Clinical correlations with disease-associated auto-antibodies in a Chinese cohort with systemic sclerosis

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To the Editor: Systemic sclerosis (SSc) is an autoimmune disease characterized by progressive skin and visceral fibrosis, microvasculopathy, and autoimmunity. Circulating auto-antibodies (AAbs) are detectable in 90% to 95% of patients with SSc.[1] It is reported that 60% to 80% of SSc patients are positive for anti-topoisomerase I antibody (ATA), anti-centromere antibody (ACA), and anti-RNA polymerase III antibody (ARA).[2] These three AAbs are the most prevalent SSc-associated AAbs, with high specificity for the diagnosis of SSc; so they have been included in the classification criteria for SSc defined by the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) in 2013. Currently, SSc-associated AAbs have been widely used in clinical practice, and the research on the clinical significance of AAbs is still in progress. In our present study, we analyzed the correlations between the SSc-associated auto-antibody profile and clinical manifestations in a well-characterized Chinese SSc cohort.

One hundred and forty-four patients enrolled between June 2018 and August 2020 in our center were included in this cross-sectional study. The inclusion criteria were as follows: (1) patients diagnosed with SSc according to 2013 ACR/EULAR classification criteria; (2) patients who had undergone chest high-resolution computed tomography (HRCT) scan; and (3) patients tested for ATA, ACA, and AAbs to nuclear-ribonuclear-protein (nRNP). This research was conducted according to the *Declaration of Helsinki,* and all procedures involving study participants were approved by the ethics committee of Huashan Hospital, Fudan University (No. 2019-191). Each participant signed an informed consent form before the research.

Through careful medical history inquiry and physical examination, we collected patient demography (age at onset, sex, smoking history, and disease duration) and clinical characteristics (disease subset, Raynaud's phenomenon [RP], digital ulcer, telangiectasia, puffy finger, arthralgia, and myalgia). The onset of disease was defined as the time when the first non-RP symptom of SSc (skin thickening, sclerodactyly, puffy finger, digital ulcer, or organ involvement) appeared. The skin fibrosis was scored according to the modified Rodnan skin thickness score (mRSS), a widely used clinical assessment of skin thickness where the examining rheumatologist records the degree of skin thickening on a scale of 0 (no involvement) to 3 (severe thickening) in 17 body areas (total score range: 0–51). Interstitial lung disease (ILD) was defined by HRCT. Pulmonary arterial hypertension (PAH) was defined as mean systolic pulmonary arterial pressure ≥40 mmHg detected by echocardiography. Scleroderma renal crisis (SRC) was defined as the acute deterioration of renal function accompanied by hypertension or corresponding renal biopsy results. All patients were classified into four disease subsets, including diffuse cutaneous SSc (dcSSc), limited cutaneous SSc (lcSSc), overlap syndrome (overlap) subsets based on the classification of LeRoy *et al,* and SSc sine scleroderma subset characterized by typical visceral involvement, vasculopathy, and serologic abnormalities without skin alterations.

AAbs were tested by immunoblotting. All the patients were tested for ATA, ACA, and AAbs to Ku, nRNP, and polymyositis (PM)-Scl. Sixty-four patients were also tested for ARA and AAbs to fibrillarin, Th/To, NOR90, and platelet-derived growth factor receptor (PDGFR). Of the 64 patients, those positive for any of the above-mentioned ten AAbs were defined to be AAb-positive SSc (SSc-AAbs [+]) patients, whereas the patients who were negative for the above-mentioned ten AAbs were defined to be AAb-negative SSc (SSc-AAbs [−]) patients.

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001935

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Chinese Medical Journal 2022;135(15)
Received: 07-02-2021; **Online:** 02-02-2022 **Edited by:** Lishao Guo

Data were analyzed by GraphPad Prism (version 8.0.2 for Windows, GraphPad Software, San Diego, California, USA). Quantitative data were analyzed using *t* test, Welchtest, and Mann–Whitney test where appropriate. The Chi-squared test or Fisher's exact test was used to evaluate categorical data. *P* value < 0.05 was considered statistically significant.

A total of 144 patients were included in our SSc cohort with a mean age at onset of 47.8 ± 13.9 years. Of all, 117 (93.5%) were female and 27 (6.5%) were male. SSc disease duration from RP onset and non-RP onset was 4.0 (1.5– 9.6) and 2.0 (1.0–5.0) years, respectively. The mean mRSS was 6.0 (2.0–12.0). There were 58 (41.1%), 61 (42.4%), 8 (5.6%), and 17 (11.8%) patients diagnosed as dcSSc, lcSSc, SSc sine scleroderma, and overlap, respectively. Ninety-three patients (64.6%) were determined with ILD.

The patients with SSc-associated AAbs in our cohort is as follows in the order: ATA 61 (42.4%), ACA 34 (23.6%), ARA 11/64 (17.2%), anti-nRNP 15 (10.6%), anti-NOR90 6/64 (9.4%), anti-fibrillarin 4/64 (6.3%), anti-Ku 5 (3.5%), anti-Th/To 2/64 (3.1%), anti-PM-Scl 3 (2.8%), and anti-PDGFR 0/64 (0%). In addition, 11/64 (17.2%) of the patients were SSc-AAbs (–) [Supplementary Table 1, http://links.lww.com/CM9/A875 and Supplementary Figure 1, http://links.lww.com/CM9/A875].

Due to the limited number of anti-fibrillarin, anti-NOR90, anti-Th/To, anti-PDGFR, and anti-PM-Scl subgroups ($n < 10$), the correlation analysis may not be convincing. Therefore, clinical associations were analyzed only among the SSc patients positive for ATA, ACA, ARA, anti-nRNP and SSc-AAbs (–) subgroups.

In our cohort, compared with the patients negative for the corresponding antibodies, patients with ATA had earlier disease onset ($P = 0.02$), higher mRSS level ($P = 0.003$), and higher proportions of ILD ($P < 0.0001$); patients with ACA had lower mRSS level ($P = 0.005$), lower proportions of male ($P = 0.04$), myalgia ($P = 0.02$), and ILD ($P < 0.0001$) or overlap ($P < 0.0001$); patients with ARA had later disease onset ($P = 0.04$); and patients with anti-nRNP had lower mRSS level ($P = 0.009$). Patients who are negative for SSc-AAbs manifested as more dcSSc than lcSSc ($P = 0.009$); patients who are positive for ACA manifested as more lcSSc than dcSSc ($P < 0.0001$) or overlap ($P < 0.0001$); and patients who are positive for anti-nRNP manifested as more overlap than dcSSc ($P = 0.0003$) or lcSSc ($P = 0.001$).

No significant correlations were found between SSc-associated AAbs and disease duration, RP, digital ulcer, arthralgia, puffy finger, telangiectasia, PAH, or SRC [Table 1, Supplementary Figure 2, http://links.lww.com/CM9/A875 and Supplementary Tables 1–3, http://links.lww.com/CM9/A875].

Table 1: Correlations of quantitative clinical variables with SSc-associated AAbs.

Quantitative data	Positive	Negative	*N*+	*N*–	*P* values
Age at onset (years), mean ± SD					
ATA	44.8 ± 13.3	50.0 ± 14.0	61	83	0.020*
ACA	50.9 ± 10.3	46.8 ± 14.8	34	110	ns*
ARA	56.0 ± 13.4	45.9 ± 15.0	11	53	0.040†
Anti-nRNP	45.6 ± 15.5	48.0 ± 13.8	15	129	ns
SSc-AAbs (+)	47.4 ± 15.8	48.8 ± 11.8	53	11	ns†
mRSS, median (IQR)					
ATA	8.0 (4.0–18.0)	4.0 (2.0–10.3)	57	64	0.003*
ACA	3.0 (2.0–8.0)	7.0 (3.0–14.5)	29	92	0.005*
ARA	9.0 (4.0–16.0)	6.0 (2.0–10.3)	11	48	ns*
Anti-nRNP	2.0 (0.5–4.0)	7.0 (2.0–12.8)	11	110	0.009*
SSc-AAbs (+)	6.0 (2.0–11.0)	8.5 (5.0–11.5)	51	8	ns*
Disease duration (years), median (IQR)					
RP disease duration					
ATA	4.0 (1.1–7.9)	3.5 (1.5–9.9)	59	82	ns*
ACA	4.0 (1.5–10.0)	3.0 (1.4–8.0)	33	108	ns*
ARA	8.0 (0.7–12.0)	2.5 (1.0–7.0)	11	53	ns*
Anti-nRNP	4.0 (2.0–9.2)	3.0 (1.0–9.5)	14	127	ns*
SSc-AAbs (+)	3.0 (1.0–9.0)	2.0 (0.6–4.5)	53	11	ns*
Non-RP disease duration					
ATA	2.0 (0.8–5.0)	2.0 (1.0–5.0)	61	83	ns*
ACA	2.9 (1.0–5.0)	2.0 (0.9–5.0)	34	110	ns*
ARA	0.8 (0.7–2.8)	2.0 (1.0–5.0)	11	53	ns*
Anti-nRNP	2.0 (0.6–3.5)	2.0 (1.0–5.0)	15	129	ns*
SSc-AAbs (+)	1.5 (1.0–5.0)	1.0 (0.8–2.0)	53	11	ns*

* *t* test. † Mann–Whitney test. *N*+ = The number of positive group patients; *N*– = The number of negative group patients. AAbs: Auto-antibodies; ACA: Anti-centromere antibody; ARA: Anti-RNA polymerase III antibody; ATA: Anti-topoisomerase I antibody; IQR: Interquartile range; mRSS: Modified Rodnan skin score; ns: Not significant; RP: Raynaud's phenomenon; SD: Standard deviation; SSc: Systemic sclerosis; SSc-AAbs (+): AAb-positive-SSc.

The present study compared the prevalence of SSc-associated AAbs in a well-described Chinese cohort and analyzed the associations between SSc-associated AAbs and clinical features, such as disease subsets and critical organ involvement.

Considering that fibrosis is one of the most important characteristics of SSc, we applied various ways to evaluate the degree of fibrosis in patients, including mRSS, disease subsets according to the extent of skin fibrosis and ILD.

In our study, the proportion of lcSSc in ACA-positive patients was much higher than that of dcSSc and overlap; also, the mRSS was significantly lower in the patients who are positive for ACA than in those who are negative for ACA. We also found that mRSS was higher in ATA-positive patients and that the subset of dcSSc was more frequently seen in SSc patients who are positive for ATA. The proportion of ILD was higher in ATA-positive patients and lower in ACA-positive patients. Since ILD is one of the major causes of death in SSc at present, and normally indicative of severer disease and worse prognosis, our findings confirmed the view that early screening for ILD is highly recommended in ATA-positive patients.[3]

Importantly, it was found in our cohort that among SSc-AAbs (-) patients, the proportion of dcSSc was higher and that of lcSSc was lower, which appeared even more remarkable than in ATA-positive patients. We speculated that there could be some existing but still unknown AAbs underlying, which could be the direction of our further research.

In summary, we have applied different methods to analyze the correlation between SSc-associated auto-antibody profile and fibrosis, and gotten consistent results, which may provide supportive and supplementary data for the previous similar study in a Chinese SSc cohort.[4]

Besides the correlation with fibrosis, we also found other clinical correlations of SSc-associated AAbs in our cohort. We found that the onset age of ATA-positive patients was relatively earlier in our cohort and that ACA was more commonly seen in females, consistent with the findings of Mierau *et al*.[5]

In terms of musculoskeletal involvement, we found that myalgia is less common in ACA-positive patients, and another study[5] also reported that ACA-positive patients had less musculoskeletal involvement. In the future, we need to add more detailed musculoskeletal parameters (such as myodynamia, electromyography, muscle biopsy, and creatine kinase level) to obtain more thorough observation on the clinical relationship between AAbs and muscle involvement.

ARA-positive patients had later disease onset. None of the ARA-positive patients in our cohort developed renal crisis so far, although ARA was reported to be highly correlated with SRC.[1] Since the number of ARA-positive patients was relatively small ($n = 15$), these findings may need further observation in a larger cohort.

Anti-nRNP is usually associated with SSc overlap,[1] which was also confirmed in our cohort.

The limitation of our current study mainly lies in the limited sample size in this cohort. A well-described cohort with larger sample size from multiple centers may help us to study the clinical associations with AAbs more comprehensively and thoroughly.

Acknowledgments

The authors appreciate and acknowledge all members of the Division of Rheumatology of Huashan Hospital; they made considerable effort in the evaluation of the patient's condition, data collection, statistical analysis, etc. The authors thank the patients for their cooperation in providing descriptions of their conditions and blood samples.

Funding

This work was supported by grants from the Youth Program of National Natural Science Foundation of China (No. 81501391), and medical and health research projects from Shanghai Baoshan Science and Technology Commission (No. 20-E-3).

Conflicts of interest

None.

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How to cite this article: Yang S, Liang M, Chen C, Ye W, Zhu X, Xue Y, Kong N, Yu Y, Xuan D, Zheng S, Yang X, Zhu Z, Zhao T, Wan W, Zou H. Clinical correlations with disease-associated auto-antibodies in a Chinese cohort with systemic sclerosis. Chin Med J 2022;135:1878–1880. doi: 10.1097/CM9.0000000000001935