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ORIGINAL ARTICLE

Antinuclear antibody-negative systemic lupus erythematosus: How many patients and how to identify?

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ABSTRACT

Objectives: This study aims to the prevalence of antinuclear antibody (ANA)-negative systemic lupus erythematosus (SLE) and their clinical characteristics in a large single-center SLE inception cohort to provide guidance for early diagnosis.

Patients and methods: Between December 2012 and March 2021, the medical records of a total of 617 firstly diagnosed SLE patients (83 males, 534 females; median age [IQR]: 33+22.46 years) who fulfilled the selection criteria were retrospectively analyzed. The patients were divided into groups with ANA-negative SLE and ANA-positive SLE, or with prolonged use of glucocorticoids or immunosuppressants (SLE-1) and without (SLE-0). Demographic, clinical characteristics, and laboratory features were collected.

Results: The total prevalence of ANA-negative SLE patients was 2.11% (13/617). The prevalence of ANA-negative SLE in SLE-1 (7.46%) was significantly higher than that in SLE-0 (1.48%) ($p<0.01$). The ANA-negative SLE patients had a higher prevalence of thrombocytopenia (84.62%) than ANA-positive SLE patients (34.27%). As with ANA-positive SLE, ANA-negative SLE also had a high prevalence of low complement (92.31%) and anti-double-stranded deoxyribonucleic acid (anti-dsDNA) positivity (69.23%). The prevalence of medium-high titer anti-cardiolipin antibody (aCL) IgG (50.00%) and anti-ß2 glycoprotein I (anti-ß2GPI) (50.00%) of ANA-negative SLE was significantly higher than that of ANA-positive SLE (11.22% and 14.93%, respectively).

Conclusion: The prevalence of ANA-negative SLE is very low, but it exists, particularly under the influence of prolonged use of glucocorticoids or immunosuppressants. Thrombocytopenia, low complement, positive anti-dsDNA, and medium-high titer antiphospholipid antibody (aPL) are the main manifestations of ANA-negative SLE. It is necessary to identify complement, anti-dsDNA, and aPL in ANA-negative patients with rheumatic symptoms, particularly thrombocytopenia.

Keywords: Anti-double-stranded deoxyribonucleic acid, antinuclear antibody, antiphospholipid antibody, complement, systemic lupus erythematosus.

As a prototypic autoimmune disease, systemic lupus erythematosus (SLE) has highly variable clinical and immunological manifestations.[1] A high rate of moderate and severe damage has been detected early in young lupus patients.[2] Among immunological manifestations, antinuclear antibodies (ANAs) have been considered a key immunological finding. Therefore, the presence of ANAs has been considered a criterion in classification criteria for SLE such as the American College of Rheumatology (ACR)[3] or the Systemic Lupus International Collaborating Clinics (SLICC) criteria set.[4] A positive ANA is even required for further consideration for classification in 2019 European League Against Rheumatism (EULAR)/ACR classification criteria for SLE.[5] However, the positive rate of ANA in SLE has not been 100% in the majority of studies, no matter which test method is used. In other words, ANA-negative SLE was present in the vast

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majority of studies regardless of race or region, although the overall prevalence was low. Those ANA-negative SLE patients would be missed, if based on the 2019 (EULAR)/ACR classification criteria for SLE, which also affected the sensitivity of the classification criteria.[5] Therefore, for ANA-negative SLE, diagnosis is required according to the SLICC classification criteria.[4] However, the SLICC classification criteria is only a classification criteria for SLE, rather than a diagnostic criteria. That is, patients who even meet the classification criteria for a diagnosis of SLE are not necessarily SLE, and patients with non-rheumatic diseases or non-SLE autoimmune inflammatory rheumatic diseases (AIIRD) need to be excluded before the diagnosis of SLE is established. This undoubtedly increases the difficulty of a diagnosis of SLE, particularly ANA-negative SLE.

Due to its low prevalence and difficulty in recognition, ANA-negative SLE is prone to be missed by either rheumatologists or other physicians, which poses a major challenge to the diagnosis of ANA-negative SLE. Currently, there are few studies on ANA-negative SLE, and the influence of glucocorticoids on ANA detection is not taken into account. Therefore, the prevalence of ANA-negative SLE without the influence of glucocorticoids is still a controversial place. Furthermore, no new studies in the past decade have detailed the characteristics and possible diagnostic recommendations of ANA-negative SLE in the context of new testing methods. Therefore, in the present study, we aimed to investigate the prevalence of ANA-negative SLE and their clinical characteristics in a large single-center SLE inception cohort to provide guidance for early diagnosis.

PATIENTS AND METHODS

This single-center, retrospective, case-control study was conducted at Fujian Medical University Union Hospital, Department of Rheumatology between December 2012 and March 2021. We retrospectively reviewed the medical records of 1,159 hospitalized Chinese Han patients with SLE. All selected patients fulfilled the 2012 SLICC SLE classification criteria[4] and patients with non-rheumatic disease or non-SLE AIIRD were excluded before the diagnosis of SLE. Exclusion criteria were as follows: *(i)* diagnosed and treated before December 2012; *(ii)* diagnosed and treated in another hospital prior to this admission; *(iii)* pregnancy; *(iv)* overlapping syndrome; *(v)* missing important data, particularly ANAs; *(vi)* antiphospholipid syndrome (APS). Patients with SLE were grouped as follows: *(i)* Based on ANA positivity, the patients were divided into two groups: ANA-positive SLE and ANA-negative SLE; *(ii)* Based on the use of glucocorticoids or immunosuppressants before the detection of highest ANA titer level, the patients were divided into two groups: SLE-0 (not used or used for less than 7 days), including ANA-positive SLE-0 and ANA-negative SLE-0, and SLE-1 (used for 7 days or more because of other diagnosed diseases such as immune thrombocytopenia (ITP) and connective tissue disease), including ANA-positive SLE-1 and ANA-negative SLE-1.

Definition of clinical data

We obtained the demographic and clinical data by means of a review of electronic medical records. We analyzed the following parameters: age at disease diagnosis, sex, disease duration between symptom onset and diagnosis, autoantibodies, clinical manifestations and laboratory examinations.

Clinical characteristics and laboratory features were defined prior to diagnosis, except for anticardiolipin antibody (aCL), anti-beta-2-glycoprotein I (anti-ß2 GPI) and direct Coombs test, which were usually detected several days before or after diagnosis.

Antinuclear antibody was detected by indirect immunofluorescence (IIF) using the Euroimmun® kit (Euroimmun Medizinische Labordiagnostika AG, Beijing, China) with HEp-2 as the substrate. The screening dilution titers are presented as 1:100, 1:320, 1:1000, and 1:3200. According to the instructions, ANA positivity is defined as the presence of nuclear IIF or pure cytoplasmic and mitotic cell patterns (CMPs) staining or mixed nuclear and CMPs staining, at a titer of ≥1:320 (above laboratory reference range 1:100, and consistent with SLICC SLE classification criteria). The highest level of ANA titer obtained at or before diagnosis was selected as patients' ANA level and basis for grouping. Negative specimens were judged by two observers. Anti-double-stranded deoxyribonucleic acid (anti-dsDNA) was detected

by radioimmunoassay (RIA) using a RIA kit for anti-dsDNA antibodies (North Biotechnology Research Institute Co., Ltd., Beijing, China) or chemiluminescence immunoassay (CIA) using a kit for detection of anti-dsDNA antibodies (Yahuilong Biological Technology Co., Ltd., Shenzhen, China). All of ANA-negative SLE patients were tested for anti-dsDNA by RIA. Anti-dsDNA positivity was defined as a level above laboratory reference range (in accordance with SLICC classification criteria).

Statistical analysis

Statistical analysis was performed using the SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). Descriptive data were expressed in median (interquartile range [IQR] or min-max) or number and frequency. Variables were compared using the chi-square test or Fisher exact test. Statistical comparison of continuous variables between the groups was performed using the Mann-Whitney U test. A *p* value of <0.05 was considered statistically significant.

RESULTS

Study population and characteristics

Of 1,159 hospitalized patients with SLE, nine patients with overlap syndrome, 14 patients with pregnancy, and 36 patients with missing data were excluded, leaving 1,100 patients. Then, after 273 cases diagnosed before December 2012 and 210 cases diagnosed and treated in another hospital prior to this admission were excluded, 617 firstly diagnosed SLE patients (83 males, 534 females; median age [IQR]: 33+22.46 years) who fulfilled the selection criteria were included in the analysis. The study included a total of 604 ANA-positive SLE patients (542 patients in ANA-positive SLE-0 group and 62 patients in ANA-positive SLE-1 group), and 13 ANA-negative SLE (eight patients in ANA-negative SLE-0 group and five patients in ANA-negative SLE-1 group). That is, there were 550 patients in SLE-0 group (542 cases in ANA-positive SLE-0 and eight in ANA-negative SLE-0) and 67 patients in SLE-1 group (62 cases in ANA-positive SLE-1 and five in ANA-negative SLE-1). The baseline demographic, clinical, and laboratory characteristics are shown in Table 1. Overall, for all SLE patients, median disease duration [IQR] was two (IQR, 0.9 to 1.2) months. Lupus nephritis, leukopenia, lymphopenia and thrombocytopenia were found in 134 of 617 (21.72%), 315 of 617 (51.05%), 171 of 616 (27.76%) and 215 of 617 (35.33%) at the time of diagnosis, respectively.

Among the patients analyzed, 427 of 605 (70.58%) had positive anti-dsDNA, 161 of 540 (29.81%) had medium-high titer aCL or increased anti-ß 2GPI, and 576 of 608 (94.74%) had low complement (C3 or C4), which was significantly higher than that of other immunological indicators (p<0.01).

Comparison of the prevalence of ANA-negative SLE between SLE-0 and SLE-1 patients

The total prevalence of ANA-negative SLE patients was 2.11% (13/617). The prevalence of ANA-negative SLE in SLE-1 (7.46%) was significantly higher than that in SLE-0 (1.48%) (p<0.01), as shown in Table 2.

Comparison of clinical, and laboratory characteristics between ANA-negative SLE and ANA-positive SLE patients

Accompanied by a lower platelet count, 84.62% (11 of 13) ANA-negative SLE and 75.00% (6 of 8) ANA-negative SLE-0 patients had thrombocytopenia, which was significantly higher than that of ANA-positive SLE (34.27%) and ANA-positive SLE-0 patients (33.21%), respectively (p<0.05).

There was no significant difference in the positive rate of anti-dsDNA by statistical significance between ANA-negative SLE (69.23%) and ANA-positive SLE patients (70.61%) or ANA-negative SLE-0 (62.50%) and ANA-positive SLE-0 patients (71.56%) (p>0.05). Meanwhile, there was a high prevalence of low C3 or C4 in ANA-negative SLE (92.31%), ANA-negative SLE-0 (87.50%), ANA-positive SLE (94.79%) and ANA-positive SLE-0 patients (95.14%). The prevalence of low C3 of ANA-negative SLE-0 (4 of 8, 50%) was significantly lower than that of ANA-positive SLE-0 patients (473 of 535, 88.41%) (p<0.05), while the prevalence of low C4 of ANA-negative SLE-0 (6 of 8,75.0%) was similar to that of ANA-positive SLE-0 patients (469 of 535,87.66%) (p>0.05). The prevalence of low C3 and C4 of ANA-negative SLE-0 patients (3 of 8, 37.5%) was significantly lower than that

Table 1. Baseline demographic, clinical, and laboratory characteristics of patients

		SLE (n=617)			SLE-0 (n=550)		
	SLE (n=617)	ANA-positive SLE (n=604)	ANA-negative SLE (n=13)	*p*	ANA-positive SLE-0 (n=542)	ANA-negative SLE-0 (n=8)	*p*
Demographic							
Sex† Female	534 (86.55)	525(86.92)	9(69.23)	0.150	471 (86.90)	4 (50.50)	0.012
Age at diagnosis (year)‡	33 [22-46]	33 [22-45]	49 [30.5-50.5]	0.017	33 [22-45]	50 [26-57]	0.102
Disease duration (month)‡	2 [0.9-12]	2 [1-12]	5 [0.35-96]	0.587	2 [0.7,8]	0.5 [0.15-4.25]	0.007
Clinical							
Fever†	204 (33.06)	201 (33.28)	3 (23.08)	0.634	191 (35.24)	1 (12.50)	0.334
Cutaneous†	258 (41.82)	256 (42.38)	2 (15.38)	0.051	234 (43.17)	2 (25.00)	0.502
Oral/Nasal ulcers†	48 (7.78)	47 (7.78)	1 (7.69)	1.000	45 (8.30)	0	1.000
Nonscarring alopecia†	90 (14.59)	87 (14.40)	3 (23.08)	0.632	82 (15.13)	2 (25.00)	0.783
Arthritis†	182 (29.50)	180 (29.80)	2 (15.38)	0.412	160 (29.52)	1 (12.50)	0.510
Serositis†	193 (31.28)	188 (31.13)	5 (38.46)	0.793	180 (33.21)	3 (37.50)	1.000
Nephritis†	134 (21.72)	132 (21.85)	2 (15.38)	0.821	126 (23.25)	2 (25.00)	1.000
Neurologic†	23 (3.73)	23 (3.81)	0	1.000	19 (3.51)	0	1.000
Hemolytic anemia†	48 (7.78)	47 (7.78)	1 (7.69)	1.000	46 (8.49)	1 (12.50)	0.513
WBC ($\times 10^9$/L)‡	3.93 [2.92-5.90]	3.92 [2.90-5.84]	4.54 [3.60-10.35]	0.172	3.82 [2.87-5.60]	5.61 [3.68-9.12]	0.334
Leukopenia†	315 (51.05)	309 (51.16)	6 (46.15)	0.721	289 (53.32)	3 (37.50)	0.594
LYM ($\times 10^9$/L)‡	1.09 [0.73-1.52]	1.08 [0.72-1.50]	1.57 [0.82-1.81]	0.158	1.07 [0.72-1.50]	1.38 [0.81-1.84]	0.254
Lymphopenia†	171 (27.76)	169 (28.03)	2 (15.38)	0.488	155 (28.65)	1 (12.50)	0.541
HGB ($\times 10^9$/L)‡	105 [87-118]	105 [87-117.5]	121 [107.50-130.00]	0.008	104 [85-117]	119 [104.25-129.75]	0.075
PLT ($\times 10^9$/L)‡	146 [65-215]	147.5 [70-216.50]	6 [1.5-61]	0.000	148 [73.75-219.25]	31 [1.25-232.25]	0.006
Thrombocytopenia†	218 (35.33)	207 (34.27)	11 (84.62)	0.001	180 (33.21)	6 (75.00)	0.035

Table 1. Continued

	SLE (n=617)	SLE (n=617)		p	SLE-0 (n=550)		p
		ANA-positive SLE (n=604)	ANA-negative SLE (n=13)		ANA-positive SLE-0 (n=542)	ANA-negative SLE-0 (n=8)	
Immunologic							
Positive anti-dsDNA†	427 (70.58)	418 (70.61)	9 (69.23)*	1.000	380 (71.56)	5 (62.50)¶	0.866
Direct coombs test†	71 (18.59)	70 (18.82)	1 (11.11)	0.881	62 (18.13)	1 (20.00)	1.000
aCL IgM (MPLU/mL)‡	4.29 [2.57-8.10]	4.3 [2.58-8.10]	4.19 [1.91-6.86]	0.589	4.35 [2.62-8.10]	4.24 [2.68-17.25]	0.918
Medium-high titer aCL IgM†	23 (4.29)	23 (4.40)	0	1.000	20 (4.20)	0	1.000
aCL IgG (GPLU/mL)‡	11.27 [7.18-18.69]	11.25 [7.20-18.01]	34.46 [6.21-82.84]	0.266	11.59 [7.28-18.69]	7.13 [6.07-207.43]	0.953
Medium-high titer aCL IgG†	64 (11.94)	59 (11.22)	5 (50.00)	0.001	53 (11.23)	2 (40.00)	0.104
Anti-ß2GPI (RU/mL)‡	11.27 [6.86-22.08]	18.6 [15.1-23.2]	31.06 [6.52-102.51]	0.118	11.28 [6.89-21.21]	43.2 [11.52-92.89]	0.077
Increased anti-ß2GPI†	138 (26.58)	133 (26.13)	5 (50.00)	0.183	116 (25.44)	3 (60.00)	0.214
Medium-high titer anti-ß2GPI†	81 (15.61)	76 (14.93)	5 (50.00)	0.010	65 (14.25)	3 (60.00)	0.025
Medium-high titer aCL or increased anti-ß2GPI†	161 (29.81)	155 (29.24)	6 (60.00)*	0.079	138 (28.99)	3 (60.00)¶	0.307
Medium-high titer aCL or anti-ß2GPI†	112 (20.74)	106 (20.00)	6 (60.00)	0.007	92 (19.32)	3 (60.00)	0.055
IgG (g/L)‡	18.55 [14.90-23.06]	11.36 [6.92-21.85]	14.60 [12.70-20.10]	0.066	19.00 [15.40-23.30]	14.50 [12.40-18.90]	0.079
C3 (g/L)‡	0.47 [0.29-0.68]	0.46 [0.28-0.68]	0.63 [0.54-0.88]	0.007	0.45 [0.27-0.67]	0.80 [0.46-0.97]	0.023
Low C3†	532 (87.50)	523 (87.90)	9 (69.23)	0.112	473 (88.41)	4 (50.00)	0.010
C4 (g/L)‡	0.08 [0.04-0.13]	0.08 [0.04-0.13]	0.11 [0.12-0.16]	0.010	0.08 [0.04-0.13]	0.13 [0.10-0.19]	0.041
Low C4†	530 (87.17)	520 (87.39)	10 (76.92)	0.485	469 (87.66)	6 (75.00)	0.592
Low C3 and C4†	486 (79.93)	479 (80.50)	7 (53.85)	0.043	433 (80.93)	3 (37.50)	0.009
Low C3 or C4†	576 (94.74)Δ	564 (94.79)	12 (92.31)	0.508	509 (95.14)	7 (87.50)	0.337

SLE: All SLE patients included in the study after being screened by inclusion and exclusion criteria; SLE-0: Patients who did not take glucocorticoids or immunosuppressants or took them for less than 7 days before the detection of highest ANA titer level; SLE: Systemic lupus erythematosus; ANA: Antinuclear antibodies; WBC: White blood cell count; LYM: Lymphocyte count; HGB: Hemoglobin; PLT: Platelet; anti-dsDNA: Anti-double-stranded DNA; aCL: Anti-cardiolipin antibody; IgM: Immunoglobulin M; IgG: Immunoglobulin G; Anti-ß2 GPI: Anti-beta-2-glycoprotein I; C: Complement; † n (%); ‡ Median [IQR]; * There was no difference by statistical significance between the two groups; ¶ There was no difference by statistical significance between the two groups; Δ Compared with other immunological indicators, p<0.01.

Table 2. Comparison of the prevalence of ANA-negative SLE between SLE-0 and SLE-1 groups

	SLE (n=617)		SLE-0 (n=550)		SLE-1 (n=67)	
	n	%	n	%	n	%
ANA-negative SLE	13	2.11	8	1.48	5*	7.46*

ANA: Antinuclear antibodies; SLE: Systemic lupus erythematosus; SLE: All SLE patients included in the study after being screened by inclusion and exclusion criteria; SLE-0: Patients who did not take glucocorticoids or immunosuppressants or took them for less than 7 days before the detection of highest ANA titer level; SLE-1: Patients who took glucocorticoids or immunosuppressants used for 7 days or more before the detection of highest ANA titer level; * Compared with the SLE-0 group, $p<0.01$.

of ANA-positive SLE-0 patients (433 of 535, 80.93%) ($p<0.05$). Meanwhile, C3 and C4 levels were higher in ANA-negative SLE than in the ANA-positive SLE ($p<0.05$).

The prevalence of medium-high titer aCL IgG of ANA-negative SLE (50.00%) was significantly higher than that of ANA-positive SLE (11.22%) ($p<0.01$). Although there was no significant difference in the increased anti-ß 2GPI between ANA-negative SLE (50.00%) and ANA-positive SLE patients (26.13%) ($p>0.05$), the prevalence of medium-high titer anti-ß2GPI (at least twice normal) of ANA-negative SLE (50.00%) and ANA-negative SLE-0 (60.00%) was significantly higher than that of ANA-positive SLE (14.93%) and ANA-positive SLE-0 (14.25%), respectively ($p<0.05$).

In the ANA-negative SLE or ANA-negative SLE-0 patients, the prevalence of medium-high titer aCL or increased anti-ß 2GPI was similar to that of anti-dsDNA positivity (60% *vs.* 69.23%, 60% *vs.* 62.50%, respectively) ($p>0.05$).

These features describe the characteristics of ANA-negative SLE patients from different aspects. Table 1 provides additional details.

DISCUSSION

Systemic lupus erythematosus is a systemic autoimmune rheumatic disease with complex pathogenesis, which can potentially cause severe physical and functional impairments, with a wide range of manifestations, ranging from relatively mild skin and joint involvement to debilitating fatigue and significant cognitive impairment. High female predominance was commonly found in cohorts with SLE worldwide, and the mean age at the time of diagnosis ranged from 27.5 to 35.3 years, with no exception of our cohorts.[1,6-8] However, the frequency of clinical and serological manifestations substantially varies between the different cohorts with SLE.[1,6-8] Although it was lower than that of the Korean study cohort,[1] hematological anomaly was higher than that of another Chinese study,[6] which may be related to the fact that the hematology specialty of our hospital is better known in the region.

Earlier studies tended to show a higher prevalence of ANA-negative SLE (e.g., 8.9%).[9-11] However, reports of ANA-negative SLE have decreased significantly in recent years. One of the most important reasons is that the substrate for ANA detection alterations.[12] Antinuclear antibody-negative SLE in the Hep-2 cell era is exceptionally rare. Cohort studies in recent years have shown that ANA has a high positive rate in SLE, ranging from 96.8 to 99.8% in different studies.[1,6-8] In other words, the prevalence of ANA-negative SLE is very low, but it exists, which poses a significant challenge to prompt diagnosis and treatment, suggesting that it is important to investigate its clinical characteristics. An international inception cohort study by Choi et al.[13] showed that, in 1,132 patients enrolled in the group, 6.2% were anti-cellular antibody negative, which is much higher than that of the other cohorts study. However, as the comments on the article by Abeles,[14] 46% of ANA-negative patients in the cohort received high-dose glucocorticoids prior to enrollment. Previous studies have shown that ANA negativity occurs in patients with established SLE.[15] In data from clinical trials of belimumab, approximately 30% of patients were serologically negative, defined as an ANA with a titer of <1:80,[16] suggesting that ANA in

patients with SLE may disappear as a result of the natural history of the disease or the influence of immunosuppressive therapy.[17,18] Therefore, antibody status at the time of study is not necessarily reflective of what their antibody status was when their illness was first identified and therapy begun. The correct assessment of the prevalence of ANA-negative SLE requires consideration of effects from prolonged use of glucocorticoids or immunosuppressants.

In our cohort study, we included patients with newly diagnosed SLE and divided them into subgroups based on the use of glucocorticoids or immunosuppressants. The study showed a low prevalence of ANA-negative SLE (2.11%) in the total SLE cohort, which is similar to most current studies. Further subgroup analysis showed a higher prevalence of ANA-negative SLE in SLE-1 (7.46%) than SLE-0 (1.48%) ($p<0.01$), which may reflect that the positive rate of ANA may be affected by prolonged use of glucocorticoids or immunosuppressants. It also suggests that we should pay attention to the influence of previous use of glucocorticoids or immunosuppressants on the diagnosis of SLE. The prevalence of ANA-negative SLE in SLE-0 (1.48%) reflects the true rate of ANA-negative SLE in firstly diagnosed SLE patients unaffected by prolonged use of glucocorticoids or immunosuppressants. On the other hand, studies have shown that ANA-negative SLE patients exist, particularly under the influence of glucocorticoids or immunosuppressants. Moreover, we do not rule out the possibility that some ANA-negative SLE patients may be missed due to under-recognition, which is also the problem we strive to solve.

Although many cohort studies, including ours, have shown that thrombocytopenia occurs less frequently than leukopenia in SLE,[1,6,7,8] ANA-negative SLE had lower levels of platelets and a higher prevalence of thrombocytopenia, compared to ANA-positive SLE ($p<0.05$). The prevalence of thrombocytopenia in ANA-negative SLE even exceeded that of leukopenia numerically, although it was not statistically significant due to the small sample size. These findings suggest that thrombocytopenia is a major manifestation of ANA-negative SLE. Immune thrombocytopenia is the main cause of thrombocytopenia in SLE, resulting from an autoimmune condition in which platelets are destroyed by immune-mediated mechanisms. Of note, ITP can be classified as primary (known as idiopathic thrombocytopenic purpura) or secondary ITP (such as SLE-ITP) based on if there is an underlying recognized disease.[19] Primary ITP remains a diagnosis of exclusion both from non-immune causes of thrombocytopenia and ITP that develops in the context of other disorders (secondary ITP). As a major disease of secondary ITP, SLE-ITP can precede the onset of other systemic disease flares of SLE by months to years and can occur after the diagnosis of SLE. Antinuclear antibody is usually a screening indicator for SLE in thrombocytopenia. When ANA is negative, follow-up immunoassay for SLE is often not performed. Therefore, ANA-negative SLE characterized by thrombocytopenia may be misclassified as primary ITP.

It is well known that hypocomplementemia occurs in the vast majority of SLE patients,[20] and is closely related to the severity and activity of SLE. As an immunological indicator of the 2012 SLICC SLE classification criteria, hypocomplementemia has an important value in the diagnosis of SLE. In our cohort, as with ANA-positive SLE (94.79%), ANA-negative SLE also had a high prevalence of hypocomplementemia (92.31%), suggesting the importance of complement in the diagnosis of ANA-negative SLE. In addition, it should be noted in the diagnosis of ANA-negative SLE that simultaneous decline of C3 and C4 are less common in ANA-negative SLE, and the level of C3 and C4 in ANA-negative SLE was higher than that in ANA-positive SLE.

The value of anti-dsDNA in the diagnosis of SLE is indisputable, as evidenced by its acceptance by many SLE classification criteria.[3-5] The methods for anti-dsDNA detection include IIF, linear Western blotting, RIA and CIA. No method is specified as a standard method among the various SLE classification criteria. Different methods have different sensitivity and specificity for SLE and give different results for the same samples. This discrepancy is due to the diversity of possible antibodies generated to this biochemically complex antigen, which may have different clinical associations.[21] Therefore, anti-dsDNA may be detected by other

methods in patients whose ANA are negative for IIF. This has been also confirmed in several studies.[13,22] Elevated anti-dsDNA were detected by CIA in 28.4% of ANA-positive SLE and 11.3% of anti-cellular antibody-negative SLE patients.[13] Thus, detection of anti-dsDNA by a single analyte tests, even in ANA negative cases, is of utmost importance. In addition to the identification of more SLE patients, the test provides help in risk assessment for clinical complications.[13,21] In our study, as with ANA-positive SLE (70.61%), ANA-negative SLE also has a high anti-dsDNA positive rate (69.23%). The positive rate is not completely consistent with previous studies, which may be related to the difference of testing methods and patients' conditions at the time of detection. Therefore, given the high positive rate of them in SLE and generalizability of testing methods for them, complement and anti-dsDNA should be routinely performed to screen for ANA-negative SLE in patients with rheumatic symptoms such as skin lesions, arthralgia, nephritis, serositis, and hematological anomaly, particularly thrombocytopenia, even if ANA is negative.

Antiphospholipid antibody (aPL) was first described in the context of SLE and there are three tests for aPL used commonly in clinical practice: the aCL enzyme-linked immunosorbent assay (ELISA), the anti-ß2GPI ELISA and the LA test.[23] As an immunological indicators for SLE classification criteria, aPL also has its value in the diagnosis of SLE. However, aPL are less positive in SLE populations than complement and anti-dsDNA in our study and others.[1,6-8] Conversely, there is a high positive rate of aPL in ANA-negative SLE, similar to anti-dsDNA, revealing a significant diagnostic value of it for ANA-negative SLE. Therefore, aPL should be used as a routine screening indicator of ANA-negative SLE, similar to complement and anti-dsDNA.

Several limitations should be noted in the interpretation of our results. First, it is a retrospective study conducted in a single institute, which could have introduced selection bias. Second, ideally, outpatients should be included in the study, but as most of them have incomplete data, they must be excluded from the study, which can also lead to data bias. Finally, due to the insufficient awareness and difficulty in identification of ANA-negative SLE, it is not excluded that very few cases may have been missed or misclassified by clinicians. Thus, the prevalence of ANA-negative SLE of our cohort might have been underestimated.

In conclusion, the prevalence of ANA-negative SLE is very low, but it exists, particularly under the influence of prolonged use of glucocorticoids or immunosuppressants, and thrombocytopenia, low complement, positive anti-dsDNA and medium-high titer aPL are the main manifestations of ANA-negative SLE. It is necessary to identify complement, anti-dsDNA, and aPL in ANA-negative patients with rheumatic symptoms, particularly thrombocytopenia.

Ethics Committee Approval: The Cohort Study has full ethical approval from the ethics board of Fujian Medical University Union Hospital under the 2021KY139 project number. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: As this study was retrospective in nature, the requirement for informed consent was waived.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Was responsible for study conception and design: H.L.; Supervised the study: S.L.; Was responsible for data collection and processing: H.L., Y.Z.; Was responsible for analysis and wrote the article: H.L., L.C.; All authors reviewed the manuscript.

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