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ORIGINAL RESEARCH

# Clinical and serological features of triple autoantibody-negative patients with systemic sclerosis: insights from the multicentric SPRING registry of the Italian Society for Rheumatology

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## ABSTRACT

**Background** Antitopoisomerase I (ATA), anticentromere (ACA) and anti-RNA polymerase III (RNAP3) antibodies are included in the 2013 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for systemic sclerosis (SSc). A subset of patients with SSc satisfy criteria but may lack these specific autoantibodies, being classified as 'triple-negative'.

**Methods** We conducted a retrospective evaluation of triple-negative patients with SSc prevalence and clinical features among the multicentric Systemic sclerosis Progression INvestiGation registry.

**Results** Out of 1480 patients with SSc, 295 (19.9%) were triple-negative, while 1185 (81.1%) had

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The classification and clinical stratification of systemic sclerosis (SSc) strongly relies on the detection of three major disease-related autoantibodies—anticentromere, antitopoisomerase I and anti-RNA polymerase III—which are included in the 2013 American College of Rheumatology/European Alliance of Associations for Rheumatology criteria; however, a subset of patients fulfilling classification criteria lacks all three prototypic antibodies and are defined as 'triple-negative'.

⇒ Little is known about the clinical profile of triple-negative SSc.

### WHAT THIS STUDY ADDS

⇒ This large, multicentre study from the Italian Systemic sclerosis Progression INvestiGation registry shows that nearly 20% of patients with SSc are triple-negative and that this subset is characterised by a higher prevalence of interstitial lung disease and myopathy, but a lower frequency of vascular complications.

⇒ These patients were more frequently treated with corticosteroids and immunosuppressants.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study confirms that triple-negative patients meet current classification criteria and represent a distinct SSc endotype, providing new insights into the possible pathogenic role of rare or untested autoantibodies.

⇒ Recognising this subset may support broader serological screening and more tailored treatment strategies.

SSc-specific antibodies: ACA (54.3%), ATA (43.6%) and RNAP3 (2.1%). The triple-negative group showed a higher prevalence of myopathy (16.7% vs 10.1%, p=0.003), suggested by higher creatine phosphokinase (CPK) levels (126.2 vs 92.5 U/mL, p=0.002), more frequent CPK increase over 2–3 times (2.4% vs 0.2%, p=0.028). Triple-negative patients also exhibited fewer vascular complications, including digital ulcers (17.3% vs 22.8%, p=0.04) and calcinosis (8.2% vs 12.8%, p=0.027), and a higher prevalence of interstitial lung disease (p<0.001). Consistently, lower diffusing capacity for carbon monoxide (66.4% vs 70.98%, p=0.004) and forced vital capacity (97.01% vs 102.92%, p<0.001) were found in the triple-negative group. Triple-negative patients more frequently received corticosteroids (79.3% vs 67.9%, p=0.003), cyclophosphamide (43.4% vs 26%, p<0.001) and azathioprine (38.5% vs 22.3%, p=0.002), while less frequently received prostanoids (71.6% vs 85.9%, p<0.001), calcium channel blockers (80.1% vs 87.7%, p=0.005) and phosphodiesterase-5 inhibitors (4% vs 20%, p<0.001).

**Conclusions** A higher prevalence of myopathy and interstitial lung disease and a reduced vascular burden were found in the triple-negative patients, suggesting that the non-specific and non-routinely tested autoantibodies may identify an SSc endotype resembling sclero-myositis.

## INTRODUCTION

Systemic sclerosis (SSc) is a severe immune-mediated disease characterised by diffuse vascular damage and aberrant activation of the immune system, resulting in inflammation and fibrosis of skin and internal organs.[1] SSc is considered to have an autoimmune aetiology due at least to the following reasons: antinuclear antibodies (ANAs) are detected in >90% of patients, different potentially pathogenic autoantibodies targeting autoantigens of various components are reported and immunosuppressive therapies are effective in most cases.[1–3]

SSc-specific autoantibodies are specifically detected in SSc and rarely found in other connective tissue diseases or in healthy subjects. The majority of patients with SSc test positive for anticentromere antibodies (ACA), anti-Scl70 or antitopoisomerase I (ATA) and anti-RNA polymerase III (RNAP3).[4] These three prototypical SSc-specific autoantibodies are indeed included in the 2013 American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) classification criteria[5] for the disease and are strongly associated with distinct clinical manifestations and prognosis.[4–6] Additional and more rarely detected SSc-related autoantibodies, including antifibrillarin, anti-U3 ribonucleoprotein (RNP), anti-Th/To, anti-U11/U12 RNP, anti-U1 RNP, antipolymyositis (PM)–scleroderma (Scl) and anti-Ku have also been identified. However, they have only recently been introduced into routine clinical practice.[2 3]

Both the mechanisms by which autoantibodies are produced and their role in the pathogenesis of SSc remain unknown. However, the identification of SSc-specific autoantibodies in each patient is clinically useful to diagnose and evaluate organ involvement, depicting a unique clinical scenario, since the type of SSc-specific antibody could be indicative of clinical features, severity and prognosis.[2]

However, a subset of patients with SSc satisfies definite SSc ACR/EULAR classification but lacks all three major SSc-specific autoantibodies, irrespective of ANA status. They are defined as 'triple-negative' patients with SSc, representing a clinically and immunologically less defined SSc endotype with poorly characterised clinical manifestations.[4 6] Triple-negative patients with SSc have been previously reported in a limited number of international cohorts, mainly from Japan, Canada and the USA, with prevalences ranging from approximately 2% to 14%, depending on the definition adopted; however, data from Italian SSc cohorts are lacking.[4 7 8] Similarly, little is known about the 'quadruple-negative' patients, that is, patients with definite SSc diagnosis but lacking ACA, ATA and RNAP3 and having ANA positivity with any pattern other than the nucleolar one. Importantly though, even the ANA nucleolar pattern alone could be of clinical relevance. Anti-U3 RNP, anti-Th/To and anti-PM–Scl, indeed, usually produce an ANA nucleolar pattern that is commonly associated with SSc and has also been described in patients with ATA or RNAP3 positivity.[2 3 9]

Thus, we aimed to evaluate the prevalence of ANA-positive and triple-negative patients with SSc in the Italian Systemic Sclerosis Progression INvestiGation (SPRING) registry, the largest national cohort of patients with SSc, and to define their clinical demographic and clinical features. We also aimed to investigate the clinical features of quadruple-negative patients with SSc in the same registry.

## METHODS

### Study design and participants

SPRING is a multicentre national no-profit cohort study, promoted by Italian Society for Rheumatology (SIR) in 2015 as part of SIR-Strategic Projects; therefore, all the Italian rheumatology centres and scleroderma referral centres were invited to participate.

Patients were consecutively screened and enrolled at each participating centre according to standardised study procedures.

For the purpose of the study, we selected only patients enrolled in the SPRING registry who were aged >18 years and classified as having definite SSc according to the SSc ACR/EULAR 2013 classification criteria.[5]

Patient's age was calculated at the SSc diagnosis at each referral centre. At the same time, patients were also classified based on the extent of skin sclerosis as limited cutaneous SSc, diffuse cutaneous SSc or sine scleroderma SSc in the complete absence of cutaneous sclerosis, as previously described.[6 10] Besides, the following SSc-related symptoms and organ involvement were evaluated according to the criteria formerly described[6 11]: skin involvement as assessed by modified Rodnan Skin Score (mRSS) and its worsening in the month before enrolment, digital ulcers, gangrene and/or osteomyelitis[11 12]; arthritis (inflammatory changes observed in more than two joints); muscle weakness with/without elevated serum creatine kinase; oesophageal involvement (dysphagia and oesophageal radiographic dysmotility); pulmonary involvement (dyspnoea, ground-glass opacities and/or bibasilar fibrosis on high-resolution CT (HRCT) of the lungs and/or restrictive lung disease on pulmonary function tests (PFTs), including decreased diffusion capacity for carbon monoxide (DLCO)), cardiac involvement (at least one of the following features: pericarditis, severe arrhythmias and/or atrioventricular conduction abnormalities at ECG, left ventricle diastolic dysfunction and/or abnormal ejection fraction <50% at Doppler echocardiography); pulmonary arterial hypertension (PAH) evaluated by means of systolic pulmonary arterial pressure at Doppler echocardiography and confirmed by right heart catheterisation according to current diagnostic criteria[13] and scleroderma renal crisis (sudden onset of severe arterial hypertension together with acute renal failure). Comorbidities were also considered, as well as the history of malignancies.[14]

Besides routine laboratory investigations, the following serological markers were detected by means of standard techniques: ANA positivity and pattern, ACA and anti-extractable nuclear antigen antibody specificities, including ATA and anti-Ro/52 antibodies, RNAP3 and total creatine phosphokinase (CPK).

At baseline and every yearly visit, all the above-mentioned features were collected, as well as previous/current treatments, including both vasoactive drugs (calcium channel blockers, intravenous prostanoids, endothelin receptor antagonists), phosphodiesterase-5 inhibitors (PDE5) and/or ACE inhibitors and immunomodulants/immunosuppressors (corticosteroids, hydroxychloroquine, cyclophosphamide, methotrexate, azathioprine and/or mycophenolate mofetil).

Available information on the variables 'ANA', 'ANA pattern', 'ACA', 'ATA', 'RNAP3' and 'other antibodies' was considered for the present study. Antibodies were tested according to local procedures at each centre. In all centres, indirect immunofluorescence staining using human epithelial type 2 cells was used to test and confirm ANA positivity and to determine ANA patterns.

'Triple-negative patients' were defined as patients with SSc with a definite diagnosis, according to classification criteria and registry data, who had any ANA status (either ANA positivity, regardless of pattern or ANA negativity) and lacked ACA, ATA and RNAP3 antibodies. An additional group of patients with SSc named as 'quadruple negative' was considered in the presence of ANA negativity or ANA positivity with any other ANA pattern different from the nucleolar pattern.

### Statistical analysis

Demographic and disease-related features were analysed through descriptive statistical results and were presented as number and percentage for categorical variables, otherwise as mean (SD) or median (IQR) according to normal or non-normal distribution. Cross-sectional comparisons between triple-negative patients with SSc and other patients with SSc, as well as between quadruple-negative patients with SSc and other patients with SSc were conducted using the $\chi^2$ test or Fisher's exact test for categorical variables, and the Mann-Whitney U test or t-test for continuous variables, contingent on the data distribution and variance homogeneity assessed by Levene's test. Statistical significance was defined as a p value <0.05 for all analyses, and all tests were two-tailed.

Multiple bias mitigation strategies were adopted to enhance the validity and reliability of this retrospective multicentre study. First, uniform methods and tools for retrospective data collection across all study centres and patients were implemented to mitigate information bias according to SPRING research protocol. Data analysis was performed using SPSS V.28.0 (SPSS, Chicago, Illinois, USA).

## RESULTS

### Demographic and clinical characteristics

We included in the study 1480 patients with SSc enrolled in the SPRING registry with diagnosis of SSc who satisfied the 2013 EULAR/ACR classification criteria and with available data on immunological profile. Patients were enrolled among the 38 participating centres of the SPRING-SIR project, as represented in figure 1.

Considering the entire cohort, the majority of patients were females (88.4%), with a mean age of 59±17 years and limited cutaneous disease in 67.1% of cases. Overall, gastrointestinal involvement was reported in 56.7% of patients, followed by interstitial lung disease (ILD) (32.4%), while 21.7% had a history of digital ulcers and 11.3% had arthritis; in the same percentage of patients, the presence of muscle weakness and increased CPK, suggestive of myopathy, was reported. PAH and renal crisis were only rarely reported.

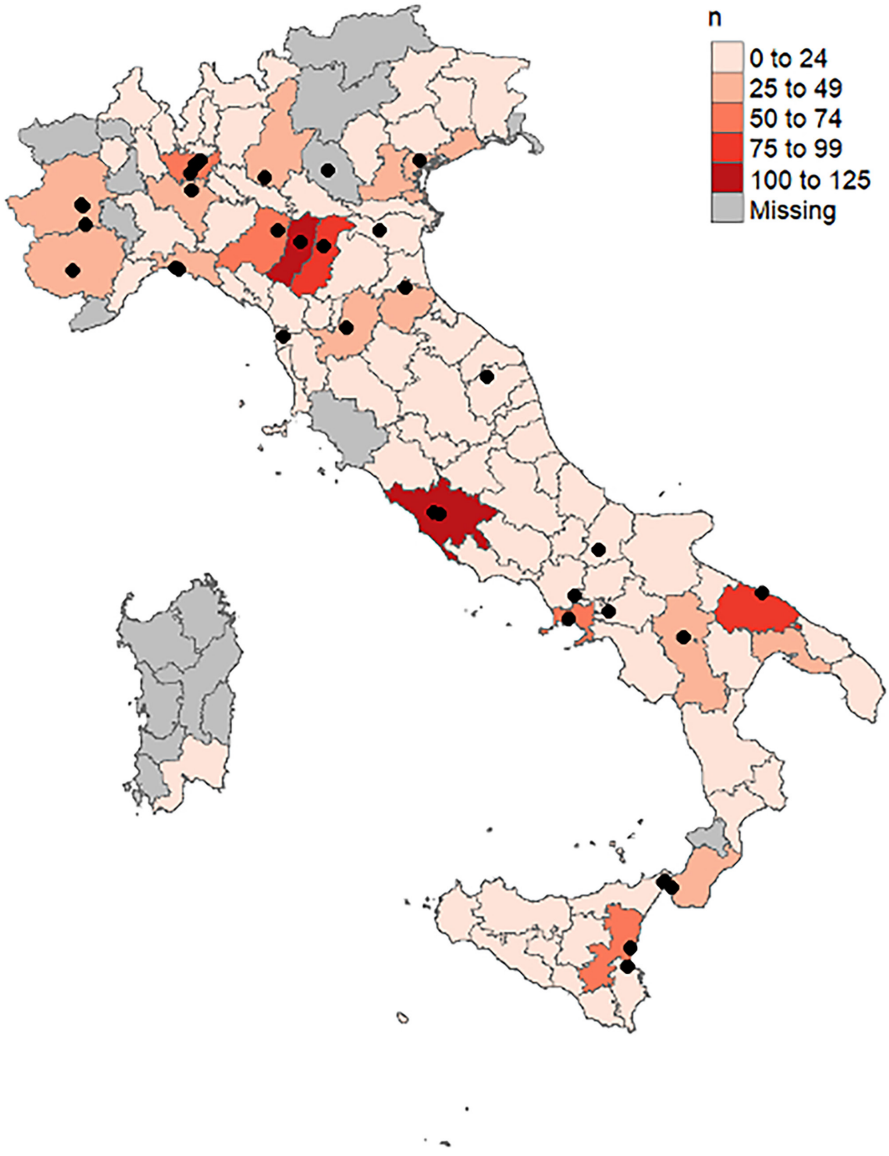


**Figure 1** Geographical distribution of the patients with systemic sclerosis (SSc) enrolled in the study. The black dots indicate the sites, that is, the Italian referral centres for SSc participating in the Systemic sclerosis Progression INvestiGation-Italian Society for Rheumatology project.

### Autoantibody profile and clinical features

Considering autoantibody profile, out of 1480 patients with SSc, 295 (19.9%) were triple-negative, while 1185 had SSc-specific antibodies, chiefly: ACA-positive (54.3%), defined as either a centromeric ANA pattern or human centromere protein positivity, ATA-positive (43.6%) and RNAP3-positive (2.1%) (table 1). Among ATA-positive patients, 21 out of 517 (4.1%) were also ACA-positive; however, these were classified as ATA-positive for the purpose of this analysis.

In the overall cohort (n=1426), triple-negative patients were more prevalent in Central Italy (24.5%) and Southern Italy (22.1%) compared with Northern Italy (16.7%) (p=0.004). ACA were most frequent in Northern Italy (47.2%), followed by Central Italy (43.5%) and Southern Italy (38.3%) (p=0.003). ATA showed a complementary distribution, being more common in the South (38.3%) than in the North (34.4%) and less frequent in the Centre (30.0%). RNAP3 antibodies positivity was rare across all geographical areas, ranging from 1.4% in the South to 2.0% in the Centre and 1.8% in the North (online supplemental table S1).

No significant differences were observed in comorbidities between the two groups, including the prevalence of traditional cardiovascular comorbidities (such as arterial hypertension, obesity, coronary artery disease, diabetes and dyslipidaemia) and neoplasms (table 1, figure 2A).

The ANA pattern was significantly different between groups, with triple-negative patients showing more frequent nucleolar patterns and speckled patterns (29.2% vs 13.1%, and 38.6% vs 12.5%, respectively; p=0.001 for both). Anti-Ro/SSA and anti-La/SSB antibodies were more frequently detected in triple-negative patients compared with non-triple-negative ones (14.6% vs 8.7% and 3.1% vs 1.2%, respectively; p=0.01; p=0.04, respectively).

**Table 1** Demographic and comorbidity characteristics of patients with SSc

| | Triple negative (n=295) | Non-triple negative (n=1185) | P value |
|---|---|---|---|
| Demographic characteristics | | | |
| Female, n (%) | 239 (81.0) | 1069 (90.2) | **<0.001** |
| Male, n (%) | 56 (19.0) | 116 (9.8) | **<0.001** |
| Age (years), mean±SD | 57.23±12.93 | 59.42±17.8 | **0.048** |
| Disease duration (years), mean±SD | 8.59±8.20 | 8.57±8.0 | 0.959 |
| Autoantibodies pattern | | | |
| ANA positivity, n (%) | 266 (90.2) | 1179 (99.3) | **<0.001** |
| Homogeneous pattern, n (%) | 54 (18.3) | 281 (23.7) | 0.052 |
| Speckled pattern, n (%) | 114 (36.8) | 148 (12.5) | **0.001** |
| Nucleolar pattern, n (%) | 86 (29.2) | 155 (13.1) | **0.001** |
| Centromeric, n (%) | 0 (0) | 615 (51.0) | **<0.001** |
| Anticentromere positivity, n (%) | 0 (0) | 644 (54.4) | – |
| Anti-SCL70 positivity, n (%) | 0 (0) | 517 (43.6) | – |
| Anti-RNA POL III positivity, n (%) | 0 (0) | 24 (2.1) | – |
| Anti-SSA/Ro positivity, n (%) | 43 (14.6) | 103 (8.7) | **0.01** |
| Anti-SSB/La positivity, n (%) | 9 (3.1) | 14 (1.2) | **0.04** |
| Disease subtype | | | |
| Sine scleroderma, n (%) | 35 (11.9) | 126 (10.9) | 0.603 |
| Limited cutaneous subset, n (%) | 199 (67.9) | 794 (68.4) | 0.888 |
| Diffuse cutaneous subset, n (%) | 59 (20.1) | 241 (20.8) | 0.872 |
| Comorbidities | | | |
| BMI, mean±SD | 23.5±3.3 | 24.2±4.6 | **0.030** |
| Heart failure, n (%) | 7 (2.4) | 33 (2.8) | 0.823 |
| Stroke, n (%) | 3 (1.0) | 10 (0.8) | 0.731 |
| Peripheral vascular disease, n (%) | 16 (5.4) | 60 (5.1) | 0.769 |
| Cerebrovascular disease, n (%) | 3 (1.0) | 24 (2.0) | 0.333 |
| Arterial hypertension, n (%) | 69 (23.4) | 285 (24.1) | 0.879 |
| Myocardial infarction, n (%) | 7 (2.4) | 33 (2.8) | 0.842 |
| Diabetes, n (%) | 6 (2.0) | 38 (3.2) | 0.343 |
| Dyslipidaemia, n (%) | 22 (7.5) | 132 (11.1) | 0.070 |
| Neoplasia, n (%) | 28 (9.5) | 82 (6.9) | 0.137 |
| Leukaemia, n (%) | 0 (0) | 2 (0.2) | 1.000 |
| Lymphoma, n (%) | 1 (0.3) | 5 (0.4) | 1.000 |

Bold indicates statistically significant results.
ANA, antinuclear antibodies; BMI, body mass index; RNA POL III, anti-RNA-polymerase III antibodies.

The triple-negative group showed a higher prevalence of myopathy compared with all other patients with SSc (16.7% vs 10.1%, p=0.003), inferred from the presence of muscle weakness and total CPK levels (126.2 vs 92.5 U/mL, p=0.002). The triple-negative patients, indeed, showed higher proportions of significant CPK elevations over 2–3 times the normal values (2.4% vs 0.2%, p=0.028) and over 3 times the normal values (3.2% vs 0.6%, p=0.035) (table 2, figure 2B).

Triple-negative patients also exhibited fewer vascular complications, such as digital ulcers (17.3% vs 22.8%, p=0.04) and calcinosis (8.2% vs 12.8%, p=0.027) in comparison with the positive group (table 2). Although the proportion of patients classified as having abnormal HRCT scans was identical between triple-negative and other patients with SSc, ILD-specific HRCT features were significantly more frequent in triple-negative patients with SSc, particularly reticulations (19.3% vs 14.3%, p=0.038) and honeycombing (10.2% vs 4.0%, p<0.001). Consistently, functional data showed that triple-negative patients had lower (%predicted) values of DLCO (66.4% vs 70.98%, p=0.004) and forced vital

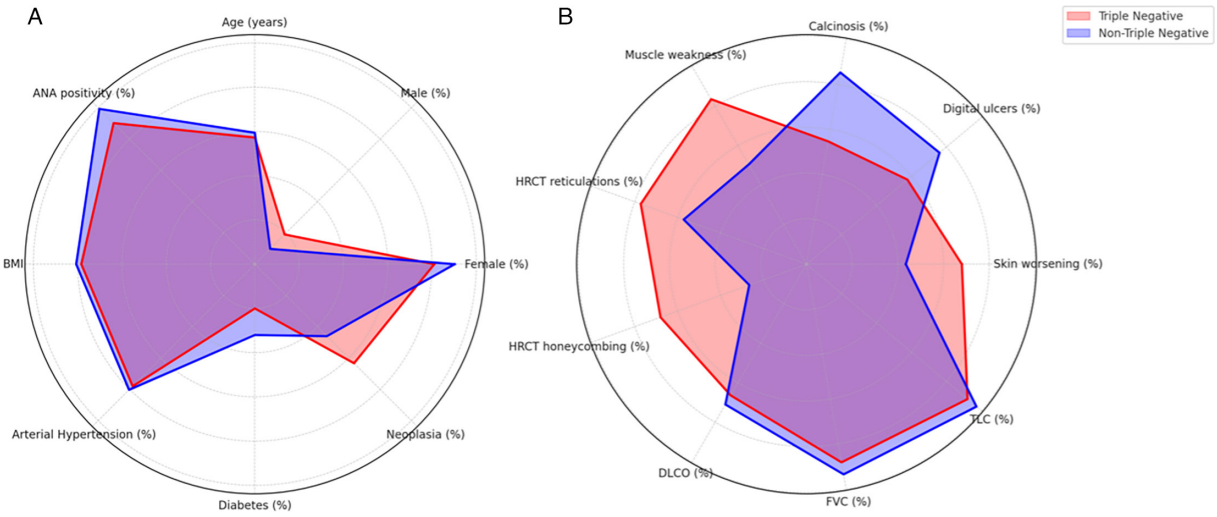


**Figure 2** Comparison of demographics, autoantibodies, comorbidities (A) and disease involvement (B) between triple-negative and non-triple-negative patients with systemic sclerosis. Panel A illustrates differences in age, sex, BMI, autoantibody profile and selected comorbidities. Panel B shows organ involvement and disease severity. Values are plotted on a uniform scale (0%–100%) for each variable to allow visual comparison across different clinical domains, regardless of their original measurement units. Each axis has been rescaled individually based on the minimum and maximum observed values for that specific variable across both groups. This approach enhances visual interpretability of relative group differences but does not reflect absolute values. ANA, antinuclear antibodies; BMI, body mass index; DLCO, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity; HRCT, high-resolution CT; TLC, total lung capacity.

capacity (FVC) (97.01% vs 102.92%, p<0.001) at PFTs (table 2, figure 2B).

Significant treatment differences among groups were also observed: triple-negative patients more frequently received corticosteroids (79.3% vs 67.9%, p=0.003), cyclophosphamide (43.4% vs 26%, p<0.001), hydroxychloroquine (58.8% vs 47.8%, p=0.03) and azathioprine (38.5% vs 22.3%, p=0.002) in comparison with all other patients with SSc. Conversely, among triple-negative patients, the use of prostanoids (71.6% vs 85.9%, p<0.001), calcium channel blockers (80.1% vs 87.7%, p=0.005) and PDE5 inhibitors (4% vs 20%, p<0.001) was less frequent, and consistent with a lower vascular involvement (table 2).

No significant differences among groups emerged regarding the remaining clinical and functional characteristics.

### Clinical features of quadruple autoantibody-negative patients with SSc

We then considered the group of patients with ACA, ATA and RNAP3 negativity and with ANA negative or any other ANA pattern different from the nucleolar one, namely 'quadruple negative'. We identified 209 such patients (14.1%). As with triple-negative patients, quadruple-negative patients more frequently had elevated CPK levels (suggestive of myopathy) and ILD on HRCT of the lungs. Specifically, these patients had significantly more reticulations (23% vs 14%, p=0.002) and a higher prevalence of honeycombing (12% vs 4%, p<0.001) on HRCT of the chest. Consistently, quadruple-negative patients also exhibited a more impaired FVC (97.10% vs 102.48%, p=0.009), DLCO (66% vs 70.7%, p=0.017) and TLC (90.4% vs 96.9%, p=0.012). Consistently, these patients more frequently received corticosteroids (80% vs 68%, p=0.004) and cyclophosphamide (46% vs 27%, p<0.001). Conversely, no differences in vascular burden in terms of digital ulcers were observed between quadruple-negative patients and all other patients with SSc (online supplemental table S2).

## DISCUSSION

In this multicentre study, we aimed to describe the unique clinical features of patients with SSc with ANA positivity who were negative for the prototypic SSc autoantibodies (ACA, ATA and RNAP3), namely the *triple-negative* patients with SSc. We also included an additional subgroup of patients defined as quadruple-negative, characterised by the absence of SSc-specific autoantibodies and either ANA negativity or ANA positivity with any pattern except nucleolar, which was the only ANA pattern considered indicative of SSc-specific reactivity.

We observed a high proportion of triple-negative patients, as almost 20% of patients included in the SPRING registry and clinically diagnosed with SSc tested negative for ACA, ATA and RNAP3.

Importantly, our data suggest that triple negativity identifies a further subset of patients with SSc with a definite diagnosis characterised by clinical features different from those of patients with SSc positive for at least one disease-specific autoantibody. Indeed, a higher prevalence of myopathy and reduced vascular complications was found in the triple-negative patients. These patients also presented a higher prevalence of ILD, as evidenced by more frequent reticulations and honeycombing at

**Table 2** Clinical characteristics of triple-negative patients with systemic sclerosis compared with patients with prototypical disease-specific antibodies

| Disease characteristics | Triple negative | | Non-triple negative | | P value |
|---|---|---|---|---|---|
| | N | | N | | |
| Skin | | | | | |
| Skin worsening in the month before enrolment, n (%) | 295 | 40 (13.6) | 1179 | 134 (8.7) | 0.313 |
| Telangiectasias, n (%) | 295 | 171 (58) | 1183 | 675 (57.1) | 0.793 |
| Sclerodactyly, n (%) | 295 | 221 (74.9) | 1181 | 829 (77.2) | 0.114 |
| mRSS, mean±SD | 252 | 6.5±7.0 | 1096 | 6.3±6.7 | 0.561 |
| Vascular | | | | | |
| Scleroderma pattern absent, n (%) | 290 | 18 (6.2) | 1162 | 58 (5.0) | 0.380 |
| Scleroderma pattern early, n (%) | 290 | 71 (24.5) | 1162 | 230 (19.8) | 0.089 |
| Scleroderma pattern active, n (%) | 290 | 118 (40.7) | 1162 | 509 (43.8) | 0.354 |
| Scleroderma pattern late, n (%) | 290 | 67 (23.1) | 1162 | 271 (23.3) | 1.000 |
| Puffy fingers, n (%) | 294 | 144 (49.0) | 1180 | 608 (51.5) | 0.473 |
| Digital ulcers, n (%) | 295 | 51 (17.3) | 1182 | 270 (22.8) | **0.040** |
| Pitting scars, n (%) | 295 | 124 (42.0) | 1179 | 544 (46.1) | 0.214 |
| Calcinosis, n (%) | 294 | 24 (8.2) | 1180 | 151 (12.8) | **0.027** |
| Gangrene, n (%) | 295 | 2 (0.7) | 1178 | 8 (0.7) | 1.000 |
| Muscular | | | | | |
| Muscle weakness, n (%) | 294 | 49 (16.7) | 1179 | 119 (10.1) | **0.003** |
| Muscle atrophy, n (%) | 294 | 10 (3.4) | 1179 | 41 (3.5) | 1.000 |
| CPK (U/mL), mean±SD | 124 | 126.2±174.1 | 483 | 92.5±83.9 | **0.002** |
| CPK normal, n (%) | 124 | 108 (87.1) | 483 | 443 (91.7) | 0.119 |
| CPK 1–2×nv, n (%) | 124 | 9 (7.3) | 483 | 36 (7.5) | 1.000 |
| CPK 2–3×nv, n (%) | 124 | 3 (2.4) | 483 | 1 (0.2) | **0.028** |
| CPK >3×nv, n (%) | 124 | 4 (3.2) | 483 | 3 (0.6) | **0.035** |
| Articular | | | | | |
| Synovitis, n (%) | 295 | 17 (5.8) | 1180 | 74 (6.3) | 0.892 |
| Clinical arthritis, n (%) | 294 | 40 (13.6) | 1172 | 126 (10.8) | 0.181 |
| Gastrointestinal | | | | | |
| Oesophageal symptoms, n (%) | 295 | 148 (50.2) | 1181 | 593 (50.2) | 1.000 |
| Gastric symptoms, n (%) | 295 | 44 (14.9) | 1178 | 244 (20.7) | **0.026** |
| Intestinal symptoms, n (%) | 295 | 53 (18.0) | 1179 | 230 (19.5) | 0.620 |
| ILD | | | | | |
| HRCT normal, n (%) | 295 | 65 (22.0) | 1185 | 261 (22.0) | 1.000 |

Continued

**Table 2** Continued

| Disease characteristics | Triple negative | | Non-triple negative | | P value |
|---|---|---|---|---|---|
| | N | | N | | |
| HRCT ground glass, n (%) | 295 | 58 (19.7) | 1185 | 215 (18.1) | 0.557 |
| HRCT reticulations, n (%) | 295 | 57 (19.3) | 1185 | 170 (14.3) | **0.038** |
| HRCT honeycombing, n (%) | 295 | 30 (10.2) | 1185 | 47 (4.0) | **<0.001** |
| Dyspnoea, n (%) | 293 | 107 (36.5) | 1175 | 410 (34.6) | 0.632 |
| DLCO (%), mean±SD | 201 | 66.4 (20.4) | 829 | 71.0 (20.3) | **0.004** |
| DLCO/alveolar volume (%), mean±SD | 171 | 76.9 (21.0) | 675 | 77.5 (18.8) | 0.710 |
| FVC (%), mean±SD | 221 | 97.0 (22.5) | 865 | 102.9 (22.9) | **<0.001** |
| $FEV_1$ (%), mean±SD | 198 | 94.2±21.4 | 792 | 97.9±21.4 | **0.029** |
| TLC, mean±SD | 109 | 92.0±19.0 | 450 | 97.1±19.0 | **0.018** |
| RV, mean±SD | 67 | 91.8±28.9 | 259 | 97.5±29.5 | 0.162 |
| Cardiopulmonary involvement | | | | | |
| Cardiopulmonary symptoms, n (%) | 294 | 88 (29.9) | 1179 | 315 (26.7) | 0.273 |
| Abnormal diastolic function, n (%) | 239 | 57 (23.8) | 938 | 206 (22.0) | 0.543 |
| Arrhythmias, n (%) | 156 | 8 (5.1) | 649 | 30 (4.6) | 0.833 |
| Bi/trigeminal rhythm, n (%) | 154 | 4 (2.6) | 645 | 9 (1.4) | 0.290 |
| PAH confirmed at RHC, n (%) | 295 | 6 (2.0) | 1185 | 15 (1.2) | 0.400 |
| ProBNP (ng/mL), mean±SD | 43 | 82.3±68.5 | 162 | 2986.2±34767.7 | 0.585 |
| NT-proBNP (ng/mL), mean±SD | 47 | 275.7±565.1 | 191 | 446.7±1616.0 | 0.476 |
| sPAP (mm Hg), mean±SD | 240 | 23.0±17.2 | 967 | 23.4±15.8 | 0.747 |
| Ejection fraction, mean±SD | 230 | 61.3±6.8 | 916 | 61.5±5.6 | 0.696 |
| Others | | | | | |
| Sicca syndrome, n (%) | 295 | 72 (24.4) | 1181 | 339 (28.7) | 0.147 |
| Renal crisis, n (%) | 295 | 6 (2.0) | 1179 | 9 (0.8) | 0.095 |
| Creatinine (mg/dL), mean±SD | 268 | 1.3±6.6 | 1070 | 1.0±3.9 | 0.403 |
| ESR (mm/1 hour), mean±SD | 243 | 20.3±19.4 | 1011 | 20.4±16.7 | 0.939 |
| C reactive protein (mg/L), mean±SD | 270 | 2.7±4.6 | 1068 | 3.0±12.7 | 0.747 |

Bold indicates statistically significant results.

lung HRCT. Consistently, functional data at PFTs showed a more impaired DLCO and FVC, suggesting a more severe ILD.

These unique clinical features, resembling sclero-myositis, could be partially responsible for different treatment strategies observed in this cohort. Triple-negative patients, indeed, more frequently received oral corticosteroids, cyclophosphamide and azathioprine in comparison with all other patients with SSc. Conversely, the use of prostacyclin, calcium channel blockers and PDE5 inhibitors was less frequent, and in keeping with the less severe vascular burden in this subgroup of patients. The same clinical features were confirmed in the quadruple-negative patients, apart from the prevalence of vascular complications.

Importantly, no significant differences among the groups were observed in gastrointestinal, renal or cardiac involvement, capillaroscopy patterns or inflammatory markers.

To the best of our knowledge, this is the largest study assessing the prevalence of triple-negative patients with SSc in the Italian population, analysing data from a large multicentre cohort of patients with SSc involving 38 Italian referral centres. Moreover, it is the first study also reporting the distinctive clinical features of quadruple-negative patients with SSc.

Depicting the clinical features of patients with a definite SSc diagnosis but lacking the three classical antibodies is of pivotal importance in clinical practice. In fact, almost all SSc case series and cohort studies have extensively characterised patients with ACA, ATA and RNAP3, providing invaluable insights into the clinical and prognostic significance of these prototypic SSc-related antibodies.[15–19] However, only a few studies have described the prevalence and clinical features of patients with SSc who lack these three prototypic antibodies, with conflicting results.[4 7 8 20–22] Within the European Scleroderma Trials and Research group cohort, Elhai *et al* primarily focused on the predictive performance of an autoantibody-based prognostic model for SSc survival but a characterisation of clinical features or organ involvement in triple-negative patients was beyond the scope of the study, and therefore not reported.[20]

Miyake *et al* identified only 5.3% of 'ANA-positive triple-negative' patients with SSc and mostly with concomitant ILD (62% of cases) and medium-to-high extent of skin fibrosis (median mRSS of 13.0); conversely, PAH was detected in only 3% of cases.[7]

Coherently with our results, a higher prevalence of ILD and the increased risk for deterioration in lung function in triple-negative patients with SSc has also been reported by Liu *et al*.[23] Accordingly, post hoc analyses from the Safety and Efficacy of Nintedanib in Systemic Sclerosis trial showed that patients with SSc-ILD negative for ACA, RNAP3 and ATA experienced a numerically greater decline in FVC compared with the overall trial population, indirectly suggesting a greater degree of pulmonary involvement in this serological subset.[21]

A lower percentage of ANA-positive and Extractable Nuclear Antigen-negative patients (1.8%) emerged in a previous study by Hudson *et al* in 874 patients; moreover, triple-negative patients had a surprisingly lower prevalence of ILD (12.5%) in this cohort.[8]

Compared with these studies from Japan and Canada, the prevalence of patients with SSc who were ANA-positive triple negative was significantly higher (14%) in a recent multicentre US cohort in agreement with our findings. Similar clinical features with respect to previous studies were reported, with 60% of patients with ILD and mild skin involvement.[4] In line with our hypothesis, the US cohort showed an elevated proportion of myositis-related antibodies: anti-RO52 autoantibodies (50%), myositis-specific antibodies (MSA, 32.5%) and myositis-associated antibodies (MAA, 30%). Among the latter, MDA5 and SAE1 were the most common, whereas Mi-2b and PM-75 were less frequent.[24] Importantly, none of the patients with myositis-related antibodies fulfilled the EULAR/ACR classification criteria for inflammatory myopathy, suggesting that patients with myositis were not misclassified as having SSc in this study.[24]

Also data from the Polish cohort underline the limitations of routine serological testing: more than half of patients initially classified as triple-negative based on the absence of ATA, ACA and RNAP3 were subsequently found to be positive for additional autoantibodies on extended serological testing, thereby reducing the prevalence of truly triple-negative SSc from 36% to approximately 15%.

These findings confirm the relevance of the triple-negative cohort among patients with SSc. In the majority of the cohorts, they confirm our data showing an increased prevalence of ILD in this subgroup and emphasise the immunological heterogeneity underlying the triple-negative definition.[22] In this context, the SPRING registry did not include data on MSA, MAA and rare SSc-associated antibodies (SAA), including U11/U12 (RNPC3), Pm–Scl, U3RNP and U1RNP (classically associated with ILD and myositis), representing one of the major limitations of our study since these missing data clearly affect study results. This limitation could be at least partially explained by the enrolment and observation period of the study, during which these autoantibody tests were not yet widely available in routine clinical practice. Importantly, in the study by Kruzer *et al*, among the SAA group, Th/To (40%) and fibrillarin (25%) were the most prevalent, followed by NOR-90, RNP-A and RNP-C (7.5% each), but none was associated with specific clinical manifestations.[4] These results indirectly support our hypothesis that triple-negative patients with SSc represent a definite subgroup with specific clinical manifestations, beside the specific SAA positivity that could eventually be found (ie, Th/To, fibrillarin, Pm–Scl, U1RNP, U3RNP, Ku). This notion is of great clinical utility since the triple negativity could be used in daily clinical practice to identify an SSc endotype resembling sclero-myositis with concomitant ILD and less vascular complication, with a potential impact on therapeutic decision and monitoring

strategies. Certainly, the identification of rare SAA in triple-negative patients with SSc is highly recommended for a precise immunological (and potentially clinical) profiling of patients with SSc. However, triple negativity for ACA, ATA and RNAP3 could have a great clinical impact and could help to identify a distinct subset of patients with SSc. Similarly, quadruple negativity, that is, the absence even of ANA with nucleolar pattern, seems to be associated with a similar endotype of patients with SSc with myositis and ILD and less severe vascular burden.

Despite the novelty of our findings, several limitations must be acknowledged, in addition to the lack of data on rare myositis and SSA. The retrospective design of our study, indeed, may introduce biases related to data collection and patient selection. The reliance on registry data means that not all clinical variables were uniformly captured, which could affect the comprehensiveness of our analysis. Even though SPRING registry enrolled only patients with diagnosis of SSc in national referral centres, we cannot completely exclude that some patients with unrecognised overlap syndromes may be included in the study. However, biases derived from data collection likely had a limited impact on study results since all data were collected in SSc referral centres with great expertise in clinical management of patients and data collection.

The strengths and limitations of SSc registry-based studies have been previously analysed.[6 25] The differences in autoantibody distribution across Italian macro-areas, such as the higher prevalence of triple-negative patients in the Centre and South, may reflect true epidemiological variability, possibly influenced by genetic and environmental factors. However, they could also be partially explained by regional disparities in diagnostic sensitivity, testing availability and laboratory protocols. Furthermore, data were collected over almost 10 years, during which monitoring approaches and therapeutic strategies evolved, influencing the therapeutic and monitoring strategies of patients with SSc. To date, the SPRING registry was initiated after the 2013 ACR/EULAR classification criteria for SSc were proposed and widely accepted[5]; therefore, the diagnostic and classification approach is expected to be homogeneous throughout the study period and even among the 38 referral centres, as indirectly supported by previous collaborative studies of the SPRING-SIR project.[6 11 26 26]

## CONCLUSIONS

Our data suggest that triple negativity identifies a further SSc subset of patients characterised by clinical features different from those of patients positive for at least one SSc-specific autoantibody. A higher prevalence of myopathy and ILD, and reduced burden of vascular complications are frequently found in the triple-negative patients. This evidence may suggest that the non-specific and not commonly assessed autoantibodies may identify an SSc endotype resembling sclero-myositis, which was more frequently treated with steroids and cyclophosphamide. Similar findings were confirmed in the quadruple-negative subgroup.

In the next future, studies on larger triple-negative cohorts are warranted to better define the clinical and serological features of this SSc subset.

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## REFERENCES

1 Denton CP, Khanna D. Systemic sclerosis. *Lancet* 2017;390:1685–99.
2 Hamaguchi Y. Autoantibody profiles in systemic sclerosis: predictive value for clinical evaluation and prognosis. *J Dermatol* 2010;37:42–53.
3 Hamaguchi Y, Takehara K. Anti-nuclear autoantibodies in systemic sclerosis: News and perspectives. *J Scleroderma Relat Disord* 2018;3:201–13.
4 Kruzer K, Goncalves Marangoni R, Heckler I, *et al*. Clinical and Autoantibody Associations in Antinuclear Antibody-Positive Systemic Sclerosis Lacking Prototypic Autoantibodies. *J Clin Rheumatol* 2023;29:47–51.
5 van den Hoogen F, Khanna D, Fransen J, *et al*. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/ European league against rheumatism collaborative initiative. *Ann Rheum Dis* 2013;72:1747–55.
6 Ferri C, Giuggioli D, Guiducci S, *et al*. Systemic sclerosis Progression INvestiGation (SPRING) Italian registry: demographic and clinico-serological features of the scleroderma spectrum. *Clin Exp Rheumatol* 2020;38 Suppl 125:40–7.
7 Miyake M, Matsushita T, Takehara K, *et al*. Clinical features of Japanese systemic sclerosis (SSc) patients negative for SSc-related autoantibodies: A single-center retrospective study. *Int J of Rheum Dis* 2020;23:1219–25.
8 Hudson M, Satoh M, Chan JYF, *et al*. Prevalence and clinical profiles of "autoantibody-negative" systemic sclerosis subjects. *Clin Exp Rheumatol* 2014;32:S–127.
9 Ho KT, Reveille JD. The clinical relevance of autoantibodies in scleroderma. *Arthritis Res Ther* 2003;5:80–93.
10 LeRoy EC, Black C, Fleischmajer R, *et al*. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988;15:202–5.
11 Cutolo M, Herrick AL, Distler O, *et al*. Nailfold Videocapillaroscopic Features and Other Clinical Risk Factors for Digital Ulcers in Systemic Sclerosis: A Multicenter, Prospective Cohort Study. *Arthritis Rheumatol* 2016;68:2527–39.
12 Giuggioli D, Manfredi A, Lumetti F, *et al*. Scleroderma skin ulcers definition, classification and treatment strategies our experience and review of the literature. *Autoimmun Rev* 2018;17:155–64.
13 Humbert M, Kovacs G, Hoeper MM, *et al*. 2022 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Heart J* 2022;43:3618–731.
14 Orlandi M, Bellando-Randone S, De Angelis R, *et al*. Towards a comprehensive approach to the management and prognosis of systemic sclerosis's patients: The role of comorbidities in the SPRING-SIR registry. *Eur J Intern Med* 2024;130:130–6.

15 De Angelis R, Giuggioli D, Bajocchi G, *et al*. Sex-related Differences in Systemic Sclerosis: A Multicenter Cross-sectional Study From the National Registry of the Italian Society for Rheumatology. *J Rheumatol* 2022;49:176–85.
16 Ferri C, De Angelis R, Giuggioli D, *et al*. Geographical heterogeneity of clinical and serological phenotypes of systemic sclerosis observed at tertiary referral centres. The experience of the Italian SIR-SPRING registry and review of the world literature. *Autoimmun Rev* 2022;21:103159.
17 De Angelis R, Ferri C, Giuggioli D, *et al*. Systemic sclerosis sine scleroderma: clinical and serological features and relationship with other cutaneous subsets in a large series of patients from the national registry "SPRING" of the Italian Society for Rheumatology. *RMD Open* 2023;9:e002890.
18 Riccieri V, Pellegrino G, Cipolletta E, *et al*. Practice pattern for the use of intravenous iloprost for the treatment of peripheral vasculopathy in systemic sclerosis: A case-control study from the Italian national multicenter "SPRING" (Systemic Sclerosis Progression InvestiGation) Registry. *J Scleroderma Relat Disord* 2024;9:38–49.
19 De Luca G, De Lorenzis E, Campochiaro C, *et al*. Rituximab retention rate in systemic sclerosis: a long term real-life multicentre study. *Rheumatology (Oxford)* 2025;64:1284–91.
20 Elhai M, Sritharan N, Boubaya M, *et al*. Stratification in systemic sclerosis according to autoantibody status versus skin involvement: a study of the prospective EUSTAR cohort. *Lancet Rheumatol* 2022;4:785–94.
21 Volkmann ER, Assassi S, Denton CP, *et al*. Outcomes in Systemic Sclerosis-Associated Interstitial Lung Disease Based on Serological Profiles With a Focus on Anticentromere and Anti-RNA Polymerase III Antibodies. *J Rheumatol* 2025;52:914–8.
22 Żebryk P, Przymuszała P, Nowak JK, *et al*. Autoantibodies and Clinical Correlations in Polish Systemic Sclerosis Patients: A Cross-Sectional Study. *J Clin Med* 2023;12:657.
23 Liu S, Chung MP, Ley B, *et al*. Peripheral blood leucocyte telomere length is associated with progression of interstitial lung disease in systemic sclerosis. *Thorax* 2021;76:1186–92.
24 Leurs A, Dubucquoi S, Machuron F, *et al*. Extended myositis-specific and -associated antibodies profile in systemic sclerosis: A cross-sectional study. *Joint Bone Spine* 2021;88:105048.
25 Galluccio F, Walker UA, Nihtyanova S, *et al*. Registries in systemic sclerosis: a worldwide experience. *Rheumatology (Oxford)* 2011;50:60–8.
26 Cacciapaglia F, De Angelis R, Ferri C, *et al*. Pulmonary Arterial Hypertension Incidence in Patients With Systemic Sclerosis Treated With Bosentan for Digital Ulcers: Evidence From the SPRING-SIR Registry. *J Rheumatol* 2025;52:375–82.