The clinical phenotype of Systemic Sclerosis patients with anti-PM/Scl antibodies: results from the EUSTAR cohort.

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Key messages:

- Muscle involvement, ILD, calcinosis and cutaneous dermatomyositis characterize the clinical phenotype of anti-PM/Scl+ in SSc
- Although frequent, ILD is characterized by a good functional outcome in anti-PM/Scl+ SSc patients
- SRC and malignancies are not part of the clinical phenotype of anti-PM/Scl+ in SSc

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ABSTRACT

Objective. To evaluate clinical associations of anti-PM/Scl antibodies in patients with Systemic Sclerosis (SSc) in a multicentre international cohort, with particular focus on unresolved issues, including scleroderma renal crisis (SRC), malignancies, and functional outcome of interstitial lung disease (ILD).

Methods. (1) Analysis of SSc patients from the EUSTAR database: 144 anti-PM/Scl+ without SSc-specific autoantibodies were compared to 7,202 anti-PM/Scl-, and then to 155 anti-Pm/Scl+ with SSc-specific antibodies. (2) Case-control study: additional data were collected for 165 anti-PM/Scl+ SSc (85 from the EUSTAR registry), and compared to 257 anti-PM/Scl- SSc controls, matched for sex, cutaneous subset, disease duration, and age at SSc onset.

Results. Patients with isolated anti-PM/Scl positivity, as compared with anti-Pm/Scl-, had higher frequency of muscle involvement, ILD, calcinosis and cutaneous signs of dermatomyositis, but similar frequency of SRC and malignancies (either synchronous with SSc onset or not). The presence of muscle involvement was associated with a more severe disease phenotype. Although very frequent, ILD had a better functional outcome in cases than in controls.

In patients with both anti-PM/Scl and SSc-specific antibodies, a higher frequency of typical SSc features than in those with isolated anti-PM/Scl was observed.

Conclusion. The analysis of the largest series of anti-PM/Scl+ SSc patients so far reported helps to delineate a specific clinical subset with muscle involvement, cutaneous dermatomyositis, calcinosis, and ILD characterized by a good functional outcome. SRC and malignancies do not seem to be part of this syndrome.

INTRODUCTION

In systemic sclerosis (SSc) antinuclear autoantibodies represent useful markers of distinct disease subsets, although their role in the pathogenic process is still debated [1,2]. The clinical significance of SSc-specific antibodies (anti-centromere (ACA), anti-topoisomerase I (anti-Topo I), anti-RNA-polymerase III (anti-RNAP3)) is now well defined, but for rarer autoantibodies further research is needed.

Antibodies against the PM/Scl complex are found not only in patients with SSc, but also in patients with Polymyositis (PM), Dermatomyositis (DM), and SSc/PM overlap syndrome [3]. The PM/Scl autoantigen is a macromolecular complex, recognized as the human exosome, involved in RNA degradation and processing. The main autoantigenic proteins were named PM/Scl-75 and PM/Scl-100, based on their apparent molecular weights, but other exosome proteins were also proven to be target autoantigens [3].

Anti-PM/Scl antibodies can be identified using nucleolar staining in indirect immunofluorescence as a screening test, and counter-immunoelectrophoresis, double immunodiffusion, immunoprecipitation or Enzyme-Linked Immunosorbent Assay (ELISA) to subsequently confirm anti-PM/Scl reactivity. The main clinical associations of anti-PM/Scl antibodies in SSc patients, including muscle involvement, calcinosis, and interstitial lung disease (ILD), were observed irrespectively of the immunoassay used, although with variable strengths of association in different studies [4-11].

Importantly, recent data have proposed other possible clinical features related to anti-PM/Scl with high translational potential for patient care, providing open questions to be investigated. First, although this antibody was generally not considered to be related with scleroderma renal crisis (SRC), a possible association was suggested [12]. Second, anti-PM/Scl antibodies were associated with a higher frequency of cancer in a single-centre SSc series [13]. This finding was particularly interesting, since a close temporal clustering between malignancies and SSc onset was previously described for anti-RNAP3+ patients, together with a possible pathogenic role of SSc autoantigens expressed by tumour cells including RNAP3 [14-17] and other

proteins [18,19]. In this light, it is noteworthy that the exosomes, the target antigens of anti-PM/Scl, are major players in cancer development and progression [20]. However, the relationship between anti-PM/Scl antibodies and cancer was never evaluated in large multicentre series and their temporal relationship was not so far clarified. Third, although an increased risk of concomitant heart involvement in SSc patients with skeletal muscle involvement was reported [21], this issue was never explored among anti-PM/Scl+ patients. Fourth, although ILD is frequently reported in anti-PM/Scl+ patients, a favourable outcome was suggested [6,8]; however, long-term data coming from multicenter studies are lacking.

Considering the low prevalence of anti-PM/Scl antibodies in SSc, only the analysis of a large, international, multicentre cohort of SSc patients, and the comparison between anti-PM/Scl+ and matched anti-PM/Scl- patients could provide further information on these clinically and pathogenic relevant issues. We aimed at this objective, taking advantage of the EUSTAR database; moreover, since some relevant clinical and laboratory variables (e.g., malignancy history) are not recorded in this database, a case-control study to cover these items was designed.

METHODS

Analysis of the EUSTAR database.

The EUSTAR registry records the minimal essential datasets (MEDS) of a longitudinally followed cohort of SSc patients [22,23]. Data were extracted from the registry in August 2017, when 14,628 patients fulfilling either the 1980 American College of Rheumatology (ACR) or the 2013 ACR/EULAR classification criteria for SSc [24,25] were included. Data on anti-PM/Scl were recorded since December 2008. Autoantibodies were detected by each center according local practice and information on autoantibody detection method is not collected in the EUSTAR database. MEDS variables were defined as described in Supplementary File 1. In particular, to evaluate the presence of muscle involvement, all the related features reported in the registry were considered (CK elevation >3 ULN, proximal muscle weakness, proximal muscle atrophy).

Patients were included in this analysis when the anti-PM/Scl status was reported at least once in the database and were excluded in case of no information or unknown status. Patients were considered positive for anti-PM/Scl when the test was positive in at least one determination, at the baseline or during the follow-up. Patients positive both for anti-PM/Scl and ACA or anti-Topo I or anti-RNAP3 were excluded from the comparison with anti-PM/Scl– patients, and separately considered. Data were included only when available in ≥70% of the patients. For each patient, a clinical manifestation was considered as present if reported in at least one of the visits recorded, except for some selected features or parameters (specifically indicated in the Tables) for which the value provided at the last available visit was considered.

Ethics approval was obtained from the respective local ethics committees [coordinator centre approval Brescia, Spedali Civili di Brescia, n.1072].

The study was conducted in accordance with Helsinki Declaration principles.

The data underlying this article are available in the article and in its online supplementary material.

Case-control study.

To collect supplementary clinical and laboratory variables not covered by the EUSTAR database, an additional dedicated form was created including: laboratory method of anti-PM/Scl detection; specific positivity for PM/Scl-75 and PM/Scl-100 autoantigens; presence of other auto-antibodies; clinical diagnosis (SSc, overlap syndrome); presence of clinical manifestations of myositis (proximal muscle weakness); muscle biopsy confirming inflammatory myositis; cutaneous manifestations of DM (see Supplementary File 1); calcinosis; data for pulmonary function tests (PFT) at the baseline, 1 year after diagnosis, and at the last visit; malignancy history. According with previous literature [12,16,26], malignancies were classified as 'synchronous' with SSc when the diagnosis was made in a period comprised between 24 months before and after SSc onset or, in a separate analysis, in a larger interval of 36 months. Overlap syndrome was defined by the treating physician as a disease occurring with SSc characteristics according to ACR/EULAR criteria, simultaneously with those of other connective tissue diseases, such as PM or DM, although it was not mandatory that patients independently fulfilled classification criteria also for these conditions [27].

Centres from the EUSTAR and the AENEAS (American and European NEtwork of Antisynthetase Syndrome) collaborative groups were contacted for this specifically designed case-control study. Centres who volunteered to participate provided retrospective data for all consecutive anti-PM/Scl+ patients fulfilling SSc classification criteria, with a follow-up of at least 2 years from SSc onset (cases), and one, or, if possible, two local anti-PM-Scl- SSc controls for each case, matched for sex, age at disease onset (by 5-years class of age), disease duration and cutaneous subset as defined by Leroy *et al* [28]. Centres were also asked to exclude SSc patients with positive anti-PM/Scl and associated SSc-specific autoantibodies (ACA, anti-Topo I, anti-RNAP3) from cases. Only patients with age >16 years at disease onset were included in this analysis.

Statistical analysis.

Frequencies and percentages were compared using Chi-square test or Fisher's exact test for categorical variables, and Student's t-tests or Mann-Whitney U tests for continuous variables, as appropriate.

To identify the clinical associations of anti-PM/Scl, multivariable logistic regression analysis was performed by selecting covariates based on the research question (clinical associations of anti-PM/Scl) and plausible independent variables which were *a priori* selected among demographical, clinical and laboratory parameters. Odds ratios (OR) were calculated with 95% confidence intervals (CI).

RESULTS

Analysis of the EUSTAR database.

Clinical associations of anti-PM/Scl antibodies.

Anti-PM/Scl status was available in 7,353 SSc patients from the EUSTAR database (whose characteristics did not differ from the entire population): 295 (4.01%) were positive for anti-PM/Scl. Among them, 151 had also one or more SSc-specific autoantibody positivity (57 ACA+, 106 anti-Topo I+, and 22 anti-RNAP3+). For the subsequent analysis, the remaining 144 patients with isolated anti-PM/Scl positivity (1.96%) were compared with 7,058 anti-PM/Scl negative patients (Supplementary Figure 1). Among them, 3,120 (44.2%) were positive for ACA, 2,361 (33.5%) for anti-Topo I, and 274 (3.88%) for anti-RNAP3.

In the univariable analysis, anti-PM/Scl positivity was associated with male sex, increased frequency of proximal muscle weakness and atrophy, serum CK elevation (>3 ULN), and lung fibrosis on imaging, while oesophageal symptoms, systemic arterial hypertension and elevated systolic pulmonary arterial pressure (sPAP) at echocardiography were less frequent. Disease duration was slightly shorter in anti-PM/Scl+ patients and death rate was lower than in anti-PM/Scl- (Table 1). In the multivariable analysis, adjusted for age at disease onset, sex and disease duration, more frequent elevation of serum CK and ILD on X-ray and/or high-resolution

chest tomography (HRCT), and less frequent oesophageal symptoms and elevated sPAP at echocardiography resulted as independently associated with anti-PM/Scl positivity (Table 1).

Scleroderma renal crisis, glucocorticoid use and anti-PM/Scl.

SRC was identified in 8 of 144 anti-PM/Scl+ SSc patients (5.56%) and in 3.12% of anti-PM/Scl- patients (p:0.140; Table 1). Notably, the frequency of SRC in anti-PM/Scl+ patients was lower than in anti-RNAP3+ (15.0% p:0.004), higher than in ACA+ (1.96%, p:0.010), and not significantly different from anti-Topo I+ patients (3.43%, p:0.167). Corticosteroids assumption was considered as a possible factor associated to SRC: anti-PM/Scl+ patients were more frequently exposed to glucocorticoids than anti-PM/Scl- (67/138 (48.6%) vs. 2523/6614 (38.2%), p:0.017, OR 95% CI 1.53, 1.09-2.14). Particularly, glucocorticoids use was more frequent than in ACA+ patients (26.7%, p<0.0001, OR 95% CI 2.6, 1.84-3.66), but not different from anti-RNAP3+ (38.1%, p:0.055) and anti-Topo I+ patients (51.7%, p:0.484).

To clarify whether SRC could be related to anti-PM/Scl positivity or to other related variables, we compared 228 patients with SRC to 6,961 patients without SRC in the EUSTAR database (Table 2). Anti-PM/Scl and SSc-specific antibodies were included in a multivariable analysis, together with corticosteroids assumption ever, muscle involvement (defined as CK elevation >3 ULN), diffuse cutaneous involvement, and pericardial effusion (Table 2). Anti-PM/Scl positivity was not independently associated with SRC (p:0.073), while corticosteroids assumption ever, diffuse cutaneous involvement, pericardial effusion and anti-RNAP3 showed a significant association.

Skeletal muscle involvement is associated with heart and other organ involvement in anti-PM/Scl+ SSc patients.

To investigate whether skeletal muscle involvement among anti-PM/Scl+ SSc patients was associated with other organ involvement, clinical and laboratory characteristics of those with raised serum CK (>3 ULN) were compared to those without CK elevation (Table 3). Both the presence of muscle and cardiac involvement were considered during the whole disease

course, as previously defined. Patients with CK elevation had a higher frequency of heart involvement (systolic and diastolic left ventricular dysfunction, any-degree conduction blocks), ILD on X-ray and/or HRCT, intestinal symptoms, joint contractures and tendon friction rubs, although multivariate analysis confirmed the independent association with conduction blocks, tendon friction rubs and intestinal symptoms only.

The clinical profile associated with anti-PM/Scl positivity is modified by the presence of SSc-specific antibodies.

To evaluate whether the clinical profile associated with anti-PM/Scl positivity is modified by the presence of SSc-specific antibodies, 144 SSc patients with anti-PM/Scl "isolated" positivity were compared to 151 patients in which anti-PM/Scl positivity was associated with one or more SSc-specific autoantibodies positivity (Table 4). Patients with "isolated" anti-PM/Scl positivity showed a significantly lower frequency of oesophageal, gastric and intestinal symptoms, diffuse cutaneous involvement, digital ulcers, joint and tendon involvement, proximal muscle weakness and elevated sPAP at echocardiogram. Multivariable analysis confirmed the lower frequency of joint synovitis and estimated pulmonary hypertension by echocardiogram in patients with "isolated" anti-PM/Scl positivity.

Case-control study.

In the complementary case-control study, retrospective data for 165 anti-PM/Scl+ SSc cases were retrieved from the participating centres (Supplementary Figure 1). Among them, 130 derived from EUSTAR centres (85 also included in the EUSTAR registry) and 35 from the AENEAS collaborative group. SSc patients deriving from these 'myositis-oriented' centres, as compared to other SSc patients, more frequently had clinical manifestations of myositis (p:0.0001), arthritis (p:0.0002) and an "overlap syndrome" phenotype (p:0.005).

A biopsy confirming the presence of inflammatory myositis was available only in 35 of 165 anti-PM/Scl+ patients, as it was performed only when requested for clinical practice.

These cases were compared with 257 anti-PM/Scl- SSc controls (32% anti-Topo I+, 42% ACA+; 39 provided by AENEAS centres), locally matched by each centre for sex, age at disease onset, disease duration and subset.

Data concerning the laboratory technique to find anti-PM/Scl positivity were available in 123 patients (74.5%): ELISA was used in 35 (28.5%), LIA in 40 (32.5%), immunoprecipitation in 19 (15.4%) and double immunodiffusion in 29 (23.6%).

The positivity of other SSc non-specific autoantibodies was detected in 17 of 165 anti-PM/Scl+ patients (10.3%): 12 anti-Ro/SSA; 2 rheumatoid factor; 1 anti-CCP, 1 anti-La/SSB and 1 anti-mitochondrial antibodies.

Anti-PM/Scl positive patients had a higher prevalence of clinical manifestations of muscle involvement, ILD on HRCT, cutaneous signs of DM and calcinosis, and a lower frequency of oesophageal symptoms, small intestine bacterial overgrowth, digital ulcers and cardiac arrhythmia requiring specific therapy than matched controls (Table 5).

Evaluation of the association of anti-PM/Scl with cancer.

The frequency of malignancies, either synchronous with SSc or not, was not significantly higher in anti-PM/Scl+ SSc patients than in anti-PM/Scl- controls (Table 5; details of malignancies synchronous to SSc onset in Supplementary Table 1).

Mean age at SSc onset was significantly higher in SSc patients with synchronous malignancies than in those without (59.9±14.7 versus 49.2±14.7 years; p:0.022), irrespective of the anti-PM/Scl status,

Outcome of ILD in anti-PM/Scl patients with Systemic Sclerosis.

In this case-control analysis, ILD on HRCT was recorded in 101/162 anti-PM/Scl+ SSc cases and 98/249 controls (62.3% vs. 39.4%, p:<0.0001; Table 5). We then conducted a sub-analysis on 81 of 101 anti-PM/Scl+ ILD cases and 78 of 98 anti-Pm/Scl- ILD controls (65.3% anti-Topo-1+) for whom longitudinal PFT data were available. The characteristics of these patients are reported in Table 6. Age at onset, gender, and disease duration at the last visit (about 10 years

in both groups) were similar between the two groups, whereas diffuse cutaneous involvement was less frequent (p:0.02) and clinical manifestations of myositis more frequent (p:<0.0001) in cases than in controls.

In anti-PM/Scl+ cases with ILD, %pFVC tended to improve from the baseline (T0) to the follow-up visit after 1 year (T1) (p:0.045), and to the last visit (LV) (p:0.057), whereas in anti-PM/Scl- controls with ILD it remained stable from T0 to T1, and declined to LV (p:0.0002). %pDLCO remained stable in anti-PM/Scl+ cases, while declined from T0 to T1 (p:0.0016) and to LV (p<0.0001) in the control group. Moreover, a higher proportion of anti-PM/Scl- than anti-PM/Scl+ patients had significant FVC and/or DLCO loss (Table 6). No difference was observed between anti-PM/Scl+ patients with or without clinical manifestations of myositis, thus suggesting that FVC improvement in PM/Scl+ patients does not merely reflect improvement in myopathy.

Clinical associations according to anti-PM/Scl specificity.

Data regarding the specificity of anti-PM/Scl positivity were available for 120 patients (72.7%). Among them, 29 (24.2%) were positive only for anti-PM/Scl-100, 33 (27.5%) only for anti-PM/Scl-75, and 58 (48.3%) for both the antigens. Patients with anti-PM/Scl-100 only as compared with the other groups had significantly more frequent calcinosis (41% vs 12.1% and 19.0%; p:0.021) and telangiectasia (65.5% vs 39.4 and 29.3%; p:0.005) (Supplementary Table 2).

DISCUSSION

We analysed the clinical associations of anti-PM/Scl in SSc, taking advantage of the EUSTAR database and network. Positivity for anti-PM/Scl was found in 4.0% of more than 7,000 SSc patients, but in half of them associated SSc-specific autoantibodies were also reported. This might be considered surprising, and even though the possibility of data imputation errors in a large multicentre registry cannot be excluded, the same finding was reported by others: 43 of 92 anti-PM/Scl+ patients in a tri-nation study and 29 of 55 patients in a Canadian cohort also had associated SSc-specific autoantibodies [9,10]. When SSc sera are analysed by novel immunoassays (e.g. LIA) for multiple autoantibodies, including rarer ones, simultaneous positivity represents a frequent issue [17; 29-31]. Therefore, the common opinion that autoantibodies in SSc are mutually exclusive, still appears valid for the three major SSc-specific autoantibodies [17, 32], while it might be challenged for the rarer ones. Noteworthy, in our study, the clinical phenotype of patients with both anti-PM/Scl and SSc-specific antibodies was characterized by a higher frequency of typical SSc features underrepresented in patients with isolated anti-PM/Scl (Table 4). This might support the hypothesis of a real co-existence of autoantibodies.

Although such co-existence of autoantibodies may confound the clinical correlations of anti-PM/Scl, in many previous studies, patients with more than one positivity were not excluded from the analysis. The present study, analysing data from the largest series so far reported of SSc patients with monospecific positivity for anti-PM/Scl autoantibodies, can help to delineate the phenotype associated with anti-PM/Scl.

Our study confirmed a higher frequency of clinical manifestations of myositis, ILD, calcinosis [4,6,10] and DM cutaneous signs in anti-PM/Scl+ patients, as previously reported [11], whereas esophageal involvement and estimated pulmonary hypertension by echocardiography had a lower frequency [6]. A higher frequency of calcinosis and telangiectasia was particularly observed in patients positive for anti-PM/Scl-100 only; a similar trend for calcinosis was previously reported [9]. The real relevance of dissecting the anti-

PM/Scl positive groups deserves therefore future studies. Notably, calcinosis is associated with anti-PM/Scl, not only in patients with SSc, but also in patients with PM and DM [33], suggesting that this feature could be related to the autoantibody regardless of the clinical setting.

The presence of muscle involvement (defined as CK elevation >3 ULN) among anti-PM/Scl+ SSc patients seems to be associated with increased frequency of heart, tendon and intestinal involvement, suggesting that this could represent a marker a more severe disease phenotype. However, on the basis of the available data, we could not define the temporal relationship between muscle and cardiac involvement, which is a relevant issue deserving additional prospective studies.

Our study also focused on some other still unresolved issues.

In the EUSTAR registry the rate of death among anti-PM/Scl+ was lower than in anti-PM/Scl- SSc patients; however, disease duration was slightly shorter in the former group. Previous studies have reported that, as compared to other SSc subsets, anti-PM/Scl+ SSc patients have a lower risk of death in the first 10 years of the disease [2,6], and a higher risk in the later phases [2]. One possible explanation might be related to the evolution of ILD in these patients [2]. In fact, a better functional outcome in anti-PM/Scl+ SSc patients with ILD as compared to anti-Topo I+ was described by single-centre longitudinal studies [2,8]. Our study confirms, in a large population with a mean follow-up of 10 years, that the functional outcome of ILD in anti-PM/Scl+ patients is less severe than in other SSc patients. However, it has been recently reported that the hazard of clinically significant ILD might increase after the first decade of the disease, differently from other SSc subsets [2].

On the other hand, the hypothesis of an association of anti-PM/Scl+ with SRC was raised when this complication was identified in 5.7% of anti-PM/Scl+ SSc patients from the Royal Free Hospital of London [12]. In our analysis, the frequency of SRC among anti-PM/Scl+ SSc patients was similar to that of the Royal Free (5.6%), and comparable to that of the whole anti-PM/Scl- group, although higher than in the ACA+ subgroup. This observation might be explained by covariates, such as muscle involvement, that more frequently requires

corticosteroids therapy. Indeed, in the multivariable analysis, SRC was associated among the others with corticosteroids assumption, but not with anti-PM/Scl antibodies, although a tendency to positive association was observed (p:0.073). Recently, an in-depth analysis of the Royal Free Hospital indicated an overall low incidence of SRC among anti-PM/Scl+ SSc patients, but differently from other SSc subsets, they did not develop SRC in the first years of disease, but later on [2]. Moreover, the role of ACE inhibitors as independent risk factors for the development of SRC in SSc patients with concomitant arterial hypertension was recently highlighted [34].

Finally, the relationship between anti-PM/Scl positivity and malignancies was analysed in detail. History of cancer was previously reported in 14 of 70 anti-PM/Scl+ SSc patients from the Royal Free Hospital cohort, which was suggested to be possibly higher than in the whole SSc population, with 5 patients having a cancer was diagnosed within 3 years of SSc diagnosis. However, the statistical significance of these data and the possible role of confounders were not evaluated in this report [12]. In another monocentric study, anti-PM/Scl positivity was found in 6 of 29 SSc patients with cancer, with a significant association in multivariable analysis. However, the temporal relationship between the two diseases was not reported [13]. Other small series (2-3 patients each) also described this possible association [35-37]. These observations led to speculations on the possible role of exosomes, the target of the anti-PM/Scl immune response, as a link between malignancies and anti-PM/Scl associated autoimmune diseases [13]. In support to this hypothesis, clinical remission of a case of anti-PM/Scl+ SSc/PM overlap syndrome was observed after curative resection of a pancreatic tumour expressing nuclear staining for PM/Scl-100 [12]. We addressed this issue through an *ad-hoc* designed case control-study. No increased frequency of malignancies, and specifically synchronous to SSc onset, was observed among anti-PM/Scl+ patients. Overall, the rate of synchronous malignancies in the present study was low, but it should be considered that patients with anti-RNAP3 autoantibodies were intentionally excluded. Significant older age of SSc onset in patients with synchronous malignancies was observed, confirming that SSc

onset in the elderly should be regarded as a condition with increased risk of concomitant malignancies, independently from the autoantibody status [26].

Limitations of our study are inherent to its nature of large, international, multicentre study, with acknowledged differences in data collection, laboratory methods for auto-antibody identification, and cancer screening. Data about malignancies and ILD progression were collected retrospectively. The presence of myositis was defined according to clinical and laboratory features, while the presence of biopsy-confirmed inflammatory myositis was not available in the EUSTAR registry and was available only in a minority of patients in the case-control study, as it was performed only according to local clinical practice. Another possible limitation is inherent to the lack of complete data regarding the timing and the effects of different kind of treatments, especially regarding the long-term outcome of ILD. In fact, on the basis of available data only exposure to corticosteroids (for the analysis on SRC in the EUSTAR registry) and cyclophosphamide (for the case control-study) could be taken in account.

Noteworthy, the majority of patients included were Caucasian, and this may limit the extension of these results to other ethnicities. Finally, in contrast with some other previous studies about anti-PM/Scl antibodies that included patients with different connective tissue disorders, the clinical associations here described should be considered applicable only to SSc individuals.

In conclusion, the analysis of the largest series of anti-PM/Scl+ SSc patients so far reported helps to delineate a peculiar subgroup of SSc patients characterized by muscle involvement, cutaneous signs of dermatomyositis, calcinosis, ILD (with a favourable functional outcome in the first decade of the disease), but less frequent oesophageal involvement and pulmonary hypertension (as estimated by echocardiography). Moreover, the presence of increased CK levels seems to define a subgroup with a more severe disease. There are no clear data to support the hypothesis that SRC and malignancies are part of this syndrome.

It has been proposed that this phenotype should be named "anti-PM/Scl syndrome" as a distinct subtype of myositis, particularly rich in extra-muscular features [11].

However, many patients with this phenotype also fulfil SSc criteria, thus suggesting that this syndrome cannot be separated from SSc.

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	Univariable analysis			Multivariable analysis	
Characteristics	**Anti-PM/Scl+**	**Anti-PM/Scl-**	**p-value**	**p-value**	**OR (95% CI)**
Age at disease onset (years, mean (SD) (n available)	45.0 (15.1) (134)	46.7 (14.5) (6100)	0.139	0.681	
Disease duration at last visit (months, mean (SD) (n available)	128.9 (100.2) (133)	150.5 (109.7) (6089)	0.013	0.535	
Male sex	31/144 (21.5)	1047/7058 (14.8)	0.033	0.971	
Caucasian ethnicity	116/144(80.6)	5618/7058 (79.6)	0.835		
Raynaud's phenomenon	140/144(97.2)	6909/7028 (98.3)	0.315		
Oesophageal symptoms	88/144 (61.1)	5563/7051 (78.9)	<0.0001	<0.0001	0.33 (0.21-0.50)
Stomach symptoms	47/144 (32.6)	2831/7038 (40.2)	0.071		
Intestinal symptoms	58/144 (40.3)	3111/7046 (44.2)	0.397		
Scleroderma renal crisis	8/144 (5.56)	220/7045 (3.12)	0.140		
Dyspnoea significant	23/137 (16.8)	1340/6615 (20.3)	0.389		
Diffuse cutaneous involvement	53/142 (37.3)	2448/7004 (34.9)	0.594		
Digital ulcers	63/120 (52.5)	4128/7045(58.6)	0.191		
Joint synovitis	33/143 (23.1)	1750/7018 (24.9)	0.696		
Joint contractures	63/143 (44.1)	2840/7005 (40.5)	0.439		
Tendon friction rubs	14/143 (9.79)	1064/7000 (15.2)	0.077		
Proximal muscle weakness	57/142 (40.1)	2210/7013 (31.5)	0.036		
Muscle atrophy	35/142 (24.7)	1153/7012 (16.4)	0.012		
CK elevation (>3 ULN)	47/134 (35.1)	920/6798 (13.5)	<0.0001	<0.0001	3.07 (2.01-4.71)
Lung fibrosis on plain X-rays	61/112 (54.5)	2462/6075 (40.5)	0.003		
Lung fibrosis on HRCT	74/107 (69.2)	2715/5442 (49.9)	<0.0001		
ILD on x-rays and/or HRCT	89/118 (75.4)	2300/5759 (59.1)	<0.0001	0.001	2.22 (1.41-3.49)
LVEF ≤ 50% (ECHO) last visit*	4/144 (4.76)	273/4463 (6.11)	0.818		
Elevated sPAP (ECHO) last visit	17/133 (12.8)	1658/6639 (25.0)	0.001	0.017	0.52 (0.30-0.89)
Pericardial effusion	13/127 (10.2)	690/6334 (10.9)	1.000		
Diastolic function abnormality	44/130 (33.9)	2392/6571 (36.4)	0.582		
Conduction blocks (any degree)	33/126 (26.2)	1513/6377 (23.7)	0.526		

Table 1. Analysis of the EUSTAR database. Univariable and multivariable analysis (adjusted on sex, age at disease onset, and disease duration) comparing anti-PM/Scl-negative (n=7,058) and anti-PM/Scl+ SSc patients (n=144). Results are presented as number/number available data (%) unless otherwise stated.
CK: creatinkinase; HRCT: high-resolution computed tomography; LVEF: left ventricular ejection fraction; ECHO: echocardiogram; sPAP: systolic Pulmonary Arterial Pressure.
*Data available <70%

	Univariable analysis			Multivariable analysis	
Characteristics	**With SRC**	**Without SRC**	**p-Value**	**p-Value**	**OR (95% CI)**
Age at disease onset (years, mean (SD) (n available)	48.7 (14.0) (201)	46.6 (14.5) (6025)	0.034		
Disease duration at last visit (months, mean (SD) (n available)	143.8 (111.8) (201)	150.3 (109.9) (6025)	0.221		
Male sex	60/228 (26.3)	1013/6961 (14.6)	<0.0001		
Caucasian ethnicity	189/228 (82.9)	5534/6961 (79.5)	0.242		
Raynaud's phenomenon	217/228 (95.2)	6824/6961 (98.0)	0.007		
Anti-PM/Scl positivity	8/228 (3.51)	136/6961 (1.95)	0.140	0.073	2.16 (0.93-4.99)
ACA positivity	61/227 (26.9)	3052/6916 (44.1)	<0.0001	0.317	
Anti-Topoisomerase I positivity	81/227 (35.7)	2278/6941 (32.8)	0.389	0.133	
Anti-RNAP3 positivity	41/211 (19.4)	232/6312 (3.68)	<0.0001	<0.0001	4.19 (2.66-6.60)
Smoker	61/157 (38.9)	1562/4712 (33.1)	0.144		
Oesophageal symptoms	194/228 (85.1)	5452/6961 (78.3)	0.014		
Stomach symptoms	118/227 (52.0)	2759/6949 (39.7)	<0.0001		
Intestinal symptoms	120/228 (52.6)	3048/6956 (43.8)	0.010		
Dyspnoea significant	81/215 (37.7)	1281/6529 (19.6)	<0.0001		
Diffuse cutaneous involvement	147/227 (64.8)	2350/6908 (34.0)	<0.0001	<0.0001	2.95 (2.05-4.24)
Corticosteroids ever	111/216 (51.4)	2476/6529 (37.9)	<0.0001	0.010	1.52 (1.11-2.10)
Digital ulcers	82/155 (52.9)	2504/4732 (52.9)	1.000		
Telangiectasia	117/156 (75.0)	3332/4705 (70.8)	0.283		
Joint synovitis	68/226 (30.1)	1713/6925 (24.7)	0.072		
Joint contractures	129/227 (56.8)	2770/6912 (40.1)	<0.0001		
Tendon friction rubs	63/227 (27.8)	1015/6907 (14.7)	<0.0001		
Proximal muscle weakness	112/226 (49.6)	2154/6919 (31.1)	<0.0001		
Muscle atrophy	77/227 (33.9)	1111/6917 (16.1)	<0.0001		
CK elevation (>3 ULN)	48/221 (21.7)	917/6702 (13.7)	0.001	0.133	
Lung fibrosis on plain X-rays	100/197 (50.8)	2421/5980 (40.5)	0.005		
Lung fibrosis on HRCT	105/177 (59.3)	2678/5361 (50.0)	0.015		
ILD on x-rays and/or HRCT	128/189 (67.7)	3254/5677 (57.3)	0.004		
LVEF ≤ 50% at ECHO last visit*	20/143 (14.0)	257/4397 (58.4)	<0.0001		
Elevated sPAP at ECHO last visit	69/218 (31.6)	1605/6544 (24.5)	0.020		
Pericardial effusion	42/203 (20.7)	660/6250 (10.6)	<0.0001	0.008	1.70 (1.15- 2.52)
Diastolic function abnormality	110/219 (50.2)	2322/6472 (35.9)	<0.0001		
Systemic arterial hypertension	180/227 (79.3)	2412/6955 (34.7)	<0.0001		
Conduction blocks (any degree)	73/210 (34.8)	1471/6284 (23.4)	<0.0001		

Table 2. Analysis of the EUSTAR database. Univariable and multivariable analysis comparing patients with SRC (n=228), and without SRC (n=6,961).
Results are presented as number/number available data (%) unless otherwise stated.
CK: creatinkinase; HRCT: high-resolution computed tomography; LVEF: left ventricular ejection fraction; ECHO: echocardiogram; sPAP: systolic Pulmonary Arterial Pressure. *Data available <70%

	Univariable analysis			Multivariable analysis	
Characteristics	**Anti-PM/Scl+ with CK elevation**	**Anti-PM/Scl+ without CK elevation**	**p-value**	**p-value**	**OR (95% CI)**
Age at disease onset (years, mean (SD)) (n available)	45.4 (17.0)(44)	44.91 (14.0)(82)	0.85	0.954	
Disease duration at last visit (months, mean (SD)) (n available)	127.2 (86.7)(43)	131.3 (107.5)(82)	0.83	0.197	
Female sex	35/47 (74.5)	70/87 (80.5)		0.863	
Diffuse cutaneous involvement	17/46 (37.0)	35/79 (44.3)			
LVEF on ECHO ≤50% last visit#	4/30 (13.3)	0/49 (0)			
Systemic arterial hypertension	17/47 (36.2)	18/87 (20.7)			
Conduction blocks (any degree)	19/44 (43.2)	14/78 (17.5)		0.041	3.05 (1.05-8.88)
Elevated sPAP on ECHO last visit	9/46 (19.6)	8/82 (9.76)			
Pericardial effusion	7/44 (15.9)	6/80 (7.50)			
Abnormal diastolic function	21/46 (45.6)	22/81 (27.2)			
Lung fibrosis on HRCT	30/37 (81.1)	38/63 (60.3)			
ILD on x-rays and/or HRCT	33/38 (86.8)	49/72 (68.1)	0.039	0.201	
Puffy fingers	26/35 (74.3)	42/66 (63.6)			
Oesophageal symptoms	32/47 (68.1)	52/87 (59.8)			
Stomach symptoms	18/47 (38.3)	28/87 (32.2)			
Intestinal symptoms	26/47 (55.3)	29/87(33.3)		0.002	4.90 (1.80-13.4)
Renal crisis	4/47 (8.51)	3/87 (3.45)			
Pitting scars	26/41(63.4)	33/70 (47.1)			
Gangrene	0/41(0)	1/73 (1.37)			
Digital ulcers	24/41 (58.5)	35/71 (49.3)			
Telangiectasia	29/40 (72.5)	47/71 (66.2)			
Joint synovitis	14/47 (29.8)	17/87 (19.5)			
Joint contractures	27/47 (57.4)	33/87 (37.9)			
Tendon friction rubs	9/47 (19.1)	5/87 (5.7)		0.039	4.96 (1.09-22.7)

Table 3. Analysis of the EUSTAR database. Univariable and multivariable analysis comparing anti-PM/Scl single positive patients with CK elevation >3 ULN (n=47) and without CK elevation (n=87). Results are presented as number/number available data (%) unless otherwise stated.

#Data available <70%

CK: creatinkinase; HRCT: high-resolution computed tomography; LVEF: left ventricular ejection fraction; ECHO: echocardiogram; sPAP: systolic Pulmonary Arterial Pressure.

	Univariable analysis			Multivariable analysis	
Characteristics	**Anti-PM/Scl single positivity**	**Anti-PM/Scl multiple positivity**	**p-value**	**p-value**	**OR (95% CI)**
Age at disease onset (years, mean (SD) (n available)	45.0 (15.1) (134)	43.5 (14.0) (134)	0.424	0.185	
Disease duration at last visit (months, mean (SD) (n available)	127.3 (100.4) (133)	142.0 (89.7) (134)	0.178	0.356	
Male sex	31/144 (21.5)	20/151 (13.2)	0.066	0.132	
Caucasian ethnicity	116/144 (80.6)	120/151 (79.5)	0.885		
Raynaud's phenomenon	140/144 (97.2)	14/151 (98.7)	0.438		
Oesophageal symptoms	88/144 (61.1)	120/151 (79.5)	0.001	0.124	
Stomach symptoms	47/144 (32.6)	76/151 (50.3)	0.002		
Intestinal symptoms	58/144 (40.3)	82/150 (54.7)	0.015		
Scleroderma renal crisis	8/144 (5.55)	7/151 (4.64)	0.795		
Dyspnoea significant	23/137 (16.8)	35/142 (24.7)	0.140		
Diffuse cutaneous subtype	53/142 (37.3)	78/150 (52.0)	0.014	0.228	
Digital ulcers	63/120 (50.7)	82/124 (66.1)	0.037	0.424	
Joint synovitis	33/143 (23.1)	58/151 (38.4)	0.005	0.026	0.48 (0.25-0.91)
Joint contractures	63/143 (44.1)	85/151 (56.3)	0.047		
Tendon friction rubs	14/143 (9.79)	34/149 (22.8)	0.003		
Proximal muscle weakness	57/142 (40.1)	85/150 (56.7)	0.005	0.110	
Muscle atrophy	35/142 (24.6)	43/150 (28.7)	0.508		
CK elevation (>3 ULN)	47/134 (35.1)	58/148 (39.2)	0.538		
Conduction blocks (any degree)	33/126 (26.2)	40/141 (28.4)	0.783		
Elevated sPAP on ECHO last visit	17/133 (12.8)	41/142 (28.9)	0.001	0.015	0.36 (0.16-0.81)
Lung fibrosis on plain X-rays	61/112 (54.5)	85/138 (61.6)	0.302		
Lung fibrosis on HRCT	74/107 (69.2)	84/129 (65.1)	0.579		
ILD on x-rays and/or HRCT	89/118 (75.4)	106/144 (73.6)	0.777		
LVEF ≤ 50% on ECHO last visit *	4/84 (4.76)	5/89 (5.61)	1.000		
Pericardial effusion	13/127 (10.2)	14/137 (10.2)	1.000		
Diastolic function abnormality	44/130 (33.8)	46/142 (32.4)	0.897		
Systemic arterial hypertension	37/144 (25.7)	55/151 (36.4)	0.059		

Table 4. Analysis of the EUSTAR database. Univariable and multivariable analysis comparing patients with anti-PM/Scl single positivity (n=144) and patients with anti-PM/Scl associated with SSc-specific autoantibodies (n=151).

Results are presented as number/number available data (%) unless otherwise stated.
CK: creatinkinase; HRCT: high-resolution computed tomography; LVEF: left ventricular ejection fraction; ECHO: echocardiogram; sPAP: systolic Pulmonary Arterial Pressure. *Data available <70%

Characteristics	Anti-PM/Scl+	Anti-PM/Scl-	p-value	OR (95% CI)
Age at disease onset (years, mean (SD) (n available); (range min-max)	48.5 (±15.9) (164); (18-82)	50.1 (±13.9) (251); (18-77)	0.29	
Disease duration at last visit (months, mean (SD) (n available)	101.2 (±76.1) (164)	106.5 (±65.8) (251)	0.45	
Female Sex	136/165(82.4)	219/257(85.2)	0.50	
Caucasian ethnicity	142/161(88.2)	213/248 (85.9)	0.55	
Smokers	51/161 (31.7)	85/254 (33.5)	0.75	
Diffuse cutaneous involvement	36/165 (21.8)	57/257 (22.2)	1.00	
Calcinosis	50/165 (30.3)	49/254 (19.3)	0.01	1.82 (1.15-2.87)
Lung fibrosis on HRCT	101/162 (62.3)	98/249(39.4)	<0.0001	2.55 (1.70-3.83)
Pulmonary Arterial Hypertension (RHC)	7/164 (4.27)	17/256 (6.64)	0.39	
Last LVEF on ECHO ≤ 50% last visit	2/106 (1.86)	4/156 (2.56)	1.00	
Arrhythmia requiring specific therapy	6/161 (3.73)	28/255 (11.0)	0.01	0.31 (0.13-0.78)
Digital ulcers	54/165 (32.7)	131/257 (51.0)	<0.0001	0.47 (0.31-0.70)
Pitting scars	48/164 (29.3)	99/256 (38.7)	0.06	
Telangiectasia	81/165 (49.1)	125/256 (48.8)	1.00	
Scleroderma Renal crisis	8/164(4.88)	4/255 (1.57)	0.07	
Systemic Arterial Hypertension	29/164 (17.7)	45/256 (17.6)	1.00	
Oesophageal involvement	99/165 (60.0)	197/257 (76.6)	<0.0001	0.46 (0.30-0.70)
Intestinal malabsorption	15/165 (9.09)	26/257 (10.1)	0.87	
Treated bacterial overgrowth	4/165 (2.42)	31/257 (12.1)	<0.0001	0.18 (0.06-0.52)
Ano-rectal incontinence	5/154 (3.25)	21/257 (8.17)	0.06	
Gastric Antral Vascular Ectasia	4/165 (2.42)	4/257 (1.56)	0.72	
Arthritis	46/165 (27.9)	65/256 (25.4)	0.57	
Clinical manifestations of myositis	84/163 (51.5)	23/215 (10.7)	<0.0001	8.88 (5.22-15.09)
CK elevation (>3 ULN)	66/165 (40.0)	24/249(9.64)	<0.0001	6.25 (3.70-10.55)
Myositis confirmed on histology	38/163 (23.3)	8/235 (3.40)	<0.0001	8.63 (3.90-19.07)
Cutaneous signs of dermatomyositis	34/163 (20.9)	6/228 (2.63)	<0.0001	9.75 (3.99-23.86)
Malignancies (any time)	20/165 (12.1)	20/253 (7.91)	0.17	
Malignancies synchronous ±36 months §	4/131 (3.05)	6/223 (3.14)	1.00	
Malignancies synchronous ± 24 months *	2/143 (1.40)	6/232 (2.59)	0.72	
Cyclophosphamide therapy	29/165 (17.6)	37/256 (14.4)	0.41	
Death	10/165 (6.06)	10/257 (3.89)	0.35	

Table 5. Case-control study. Univariable analysis comparing anti-PM/Scl single positive (n= 165; without SSc-specific autoantibodies) with anti-PM/Scl negative patients (n=257), matched for sex, age at disease onset (±5 years), disease duration (±24 months) and cutaneous involvement (limited or diffuse or sine scleroderma). Results are presented as number/number available data (%) unless otherwise stated.

CK: creatinkinase; HRCT: high-resolution computed tomography; LVEF: left ventricular ejection fraction; ECHO: echocardiogram; sPAP: systolic Pulmonary Arterial Pressure; RHC: right heart catheterism.

§ only patients with ≥ 36 months of follow-up were considered * only patients with ≥ 24 months of follow-up were considered

Characteristics	SSc-ILD anti-PM/Scl+	SSc-ILD anti-PM/Scl-	p-value	OR (95% CI)
Age at disease onset (years, mean (SD)	47.2 (14.7)	48.4 (13.7)	0.59	
Disease duration at LV (months, mean (SD)	111.7 (81.0)	115.0 (64.3)	0.77	
Female Sex	64/81(79.0)	64/78(82.1)	0.78	
Caucasian ethnicity	68/81(84.0)	68/78 (87.2)	0.72	
Smokers	29/81 (35.8)	26/78 (33.3)	0.87	
Diffuse cutaneous involvement	22/81 (27.2)	35/78 (44.9)	0.02	0.46 (0.24-0.89)
Cyclophosphamide therapy	24/81 (29.6)	25/78 (32.1)	0.87	
Pulmonary Arterial Hypertension (RHC)	5/81 (6.17)	6/78 (7.69)	0.76	
Clinical manifestations of myositis	38/81 (46.9)	7/78 (9.00)	<0.0001	8.96 (3.68-21.8)
Death	3/81 (3.70)	3/78 (3.84)	1.00	
Mean %pFVC T0	85.1 (18.3)	90.4 (18.5)	0.07	
Mean %pFVC T1	89.5 (16.5)	91.1 (16.5)	0.59	
Mean %pFVC LV	87.9 (16.9)	85.0 (18.0)	0.30	
Mean %pDLCO T0	60.5 (16.8)	67.0 (18.9)	0.02	
Mean %pDLCO T1	60.1 (17.6)	62.7 (18.2)	0.40	
Mean %pDLCO LV	60.4 (16.9)	59.6 (18.4)	0.78	
Delta %pFVC (T1-T0)	3.60 (11.6)	-0.19 (11.0)	0.05	
Delta %pFVC (LV-T0)	2.85 (11.3)	-5.42 (13.4)	0.0004	
Delta %pDLCO (T1-T0)	-2.94 (17.9)	-5.16 (12.0)	0.40	
Delta %pDLCO (LV-T0)	-0.13 (10.8)	-7.38 (14.6)	0.0015	
%pFVC T0 <70%	16/81 (19.8)	10/78 (12.8)	0.33	
%pFVC T1 <70%	7/70 (10.0)	8/67 (11.9)	0.78	
%pFVC LV <70%	9/81 (11.1)	16/78 (20.5)	0.13	
%pDLCO T0 <50%	21/81 (25.9)	11/78 (14.1)	0.08	
%pDLCO T1 <50%	21/68 (30.9)	16/67 (23.9)	0.44	
%pDLCO LV <50%	25/81 (30.9)	24/78 (5.13)	1.00	
Delta %pFVC (T1-T0) ≥10%	8/70 (11.4)	9/67 (13.4)	0.80	
Delta %pFVC (LV-T0) ≥10%	10/81 (12.3)	31/78 (39.7)	<0.0001	0.21 (0.10-0.48)
Delta %pDLCO (T1-T0) ≥10%	14/70 (20.0)	20/67 (29.9)	0.33	
Delta %pDLCO (LV-T0) ≥10%	11/81 (13.6)	33/78 (42.3)	<0.0001	0.21 (0.10-0.47)
Delta %pFVC (T1-T0) ≥5% AND Delta %pDLCO (T1-T0) ≥15%	3/70 (4.3)	6/67 (9.0)	0.32	
Delta %pFVC (LV-T0) ≥5% AND Delta %pDLCO (LV-T0) ≥15%	4/81 (4.9)	16/78 (20.5)	0.004	0.20 (0.06-0.63)
Delta %pFVC (T1-T0) ≥10% OR Delta %pFVC (T1-T0) ≥5% AND Delta %pDLCO (T1-T0) ≥15%	8/70 (11.4)	9/67 (13.4)	0.80	
Delta %pFVC (LV-T0) ≥10% OR Delta %pFVC (LV-T0) ≥5% AND Delta %pDLCO (LV-T0) ≥15%	13/81 (16.0)	33/78 (42.3)	<0.0001	0.26 (0.12-0.55)

Table 6. Sub-analysis of the case-control study on ILD outcome, comparing anti-PM/Scl positive SSc patients with ILD (n= 81) to anti-PM/Scl negative patients with ILD (n=78).
%pFVC: Forced Vital capacity (% of predicted); %pDLCO: Diffusion Lung for CO (% of predicted); T0: baseline; T1: follow-up after 1 year; LV: last visit available.