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Clinicopathologic Characteristics and Outcomes of Lupus Nephritis With Antineutrophil Cytoplasmic Antibody

A Retrospective Study

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Abstract: Few studies have analyzed the clinicopathologic characteristics and outcomes of lupus nephritis (LN) patients with antineutrophil cytoplasmic antibody (ANCA).

The clinical and renal histopathologic data of 154 patients with biopsy-proven LN from 2011 to 2013 were analyzed retrospectively. The patients were followed up for a median period of 16.8 ± 9.4 months, and their outcomes were analyzed. Multivariate Cox analysis was used to evaluate the independent factors for poor outcomes.

Among the 154 LN patients, 26 (16.88%) were seropositive for ANCA. The incidences of alopecia, oral ulcer, photosensitivity and skin lesion, and psychosomatic manifestations in the ANCA-positive group were significantly higher than in the ANCA-negative group ($P = 0.007$, 0.02, 0.02, and 0.03, respectively). Compared with the ANCA-negative group, the ANCA-positive group had significantly lower levels of complement C3 ($P = 0.03$). Additionally, the positive rate of antinucleosome antibodies, antihistone antibodies, antimitochondrial antibody M2, and anticardiolipin antibodies were higher significantly in the ANCA-positive patients than in the ANCA-negative patients ($P = 0.001$, 0.001, 0.03, 0.005, respectively). The ANCA-positive group had a notably higher chronic index than the ANCA-negative group ($P = 0.01$). During the follow-up, the complete remission rate in the ANCA-negative group was higher than that in the ANCA-positive group ($P = 0.01$). The cumulative renal survival rate in the ANCA-positive group was significantly lower than in the ANCA-negative group (log-rank = 6.59, $P = 0.01$). Multivariate Cox analysis revealed that the reduced estimated glomerular filtration rate (HR, 1.02; 95% confidence interval, 1.01 to 1.03; $P = 0.005$), NLR (HR, 1.20; 95% confidence interval, 1.02 to 1.40; $P = 0.03$), and ANCA (HR, 3.37; 95% confidence interval, 1.12 to 10.09; $P = 0.03$) were independent risk factors for patients' renal survival after adjusting for age, sex, crescent formation, and glomerulosclerosis.

The study found ANCA in LN patients is not rare, and patients with ANCA present with more severe clinicopathologic injuries. Thus, ANCA is an independent risk factor for poor renal outcomes in LN patients.

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Abbreviations: AI = activity index, ANCA = antineutrophil cytoplasmic antibody, CI = chronic index, CKD = chronic kidney disease, eGFR = estimated glomerular filtration, ELISA = enzyme-linked immunosorbent assay, ESRD = end-stage renal disease, HR = hazard ratio, IIF = indirect immunofluorescence, IQR = interquartile range, LN = lupus nephritis, MPO = myeloperoxidase, NET = neutrophil extracellular trap, NLR = neutrophil to lymphocyte ratio, PR3 = proteinase-3, SCr = serum creatinine, SLE = systemic lupus erythematosus, SVV = small vessel vasculitides, TIL = tubular interstitial lesion.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease that can affect essentially any organ or tissue. Lupus nephritis (LN) is one of the most serious complications of SLE because it is the major predictor of poor prognosis. The presence of autoantibodies directed against several cytoplasmic and nuclear antigens is a characteristic feature of SLE and plays a very important role in the pathogenesis of LN.[1] Antineutrophil cytoplasmic autoantibodies (ANCA) constitute a distinct class of autoantibodies that are directed against cytoplasmic constituents of human neutrophils, and they are mainly associated with small vessel vasculitides (SVVs), such as microscopic polyangiitis, granulomatosis with polyangiitis, and eosinophilic granulomatosis with polyangiitis.[2] According to the pattern of indirect immunofluorescence (IIF) staining, ANCA can be divided into the cytoplasmic staining pattern (c-ANCA) which representative target antigen is proteinase-3 (PR3) and the perinuclear staining pattern (p-ANCA) which representative target antigen is myeloperoxidase (MPO). The subtype of ANCA was determined by antigen-specific enzyme-limked immunosorbent assay (ELISA).[3] ANCA were first described in patients with necrotizing glomerulonephritis.[4] Until now, ANCA have been detected in infectious, neoplastic, and inflammatory diseases.[5,6] Circulating ANCA in serum is also present in connective tissue diseases such as SLE.[7] However, because of there are a broad range of autoantibodies in the SLE, and some are closely associated with specific clinical manifestations and disease activity, the presence of ANCA has not received the same attention as in SVV.[8]

Recently, a number of studies concerning ANCA prevalence in SLE have appeared. However, these reports have been controversial about the clinical relevance and disease activity of ANCA,[9–14] and the role of ANCA in SLE remains unclear. Moreover, only rare investigations have addressed the renal outcomes of LN patients with ANCA. Therefore, we retrospectively analyzed the baseline characteristics and outcomes of 154 patients with LN and evaluated the risk factors for adverse renal outcomes.

METHODS

Patients

A total of 154 Chinese patients, from January 2011 to December 2013, who fulfilled at least 4 of the 2012 American College of Rheumatology revised criteria for the diagnosis of SLE[15] and had renal involvement confirmed by renal biopsy, were analyzed retrospectively. Renal biopsy was performed at the time of diagnosis and before immunosuppressive therapy was initiated. Drug-induced LN and patients with other autoimmune diseases were excluded. None of the patients had a history of propylthiouracil, isoniazide, or hydralazine use, which could cause ANCA positivity. The details of the recruitment process are shown in Figure 1.

This study was in compliance with the Declaration of Helsinki and was approved by the Medical Ethical Committee of the Xinqiao Hospital, Third Military Medical University. The requirement for patient consent was waived by the Medical Ethical Committee because this study involved no more than minimal risk as a retrospective study, and no identifiable information was used.

Measurements

The patients' demographic and clinical data were reviewed retrospectively for age, sex, medical history, medications, and follow-up duration by medical records. Clinical features included butterfly erythema, fever, oral ulcer, arthritis, nervous system disorder, serositis, alopecia, and photosensitivity. Laboratory data consisted of levels of erythrocytes, leukocytes, platelets, hemoglobin, albumin, erythrocyte sedimentation rate,

serum creatinine (SCr), uric acid, complement C3, serum IgG, 24-h proteinuria, and autoantibodies. The neutrophil to lymphocyte ratio (NLR) and the estimated glomerular filtration rate (eGFR) were also included in the study. eGFR was estimated according to the Chronic Kidney Disease Epidemiology Collaboration 2-level race equation[16] Laboratory data regarding the presence of autoantibodies, including anti-dsDNA, anti-Sm, anti-SSA, anti-SSB, anti-Ro-52, antiribosomal P, anti-U1RNP, antihistone, antinucleosome, anticardiolipin, antimitochondrial antibody M2 and ANCA, were collected from the Department of Clinical Laboratory. Serum antinuclear antibodies were detected using the indirect immunofluorescence assay (EUROIMMUN; Lübeck, Germany) and anti-dsDNA antibodies were detected using the Crithidia luciliae indirect immunofluorescence test (EUROIMMUN; Lübeck, Germany). Antiextractable nuclear antigen antibodies, including anti-Sm, anti-SSA, anti-SSB, anti-Ro-52, antiribosomal P, anti-U1RNP, antihistone, antinucleosome, and antimitochondrial antibody M2 antibodies, were detected using the immunodotting assay (EUROIMMUN; Lübeck, Germany). Anticardiolipin antibodies were detected using ELISA (EUROIMMUN; Lübeck, Germany) PR3-ANCA and MPO-ANCA were evaluated using EUROBlot (EUROIMMUN; Lübeck, Germany) and IIF (EUROIMMUN; Lübeck, Germany), according to the protocols provided by the manufacturer.

The renal biopsies were processed according to standard techniques for light microscopy, immunofluorescence (IF), and electron microscopy. For light microscopy, paraffin sections were stained with hematoxylin and eosin, Masson's trichrome, periodic acid-Schiff, and silver methenamine. Glomerular sclerosis, crescent formation, fibrinoid necrosis, microthrombosis, tubular atrophy, and interstitial fibrosis were observed. For renal tissue with IF staining, the presence of IgA, IgG, IgM, and C3 was documented (estimated semiquantitatively on a scale of 0–4 [0 for absence, 1 for mild, 2 for moderate, 3 for severe, 4 for very severe]). Localization of deposits was verified using electron microscopy. LN was classified according to the International Society of Nephrology/Renal Pathology Society classes.[17] Pathological parameters, such as the activity index (AI), chronic index (CI), and tubular interstitial lesion (TIL), were assessed using a modification of the criteria[18] involving
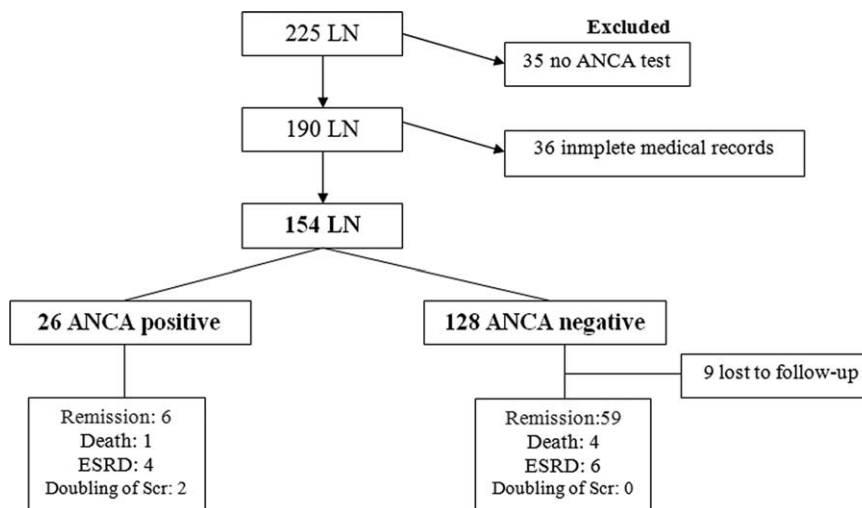

FIGURE 1. Flow diagram of the included patients. ANCA = antineutrophil cytoplasmic antibody; ESRD = end-stage renal disease; LN = lupus nephritis; SCr = serum creatinine.

semiquantitative scoring of specific renal pathological features by 2 renal pathologists. AI included endocapillary hypercellularity, leukocyte infiltration, cellular crescents, karyorrhexis, fibrinoid necrosis, formation of wire loops, and interstitial inflammatory cell infiltration. CI included glomerular sclerosis, fibrous crescents, interstitial fibrosis, and tubular atrophy. TIL included tubule degeneration and necrosis, tubular atrophy, interstitial inflammatory cell infiltration, and interstitial fibrosis. Differences in scoring between the 2 pathologists were resolved by re-reviewing the biopsies and reaching a consensus.

Outcomes

The patients were followed up in an outpatient clinic specified for patients with LN. The primary end point was defined as death, and the secondary end point was defined as end-stage renal disease (ESRD) or the doubling of SCr. The combined end point was defined as a composite outcome of death, ESRD or the doubling of SCr. ESRD was defined as maintenance hemodialysis, renal transplantation and eGFR ≤ 15 mL/min/1.73 m^2. Remission of LN was defined as protein-negative urine and normal or near-normal (within 10% of normal eGFR if previously abnormal) eGFR.[19] If patients were lost to follow-up during the study, they were followed until the last recorded visit.

Statistical Analysis

All statistical analyses were performed using SPSS, version 20.0 (SPSS, Chicago, IL). For continuous variables, the results are expressed as the mean ± standard deviation, median (range), or median (interquartile range, IQR), as appropriate. Categorical variables are reported as the number and frequency. The 1-sample K-S test was used to determine the normality of the data distribution. According to their normality, continuous variables were compared with the independent samples t test, the paired samples t test or the Mann–Whitney U test. Comparisons were based on the Chi-square test or Fisher's exact test for categorical variables. Kaplan–Meier analysis was used to compare survival in the ANCA-positive and ANCA-negative groups. A multivariate Cox regression model was used to evaluate risk factors for poor outcomes. Relevant variables that were significantly associated with poor outcomes by univariate analysis were included in multivariate models. All tests were 2-sided, and $P < 0.05$ was considered significant.

RESULTS

General Patient Data

Among the 154 patients with LN enrolled in this study, 26 (16.88%) were seropositive for ANCA, with MPO-ANCA presenting in 24 (92.31%) patients, whereas 2 (7.69%) patients showed PR3-ANCA. In the ANCA-positive group, 4 patients (15.38%) were men, and 22 (84.62%) were women, with a median age of 35 (IQR, 23–51) years old at diagnosis. There were no significant differences in sex, age, or disease duration between the ANCA-positive and ANCA-negative groups (Table 1).

Clinical and Laboratory Parameters

The clinical and laboratory features of the patients in the 2 groups are shown in Table 1. The incidences of alopecia, oral ulcers, photosensitivity and skin lesions, and psychosomatic manifestations in the ANCA-positive group were significantly

higher than in the ANCA-negative group (19.23% vs 3.13%, $P = 0.007$; 15.38% vs 2.34%, $P = 0.02$; 19.23% vs 7.03%, $P = 0.02$; and 7.69% vs 0.00%, $P = 0.03$, respectively). Compared with the ANCA-negative group, the ANCA-positive group had significantly lower levels of complement C3 (0.3 [IQR, 0.2–0.5] vs 0.4 [IQR, 0.3–0.6] g/L; $P = 0.03$). Additionally, the positive rates of antinucleosome antibodies, antihistone antibodies, antimitochondrial antibody M2, and anticardiolipin antibodies were significantly higher in the ANCA-positive patients than in the ANCA-negative patients (53.84% vs 17.97%, $P < 0.001$; 53.84% vs 20.31%, $P < 0.001$; 19.23% vs 5.47%, $P = 0.03$; 30.77% vs 8.59%, $P = 0.005$, respectively).

Renal Histopathology

The parameters of renal histopathology of the LN patients with and without ANCA are listed in Table 2. We observed that the distributions of LN classifications were similar in the 2 groups. The incidence and proportion of glomerular sclerosis were higher in the ANCA-positive group than in the ANCA-negative group (53.85% vs 25.00%, $P = 0.003$; 6.27% vs 0.00%, $P = 0.004$, respectively). There were no significant differences in the SLEDAI, AI, CI, or TIL scores between the 2 groups, whereas the ANCA-positive group had a notably higher chronic index score than the ANCA-negative group (1 [IQR, 0–2.5] vs 0 [IQR, 0–1], respectively, $P = 0.01$).

Treatment and Outcome

The therapies for LN patients with and without ANCA were compared, and there were no significant differences in therapy between the 2 groups (Table 3).

All 26 of the ANCA-positive patients were followed up for 1 to 38 months (mean 15.0 ± 10.6 months). At the end of the study, 1 patient died, 2 patients underwent maintenance hemodialysis (one of whom received renal transplantation after 11 months), 2 patients developed the fifth stage of chronic kidney disease (CKD), another 2 patients experienced SCr doubling, and 6 patients achieved remission. In the ANCA-negative group, 9 patients were lost to follow-up, and the remaining 119 patients were followed up for 1 to 40 months (mean 17.9 ± 9.8 months). Four patients died, 3 patients underwent maintenance hemodialysis, 3 patients developed the fifth stage of CKD, and 59 patients achieved remission. The mortality rates between the 2 groups had no significant difference ($P > 0.99$), but the complete remission rate in the ANCA-negative group was higher than that in the ANCA-positive group (49.58% vs 23.08%, respectively, $P = 0.01$). The cumulative renal survival rate in the ANCA-positive group was significantly lower than that in the ANCA-negative group (71.26% vs 91.48%, log-rank $= 6.59$, $P = 0.01$; Figure 2).

Univariate Cox regression analysis showed that reduced eGFR (hazard ratio [HR], 1.02; 95% confidence interval, 1.01 to 1.04; $P < 0.001$), NLR (HR, 1.29; 95% confidence interval, 1.12 to 1.47; $P < 0.001$), ANCA (HR, 3.25; 95% confidence interval, 1.24 to 8.55; $P = 0.02$), crescent formation (HR, 2.89; 95% confidence interval, 1.00 to 7.82; $P = 0.04$), and glomerulosclerosis (HR, 2.72; 95% confidence interval, 1.05 to 7.05; $P = 0.04$) were risk factors for LN renal survival. Multivariate Cox analysis revealed that reduced eGFR (HR, 1.02; 95% confidence interval, 1.01 to 1.03; $P = 0.005$), NLR (HR, 1.20; 95% confidence interval, 1.02 to 1.40; $P = 0.03$), and ANCA (HR, 3.37; 95% confidence interval, 1.12 to 10.09; $P = 0.03$) remained independent risk factors for the patients'

TABLE 1. Comparison of Clinical and Laboratory Parameters Between LN Patients With and Without ANCA

Parameters	ANCA-Positive Group (n = 26)	ANCA-Negative Group (n = 128)	*P* Value
Male/female, number	4/22	7/121	0.09
Age, years	35 (23−51)	36 (24−45)	0.52
Disease duration, months	4 (1−12)	3 (1−24)	0.57
Clinical features			
Fever	2 (7.69%)	8 (6.25%)	0.68
Edema	15 (57.69%)	84 (65.63%)	0.44
Malar rash	4 (15.38%)	15 (11.72%)	0.53
Photosensitivity	5 (19.23%)	9 (7.03%)	0.02
Oral ulcerations	4 (15.38%)	3 (2.34%)	0.02
Alopecia	5 (19.23%)	4 (3.13%)	0.007
Arthritis	5 (19.23%)	15 (11.72%)	0.34
Serositis	3 (11.54%)	14 (10.94%)	>0.99
Psychomatic features	2 (7.69%)	0 (0.00%)	0.03
Laboratory measurements			
WBC, $\times 10^9$/L	4.43 (3.08−6.41)	4.63 (3.47−6.45)	0.68
RBC, $\times 10^{12}$/L	3.17 (2.48−3.91)	3.55 (2.78−4.09)	0.13
PLT, $\times 10^9$/L	140 (102−173)	143 (102−199)	0.57
Neutrophil, $\times 10^9$/L	2.87 (2.09−4.32)	2.93 (2.18−4.47)	0.58
Lymphocyte, $\times 10^9$/L	0.90 ± 0.49	1.01 ± 0.46	0.26
NLR	4.40 (2.68−5.45)	3.33 (2.22−5.07)	0.31
HB, g/L	91 (75−113)	100 (80−115)	0.34
ESR, mm/h	51.50 (26.50−79.75)	39.00 (20.00−66.75)	0.17
C3, g/L	0.28 (0.18−0.47)	0.41 (0.27−0.60)	0.03
IgG, g/L	13.45 (8.50−19.20)	11.70 (8.97−16.53)	0.35
Albumin, g/L	26.70 (22.8−32.28)	26.05 (21.90−33.25)	0.79
SCr, μmol/L	80.00 (50.58−194.55)	62.90 (46.73−101.38)	0.15
UA, μmol/L	409 (299−507)	362 (283−454)	0.21
24-h proteinuria, g/d	2.30 (0.76−4.59)	1.79 (0.86−3.79)	0.36
eGFR, mL/min/1.73 m^2	78.07 (38.23−122.41)	109.07 (57.41−126.08)	0.15
AntidsDNA antibody	13 (50.00%)	46 (35.94%)	0.18
Antinucleosome antibody	14 (53.84%)	23 (17.97%)	<0.001
Antihistone antibody	14 (53.84%)	26 (20.31%)	<0.001
Antiribosomal P antibody	12 (46.15%)	39 (30.47%)	0.12
Antimitochondrial antibody M2	5 (19.23%)	7 (5.47%)	0.03
AntiSm antibody	7 (26.92%)	17 (13.28%)	0.13
AntiSSA antibody	15 (57.69%)	79 (61.72%)	0.70
AntiRo-52 antibody	11 (42.31%)	32 (25%)	0.07
AntiSSB antibody	3 (11.54%)	23 (17.97%)	0.57
Anti-UIRNP antibody	12 (46.15%)	56 (43.75%)	0.82
Anticardiolipin antibody	8 (30.77%)	11 (8.59%)	0.005

Values for categorical variables are given as numbers (percentages); values for continuous variables are given as the means ± standard deviations or medians (interquartile ranges).

ANCA = antineutrophil cytoplasmic antibody; eGFR = estimated glomerular filtration; ESR = erythrocyte sedimentation rate; HB = hemoglobin; LN = lupus nephritis; NLR = neutrophil to lymphocyte ratio; PLT = platelets; RBC = red blood count; SCr = serum creatinine; UA = uric acid; WBC = white blood count.

renal survival after adjusting for age, sex, crescent formation, and glomerulosclerosis (Table 4).

DISCUSSION

Our study found that the prevalence of ANCA in LN was 16.9% (26 of 154 patients). The predominant ANCA pattern observed was p-ANCA, of which 92.31% of cases had anti-MPO antibodies. Regarding clinical features, this study demonstrated that more multisystem damage occurred in ANCA-positive LN patients than in ANCA-negative LN patients. Among the studied laboratory parameters, the frequencies of

antinucleosome antibody, antihistone antibody, antimitochondrial antibody M2, and anticardiolipin antibody were significantly higher in the ANCA-positive group than in the ANCA-negative group, whereas the level of complement C3 was lower in the ANCA-positive group. Moreover, ANCA-positive LN patients showed high scores on the pathological chronic index.

Several previous studies have investigated the role of ANCA in SLE.[8–14,20–25] Some reported no associations between ANCA positivity and disease activity, and the clinical picture and antibody profile were similar in ANCA-positive and ANCA-negative SLE patients. These studies also found no

TABLE 2. Comparison of Pathological Parameters and Disease Activity Between LN Patients With and Without ANCA

Parameters	ANCA-Positive Group (n = 26)	ANCA-Negative Group (n = 128)	*P* Value
WHO classifications			0.67
I type	0 (0.00%)	7 (5.47%)	
II type	3 (11.54%)	16 (12.50%)	
III type	6 (23.08%)	19 (14.84%)	
IV type	4 (15.38%)	11 (8.59%)	
V type	3 (11.54%)	23 (17.99%)	
III + V type	5 (19.23%)	23 (17.99%)	
IV + V type	5 (19.23%)	29 (22.65%)	
Pathology reports			
Glomerulosclerosis	14 (53.85%)	32 (25.00%)	0.003
Glomerulosclerosis rate	6.27% (0–12.78%)	0% (0–28.85%)	0.004
Cellular crescent formation	3 (11.54%)	19 (14.84%)	>0.99
Fibrous crescent formation	2 (7.69%)	7 (5.47%)	0.65
Fibrinoid necrosis	6 (23.08%)	27 (21.09%)	0.82
Microthrombosis	1 (3.85%)	5 (3.91%)	>0.99
Wire loop lesions	1 (3.85%)	2 (1.56%)	0.43
Tubular atrophy	6 (23.08%)	30 (23.44%)	0.97
Interstitial fibrosis	8 (30.78%)	24 (18.75%)	0.17
SLEDAI	13 (10–16)	12 (10–14)	0.11
AI	1 (0–3)	1 (0–2)	0.64
CI	1 (0–2.5)	0 (0–1)	0.01
TIL	1 (1–2)	1 (1–3)	0.57

Values for categorical variables are given as numbers (percentages); values for continuous variables are given as medians (interquartile ranges).
AI = active index; ANCA = antineutrophil cytoplasmic antibody; CI = chronic index; LN = lupus nephritis; SLEDAI = systemic lupus erythematosus disease activity index; TIL = tubular interstitial lesion.

association of ANCA with renal involvement. However, other studies have reported a correlation. Lee et al[9] reported a significant correlation between the presence of ANCA and crescent formation in patients with World Health Organization (WHO) class IV glomerulonephritis but not with the overall presence of renal involvement. Chin et al[23] also studied the relationship between ANCA and nephritis. They found that p-ANCA was significantly associated with the presence of nephritis, particularly with WHO class IV, and with the presence of

anti-dsDNA antibody. In addition, they reported an association between the presence of ANCA and deterioration of renal function. Pan et al[13] conducted a study among patients with new-onset SLE and identified correlations between ANCA positivity and the presence of various clinical manifestations of SLE, anti-dsDNA antibody, and anti-Sm antibody. Many factors could be involved in these disparities, such as demographic characteristics, the application of different classifications, and the patient selection criteria. Our study found

TABLE 3. Comparison of Treatment Data Between LN Patients With and Without ANCA

Parameters	ANCA-Positive Group (n = 26)	ANCA-Negative Group (n = 128)	*P* Value
Treatment			
Steroid only	10 (38.46%)	33 (25.78%)	0.19
Steroid + other immunosuppressors			
Cyclophosphamide	11 (42.31%)	36 (28.13%)	0.15
Tacrolimus	1 (3.85%)	25 (19.53%)	0.08
Mycophenolate mofetil	3 (11.54%)	16 (12.50%)	>0.99
Intravenous immunoglobulin	1 (3.85%)	11 (8.59%)	0.69
Plasma exchange	1 (3.85%)	1 (0.78%)	0.31
Response[*]			
Remission	6 (23.08%)	59 (49.58)	0.01
Death	1 (3.85%)	4 (3.36%)	>0.99
ESRD	4 (15.38%)	6 (5.04%)	0.08
Doubling of SCr	2 (7.69%)	0 (0.00%)	0.03

Values for categorical variables are given as numbers (percentages).
ANCA = antineutrophil cytoplasmic antibody; ESRD = end-stage renal disease; LN = lupus nephritis; SCr = serum creatinine.
[*] The ANCA-negative group had 119 patients (9 patients were lost to follow-up).
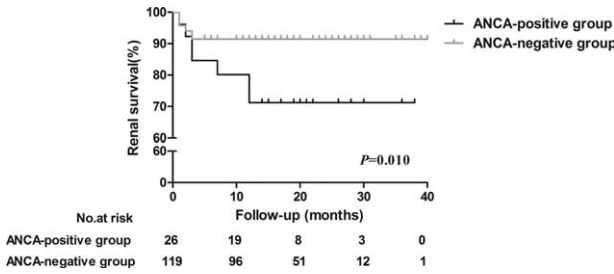
FIGURE 2. Comparison of renal survival between LN patients with and without antineutrophil cytoplasmic antibody (ANCA). Kaplan–Meier analysis was used to calculate the renal cum survivals in ANCA-positive and ANCA-negative groups, and the rates were 71.26% and 91.48%, respectively. The renal survival rate in the ANCA-positive group was significantly lower than that in the ANCA-negative group (log-rank = 6.59, P = 0.01). ANCA = antineutrophil cytoplasmic antibody; LN = lupus nephritis.

some correlations of ANCA with severe clinicopathologic features, which suggests that ANCA could play a role in the pathogenesis of LN.

Previous studies have focused on the clinical manifestations of LN patients with ANCA, but few have investigated the outcomes of these patients. Yu et al[14] reported that the mortality rate in the ANCA-positive group was higher than in the ANCA-negative group and that ANCA was a risk factor for mortality, but the renal outcomes of the ANCA-positive LN patients were not mentioned. In our study, the remission rate in the ANCA-positive group was lower than in the ANCA-negative group, and ANCA was an independent risk factor for poor renal outcomes. In addition, we found that 2 additional factors, reduced eGFR and NLR, were also independent risk factors for poor renal outcomes. Decreases in eGFR are strongly and consistently associated with the risks of ESRD and mortality,[26] so eGFR could predict the prognosis of LN patients. NLR is an important measurement of systemic inflammation because it is

cost effective and readily available, and it can be calculated easily.[27] Many recent studies have suggested that an elevated NLR is associated with poor survival of subjects with cancer,[28] but its value in LN has been uncertain. The contribution of elevated NLR in LN patients must be confirmed by prospective studies.

We found that ANCA was associated with poor renal outcomes of LN patients, but the pathogenesis of ANCA in LN patients is far from clear. Hervier et al[29] found that ANCA, usually MPO-ANCA, occurs in a small minority of patients with SLE who seemed to have vasculitic features of ANCA-associated disease, which is considered to be an overlapping syndrome. This finding could constitute proof that ANCA participates in the pathogenesis of LN. In the ANCA-positive group, we observed that other autoantibodies appeared more frequently, perhaps due to neutrophil extracellular trap (NET) formation. NETs, which are released via a unique form of cell death called NETosis, consist of decondensed chromatin DNA in association with histones, granular proteins, and a few cytoplasmic proteins.[30–32] These NETs might provide a novel source of autoantigens. Autoantigens can be recognized by the immune system, producing pathogenic antibodies to accelerate the progression of LN. In addition to NETs, ANCA might activate the complement pathway to participate in the pathogenesis of LN. Recent studies have provided strong evidence that complement system activation, particularly through an alternative pathway, is involved in the pathogenesis of ANCA-associated vasculitides,[33] and complement system activation also plays an important role in the pathogenesis of LN.[34] In addition, our research found that LN patients with ANCA had lower levels of complement C3 than those without ANCA. Collectively, we hypothesize that the occurrence of ANCA in LN is a multifactorial process. The exact mechanism is of great interest for further investigations.

However, healthy individuals also have circulating autoantibodies against MPO and PR3.[33] Compared with pathogenic MPO-ANCA, natural MPO-ANCA has lower titers, lower avidity, less subclass diversity, and less capability to activate

TABLE 4. Univariate and Multivariate Cox Regression Analysis of Factors at Baseline that Influence Poor Renal Outcomes

Characteristics	Univariate		Multivariate	
	HR (95% CI)	P Value	HR (95% CI)	P Value
Age, per 1 year older	0.99 (0.96 to 1.03)	0.76	0.97 (0.94 to 1.01)	0.15
Sex, male vs female	1.32 (0.30 to 5.78)	0.71	0.92 (0.20 to 4.31)	0.92
eGFR, per 1 mL/min/1.73 m^2 lower	1.02 (1.01 to 1.04)	<0.001	1.02 (1.01 to 1.03)	0.005
24-h proteinuria, per 1 g/d higher	1.04 (0.88 to 1.22)	0.68		
Albumin, per 1 g/L higher	0.99 (0.93 to 1.06)	0.73		
NLR, per 1 higher	1.29 (1.12 to 1.47)	<0.001	1.20 (1.02 to 1.40)	0.03
C3, per 1 g/L higher	1.28 (0.20 to 8.30)	0.80		
ANCA, positive vs negative	3.25 (1.24 to 8.55)	0.02	3.37 (1.13 to 10.09)	0.03
Anti-nucleosome antibody, positive vs negative	0.80 (0.32 to 3.00)	0.97		
Anti-histone antibody, positive vs negative	1.54 (0.57 to 4.16)	0.40		
antimitochondrial antibody M2, positive vs negative	0.63 (0.08 to 4.77)	0.66		
Anti-cardiolipin antibody, positive vs negative	0.44 (0.06 to 3.28)	0.42		
Crescent formation, yes or no	2.89 (1.01 to 7.82)	0.04	0.82 (0.26 to 2.58)	0.74
Fibrinoid necrosis, yes or no	1.53 (0.54 to 4.33)	0.43		
Glomerulosclerosis, yes or no	2.72 (1.05 to 7.05)	0.04	1.62 (0.58 to 4.52)	0.36

ANCA = antineutrophil cytoplasmic antibody; CI = confidence interval; eGFR = estimated glomerular filtration; HR = hazard ratio; NLR = neutrophil to lymphocyte ratio.

neutrophils *in vitro*.[35] In addition, Roth et al[36] found that certain MPO epitopes were specific for active disease, and others remained present during remission or were also present in healthy individuals. The high sensitivity and specificity of ANCA detection should be examined in further studies, and the epitopes should be determined.

Our study has several limitations. The major limitation of this study was that the follow-up time was limited. Therefore, we could not effectively evaluate the renal outcomes. In addition, due to limitations based on the relatively small sample size in each subgroup of LN WHO classifications, the results of the renal outcomes of the LN patients among different categories must be validated in larger cohorts. Finally, although we speculate that ANCA could induce NETs and active complement system to participate in the pathogenesis of LN, such experiments were not performed because this study was retrospective.

In conclusion, ANCA in LN patients is not rare, and LN patients with ANCA present with more severe clinicopathologic injuries. ANCA is an independent risk factor for poor renal outcomes in LN patients.

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REFERENCES

1. Borchers AT, Leibushor N, Naguwa SM, et al. Lupus nephritis: a critical review. *Autoimmun Rev.* 2012;12:174–194.

2. Furuta S, Jayne DRW. Antineutrophil cytoplasm antibody-associated vasculitis: recent developments. *Kidney Int.* 2013;84:244–249.

3. Chen M, Zhao M, Zhang Y, et al. Antineutrophil cytoplasmic autoantibodies in patients with systemic lupus erythematosus recognize a novel 69 kDa target antigen of neutrophil granules. *Nephrology.* 2005;10:491–495.

4. Davies DJ, Moran JE, Niall JF, et al. Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *Br Med J (Clin Res Ed).* 1982;285:606.

5. Savige JA, Davies DJ, Gatenby PA. Anti-neutrophil cytoplasmic antibodies (ANCA): their detection and significance: report from workshops. *Pathology.* 1994;26:186–193.

6. Ardiles LG, Valderrama G, Moya P, et al. Incidence and studies on antigenic specificities of antineutrophil-cytoplasmic autoantibodies (ANCA) in poststreptococcal glomerulonephritis. *Clin Nephrol.* 1997;47:1–5.

7. Sen D, Isenberg DA. Antineutrophil cytoplasmic autoantibodies in systemic lupus erythematosus. *Lupus.* 2003;12:651–658.

8. Manolova I, Dancheva M, Halacheva K. Antineutrophil cytoplasmic antibodies in patients with systemic lupus erythematosus: prevalence, antigen specificity, and clinical associations. *Rheumatol Int.* 2001;20:197–204.

9. Lee SS, Lawton JW, Chan CE, et al. Antilactoferrin antibody in systemic lupus erythematosus. *Br J Rheumatol.* 1992;31:669–673.

10. Galeazzi M, Morozzi G, Sebastiani GD, et al. Anti-neutrophil cytoplasmic antibodies in 566 European patients with systemic lupus erythematosus: prevalence, clinical associations and correlation with other autoantibodies. European Concerted Action on the Immunogenetics of SLE. *Clin Exp Rheumatol.* 1998;16:541–546.

11. Fauzi AR, Kong NC, Chua MK, et al. Antibodies in systemic lupus antineutrophil cytoplasmic erythematosus: prevalence, disease activity correlations and organ system associations. *Med J Malaysia.* 2004;59:372–377.

12. Pradhan VD, Badakere SS, Bichile LS, et al. Anti-neutrophil cytoplasmic antibodies (ANCA) in systemic lupus erythematosus: prevalence, clinical associations and correlation with other autoantibodies. *J Assoc Physic India.* 2004;52:533–537.

13. Pan HF, Fang XH, Wu GC, et al. Anti-neutrophil cytoplasmic antibodies in new-onset systemic lupus erythematosus and lupus nephritis. *Inflammation.* 2008;31:260–265.

14. Yu YW, Liu ZR, Xie D, et al. Clinical significance of antineutrophil cytoplasmic antibodies in patients with lupus nephritis. *Nan Fang Yi Ke Da Xue Xue Bao.* 2006;26:833–836.

15. Petri M, Orbai A, Alarcón GS, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum.* 2012;64:2677–2686.

16. Kong X, Ma Y, Chen J, et al. Evaluation of the Chronic Kidney Disease Epidemiology Collaboration equation for estimating glomerular filtration rate in the Chinese population. *Nephrol Dial Transplant.* 2013;28:641–651.

17. McClain MT, Arbuckle MR, Heinlen LD, et al. The prevalence, onset, and clinical significance of antiphospholipid antibodies prior to diagnosis of systemic lupus erythematosus. *Arthritis Rheum.* 2004;50:1226–1232.

18. Hill GS, Delahousse M, Nochy D, et al. A new morphologic index for the evaluation of renal biopsies in lupus nephritis. *Kidney Int.* 2000;58:1160–1173.

19. Bertsias GK, Tektonidou M, Amoura Z, et al. Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. *Ann Rheum Dis.* 2012;71:1771–1782.

20. Schnabel A, Csernok E, Isenberg DA, et al. Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. Prevalence, specificities, and clinical significance. *Arthritis Rheum.* 1995;38:633–637.

21. Spronk Pe, Bootsma H, Horst G, et al. Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. *Rheumatology.* 1996;35:625–631.

22. Nishiya K, Chikazawa H, Nishimura S, et al. Anti-neutrophil cytoplasmic antibody in patients with systemic lupus erythematosus is unrelated to clinical features. *Clin Rheumatol.* 1997;16:70–75.

23. Chin HJ, Ahn C, Lim CS, et al. Clinical implications of antineutrophil cytoplasmic antibody test in lupus nephritis. *Am J Nephrol.* 2000;20:57–63.

24. Pradhan VD, Badakere SS, Iyer YS, et al. A study of anti-neutrophil cytoplasm antibodies in systemic vasculitis and other related disorders. *J Postgrad Med.* 2003;49:5–9.

25. Yu F, Tan Y, Liu G, et al. Clinicopathological characteristics and outcomes of patients with crescentic lupus nephritis. *Kidney Int.* 2009;76:307–317.

26. Coresh J, Turin TC, Matsushita K, et al. Decline in estimated glomerular filtration rate and subsequent risk of end-stage renal disease and mortality. *JAMA.* 2014;311:2518.

27. Imtiaz F, Shafique K, Mirza SS, et al. Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. *Int Arch Med.* 2012;5:2.

28. Templeton AJ, McNamara MG, Eruga B, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J Nat Cancer Inst.* 2014;106:u124.

29. Hervier B, Hamidou M, Haroche J, et al. Systemic lupus erythematosus associated with ANCA-associated vasculitis: an overlapping syndrome? *Rheumatol Int.* 2012;32:3285–3290.

30. Fuchs TA, Abed U, Goosmann C, et al. Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol.* 2007;176: 231–241.

31. Steinberg BE, Grinstein S. Unconventional roles of the NADPH oxidase: signaling, ion homeostasis, and cell death. *Sci STKE.* 2007;2007:e11.

32. Urban CF, Ermert D, Schmid M, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. *PLoS Pathog.* 2009;5:e1000639.

33. Jennette JC, Falk RJ. Pathogenesis of antineutrophil cytoplasmic autoantibody-mediated disease. *Nat Rev Rheumatol.* 2014;10: 463–473.

34. Mohan C, Putterman C. Genetics and pathogenesis of systemic lupus erythematosus and lupus nephritis. *Nat Rev Nephrol.* 2015.

35. Xu PC, Cui Z, Chen M, et al. Comparison of characteristics of natural autoantibodies against myeloperoxidase and anti-myeloperoxidase autoantibodies from patients with microscopic polyangiitis. *Rheumatology.* 2011;50:1236–1243.

36. Roth AJ, Ooi JD, Hess JJ, et al. Epitope specificity determines pathogenicity and detectability in ANCA-associated vasculitis. *J Clin Invest.* 2013;123:1773–1783.