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Evaluation of a commercial immunoassay for autoantibodies in Chinese Han systemic sclerosis population

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ABSTRACT

Objective: The autoantibody profile, which varies between races, has rarely been reported in Chinese Han systemic sclerosis (SSc) patients. The present study aims to investigate the autoantibody profile of Chinese Han SSc patients using a commercial line immunoassay.

Methods: The prevalence of autoantibodies to Ro-52, PDGFR, Ku, PM75, PM100, Th/To, NOR90, Fib, RP155, RP11, CENP-B, CENP-A and Scl-70 were analyzed in serum samples obtained from healthy controls (n = 30), SSc patients (n = 320) and non-SSc connective-tissue disease patients (n = 100) using an Euroline Systemic Sclerosis Profile kit.

Results: SSc patients had increased prevalence of anti-RP11 (P = 0.032), anti-CENPB (P < 0.001), anti-CENPA (P = 0.001) and anti-Scl-70 (P < 0.001), but had decreased prevalence of anti-Ro-52 (P = 0.004), when compared to non-SSc CTDs. Furthermore, SSc patients had increased prevalence of anti-Ro-52 (P = 0.003), anti-CENPB (P = 0.034), anti-CENPA (P = 0.034) and anti-Scl-70 (P < 0.001), when compared to HCs. In addition, SSc patients with interstitial lung disease (ILD) had increased prevalence of anti-Ro-52 (P = 0.046) and anti-Scl-70 (P = 0.035), but had decreased prevalence of anti-CENPB (P < 0.001) and anti-CENPA (P < 0.001). Moreover, SSc patients with pulmonary arterial hypertension (PAH) had increased prevalence of anti-Ro-52 (P = 0.013) and decreased prevalence of anti-Scl-70 (P = 0.001). Anti-Ro-52 and anti-Scl-70 had increased values for predicting ILD in SSc patients, while anti-Ro-52 had increased values for predicting PAH in SSc patients.

Conclusion: A commercial line immunoassay was used to evaluate the diagnostic value of autoantibodies in Chinese Han SSc patients. The results clearly indicate that the immunoblot assay has good diagnostic utility for SSc. Anti-Ro-52, anti-RP11, anti-CENPB, anti-CENPA and anti-Scl-70 are useful for diagnosing SSc in the Chinese Han population. Anti-Ro-52, anti-CENPB, anti-CENPA and anti-Scl-70 have a potential value for diagnosing ILD in SSc patients. Furthermore, anti-Ro-52 and anti-Scl-70 may be used to diagnose PAH in SSc patients.

1. Introduction

Systemic sclerosis (SSc) is a systemic connective-tissue disease (CTD) characterized by autoimmune disorders, which lead to vasculopathy, tissue fibrosis and autoantibodies [1–3]. SSc affects not only the skin [2], but also the internal organs, such as the heart [4–6], lungs [7–9] and kidneys [10–12]. Basically, cardiopulmonary involvement is the leading cause of early death in SSc, and lung involvement, including interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH), has been identified as the main cause of SSc-related death [13–15].

It has been well-documented that the autoantibodies profile could be used as key classification criteria for SSs patients [16]. The American College of Rheumatology/European League Against Rheumatism (ACR/

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Table 1
The prevalence of autoantibodies in SSc, non-SSc CTDs, HCs, and SSc with or without ILD and PAH[a].

	1.SSc	2.non-SSc CTDs	3.HCs	*P*-value[b]		4.SSc with ILD	5.SSc without ILD	*P*-value[b]	6.SSc with PAH	7.SSc without PAH	*P*-value[b]
Autoantibodies	%	%	%	1 *vs.* 2	1 *vs.* 3	%	%	4 *vs.* 5	%	%	6 *vs.* 7
Anti-Ro-52	33.13%	49.00%	6.67%	**0.004**	**0.003**	37.13%	26.27%	**0.046**	46.67%	30.00%	**0.013**
Anti-PDGFR	0.00%	0.00%	0.00%	NA	NA	0.00%	0.00%	NA	0.00%	0.00%	NA
Anti-Ku	1.88%	3.00%	0.00%	0.778	1.000	0.99%	3.39%	0.271	1.67%	1.92%	1.000
Anti-PM75	4.06%	4.00%	3.33%	1.000	1.000	4.46%	3.39%	0.863	3.33%	4.23%	1.000
Anti-PM100	1.25%	2.00%	0.00%	0.945	1.000	1.98%	0.00%	0.309	0.00%	1.54%	1.000
Anti-Th/To	1.56%	1.00%	0.00%	1.000	1.000	1.98%	0.85%	0.748	3.33%	1.15%	0.237
Anti-NOR90	2.19%	2.00%	3.33%	1.000	0.515	2.48%	1.69%	0.949	3.33%	1.92%	0.854
Anti-Fib	1.88%	0.00%	0.00%	0.370	1.000	1.98%	1.69%	1.000	1.67%	1.92%	1.000
Anti-RP155	9.06%	5.00%	0.00%	0.194	0.169	7.43%	11.86%	0.182	6.67%	9.62%	0.437
Anti-RP11	8.13%	2.00%	0.00%	**0.032**	0.208	6.44%	11.02%	0.148	8.33%	8.08%	1.000
Anti-CENPB	16.25%	2.00%	0.00%	**< 0.001**	**0.034**	9.41%	27.97%	**< 0.001**	20.00%	15.38%	0.382
Anti-CENPA	16.25%	3.00%	0.00%	**0.001**	**0.034**	10.40%	26.27%	**< 0.001**	18.33%	15.77%	0.627
Anti-Scl-70	40.63%	3.00%	0.00%	**< 0.001**	**< 0.001**	45.05%	33.05%	**0.035**	21.67%	45.00%	**0.001**
Anti-RP155 &[c] Anti-RP11	5.94%	1.00%	0.00%	0.079	0.342	4.46%	8.47%	0.142	5.00%	6.15%	0.970
Anti-CENPB &[c] Anti-CENPA	15.31%	2.00%	0.00%	**< 0.001**	**0.042**	9.41%	25.42%	**< 0.001**	18.33%	14.62%	0.471
Anti-PM75 &[c] Anti-PM100	0.31%	0.00%	0.00%	0.762	0.914	0.50%	0.00%	0.631	0.00%	0.38%	0.812

P-value < 0.05 was considered statistically significant.
[a] SSc = systemic sclerosis; CTDs = connective-tissue diseases; HCs = healthy controls; ILD = interstitial lung disease; PAH = pulmonary arterial hypertension; NA = not available.
[b] The *P*-value was calculated under Chi-square test analysis.
[c] & = combined.

EULAR) published the latest standard classification criteria for SSc in 2013 [2]. In this ACR/EULAR standard, SSc is diagnosed using an SSc score, which is basically calculated based on the autoantibodies profile (consists of anti-Scl-70, anti-centromere [CENP] and anti-RNA polymerase III) of patients. Indeed, in addition to the three autoantibodies used in the ACR/EULAR standard, a bunch of autoantibodies, such as anti-Ro-52 (TRIM21) [17], anti-Platelet Derived Growth Factor Receptor (PDGFR) [18], anti-Ku [19], anti-PMScl [20], anti-Th/To [21], anti-human Upstream Binding Factor (hUBF/NOR-90) [22], and anti-U3RNP (Fib) [23], have also been identified in SSc patients.

Although it has been well-accepted that the prevalence of SSc-associated autoantibodies varies among races [24–27], the autoantibodies profile of Chinese Han SSc patients has rarely been reported. Thus, the present study was conducted to investigate the autoantibodies profile of Chinese Han SSc patients using a commercial line immunoassay. The diagnostic utility of autoantibodies in this Chinese Han SSc population was evaluated. Furthermore, the differences in autoantibody profiles were also analyzed between SSc and non-SSc CTD patients (CTDs), between SSc and healthy controls (HCs), between SSc with ILD and SSc without ILD, and between SSc with PAH and SSc without PAH.

2. Materials and methods

2.1. Patients

The present study was approved by the Ethics Committee at Peking Union Medical College Hospital, and all participants provided an informed consent. A total of 450 subjects (HCs, $n = 30$; SSc, $n = 320$; non-SSc CTDs, $n = 100$) were recruited from Peking Union Medical College Hospital (Beijing, China), and another 21 centers in China, which included 175 SSc samples and 130 control samples from PUMCH, and 145 SSc samples from the other 21 centers. Patients were classified as diffuse cutaneous systemic sclerosis (dcSSc) and limited cutaneous systemic sclerosis (lcSSc), according to the LeRoy criteria [1]. Patients ($n = 320$, 43 males and 277 females, with a mean age of 48.20 ± 12.92 years old; 108 patients had dcSSc and 169 patients had lcSSc), were diagnosed for SSc based on the 2013 ACR/EULAR standard, diagnosed for ILD by high-resolution computed tomography, and diagnosed for PAH by right heart catheterization (PAH was diagnosed with a mean pulmonary pressure of ≥25 mmHg). Patients ($n = 20$) were diagnosed for Sjögren's syndrome (SS) according to the American-European consensus group classification criteria [28]. Rheumatoid arthritis (RA) patients ($n = 20$) were selected based on the 1987 American College of Rheumatology (ACR) revised classification criteria [29]. Systemic lupus erythematosus (SLE) patients ($n = 20$) were selected based on the 1997 ACR revised classification criteria [30]. Dermatomyositis (DM, $n = 20$) and polymyositis (PM, $n = 20$) patients were diagnosed according to Bohan and Peter criteria [31,32]. Serum samples were obtained from all 450 subjects, and stored at −80 °C until analysis.

2.2. The testing of autoantibodies

A line immunoblot assay (LIA) was conducted to analyze the profile of serum autoantibodies using a commercially available kit (Euroline Systemic Sclerosis [Nucleoli] Profile [IgG] kit) obtained from Euroimmun AG (Lübeck, Germany). The SSc autoantibody profile consisted of anti-Ro-52, anti-PDGFR, anti-Ku, anti-PM75, anti-PM100, anti-Th/To, anti-NOR90, anti-Fib, anti-RP155, anti-RP11, anti-CENPB, anti-CENPA and anti-Scl-70. The line immunoblot assay was performed according to manufacturer's instructions. The results (negative, borderline, positive and strong positive) were interpreted using the EUROScan software (Euroimmun AG, Lübeck, Germany). The borderline was considered as negative in the present study.

2.3. Statistical analysis

Data were analyzed by SPSS version 19.0 for Windows (SPSS Inc., Chicago, IL, USA). The frequencies of categorical variables were compared using Pearson χ^2 or Fisher's exact test, when appropriate. The sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV), positive likelihood ratio (LR+) and negative likelihood ratio (LR-) were calculated, accordingly. A *P*-value < .05 was considered statistically significant.

3. Results

3.1. Prevalence of Autoantibodies in SSc, Non-SSc CTDs, HCs, and SSc with or without ILD and PAH

As shown in Table 1, SSc patients had increased prevalence of anti-RP11 (8.13% *vs.* 2%, P = 0.032), anti-CENPB (16.25% *vs.* 2%, P < 0.001), anti-CENPA (16.25% *vs.* 3%, P = 0.001) and anti-Scl-70 (40.63% *vs.* 3%, P < 0.001), but had decreased prevalence of anti-Ro-52 (33.13% *vs.* 49%, P = 0.004), when compared to non-SSc CTDs. Furthermore, SSc patients had increased prevalence of anti-Ro-52 (33.13% *vs.* 6.67%, P = 0.003), anti-CENPB (16.25% *vs.* 0%, P = 0.034), anti-CENPA (16.25% *vs.* 0%, P = 0.034) and anti-Scl-70 (40.63% *vs.* 0%, P < 0.001), when compared to HCs. In addition, SSc patients had increased prevalence of anti-CENPA combined anti-CENPB (15.31% *vs.* 2%, P < 0.001; 15.31% *vs.* 0%, P = 0.042), when compared to non-SSc CTDs and HCs, respectively. No difference (P > 0.05) was observed on anti-PDGFR, anti-Ku, anti-PM75, anti-PM100, anti-Th/To, anti-NOR90, anti-Fib, and anti-RP155 among the three groups.

Furthermore, SSc patients with ILD (n = 202) had an increased prevalence of anti-Ro-52 (37.13% *vs.* 26.27%, P = 0.046) and anti-Scl-70 (45.05% *vs.* 33.05%, P = 0.035), but had a decreased prevalence of anti-CENPB (9.41% *vs.* 27.97%, P < 0.001), anti-CENPA (10.4% *vs.* 26.27%, P < 0.001) and anti-CENPB combined anti-CENPA (9.41% *vs.* 25.42%, P < 0.001), when compared to SSc patients without ILD (n = 118). In addition, SSc patients with PAH (n = 60) had an increased prevalence of anti-Ro-52 (46.67% *vs.* 30%, P = 0.013) and a decreased prevalence of anti-Scl-70 (21.67% *vs.* 45%, P = 0.001), when compared to SSc patients without PAH (n = 260). No difference (P > 0.05) was observed on anti-PDGFR, anti-Ku, anti-PM75, anti-PM100, anti-Th/To, anti-NOR90, anti-Fib, anti-RP155 and anti-RP11 among the three groups.

3.2. Autoantibodies in Distinguishing SSc from Non-SSc CTDs

As illustrated in Table 2, for distinguishing SSc from non-SSc CTDs, high sensitivities were recorded on anti-Ro-52 (34.19%) and anti-Scl-70 (40.63%). The specificities of anti-Ro-52 and anti-Scl-70 were 51% and 97%, respectively. Anti-Scl-70 had the highest LR+ (13.54), when compared with the other autoantibodies. The third highest sensitivities were recorded on anti-CENPB and anti-CENPA (16.25%). Anti-CENPB combined anti-CENPA also exhibited a high sensitivity (15.31%) and high LR+ (7.66). For anti-RP155, the sensitivity, specificity, PPV and NPV were 9.06%, 95%, 85.29% and 24.61%, respectively. For anti-RP11, the sensitivity, specificity, PPV and NPV were 8.13%, 98%, 92.86% and 25%, respectively. For the other measured autoantibodies, although high specificities (> 95%) were recorded, the sensitivities remained low (< 5%). Moreover, anti-RP11, anti-CENPB, anti-CENPA, anti-Scl-70, anti-PM75 and anti-PM100, anti-RP155 and anti-RP11, and anti-CENPB and anti-CENPA exhibited with high PPV, demonstrating that these autoantibody tests had good accuracy.

Table 2
Evaluation of autoantibodies in distinguishing SSc from non-SSc CTDs[a].

Autoantibodies	SEN	SPE	PPV	NPV	LR+	LR-
Anti-Ro-52	**34.19%**	**51.00%**	68.39%	20.00%	0.70	1.29
Anti-PDGFR	0.00%	100.00%	NA	23.81%	NA	1.00
Anti-Ku	1.88%	97.00%	66.67%	23.60%	0.62	1.01
Anti-PM75	4.06%	96.00%	76.47%	23.82%	1.02	1.00
Anti-PM100	1.25%	98.00%	66.67%	23.67%	0.62	1.01
Anti-Th/To	1.56%	99.00%	83.33%	23.91%	1.56	0.99
Anti-NOR90	2.19%	98.00%	77.78%	23.84%	1.09	1.00
Anti-Fib	1.88%	100.00%	100.00%	24.15%	NA	0.98
Anti-RP155	9.06%	95.00%	85.29%	24.61%	1.81	0.96
Anti-RP11	8.13%	98.00%	92.86%	25.00%	4.06	0.94
Anti-CENPB	16.25%	98.00%	96.30%	26.78%	8.12	0.85
Anti-CENPA	16.25%	97.00%	94.55%	26.58%	5.42	0.86
Anti-Scl-70	**40.63%**	**97.00%**	97.74%	33.80%	**13.54**	0.61
Anti-PM75 &[b] Anti-PM100	0.31%	100.00%	100.00%	23.87%	NA	1.00
Anti-RP155 &[b] Anti-RP11	5.94%	99.00%	95.00%	24.75%	5.94	0.95
Anti-CENPB &[b] Anti-CENPA	15.31%	98.00%	96.08%	26.56%	7.66	0.86

Anti-Scl-70 performed the highest sensitivity and LR+, and anti-Ro-52 performed the second sensitivity.

[a] SSc = systemic sclerosis; CTDs = connective-tissue diseases; SEN = sensitivity; SPE = specificity; PPV = positive predictive value; NPV = negative predictive value; LR+ = positive likelihood ratio; LR- = negative likelihood ratio; NA = not available.

[b] & = combined.

3.3. Autoantibodies in predicting ILD and PAH in SSc

Table 3 clearly shows that anti-Ro-52 (sensitivity, 37.13%; specificity, 73.73%) and anti-Scl-70 (sensitivity, 45.05%; specificity, 66.95%) had increased values for predicting ILD in SSc patients, when compared to the other measured autoantibodies (sensitivity < 10.4%). As presented in Table 3, anti-Ro-52 (sensitivity, 46.67%; specificity, 70%) had elevated values for predicting PAH in SSc patients, when compared to the other measured autoantibodies (sensitivity < 21.67%).

3.4. Prevalence of Autoantibodies in dcSSc and lcSSc

As shown in Supplementary Table 1, 108 (33.75%) patients had dcSSc, 169 (52.81%) patients had lcSSc, and some data (43, 13.44%) on SSc clinical subsets were missing from the medical records due to the retrospective nature of the present study. DcSSc patients (n = 108) had an increased prevalence of anti-Scl-70 (48.15% *vs.* 33.73%, P < 0.001), but had a decreased prevalence of anti-CENPB (5.56% *vs.* 24.85%, P < 0.001), anti-CENPA (4.63% *vs.* 25.44%, P < 0.001) and anti-CENPB combined anti-CENPA (4.63% *vs.* 24.26%, P < 0.001), when compared to lcSSc patients (n = 169). No difference (P > 0.05) was observed on other autoantibodies between dcSSc and lcSSc.

3.5. Coexistence of Autoantibody in SSc

As shown in Supplementary Table 2, 96.87% (310/320) of SSc patients had at least one positive autoantibody, and 15.62% (50/320) of SSc patients were negative for all the tested autoantibodies. The most common coexisting autoantibodies were anti-CENPA and anti-CENPB (49/320), followed by anti-Ro-52 and anti-Scl-70 (31/320). Anti-Scl-70 was the most frequent monospecific autoantibody (85/320), and anti-PDGFR, anti-Th/To and anti-CENPA alone did not appear to be positive. In addition, 63.43% (203/320) of SSc patients were positive for at least one of the three autoantibodies (anti-Scl-70, anti-centromere and anti-RNA polymerase III) used in the ACR/EULAR 2013 standard (two patients were positive for all three autoantibodies).

4. Discussion

The present study is the first to report the autoantibody profile of the Chinese Han SSc population based on line immunoblot technology. Anti-Scl-70, which was the most frequent autoantibody in the present study, presented in 40.63% (130/320) of SSc patients. The major autoantigen of anti-Scl-70 was topoisomerase I (topo I), which is a 70-kDa protein [33]. Similar to the present results, it was reported that the prevalence of anti-Scl-70 ranged from 30.9% to 32.3% in Asia [27,34,35]. In addition, the highest LR+ (the ratio of the true positive rate to the false positive rate) was recorded on anti-Scl-70. Thus, the correctness of the immunoblot test for anti-Scl-70 determines that the positive possibility is the multiple probabilities of a false positive judgment. A study reported that when SSc and non-SSc CTDs were compared, the sensitivity, specificity, LR+ and LR- of aiti-Scl-70 were

Table 3
Evaluation of autoantibodies in predicting ILD and PAH in SSc[a].

	SSc with ILD *vs.* SSc without ILD						SSc with PAH *vs.* SSc without PAH					
Autoantibodies	SEN	SPE	PPV	NPV	LR+	LR-	SEN	SPE	PPV	NPV	LR+	LR-
Anti-Ro-52	**37.13%**	**73.73%**	70.75%	40.65%	1.41	0.85	**46.67%**	70.00%	26.42%	85.05%	1.56	0.76
Anti-PDGFR	0.00%	100.00%	NA	36.88%	NA	1.00	0.00%	100.00%	NA	81.25%	NA	1.00
Anti-Ku	0.99%	96.61%	33.33%	36.31%	0.29	1.02	1.67%	98.08%	16.67%	81.21%	0.87	1.00
Anti-PM75	4.46%	96.61%	69.23%	37.13%	1.31	0.99	3.33%	95.77%	15.38%	81.11%	0.79	1.01
Anti-PM100	1.98%	100.00%	100.00%	37.34%	NA	0.98	0.00%	98.46%	0.00%	81.01%	0.00	1.02
Anti-Th/To	1.98%	99.15%	80.00%	37.14%	2.34	0.99	3.33%	98.85%	40.00%	81.59%	2.89	0.98
Anti-NOR90	2.48%	98.31%	71.43%	37.06%	1.46	0.99	3.33%	98.08%	28.57%	81.47%	1.73	0.99
Anti-Fib	1.98%	98.31%	66.67%	36.94%	1.17	1.00	1.67%	98.08%	16.67%	81.21%	0.87	1.00
Anti-RP155	7.43%	88.14%	51.72%	35.74%	0.63	1.05	6.67%	90.38%	13.79%	80.76%	0.69	1.03
Anti-RP11	6.44%	88.98%	50.00%	35.71%	0.58	1.05	8.33%	91.92%	19.23%	81.29%	1.03	1.00
Anti-CENPB	9.41%	72.03%	36.54%	31.72%	0.34	1.26	20.00%	84.62%	23.08%	82.09%	1.30	0.95
Anti-CENPA	10.40%	73.73%	40.38%	32.46%	0.40	1.22	18.33%	84.23%	21.15%	81.72%	1.16	0.97
Anti-Scl-70	**45.05%**	**66.95%**	70.00%	41.58%	1.36	0.82	21.67%	55.00%	10.00%	75.26%	0.48	1.42

Anti-Ro-52 (sensitivity, 37.13%; specificity, 73.73%) and anti-Scl-70 (sensitivity, 45.05%; specificity, 66.95%) had increased values for predicting ILD in SSc patients.

[a] SSc = systemic sclerosis; ILD = interstitial lung disease; PAH = pulmonary arterial hypertension; SEN = sensitivity; SPE = specificity; PPV = positive predictive value; NPV = negative predictive value; LR+ = positive likelihood ratio; LR- = negative likelihood ratio; NA = not available.

26%, 99.5%, 52 and 1.5, respectively [36]. Together with the present results (anti-Scl-70: sensitivity, 40.63%; specificity, 97%; LR+, 13.54), it would be safe to propose anti-Scl-70 as a potential factor for diagnosing SSc. In addition, in the present study, SSc patients had increased prevalence of anti-Scl-70, when compared to both non-SSc CTDs and HCs. In addition, dcSSc patients had increased anti-Scl-70, when compared to lcSSc, which is similar to those repeatedly reported by many studies [24]. As for the clinical manifestations, the prevalence of anti-Scl-70 was significantly higher in SSc patients with ILD, when compared to patients without ILD. This is similar to existing publications [37]. Interestingly, the prevalence of anti-Scl-70 was significantly lower in SSc patients with PAH, when compared to patients without PAH. To our knowledge, very limited information could be found with regard to the potential use of anti-Scl-70 for PAH diagnosis in SSc patients. However, the associations between autoantibodies and clinical manifestations may be different according to the various methods used. Hence, further studies are needed.

In the present study, following anti-Scl-70, anti-Ro-52 was the second frequent antibody found in SSc patients. Although this was not specific for SSc, it also occurred in non-SSc CTDs, including Sjögren's syndrome, systemic lupus erythematosus, rheumatoid arthritis and so on [38,39]. According to these present results, all autoantibodies, except anti-Ro-52, had high specificities when using other CTD comparators. These results are consistent with a literature [27]. In that literature, anti-Ro-52 was proven to be associated with pulmonary involvement in SSc [35]. Similarly, the present results indicate that anti-Ro-52 is effective in diagnosing PAH in SSc patients.

Anti–RNA polymerase III is an autoantibody associated with renal crisis in SSc patients [40]. In the present analysis, merely nine of 320 SSc patients developed renal crisis (four of them were RP155 positive and three of them were RP11 positive). Both RP155 and RP11 are subunits of human RNA Polymerase III. Interestingly, merely anti-RP11 was statistically valuable in distinguishing SSc from non-SSc CTDs, and both anti-RP155 and anti-RP11 were not significantly different between SSc and HCs.

Anti-CENPB and anti-CENPA were present in 16.25% (52/320) of the SSc patients, and 49 of them coexisted in the present study. Both CENPB and CENPA were centromere protein subunits. In a large Canadian cohort, anti-CENPB and anti-CENPA exhibited similar clinical profiles with the anticentromere antibody (ACA) [17]. The prevalence of anti-CENP was very different among races. For example, 7% was reported in Thailand [24], 22.1% was reported in Singapore [27], and 36.9% was reported in Japan [34]. It has been reported that in comparing SSc and non-SSc CTDs, the sensitivity, specificity, LR+, LR- of ACA were 31%, 97%, 12.5 and 0.7, respectively [36]. In the present study, sensitivity and LR+ were lower. Furthermore, SSc patients had increased prevalence of anti-CENPB, anti-CENPA, and anti-CENPB combined anti-CENPA, when compared to non-SSc CTDs and HCs. These results suggest anti-CENPB and anti-CENPA are useful for SSc identification. Furthermore, lcSSc patients had increased anti-CENPB and anti-CENPA when compared to dcSSc, which is similar to those repeatedly reported by many studies [25]. The prevalence of anti-CENPB and anti-CENPA were significantly lower in SSc patients with ILD, when compared to patients without ILD. It is noteworthy that although anti-CENPB and anti-CENPA have been repeatedly reported in SSc-associated PAH, in the present study, no distinctions were observed in anti-CENPB and anti-CENPA between SSc patients with PAH and SSc patients without PAH.

Danilo et al. [41] found that SSc-associated autoantibodies were usually mutually exclusive. Interestingly, the present results revealed that the coexistence of autoantibodies was common in the Chinese Han SSc population. In addition, it was found that anti-CENPA and anti-CENPB were the most common coexisting autoantibodies. Furthermore, similar results were previously reported [41].

In the present study, a commercial line immunoassay was used to evaluate the diagnostic value of autoantibodies in Chinese Han SSc patients. According to the literature [27], both enzyme linked immunosorbent assay (ELISA) and the immunoblot assay are very reliable methods for selecting antibodies. These results clearly indicate that the immunoblot assay, which has been widely accepted as a technology for antibody analysis, has good diagnostic utility for SSc [27]. Since the immunoblot assay was cheaper than ELISA and as effective as ELISA, this test was cost effective. According to our findings, we think anti-Scl-70, anti-CENPB and anti-CENPA may be suitable for clinical use.

However, there were some limitations in the present study. First, due to the retrospective nature of this study, the autoantibody profile of SSc patients were not followed-up or repeated, and the changes in serum antibody conversion and clinical characteristics could not be provided. Second, other techniques to confirm the level of autoantibodies were not used.

This could be concluded as follows: (1) anti-Ro-52, anti-RP11, anti-CENPB, anti-CENPA and anti-Scl-70 are useful for diagnosing SSc in the Chinese Han population; (2) anti-Ro-52, anti-CENPB, anti-CENPA and anti-Scl-70 have potential value for diagnosing ILD in SSc patients; (3) anti-Ro-52 and anti-Scl-70 may be used to diagnose PAH in SSc patients; (4) the coexistence of autoantibodies in SSc is common.

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Conflicts of interest

The authors have no conflicts of interest to declare.

Author contributions

CL and YH contributed equally to the present study. YL conceived the project. CL performed the experiments and wrote the manuscript. YH designed the experiment and revised the manuscript. YY and DX provided help in the language. LL, JL and XW collected the samples. XZ and FZ provided the valuable discussion.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.01.020.

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