Overlap of ACA-positive systemic sclerosis and Sjögren's syndrome: a distinct clinical entity with mild organ involvement but at high risk of lymphoma

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Abstract

Objectives

We aimed to assess the prevalence of patients with either primary Sjögren's syndrome (pSS) and positive anticentromere antibodies (ACA) and secondary Sjögren's syndrome (sSS) and limited cutaneous ACA-positive systemic sclerosis (SSc) in two large cohorts of patients with pSS and SSc, and also to compare the clinical features of these two subsets with those of patients affected by "ACA-positive SSc without sicca symptoms" and "pSS".

Methods

In this retrospective monocentric study, the case records of "overlap" patients fulfilling both the classification criteria for SS and the LeRoy criteria for early SSc were identified from two datasets of patients with limited cutaneous ACA-positive SSc (209 subjects) and with pSS (402 subjects) who attended our Rheumatology Unit in the years between 1989 and 2011. Control groups were represented by SSc subjects without sicca symptoms ("SSc group") and ACA-negative pSS patients ("pSS group"). SSc patients with sicca symptoms ("Sicca-SSc group") who did not complete the diagnostic algorithm for SS were excluded from the analysis. Demographic, clinical and immunological data of the patients enrolled were collected cumulatively over the entire follow-up period. Statistical analysis was performed using SPSS 13 (SPSS Inc., Chicago IL, USA)

Results

Out of the two datasets 41 "overlap" patients were selected. The control groups were represented by 102/209 SSc subjects without sicca symptoms ("SSc group") and 387/402 pSS patients ("pSS group"). Eighty-one "sicca-SSc" with an incomplete work-up for SS were excluded from the analysis. The prevalence of ACA-positive pSS patients among pSS was 3.7% (15/402), while the frequency of patients with definite sSS in the SSc cohort was 20% (26/128). No differences were detected between "overlap" patients and control groups, relatively to demographic characteristics. "Overlap patients" were characterised by a milder SSc disease (i.e. lower frequency of sclerodactily, negative evolution of the capillaroscopy pattern or absence of severe systemic involvement) whereas, as far as the SS-related manifestations were concerned, although often lacking in specific autoantibodies (i.e. rheumatoid factor, anti-Ro/SSA, anti-La/SSB), the "overlap patients" displayed a full blown SS phenotype with recurrent salivary gland enlargement, purpura, fatigue, arthralgias, and leukocytopenia. It is noteworthy that the prevalence of non-Hodgkin's lymphoma in the "overlap patients" was higher than in pSS.

Conclusions

Taken together, the results of our work emphasise the existence of a novel distinct clinical entity which might tentatively be called "ACA-positive limited scleroderma/SS overlap syndrome" characterised by a benign SSc clinical course but at a high risk of non-Hodgkin's lymphoma.

Key words

systemic sclerosis, Sjögren's syndrome, lymphoma

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Introduction

Systemic autoimmune diseases may present with a clinical picture varying from undifferentiated to overlapping diseases (1-6). Sjögren's syndrome (SS) is a systemic autoimmune disease characterised by a progressive hypofunction of the salivary and lachrymal glands, frequently associated with a variety of extra-glandular manifestations, including malignant lymphoproliferative disorders (7, 8). The disease may occur alone as primary SS or in association with other connective tissue disorders as secondary SS. In particular, the association between systemic sclerosis (SSc) and SS has been described since 1965 when Bloch *et al*. (9) first reported the cases of three patients with SS and progressive SSc. Afterwards, several studies have confirmed the existence of a full SS clinical phenotype in patients with SSc, especially in those presenting a limited SSc with positive anticentromere antibodies (ACA) (10-20). On the other hand, an increasing number of reports have described the existence of a subgroup of SS patients with positive ACA regarded as a distinct clinical variant of pSS (13, 17, 18, 21-24). Thus, the interplay between SS and SSc seems to be quite complex encompassing a wide spectrum of clinical intermediate and overlapping phenotypes which might make it challenging to differentiate one from the others in the absence of novel biomarkers (25-36). In the present study we analysed the prevalence and clinical profile of patients classified as affected by either "ACA-positive limited cutaneous SSc and concomitant sSS" or "ACA-positive pSS". The features of these patients were compared with those of both the patients affected by "ACA-positive SSc without SS" (from now on called SSc) and the "ACA-negative pSS" patients (from now on called pSS).

Patients and methods

This study was a single-centre, retrospective, observational study carried out at a tertiary-referral Rheumatology centre.

Patient selection

The case records of all the patients with a diagnosis of limited cutaneous ACA-positive SSc and/or pSS attending the Rheumatology Unit of the University of Pisa in the years between 1999 and 2011 were reviewed in order to identify patients fulfilling both the classification criteria for ACA-positive SSc and for SS. The diagnosis of SSc was made according to the "American Rheumatism Association criteria" (37) and LeRoy *et al* "early SSc criteria" (38); the diagnosis of SS followed the "American-European consensus group" (AECG) criteria (39). Criteria for pre-scleroderma or very early SSc, as recently put forward by the European League Against Rheumatism Scleroderma Trials and Research Group (EUSTAR) (40) was also assessed in all the cases. Overall, 611 patients were enrolled in this study: 209 with a diagnosis of limited cutaneous ACA-positive SSc and 402 with a diagnosis of pSS.

Data collection

Demographic, clinical and immunological data were collected cumulatively over the entire follow-up period for all patients of each group. According to the most recent literature studies, disease onset was defined by the appearance of Raynaud's Phenomenon, sicca manifestations, salivary gland enlargement, arthritis, purpura, puffy fingers, sclerodactily, digital ulcers, calcinosis, dysphagia, gastroesophageal reflux, pulmonary arterial hypertension or lung fibrosis (41-43).

Clinical and laboratory assessment for the SS component included data on subjective dry eyes and mouth, parotid gland enlargement and previous or present extra-glandular manifestations as defined in the recently published activity and damage criteria for the disease and in the largest case series of the literature (8, 44-48). Objective xerostomia and keratoconjunctivitis sicca were assessed according to the AECG criteria. Biopsy results of minor salivary glands were classified according to the focus scoring system and/or the Chisholm and Mason score (39, 49). Diagnosis of lymphoma required histological confirmation. For the SSc component we collected data on the presence of sclerodactily, Raynaud's phenomenon, telangiectasia,

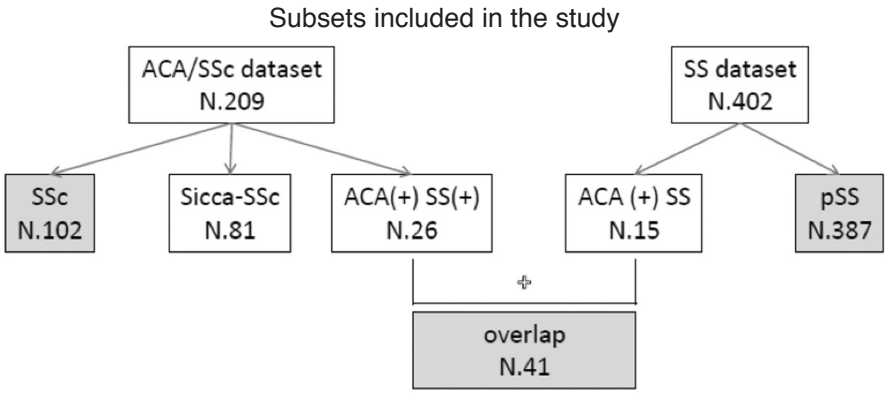


Fig. 1. Displays the subsets included in the study. Two hundred and nine from our Rheumatology Unit from 1989 to 2011. Out of the two datasets of patients with a diagnosis of limited cutaneous ACA-positive SSc (n=209) and with pSS (n=402) we selected 102/209 subjects with limited cutaneous SSc without sicca symptoms (SSc group), 387/402 patients with ACA-negative pSS (pSS group) and 41 patients who fulfilled both the LeRoy early SSc diagnostic criteria and the diagnostic criteria for SS ("overlap" group). This "overlap" group consisted of 26 patients originally classified among the 209 SSc patients and of 15 patients previously included in the pSS dataset. Eighty-one SSc patients with sicca symptoms ("sicca SSc").were excluded from the analysis since either the diagnosis of sSS was not substantiated by a minor salivary gland biopsy or they did not complete the algorithm for sSS diagnosis as required by the AECG criteria 2002.
Note: *SSc*: patients with ACA(+)SSc and no sicca symptoms; *Sicca-SSc*: SSc patients with sicca symptoms an incomplete work up SS; *Overlap*: patients fulfilling both the criteria for SSc and SS; *pSS*: patients with primary SS.

eosophageal involvement, lung interstitial fibrosis, pulmonary hypertension, and digital ulcerations. In particular pulmonary hypertension was defined by an increase in mean pulmonary arterial pressure (PAP) 25 mmHg at rest as assessed by right heart catheterisation (50); if a right heart catheterisation had not been performed, a pulmonary artery systolic pressure >40 mmHg on the heart Doppler echocardiography was considered diagnostic. Lung interstitial involvement was documented by chest x-ray and when required by high resolution computed tomography. As far as laboratory tests were concerned, white blood cell count <4000/mm^3, C3 <80 mg/dl, C4 <10 mg/dl, IgG globulin >1.6 g/dl, creatinine clearence <60 ml/min, proteinuria >300 mg/day and urinary pH >6 were considered abnormal. Immunological data included antinuclear antibodies (detected by indirect immunofluorescence), anti Ro/SSA, La/SSB antibodies (by counterimmunoelectrophoresis), and rheumatoid factor (RF, by nephelometry). ACA were detected by indirect immunofluorescence. Data on mixed cryoglobulinemia and monoclonal antibodies were not available for many of the patients enrolled in this study and so were not included in the analysis.

Statistical analysis

Data were expressed as mean±SD for continuous variables and as absolute frequencies and percentages for nominal variables. Chi-Square, ANOVA and Logistic regression were performed. Patients with missing data were excluded from the respective analysis. Statistical analysis was performed using SPSS 13 (SPSS Inc., Chicago IL, USA)

Results

Study population

Two hundred and nine patients with a diagnosis of limited cutaneous ACA-positive SSc and 402 patients with pSS attended our Rheumatology Unit from 1989 to 2011. Out of the two datasets, we selected 102/209 subjects with limited cutaneous SSc without sicca symptoms (SSc group), 387/402 patients with ACA-negative pSS (pSS group) and 41 patients who fulfilled both the LeRoy early SSc diagnostic criteria and the diagnostic criteria for SS (from now on called "overlap" group). Eighty-one SSc patients with sicca symptoms were excluded from the analysis since either the diagnosis of sSS was not substantiated by a minor salivary gland biopsy or they did not complete the algorithm for sSS diagnosis as required by the AECG criteria 2002 (from now on called "sicca SSc"). Figure 1 summarises the different subsets included in the study. It can be observed that the prevalence of ACA(+) SS patients was 3.7% (15/402) while the frequency of patients with sicca symptoms and/or definite sSS in the SSc cohort were 51.2% (107/209) and 20% (26/128), respectively. Table I summarises the epidemiologic features of the three subgroups of patients enrolled and namely: the "overlap group" (n=41), the "SSc group" (n=102) and the "pSS group" (n=387). No differ-

Table I. Epidemiologic features of the patients enrolled in the study.

Epidemiologic features	Overlap* (n=41)	SSc (n=102)	pSS (n=387)	*p*-value	sicca-SSc** (n=81)
Sex	41 F:0 M	95 F:7 M	376F:11 M	NS	80F:1M
Age at the onset (mean ±SD, yrs)	45 ± 13	46 ± 15	49 ± 14	NS	48 ± 16
Age at diagnosis of SSc (mean ±SD, yrs)	52 ± 12	54 ± 14	n.a.	NS	58 ± 12
Age at the inclusion (mean ±SD, yrs)	59 ± 12	60 ± 14	59 ± 14	NS	64 ± 11
Disease duration from the onset (mean ±SD, yrs)	13.6 ± 9.4	15 ± 1.6	14 ± 10	NS	16 ± 13
Length of follow-up (mean ±SD, yrs)	6 ± 5	6 ± 7	7 ± 7	NS	6 ± 7
Menopausal status (+)	32 (78%)	78 (77%)	270 (75%)	NS	60 (74%)

Notes.*_overlap_: patients with both SSc and SS; **_sicca-SSc_: SSc patients with sicca symptoms for whom the diagnosis of sSS was not substantiated by a minor salivary gland biopsy or by a complete diagnostic algorithm as required by the AECG criteria 2002.

ences were detected between the three groups as far as the epidemiologic and demographic characteristics were concerned. In particular, the three groups did not differ for sex ratio, age at the onset of the disease, age at the diagnosis of SSc, age at the inclusion, menopausal status and follow-up length.

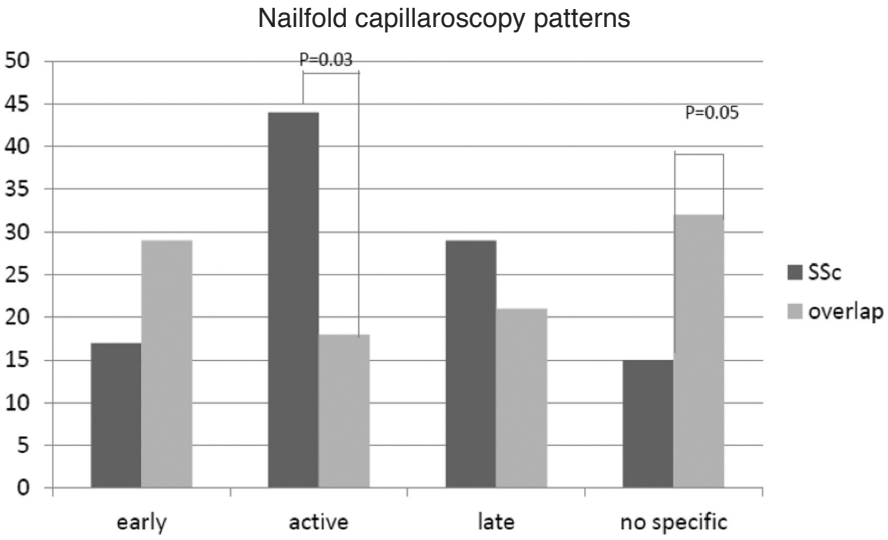
SSc-related manifestation in "overlap patients" vs. "SSc patients"

The frequency of "SSc-related clinical manifestations" is summarised in Table III. All the patients included in the study satisfied the Le Roy criteria and the VEDOSS criteria whereas the ARA criteria were fulfilled by 88% of the "SSc patients" and by 61% of the "overlap patients" with a statistically significant difference between the two groups (p<0.0001). Although all the "overlap patients" fulfilled at least the classification criteria for early SSc, the comparative analysis shows that these patients had a less severe systemic involvement than the "SSc patients". Raynaud's phenomenon represented the first symptom for the vast majority of both "SSc patients" and "overlap patients" (96% *vs*. 85% respectively, p=0.07). Nonetheless, scleroderma-type nailfold capillary patterns were detected more frequently in the former subgroup rather than in the latter (Fig. 2). In particular, we found an unspecific abnormal capillaroscopy pattern in the 32% of the "overlap patients" *vs*. 15% of the "SSc patients" (p-value=0.05) and an active pattern in 18% of the "overlap patients" *vs*. 44% of the "SSc patients" (p-value=0.03). The onset of subjective sicca symptoms was apparently concomitant to Raynaud's phenomenon in 21/41 (51%) of the "overlap patients" whereas dry eye and dry mouth were referred later during the follow-up by 18/41 (44%) patients, with a mean latency from the first symptom of 7.9±7.3 years. Only in 2/41 (5%) of the cases sicca symptoms preceded the diagnosis of SSc. As far as internal organ involvement was related, when compared with patients with SSc alone, the "overlap patient" subgroup seemed to evolve to a less serious SSc disease. More specifically, "overlap patients" presented a higher prevalence of teleangectasia (43.7% *vs*. 3.4%, p=0.02) but a lower prevalence of both digital necrosis and ulcers (12.2% *vs*. 49%, p<0.0001) and sclerodactily (51.2% *vs*. 87.3%, p<0.0001), with 20 out of the 41 the "overlap patients" not

Table II. Clinical pattern of the "SSc-related manifestations".

Features	Overlap * (n=41)	SSc (n=102)	p-value	sicca-SSc** (n=81)
ARA criteria	25 (61%)	90 (88%)	<0.0001	66 (81%)
Le Roy criteria	41 (100%)	102 (100%)	NS	81 (100%)
Capillaroscopy (specific pattern)	29 (72.5%)	90 (92.8%)	0.004	62 (76%)
Difficulty in swallowing/Dysphagia	28 (68.3%)	76 (74.5%)	NS	50 (61%)
Esophageal involvement	22 (53.7%)	72 (70.6%)	0.07	43 (53%)
Sclerodactyly	21 (51.2%)	89 (87.3%)	<0.0001	65 (81%)
Teleangiectasia	22 (43.7%)	32 (31.4%)	0.02	28 (34%)
Melanoderma	3 (7.3%)	9 (8.8%)	NS	10 (13%)
Calcinosis	1 (2.4%)	7 (6.9%)	NS	3 (4%)
Digital ulcers	5 (12%)	50 (49%)	<0.0001	29 (36%)
Pulmonary arterial hypertension	2 (4.9%)	16 (15.7%)	0.09	6 (8%)
Heart involvement	4 (9.8%)	18 (17.6%)	NS	12 (14%)
Lung fibrosis	6 (14.6%)	26 (25.5%)	NS	8 (10%)
Renal involvement	0 (0%)	2 (2%)	NS	0 (0%)
Primary Biliary cirrosis	2 (4.9%)	3 (3%)	NS	5 (6%)
Thyroiditis	15 (36.6%)	34 (33.3%)	NS	30 (37%)

Notes.*_overlap_: patients with both SSc and SS; **_sicca-SSc_: SSc patients with sicca symptoms for whom the diagnosis of sSS was not substantiated by a minor salivary gland biopsy or by a complete diagnostic algorithm as required by the AECG criteria 2002.



	SSc (%)	Overlap (%)
early	17	29
active	44	18
late	29	21
no specific	15	32

Fig. 2. Shows capillaroscopy patterns in the SSc group and in the overlap group. Scleroderma-type nailfold capillary patterns were detected more frequently in the SSc group rather than in the overlap group.

Table III. A. Clinical pattern of the "SS-related manifestations".

Features	Overlap* (n=41)	SSc (n=102)	*p*-value	*sicca-SSc*** (n=81)
Xerophtalmia	41 (100%)	0	<0.0001	75 (93%)
Xerostomia	40 (97.5%)	0	<0.0001	74 (92%)
Parotid enlargement	13 (31.7)	1 (1%)	<0.0001	3 (4%)
Articular involvement	19 (46.3%)	17 (16.7%)	<0.0001	24 (29%)
Purpura	6 (14.6%)	0	0.0004	2 (3%)
PNS	1 (2.4%)	0	NS	1 (1%)
Fatigue	16 (39%)	3 (2.9%)	<0.0001	17 (21%)
Lymphoma	6 (14.6%)	1 (1%)	0.002	1 (1%)
Low C3 levels	7 (17.9%)	22 (22%)	NS	19 (24%)
Low C4 levels	3 (7.7%)	4 (4%)	NS	4 (5%)
Leukocytopenia	8 (19.5%)	1 (1%)	<0.0001	11 (13%)
Hypergammaglobulinaemia	8 (20%)	2 (2%)	0.001	6 (8%)
ESR	7 (17.1%)	6 (6%)	0.05	15 (18%)
Anti-Ro/SSA	9 (22%)	0 (0%)	<0.0001	0 (0%)
Anti-La/SSB	3 (7.3%)	0 (0%)	0.02	0 (0%)
Rheumatoid factor (RF)	17 (41.5%)	11 (11.3%)	<0.0001	19 (24%)

Notes.*_overlap_: patients with both SSc and SS; **_sicca-SSc_: SSc patients with sicca symptoms for whom the diagnosis of sSS was not substantiated by a minor salivary gland biopsy or by a complete diagnostic algorithm as required by the AECG criteria 2002.

Table III. B. Clinical pattern of the "SS-related manifestations".

Features	Overlap* (n=41)	pSS (n=387)	*p*-value	Sicca-SSc** (n 81)
Xerophtalmia	41 (100%)	346 (89.4%)	0.02	75 (93%)
Xerostomia	40 (97.5%)	344 (89)	NS	74 (92%)
Parotid enlargement	13 (31.7)	52 (13.6%)	0.005	3 (4%)
Articular involvement	19 (46.3%)	233 (60%)	NS	24 (29%)
Purpura	6 (14.6%)	20 (5%)	0.02	2 (3%)
PNS	1 (2.4%)	2 (0.5%)	0.02	1 (1%)
Fatigue	16 (39%)	85 (22%)	0.01	17 (21%)
Lymphoma	6 (14.6%)	12 (3%)	0.004	1 (1%)
Low C3 levels	7 (17.9%)	44 (14%)	NS	19 (24%)
Low C4 levels	3 (7.7%)	23 (7%)	NS	4 (5%)
Leukocytopenia	8 (19.5%)	80 (21%)	NS	11 (13%)
Hypergammaglobulinaemia	8 (20%)	211 (56%)	<0.0001	6 (8%)
Anti-Ro/SSA	9 (22%)	292 (75%)	<0.0001	15 (18%)
Anti-La/SSB	3 (7.3%)	139 (36%)	<0.0001	0 (0%)
Rheumatoid factor (RF)	17 (41.5%)	241 (64%)	0.01	0 (0%)

Notes.*_overlap_: patients with both SSc and SS; **_sicca-SSc_: SSc patients with sicca symptoms for whom the diagnosis of sSS was not substantiated by a minor salivary gland biopsy or by a complete diagnostic algorithm as required by the AECG criteria 2002.

developing skin sclerosis over a mean follow-up of 3.3±3.5 yrs. In addition, although not statistically significant, heart involvement, gastrointestinal tract involvement and lung fibrosis occurred more frequently in the group of patients with SSc alone rather than in the overlap patients. It is noteworthy that, pulmonary arterial hypertension estimated on the basis of echocardiography was observed in 16/102 patients with SSc and solely in 2/41 "overlap" patients (15.7% *vs*. 4.9%, p=0.09). Right heart catheterisation confirmed a pulmonary arterial hypertension diagnosis in 7/102 "SSc patients" and only in 1/41 of the "overlap patients". Noteworthy, the 81 sicca-SSc patients showed an intermediate clinical profile between the SSc and the "overlap patient" groups. More specifically, in comparison with "overlap patients" they presented an higher frequency of sclerodactily (81% *vs*. 51.2%) and digital ulcers (36% *vs*. 12%). On the other hand, when compared to patients with SSc alone they presented a lower prevalence of lung fibrosis (10% *vs*. 25.5%) (Table II).

SS-related manifestation in "overlap patients" vs. "SSc patients" and "pSS patients"

The frequency of the "SS-related clinical manifestations" in the three groups is summarised in Table III A and Table III B. Despite excluded from the statistical analysis, we also reported in Tables III A and III B the frequency of the "SS-related clinical manifestations" of the 81 "sicca-SSc patients". All the "overlap patients" satisfied the AECG criteria for SS and had a focus score ≥1 at the minor salivary gland biopsy. As far as glandular manifestations was related, subjective and objective evidence of sicca syndrome was detected almost in all the patients. In addition, we also found that "overlap patients" in comparison to the "SSc patients" presented a higher frequency of parotid swelling (31.7% *vs*. 1% p<0.0001), arthralgias (46.3% *vs*. 16.7%, p<0.0001), purpura (14.6 *vs*. 0, p=0.0004), leukocytopenia (19.5% *vs*. 1%, p<0.0001) and fatigue (39% *vs*. 2.9% p<0.0001). Surprisingly, parotid salivary gland enlargement, purpura, peripheral nervous system involvement and fatigue were also more common in the "overlap patient group" than in patients with pSS. Nonetheless, in spite of their clinical presentation, when compared to pSS patients, the "overlap patients" more infrequently showed hypergammaglobulinaemia (p<0.0001) RF positivity (p=0.01), anti Ro/SSA (p<0.0001) and anti-La/SSB autoantibodies (p<0.0001). Regarding SS-related long-term complications 6/41 "overlap patients" and 1 of the SSc patients developed a non-Hodgkin's lymphoma (p<0.0001). The frequency of lymphoma in the overlap patients was even higher than the one detected in the pSS group (6/41 *vs*. 12/387, p=0.004).

Prevalence and risk factors for non-Hodgkin's lymphoma in overlap patients

In our series we documented 20 cases of non-Hodgkin's lymphoma: 6/20 cases occurred in the overlap subset, 1/20 in the "SSc group" (p=0.002), 1/20 in the group of the "sicca-SSc patients" and the remaining 12/20 in pSS patients. The histology features of the 12

non-Hodgkin's lymphomas occurring in patients with pSS have been previously described separately (8). Out of the 8 non-Hodgkin's lymphoma occurring in the other groups, 4/8 were MALT-type lymphomas, whereas the other 4/8 were diffuse large B cell lymphomas. The mean latency between the onset of the SSc disease and lymphoma development was 18.6±10.6 years. No differences were observed in the disease duration and length of follow-up between pSS patients and the overlap patient group. When we analysed clinical and serological features potentially associated to lymphoma development in the overlap patient group (Table IV) we found that fulfillment of the AECG criteria for SS, parotid enlargement, purpura, peripheral nervous system involvement, hypergammaglobulinaemia and leukocytopenia represented significant risk factors for non-Hodgkin's lymphoma. No significant relationship was found between lymphoma development, hypocomplementemia, positivity for Rheumatoid factor, anti-Ro/SSA and anti-La/SSB. We have previously identified parotid enlargement and low C4 levels as a independent risk factors for lymphoma development in pSS patients (8). By contrast, in this study we observed that while parotid gland enlargement was still a prognosis factor for lymphoma development, low C4 levels did not seem to be correlated with lymphoma in the "overlap patient group".

Table IV. Epidemiologic, clinical and serologic features associated with lymphoma development in the overlap patient group.

Variables	Lymphoma		*p* (OR)
	Yes (n=7)	No (n=136)	
AECG criteria	6 (85.7%)	35 (29.7%)	0.002
sex (male)	0 (0%)	7 (5.1%)	NS
Salivary gland enlargement (recurrent)	5 (71.4%)	9 (6.6%)	<0.0001
Age at the onset (mean±SD, yrs)	40 ± 12	46 ± 15	NS
Age at SSc diagnosis (mean±SD, yrs)	52 ± 11	53 ± 14	NS
Disease duration from the onset (mean±SD, yrs)	18.6 ± 10.6	14.4 ± 11	NS
Esophageal involvement	5 (71.4%)	89 (65.4%)	NS
Articular involvement	3 (42.8%)	33 (24.3%)	NS
Purpura	3 (42.8%)	3 (2.2%)	0.001
Sclerodactyly	5 (71.4%)	105 (77.2%)	NS
Teleangectasia	5 (71.4%)	49 (36%)	NS
Pulmonary arterial hypertension	0 (0%)	18 (13.2%)	NS
Digital ulcers	2 (28.6%)	53 (38.6%)	NS
Peripheral nervous system	1 (14.3%)	0 (0%)	0.05
Renal involvement	0 (0%)	2 (1.5%)	NS
Lung fibrosis	2 (28.6%)	30 (22%)	NS
Low C4 levels	1 (14.3%)	6 (4.4%)	NS
Low C3 levels	1 (14.3%)	28 (20.6%)	NS
Leukocytopenia	3 (42.8%)	6 (4.4%)	0.005
Hypergammaglobulinaemia	3 (42.8%)	7 (5.1%)	0.008
Rheumatoid factor	3 (42.8%)	25 (18.4%)	NS
Increased ESR	1 (14.3%)	12 (8.8%)	NS
Anti-Ro/SSA	2 (28.6%)	7 (5.1%)	NS
Anti/La-SSB	1 (14.3%)	2 (1.5%)	NS

Note: NS: not significant.

Table V. Treatment of the patients enrolled in the study.

Treatment	Overlap* (n=41)	SSc (n=102)	*p*-value	pSS (n=387)	*p*-value
Calcium channel blockers	15 (37%)	50 (49%)	NS	N.A	N.A
Glucocorticosteroids	19 (46.3%)	14 (13.9%)	<0.0001	147 (38%)	NS
Hydroxychloroquine	16 (39%)	5 (5%)	<0.0001	170 (44%)	NS
Prostacyclin	5 (12.2%)	27 (26.7%)	0.07	N.A	N.A
DMARDs	2 (4.9%)	7 (7%)	NS	39 (10%)	NS

Notes.**overlap*: patients with both SSc and SS; NS: not significant, N.A.: not applicable.

Treatment of patients subgroups

Therapeutic strategies adopted over the follow-up are stratified according to the clinical subsets in Table V. No differences were observed between the "SSc group" and the "overlap subset" relatively to the use of calcium channel blockers as well as DMARDs. Nonetheless, we noticed that even if not statistically significant, the use of prostacyclin was more common in the "SSc group" (26.7%) than in the "overlap group" (12.2%), thus supporting the hypothesis of a more severe microvascular involvement in "SSc patients" in comparison with "overlap patients". Intriguingly, we found that the latter were treated more frequently with low-dose prednisolone and hydroxychloroquine, in that closely reflecting the therapeutic strategies adopted for pSS patients. The usage of glucocorticosteroids and antimalarial drugs was strictly related to the presence of parotid gland enlargement.

Discussion

The present study supports the hypothesis that patients with "ACA-positive pSS" or "ACA-SSc with sSS" are a unique distinct overlap clinical subset since these patients fulfil definite criteria for both diseases. In our experience this subset might represent 3% of patients attending a SS clinic and 20% of patient attending an SSc clinic. This overlap syndrome is characterised by some key distinctive features with respect to classical limited SSc or to pSS. As far as SSc-related manifestations are concerned, these patients presented a milder clinical form of SSc, with a considerable proportion of the "overlap" patients not showing any trend in developing sclerodactyly, negative evolution of the capillaroscopy pattern or severe systemic involvement. From this perspective, it is to be noted that only 1/41 developed an overt pulmonary arterial hypertension. As far as pSS is concerned, in spite of the scarce positivity for the pSS specific serologic abnormalities, surprisingly these pa-

tients showed a more pronounced parotid gland enlargement, purpura and peripheral nervous systemic involvement, and above all their risk for lymphoma was apparently higher than that presented by pSS patients.

These data only partially agree with the existing literature. More specifically, the prevalence of this overlap entity was consistent with previously reported data (Table VI A, VI B) (10-24). In fact, studies aimed at assessing the frequency of concomitant SS in SSc appeared quite heterogeneous due to the fact that the analysis was performed in both limited and diffuse series irrespectively for ACA positivity. To date, only two studies by Avouac *et al*. (10), Salliot *et al*. (12) have specifically assessed the frequency of sSS in ACA- SSc patients reporting a prevalence of 31%, and 22.3%, respectively, similarly to our findings. In our study, the frequency of "overlap patients" might be even higher considering that a consistent number of patients with SSc and sicca symptoms were incompletely characterised for SS. Similarly, the frequency of the ACA/pSS in the literature datasets of pSS is generally assessed as ~5% which is very close to the prevalence of 3.7% that we found in this study (Table VI B) (13, 19, 21, 23).

As far as clinical manifestations are concerned, there is also a general agreement that in patients with overlap syndrome, SSc is generally less severe whereas pSS glandular manifestations tend to be fully expressed (10, 12, 15). In other words, secondary or associated SS seemed to have a favourable impact on SSc long term outcomes supporting the hypothesis that SS might temper the clinical presentation of SSc, at the same time fostering a spreading of autoimmunity. Interestingly, from this point of view, Bournia *et al*. in their study concluded that ACA-positive SS patients represented a subset of patients with an intermediate phenotype between pSS and SSc and a little tendency to evolve to SSc (21). Similarly, Manoussakis *et al*. (51) showed that also SLE seemed to be less serious when it was associated with SS, featuring a low occurrence of thrombocytopenia and lymphadenopathic and renal involvement.

Table VI.A. Prevalence of sicca symptoms and secondary Sjögren's syndrome in systemic sclerosis: review of the literature.

Author	Year	n. of pts	Prevalence of sicca (%)	Prevalence of sSS (%)	Impact of SS on SSc
Miyawaki (10)	2005	108	n.r.	29.7	
Avouac (3)	2006	133 (63*)	68	14 (12)	Lower prevalence of lung fibrosis
Avouac (4)	2010	547 585	17 7.5		
Salliot (5)	2007	121*	n.r.	23	Lower prevalence of lung fibrosis; Greater frequency of additional autoantibodies
Swaminathan (7)	2008	117	59	n.r.	
Hashimoto (8)	2011	127	n.r.	12.6	Lower mortality rate

Note:* Subanalysis on anticentromere positive-limited cutaneous systemic sclerosis; n.r.: not reported.

Table VI.B. Prevalence of ACA-positive Sjögren's syndrome in pSS datasets: review of the literature.

Author	Year	n. of pts	Prevalence of ACA(+) pSS (%)	ACA(+) pSS *vs*. pSS
Vlachoyiannopoulos (11)	1993	41	17	Lower incidence gland enlargement and anti-La (SSB) antibodies
Caramaschi (12)	1995	44	4.5	
Miyawaki (10)	2005	108	27.7	
Salliot (6)	2007	212	4.7	Greater frequency of Raynaud's phenomenon, objective xerophthalmia, peripheral neuropathy, and additional autoimmune disorders; less frequent anti-SSA or anti-SSB antibodies
Ramos-Casals (16)	2006	402	2	Greater frequency of Raynaud's phenomenon but no clinical data suggestive of sclerodactily
Bournia (14)	2010	535	3.7	Lower prevalence of dry eyes, hypergammaglobulinaemia, anti-Ro and anti-La antibodies and a higher prevalence of Raynaud's phenomenon and dysphagia
Kitagawa (17)	2012	64	17	Similar impairment of the lachrymal secretion; similar lymphocytic infiltration and tissue destruction in minor salivary gland biopsies

In our series we indeed confirm that the association of SS with SSc may modify the severity of SSc but differently from the studies which supported the hypothesis of a spreading of the autoimmunity in the overlap syndrome, we did not find any difference in the prevalence of autoimmune thyroiditis or primary biliary cirrhosis between the groups enrolled. On the contrary, we found that microvascular involvement was less severe in overlap patients in terms of nailfold capillaroscopy abnormalities and digital ulcers. This observation was indirectly supported by the fact that prostacyclin was used less frequently in the overlap subset than in patients with SSc alone. In addition we found that sclerodactily was more common in SSc patients and that in general, although not statistically significant, heart involvement, gastrointestinal tract involvement and lung fibrosis occurred more frequently in the group of patients with SSc alone rather than in the subset of patients with SSc and concomitant SS. In particular, pulmonary arterial hypertension apparently occurred less frequently in the "overlap patients" rather than in the "SSc group".

On the other hand, specifically focusing on the SS-related clinical manifestations in the "overlap patient group" with in comparison with pSS patients we observed that although often lacking of specific autoantibodies (*i.e.* Rheumatoid Factor, anti-Ro/SSA, anti-La/SSB), the "overlap patients" displayed a full blown SS phenotype with recurrent salivary gland enlargement, purpura, fatigue, arthralgias, and "typical" SS-related haematological abnormalities (*i.e.* leukocytopenia). Again, these results reflect the available literature on the topic (13, 17-19, 21, 23, 24). Consequently, similarly to pSS, the overlap patients were treated more frequently with low-dose steroids and antimalarial drugs. In particular, we observed a statistically significant association between steroids, hydroxychloroquine and salivary gland enlargement. Above all, similarly to pSS, non-Hodgkin's lymphoma represented a significant late onset complication of the "overlap patients" present in as much as 14% of these patients. This prevalence is comparable as and/or even higher than the frequency of lymphoma that we detected in pSS (~3%). In addition, MALT-lymphomas of the salivary glands and diffuse large cell lymphomas represented the more frequently detected type of lymphoma at the histopathological examination, thus reflecting the lymphoproliferation model of pSS (52). Surprisingly the association between non-Hodgkin's lymphoma and "overlap syndrome" was not emphasised in the already published largest cohorts described in the literature with few exceptions. In fact recently, Gulati *et al*. (53) have described the presence of ACA antibodies in two pSS patients with small vessel cutaneous vasculitis, parotid enlargement, low C4 complement levels, positive rheumatoid factor and lymphoma. In addition, Bournia *et al*. (21) in assessing the long-term outcomes of ACA-positive pSS patients even if not reporting an increased lymphoproliferative risk in their population cohort, however, observed two lymphoma cases in patients with SSc and sicca manifestations and no cases in patients with SSc alone. We have also searched for clinical and serological risk factors which may be associated to the development of lymphoma. Serum levels of cytokines traditionally implicated in B cell ontogenesis and proliferation (*i.e.* Fms-like tyrosine kinase 3 ligand, BlyS) (54, 55) were not investigated in this study. We have found that similarly to pSS, salivary gland enlargement, purpura, leukocytopenia and hypergammaglobulinaemia were among the most significant clinical and serologic biomarkers for lymphoproliferative complications (8, 56-58). In fact, in a previous work searching for independent risk factors for lymphoma development in pSS, we demonstrated that salivary gland enlargement and low C4 were the most predictive factors at the multivariate analysis (8). It might be noteworthy that salivary gland enlargement still represented a strong predictor of lymphoproliferative evolution in the "overlap patient group" while low C4 apparently was not related to non-Hodgkin's lymphoma development, thus suggesting that several pathogenetic mechanisms might be involved in the lymphoproliferation model of this complex phenotype of SSc/SS.

In summary, taken together, the results of our work emphasises the existence of a novel distinct clinical entity which might be called temptatively "ACA-positive limited scleroderma/SS overlap syndrome" characterised by a benign SSc clinical course but at a high risk of non-Hodgkin's lymphoma. Given the high risk of lymphoproliferative complications, the present study emphasised that in clinical practice patients with ACA-positive limited cutaneous SSc and sicca symptoms should undergo a complete work up for SS, and viceversa ACA-positive pSS should be screened for SSc and tightly monitored over the time. Further studies are ongoing to verify whether this novel clinical entity might involve also distinct genetic or pathophysiologic mechanisms with respect to the corresponding typical phenotypes of SSc or pSS.

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