Systemic Sclerosis Patients With Negative Antinuclear Antibodies Have Distinctive Clinical Manifestations: a Multicenter CRDC Cohort in China

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Abstract

Objective: The presence of circulating antinuclear antibodies (ANAs) is a hallmark of immune dysregulation in patients with systemic sclerosis (SSc). A variety of ANAs are associated with unique sets of disease manifestations and are widely used in clinical practice for diagnosis, clinical subgrouping, and prediction of future organ involvement and prognosis 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: Based on the CRDC database, 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% patients were negative. There was a significant difference between patients negative and positive for ANAs based on sex (29/60 vs 294/1746, p□0.001). The presence of Raynaud's phenomenon was less common (71.8% vs 91.8%, p□0.001) in the ANA-negative patients. In addition, 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 ESR (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.

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 18–20 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 facts 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 differs from 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. The diagnosis of SSc was performed according to the 2013 American College of Rheumatology (ACR)/the European League Against Rheumatism (EULAR) classification criteria[6]. All patients were classified into two subsets, including limited cutaneous systemic sclerosis (lcSSc) and diffuse cutaneous systemic sclerosis (dcSSc), with systemic sclerosis sine scleroderma (SSSSc) considered to be lcSSc. Disease onset was defined as the time of appearance of the first non-Raynaud phenomenon symptoms. The occurrence of organ involvement was assessed both at the initial visit and during regular follow-up. Demographic, clinical and laboratory data were collected prospectively utilizing a standard form at the time of entry. Disease duration was defined as the interval between the onset of the first non-Raynaud phenomenon symptoms and entry. Global assessment of disease severity was reported by clinical physicians using 0–3 numerical rating scales. The presence of interstitial lung disease (ILD) was determined by 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. Identification of PAH was based on the 2015 European Society of Cardiology/European Respiratory Society guidelines[7] as follows: the mean pulmonary arterial pressure (mPAP) $\geq$ 25 mmHg at rest; pulmonary artery wedge pressure (PAWP) $\leq$ 15 mmHg; and pulmonary vascular resistance (PVR) > 3

Wood units in right heart catheterization (RHC) assessment or an estimated systolic pulmonary artery pressure (SPAP) ≥ 45 mmHg on echocardiography when RHC was not available. Ethics committee approval was obtained for the CRDC study under number S-478. Written informed consent was obtained from each patient before enrollment.

Autoantibody analysis

The presence of antinuclear antibodies (ANAs) was investigated in all patients, and indirect immunofluorescence was performed as a screening method for the detection of ANAs using HEp-2 cells. All ANA titers were determined by experienced investigators from each center, 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. 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 method or chemiluminescence method. To be considered ANA negative, results for both ANA and all other autoantibodies listed above had to be negative. The control group comprised patients with ANA positivity. Since the technique and assays were developed in recent years in our country, anti-RNA polymerase III antibodies could be detected in only a few centers. Because 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.

Statistical analysis

Continuous variables were compared between groups using Student's t test and the results are expressed as the mean ± SD. Categorical variables are presented as numbers and percentages (%); clinically relevant differences were evaluated by the chi-square test or Fischer'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.

Result

Demographic characteristics

In total, 2809 patients were prospectively recruited from 154 clinical centers, covering 29 provinces, based on the CRDC database. Antinuclear antibodies were detected in 2129 patients, of whom 89 (4.2%) were ANA negative. 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 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.

Table 1
Demographic characteristics in systemic sclerosis (SSc) patients with negative and positive ANAs.

	ANA negative (n = 89)	ANA positive (n = 2040)	p
Age at disease onset, y	34.6 ± 11.9	41.4 ± 13.0	<0.001
Gender, male (%)	29 (32.6)	294 (14.4)	<0.001
Disease duration to diagnosis, m	18.0 (0, 240)	24.0 (0, 601)	0.487
Disease duration to enrollment, m	63.0 (5, 403)	43.0 (0, 617)	0.026
Disease type, diffuse (%)	33 (37.1)	868/1789 (48.5)	0.035
Overlap syndrome (%)	6 (6.7)	183 (9.0)	0.469
* Disease duration were reported as median and interquartile range.			

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.

Table 2
Prevalence of autoantibodies in different disease subsets in 2129 scleroderma patients

Autoantibodies	All patients (n = 2129)	Limited (n = 976)	Diffuse (n = 901)	p
Antinuclear 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-rRNP, 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
ANuA, 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
Abbreviations: ANA = antinuclear antibodies, ANuA = anti-nucleosome antibody, AHA = Anti-histone antibody				

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 were also found to have a significantly higher prevalence of tendon friction rubs (2.2% vs. 0.1%, $p < 0.001$), and ANA-negative patients experienced markedly fewer digital pits (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.

Table 3
Comparisons of clinical parameters in systemic sclerosis (SSc) patients with negative and positive ANAs.

	ANA negative (n = 89)	ANA positive (n = 2040)	p
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
Tendon friction rubs, n (%)	2 (2.2)	3 (0.1)	<0.001
Calcinosis, n (%)	0 (0)	16 (0.8)	0.402
Myositis, n (%)	4 (4.5)	186 (9.1)	0.134
Arthritis, n (%)	10 (11.2)	291 (14.3)	0.422
GI involvement, n (%)	9/9 (100)	486/505 (96.2)	0.553
Gastroesophageal reflux, n (%)	5 (5.6)	378 (18.5)	0.002
Interstitial lung disease, n (%)	43/66 (65.2)	1400/1798 (77.9)	0.015
Pulmonary arterial hypertension, n (%)	6/52 (11.5)	416/1433 (29.0)	0.006
Myocardial involvement, n (%)	0 (0)	8 (0.4)	0.530
Left ventricular diastolic dysfunction, n (%)	4/12 (33.3)	116/454 (25.6)	0.543
Pericardial effusion, n (%)	1/11 (9.1)	78/451 (17.3)	0.475
Valve lesions, n (%)	3/11 (27.3)	162/451 (35.9)	0.554
Arrhythmia, n (%)	0 (0)	50/157 (31.8)	0.238
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

Abbreviations: ANA = antinuclear antibodies, GI = gastrointestinal, ESR = erythrocyte sedimentation rate, DLCO = diffusion capacity of carbon monoxide, FVC = forced vital capacity

	ANA negative (n = 89)	ANA positive (n = 2040)	p
DLCO, % predicted	61.0 ± 22.5	60.5 ± 20.8	0.951
FVC, % predicted	76.9 ± 21.8	78.6 ± 19.9	0.804

Abbreviations: ANA = antinuclear antibodies, GI = gastrointestinal, ESR = erythrocyte sedimentation rate, DLCO = diffusion capacity of carbon monoxide, FVC = forced vital capacity

Predictive factor

The results of univariate logistic regression analysis verified that ANA correlated with Raynaud's phenomenon (OR 4.40; CI = 2.56–7.57; p < 0.001), digital pits (OR 1.74; CI = 1.00–3.01; p = 0.049), tendon friction rubs (OR 0.06; CI = 0.01–0.39; p = 0.003), gastroesophageal reflux (OR 3.82; CI = 1.54–9.48; p = 0.004), interstitial lung disease (OR 1.88; CI = 1.12–3.16; p = 0.017), and pulmonary arterial hypertension (OR 3.14; CI = 1.33–7.40; p = 0.009), as well as elevation of ESR (OR 1.90; CI = 1.13–3.19; p = 0.015) and IgG (OR 3.53; CI = 1.48–8.44; p = 0.005) (Table 4). As illustrated in Table 5, when examined by the multivariable analysis model, Raynaud's phenomenon (OR 4.31; CI = 2.11–8.79; p < 0.001), pulmonary arterial hypertension (OR 5.89; CI = 1.39–24.90; p = 0.016) and elevated ESR (OR 2.01; CI = 1.01–4.02; p = 0.048) still showed statistically 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
Univariable analysis of clinical parameters in systemic sclerosis (SSc) patients with negative- compared with positive ANAs.

	OR	95%CI	p
Raynaud's phenomenon	4.403	2.559, 7.567	<0.001
Digital ulcers	1.715	0.942, 3.123	0.078
Digital pits	1.735	1.001, 3.006	0.049
Tendon friction rubs	0.064	0.011, 0.388	0.003
Gastroesophageal reflux	3.821	1.539, 9.484	0.004
Interstitial lung disease	1.882	1.120, 3.160	0.017
Pulmonary arterial hypertension	3.136	1.329, 7.399	0.009
ESR > 20 mm/h	1.901	1.134, 3.186	0.015
IgG elevation	3.529	1.477, 8.437	0.005
Abbreviations: ESR= Erythrocyte sedimentation rate, OR=Odds ratio, 95%CI=95% confidence interval			

Table 5

Multivariable analysis of clinical parameters in systemic sclerosis (SSc) patients with negative- compared with positive ANAs.

	OR	95%CI	p
Raynaud's phenomenon	4.310	2.113, 8.790	□0.001
Digital ulcers	0.745	0.338, 1.638	0.463
Tendon friction rubs	NA	NA	1.000
Gastroesophageal reflux	1.706	0.582, 5.000	0.330
Interstitial lung disease	1.233	0.618, 2.463	0.553
Pulmonary arterial hypertension	5.889	1.393, 24.903	0.016
ESR□20mm/h	2.013	1.007, 4.023	0.048
Abbreviations: ESR= Erythrocyte sedimentation rate, OR=Odds ratio, 95%CI=95% confidence interval			

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% SSc subjects in the Japanese population. In a recent cohort study, data from a multicenter registry on 3,249 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 those SSc-specific autoantibodies vary among different study populations, suggesting racial divergence[14]. Moreover, encouragingly supported by our study and previous one, autoantibodies tend to be specific for clinical characteristics.

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. 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, though dcSSc was more common in the ANA-negative group, the skin fibrosis severity evaluated by mRSS was actually lower in 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 tend to be less severe. In addition to the skin, fibrosis commonly occurs in the lung in systemic sclerosis, leading to aggravations of respiratory dysfunctions 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 microvasculopathy, such as Raynaud's phenomenon, digital pits and PAH, were remarkably less common in ANA-negative patients but that digital ulcers tended to be less prevalent in these patients, though with no significant differences. Similar to our results, several previous studies have indicated that ANA-negative patients less commonly display vasculopathic features, which may implicate 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 negative ANA are less likely to present typical manifestations, such as Raynaud's phenomenon, GERD, ILD, PAH, 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. 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. For a profounder comprehension of ANA-negative SSc, long-term follow-up in ANA-negative SSc patients is required in future studies to investigate the overall survival of this subgroup and identify the prognostic role of ANAs.

Conclusion

Our study suggests that patients negative for ANAs comprise a rare and distinctive subgroup of SSc cases, 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 for the role of ANA in the pathophysiology of the disease.

Declarations

Ethical Approval and Consent to participate

This study was approved by Chinese Rheumatism Data Center (CRDC) with the number S-478. Informed consent was obtained.

Consent for publication

Not applicable

Availability of supporting data and materials

All data generated or analyzed during this study are included in the article.

Competing interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Authors' contributions

Min Hui and Jiaxin Zhou: Data curation, Writing-Original draft preparation.

Liyun Zhang and Xinwang Duan: Supervision.

Mengtao Li and Qian Wang: Visualization, Investigation.

Jiuliang Zhao: Conceptualization, Methodology, Software.

Yong Hou: Software, Validation.

Dong Xu and Xiaofeng Zeng: Writing- Reviewing and Editing.

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