuse SSc. One patient was weakly positive for anti-myeloperoxidase antibodies in the absence of renal involvement and the other was strongly positive for anti-proteinase 3 antibodies and had rapidly progressive skin and lung involvement [124]. Whether or not this autoantibody system has any relevance to SSc needs further study.

Autoantibodies against endothelial cell antigen have been described in patients with SSc, supporting the hypothesis that endothelial cell dysfunction and vascular injury are required in the development of scleroderma. Anti-endothelial cell antibodies were found to be correlated with pulmonary fibrosis in patients with SSc in one study [125] but not in another [126]. Anti-endothelial cell antibodies have also been found in association with alveolo-capillary involvement, pulmonary arterial hypertension, digital ulcers and ischemia, severe Raynaud's phenomenon and capillaroscopic abnormalities [126–128]. In addition, these autoantibodies might provide useful information on prognosis because there seems to be a trend toward more severe disease and the presence of anti-endothelial antibodies [128]. This autoantibody system clearly needs further study.

A small number of patients with SSc develop autoantibodies against the centrioles and mitotic apparatus, such as the centrosomes [129,130]. Anti-centriole antibodies are seen in association with primary Raynaud's phenomenon and scleroderma [131,132]. Anti-p80-coilin antibodies have been isolated from the sera of five patients from a serum bank of 810 Japanese patients with 'collagen diseases'. Of these, four had localized scleroderma and one had primary Raynaud's phenomenon [133]. The significance of this autoantibody remains to be determined, although its low prevalence makes it unlikely to be important in the pathogenesis of SSc.

Antibodies against fibrillin-1 protein, an extracellular matrix microfibrillar protein, have been found to be highly associated with SSc in most ethnic groups. In addition, patients with diffuse SSc and CREST also had significantly higher frequencies of anti-fibrillin-1 antibodies than did their controls or other CTD patients [134].

Anti-histone antibodies can be seen in a variety of conditions, including SSc. In one study, limited SSc was associated with the presence of IgM antibodies against histone H1, whereas diffuse SSc was related to the presence of IgG antibodies against the inner core molecules such as H2B [135]. Given the low diagnostic value that anti-histone antibodies have in other CTD (with the highest

Table 5

Autoantibodies in systemic sclerosis

Autoantibody	Methods of testing	Prevalence in SSc	Clinical and serologic associations	Prognosis
Anti-centromere	IIF IB ELISA	20–30%	CREST lcSSc ↓Pulmonary fibrosis Pulmonary hypertension	Better prognosis than anti-Scl-70 ↑Survival compared with anti-Scl-70 or anti-nucleolar antibodies No benefit in following levels over time
Anti-Scl-70	ID CIE IB ELISA	~15–20%	Mutually exclusive with ACA dcSSc Pulmonary fibrosis and secondary cor pulmonale	Worse prognosis ? Levels by ELISA fluctuate with extent of disease involvment
Anti-PM-Scl	ID IP	~3% (Rare in Japanese)	lcSSc PM/SSc overlap	Benign/chronic course with better response to steroids
Anti-Th/To	IP	~2–5% (More common in Japanese)	Mutually exclusive with ACA lcSSc ↓Joint involvement ↑puffy fingers, small bowel involvement, hypothyroidism	Worse prognosis with reduced 10-year survival
AFA	IP	~4% 16–22% in patients of African descent 4% in Caucasians	Mutually exclusive with ACA, anti-Scl-70, anti-RNAP dcSSc Myositis, pulmonary hypertension, renal disease	Seen in younger patients with greater internal organ involvement
Anti-RNAP	IP	~20%	dcSSc Anti-RNAP II with ↓lung function Cor pulmonale unrelated to pulmonary fibrosis	Increased mortality
Anti-Ku	IB IP ELISA	Infrequent	Overlap syndrome with scleroderma features	
Anti-Ro	ID ELISA	Infrequent	Seen in one-third to one-half of SSc patients with sicca complex	
Anti-Sm	IIF IP/CIE/ID ELISA HA	Rare Lupus nephritis, renal crisis Pulmonary hypertension	SLE overlap	Poor prognosis

Continued

Table 5

Continued

Autoantibody	Methods of testing	Prevalence in SSc	Clinical and serologic associations	Prognosis
Anti-ribonucleoprotein	IIF IP/CIE/ID ELISA HA	~8%	MCTD Less CNS and renal diseases Raynaud's, puffy hands, sicca, myositis, esophageal disease lcSSc Septal hypertrophy Cor pulmonale secondary to pulmonary hypertension Negatively correlates with dsDNA and low complement glomerulonephritis in those with SLE overlap	More benign prognosis with favorable response to steroids
Anti-phospholipid antibodies	ELISA	~20–25% with <1% SSc with APS	Mutually exclusive with anti-centromere antibodies aCL with ↑ disease severity and ↓ proximal skin involvement, scarring, esophageal hypomotility in one study $\beta_2$gp/aCL with pulmonary hypertension Associations inconsistent – needs further study	Associations inconsistent – needs further study

ACA, anti-centromere antibodies; aCL, anticardiolipin antibodies; AFA, antifibrillarin/anti-U3-ribonucleoprotein; $\beta_2$gp, β_2 glycoprotein antibodies; CIE, counterimmunoelectrophoresis; CNS, central nervous system; dcSSc, diffuse cutaneous systemic sclerosis; dsDNA, double-stranded DNA; ELISA, enzyme-linked immunosorbent assay; HA, hemagglutination; IB, immunoblotting; ID, immunodiffusion; IIF, indirect immunofluorescence; IP, immunoprecipitation; lcSSc, limited cutaneous systemic sclerosis; MCTD, mixed connective tissue disease; PM/SSc, myositis/scleroderma overlap; SLE, systemic lupus erythematosus.

prevalence in drug-induced SLE), this finding needs to be confirmed further.

Summary

Significant serologic heterogeneity is well known to occur in SSc. Although it remains controversial whether autoantibodies seen in patients with SSc have an actual role in pathogenesis, these serologic markers are useful in the diagnosis and clinical management of scleroderma patients (Table 5). ACA are most often found in Caucasians and in association with limited cutaneous involvement, CREST, and isolated pulmonary hypertension. In contrast, they are infrequently found in patients with pulmonary fibrosis. ACA seem to be a marker for a better prognosis, whereas anti-Scl-70 antibodies, found in patients with dcSSc and pulmonary fibrosis, portend a poor prognosis with increased SSc-related mortality. The following of ACA and anti-Scl-70 levels over time has not been shown to have clinical utility. Of the ANoA, anti-PM-Scl and anti-Th/To antibodies are associated chiefly with lcSSc (with anti-PM-Scl antibodies associated with an overlap syndrome), whereas AFA and anti-RNAP are seen with dcSSc. Anti-Th/To, anti-RNAP and AFA are associated with a less favorable prognosis with a higher frequency of organ involvement, contrary to what is seen in those with anti-PM-Scl antibodies.

Anti-Ku antibodies might have a role in identifying CTD patients with overlap syndrome involving features of scleroderma in the absence of other autoantibodies such as anti-PM-Scl or anti-U1-RNP antibodies. Anti-Ro antibodies are identified in the sera of SSc patients with Sjögren syndrome. Anti-Sm antibodies are rarely seen in patients with SSc unless there are features of SLE overlap. When present, they predict a poor prognosis with frequent renal involvement. Anti-U1-RNP antibodies are usually seen in association with CTD overlaps, specifically with Raynaud's phenomenon, joint involvement, myositis, lcSSc, and a more favorable outcome. Although not seen in association with thrombosis in patients with SSc, inconsistent findings of associations with myocardial ischemia and pulmonary hypertension indicate a need for further study before any clear place of aPL determinations in patients with SSc can be recommended. Similarly, the clinical relevance of more newly recognized autoantibody systems in patients with SSc, particularly ANCA, anti-endothelial cell antibodies and anti-fibrillin-1, needs more study.

Much like the SSc, these disease-associated autoantibodies differ in their frequencies, associated clinical manifestations, pathophysiology, and ethnic and genetic associations. When used correctly they can be a clinically relevant and useful tool in patient management.

Competing interests

None declared.

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