

Clinical and Serological Hallmarks of Systemic Sclerosis Overlap Syndromes

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ABSTRACT. *Objective.* To determine the prevalence of systemic sclerosis (SSc) overlap syndrome and autoantibody profile in a large single-center cohort.

Methods. SSc diagnoses, subsets, and autoantibody profiles were obtained from clinical records of patients attending the Centre for Rheumatology, Royal Free Hospital, between September 1999 and February 2007.

Results. In total, 332 (20%) of 1700 patients with SSc had overlap syndrome. This comprised myositis (42.8%), rheumatoid arthritis (RA; 32%), Sjögren's syndrome (SS; 16.8%), and systemic lupus erythematosus (SLE; 8.4%). Antinuclear antibody was positive in 96.6% of patients. Anticentromere antibody (ACA) was exclusively present in limited cutaneous SSc (lcSSc) overlap cases (22%), and more common in SSc/SS overlap (44.7%), whereas no difference was found in the prevalence of Scl-70 autoantibody between lcSSc and diffuse cutaneous SSc overlap groups. U1RNP was more frequent in SSc/SLE (44%), while Ro antibody was more likely to be found in SSc/SS (29.8%). ACA was absent and anti-Scl-70 was infrequent in SSc/myositis; polymyositis-scleroderma antibody was more frequent in this group (33.1%). About 50% of patients had raised rheumatoid factor (RF), with no difference between overlap groups irrespective of RF titer. In contrast, anticyclic citrullinated peptide antibody was more frequent in patients with RA features.

Conclusion. About one-fifth of SSc cases had overlap features. There were distinct serological features that may predict specific clinical presentation and disease course. (J Rheumatol First Release Aug 15 2011; doi:10.3899/jrheum.101248)

Key Indexing Terms:

SYSTEMIC SCLEROSIS

OVERLAP SYNDROME

Systemic sclerosis (SSc) is a multisystem rheumatic disorder with a wide variety of clinical and serological manifestations. Two major subsets are designated limited cutaneous (lcSSc) and diffuse cutaneous (dcSSc)^{1,2}. In some cases of SSc, the disease may overlap with another connective tissue disease (CTD), such as myositis, rheumatoid arthritis (RA), Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), and others³. Although there is no firm definition of overlap features this is generally considered when manifestations of myositis, arthritis, or other autoimmune rheumatic disease are substantially greater than normally observed in SSc.

Autoantibody testing in SSc often reveals autoantibodies specific to SSc. These are generally mutually exclusive for the hallmark SSc-specific reactivities and have particular clinical associations⁴. Thus, many patients with lcSSc carry anticentromere antibodies (ACA) while antitopoisomerase I antibody

(anti-Scl-70) is frequently found in those with dcSSc⁵. Certain internal organ complications appear to occur with increased frequency in different serological reactivities. Other autoantibodies are also reported and these may be associated with clinical features of overlap CTD. The purpose of our study was to determine the prevalence of clinical features of overlap CTD in patients with definite SSc, and to describe the frequency of autoantibodies.

MATERIALS AND METHODS

SSc diagnoses were obtained from clinical records of patients attending the Centre for Rheumatology at the Royal Free Hospital between September 1999 and February 2007. Diagnoses, subsets, and autoantibody profiles were systematically recorded in our research database; missing data were retrieved from medical notes. Overlap syndrome was defined as a disease occurring with clinical aspects of SSc [according to the American College of Rheumatology (ACR) criteria⁶ or main symptoms of SSc simultaneously with those of other CTD such as myositis, RA, SS, and SLE. Arthritis was diagnosed according to the ACR criteria of 1987⁷. Myositis was diagnosed using the Bohan and Peter criteria⁸. According to skin involvement, SSc patients were divided to lcSSc and dcSSc subgroups. In the former, skin involvement is limited to areas distal to knees and elbows, while the latter affects the proximal extremities and trunk. Autoantibodies were measured in an accredited institutional autoimmune serology laboratory using validated commercial tests with appropriate quality control and blinded assessment of the results at time of reading⁹. In brief, antinuclear antibody (ANA) pattern was screened by indirect immunofluorescence using a HEp-2 cell substrate (Bion Inc., Park Ridge, IL, USA) with rabbit antihuman polyvalent FITC con-

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jugate (F0200; Dako, Ely, Cambridgeshire, UK). Further characterization of defined extractable nuclear antigen (ENA) was by counterimmunoelectrophoresis for anti-ENA using soluble extracts from human spleen and rabbit thymus acetone powder (Pelfreez Biologicals, Rogers, AR, USA) as antigen. ELISA was used for further determination of some reactivities including anti-RNA polymerase III (RNAP), and for anticitrullinated protein antibodies (ACPA) detection a commercial fluorescence enzyme immunoassay was used (EliA CCP on the ImmunoCAP 250, Phadia, Freiburg, Germany). Rheumatoid factor (RF) IgM level was determined using nephelometry. Our laboratory has previously verified that this ELISA robustly predicts positive results for RNAP by immunoprecipitation¹⁰. Statistical evaluation included chi-square tests to describe significant differences or associations.

All patients included in this study signed informed consent for their clinical and laboratory data to be used in this clinical research project.

RESULTS

At the time of this analysis, a total of 1700 patients with SSc had been entered into the clinical research database. The most frequent subset was lcSSc (970, 57%), followed by dcSSc (398, 23%) and SSc overlap syndrome (332, 20%). Overlap features were equally distributed between diffuse and limited subsets. In SSc overlap syndrome, 35 patients had 3 or more CTD. Out of 297 patients with 2 overlapping CTD, 213 (71.7%) had the limited and 81 (27.3%) had the diffuse subset. Subset data was not available for 3 patients (SSc/myositis group). The most common SSc overlap syndrome was myositis (127, 42.8%), followed by RA (95, 32%), SS (50, 16.8%), and SLE (25, 8.4%). Interestingly, the prevalence of the diffuse subset was relatively high in those with SSc/myositis overlap syndrome compared to other SSc overlap groups (40% vs 17%–26%, respectively).

The frequency of autoantibodies was determined in SSc overlap syndromes (Table 1). ANA was positive in 96.6% of all cases of SSc overlap syndrome; however, data were missing in 5 cases (1.7%). ACA was observed in 22% of lcSSc overlap cases (13.8% of all SSc overlap), while it was virtually absent in dcSSc overlap syndrome. ACA was significantly most common in SSc/SS compared to SSc/RA and SSc/SLE

(44.7% vs 27.4% and 8.7%, respectively; $p < 0.05$). No patient had ACA in the SSc/myositis group. We found 17% of all patients carried anti-Scl-70 autoantibody. There was no statistical significance in the prevalence of anti-Scl-70 between the lcSSc and dcSSc groups (10.3% and 18.5%, respectively) or between subsets. No individual had both ACA and anti-Scl-70 autoantibodies. U1RNP was significantly more frequent in SSc/SLE patients (44% vs 15.7%, 11.5% and 14.6%; SSc/SLE vs SSc/myositis, SSc/RA, and SSc/SS, respectively; $p < 0.05$). Antibodies against Ro were significantly more likely in SSc/SS compared to SSc/myositis and SSc/RA (29.8% vs 6.3% and 12.6%, respectively; $p < 0.01$ and $p < 0.05$). The most frequently appearing autoantibody in SSc/myositis was the polymyositis-scleroderma (PM/Scl) antibody, statistically more prevalent compared to SSc/RA or SSc/SS (33.1% vs 10.3% and 2.1%, respectively; $p < 0.01$), whereas it was virtually absent in SSc/SLE. No other coexisting autoantibody than PM/Scl was detected in 94% of the cases. In addition, anti-Jo-1 antibodies were found in 8 (6.3%) patients with SSc/myositis. RNAP and U3RNP antibodies (present in 0–4.2% and 0–6.2% of overlap subsets, respectively) were not significantly more common in any of the overlap groups. Anti-Ku antibodies were uncommon, being detected in 2.3% of SSc/myositis and 1% of SSc/RA.

Of 297 patients, 245 had RF tested, of which 50.6% had raised RF (65.5% of SSc/RA, 58.7% of SSc/SS, 50% of SSc/SLE, and 33.3% of SSc/myositis; Table 2). Among the seropositive cases, RF was strongly positive (3 times the upper limit of normal) in 58.2% of SSc/RA, 48.1% of SSc/SS, 38.7% of SSc/myositis, and 36.4% of SSc/SLE (no statistical difference). In addition, the ACPA test was carried out in one-third of SSc/RA overlap cases. ACPA antibody was found in 18 of 31 SSc/RA patients (58%), while it was significantly less frequent in the other 3 overlap groups (2 of 15, 13.3%; $p < 0.01$). Elevated levels of RF were observed in these cases.

Table 1. Autoantibody profile in SSc overlap syndrome.

	SSc/Myositis		SSc/RA		SSc/SS		SSc/SLE	
	lcSSc, n (%)	dcSSc, n (%)						
Patients	73 (57.5)	51 (40.2)	80 (84.2)	15 (15.8)	41 (82)	9 (18)	19 (76)	6 (24)
ANA	73 (100)	50 (98)	76 (95)	14 (93.3)	39 (95.1)	9 (100)	19 (100)	6 (100)
ACA	—	—	23 (28.8)	—	21 (51.2)	—	2 (10.5)	—
Scl-70	4 (5.5)	6 (11.7)	13 (16.3)	4 (26.7)	3 (7.3)	5 (55.6)	3 (15.8)	—
U1RNP	14 (19.2)	6 (11.7)	9 (11.3)	—	6 (14.6)	—	8 (42.1)	3 (50)
Ro	5 (6.8)	3 (5.9)	7 (8.8)	4 (26.7)	13 (31.7)	1 (11.1)	2 (10)	1 (16.7)
La	1 (1.4)	1 (2)	4 (5)	—	5 (12.2)	—	—	—
PM/Scl	25 (34.2)	17 (33.3)	7 (8.8)	2 (13.3)	—	1 (11.1)	—	—
Jo-1	7 (9.6)	1 (2)	—	1 (6.7)	—	—	—	—
RNAP	1 (1.4)	3 (5.9)	3 (3.8)	1 (6.7)	—	1 (11.1)	—	—
U3RNP	2 (2.8)	6 (11.7)	1 (1.3)	—	—	—	—	1 (16.7)

SSc: systemic sclerosis; RA: rheumatoid arthritis; SS: Sjögren's syndrome; SLE: systemic lupus erythematosus; lcSSc: limited cutaneous systemic sclerosis; dcSSc: diffuse cutaneous systemic sclerosis; ANA: antinuclear antibody; ACA: anticentromere antibody; Scl-70: antitopoisomerase I Antibody; RNAP: RNA polymerase antibody.

Table 2. Rheumatoid factor (RF) in SSc overlap syndrome.

	SSc/Myositis		SSc/RA		SSc/SS		SSc/SLE	
	lcSSc, n (%)	dcSSc, n (%)						
Patients	73 (57)	51 (40.2)	80 (84.2)	15 (15.8)	41 (82)	9 (18)	19 (76)	6 (24)
RF+	17 (23.3)	13 (26)	48 (60)	7 (46.7)	24 (58.5)	3 (33.3)	9 (47.4)	2 (33.3)
RF-	37 (50.7)	24 (48)	24 (30)	5 (33.3)	13 (31.7)	6 (66.6)	8 (42.1)	3 (50)
RF unknown	19 (26)	14 (28)	8 (10)	3 (20)	4 (9.8)	—	2 (10.5)	1 (16.7)

Definitions as given in Table 1.

DISCUSSION

Compared to other CTD, SSc is relatively rare, and few studies have analyzed disease presentation in SSc overlap syndrome on a large scale. Caramaschi and co-workers reported 32.2% of 118 Italian patients with SSc had at least 1 concomitant autoimmune disease, most commonly autoimmune thyroiditis (14.4%) and SS (8.4%)¹¹. On a larger scale, a recent study of 719 patients with SSc from Canada and Colombia described additional autoimmune features in 38% of the cases, most frequently autoimmune thyroiditis (38%), RA (21%), SS (18%), and primary biliary cirrhosis (4%)¹². In the registry of the German SSc Network, which included 1483 patients from 27 centers, 10.9% of SSc patients had overlap syndrome¹³. Our study was based on a large single-center cohort including 1700 patients with SSc, from which we identified 332 (20%) with SSc overlap syndrome, indicating that SSc overlap syndrome is a relatively common condition. The distribution between limited or diffuse subsets in the SSc overlap group was similar to the SSc population without overlap features; however, an increased prevalence of the diffuse subset in SSc/myositis (40%) was observed. The most common overlap syndrome was SSc/myositis (42.8%), followed by SSc/RA (32%), SSc/SS (16.8%), and SSc/SLE (8.4%).

Autoantibody testing in SSc usually reveals antibodies associated more often with one or another subset. They are not only disease-specific but also strong predictors of the disease severity and clinical presentation⁴. The frequency of ACA in patients with SSc has been reported to be 16%–39% and is strongly associated with the occurrence of lcSSc (up to 60%)^{5,14,15}. In our cohort, 96.6% of patients had ANA; ACA was exclusively present in lcSSc overlap cases (22%), significantly more common in SSc/SS overlap (44.7%). Previously, Scl-70 antibodies were found in about 9%–39% of patients with SSc, with slight predominance in the diffuse subset¹⁵. Our data showed no difference in the prevalence of Scl-70 autoantibody between lcSSc and dcSSc overlap groups. In the SSc/myositis group, ACA was absent and Scl-70 autoantibody was infrequent (7.8%). On the other hand, the PM/Scl antibody, which is considered to be less specific to SSc¹⁶ and is present in 0–6% of the SSc population¹⁵, was carried by one-third of SSc/myositis patients. Even though autoantibodies against U1RNP and Ro were generally associated with all overlap syndromes, anti-U1RNP was more frequent in

SSc/SLE (44%), while anti-Ro was common in SSc/SS (29.8%). Interestingly, anti-RNAP antibodies, which are highly specific to SSc and are predictors of SSc renal crisis, were infrequently present in the overlap cohort (0–4.2%), while previous studies suggested a higher incidence in the general SSc population (4%–25%)¹⁵.

In the future it may be possible to use serological testing to better define overlap SSc, but this will require further validation perhaps in a well-documented prospective study. Our report provides some support for the validity of such an approach.

In a recent study by Ueda-Hayakawa and co-workers, where the incidence of SSc/RA overlap was 7.5% (11/146), RF was detected in 73% of SSc/RA overlap patients, and similarly, ACPA was found in 63.3% of SSc/RA overlap cases¹⁷. In our cohort, about half of all SSc overlap cases had positive RF. Although most frequently present in SSc/RA patients (65.5%), no statistical difference was found in the prevalence of RF between groups, even when detected in high titers, suggesting poor specificity. Further, we showed that ACPA were positive in 18 of 31 patients in the RA overlap group (58%), but less frequently detected in patients with other SSc overlap syndromes (13.3%). These findings suggest the ACPA test may be useful to detect RA in patients with SSc.

The main limitation of our study is that the information on overlap features based on expert clinician diagnosis was obtained retrospectively from research database and medical notes. In addition, ACPA serology was missing in a number of cases; hence one must consider that the tested individuals might represent a randomly or rather carefully selected population. Finally, although this study highlights the important associations between autoantibodies and overlap features, in terms of autoantibody frequencies in the nonoverlap cohort we relied on previously published data.

There is a high prevalence of overlap features in patients with SSc. Our study suggests that clinically different overlap phenotypes could be indicated by the presence of the autoantibodies. There were distinct serological features in our study group and early identification may permit better management of the non-SSc aspects of disease. Overlap SSc may represent an important clinical subgroup, and risk factors of developing pulmonary, renal, and cardiovascular complications should be defined. In addition, investigation of overlap SSc may dis-

close pathogenic processes common to different autoimmune rheumatic diseases. Whether overlap cases should be excluded from interventional clinical trials is not clear and will depend upon better characterization in future studies. Operationally, the overlap features may have substantial clinical effects and might be more treatable than the associated SSc, or result in modification of treatments such as avoiding high-dose corticosteroid therapy because of the risk of scleroderma renal crisis.

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