Clinical features and outcomes of patients with antineutrophil cytoplasmic antibody-positive systemic lupus erythematosus

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Research Article

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Abstract

Objective: To investigate the clinical characteristics, pathological features, and outcomes of patients with antineutrophil cytoplasmic antibodies (ANCA)-positive systemic lupus erythematosus (SLE) in northwest China.

Methods: We conducted a retrospective study of an ANCA-positive SLE cohort from 2012 to 2019. Clinical characteristics, laboratory data, renal histological features, and outcomes were analyzed. Patients diagnosed with antineutrophil cytoplasmic antibody-associated vasculitis (AAV) with renal involvement, excluding those with SLE and ANCA-negative lupus nephritis (LN), were randomly selected as controls.

Results: A total of 49 ANCA-positive LN patients were included in this study. The enrolled patients had a significantly lower estimated glomerular filtration rate (eGFR) than ANCA-negative LN patients and had a higher eGFR than AAV patients. SLE patients with MPO-ANCA had higher levels of serum creatinine (CREA) and blood urea nitrogen (BUN) than those with PR3-ANCA (CREA:156.5 μmol/L vs. 45.5 μmol/L, $P = 0.005$; BUN: 12.77 μmol/L vs 4.27 μmol/L, $P = 0.005$, respectively). ANCA titers positively correlated with serum complement levels in patients with SLE. ANCA-positive LN cases had a significantly higher percentage of tubulointerstitial fibrosis (84.6% vs. 14.3%, $P < 0.001$) and higher median glomerular chronicity index (2.1 vs. 0.9, $P = 0.017$) than ANCA-negative LN patients. During follow-up, there were significant differences in the proteinuria remission rates between the two matched groups when using propensity scoring ($P = 0.008$).

Conclusion: ANCA-positive SLE patients may have worse baseline renal function (especially those with MPO-ANCA positivity) and lower protein remission rates. During the follow-up period, a decrease in ANCA titer seems to correlate with renal function improvement. ANCA titers should be regularly monitored throughout the follow-up period of SLE patients, especially when there is renal involvement.

1. Introduction

Systemic lupus erythematosus (SLE) is a common, complex, multi-system autoimmune disease that can involve multiple organs. Lupus nephritis (LN) is one of the most frequent and serious complications of SLE that affects 30–60% of SLE patients [1]. LN is characterized by a series of inflammatory responses triggered by the deposition of immune complexes in the glomeruli.

Antineutrophil cytoplasmic autoantibodies (ANCAs) are unique autoantibodies that target cytoplasmic components of human neutrophils, including protease (PR3) and myeloperoxidase (MPO) [2], and it is primarily associated with small-vessel vasculitis [3]. ANCAs are detectable in 15–20% of SLE patients [4], especially among those with LN [5]. Several studies have suggested that ANCAs are associated with diffuse proliferative LN, especially in class IV segment-type LN [6]. However, the role of ANCAs in LN and its association with LN activity, histological features, and prognosis remain controversial [7–9].

In our study, we summarized the clinical characteristics and outcomes of ANCA-positive SLE patients. Furthermore, the comparisons between subgroups, which were categorized according to the ANCA serotype and renal involvement, were analyzed.

2. Methods

2.1 Patients

A total of 49 patients with confirmed ANCA-positive SLE at the First Affiliated Hospital of Xi'an Jiaotong University from January 2012 to October 2019 were included. The inclusion criteria were as follows: (1) SLE grading criteria of the American College of Rheumatology were met [10]; (2) positivity for anti-MPO and anti-PR3 titers by enzyme-linked immunosorbent assay (ELISA) [11]; and (3) renal involvement as confirmed by renal biopsy for LN. Those with drug-induced

LN and other autoimmune diseases and tumors were excluded, as were ANCA-positive patients caused by drugs. The eligible patients were further divided into those with and without renal involvement based on the 2021 KDIGO guidelines [12]. Patients with ≥ 2 + proteinuria assessed by dipstick protein, spot PCR > 500 mg/g, or confirmed by kidney biopsy were grouped into the renal involvement group. According to ANCA serology, the patients were divided into MPO-positive and PR3-positive groups. Simultaneously, 56 patients with renal involvement with ANCA-associated vasculitis (AAV) and 163 ANCA-negative renal biopsy-proven LN cases were included in the control group. The group characteristics are shown in Table 1.

Table 1
Patient characteristics at the time of renal biopsy

	AAV	ANCA negative LN	SLE with ANCA positive
Numbers(n)	56	163	49
ANCA serology(n)	MPO positive:33		MPO:16
	PR3 positive:8		PR3:30
			MPO and PR3:3
Renal involvement(n)	56	163	ANCA positive LN:27
			ANCA positive SLE without renal involvement:22

2.2 Clinical and laboratory data

Demographic and clinical data, including age, sex, medical history, medication status, and follow-up duration, were retrospectively analyzed. Laboratory data collected included hemoglobin (HGB) level, white blood cell (WBC) count, C-reactive protein (CRP) concentration, erythrocyte sedimentation rate, serum albumin and serum creatinine (CREA) levels, blood urea nitrogen, 24-hour urinary protein quantification, serum immunoglobulin G (IgG) concentration, complement C3 and complement C4 levels, serum antinuclear antibody level, serum anti-dsDNA antibody and titer, and ANCA serotype. The estimated glomerular filtration rate (eGFR) was determined using the Chronic Kidney Disease Epidemiology Partnership study equation [13]. Disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index [14]. The follow-up duration was from the first hospitalization due to ANCA-positive SLE to the date of last follow-up (up to August 2, 2021). The primary endpoint was death associated with SLE and end-stage renal disease (ERSD), and the secondary endpoint was the urinary protein remission rate. ERSD was defined as requiring continuous dialysis or kidney transplantation, or having an eGFR ≤ 15 mL/min/1.73 m^2. The urinary protein remission rate was defined as the remission of proteinuria by greater than 50% from the baseline value or a final proteinuria level of less than 0.5 g/24 hours [15]. Death and ERSD were defined as the composite endpoints. If a patient was lost to follow-up, the follow-up duration was based on the last medical record.

2.3 Renal histology

Renal tissue samples were obtained using ultrasound-guided percutaneous renal biopsy. Under a light microscope, paraffin sections were stained with hematoxylin and eosin, Masson's trichrome stain, periodic acid-Schiff, and periodic acid silver methenamine. Glomerular hyperplasia, necrosis and exudative lesions, the basement membrane, glomerular thrombosis, and tubulointerstitial inflammation were observed. The renal cortex was examined by electron microscopy to verify the location of the deposits. The LN classification criteria were defined by the International Society of Nephrology and the Society of Renal Pathology, and LN activity and chronic index (CI) were assessed.

2.4 Statistical analysis

Statistical analyses were performed using SPSS (version 26.0; IBM SPSS Statistics) and GraphPad Prism 8 (version 8.0.2; GraphPad Software). Continuous variables were expressed as mean ± standard deviation and median (interquartile range, IQR). Classified variables were described in terms of quantity and frequency. The normality of data distribution was determined using a single-sample Kolmogorov–Smirnov test. The independent sample t-test and Mann–Whitney U test were used to compare the two groups. The Chi-square test or Fisher's exact test was used to compare classified variables, where appropriate. Spearman's rank correlation coefficient was used for correlation analysis between ANCA titers and clinical characteristics. Kaplan–Meier survival analysis was used to compare patient survival, with propensity score matching for age, HGB level, and baseline eGFR. Patients were derived using a 1:1 greedy nearest neighbor matching with a PS score of 0.02. Statistical significance was set at $P < 0.05$.

3. Results

3.1 Comparison of clinicopathological features between ANCA-positive LN, AAV, ANCA-negative LN

The clinical and laboratory features of the patients in the three groups are shown in Table 2. Compared with the ANCA-negative LN group, the ANCA-positive LN group was older (46.41 ± 14.33 vs. 35.09 ± 12.63 years; $P < 0.001$) and had significantly lower levels of HGB (91.68 ± 22.23 vs 101.24 ± 21.49 g/L; $P = 0.041$), lower eGFR (47.76 [IQR, 16.00–113.88] vs. 105.64 [IQR, 54.95–124.70] mL/min/1.73 m^2; $P = 0.007$), and higher levels of IgG (18.38 ± 9.60 vs 12.41 ± 5.62, g/L; $P = 0.004$). Both ANCA-positive and -negative LN groups had low complement C4 levels, whereas there were higher levels of complement C4 in the ANCA-positive LN group (0.15 ± 0.11 vs. 0.10 ± 0.07 g/L; $P = 0.048$). The positivity rate of antinucleosome antibodies was significantly higher in the ANCA-positive LN group than in the ANCA-negative LN group (55.6% vs. 33.6%, $P = 0.034$).

Table 2
Comparison of clinicopathological features between ANCA positive LN, ANCA-associated vasculitis and ANCA negative LN

Variable	ANCA positive LN N = 27	ANCA negative LN N = 163	ANCA-associated vasculitis N = 56	P^a-value	P^b-value
Age(years)	46.41 ± 14.33	35.09 ± 12.63	61.54 ± 10.15	< 0.001	< 0.001
Male, n(%)	1(3.7%)	25(15.3%)	35(62.5%)	0.134*	< 0.001
White blood cell (⊆109/L)	4.06(2.81,5.54)	4.53(3.48,7.02)	7.06(5.50,9.79)	0.232	0.001
Hemoglobin (g/L)	91.68 ± 22.23	101.24 ± 21.49	89.22 ± 18.44	0.041	0.607
Albumin (g/L)	36.47 ± 12.86	33.90 ± 12.96	37.57 ± 9.22	0.341	0.658
C-reaction protein(mg/L)	9.90(9.90,19.10)	9.90(9.90,9.90)	45.80(19.70,79.60)	0.023	< 0.001
Erythrocyte sedimentation rate(mm/h)	59.82 ± 35.75	46.92 ± 30.84	86.33 ± 26.46	0.141	0.017
Creatinine (umol/L)	111.00(51.00,272.00)	66.00(48.00,114.00)	266.50(174.25,534.75)	0.094	0.001
eGFR(ml/min/1.73m2)	47.76(16.00,113.88)	105.64(54.95,124.70)	17.89(8.17,34.20)	0.007	0.001
24h urine protein (g)	1.28(0.52,3.30)	2.24(1.23,3.75)	1.37(0.79,2.39)	0.027	0.973
IgE,g/L	72.00(19.88,113.75)	53.50(19.25,183.25)	51.50(16.00,191.75)	0.849	0.805
IgG,g/L	18.38 ± 9.60	12.41 ± 5.62	15.01 ± 5.43	0.004	0.103
IgM,g/L	0.83(0.46,1.47)	0.97(0.61,1.43)	0.91(0.65,1.33)	0.651	0.730
IgA,g/L	2.97 ± 1.34	2.53 ± 1.20	2.67 ± 0.82	0.094	0.307
C3,g/L	0.57 ± 0.30	0.49 ± 0.27	1.02 ± 0.21	0.172	< 0.001
C4,g/L	0.15 ± 0.11	0.10 ± 0.07	0.29 ± 0.08	0.048	< 0.001
Anti-dsDNA, n(%)	12(50.0%)	25(53.2%)	/	0.799	/
Anti-dsDNA titer	6.63(2.29,100.00)	79.70(9.79,100.00)	/	0.120	/
Anti-SmD1, n(%)	11(47.8%)	17(36.2%)	/	0.350	/
Anti-ANA, n(%)	15(55.6%)	39(33.6%)	1(2.3%)	0.034	< 0.001
Anti-MPO, n(%)	13(48.1%)	/	33(76.7%)	/	0.014
Anti-PR3, n(%)	16(59.3%)	/	8(18.6%)	/	< 0.001

a the comparison of ANCA positive LN and ANCA negative LN

b the comparison of ANCA positive LN and AAV

* Fisher test;

Variable	ANCA positive LN N = 27	ANCA negative LN N = 163	ANCA-associated vasculitis N = 56	P^a-value	P^b-value
Anti-MPO titer	73.94(64.98,127.64)	/	159.42(106.28,212.09)	/	0.012
Anti-PR3 titer	56.70(31.98,85.14)	/	298.78(138.05,390.77)	/	0.001
a the comparison of ANCA positive LN and ANCA negative LN					
b the comparison of ANCA positive LN and AAV					
* Fisher test;					

Compared with the AAV group, the ANCA-positive LN group was younger (46.41 ± 14.33 vs. 61.54 ± 10.15 years; $P < 0.001$) and had fewer males (3.7% vs. 62.5%; $P < 0.001$). The ANCA-positive LN group had significantly lower levels of CREA (111.00 [IQR, 51.00–272.00] vs. 266.50 [IQR, 174.25–534.75] μmol/L; $P = 0.001$), complement C3 (0.57 ± 0.30 vs. 1.02 ± 0.21 g/L; $P < 0.001$), and complement C4 (0.15 ± 0.11 vs. 0.29 ± 0.08 g/L; $P < 0.001$) than the AAV group. Additionally, the positivity rates of antinucleosome antibodies and anti-PR3 were significantly higher in the ANCA-positive LN group than in the AVV group (55.6% vs. 2.3%, $P < 0.001$; 59.3% vs. 18.6%, $P < 0.001$, respectively). However, the positivity rate of anti-MPO was lower in the ANCA-positive LN group than in the AVV group (48.1% vs. 76.7%, $P = 0.014$). The levels of anti-MPO and anti-PR3 were significantly lower in the ANCA-positive LN group than in the AVV group (73.94 [IQR, 64.98–127.64] vs. 159.42 [IQR, 106.28–212.09] RU/mL; $P = 0.012$; 56.70 [IQR, 31.98–85.14] vs. 298.78 [IQR, 138.05–390.77] RU/mL; $P = 0.001$, respectively).

3.2 Comparison of clinicopathological features between ANCA-positive LN and ANCA-positive SLE without renal involvement

Three patients with ANCA-positive SLE were excluded as both the MPO-ANCA and PR3-ANCA were positive. The clinical and laboratory features of the patients in the abovementioned two groups are shown in Supplementary Table S1. The ANCA-positive LN group had significantly higher levels of CREA (104.90 [IQR, 48.50–245.00] vs. 43.00 [IQR, 37.51–53.00] μmol/L; $P = 0.002$, respectively), higher levels of 24-h urine protein (1.36 [IQR, 0.66–3.39] vs 0.10 [IQR, 0.05–0.25] g/L; $P < 0.001$, respectively), lower eGFR (69.41 ± 50.07 vs. 118.70 ± 35.58 mL/min/1.73 m^2; $P < 0.001$, respectively), lower levels of IgM (0.84 [IQR, 0.43–1.41] vs. 1.39 [IQR, 1.10–1.91] g/L; $P = 0.003$, respectively), and lower levels of complement C3 (0.55 ± 0.28 vs. 0.75 ± 0.26] g/L; $P = 0.016$, respectively) than the ANCA-positive non-LN group. Additionally, the positivity rate of anti-ribonucleoprotein antibody was significantly lower in the ANCA-positive LN group than in the ANCA-positive non-LN group (28.6% vs. 66.7%, $P = 0.040$, respectively).

3.3 Characteristics and pathological classification of ANCA-positive SLE patients based on ANCA serotypes

The serum CREA and blood urea nitrogen values in the MPO-ANCA group were higher than those in the PR3-ANCA group (156.5 [IQR, 45.75–350.75] vs. 45.50 [IQR, 37.76–58.60] μmol/L; $P = 0.005$; (12.77 [IQR, 4.55–19.34] vs. 4.27 [IQR, 3.48–5.75] μmol/L; $P = 0.005$, respectively), while eGFR and IgG levels in the MPO-ANCA group were lower than those in the PR3-ANCA group (36.15 [IQR, 12.43–126.40] vs. 114.11 [IQR, 100.35–131.54] mL/min/1.73 m^2; $P = 0.015$; 13.80 [IQR, 7.70–23.70] vs. 22.50 [IQR, 14.30–29.10] μmol/L; $P = 0.034$, respectively). The levels of complement C4 in the MPO-ANCA group was higher than that in the PR3-ANCA group (0.17 ± 0.08 vs. 0.12 ± 0.08] g/L; $P = 0.037$, respectively) (Table 3).

Table 3
Comparison of clinicopathological features between MPO-ANCA positive group and PR3-ANCA positive group in the ANCA positive SLE.

	MPO-ANCA positivity N = 16	PR3-ANCA positivity N = 30	P-value
Age(years)	47.88 ± 17.23	41.57 ± 12.09	0.154
Male, n(%)	2(12.5%)	0(0.0%)	0.116*
diagnosis of SLE to ANCA positive(months)	13.50(1.75,114.00)	12(4.50,72.00)	0.917
White blood cell (⊆109/L)	3.72(2.81,5.69)	4.32(2.73,5.80)	0.517
Hemoglobin (g/L)	92.29 ± 23.23	98.21 ± 18.02	0.364
Albumin (g/L)	39.39 ± 14.90	36.74 ± 9.86	0.529
C-reaction protein(mg/L)	14.20(9.90,22.75)	9.90(9.90,14.95)	0.127
Erythrocyte sedimentation rate(mm/h)	57.33 ± 37.47	53.74 ± 32.11	0.788
Creatinine (umol/L)	156.50(45.75,350.75)	45.50(37.76,58.60)	0.005
Urea nitrogen (umol/L)	12.77(4.55,19.34)	4.27(3.48,5.75)	0.005
eGFR(ml/min/1.73m2)	36.15(12.43,126.40)	114.11(100.35,131,54)	0.015
24h urine protein (g)	0.96(0.12,1.48)	0.40(0.08,1.87)	0.340
IgE,g/L	35.00(16.00,108.75)	82.00(22.90,310.00)	0.210
IgG,g/L	13.80(7.70,23.70)	22.50(14.30,29.10)	0.034
IgM,g/L	0.89(0.41,1.27)	1.27(0.85,1.70)	0.053
IgA,g/L	2.56 ± 1.16	3.44 ± 1.54	0.058
C3,g/L	0.64 ± 0.25	0.64 ± 0.31	0.977
C4,g/L	0.17 ± 0.08	0.12 ± 0.08	0.037
Anti-dsDNA, n(%)	8(57.1%)	13(54.2%)	0.859
Anti-dsDNA titer	2.76(0.79,52.98)	10.92(1.49,85.62)	0.604
Anti-SmD1, n(%)	5(35.7%)	15(65.2%)	0.081
Anti-ANA, n(%)	8(50.0%)	21(70.0%)	0.181
Anti-RNP, n(%)	4(57.1%)	10(45.5%)	0.458*
Anti-MPO titer(RU/mL)	83.94 ± 50.14	/	/
Anti-PR3 titer(RU/mL)	/	56.70(32.95,83.27)	/
*fisher test			

3.4 Renal pathological evaluation

The characteristics of renal histopathology of LN patients with and without ANCA are shown in Fig. 1. There were no significant differences in the histopathological features of LN in the ANCA-positive group compared with that in the ANCA-

negative group (Fig. 1a). Comparing the biopsy specimens with proliferative LN (i.e., class III and class IV) between the two groups, focal endocapillary hypercellularity was not observed in the ANCA-positive LNs; however, it was seen in 35.7% of the ANCA-negative LNs ($P < 0.01$, Fig. 1b). The proportion of tubulointerstitial fibrosis were higher in the ANCA-positive group than in the ANCA-negative group (84.6% vs. 14.3%, $P < 0.001$, Fig. 1f). Compared with the ANCA-negative group, a higher proportion of biopsy specimens in the ANCA-positive group had necrosis (23.0% vs. 0%), crescent formation (22.5% vs. 13.0%), and glomerulosclerosis (7.3% vs. 2.1%); however, the difference was not statistically significant (Fig. 1c-e). The ANCA-positive LN patients had higher median CI scores (2.1 vs. 0.9, $P = 0.017$, Fig. 1g).

ANCA serology and correlation of ANCA titers and clinical characteristics

The change in ANCA titers during treatment was monitored, and the anti-MPO and anti-PR3 titers in most of the patients decreased to within normal range (20 RU/mL) within six months (Supplementary Figure S2a, S2e). In one patient, high MPO titers recurred one year after self-discontinuation of treatment (Figure S2a). The ds-DNA titer and serum CREA level were also collected during the same period (Supplementary Figure S2b, S2c, S2f, S2g).

Anti-MPO titers were correlated with levels of complement C3 and C4. Anti-PR3 titers correlated with levels of complement C4. However, there was no correlation between the ANCA titers and antibody seropositivity in the ANCA-positive group. Meanwhile, ds-DNA titers were correlated with antibody seropositivity in the ANCA-positive group (Supplementary Figure S3).

3.5 Long-term outcomes

In the ANCA-positive group, seven patients were lost to follow-up, and thirty-nine patients were followed-up for 3 to 95 months (mean, 42.42 ± 29.32 months). At the end of the study, four patients died, and three patients underwent maintenance hemodialysis. The time to reach composite endpoints between the ANCA-positive and ANCA-negative LN groups was not significantly different ($P = 0.332$) (Fig. 2a). In the MPO-ANCA and PR3-ANCA groups, there was no significant difference in the long-term outcomes ($P = 0.552$) (Fig. 2b). To compare the outcomes of the ANCA-positive LN patients with those of the ANCA-negative LN patients with a similar risk of poor outcome, patients were matched for age, HGB level, and baseline eGFR using propensity scoring at a 1:1 ratio of ANCA-negative to ANCA-positive LN patients. The proteinuria remission rate in ANCA-positive LN patients was lower than that in ANCA-negative LN patients ($P = 0.008$, Fig. 3).

4. Discussion

In this study, we showed that ANCA-positive LN had worse baseline renal function and a lower proteinuria remission rate than ANCA-negative LN; the decrease in ANCA titers seems to be consistent with an improvement in renal function. However, ANCA positivity was not associated with poor outcomes in LN patients.

The age of onset of ANCA-positive LN patients was higher than that in ANCA-negative LN patients but lower than that in AAV patients. Older people are known to be more susceptible to AAV, but studies have not determined a difference in the age of onset between ANCA-positive and ANCA-negative LN patients [7, 16–18]. ANCA-positive LN patients are more likely to have a worse baseline renal function (lower eGFR) than ANCA-negative LN patients, which is similar with those of previous studies [7, 19]. In addition, the follow-up data showed that ANCA titers seemed to decrease concomitantly with renal function improvement. Therefore, treatment options for LN patients with ANCA-positivity should be carefully chosen. However, the association of ANCA positivity in LN and poor prognosis remains controversial [16–18, 20]. Wang et al. showed that the ANCA-positive group had a lower remission rate than the ANCA-negative group, and that ANCA was an independent risk factor for poor renal outcome [16]. Li et al. reported that ANCA-positive LN patients have advanced renal insufficiency; however, the renal outcomes of ANCA-positive and ANCA-negative LNs were not statistically significant [7]. Our survival analysis suggests that the time to reach the endpoint between the ANCA-positive and ANCA-negative LN patients was not

significantly different, although we found a lower proteinuria remission rate in ANCA-positive LN patients than in ANCA-negative LN patients. Another study found that ANCA-positive and negative LN patients had significant improvement in proteinuria during the first six months post-biopsy [19]. It is known that neutrophils, NETosis, and complement play a role in tissue damage in SLE. Neutrophil phenotype and function were significantly abnormal in SLE patients, and neutrophil death was enhanced by apoptosis and NETosis [21]. ANCA has been shown to induce endothelial cell injury by stimulating neutrophils and monocytes to release granules, and ANCA is involved in the acceleration of neutrophil apoptosis [4]. These studies suggest a potential pathogenic role of ANCA in SLE. Therefore, we hypothesized that ANCA can be used as an indicator of SLE disease activity; however, further research will be required to confirm this. Regardless, we recommend that all patients with SLE be dynamically monitored for ANCA during treatment follow-up.

AAV patients have normal complement levels; both ANCA-positive and -negative LN patients have decreased complement levels, whereas ANCA-negative patients have lower C4 levels than ANCA-positive patients. Other reports have shown much lower C3 and C4 levels in ANCA-positive LN patients than in ANCA-negative LN patients [16]. These disparities may be due to the selection of patients with different disease activities. To our knowledge, serum total complement levels may not necessarily reflect complement activation. In general, circulating C3 and C4 levels are normal in AAV [22]. In SLE, low C3 and C4 levels are commonly due to C3 and C4 consumption, which is thought to be associated with activation of the classical complement system by autoantibodies and immune complexes. Previous studies have reported that decreases in serum C3 and C4 levels correlate with SLE disease activity; however, there are several contentions against these results [23]. The main reason was that standard laboratory tests measure the concentration of parental C3 and C4 molecules rather than their activation products, and the acute phase response during inflammation may lead to an increase in C4 and C3 synthesis, which can balance the activation and increased consumption of these proteins.

SLE is a prototypic systemic autoimmune disease characterized by the presence of a variety of autoantibodies, including those directed towards DNA, chromatin, histones, and ribonucleoproteins. Jethwa et al. found that elevated levels of anti-dsDNA antibodies in the serum may result in false-positive results on the MPO-ANCA test due to the charge interaction between DNA and MPO in the serum [24]. We analyzed whether cross-reacting autoantibodies in SLE would result in a correlation between ANCA and autoantibody seropositivity and titer. We found that there was no significant correlation between the ANCA titer and antibody seropositivity in the ANCA-positive SLE group, and elevated ds-DNA levels were not associated with elevated ANCA titer levels. However, the ds-DNA titer positively correlated with the number of autoantibodies. Therefore, we speculated that there was no ANCA cross-reaction in this study, but further experimental verification is still needed.

In our study, we analyzed 16 (34.8%) cases of MPO-ANCA and 30 (65.2%) cases of PR3-ANCA in ANCA-positive SLE patients. Previous epidemiological investigations have shown that MPO-ANCA vasculitis is more common in southern Europe, the southern United States, and Asia, whereas PR3-ANCA vasculitis is more common in Northern Europe, northern North America, and Australia [25]. It is not clear whether this is due to genetic differences or other environmental factors, such as vitamin D levels and sun exposure. In China, the incidence of MPO-ANCA-positive AAV ranges from 80–95% [26]. In a large study, ANCA was detected by indirect immunofluorescence in 16.4% of 566 European SLE patients; ELISA detection of MPO-ANCA and PR3-ANCA accounted for 9.3% and 1.7%, respectively [27]. Li et al. found that 3.5% of 110 Chinese LN patients were ANCA positive; 95 eligible patients were selected, which were comprised of 26 (27.4%) who were PR3-ANCA-positive and 69 (72.6%) who were MPO-ANCA positive [28]. Our study results were inconsistent with those of previous studies in a number of aspects. First, Harper and Savage found that serum MPO-ANCA was more prevalent in older ANCA patients than in younger patients [29]; a report from a Chinese population study was consistent with the results of this study [26]. In our study, ANCA-positive SLE patients were younger (43.27 ± 13.98 years) than those in previous studies. Second, majority of the patients in our study were treated with glucocorticoids and immunosuppressants for SLE; at the time of kidney biopsy, we also detected ANCA serology. Recent reports indicate that MPO-ANCA-positive LN patients show poor renal function and a high level of activity on renal histological examination [30, 31]. Although the ratio of MPO-positive cases

to PR3-positive cases was lower in our study, MPO-ANCA-positive SLE patients were more likely to have worse baseline renal function. The rate of each composite endpoint (death rate and ESRD) of the two groups were not significantly different, probably due to the limitation of the small study population. A Chinese LN cohort showed that MPO-positive patients had worse renal outcomes compared with PR3-positive patients [28].

ANCA mediates acute injury and induces a chronic response to injury [32]. Our results showed that ANCA-positive LN patients were more likely to have severe interstitial fibrosis and higher chronicity index (CI) scores on renal biopsy specimens than ANCA-negative LN patients. Li et al. found that ANCA may be associated with a higher chronicity index in LN patients [7], and Pyo et al. discovered that ANCA positivity was associated with the chronic index [18]. These findings support the increase in chronic inflammation of ANCA-positive LNs due to ANCA. Other studies have reported that ANCA-positive LN patients are more likely to have segmental endocapillary hypercellularity [17, 19]. However, in our study, LN was more likely to result in segmental endocapillary hypercellularity in ANCA-positive patients than in ANCA-negative patients, albeit without statistical significance. Further studies are needed to examine the pathogenesis of ANCA in LN.

This study has several limitations. First, this was a single-center study, and the number of patients was not large enough to conduct a multivariable analysis. Second, although we followed-up the change in ANCA titers during the treatment period to determine whether the dynamic evolution of ANCA is related to LN activity, the continuity of variables is not sufficient due to the limitations of a retrospective study design.

In conclusion, our study found that ANCA was associated with a poor baseline renal function in LN and delayed urinary protein remission; however, prospective studies are needed to confirm whether ANCA has a pathogenic effect on LN and to determine the specific mechanisms involved to guide therapy. We also recommend regular monitoring of ANCA titers in patients with ANCA-positive SLE, especially in those with renal improvement.

Declarations

Ethical approval

This study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (XJTU1AF2021LSK-214). Informed consent was waived by the Research Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University, Shaanxi, China because this study involved retrospective review of existing data.

Author contributions

Xinfang Xie and Wanhong Lu designed the study. Ying Wang and Xiaoyang Yu wrote the initial draft of the paper. Wei Yang and Yu Liang collected the data. Huixian Li and Ying Wang analyzed the statistics.

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Availability of data and materials

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

Disclosure

All the authors declared no competing interests.

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Figures

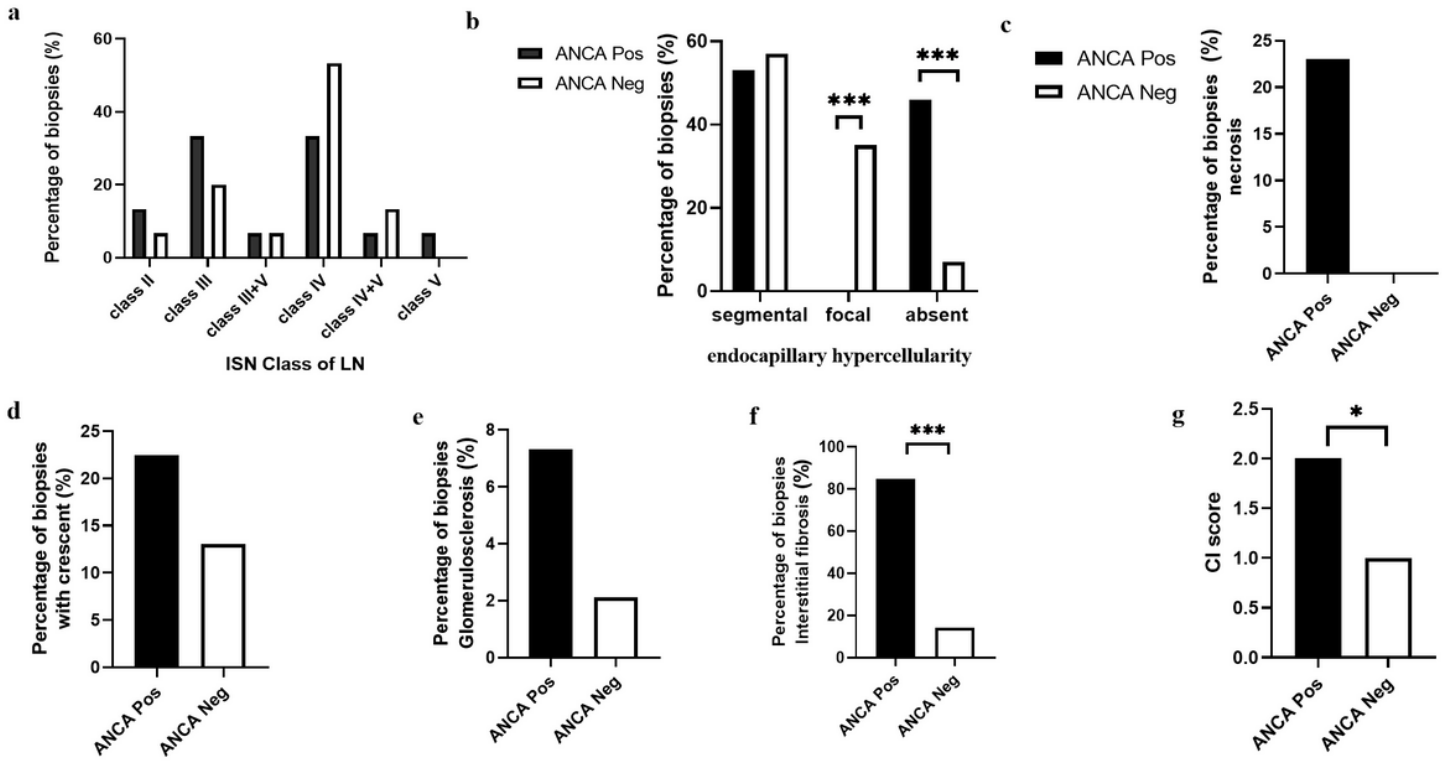


Figure 1

Histopathological features of LN. (a)Percentage of biopsy specimens for each type of LN with ANCA -positive and ANCA-negative. (b-e) include only Class III and IV LN (i.e., proliferative LN). Compare the pattern of endocapillary hypercellularity(b), the proportion of biopsy specimens with necrosis (c), the proportion of biopsy specimens with crescents(d), the proportion of biopsy specimens with glomerulosclerosis(e), the proportion of biopsy specimens with interstitial fibrosis(f). Compare the CI scores(g) *P < 0.05; ***P < 0.01.

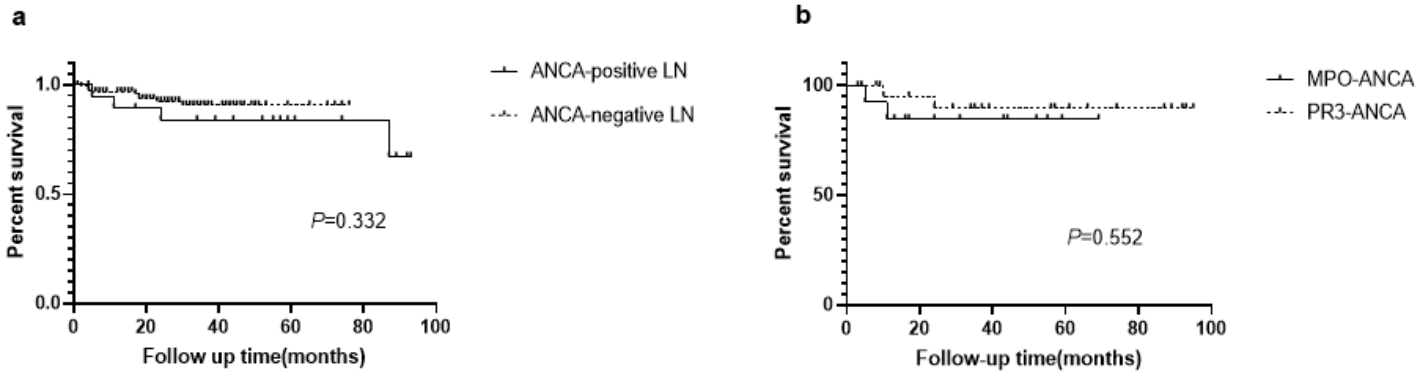
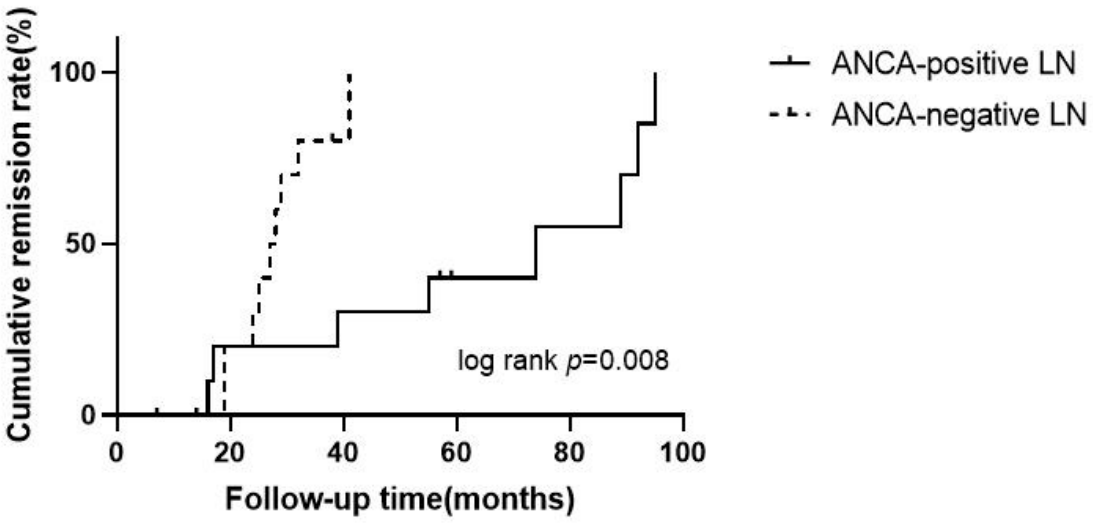


Figure 2

(a) Kaplan–Meier survival curve of the survival between the ANCA-positive LN group and ANCA -negative LN group. (b). Kaplan–Meier survival curve of the survival between MPO-ANCA and PR3-ANCA in SLE.



Number at remission

Months	0	12	24	36	48	60	72	84	96
ANCA-Pos	10	10	29	29	37	62	73	77	83
ANCA-Neg	12	24	53	58	81	81	81	81	81

Figure 3

Kaplan–Meier survival curve of the proteinuria remission rate between the ANCA-positive LN group and ANCA -negative LN group.

Supplementary Files

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