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RESEARCH ARTICLE

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Analysis of antinuclear antibody titers and patterns by using HEp-2 and primate liver tissue substrate indirect immunofluorescence assay in patients with systemic autoimmune rheumatic diseases

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Abstract

Background: Indirect immunofluorescence assay (IIFA) is viewed as a preliminary standard to assess antinuclear antibodies (ANAs). Our aim was to explore ANA positivity rate, titers, and patterns in patients with systemic autoimmune rheumatic diseases (SARD), including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), primary Sjögren's syndrome (pSS), systemic sclerosis (SSc), and mixed connective tissue disease (MCTD), compared with healthy controls (HC).

Methods: Assess antinuclear antibody titers and patterns were retrospectively identified and compared by IIFA using human epithelial cells (HEp-2) and primate liver tissue substrate according to international consensus in SARD. Serum complement 3 (C3), C4, and immunoglobulin G were compared among subgroups with different ANA titers. The positive predictive values (PPV) for different ANA titers were calculated.

Results: There were a total of 3510 samples, including 2034 SLE, 973 RA, 155 SSc, 309 pSS, and 39 MCTD cases. There was no difference in age between HC and SARD, excluding RA. ANA positivity rate in SARD and HC was 78.7% and 12.2%, respectively. A titer of ≥1:320 revealed a PPV of 84.0% in SARD. SLE patients with ANA titers ≥1:320 had significantly lower levels of C3 and C4. AC-4 (31.2%) was the major pattern in patients with SARD, followed by AC-5 (23.9%) and AC-1 (18.8%). SLE mostly presented with AC-4 (30.3%). Several mixed patterns provided a significant hint for SSc and SLE. The major pattern in HC was AC-2 (12.2%).

Conclusions: Assess antinuclear antibody positivity, titers, and patterns display differences in various SARD, contributing to the classification of SARD.

Qiujing Wei and Yutong Jiang should be considered joint first author.

KEYWORDS
antinuclear antibodies, autoimmune diseases, Sjögren's syndrome, systemic lupus erythematosus, systemic sclerosis

1 | INTRODUCTION

Antinuclear antibodies (ANAs) work as a critical biomarker in the diagnosis and differential diagnosis, disease monitoring, and efficacy observation in systemic autoimmune rheumatic diseases (SARD).[1] ANA screening is a standard and economical test used for rheumatologic and non-rheumatologic diseases,[2] with high sensitivity in systemic lupus erythematosus (SLE), primary Sjögren's syndrome (pSS), systemic sclerosis (SSc), and mixed connective tissue disease (MCTD).[3] Other autoimmune diseases, including rheumatoid arthritis (RA), and various non-rheumatologic conditions, for example, chronic infection and healthy individuals, can exhibit a positive ANA.[4]

Antinuclear antibodies can be evaluated by using several techniques,[5] with the indirect fluorescence assays (IIFA) using human epithelial cells (HEp-2) regularly regarded as the gold standard. The primate liver not only contributes to the confirmation of results between the two substrates but also helps to establish titer levels as well. Staining patterns and titers can be determined by skilled examiners using this method.[6] In order to unify the classification and interpretation of the fluorescence intensity and staining patterns of antibodies, the International Consensus on Antinuclear Antibody Pattern (ICAP) defined and described the patterns in detail online (www.ANApatterns.org). IIFA has the value assessing both the titers and the fluorescence patterns of the autoantibodies, which display consistency with clinical relevance.[7]

Previous studies have demonstrated that positive ANA could exist not only in diverse patient populations but also in the general population as well.[8] Elevated ANA titers provide a hint for SARD and imply more likelihood to detect autoantigens in follow-up testings.[9] Nevertheless, some laboratories lack essential techniques and reasonable interpretation of the results. ANA testing is suggested to be performed in patients with clinically suspected SARD, especially in patients with multiple organ involvement. It is also recommended for screening for healthy people with high risks, such as women of childbearing age, family members of SARD patients, and those with abnormal immune function.[10]

Antinuclear antibodies testing is an affordable test and helps the clinicians to distinguish SARD by ordering further autoantibody tests for possible patients; however, the information which ANA patterns and titers could provide has not been explored thoroughly in SARD patients as it is a group of diseases with a relatively low prevalence. Being significant indicators for monitoring disease status of SARD, serum complement 3 (C3), C4, and immunoglobulin G (IgG) have been reported to relate to the production of ANA.[11] The objective of this study was to investigate (a) the performance of ANA positivity and ANA patterns in SARD patients and healthy individuals; (b) the relationship between C3, C4, IgG, and ANA titers; and (c) the positive predictive values (PPV) for different levels of ANA titers in patients with SARD.

2 | MATERIALS AND METHODS

2.1 | Study population

Blood samples of SARD patients examined at the rheumatology laboratory of the Third Affiliated Hospital of Sun Yat-sen University were collected from 2016 to 2018. The diagnosis of SARD was confirmed by the rheumatologists of our hospital following each classification criteria of the diseases, involving SLE,[12] RA,[13] pSS,[14] SSc,[15] and MCTD.[16] Patients with overlap syndrome, tumor, and pregnancy were excluded. Information on the participants' age, sex, and diagnosis was acquired.

Healthy individuals were recruited from among the employees of our hospital. People with confirmed SARD or SARD-related symptoms such as chronic fever of unknown reasons, persistent joint pain, oral ulcer and skin rash, other chronic systemic diseases, other immunological diseases, tumor, acute or chronic infection, and family history of SARD were excluded. The participants were informed of our study and completed informed consent forms. The study was approved by the ethics committee of our hospital and performed under the ethical standards in the Declaration of Helsinki.

2.2 | ANA testing

Indirect fluorescence assays using HEp-2 and monkey liver tissue substrate (EUROIMMUN) was applied after the serum samples were diluted into 1:100, 1:320, 1:1000, and 1:3200. Serum diluted by phosphate-buffered saline (PBS) was overlaid onto fixed HEp-2 cells and substrate for half an hour at room temperature as indicated.[17] Wash the slides twice with PBS, then overlay them with fluorescence-labeled anti-human globulin, and incubate for half an hour. After washing twice again with PBS, place the embedding medium onto a cover glass. All the procedures were completed under the manufacturers' protocol by three experienced technicians, and then, the slides were read by two experts using a fluorescence microscope (EUROStar III Plus; EUROIMMUN) at ×40 power. ANA would be reported positive if the fluorescent signal was noticed with a serum dilution ratio of no less than 1:100. The fluorescence patterns were differentiated following the recommendations of ICAP[18] and stated previously.[19] The mixed patterns in our study referred to the existence of two or more patterns, while the other patterns involved the AC-22, AC-23, AC-24, AC-25, and AC-29 patterns in the current study. The levels of serum C3, C4, and IgG were measured

using immunoscattering turbidimetry by the automatic biochemical analyzer (HITACHI 7600-020) in patients with SLE.

2.3 | Statistical analysis

First of all, we conducted a descriptive analysis of the participants. Continuous variables were stated using mean ± SD or the median value (interquartile range) according to whether they met normal distribution. The participants were divided into subgroups. Intergroup comparisons were performed in subgroups based on various SARD and levels of ANA titers by using the independent t test, the Mann-Whitney U test, and the chi-square test. Non-continuous data were represented by a percentage (rate). The PPV for specific ANA titers were studied as described.[20] They were calculated as the number of patients divided by that of the entire participants. Statistical significance was set at two-sided $P < .05$. The IBM Statistical Package for Social Sciences (SPSS, version 20) software was employed for data analyses.

3 | RESULTS

3.1 | Demographic variables of the participants

There were 3510 cases with a confirmed diagnosis of SARD, involving 2034 patients with SLE, 973 patients with RA, 309 patients with pSS, 155 patients with SSc, and 39 patients with MCTD included in the study (Table 1). 3088 (88.0%) of the patients were females. All the participants were Chinese. The mean age of the study population was 41.5 ± 9.0 years old. 723 (20.6%) of the participants were naïve to treatment. There was no difference in age between HC and SARD patients, except for RA.

3.2 | The ANA positivity rate in SARD and HC

Positive ANA (a titer ≥ 1:100) was observed in 2762 (78.7%) SARD patients, which was mainly observed in 92.3% of MCTD, 90.7% of SLE, and 86.4% of pSS patients (Table 2). The rate of ANA positivity was significantly lower in RA (48.8%) compared to other SARD ($P < .001$). Comparatively, ANA positivity was seen in 131 (12.2%) healthy individuals.

3.3 | Different levels of ANA titers in various SARD and HC

The median value of ANA titers was 1:320 in SARD except for RA, while an ANA titer was majorly less than 1:100 in HC (Figure 1). 83.6% of RA patients had a titer less than 1:100 in the current study. The median ANA titer in MCTD patients was significantly higher than that seen in other SARD ($P < .001$). ANA titers in RA were comparatively lower than other SARD but still higher than those of HC ($P < .001$). 43.4% (1522/3510) of SARD patients had a titer ≥1:320.

3.4 | The comparison of C3, C4, and IgG among SLE patients with different ANA titers

To further evaluate ANA and immune indicators, we examined ANA, serum C3, C4, and IgG simultaneously in 2034 cases of SLE. The levels of C3, C4, IgG, and ANA titers were compared and analyzed in SLE patients with different levels of ANA titers. We found that serum IgG was elevated, while C3 and C4 values were decreased significantly as ANA titers went up (Table 3). When an ANA titer was higher than 1:320, the levels of C3 and C4 became significantly inferior to those of the subgroup with negative ANA ($P < .001$).

3.5 | The PPV of ANA titers in SLE and SARD excluding RA

The PPV of a ANA titer of ≥1:100 was 51.5%, ≥1:320 was 67.0%, ≥1:1000 was 70.9%, and ≥1:3200 was 74.3% in SLE patients. The PPV of a titer of ≥1:100 was 63.8%, ≥1:320 was 84.0%, ≥1:1000

TABLE 1 Demographic and clinical variables of patients with SARD

	SARD	SLE	RA	pSS	SSc	MCTD
Number	3510	2034	973	309	155	39
Age (years)	41.5 ± 9.0	34.1 ± 12.7	50.4 ± 14.2	44.7 ± 14.5	43.2 ± 15.4	36.0 ± 12.1
Sex (Female), n (%)	3273 (93.2)	1845 (90.7)	805 (82.7)	281 (90.9)	120 (77.4)	37 (94.8)
Disease duration (years)	5.0 ± 2.1	3.9 ± 2.4	8.3 ± 5.6	3.2 ± 1.3	4.6 ± 2.1	3.4 ± 1.3
Treatment (n, %)						
Naïve to treatment	723 (20.6)	318 (15.6)	309 (31.8)	52 (16.8)	33 (21.3)	11 (28.2)
Glucocorticoids (ever)	2353 (67.0)	1702 (83.7)	320 (32.9)	189 (61.1)	115 (74.2)	27 (69.2)
DMRADs (ever)	2454 (69.9)	1653 (81.3)	593 (60.9)	104 (33.7)	83 (53.5)	21 (53.8)

Note: Data with normal distribution were represented using mean ± SD.

Abbreviations: DMARDs, disease-modifying antirheumatic drugs; MCTD, mixed connective tissue disease; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SARD, systemic autoimmune rheumatic diseases; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

TABLE 2 ANA positivity and ANA titers in SARD patients and healthy individuals

Diseases	ANA titers				
	Negative (<1:100)	≥1:100 (and < 1:320)	≥1:320 (and < 1:1000)	≥1:1000 (and < 1:3200)	≥1:3200
SLE	190 (9.3)	758 (37.3)	433 (21.3)	286 (14.1)	367 (18.0)
RA	498 (51.2)	315(32.4)	87 (8.9)	41 (4.2)	32 (3.3)
pSS	42 (13.6)	125 (40.5)	59 (19.1)	38 (12.3)	45 (14.6)
SSc	15 (9.7)	39 (25.2)	35 (22.6)	35 (22.6)	31 (20.0)
MCTD	3 (7.7)	3 (7.7)	7 (17.9)	11 (28.2)	15 (38.5)
HC	942 (87.8)	119(11.1)	10 (0.9)	2 (0.2)	0

Note: Data were expressed as number (percentage).

Abbreviations: ANA, antinuclear antibodies; HC, healthy controls; MCTD, mixed connective tissue disease; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SARD, systemic autoimmune rheumatic diseases; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

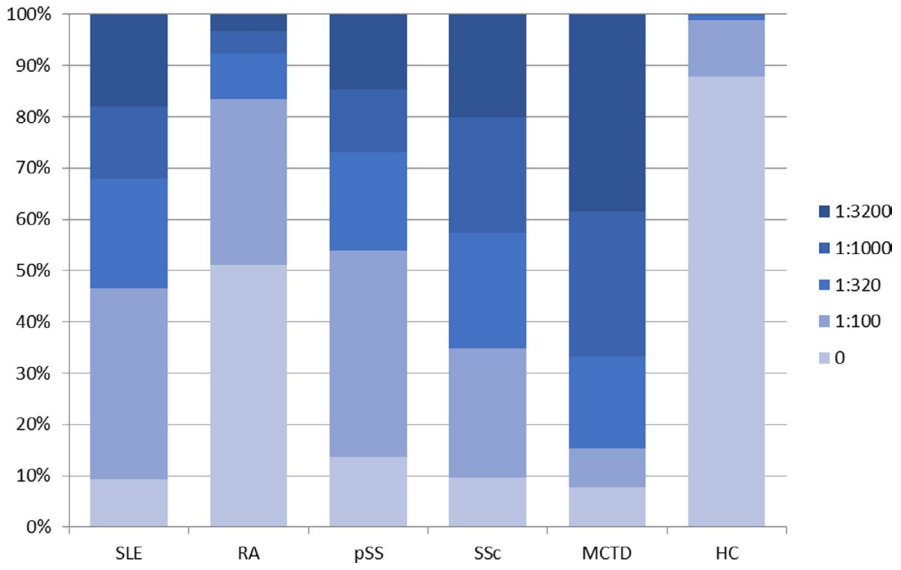

FIGURE 1 ANA titers determined by IIFA in SARD and HC. Data were presented as percentage. ANA, antinuclear antibodies; HC, healthy controls; IIFA, the indirect immunofluorescence assay; MCTD, mixed connective tissue disease; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SARD, systemic autoimmune rheumatic diseases; SLE, systemic lupus erythematosus; SSc, systemic sclerosis

was 89.9%, and ≥1:3200 was 92.5% in SARD patients excluding RA, which is not an ANA-associated disease. Using higher-titer cutoffs yielded slightly higher PPVs, but a titer of ≥1:320 revealed the PPV of 84.0% in SARD except for RA could function as a threshold to differentiate SARD from HC.

3.6 | The ANA patterns in SARD and HC

We described the ANA patterns observed in various SARD and healthy population (Table 4). The most frequent ANA pattern seen in SARD was AC-4 with a percentage of 31.2, followed by AC-5 with a percentage of 23.9% and AC-1 with a percentage of 18.8% (Figure 2). The most frequent ANA pattern observed in MCTD was AC-5 (82.1%). The most frequent pattern in SSc was AC-8/9, seen in 35.5% (55/155) of SSc patients, which was not found in patients with MCTD. 2.8% (100/3510) of the patients exhibited the mixed patterns, while 0.7% (23/3510) exhibited the other patterns. In contrast, the majority (87.8%) of HC had negative ANA, followed by the AC-2 pattern (5.9%), and the percentage was significantly higher than that of SARD ($P < .001$).

In the cases with AC-1 positivity, SLE (26.7%) accounted for the main proportion compared to other diseases, while AC-2 pattern majorly consisted of HC (87.2%) (Figure 3). AC-3 was primarily found in SSc (56.8%), and AC-4 was commonly observed in pSS (43.8%) (Figure 4). AC-5 positivity majorly contained MTCD (64.9%), while AC-6 frequently existed in SLE (61.5%) and RA (38.5%). AC-8/9 (78.3%) and AC-11/12 (43.0%) were mostly observed in SSc, while AC-15 and AC-19 were exclusively found in patients with SLE in the current study. 20% of SLE patients who exhibited AC-15 and AC-21 positivity had current hepatic involvement characterized by impaired liver function. 59% of pSS displayed AC-21 positivity. The mixed patterns provided a hint for SSc (43.4%) and SLE (27.8%), while the other patterns could be found in patients with SSc (33.5%), pSS (25.2%), RA (21.3%), and SLE (12.8%), but on in MCTD (0%).

4 | DISCUSSION

Antinuclear antibodies screening is a recommended gold test during the diagnosis of SARD. Given the featured location of target autoantigens which function as a clue to specific autoantigens, the fluorescence patterns observed in IIFA may shed light on the specificity of ANA.[6] We firstly reported the titers and the ANA pattern characteristics involving a large SARD population, revealing different SARD having unique ANA patterns.

Recognition of particular patterns may help the diagnosis of certain autoimmune diseases.[21] We found that ANA positivity was much more frequently seen in SARD cases (78.7%), especially in MCTD (92.3%) and SLE (90.7%), compared to healthy individuals (12.2%). The fluorescence pattern of ANA provides additional diagnostic information. For example, the AC-4 pattern was commonly seen in pSS, and the AC-5 pattern was majorly seen in MTCD. AC-8/9 and AC-11/12 patterns were often found in SSc. Moreover, AC-15 and AC-21 patterns can provide information for liver involvement.[19] These two patterns were also found in SLE patients who had hepatic involvement. Our findings could aid to clarify the role of IIFA patterns in differentiating various SARD.

Antinuclear antibodies titers may also contribute to the classification of SARD. ANA titers in RA were comparatively inferior to the titers of other SARD. Besides, a titer of <1:320 reduces the possibility of the diagnosis of treatment-naïve SARD, except for RA, based on the PPV and the experience of our rheumatologists and technicians. What's more, we found that an ANA titer ≥1:320 correlated with low levels of C3 and C4. One of the reasons could be that the combination of excessive immune complexes and complement lead to the consumption of C3 and C4. Since the levels of C3, C4, and IgG are closely related to the production of ANA, they have also become significant indicators for monitoring disease status of SARD.[11] A recent study also stated that ANA's titer higher than 1:320 was predictive of the diagnosis of connective tissue disease (OR = 14.4).[22] Setting cutoffs for ANA testing is comparatively difficult for SARD, as most healthy individuals have negative ANA. By using a cohort of patients with SARD, we can establish diagnostic cutoff values that may be more beneficial to identify patients. To be mentioned, the cutoff titers vary between laboratories depending on the microscope equipment and technicians' experience. Each laboratory needs to set up its own cutoff values based on the 95th percentile of healthy blood donors or, as in this case, a patient cohort.

Because HEp-2 cell lines obtained from cultured human laryngeal epithelial carcinoma have high sensitivity rates to the presence

TABLE 3 The comparison of C3, C4, and IgG in SLE patients with different ANA titers

ANA titers	N	C3	C4	IgG (g/L)
1:100	758	0.86 ± 0.31	0.19 ± 0.07	14.25 ± 4.00
1:320	433	0.75 ± 0.32	0.19 ± 0.06	16.07 ± 4.97
1:1000	286	0.62 ± 0.30	0.17 ± 0.07	18.67 ± 3.85
1:3200	367	0.53 ± 0.21	0.16 ± 0.09	18.17 ± 4.06
Negativity	190	0.95 ± 0.24	0.22 ± 0.07	12.57 ± 3.00
F value		114.09	35.18	119.57
P value		<.001**	<.001**	<.001**

Note: Data with normal distribution were represented using mean ± SD. Chi-square was employed for comparing multiple groups.

Abbreviations: C3, complement 3; C4, complement 4; IgG, immunoglobulin G; SLE, systemic lupus erythematosus.

**$P < .001$.

TABLE 4 The ANA patterns in SARD patients and healthy individuals

Group		SLE	RA	pSS	SSc	MCTD	HC
ANA pattern	AC-1	415 (20.4)	198 (20.3)	34 (11.0)	24 (15.5)	3 (7.7)	16 (1.5)
	AC-2	5 (0.2)	6 (0.6)	0	0	0	63 (5.9)
	AC-3	23 (0.1)	16 (1.6)	13 (4.2)	21 (13.6)	1 (2.6)	8 (0.8)
	AC-4	617 (30.3)	187 (19.2)	155 (50.2)	20 (12.9)	0	20 (1.9)
	AC-5	536 (26.4)	28 (2.9)	32 (10.4)	7 (4.5)	32 (82.1)	2 (0.2)
	AC-6	10 (0.5)	3 (0.3)	0	0	0	0
	AC-8/9	50 (2.6)	22 (2.3)	12 (3.9)	55 (35.5)	0	13 (1.2)
	AC-11/12	9 (0.4)	2 (0.2)	3 (1.0)	2 (1.3)	0	1 (0.1)
	AC-15	3 (0.2)	0	0	0	0	0
	AC-19	75 (3.7)	0	0	0	0	0
	AC-21	13 (0.6)	2 (0.2)	5 (1.7)	0	0	3 (0.3)
	Mixed	78 (3.8)	3 (0.3)	10 (3.2)	9 (5.8)	0	2 (0.2)
	Other	10 (0.5)	8 (0.8)	3 (1.0)	2 (1.3)	0	3 (0.3)
ANA negativity		190 (9.3)	498 (51.2)	42 (13.6)	15 (9.7)	3 (7.7)	942 (87.8)

Note: Data were expressed as number (percentage).

Abbreviations: ANA, antinuclear antibodies; HC, healthy controls; MCTD, mixed connective tissue disease; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SARD, systemic autoimmune rheumatic diseases; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

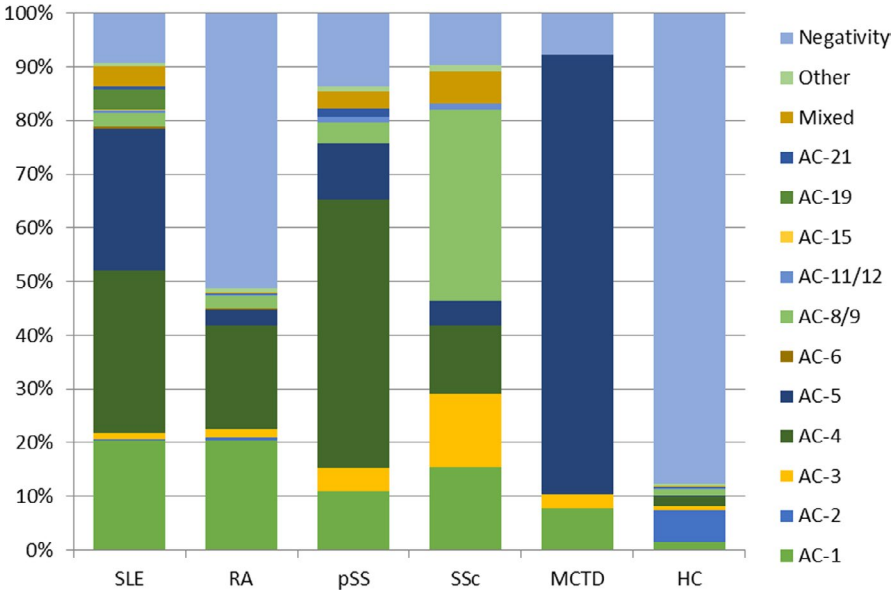

FIGURE 2 The percentage of individual ANA patterns observed in SARD and HC. Data were presented as percentage. ANA, antinuclear antibodies; HC, healthy controls; MCTD, mixed connective tissue disease; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SARD, systemic autoimmune rheumatic diseases; SLE, systemic lupus erythematosus; SSc, systemic sclerosis

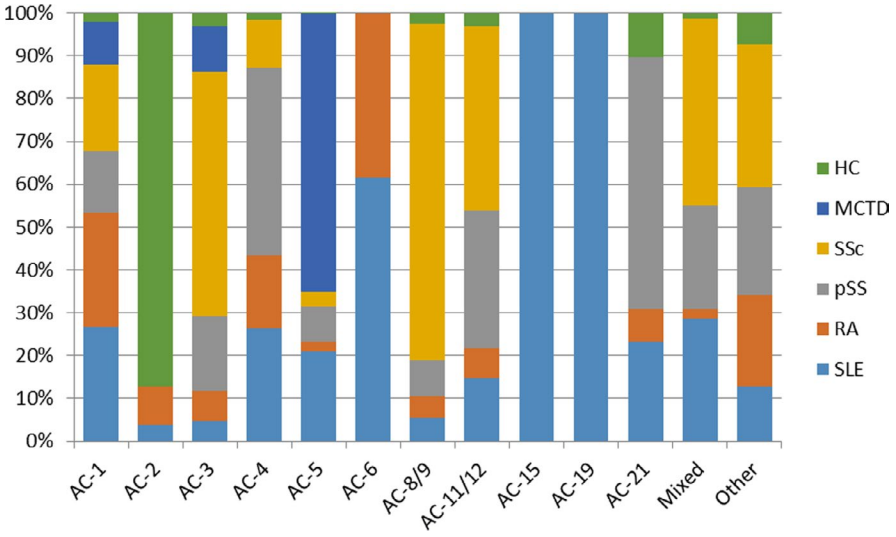

FIGURE 3 The percentage of SARD and HC distributed in each ANA pattern. Data were presented as percentage. ANA, antinuclear antibodies; HC, healthy controls; MCTD, mixed connective tissue disease; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis

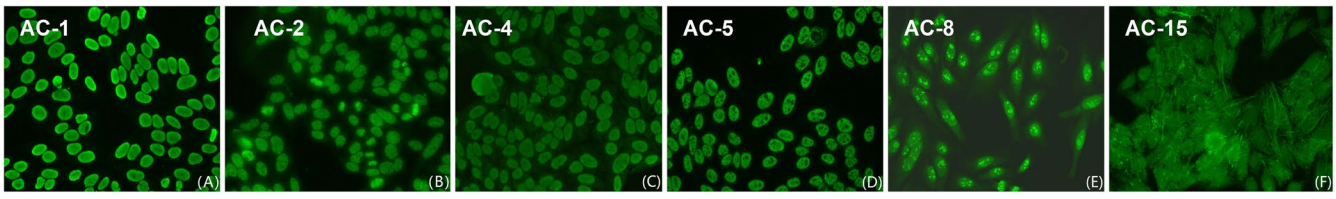

FIGURE 4 Several common ANA patterns observed in patients with SARD. ANA, antinuclear antibodies; SARD, systemic autoimmune rheumatic diseases

of ANA, a high false-positive rate for ANA can be expected. To solve this problem, incorporating HEp-2 cells and primate liver has been applied in the market.[23] We found that primate liver tissue substrate can help identify some ANA patterns. For instance, it can show T or L fluorescence in monkey liver tissue if there is anti-actin autoantibody. Second, it can help to determine ANA titers. If it presents intense fluorescence in monkey liver tissue, but very week in HEp-2 cells, one of the reasons being that the concentration of the antibody is too high, which needs to be diluted before the experiment. Moreover, it can contribute to the discovery of some anti-liver tissue antibodies, such as anti-liver membrane antibody, anti-liver-specific protein antibody, anti-soluble liver antigen antibody, and anti-liver cytosol antibody.

To be mentioned, the simultaneous occurrence of AC-3 and AC-6 could lead to difficulty in the differentiation. To solve this problem, we offered three possible solutions to identify AC-6 according to our experience. First, the fluorescence intensity of part of the stained nuclear dot in the dividing phase is enhanced. Second, the nuclear

fluorescence pattern can be easily observed in the monkey liver tissue. Third, target antigen confirmation experiments provide evidence of anti-CENP antibodies instead of anti-Sp100 antibodies' positivity in the AC-6 pattern.

In China, the rheumatologists and laboratory which performs specific autoantibody testing are extremely insufficient, leading to the consequence that ANA positivity may be an obstacle to clinical diagnosis. Due to a lack of uniform titration dilution system and standardized reporting procedures in the country, different ANA titers, together with individual patterns, need to be carefully interpreted in the process of differential diagnosis. Our study offers new information on the ANA patterns and titers through the results of a large number of patients with various SARD. Noticeably, ANA is not the sole decisive factor to make the diagnosis of SARD as many other conditions can lead to ANA positivity. Nevertheless, supported by a relevant medical history, a positive ANA still makes it possible to reach an accurate diagnosis.[24] Our study revealed that 12.2% of healthy individuals had positive ANA, which was consistent with previous findings.[25]

Quantitative enzyme immunoassays have been established in some modern laboratories, but they also have limitations of the relatively few antigens involved in the assays.[3] Clinicians should cautiously assess the patients' condition before ordering further tests, as only a minority of the autoantibodies have been certified to participate in the pathogenesis of SARD.[26] Thus, an economical ANA testing having pattern recognition and titer determination proves to be very practical in the use of the classification of SARD. Prominently, ANA testing is ordinarily conducted manually and thus time-consuming. Meanwhile, its interpretation depends on qualified laboratory personnel.[27] What's more, titers may fluctuate between different laboratories due to the substrates applied, and consequently, the specific reference values should be distinguished in each laboratory.[7] Domestic and International recommendations suggested that the laboratories set their dilution system and values.[28] Our study used the system from EUROIMMUN with a dilution factor of 3.2, whose advantages lied in not being overly exact than quadratic dilutions and meanwhile not as inexact as fourfold dilutions and possibly helping to reduce the requirement for the technician's experience and outstanding recognition ability.

The current study has some limitations. First of all, decreased disease activity can result in lower titers of ANA, which may be one of the explanations why only 46.6% of SLE patients had ANA titers ≥1:320 in our participants. We failed to combine treatment with the ANA results, thus resulting in a weak interpretation. Using a cohort of patients naïve to treatment would provide more information on disease status and ANA titers. Besides, there were no follow-up data on the participants who might develop SARD or have altered ANA patterns and titers.[29] The last but not the least, our ANA testing was using a different dilution system compared with some other countries, leading to the difficulty in comparing the results among different laboratories.

To conclude, we analyzed ANA patterns and titer distribution of various SARD, showing that each ANA pattern had a unique presence in different SARD. ANA testing by IIFA is recommended for the classification of SARD due to its comprehensive value.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Q. W, Y. J, and J. G designed the study. J. X, Q. L, and Y. X did the data collection and assembly. Q. W and J. X read the patterns. Z. W, L. T, and M. X conducted the analysis and interpreted the data. Q. W and Y. J wrote the final manuscript with help from M. X and J. G. All authors approved the final manuscript.

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