The clinical phenotype of Systemic Sclerosis patients with anti-Pm/Scl antibodies: results from the European Scleroderma Trials and Research group (EUSTAR) registry and case-control study.

Maria-Grazia Lazzaroni[1,2], Emiliano Marasco[3], Corrado Campochiaro[4,5], Jeska DeVries-Bouwstra[6], Montserrat-Ixchel Gonzalez-Perez[7], Jorge Rojas-Serrano[7], Eric Hachulla[8], Elisabetta Zanatta[9], Simone Barsotti[10], Federica Furini[11], Konstantinos Triantafyllias[12], Giuseppina Abignano[13], Marie-Elise Truchetet[14], Giacomo De Luca[4], Ellen De Langhe[15], Roger Hesselstrand[16], Francesca Ingegnoli[17], Eugenia Bertoldo[18], Vanessa Smith[19], Silvia Bellando-Randone[20], Hadi Poormoghim[21], Enrico Colombo[22], Angela Ceribelli[23], Alessio Furloni[2], Stefania Zingarelli[1], Franco Franceschini[1,2], Francesco Del Galdo[13], Christopher-Paul Denton[5], Lorenzo Cavagna[3], Oliver Distler[24], Yannick Allanore[25], Paolo Airò[1] on behalf of the EUSTAR co-authors

1 Rheumatology and Clinical Immunology Unit, ASST Spedali Civili of Brescia, Brescia, Italy
2 Department of Clinical and Experimental Sciences, University of Brescia, Italy
3 Division of Rheumatology, Hospital IRCCS Policlinico S. Matteo Foundation of Pavia, University of Pavia, Pavia, Italy
4 Unit of Immunology, Rheumatology, Allergy and Rare Diseases (UnIRAR), San Raffaele Scientific Institute, Milan, Italy
5 Royal Free Hospital and University College London Medical School, London, UK
6 Department of Rheumatology, Leiden University Medical Centre (LUMC), Leiden, Netherlands
7 Interstitial Lung Disease and Rheumatology Units, Instituto Nacional de Enfermedades Respiratorias, Ismael Cosío Villegas, México City, México
8 Department of Internal Medicine, University Lille Nord-de-France, Lille, France
9 Rheumatology Unit, Department of Medicine-DIMED, University of Padova, Padova, Italy
10 Division of Rheumatology, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
11 UOC Reumatologia, Azienda Ospedaliero Universitaria S. Anna, University of Ferrara, Ferrara, Italy
12 ACURA Centre for Rheumatic Diseases, Baden-Baden, Germany
13 Leeds Institute of Rheumatic and Musculoskeletal Medicine, and NIHR Biomedical Research Centre, University of Leeds, Leeds, UK
14 Division of Rheumatology and ImmunoConcept, University Hospital, Bordeaux, France
15 Department of Rheumatology, University Hospitals Leuven, Leuven, Belgium
16 Department of Clinical Sciences, Section of Rheumatology, Lund University, Lund, Sweden
17 Division of Clinical Rheumatology, ASST Pini-CTO; Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milano, Italy
18 Rheumatology Unit, Azienda Ospedaliero Universitaria Integrata, Verona, Italy
19 Rheumatology, Ghent University Hospital, Ghent University, Ghent, Belgium
20 Department of Biomedicine, Division of Rheumatology AOUC and Department of Clinical and Experimental Medicine, University of Florence, Florence, Italy
21 Scleroderma Study group, Department of Rheumatology. Firoozgar Hospital, Tehran, Iran
22 University of Sassari, Sassari, Italy
23 Rheumatology and Clinical Immunology Unit, Humanitas Clinical and Research Centre-IRCCS, Rozzano, Italy
24 Division of Rheumatology, University Hospital Zurich, Zurich, Switzerland
25 Department of Rheumatology, University of Paris, Cochin Hospital and INSERM U1016, Paris, France

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EUSTAR collaborators (in ascending centre order):

Gemma Lepri (Dept of Medicine, Section of Rheumatology, University of Florence, Florence, Italy); Veronika Jaeger, Ulrich Walker (Unispital Basel, Department of Rheumatology, Basel, Switzerland); Florenzo Iannone, Fabio Cacciapaglia (Rheumatology Unit-DiMIMP, School of Medicine University of Bari, Italy); Michal Tomčík, Radim Becvar (Institute of Rheumatology and Department of Rheumatology, 1st Medical School, Charles University, Prague, Czech Republic); Simona Rednic, Ana Petcu, Iulia Szabo (Rheumatology Clinic, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania); Veronica Codullo, Roberto Caporali, Carlomaurizio Montecucco (Unita' Operativa e Cattedra di Reumatologia, IRCCS Policlinico S Matteo, Pavia, Italy); Patricia Carreira, Beatriz Ioven (Rheumatology Department, Hospital Universitario 12 de Octubre, Madrid, Spain); Tunde Minier, László Czirják (Department of Rheumatology and Immunology, Medical Centre, University of Pécs, Pécs, Hungary); Carlo Chizzolini, Daniela Allai (Immunology and Allergy, University Hospital Geneva, Switzerland); Andrea Doria (Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Padova, Padova, Italy); Armando Gabrielli (Istituto di Clinica Medica, Università Politecnica delle Marche Polo Didattico University of Ancona, Ancona, Italy); Paolo Airò, Maria-Grazia Lazzaroni (Spedali Civili di Brescia, Servizio di Reumatologia Allergologia e Immunologia Clinica, Brescia, Italy); Mislav Radić, Duska Martinovic (Department of Internal Clinic. Clinical Hospital of Split, Split, Croatia); Yolanda Braun-Moscovici, Alexandra Balbir-Gurman (B. Shine Rheumatology Unit, Rambam Health Care Campus, Rappaport Faculty of Medicine, Technion, Haifa, Israel); Nicolas Hunzelmann (Universitätshautklinik Köln, Köln, Germany); Paola Caramaschi (Università degli Studi di Verona, Dipartimento di Medicina Clinica e Sperimentale, Rheumatology Unit, Policlinico GB Rossi, Verona, Italy); Jadranka Morovic-Vergles (Division of Clinical Immunology and Rheumatology, Department of Internal Medicine, Dubrava University Hospital, Zagreb, Croatia); Christopher Denton (Centre for Rheumatology, Royal Free and University College London Medical School, London, United Kingdom); Vera Ortiz Santamaria (Rheumatology Granollers General Hospital, Barcelona, Spain); Stefan Heitmann (Department of Rheumatology, Marienhospital Stuttgart, Stuttgart, Germany); Dorota Krasowska, Malgorzata Michalska-Jakubus (Department of Dermatology, Venereology and Pediatric Dermatology, Medical University of Lublin, Lublin, Poland); Matthias Seidel (Medizinische Universitäts-Poliklinik, Departent of Rheumatology, Bonn, Germany); Ivan Foeldvari, Nicola Helmus (Hamburger Zentrum für Kinder- und Jugendrheumatologie, An der Schön Klinik Hamburg Eilbek, Hamburg, Germany); Maria João Salvador (Rheumatology Department, Hospitais da Universidade, Coimbra, Portugal); Bojana Stamenkovic, Aleksandra Stankovic (Institute for prevention, treatment and rehabilitation rheumatic and cardiovascular disease, Niska Banja, Serbia and Montenegro); Lidia Ananieva (Laboratory of microcirculation and inflammation, VA Nasonova Institute of Rheumatology, Moscow, Russian Federation); Ariane Herrick (Salford Royal Hospital - University of Manchester, Salford, United Kingdom); Merete Engelhart (Department of Rheumatology, University Hospital of Gentofte, Hellerup, Denmark); Carlos De La Puente (Servicio de Reumatología, Hospital Ramon Y Cajal, Madrid, Spain); Anna-Maria Hoffmann-Vold, Øyvind Midtvedt (Department of Rheumatology, Rikshospitalet University Hospital, Oslo, Norway); David Launay, Vincent Sobanski (Department of Internal Medicine and Clinical Immunology, Huriez Hospital, University of Lille, Lille, France); Valeria Riccieri (Rheumatology Dept. Of Internal Medicine and Medical Specialities “Sapienza” University of Rome, Rome, Italy); Daniela Opris-Belinski, Laura Groseanu, Ruxandra Maria Ionescu (Department of Rheumatology - St. Maria Hospital, Carol Davila, University of Medicine and Pharmacy, Bucharest, Romania); Mihai Bojinca (Department of Rheumatology - Cantacuzino Hospital, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania); Cord Sunderkötter (Department of Dermatology, University of Münster, Münster, Germany); Jörg Distler (Department of Internal Medicine 3, Universitätsklinikum Erlangen, Erlangen, Germany); Francesca Ingegnoli (Division of Rheumatology, Istituto Gaetano Pini, Department of Clinical Sciences & Community Health, University of Milano, Milano, Italy); Amber van der Haecke (University of Ghent, Department of Rheumatology, Gent, Belgium); Susanne Ullman (University Hospital of Copenhagen, Department of Dermatology D-40, HS-Bispebjerg Hospital, Copenhagen); Maria Rosa Pozzi (Dipartimento di Medicina, Ospedale San Gerardo, Monza, Italy); Kilian Eyerich (Department of Dermatology and Allergy of the TU Munich, Munich, Germany); Marie Vanthuyne (Université Catholique de Louvain, Cliniques Universitaires

St-Luc, Bruxelles, Belgium); Anne Erler, Martin Aringer (Division of Rheumatology, Department of Medicine II, University Medical Center Carl Gustav Carus, Technical University of Dresden, Dresden, Germany); Ellen De Langhe (Department of Rheumatology , University Hospital Leuven, Leuven, Belgium); Marko Baresic, Miroslav Mayer, Branimir Anic (Division of Clinical Immunology and Rheumatology, Department of Internal Medicine, University of Zagreb, School of Medicine, Zagreb, Croatia); Sule Yavuz (Istanbul Bilim University, Dept. of Rheumatology, Istanbul, Turkey); Brigitte Granel (Service de Médecine Interne, Hôpital Nord de Marseille, Marseille, France); Sergei Popa, Svetlana Agachi (Department of Rheumatology&Nephrology , SUMF "N. Testemitanu", Chisinau, Republic of Moldova); Thierry Zenone (Department of Medicine, Unit of Internal Medicine, Valence, France); Alessandro Mathieu, Alessandra Vacca (II Chair of Rheumatology, University of Cagliari-Policlinico Universitario, Monserrato, Italy); Kamal Solanki (Waikato University Hospital, Rheumatology Unit, Hamilton City, New Zealand); Douglas Veale (Department of Rheumatology, Bone and Joint Unit, St. Vincent's University Hospital, Dublin, Ireland); Esthela Loyo, Carmen Tineo (Hospital Regional Universitario Jose Ma. Cabral y Baez, Santiago, Dominican Republic); Antonetta Gigante, Edoardo Rosato (Sapienza Università di Roma, Dipartimento di Medicina Clinica, UOC di Medicina Interna e Nutrizione Clinica-Scleroderma Unit., Università La Sapienza, Policlinico Umberto I, Roma, Italy); Fahrettin Oksel, Figen Yagurcu (Ege University, Faculty of Medicine, Dept. of Internal Medicine, Division of Rheumatology, Izmir, Turkey); Cristina-Mihaela Tănăseanu (Clinical Emergency Hospital St. Pantelimon, Bucharest, Romania); Elisa Visalli, Alessia Benenati, Rosario Foti (Rheumatology Unit; A.O.U. Policlinico V.E., Catania Italy); Codrina Ancuta (Clinical Rehabilitation Hospital, Rheumatology & Rehabilitation, 'Grigore T. Popa' University of Medicine and Pharmacy Iasi, Romania); Diana Dan, Sabine Adler, Peter Villiger (Department of Rheumatology and Clinical Immunology/Allergology, Inselspital, University of Bern, Bern, Switzerland); Nihal Fathi (Assiut and Sohage University Hospital, Rheumatology Department Assiut University Hospital, Assiut, Egypt); Paloma García de la Peña Lefebvre, Jorge Juan González Martín (Hospital Universitario Madrid Norte Sanchinarro, Madrid, Spain); Emmanuel Chatelus, Jean Sibilia (University Hospital of Strasbourg-Department of Rheumatology, Hôpital de Hautepierre, Service de Rhumatologie, Strasbourg, France); Ira Litinsky (Rheumatology Institute, Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine,Tel Aviv University, Tel Aviv, Israel); Francesco Del Galdo (Scleroderma Programme, Institute of Molecular Medicine, Division of Musculoskeletal Diseases, University of Leeds, Chapel Allerton Hospital, Leeds, United Kingdom); Lesley Ann Sakettkoo (Tulane University Lung Center, Tulane / University Medical Center , Scleroderma and Sarcoidosis Patient Care and Research Center, New Orleans, Lousiana, USA); Eduardo Kerzberg (Osteoarticular Diseases and Osteoporosis Centre, Pharmacology and Clinical Pharmacological Research Centre, School of medicine - University of Buenos Aires, Rheumatology and Collagenopathies Department, Ramos Mejía Hospital, Buenos Aires, Argentina); Washington Bianchi, Breno Valdetaro Bianchi (Department of Rheumatology-Santa Casa da Misericórdia do Rio de Janeiro, Rio de Janeiro, Brasil); Ivan Castellví (Hospital de la Santa Creu i Sant Pau, Barcelona, Spain); Massimiliano Limonta (USSD Reumatologia, Ospedali Riuniti di Bergamo, Bergamo, Italy); Doron Rimar (Rheumatology Unit Bnai Zion Medical Center, Haifa, Israel); Maura Couto (Unidade de Reumatologia de Viseu, Centro Hospitalar Tondela-Viseu, Viseu, Portugal); Camillo Ribi, François Spertini (Department of Rheumatology, Clinical Immunology and Allergy, Lausanne, Switzerland); Sarah Kahl (Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Innere Medizin/Rheumatologie/Immunologie, Rheumaklinik Bad Bramstedt, Bad Bramstedt, Germany); Vivien Hsu (Rutgers- RWJ Scleroderma Program, Rutgers-RWJ Rheumatology Fellowship Program, New Brunswick, New Jersey, USA); Vincent Poindron, Kilifa Meghit, Thierry Martin (Clinical Immunology Internal Medicine, National Referral Center for Systemic Autoimmune Diseases, Nouvel Hopital Civil 1, Strasbourg, France); Kathleen Kolstad, Lorinda Chung (Department of Dermatology Stanford University School of Medicine, Redwood City, California, USA); Astrid Thiele, Tim Schmeiser (Krankenhaus St. Josef, Wuppertal-Elberfeld, Germany); Zbigniew Zdrojewski (Department of Internal Medicine, Connective Tissue Diseases and Geriatrics, University Clinical Centre, Medical University of Gdansk, Gdansk, Poland); Gabriela Riemekasten (Universitätsklinik Lübeck, Lübeck, Germany); Yair Levy (Meir Medical Center, Kfar-Saba, Israel); Anca Cardoneanu, Alexandra Burlui, Elena Rezus (Division of Rheumatology & Rehabilitation , University of Medicine and Pharmacy "Grigore T. Popa" Iasi, Rehabilitation Hospital, Iasi, Romania); Omer Nuri Pamuk (Trakya University Medical Faculty, Department of Internal Medicine, Division of Rheumatology, Edirne, Turkey); Rossella Talotta, Sara Bongiovanni, Piercarlo Sarzi Puttini (University Hospital Luigi Sacco, Milano, Italy).

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 myositis, 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.

Conclusions. The analysis of the largest series of anti-PM/Scl+ SSc patients so far reported helps to delineate a specific clinical subset with myositis, 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). If 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, for rarer autoantibodies further research is needed.

Antibodies against the PM/Scl complex are found not only in patients with SSc, but also 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 then counter-immunoelectrophoresis, double immunodiffusion, immunoprecipitation or Enzyme-Linked Immunosorbent Assay (ELISA) to 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 irrespective of the immunoassay used, although with various strength of association among different studies (4-11).

Very recently, important new data have proposed potential clinical associations of 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 recently 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 so far not clarified. Third, although an increased risk of concomitant heart involvement in SSc patients with skeletal muscle 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 and developing a specifically dedicated case-control study.

METHODS

Analysis of the EUSTAR database.

The EUSTAR registry is based on the records of 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 from 149 centres were included and fulfilled either the 1980 American College of Rheumatology (ACR) or the 2013 ACR/EULAR classification criteria for SSc (24,25). Data on anti-PM/Scl have been recorded since December 2008. MEDS variables were defined as previously reported (22,23).

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 between anti-PM/Scl+ and anti-PM/Scl– patients, and considered separately. Data from all available visits for each patient were included when a specific variable was evaluated as “ever” or “never” present. For all the other variables, data reported at the last available visit were considered.

Ethics approval was obtained from the respective local ethics committees by all participating centres, and patients included in the database gave their written informed consent.

The study was conducted in accordance with Helsinki Declaration principles.

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 (according to the local practice at the participating centre); specific positivity for PM/Scl-75 and PM/Scl-100 autoantigens; presence of other auto-antibodies; clinical diagnosis (SSc, overlap syndrome); muscle biopsy confirming myositis; cutaneous manifestations of DM; 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. Participating centres provided retrospective data for all the anti-PM/Scl+ patients in their SSc cohort 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 asked to exclude SSc patients with positive anti-PM/Scl and associated SSc-specific autoantibodies from cases, and patients with positive anti-RNAP3 antibodies from controls, because of their known increased risk of synchronous associated malignancies (16,26). 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 (adjusted for sex, age at onset, and disease duration) were performed with calculation of odds ratios (OR) and 95% confidence intervals (CI); besides *a priori* potential confounders, variables associated with $p<0.05$ in univariable analysis were considered in these analysis.

To explore the association of anti-PM/Scl with SRC, a multivariable logistic regression analysis was performed; besides anti-PM/Scl and SSc-specific antibodies, other clinical variables with well-known association with SRC from previous literature were considered in this analysis.

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 muscle involvement 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 muscle involvement 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, corticosteroid 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

had more frequent ever corticosteroids exposure 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, corticosteroids 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). At the univariable analysis, anti-RNAP3, corticosteroids assumption ever, muscle involvement, gastro-intestinal involvement, ILD, tendon friction rubs, pericardial effusion and diffuse cutaneous involvement were positively associated with SRC. A negative association was observed for ACA. Anti-PM/Scl and SSc-specific antibodies were included in a multivariable analysis, together with corticosteroids assumption ever, muscle involvement (CK elevation), diffuse cutaneous involvement, and pericardial effusion (Table 2). Anti-PM/Scl were 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 involvements in anti-PM/Scl+ SSc patients.

To investigate whether skeletal muscle involvement among anti-PM/Scl+ SSc patients was associated with other organ involvements, clinical and laboratory characteristics of those with raised serum CK were compared to those without CK elevation (Table 3, Group 1 vs. Group 2). Patients with CK elevation had a higher frequency of heart involvement (systolic and diastolic left ventricular dysfunction, 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.

Moreover, anti-PM/Scl+ patients with CK elevation were compared to anti-PM/Scl- with CK elevation (Table 3, Group 1 vs. Group 3). Among anti-PM/Scl+ patients, a higher frequency

of ILD on X-ray and/or HRCT, and lower frequencies of diffuse cutaneous involvement and oesophageal symptoms were demonstrated by multivariable analysis.

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, muscle weakness and elevated sPAP at echocardiogram. Multivariate 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. As expected, SSc patients deriving from these 'myositis-oriented' centres as compared to other SSc patients, more frequently had clinically signs of myositis (p:0.0001) arthritis (p:0.0002) and an "overlap syndrome" phenotype (p:0.005).

These cases were compared with 257 local anti-PM/Scl- SSc controls (32% anti-Topo I+, 42% ACA+; 39 provided by AENEAS centres), matched 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%).

Anti-PM/Scl positive patients had a higher prevalence of myositis, ILD on HRCT, cutaneous signs of dermatomyositis 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, neither considering a period of 24 months before and after SSc onset, or a larger interval of 36 months (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), irrespectively 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 reported 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 were similar between the two groups, whereas diffuse cutaneous involvement was less frequent (p:0.02) and 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).

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 evaluable 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, it should be considered that the same finding was reported in 43 of 92 anti-PM/Scl+ patients a tri-nation study (9), and in 29 of 55 in a Canadian cohort (10). 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 that were underrepresented in patients with isolated anti-PM/Scl (Table 4). This seems to 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. Having the chance to analyse data from 144 SSc patients with monospecific positivity for anti-PM/Scl autoantibodies, the present study is the largest so far reported on this topic and can help to delineate the phenotype associated with anti-PM/Scl.

Our data confirmed the previously reported clinical associations of anti-PM/Scl with muscle involvement, ILD, calcinosis (4,6,10) and cutaneous signs of dermatomyositis (11), whereas oesophageal involvement and estimated pulmonary hypertension by echocardiogram were less frequent in these patients (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.

Muscle involvement in anti-PM/Scl+ SSc patients was associated with increased frequency of heart, tendon and intestinal involvement as compared to anti-Pm/Scl- without muscle involvement. Moreover, as compared to anti-Pm/Scl- patients with muscle involvement they

had a significantly higher frequency of ILD. Altogether, these observations suggest that the presence of muscle involvement (defined as increased CK levels) in anti-Pm/Scl+ patients defines a subgroup with a more severe disease phenotype.

Our study also focused on some other still unresolved issues.

A lower rate of death among anti-PM/Scl+ than anti-PM/Scl- SSc patients was observed in the EUSTAR registry, but disease duration was slightly shorter in the former group. As compared to other SSc subsets, anti-PM/Scl+ SSc patients were reported to 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 of this observation 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 with ILD. 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, contrary to the prevailing view, 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 of the EUSTAR registry, 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). Importantly, anti-Pm/Scl+ patients represented the smaller auto-antibody subgroup in this analysis, possibly leading to a sample size issue. 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).

Finally, the relationship between anti-PM/Scl positivity and malignancies was analysed in detail. History of cancer was found 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 populations, and in 5 patients 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 (29-31). 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). Remarkably, in support to this hypothesis, in a case of anti-PM/Scl+ SSc/PM overlap syndrome, clinical remission of the autoimmune disease was observed after curative resection of a pancreatic tumour expressing nuclear staining for PM/Scl-100 (12). We have addressed this issue through an *ad-hoc* designed case control-study. No increased frequency of malignancies, and in particular of malignancies 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 from our case-control study. Interestingly, a 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. Moreover, the large majority of patients included in the EUSTAR registry and in the case-control study were Caucasians, 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 as the “anti-PM/Scl syndrome” (11). As many of its features are typical of SSc, this study suggests considering it as a distinct subset of SSc and not as a separate entity. Further research is required to better define this syndrome, in particular in patients with anti-PM/Scl positivity without SSc criteria (e.g. pure PM/DM patients).

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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 (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)	2528/4773 (53.0)	0.927		
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		
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	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) at last visit	4/144 (4.76)	273/4463 (6.11)	0.818		
Elevated sPAP (ECHO)	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		
Systemic arterial hypertension	37/144 (25.7)	2557/7043 (36.3)	0.008		
Conduction blocks	33/126 (26.2)	1513/6377 (23.7)	0.526		
Death	3/144 (2.08)	610/7058 (8.64)	0.002		

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.

	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 (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		
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	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	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.

Characteristics	Group 1	Group 2	Group 3	Group 1 vs Group2		Group 1 vs Group 3	
Anti-Pm/Scl status (+/-) CK elevation (present/absent)	**Anti-PM/Scl+ present**	**Anti-PM/Scl+ absent**	**Anti-PmScl- present**	**p-value***	**p-value****	**p-value***	**p-value****
Age at disease onset (years, mean (SD)) (n available)	45.4 (17.0)(44)	44.91 (14.0)(82)	46.0 (14.4) (834)	0.85	0.954	0.81	0.857
Disease duration (months, mean (SD)) (n available)	127.2 (86.7)(43)	131.3 (107.5)(82)	137.8 (101.0)(832)	0.83	0.197	0.53	0.217
Female sex	35/47 (74.5)	70/87 (80.5)	679/920 (73.8)	0.51	0.863	1.00	0.726
Diffuse cutaneous involvement	17/46 (37.0)	35/79 (44.3)	457/915 (49.9)	0.457		0.10	0.012[d]
LVEF on ECHO ≤50%	4/30 (13.3)	0/49 (0)	46/571 (8.06)	0.018		0.31	
Systemic arterial hypertension	17/47 (36.2)	18/87 (20.7)	382/917 (41.7)	0.064		0.54	
Conduction blocks	19/44 (43.2)	14/78 (17.5)	286/866 (33.0)	0.005	0.041[a]	0.19	
Elevated sPAP (ECHO)	9/46 (19.6)	8/82 (9.76)	254/888 (28.6)	0.173		0.24	
Pericardial effusion	7/44 (15.9)	6/80 (7.50)	119/854 (13.9)	0.219		0.66	
Abnormal diastolic function	21/46 (45.6)	22/81 (27.2)	350/874 (40.0)	0.050		0.45	
Lung fibrosis on HRCT	30/37 (81.1)	38/63 (60.3)	414/764 (54.2)	0.045		0.001	
ILD on x-rays and/or HRCT	33/38 (86.8)	49/72 (68.1)	501/815 (61.5)	0.039	0.201	0.001	0.003[e]
Puffy fingers	26/35 (74.3)	42/66 (63.6)	503/713 (70.5)	0.373		0.71	
Oesophageal symptoms	32/47 (68.1)	52/87 (59.8)	771/918 (84.0)	0.357		0.008	0.016[f]
Stomach symptoms	18/47 (38.3)	28/87 (32.2)	436/917 (47.5)	0.568		0.23	
Intestinal symptoms	26/47 (55.3)	29/87(33.3)	447/917 (48.7)	0.017	0.002[b]	0.46	
Renal crisis	4/47 (8.51)	3/87 (3.45)	44/918 (4.79)	0.240		0.29	
Pitting scars	26/41(63.4)	33/70 (47.1)	387/633 (61.1)	0.117		0.87	
Gangrene	0/41(0)	1/73 (1.37)	31/661 (4.69)	1.000		0.25	
Digital ulcers	24/41 (58.5)	35/71 (49.3)	373/652 (57.2)	0.433		1.00	
Telangiectasia	29/40 (72.5)	47/71 (66.2)	447/646 (69.2)	0.531		0.73	
Joint synovitis	14/47 (29.8)	17/87 (19.5)	310/917 (33.8)	0.202		0.64	
Joint contractures	27/47 (57.4)	33/87 (37.9)	458/911 (50.3)	0.045		0.37	
Tendon friction rubs	9/47 (19.1)	5/87 (5.7)	220/910 (24.2)	0.034	0.039[c]	0.49	
Death	0/47 (0)	2/87 (2.30)	78/920 (8.47)	0.541		0.03	

Table 3. Analysis of the EUSTAR database. Univariable and multivariable analysis comparing anti-PM/Scl single positive patients with CK elevation (n=47) and without CK elevation (n=87) and univariable and multivariable analysis comparing anti-PM/Scl single positive patients with CK elevation (n=47) and anti-PM/Scl negative patients with CK elevation (n=920).

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.

a OR (95% CI) 3.05 (1.05-8.88);

b: OR (95% CI) 4.90 (1.80-13.4);

c: OR (95% CI) 4.96 (1.09-22.7);

d: OR (95% CI) 0.37 (0.17-0.81);

e: OR (95% CI) 3.65 (1.54-8.64);

f: OR (95% CI) 0.38 (0.18-0.84)

	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 (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 (52.5)	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		
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	47/134 (35.1)	58/148 (39.2)	0.538		
Conduction blocks	33/126 (26.2)	40/141 (28.4)	0.783		
Elevated sPAP (ECHO)	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% (ECHO) at 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.

Characteristics	Anti-PM/Scl+	Anti-PM/Scl-	p-value	OR (95% CI)
Age at disease onset (years, mean (SD) (n available)	48.5 (±15.9) (164)	50.1 (±13.9) (251)	0.29	
Disease duration (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%	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 symptoms of myositis	84/163 (51.5)	23/215 (10.7)	<0.0001	8.88 (5.22-15.09)
CK elevation (>x3 ULT)	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 (ever)	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 ever	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 the 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 ever	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 signs 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.

Auto-antibody	Neoplasia	Age at SSc onset (years)	Sex	Smoke (ever)	Myositis	Lung fibrosis (HRCT)	Cutaneous subset	CYC therapy
Anti-PM/Scl	Cholangio-carcinoma	75	Female	YES	YES	YES	Unclassified	NO
Anti-PM/Scl	Breast cancer	67	Female	NO	NO	YES	Limited	YES
Anti-PM/Scl	Parotid cancer	64	Male	YES	NO	YES	Diffuse	YES
Anti-PM/Scl	Colon cancer	70	Female	YES	NO	NO	Limited	NO
Other	Breast cancer	63	Female	YES	NO	NO	Limited	NO
Anti-Topo I	Ovarian cancer	27	Female	NO	NO	YES	Limited	NO
ACA	Breast cancer	52	Female	NO	NO	NO	Limited	NO
ACA	Lymphoma	45	Female	YES	NO	NO	Limited	NO
Anti-Topo I	Lung cancer	72	Female	NO	NO	YES	Diffuse	NO
Other	Multiple myeloma	64	Female	NO	YES	YES	Diffuse	YES

Supplementary Table 1. Case-control study. Characteristics of the 10 patients with malignancies synchronous to SSc onset (±36 months) in the whole cohort (cases and controls).
Results are presented as number/number available data (%) unless otherwise stated.
HRCT: high-resolution computed tomography; anti-Topo I: anti-Topoisomerase I; ACA: anti-centromere; Other: ACA-, anti-Topo I-, anti-RNAP3-, anti-/PM/Scl- CYC: cyclophosphamide.

Characteristics	Anti-PM/Scl75	Anti-PM/Scl100	Anti-PM/Scl 75+100	p-value
Female Sex	30/33 (90.9)	25/29 (86.2)	45/58 (77.6)	0.227
Caucasian ethnicity	25/33 (75.8)	28/29 (96.6)	51/58 (87.9)	0.055
Smokers (ever)	9/31 (29.0)	7/29 (24.1)	19/58 (32.8)	0.727
Death	1/33 (3.03)	1/29 (3.44)	3/58 (5.17)	0.999
Diffuse cutaneous involvement	9/33 (27.3)	5/28 (17.9)	12/57 (21.1)	0.657
Calcinosis	4/33 (12.1)	12/29 (41.4)	11/58 (19.0)	**0.021**
Lung fibrosis on HRCT	21/33 (63.6)	17/29 (58.6)	32/58 (55.2)	0.733
Pulmonary Arterial Hypertension (RHC)	0/32 (0)	1/29 (3.44)	5/58 (8.62)	0.226
Last LVEF on ECHO ≤50%	0/17 (0)	0/18 (0)	1/41 (2.44)	0.999
Arrhythmia requiring specific therapy	0/31 (0)	1/29 (3.44)	2/58 (3.44)	0.613
Digital ulcers	12/33 (36.4)	10/29 (34.5)	19/58 (32.8)	0.941
Pitting scars	8/33 (24.2)	8/28 (28.6)	12/58 (20.7)	0.719
Telangiectasia	13/33 (39.4)	19/29 (65.5)	17/58 (29.3)	**0.005**
Scleroderma Renal crisis	0/33 (0)	1/29 (3.44)	4/58 (6.90)	0.306
Systemic Arterial Hypertension	4/32 (12.5)	6/29 (20.7)	11/58 (19.0)	0.670
Oesophageal involvement	19/33 (57.6)	19/29 (65.5)	36/58 (62.1)	0.832
Intestinal malabsorption	2/33 (6.06)	2/29 (6.90)	7/58 (12.1)	0.712
Treated bacterial overgrowth	1/33 (3.03)	0/29 (0)	3/58 (5.17)	0.807
Ano-rectal incontinence	0/29 (0)	0/29 (0)	1/53 (1.89)	0.999
Gastric Antral Vascular Ectasia	0/33 (0)	0/28 (0)	1/58 (1.72)	1.000
Arthritis	11/33 (33.3)	5/29 (17.2)	22/58 (37.9)	0.141
Clinical symptoms of myositis	20/33 (60.6)	12/29 (41.4)	36/58 (62.1)	0.182
CK elevation (>x3 ULT)	12/33 (36.4)	12/29 (41.4)	28/58 (48.2)	0.739
Myositis confirmed on histology	7/32 (21.8)	8/28 (28.6)	10/58 (17.2)	0.482
Cutaneous signs of dermatomyositis	4/33 (12.1)	4/29 (13.8)	18/58 (31.0)	0.064
Malignancies (ever)	6/33 (18.2)	4/29 (13.8)	4/58 (6.90)	0.229
Malignancies synchronous ±36 months §	0/33 (0)	0/29 (0)	0/58 (0)	1.000
Malignancies synchronous ± 24 months *	0/33 (0)	0/29 (0)	0/58 (0)	1.000
Cyclophosphamide therapy ever	5/33 (15.2)	4/29 (13.8)	5/58 (8.62)	0.576

Supplementary Table 2. Case-control study. Univariable analysis comparing anti-PM/SCl 75 (n=33) to anti-PM/Scl 100 (n=29) and to anti PM/Scl-75+100 (n=58) positive patients.

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

Supplementary Figure 1.