Systemic sclerosis patients with negative antinuclear antibodies have distinctive clinical manifestations: a multicenter CRDC cohort in China

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Abstract

Background: The presence of circulating antinuclear antibodies (ANAs) is a hallmark of immune dysregulation in patients with systemic sclerosis (SSc).

Objective: A variety of ANAs are associated with unique sets of disease manifestations and are widely used in clinical practice in SSc. This study aimed to investigate the clinical features of SSc patients negative for ANAs in a Chinese Rheumatism Data Center (CRDC) multicenter cohort in China.

Methods: Patients were prospectively recruited between April 2008 and June 2019 from 154 clinical centers nationwide, and all cases fulfilled the 2013 ACR/EULAR classification criteria for systemic sclerosis. Results for antinuclear antibodies were intensively collected. Demographic, clinical, and laboratory data were compared between ANA-positive SSc patients and those negative for ANAs.

Results: Antinuclear antibodies were detected in 2129 of 2809 patients enrolled in the study; 4.2% of patients were negative. There were more males among ANA-negative SSc patients (29.6% vs. 29.4%, p = 0.001). The incidence of certain critical organ involvement, including gastroesophageal reflux (5.6% vs. 18.5%, p = 0.002), interstitial lung disease (65.2% vs. 77.9%, p = 0.015), and pulmonary arterial hypertension (11.5% vs. 29.0%, p = 0.006) was significantly lower in ANA-negative patients than in ANA-positive patients. The proportion of abnormal erythrocyte sedimentation rate (32.4% vs. 47.6%, p = 0.013) and IgG elevation (14.3% vs. 37.0%, p = 0.003), an indicator of disease activity, was significantly lower in ANA-negative patients than in ANA-positive patients.

Conclusion: Antinuclear antibodies are strongly associated with the clinical manifestations of systemic sclerosis, with ANA-negative SSc patients tending to exhibit relatively milder disease.

Keywords
Autoantibody · Scleroderma · Disease phenotype · Prospective registry study · Organ involvement
Introduction

Systemic sclerosis (SSc) is a chronic connective tissue disorder that is characterized by microvascular injury and dysregulation of the immune system that consequently leads to atrophy, fibrosis, and vascular obliteration of the skin and multiple internal organs. The incidence of systemic sclerosis is between 18 and 20 individuals per million of the population per year, and the prevalence ranges from 0.1 to 13.8 per 100,000 [1].

With the help of modern standardized assays, autoantibodies can be detected in approximately 90–95% of SSc subjects [2]. Indeed, as a marker of immunological dysregulation, the presence of circulating autoantibodies is one of the hallmarks and prominent early features of systemic sclerosis. The occurrence of different types of antinuclear antibodies (ANAs) is usually disease specific and mutually exclusive, correlating with particular manifestations, unique syndromes, distinct disease subtypes, disease activity, and prognosis. The most specific ANAs are anti-topoisomerase I (anti-Scl70) antibodies, anti-centromere antibodies (ACAs), and anti-RNA polymerase III antibodies. The occurrence of anti-Scl-70 antibodies is a marker of more extensive skin fibrosis and clinically significant pulmonary fibrosis, which predicts a poor prognosis. Conversely, anti-centromere antibodies are typically associated with localized cutaneous systemic sclerosis (lcSSc), infrequent lung, heart, and kidney involvement, and late onset of pulmonary arterial hypertension (PAH), representing an overall good prognosis [3].

Based on the fact that autoantibodies are usually produced prior to the onset of clinical manifestations and play important role in pathogenesis, ANAs have been studied in considerable detail and extensively used in clinical practice for diagnosis, clinical subgrouping, and prediction of future organ involvement and prognosis in systemic sclerosis [4, 5]. Although the typical clinical presentations of the different subsets of ANA-positive patients have been extensively explored, approximately 5–10% of SSc subjects have been reported to be ANA negative. Whether the pathogenesis, as well as the demographic and clinical characteristics, of ANA-negative SSc patients differ from ANA-positive patients is still unknown.

This study aimed to investigate the clinical features of ANA-negative subjects in a Chinese Rheumatism Data Center (CRDC) multicenter cohort in China by determining their demographic and clinical differences compared to ANA-positive patients. We sought to demonstrate a relationship between ANA profiles and clinical manifestations, organ involvement, and laboratory parameters to facilitate a strategy of risk stratification.

Materials and methods

Study population

Systemic sclerosis patients prospectively recruited based on a CRDC multicenter cohort from 154 clinical centers nationwide between April 2008 and June 2019 were included in this study. Authors had access to information that could identify individual participants during or after data collection. The diagnosis of SSc was performed according to the 2013 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria [6]. Systemic sclerosis (SSc) overlap syndrome was defined as SSc with another one or more coexisting connective tissue diseases, which were also included in the study population. All patients were classified into two subsets, including limited cutaneous systemic sclerosis (lcSSc) and diffuse cutaneous systemic sclerosis (dcSSc), with systemic sclerosis sine scleroderma (SSSc) considered to be lcSSc. The pattern of skin involvement, history of underlying diseases, chemical exposures, and patient demographics are helpful factors in differential diagnosis of scleroderma mimics, including morphea, scleredema, diabetic cheiroarthropathy, scleromyxedema, nephrogenic systemic fibrosis, and eosiophilic fasciitis. Demographic, clinical, and laboratory data were collected prospectively using a standard form at the time of entry. Ethics committee approval was obtained for the CRDC study under number S-478. Written informed consent was obtained from each patient before enrollment.

Clinical features

Disease duration was defined as the interval between the onset of the first non-Raynaud phenomenon symptoms and entry. The occurrence of organ involvement was assessed both at the initial visit and during regular follow-up according to the following criteria: (1) calcinosis: subcutaneous calcifications observed either by physical examination or radiography; (2) telangiectasia: visible dilated superficial blood vessels, with collapse upon pressure and slowly refilling when released; (3) digital ulcers/pits: ulcers or scars distal to or at the proximal interphalangeal joint; (4) gastrointestinal involvement: history of esophageal reflux, diarrhea, or constipation; (5) interstitial lung disease (ILD): imaging changes consistent with scleroderma-related fibrosis, including honeycombing, increased interstitial markings or ground glass opacity on high-resolution computed tomographic (HRCT) scans of the chest; (6) PH: the mean pulmonary arterial pressure (mPAP) ≥ 25 mm Hg at rest; pulmonary artery wedge pressure (PAWP) ≤ 15 mm Hg; and pulmonary vascular resistance (PVR) > 3 Wood units in right heart catheterization (RHC) assessment based on the 2015 European Society of Cardiology/European Respiratory Society guidelines [7], or an estimated systolic pulmonary artery pressure (SPAP) ≥ 45 mm Hg on echocardiography when RHC was not available; (7) cardiac involvement: defined when any rhythm disturbances, right or left ventricular dysfunction, congestive heart failure, or pericardial effusion was detected in cardiogram (ECG) and echocardiography; (8) renal crisis: malignant hypertension and/or rapidly progressive renal insufficiency and/or microangiopathic hemolytic anemia.

Antinuclear antibody analysis

Antinuclear antibodies (ANAs) were investigated in all patients, and indirect immunofluorescence (IIF) was performed with serial dilutions ranging from 1:40 to 1:1280 using HEp-2 substrate that included fluorescein-conjugated goat antibodies to human IgG. All ANA titers and IIF patterns were determined by two independent
experienced investigators, and a titer of >1:80 dilution was considered positive. Anti-centromere antibodies (ACA) were determined based on the pattern of immunofluorescence staining of HEp-2 cells, while both CENP-A and CENP-B were detected by a line immunoassay. Anti-Scl70, anti-Sm, anti-RNP, anti-SSA (anti-Ro60), anti-SSB (anti-La), anti-PM-Scl, and anti-Jo-1 antibodies were determined by the line blot or chemiluminescence method. Antibodies to RNA polymerase III were detected by enzyme-linked immunosorbent assay (ELISA). Since the technique and assays have been developed in recent years in our country, anti-RNA polymerase III antibodies could be detected in only a few centers. Since ANA should also be positive when anti-RNA polymerase III antibodies are present, this definition excludes SSc patients with anti-RNA polymerase III antibody positivity. Multicenter detection of antibodies had been conducted locally, but consistency was ensured by strict quality control.

Statistical analysis

Variables were tested to determine whether they had a normal distribution using the Shapiro–Wilks test and the Kolmogorov–Smirnov test. Continuous variables are expressed as mean ± standard deviation, nonnormally distributed data as median (range), and were compared between groups using Student’s t-test or the nonparametric Mann–Whitney U test. Categorical variables are presented as numbers and percentages (%); clinically relevant differences were evaluated by the chi-squared or Fisher’s exact test, as appropriate. Univariate and multivariate logistic regression analyses adjusting for potential confounders, specifically age at enrollment, disease duration, disease type (limited or diffuse cutaneous disease), and sex, were performed to identify whether the presence of ANAs is a potential independent risk factor for specific clinical manifestations or organ involvement. The statistical analyses were performed using SPSS statistics version 24.0 (IBM, Armonk, NY, USA). A p-value < 0.05 was considered statistically significant.

Results

Demographic characteristics

In total, 2809 patients were prospectively recruited from 154 clinical centers, covering 29 provinces, based on the CRDC database. Antinuclear antibodies were tested in 2129 patients, of whom 89 (4.2%) were ANA negative (there was only a single case with ANA negative and anti-SSA antibody weekly positive, and this does not change the results). The demographic characteristics of patients positive or negative for ANA are shown in Table 1. The mean age at disease onset in ANA-negative patients was significantly younger than that in the ANA-positive group (34.6 ± 11.9 years vs. 41.4 ± 13.0 years, p < 0.001). There were more males among ANA-negative SSc patients (32.6% vs. 14.4%, p < 0.001). No significant difference in the median duration from disease onset to the confirmed diagnosis was observed between the two groups, while the median disease duration was significantly longer in the ANA-negative group (63.0 months vs. 43.0 months, p = 0.026). Of the 89 ANA-negative SSc patients, 37.1% were classified as having dcSSc, whereas a significantly greater proportion of ANA-positive patients (48.5%) had dcSSc (p = 0.035). In addition, 6.7% of ANA-negative SSc patients and 9.0% of ANA-positive patients had an overlap syndrome, but no notable differences were found.

Antibody profiles

The clinical autoantibody profile of the patients included in this study is shown in Table 2. Overall, ANAs were detected in 95.8% of SSc patients. A total of 97.4% of lcSSc patients and 94.8% dcSSc were ANA positive. Among the three disease-specific autoantibodies, anti-Scl-70 was observed in 46.4% (947/2041) of patients, exhibiting the most significant positivity, followed by an ACA prevalence of 20.0% (378/1890). Anti-RNA polymerase III antibody was the rarest, at only 10.0% (45/448) of SSc patients.

Clinical features

The comparison of clinical features in SSc patients positive or negative for ANAs is shown in Table 3. The presence of Raynaud’s phenomenon tended to be less common (71.8% vs. 99.8%, p < 0.001) in the ANA-negative patients. ANA-negative patients experienced markedly fewer digital pitting (18.0% vs. 27.6%, p = 0.047) than ANA-positive patients. Some critical organ involvement, including gastroesophageal reflux (5.6% vs. 18.5%, p = 0.002), interstitial lung disease (65.2% vs. 77.9%, p = 0.015), and pulmonary arterial hypertension (11.5% vs. 29.0%, p = 0.006), occurred at significantly lower rates in ANA-negative patients than in ANA-positive patients. Significantly lower proportions of elevated ESR (32.4% vs. 47.6%, p = 0.013) and IgG (14.3% vs. 37.0%, p = 0.003) were observed in ANA-negative patients.

Predictive factors

The results of univariate logistic regression analysis verified that ANA correlated with Raynaud’s phenomenon, digital pits, gastroesophageal reflux, interstitial lung disease, and pulmonary arterial hypertension, as well as with elevation of ESR and IgG. When examined by the multivariable analysis model, Raynaud’s phenomenon, pulmonary arterial hypertension, and elevated ESR still showed statistically significant.
Table 3  Prevalence of autoantibodies in different disease subsets in 2129 scleroderma patients

| Autoantibodies                                                                 | All patients (n = 2129) | Limited (n = 976) | Diffuse (n = 901) | p-value |
|--------------------------------------------------------------------------------|-------------------------|-------------------|-------------------|---------|
| Anti-nuclear antibodies, n (%)                                                  | 2040 (95.8)             | 951 (97.4)        | 854 (94.8)        | 0.973   |
| Anti-ds-DNA, n (%)                                                             | 56/946 (5.9)            | 23/477 (4.8)      | 14/334 (4.2)      | 0.874   |
| Anti-Sm, n (%)                                                                 | 48/955 (5.0)            | 27/478 (5.6)      | 12/333 (3.6)      | 0.879   |
| Anti-SSA, n (%)                                                                | 30/923 (3.3)            | 12/469 (2.6)      | 12/330 (3.6)      | 0.522   |
| Anti-SSB, n (%)                                                                | 219/956 (22.9)          | 111/478 (23.3)    | 74/333 (22.2)     | 0.545   |
| Anti-RNP, n (%)                                                                | 210/958 (21.9)          | 129/479 (26.9)    | 60/334 (18.0)     | 0.207   |
| Anti-riRNP, n (%)                                                              | 17/952 (1.8)            | 11/476 (2.3)      | 5/332 (1.5)       | 0.129   |
| Anti-Scl-70, n (%)                                                             | 947/2041 (46.4)         | 358/954 (37.5)    | 485/883 (54.9)    | 0.332   |
| Anti-centromere, n (%)                                                         | 378/1890 (20.0)         | 209/904 (23.1)    | 127/818 (15.5)    | 0.153   |
| Anti-RNA polymerase III, n (%)                                                  | 45/448 (10.0)           | 23/180 (12.8)     | 19/255 (7.5)      | 0.849   |
| Anti-Jo-1, n (%)                                                               | 11/953 (1.2)            | 6/476 (1.3)       | 2/333 (0.6)       | 0.112   |
| Anti-mitochondrial M2, n (%)                                                   | 86/1676 (5.1)           | 50/817 (6.1)      | 31/718 (4.3)      | 0.963   |
| Anti-PM-Scl, n (%)                                                             | 54/1766 (3.1)           | 24/850 (2.8)      | 29/757 (3.8)      | 0.493   |
| ANA, n (%)                                                                     | 13/944 (1.4)            | 6/466 (1.3)       | 4/334 (1.2)       | 0.568   |
| AHA, n (%)                                                                     | 16/944 (1.7)            | 7/466 (1.5)       | 8/334 (2.4)       | 0.081   |
| Anti-Ro-52, n (%)                                                              | 152/945 (16.1)          | 84/466 (18.0)     | 42/334 (12.6)     | 0.574   |

ANuA anti-nucleosome antibody, AHA anti-histone antibody

Table 4  Comparisons of clinical parameters in systemic sclerosis (SSc) patients with negative and positive ANAs

| Clinical parameter                                      | ANA negative (n = 89) | ANA positive (n = 2040) | p-value |
|--------------------------------------------------------|-----------------------|-------------------------|---------|
| Raynaud’s phenomenon, n (%)                            | 51/71 (71.8)          | 1729/1883 (91.8)        | < 0.001 |
| Sclerodactyly, n (%)                                   | 80/81 (98.8)          | 1888/1959 (96.4)        | 0.253   |
| Digital ulcers, n (%)                                  | 13/86 (15.1)          | 457/1953 (23.4)         | 0.074   |
| Digital pits, n (%)                                    | 16 (18.0)             | 562/2039 (27.6)         | 0.047   |
| Telangiectasias, n (%)                                 | 19/81 (23.5)          | 545/1829 (29.8)         | 0.221   |
| Myositis, n (%)                                         | 4 (4.5)               | 186 (9.1)               | 0.134   |
| Arthritis, n (%)                                        | 10 (11.2)             | 291 (14.3)              | 0.422   |
| Gastroesophageal reflux, n (%)                         | 5 (5.6)               | 378 (18.5)              | 0.002   |
| Interstitial lung disease, n (%)                       | 6/52 (11.5)           | 416/1433 (29.0)         | 0.006   |
| Pulmonary arterial hypertension, n (%)                 | 4/12 (33.3)           | 116/454 (25.6)          | 0.534   |
| Left ventricular diastolic dysfunction, n (%)          | 1/11 (9.1)            | 78/451 (17.3)           | 0.475   |
| Pericardial effusion, n (%)                            | 3/11 (27.3)           | 162/451 (35.9)          | 0.554   |
| Renal crisis, n (%)                                    | 2 (2.2)               | 35 (1.7)                | 0.707   |
| Modified Rodnan skin score                             | 5.0 (0.48)            | 6.0 (0.48)              | 0.864   |
| Global assessment of severity                          | 1.2 ± 0.9             | 1.0 ± 0.8               | 0.058   |
| ESR > 20 mm/h, n (%)                                   | 22/68 (32.4)          | 840/1764 (47.6)         | 0.013   |
| IgG elevation, n (%)                                   | 6/42 (14.3)           | 490/1323 (37.0)         | 0.003   |
| Hypocomplementemia, n (%)                              | 6/38 (15.8)           | 229/1155 (19.8)         | 0.538   |

ESR erythrocyte sedimentation rate

Discussion

The presence of circulating antinuclear antibodies (ANAs) is a hallmark of immune dysregulation in patients with systemic sclerosis (SSc). In this Chinese cohort of SSc subjects, 4.2% of subjects were negative for ANA, which is approximately in agreement with published findings. EUSTAR has also identified their subjects without ANA or Raynaud’s phenomenon as a very small subgroup of SSc [8]. Hamaguchi and colleagues [9] reported that the absence of ANA was found in 5% of SSc subjects in the Japanese population. In a recent cohort study, data from a multicenter registry on 3249 SSc patients in North America were collected, and only 6.4% were negative for ANA [10]. Additionally, a German network reported that 50/863 (5.8%) SSc patients showed ANA negativity [5]. In light of previous studies, autoantibodies, including anti-centromere (ACA), anti-topoisomerase I (Scl-70), and anti-RNA polymerase III (RNP III), can be grouped into SSc-specific ANAs. Detection of SSc-specific ANAs is not only beneficial for diagnosis but is also clinically useful for classifying SSc subtypes that are exclusively associated with characteristic clinical phenotypes [11]. According to the study of Cristinane et al. on an African-Brazilian population [12], anti-Scl-70 was present in 38.1% of patients with dcSSc and 25.0% of patients with lcSSc; in another cohort study, ACA was present in 9% of dcSSc patients and 38% of lcSSc patients [3]. Bardoni and colleagues [13] indicated that 7.8% of SSc patients had anti-RNP III antibodies. We detected anti-Scl-70 positivity in 46.4% of SSc patients (37.5% in lcSSc, 54.9% in dcSSc), along with anti-ACA positivity of 20.0% (23.1% in lcSSc, 15.5% in dcSSc); anti-RNP III antibody was found in 10.0% of SSc patients. Positive rates of these SSc-specific autoantibodies vary among different study populations, suggesting racial divergence [14]. Moreover, encouragingly supported by our study and a previous one, autoantibodies

significant differences after adjusting for confounders, including age, sex, and disease duration. Thus, the presence of ANA is a potential independent risk factor for these clinical features (Table 4).
tend to be specific for clinical characteristics. It was seen that the positive rate of anti-Sm antibody in our study population was considerably higher than that in the German network registry, which may be due to the inclusion of some SSc-lupus overlap patients.

Only a few studies have previously described the clinical characteristics of ANA-negative patients. In existing literature, the peak age of onset for SSc is 55–69 years [15]. Interestingly, we found that patients with ANA negativity had a much earlier onset compared with ANA-positive subjects, which had not been analyzed or shown any marked differences in the previous cohorts. In general, rather young age at onset along with negative antibodies would bring great challenges to the diagnosis and early treatment of these patients. Although SSc is relatively more frequent in females [16], there was a much higher proportion of male ANA-negative patients. In addition, we observed a lower percentage of diffuse disease in the ANA-negative group, which seems to be different from the American study [10]. In that study, although dCSSc was more common in the ANA-negative group, the skin fibrosis severity evaluated by mRSS was actually lower in the ANA-negative group after adjusting for potential confounders. An identical tendency was also found by Poormoghim et al. [17], whereby milder skin involvement was accompanied by the absence of ANAs. Therefore, in general, skin fibrosis in ANA-negative SSc patients tends to be less severe. In addition to the skin, fibrosis commonly occurs in the lung in systemic sclerosis, leading to aggravations of respiratory dysfunction and consequently mortality [18, 19]. In this study, a lower incidence of ILD was observed in the ANA-negative group, which agreed with data from Hamaguchi et al. [9] based on 203 Japanese SSc patients. Despite being a multifactorial process, the etiology of GERD in SSc has been suggested to involve T lymphocyte-mediated activation of myofibroblasts through cytokines and growth factors, resulting in excessive collagen production, which causes structural damage and fibrosis of normal esophageal tissues and also leads to dysmotility [20]. Our study showed that ANA-negative SSc patients were less likely to develop GERD. According to Robinson’s study [21], ACA is relevant to esophageal involvement, which suggests that SSc patients negative for ANAs have a reduced risk of esophageal lesions.

A hallmark of systemic sclerosis is vascular dysfunction, which is thought to occur early and play a central role in disease pathogenesis [18, 22]. In this study, we observed that clinical manifestations of widely recognized microvascularopathy, such as Raynaud’s phenomenon, digital pits, and PAH, were remarkably less common in ANA-negative patients, and digital ulcers tended to be less prevalent in these patients, though with no significant differences. For subjects with no Raynaud phenomenon and testing negative for ANA, confirmation of systemic sclerosis and exclusion of scleroderma mimics became challenging, which calls for the widespread application of nailfold capillary microscopy. Similar to our results, several previous studies have indicated that ANA-negative patients less commonly display vasculopathic features, which may imply unknown pathophysiological etiologies [5, 10, 23, 24]. The pathophysiological mechanisms of pulmonary arterial hypertension in systemic sclerosis are unclear [25]. Based on idiopathic PAH, microvascular inflammation originating from the activation of B lymphocytes has come to light [26]. It is probable that the lower frequency of PAH in ANA-negative patients predicts a favorable prognosis. Our results are generally consistent with these findings, suggesting that ANA-negative subjects have fewer microangiopathies and critical organ involvement, such as interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH), which has been reported as the leading cause of mortality (43–62.1%) in recent studies [27–31]. In addition, we found a significantly lower frequency of abnormal ESR and IgG elevation in ANA-negative patients, indicating that the presence of ANA may correlate more with severe inflammation and intense immune reaction. Uniformly, Yamane et al. [32] found that elevated ESR and increased IgG were common features of scleroderma patients with PAH. Taken together, the results of this study suggest that ANA-negative SSc subjects constitute a distinct subset of SSc with distinct demographic and clinical manifestations and that their disease is generally milder.

Systemic sclerosis is a devastating disease that has a profound impact on life expectancy [33]. Early diagnosis, accurate stratification, and preemptive therapy might improve patient outcomes [34]. However, SSc patients with ANA negativity are less likely to present typical manifestations, such as Raynaud’s phenomenon, GERD, ILD, PAH, and elevated ESR and IgG, leading to impediment and delay in disease recognition, which is not conducive to early diagnosis [35, 36]. Therefore, more effective screening algorithms should be addressed in future longitudinal studies.

This study is the first to specifically focus on the demographic and clinical characteristics of ANA-negative SSc in a large multicenter Chinese cohort. Nevertheless, the present study has some limitations. There were considerably high numbers of missing values for some parameters due to the observational nature of the registry, such as calcinosis, myocardial involvement, and forced vital capacity etc., data on which

| Table 4 | Univariable and multivariable analysis of clinical parameters in systemic sclerosis (SSc) patients with negative and positive ANAs |
|---------|---------------------------------------------------|
|          | OR     | 95% CI | p-value | OR     | 95% CI | p-value |
| Raynaud’s phenomenon | 4.40   | 2.56–7.57 | <0.001 | 4.31   | 2.11–8.79 | <0.001 |
| Digital ulcers | 1.72   | 0.94–3.12 | 0.078 | 0.75   | 0.34–1.64 | 0.463 |
| Digital pits | 1.74   | 1.00–3.00 | 0.049 | –      | –       | –       |
| Gastroesophageal reflux | 3.82   | 1.54–9.48 | 0.004 | 1.71   | 0.58–5.00 | 0.330 |
| Intestinal lung disease | 1.88   | 1.12–3.16 | 0.017 | 1.23   | 0.62–2.46 | 0.553 |
| Pulmonary arterial hypertension | 3.14   | 1.33–7.40 | 0.009 | 5.89   | 1.39–24.90 | 0.016 |
| ESR > 20 mm/h | 1.90   | 1.13–3.19 | 0.015 | 2.01   | 1.01–4.02 | 0.048 |
| IgG elevation | 3.53   | 1.48–8.44 | 0.005 | –      | –       | –       |

OR odds ratio, 95% CI 95% confidence interval, ESR erythrocyte sedimentation rate
could not be considered for this analysis. Besides, filtration of original data and re-definition of organ involvement relying on standard criteria may also lead to data missing, which could explain the relatively low prevalence. Detections of autoantibodies were performed at different centers, and discrepancies could not be avoided. In our study, ANA negativity was defined as no presence of currently detectable autoantibodies in the ANA profile. However, in a considerable number of patients, anti-RNA polymerase III antibodies were not tested. On the other hand, it is also possible that ANA-negative patients produce other antibodies that are not currently detected by our traditional assays, which caused the observed clinical differences or vascular damage. Patients who had diffused skin fibrosis in the early stage but later got spontaneously remised before baseline were likely to be misclassified into the limited cutaneous subtype, which may explain the high proportion of anti-ScI70-positive patients in the limited cutaneous subgroup. In addition, since some centers participating in CRDC were not qualified to perform right heart catheterization (RHC), 140 out of the 422 patients identified with pulmonary arterial hypertension were diagnosed according to the results of echocardiography. Therefore, these results should be interpreted with caution.

In conclusion, our study suggests that patients negative for ANAs comprise a rare and distinctive subgroup of SSC, which has barely been discussed previously. A considerably lower prevalence of Raynaud's phenomenon, certain critical organ involvement, and inflammation indicator anomalies were observed, demonstrating that ANA-negative SSC patients tend to have relatively milder clinical conditions. It is important to understand the clinical characteristics of ANA-negative SSC, because this will allow further exploration of the role of ANA in the pathophysiology of the disease.

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**Declarations**

**Conflict of interest.** M. Hui, X. Wang, J. Zhou, L. Zhang, X. Duan, M. Li, Q. Wang, J. Zhao, Y. Hou, D. Xu, and X. Zeng declare that they have no competing interests.

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Zusammenfassung

Patienten mit systemischer Sklerose und negativen antinukleären Antikörpern haben ausgeprägte klinische Symptome: eine multizentrische CRDC-Kohorte in China

Hintergrund: Das Vorhandensein von zirkulierenden antinukleären Antikörpern (ANA) ist ein Merkmal der Immundysregulation bei Patienten mit systemischer Sklerose (SSc).

Zielsetzung: Eine Vielzahl unterschiedlicher ANA wird mit einzigartigen Krankheitsmanifestationen in Verbindung gebracht und ist bei SSc in der klinischen Praxis weit verbreitet. Ziel dieser Studie war es, die klinischen Merkmale von SSc-Patienten zu untersuchen, die in einer multizentrischen Kohorte des Chinese Rheumatism Data Center (CRDC) in China negativ auf ANA reagieren.

Methoden: Die Patienten wurden zwischen April 2008 und Juni 2019 aus 154 klinischen Zentren im ganzen Land prospektiv rekrutiert, und alle Fälle erfüllten die ACR/EULAR-Klassifikationskriterien für systemische Sklerose von 2013. Die Ergebnisse für antinukleäre Antikörper wurden erfasst. Demografische, klinische und Labordaten von ANA-positiven und ANA-negativen Patienten wurden verglichen.

Ergebnisse: Antinukleäre Antikörper wurden bei 2129 von 2809 in die Studie aufgenommenen Patienten nachgewiesen; 4,2% der Patienten waren negativ. Unter den ANA-negativen SSc-Patienten waren mehr Männer (29/60 vs. 294/1746; \( p < 0,001 \)). Die Inzidenz bestimmter kritischer Organbeteiligungen, darunter gastroösophagealer Reflux (5,6% vs. 18,5%; \( p = 0,002 \)), interstitielle Lungenerkrankung (65,2% vs. 77,9%, \( p = 0,015 \)) und pulmonalearterielle Hypertonie (11,5% vs. 29,0%; \( p = 0,006 \)), war bei ANA-negativen Patienten signifikant niedriger als bei ANA-positiven Patienten. Die Anteil der abnormen Erythrozytensedimentationsrate (ESR; 32,4% vs. 47,6%; \( p = 0,013 \)) und IgG-Erhöhung (14,3% vs. 37,0%, \( p = 0,003 \)), ein Indikator für Krankheitsaktivität, war bei ANA-negativen Patienten signifikant niedriger als bei ANA-positiven Patienten.

Schlussfolgerung: Antinukleäre Antikörper stehen in engem Zusammenhang mit den klinischen Manifestationen der systemischen Sklerose, wobei ANA-negative SSc-Patienten in der Regel eine relativ mildere Erkrankung aufweisen.

Schlüsselwörter
Autoantikörper · Sklerodermie · Phänotyp der Krankheit · Voraussichtliche Registerstudie · Organbeteiligung