Analysis of anti-RNA polymerase III antibodies in Chinese Han systemic sclerosis patients

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Abstract

Objectives This study aimed to assess the prevalence and clinical correlation of anti-RNA polymerase III antibodies (anti-RNAP III) in Chinese Han systemic sclerosis (SSc) patients.

Methods Serum samples from 236 patients with SSc, 125 patients with connective tissue diseases (CTD), and 166 healthy controls (HCs), recruited from Peking Union Medical College Hospital and 21 other medical centers in China, were tested for antibodies to RNA polymerase III by means of a line immunoassay (LIA) or an enzyme-linked immunosorbent assay (ELISA) kit.

Results Anti-RNAP III antibodies were found in 14/236 SSc patients (5.93%), 1/125 (0.80%) CTD patients, and 0/166 (0.00%) HCs. The prevalence of anti-RNAP III was higher in SSc patients than in the CTD and HC groups ($p = 0.02$, $p = 0.001$, respectively). Renal crisis was significantly more common in patients with anti-RNAP III than patients without anti-RNAP III (42.9 vs. 4.1%, $p < 0.0001$). Gastrointestinal involvement was significantly more common in patients without anti-RNAP III than patients with anti-RNAP III (53.6 vs. 21.4%, $p = 0.039$). There was good agreement between the ELISA and line immunoassay (LIA) detection capabilities for anti-RNAP III.

Conclusions The anti-RNAP III antibody, which was detected by ELISA, has diagnostic value for SSc and predictive value for SSc-related renal crisis. Both ELISA and LIA are very reliable methods for anti-RNAP III.

Key Points

- The prevalence of anti-RNAP III antibody was determined in Chinese SSc patients and performed ethnic differences.
- The clinical association between anti-RNAP III antibody and Chinese SSc patients was evaluated in this research.
- Methodological consistency of detection of anti-RNAP III antibody using commercial ELISA and LIA methods was evaluated in this research.

Keywords Autoantibodies · Connective tissue disease · Enzyme-linked immunosorbent assay · RNA polymerase III · Systemic sclerosis

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Introduction

Systemic sclerosis (SSc) is an autoimmune disease characterized by vasculopathy, tissue fibrosis, and the production of autoantibodies [1, 2]. The clinical manifestations of SSc are patient-specific, and most SSc patients will develop skin thickening with variable organ involvement, including interstitial lung disease (ILD), pulmonary arterial hypertension (PAH), and renal crisis [3–5]. As abnormal immunity is activated, multiple autoantibodies can be detected in the serum of SSc patients, such as anti-nuclear antibodies (ANA), anti-topoisomerase I antibodies (ATA, also known as anti-Scl-70 antibodies), and anti-centromere antibodies (ACA). Anti-topoisomerase I antibodies are classically associated with ILD and the diffuse cutaneous form of SSc, while anti-centromere antibodies are typically associated with PAH and the limited cutaneous form of SSc [6].

Anti-RNA polymerase III antibody (anti-RNAP III) is the third type of antinuclear autoantibody detected in patients with SSc. It was first shown to be associated with SSc in 1993 when Okano et al. discovered the anti-RNAP III antibody using radioimmunoprecipitation technology [7]. The RNA polymerase III antibody is a multi-protein complex that is located in the nucleoplasm. RP155 was identified as the major epitope of RNA polymerase III recognized by SSc serum [8]. In recent years, foreign studies have identified that the prevalence of anti-RNAP III in SSc ranges from 10 to 25% in American SSc patients [9, 10], nearly 6% in Japanese SSc patients [11, 12], 15.3% in Australian SSc patients [13], and 19.4% in Canadian SSc patients [14], which has been linked to ethnic variations. Currently, there are few reports on the prevalence of anti-RNAP III in Chinese SSc patients and its correlation with the clinical features of SSc. Hence, we aimed to evaluate the prevalence and predictive value of anti-RNAP III in Chinese Han SSc patients.

Materials and methods

Patients

The study population included a total sample of 527 patients, including 236 SSc patients (28 males and 208 females, mean age 48.3 ± 12.5 years) who fulfilled the 2013 ACR/EULAR classification criteria for SSc. ILD was diagnosed by the high-resolution computed tomography in the SSc patients. PAH was diagnosed by mean pulmonary pressure values ≥ 25 mmHg in SSc patients using the right heart catheterization. Gastrointestinal involvement related to SSc included reflux esophagitis, esophageal dysmotility, dysphagia, and others. Cardiac involvement included SSc-induced systolic or diastolic dysfunction. Renal crisis was defined as severe hypertension and rapid renal insufficiency. Some data about clinical

manifestations were missing from the medical records. In addition, 30 patients were unclassified due to missing clinical data.

In addition, 125 control serum samples (mean age 46.0 ± 15.5 years, matched for age and sex with SSc patients) were obtained from 25 primary Sjögren's syndrome (pSS) patients who fulfilled the American-European consensus group classification criteria [15], 25 rheumatoid arthritis (RA) patients who fulfilled the 1987 American College of Rheumatology (ACR) revised classification criteria [16], 25 systemic lupus erythematosus (SLE) patients who fulfilled the 1997 ACR revised classification criteria [17], and 25 dermatomyositis (DM) and 25 polymyositis (PM) patients diagnosed according to the Bohan and Peter criteria [18, 19], were studied. Also, 166 healthy subjects (mean age 47.6 ± 12.5 years, matched for age and sex with SSc patients) admitted to the Peking Union Medical College Hospital Health Examination Center for physical examination were also recruited for this study. All serum samples were stored at -80°C until measurement. All the samples were obtained from patients recruited from the Peking Union Medical College Hospital (PUMCH) and other 21 health centers in China. The study was approved by the Ethics Committee of the Peking Union Medical College Hospital, and all recruited participants gave informed consent.

Measurement of anti-RNAP III

We used an anti-RNA polymerase III enzyme-linked immunosorbent assay (ELISA) kit for IgG (Medical and Biological Laboratories (MBL) Co. Ltd, Nagano, Japan) to retrospectively analyze 527 serum samples. The antigen was the recombinant immunodominant epitope based on the RP155 subunit of RNA polymerase III. The assay was performed according to the manufacturer's instructions. The values that were ≥ 28 U/ml were considered positive for anti-RNAP III. In addition, we also used a line immunoassay (LIA) to analyze anti-RNAP III with a commercially available kit (Euroline Systemic Sclerosis Nucleoli Profile IgG kit, Euroimmun AG, Lübeck, Germany) in 236 SSc patient serum samples. The assay was performed according to the manufacturer's instructions. The results (negative, borderline, positive, and strong positive) were interpreted using the EUROScan software (Euroimmun AG, Lübeck, Germany), and borderline was considered as negative in this study.

Statistical analysis

The data were analyzed by SPSS version 19.0 for Windows (SPSS Inc, Chicago, IL, USA). Figure 1 was generated by GraphPad Prism 7.0 (La Jolla, CA). The frequencies of categorical variables were compared using Pearson's χ^2 or Fisher's exact test, when appropriate. Spearman correlation analysis was used to assess the relationship of anti-RNAP III levels using ELISA with anti-RNAP III levels using LIA. The

Fig. 1 Prevalence of anti-RNAP III antibodies in SSc patients, CTD patients, and HCs. *SSc* systemic sclerosis, *Anti-RNAP III* anti-RNA polymerase III, *CTD* connective tissue diseases, *HCs* healthy controls

SSc = systemic sclerosis; Anti-RNAP III = Anti-RNA polymerase III; CTD = connective tissue diseases; HCs = healthy controls

sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV), positive likelihood ratios (LR+), and negative likelihood ratios (LR−), were calculated accordingly. *p* values that were less than 0.05 were considered statistically significant.

Results

Prevalence of anti-RNAP III antibodies in various groups

Serum samples from 236 SSc patients, 125 CTD controls, and 166 healthy controls were analyzed for anti-RNAP III by an MBL ELISA (Fig. 1). The prevalence of anti-RNAP III antibodies in the SSc, CTD, and HC groups were 5.93% (14/236), 0.80% (1/125), and 0.00% (0/166), respectively. The prevalence of anti-RNAP III was significantly higher in SSc, compared with the CTD group ($p = 0.020$). When comparing the SSc with HC groups, the prevalence of anti-RNAP III was significantly higher in SSc ($p = 0.001$), suggesting that anti-RNAP III is useful for diagnosing SSc. The sensitivity and specificity of anti-RNAP III by the MBL ELISA in Chinese SSc patients were 5.93 and 99.2%, respectively. The PPV and NPV were 93.4 and 52.5%, respectively. The LR+, a multiple between the probability and a true positive judgement to the probability of a false positive judgment, was 7.41, and LR− was 0.95. The high LR+ demonstrated that the accuracy of the ELISA results for anti-RNAP III is high.

Clinical characteristics of SSc patients and clinical associations of anti-RNAP III

The clinical characteristics of SSc patients and associations of anti-RNAP III are summarized in Table 1. Patients with anti-

RNAP III were not different from patients without anti-RNAP III considering gender (female, 78.6 vs. 88.7%) and disease subtype (diffuse cutaneous, 50 vs. 37.1%). No significant differences were discovered for disease duration (11.2 ± 8.1 vs. 11.4 ± 5.8 , $p = 0.87$), joint involvement (35.7 vs. 50.5%, $p = 0.427$), ILD (50.0 vs. 67.1%, $p = 0.307$), PAH (35.7 vs. 19.8%, $p = 0.279$), or cardiac involvement (42.9 vs. 34.7%, $p = 0.739$) in the patients with anti-RNAP III and without anti-RNAP III, respectively. Patients with anti-RNAP III were older than patients without anti-RNAP III (55.4 ± 8.2 vs. 47.9 ± 12.9 , $p = 0.005$). Renal crisis was significantly more common in patients with anti-RNAP III than patients without anti-RNAP III (42.9 vs. 4.1%, $p < 0.0001$). The odds ratio (OR) was 17.8 with a 95% confidence interval of 5.1 to 62.0. Gastrointestinal involvement was significantly more common in patients without anti-RNAP III than patients with anti-RNAP III (53.6 vs. 21.4%, $p = 0.039$).

Evaluation of anti-RNAP III in predicting clinical manifestations of SSc

Anti-RNAP III was evaluated on its ability to predict the clinical manifestations of SSc (Table 2). Anti-RNAP III had a low PPV and NPV, especially when considering renal involvement (NPV 95.95%). In addition, the LR+ and nLR− were universally low except for renal crisis (LR+ 11.05).

Agreement between ELISA and LIA of detection with anti-RNAP III

There was a good agreement between ELISA and LIA of detection with anti-RNAP III (Table 3). The κ was 0.799, representing a significant consistency. The χ^2 was 144.26, and the p value was less than 0.001, suggesting that the relevance of these two methods is very significant. The anti-

Table 1 Clinical characteristics of SSc patients and clinical associations of anti-RNAP III

Characteristics	Anti-RNAP III + (<i>n</i> = 14)		Anti-RNAP III – (<i>n</i> = 222)		<i>p</i> value
	Total ^a	<i>n</i> (%)	Total	<i>n</i> (%)	
Gender					
Female	14	11 (78.6%)	222	197 (88.7%)	0.475
Male	14	3 (21.4%)	222	25 (11.3%)	
Age (years)	14	55.4 ± 8.2	222	47.9 ± 12.9	0.005
Disease duration (years)	14	11.2 ± 8.1	222	11.4 ± 5.8	0.87
Disease subtype					
Diffuse	12	6 (50%)	194	72 (37.1%)	0.558
Limited	12	6 (50%)	194	122 (62.9%)	
Clinical manifestations					
Joint involvement	14	5 (35.7%)	222	112 (50.5%)	0.427
ILD	14	7 (50%)	222	149 (67.1%)	0.307
PAH	14	5 (35.7%)	222	44 (19.8%)	0.279
Gastrointestinal involvement	14	3 (21.4%)	222	119 (53.6%)	0.039
Cardiac involvement	14	6 (42.9%)	222	77 (34.7%)	0.739
Renal crisis	14	6 (42.9%)	222	9 (4.1%)	< 0.0001

SSc systemic sclerosis, *Anti-RNAP III* anti-RNA polymerase III, *ILD* interstitial lung disease, *PAH* pulmonary arterial hypertension

^a Total number of SSc patients whose data were available for analysis

RNAP III values using ELISA in SSc patients had a significantly positive correlation with anti-RNAP III values using LIA (Fig. 2; $r = 0.824$, $p < 0.0001$).

Discussion

This study aimed to determine the prevalence of anti-RNAP III in Chinese SSc patients. The reported data of pooled prevalence of anti-RNAP III was 11% (95% CI 8 to 14%) in a transnational meta-analysis [20], which was consistent with the findings from Koenig et al.

[21]. However, our data was slightly lower with only 5.93% of Chinese SSc patients expressing anti-RNAP III because of the racial variation. From these data, we believe that the prevalence of anti-RNAP III is higher in Caucasian SSc patients and lower in Asian SSc patients, especially in patients from China and Japan.

Anti-RNAP III is known to be associated with some clinical manifestations in SSc, including diffuse cutaneous involvement and renal crisis [6]. Furthermore, Nikpour et al. [13] reported that anti-RNAP III was associated with diffuse disease, renal crisis, joint contractures, and higher modified Rodnan skin scores ($p < 0.0001$). Patients with anti-RNAP III

Table 2 Evaluation of anti-RNAP III in predicting clinical manifestations in SSc

	SEN (%)	SPE (%)	PPV (%)	NPV (%)	LR+	LR–
Diffuse subtype	7.69	95.31	50.00	62.89	1.64	0.97
Limited subtype	4.69	92.31	50.00	37.11	0.61	1.03
Joint involvement	4.27	92.44	35.71	49.55	0.57	1.04
ILD	4.49	91.25	50.00	32.88	0.51	1.05
PAH	10.20	95.19	35.71	80.18	2.12	0.94
Gastrointestinal involvement	2.46	90.35	21.43	46.40	0.25	1.08
Cardiac involvement	7.23	94.77	42.86	65.32	1.38	0.98
Renal crisis	40.00	96.38	42.86	95.95	11.05	0.62

SSc systemic sclerosis, *PAH* pulmonary arterial hypertension, *SEN* sensitivity, *SPE* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *LR+* positive likelihood ratio, *LR–* negative likelihood ratio

Table 3 Agreement between ELISA and LIA of detection with anti-RNAP III

Detection method	TP, <i>n</i>	TN, <i>n</i>	FP, <i>n</i>	FN, <i>n</i>	Agreement, <i>n/n</i> (%)	χ^2	κ	<i>p</i> value
ELISA vs. LIA	13	217	6	0	230/236 (97.5%)	144.26	0.799	< 0.001

TP true positive, i.e., ELISA positive and LIA positive; *TN* true negative, i.e., ELISA negative and LIA negative; *FP* false positive, i.e., LIA positive and ELISA negative; *FN* false negative, i.e., LIA negative and ELISA positive; *ELISA* enzyme-linked immunosorbent assay; *LIA* line immunoassay

had a higher risk of malignancies diagnosed within 5 years of the onset of the SSc skin disease. Motegi et al. [11] also reported the diagnostic usefulness and risk stratification of anti-RNAP III for severe skin sclerosis and renal crisis. Steen [22] found that SSc patients with anti-RNAP III had a lower frequency of severe ILD. In this study, we found a significant difference in the frequency of renal crisis and gastrointestinal involvement between the patients with and without anti-RNAP III. However, there was no association found with the diffuse cutaneous form as well as other clinical manifestations in our study. Probably the predictive power of anti-RNAP III antibodies was limited by the low sensitivity, except for renal crisis, as expected.

Recent studies have shown that cardiopulmonary and kidney involvement accounts for most SSc-related deaths with ILD and PAH being the primary causes of deaths [23, 24]. Interestingly, anti-RNAP III was not associated with ILD or PAH. Therefore, the early diagnosis and treatment is essential for improving the prognosis of patients with SSc.

It has been reported that 2–15% of patients with SSc will develop renal crisis, and the SSc patients with renal crisis have 5-year survival rates of 50–60% [25]. Patients with scleroderma renal crisis may develop malignant hypertension, anuria, and renal failure. These severe complications exacerbate the disease progression and lead to death in some cases. In our study, SSc patients with anti-RNAP III had a higher risk of renal crisis than patients without anti-RNAP III, with an OR of 17.8 and 95%

confidence interval 5.1 to 62.0. In the SSc patients, the frequency of renal crisis was 15/236 (6.4%), which increased to 6/14 (42.9%) in the anti-RNAP III-positive SSc patients. The LR+ was 11.05 and the anti-RNAP III showed good predictive value for renal crisis. For SSc patients who are anti-RNAP III-positive, physicians should be alerted to the possibility of patients developing renal crisis. In return, the patients can receive timely treatment to avoid high-dose hormone therapy. Therefore, SSc patients positive for anti-RNAP III should regularly monitor their blood pressure, creatinine levels, and urine protein levels. For those SSc patients showing signs of renal crisis, treatment with an angiotensin-converting enzyme inhibitor (ACEI) can lead to improved prognoses [26]. However, despite the controversy of the finding, there has been reported that treatment with ACEI may be associated with an increased risk of dialysis or death before the abrupt rise in blood pressure and creatinine levels [27]. Notably, corticosteroid is commonly used for the treatment of the inflammatory symptom in SSc, but it is a risk factor for renal crisis [28]. Some studies have reported that SSc patients treated with moderate-to-high doses of corticosteroids performed an increased risk of renal crisis [29]. It is crucial to limit the corticosteroids use to low dose and the short duration possible.

As we all know, the immunoprecipitation (IP) assay is the gold standard for detecting anti-RNAP III antibodies. Previously, it was reported that the highly sensitive and specific ELISA system showed similar results to those of the IP assay [30]. While the agreement between the detection values from

Fig. 2 Correlation between ELISA and LIA of detection with anti-RNAP III among SSc patients (*n* = 236). SSc systemic sclerosis, *ELISA* enzyme-linked immunosorbent assay, *LIA* line immunoassay

SSc = systemic sclerosis; ELISA = enzyme-linked immunosorbent assay; LIA = line immunoassay.

ELISA and LIA of detection was high ($\kappa = 0.799$), there were still several false-positive results with the LIA kit, suggesting that the assay needs to be improved before its clinical use.

In this study, gastrointestinal involvement was significantly more common in patients without anti-RNAP III than patients with anti-RNAP III. Previously, Ingraham et al. [31] reported an association between anti-RNAP III and gastric antral vascular ectasia (GAVE), another type of gastrointestinal involvement related to SSc, and the absence of anti-Scl-70 was useful for identifying the risk of developed SSc-induced GAVE. This finding was later verified by another research group [32]. However, Hung et al. [33] did not find the association between anti-RNAP III and GAVE, but they confirmed the negative association between anti-Scl-70 and GAVE. Thus, it can be seen that the results can vary between groups, suggesting that more rigorous multi-center large population trials are needed to investigate the relationship between anti-RNAP III and gastrointestinal diseases. In conclusion, the ELISA-detected anti-RNAP III antibody has diagnostic value for SSc and predictive value for SSc-related renal crisis.

However, there were some limitations in the present study. Some data about clinical manifestations were insufficient, so the pulmonary function tests (PFTs) and Modified Rodnan Skin Score (MRSS) could not be provided because of the characteristics of retrospective study.

In conclusion, a commercial enzyme-linked immunosorbent assay (ELISA) kit for IgG was used to evaluate the diagnostic value of anti-RNA polymerase III in Chinese SSc patients in this study. There was a good agreement between ELISA and LIA of detection with anti-RNAP III. The ELISA-detected anti-RNAP III antibody has diagnostic value for SSc and predictive value for SSc-related renal crisis.

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Compliance with ethical standards The study was approved by the Ethics Committee of the Peking Union Medical College Hospital, and all recruited participants gave informed consent.

Disclosures None.

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