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Original Article

Clinicopathological characteristics and outcomes of patients with ANCA-positive lupus nephritis: a large cohort study from a single Chinese center

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Abstract: Very limited clinicopathological and clinical outcome data are available regarding lupus nephritis (LN) patients with antineutrophil cytoplasmic antibodies (ANCAs). The present study therefore investigated such data in a large cohort of LN patients in China. 478 patients with renal biopsy-proven lupus nephritis diagnosed in The First Affiliated Hospital of Sun Yat-sen University from January 1998 to December 2011 were enrolled. The primary endpoint was the composite of a doubling of baseline serum creatinine, end-stage renal disease, or death. The prevalence of ANCA seropositivity in this cohort was 14.0% (67/478). At the time of biopsy, ANCA-positive patients had higher CRP levels, higher ESR values, and were more frequently positive for anti-cardiolipin antibodies. However, they had lower eGFR values, and lower levels of HDL-C, LDL-C, and hemoglobin when compared with ANCA-negative patients. ANCA-positive patients had a higher prevalence of 2003 ISN/RPS class IV LN, and higher scores for karyorrhexis/fibrinoid necrosis, cellular glomerular crescents, interstitial inflammation, and tubular atrophy. At a median follow-up time of 56 months (range, 3-162 months), the event-free survival rate for a composite outcome was significantly lower among patients with ANCAs than patients without (P = 0.015). Multivariate Cox regression analysis revealed that initial serum creatinine and CI score were the most important risk factors significantly affecting the primary outcome. Notably, ANCA was not independently associated with the primary outcome. Patients with ANCA-positive LN have more severe kidney injury and a worse long-term outcome compared with patients with ANCA-negative LN.

Keywords: Antineutrophil cytoplasmic antibodies, lupus nephritis, clinical outcomes

Introduction

Renal involvement significantly contributes to the morbidity and mortality among patients with systemic lupus erythematosus (SLE) [1, 2]. Moreover, the clinical manifestations of SLE and various histopathological characteristics of its associated renal lesions are closely related to the therapeutic responses and clinical outcomes achieved when treating patients with lupus nephritis (LN) [3], which is the most prevalent pathologic type (54.3%) of secondary glomerulonephritis diagnosed in China [4].

Antineutrophil cytoplasmic antibodies (ANCAs) constitute a class of autoantibodies directed against the cytoplasmic constituents of primary granules often found in the lysosomes of neutrophils and monocytes [5]. Davies et al. [6] first described ANCAs while conducting studies on segmental necrotizing glomerulonephritis. We now know that ANCAs are not only found in cases of ANCA-associated vasculitis (AAV), but can also be detected in non-vasculitic diseases such as infections, inflammatory bowel disease and autoimmune disorders [7-10]. The reported prevalence of ANCAs among SLE patients is 15-20% as determined by IIF assays [11-13], and 22-37% among patients with LN [14, 15]. Although ANCAs are frequently detected in cases of SLE and LN, the clinical implications of their presence remain controversial [12, 15-17], and only limited reports are available concerning the long-term outcomes of ANCA-positive LN patients. We conducted the current study to examine the prevalence of ANCAs in Chinese LN patients. We also evaluated the clinical manifestations, laboratory characteristics, pathological features, and the clinical outcomes of a

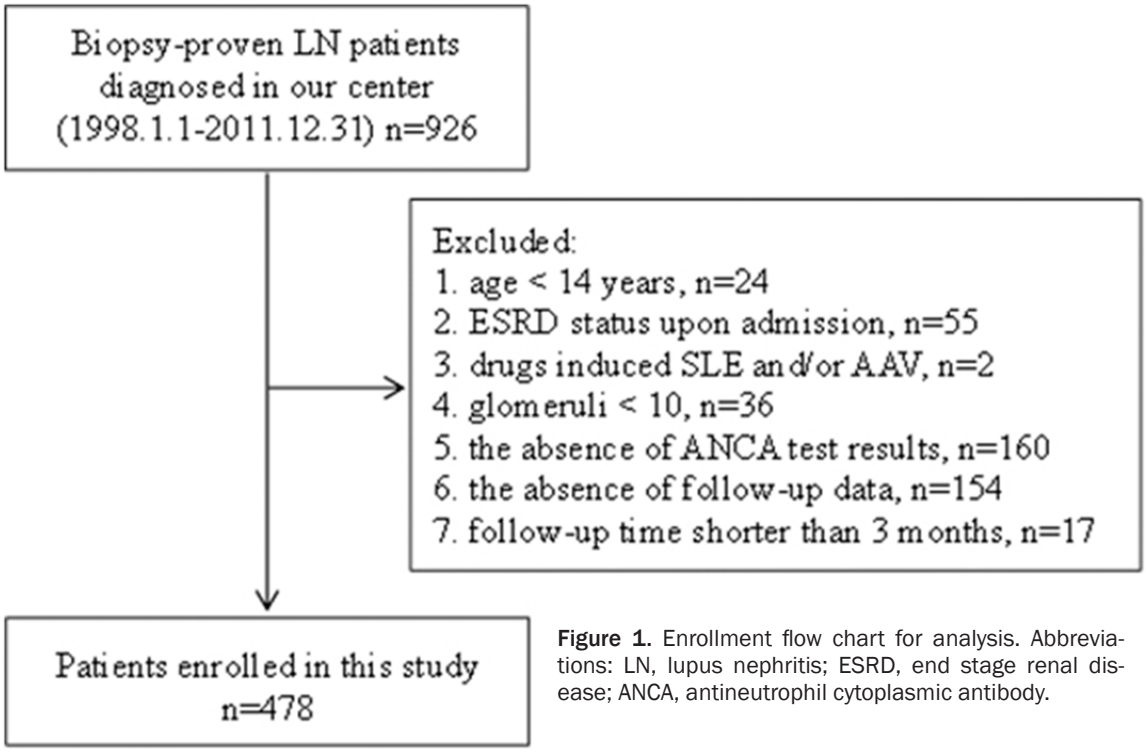

Figure 1. Enrollment flow chart for analysis. Abbreviations: LN, lupus nephritis; ESRD, end stage renal disease; ANCA, antineutrophil cytoplasmic antibody.

large cohort of ANCA-positive LN patients in China.

Patients and methods

The clinical data, pathological features, and clinical outcomes of 478 patients with renal biopsy-proven lupus nephritis diagnosed at The First Affiliated Hospital of Sun Yat-sen University between January 1998 and December 2011 were retrospectively analyzed for this study. Additionally, renal histopathology data were reviewed and reclassified according to the International Society of Nephrology and Renal Pathology Society (ISN/RPS) 2003 classification [18].

Each study patient satisfied the revised criteria for SLE developed in 1997 by the American College of Rheumatology [19]. The study exclusion criteria were as follows: (1) age < 14 years; (2) dialysis dependence upon admission; (3) drugs (such as hydralazine, propylthiouracil or thioridazine etc.) induced SLE and/or AAV; (4) the absence of ANCAs test results; (5) glomeruli < 10; (6) the absence of follow-up data or follow-up time shorter than 3 months. The flow chart of this study is shown in **Figure 1**. The conduct of this study complied with ethical principles outlined in the Helsinki Declaration, and the Human Ethics Committees of Sun Yat-sen University reviewed and approved the study protocol. All study participants provided a signed written Informed Consent prior to enrollment.

Clinical data and laboratory tests

Baseline demographic and clinical data for each patient were collected immediately prior to biopsy. Each patient's estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation [20]. Disease activity was classified according to guidelines in the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [21].

ANCAs testing were conducted with an indirect immunofluorescence (IIF) assay and/or an enzyme-linked immunosorbent assay (ELISA). Standard IIF assays were performed in accordance with instructions provided by the manufacturer of the assay kit (EUROIMMUN; Lübeck, Germany). The human neutrophil ANCAs antigens proteinase 3 (PR3) and myeloperoxidase (MPO) were purified as previously reported [13], and used as solid-phase ligands in the antigen-specific ELISAs. A subset of the patients enrolled in this study had been tested for ANCAs only with either an IIF assay or ELISA rather than with both assays. Therefore, patients who

tested positive for ANCAs with either type of assay were classified ANCA positive. Furthermore, it has been reported that ~5% of serum samples are positive by ELISA only [5].

Renal histopathology

Each renal biopsy specimen was examined by both light microscopy and direct immunofluorescence.

Light microscopy examination

Renal biopsy specimens for light microscopy were fixed in 4% buffered formaldehyde, serially cut into consecutive 2 μm sections, and then stained with either hematoxylin and eosin (H&E), periodic acid-Schiff, silver methenamine, or Masson's trichrome. Next, renal pathologists evaluated the stained sections for various pathological parameters including activity indices (AIs) and chronicity indices (CIs); after which, specific biopsy features were semi-quantitatively scored as described in previous studies [13, 22, 23].

Direct immunofluorescence examination

The intensity of immunofluorescence indicating immunoglobulin deposition (IgG, IgA, IgM, C3 or C1q) or the presence of fibrin deposits was semi-quantitatively graded on a scale of 0 to 4+ [24].

Patient follow-up and clinical outcomes

All enrolled patients participated in follow-up visits until one of the following events occurred: (1) doubling of baseline serum creatinine (D-SCr), (2) progression to ESRD, (3) all-cause death or (4) the date of December 31, 2012. If patients were lost to follow-up, they were followed until the last recorded visit. The primary end point was the composite of D-SCr, the onset of ESRD, or patient mortality. ESRD was defined as the initiation of maintenance dialysis or renal transplantation [25, 26]. Survival time was defined as the time elapsed between a patient's enrollment in the study and the start of D-SCr, permanent dialysis, a renal transplantation, the occurrence of patient death or the date of the last follow-up; whichever occurred first.

Statistical analysis

All study data were analyzed using SPSS for Windows, Version 13.0. Chicago, IL: SPSS Inc. Continuous variables are expressed as the mean ± standard deviation (SD) or a median value with the interquartile range. Categorical data are presented as the frequency and percentage. Clinical, laboratory, and pathology data were compared using the Student's *t*-test, non-parametric Mann-Whitney test or chi-square test.

A Kaplan-Meier survival curve was created and used to calculate the cumulative event-free survival rates, and the log-rank test was used to compare survival differences. Risk factors which might affect clinical outcomes were evaluated using univariate analysis followed by a multivariate Cox proportional hazards regression model. The following variables were examined in the multivariate analysis: ANCA, sex, age, SLEDAI, systolic blood pressure, 24-h urine protein, hemoglobin, creatinine, HDL-C, C-reactive protein, AI score, CI score, and immunosuppressive therapy. The results of these regression analyses are expressed as hazard ratios (HRs) with 95% confidence intervals (CIs). Two-tailed *P*-values < 0.05 were considered statistically significant.

Results

Baseline patient demographic and clinical characteristics

Among the 478 subjects, 14.0% (67/478) were ANCA positive, 16.9% were male, and the median (25th, 75th quartiles) age was 28 years (20, 38). The mean SLEDAI score was 15 ± 5, and the median (25th, 75th) eGFR and 24-h urine protein values were 90.13 (49.41, 120.21) ml/min/1.73 m^2 and 1.61 (0.76, 3.10) g/d, respectively. Among the subjects, 45.0% (215/478) were classified as Class IV LN (2003 ISN/RPS), and the median scores for AI and CI were 7 (4, 10) and 2 (0, 3), respectively. With regards to medications, 98.3% (470/478) of the patients had received oral prednisone, and 61.1% (292/478) had received an additional immunosuppressive agent; e.g., 42.7% (204/478) had received cyclophosphamide (i.v.; 800-1000 mg/month). At the median follow-up time of 56 months (range, 3-162 months), 69 patients reached a composite outcome, the 1-, 3-, 5- and 10-year cumulative event-free survival rates were 94.5%, 91.0%, 87.8% and 75.3%, respectively.

Table 1. Comparison of baseline clinical characteristics of patients with ANCA-negative or ANCA-positive lupus nephritis

Parameters	Total	ANCA-negative	ANCA-positive	*P*-value
Number of patients	478	411	67	
Male, n (%)	81 (16.9)	70 (17.0)	11 (16.4)	0.901
Age (years)	28 (20, 38)	27 (20, 38)	30 (22, 39)	0.420
Arthralgia, n (%)	153 (32.0)	126 (30.7)	27 (40.3)	0.117
AKI, n (%)	84 (17.6)	72 (17.5)	12 (17.9)	0.938
SLEDAI	15 ± 5	15 ± 5	16 ± 5	0.347
Systolic BP (mmHg)	129 ± 19	128 ± 19	129 ± 21	0.702
Diastolic BP (mmHg)	82 ± 13	82 ± 13	81 ± 13	0.769
eGFR (mL/min/1.73 m^2)	90.13 (49.41, 120.21)	98.32 (51.14, 121.23)	66.14 (26.54, 103.89)	0.001
Hemoglobin (g/L)	100 ± 23	103 ± 23	88 ± 20	< 0.001
Albumin (g/L)	27 (22, 32)	26 (21, 32)	28 (23, 31)	0.593
Globulin (g/L)	26 (22, 32)	26 (21, 31)	31 (25, 36)	< 0.001
HDL-C (mmol/L)	0.96 (0.73, 1.35)	0.99 (0.73, 1.40)	0.82 (0.68, 1.12)	0.005
LDL-C (mmol/L)	3.20 (2.38, 4.28)	3.25 (2.44, 4.42)	2.85 (2.09, 3.55)	0.002
Creatinine (mg/dL)	0.92 (0.68, 1.49)	0.86 (0.67, 1.45)	1.11 (0.81, 2.41)	0.001
Urine protein (g/24 hours)	1.61 (0.76, 3.10)	1.60 (0.71, 3.13)	1.70 (0.98, 2.61)	0.709
Hematuria, n (%)	335 (70.1)	288 (70.1)	47 (70.1)	0.990
Positive ANA, n (%)	465 (97.3)	399 (97.1)	66 (98.5)	0.794
Positive anti-ds-DNA, n (%)	391 (81.8)	331 (80.5)	60 (89.6)	0.076
Positive aCL, n (%)	162 (38.7)	133 (36.2)	29 (55.8)	0.007
C3 (g/L)	0.43 (0.30, 0.66)	0.44 (0.31, 0.67)	0.38 (0.27, 0.54)	0.058
C4 (g/L)	0.10 (0.06, 0.16)	0.10 (0.06, 0.16)	0.10 (0.06, 0.16)	0.272
C-reactive protein (mg/L)	1.96 (0.81, 5.97)	1.70 (0.81, 5.73)	2.64 (0.99, 11.41)	0.024
ESR (mm/h)	36 (18, 62)	34 (18, 59)	53 (26, 79)	0.003
Treatment				
Oral prednisone, n (%)	470 (98.3)	404 (98.3)	66 (98.5)	1.000
Cyclophosphamide, n (%)	204 (42.7)	178 (43.3)	26 (38.8)	0.490
MMF, n (%)	70 (14.6)	57 (13.9)	13 (19.4)	0.235
Cyclosporin A, n (%)	11 (2.3)	10 (2.4)	1 (1.5)	0.971
Tacrolimus, n (%)	7 (1.5)	6 (1.5)	1 (1.5)	1.000

Notes: Data are presented as mean ± SD, median (25th, 75th) or number (%). AKI: acute kidney injury; SLEDAI: systemic lupus erythematosus disease activity index; BP: blood pressure; eGFR: estimated glomerular filtration rate; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; aCL: anticardiolipin; creatinine: 1 mg/dL = 88.41 μmol/L.

Each patient in the ANCA-positive group was identified by an ANCA positive ELISA or IIF test result. Nearly all ANCA-positive patients (64/67) had been tested for ANCAs with an IIF assay, and the results showed that 60.9% (39/64) were p-ANCA positive, 12.5% (8/64) were c-ANCA positive, 4.7% (3/64) were both p-ANCA and c-ANCA positive, and 21.9% (14/64) were IIF negative. Among patients in the ANCA-positive cohort, 79.1% (53/67) had been tested by ELISA, and those results showed that 37.7% (20/53) were MPO-ANCA positive, 15.1% (8/53) were PR3-ANCA positive, 24.5% (13/53) were both MPO-ANCA and PR3-ANCA positive, and 22.7% (12/53) were ELISA negative.

Comparison of baseline characteristics of patients with ANCA-negative and ANCA-positive LN

Table 1 shows clinical characteristics of the 478 patients with LN after they were divided into two groups consisting of ANCA-negative and ANCA-positive patients. At the time of renal biopsy, patients in the ANCA-positive groups had higher levels of serum creatinine, globulin, and C-reactive protein (CRP), higher ESR values, and were more likely to test positive for anticardiolipin (aCL) antibodies. However, the ANCA-positive patients had lower eGFR values, and lower levels of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein choles-

Table 2. Comparison of renal pathological data from groups of patients with ANCA-negative or ANCA-positive lupus nephritis

Parameters	Total	ANCA-negative	ANCA-positive	*P*-value
Number of biopsies	478	411	67	
Pathological classification				0.017
Class I/II, n (%)	48 (10.0)	44 (10.7)	4 (6.0)	
Class III, n (%)	49 (10.3)	43 (10.5)	6 (9.0)	
Class IV, n (%)	215 (45.0)	174 (42.3)	41 (61.2)	0.004*
Class V, n (%)	69 (14.4)	64 (15.6)	5 (7.4)	
Class VI, n (%)	7 (1.5)	4 (1.0)	3 (4.4)	
Class V + III, n (%)	33 (6.9)	30 (7.3)	3 (4.5)	
Class V + IV, n (%)	57 (11.9)	52 (12.6)	5 (7.5)	
AI score	7 (4, 10)	7 (4, 10)	7 (5, 10)	0.061
Endocapillary hypercellularity	2 (1, 3)	2 (1, 3)	2 (2, 3)	0.829
Subendothelial hyaline deposits	0 (0, 1)	0 (0, 1)	0 (0, 1)	0.969
Karyorrhexis/fibrinoid necrosis	2 (0, 2)	0 (0, 2)	2 (0, 2)	0.041
Cellular crescent	0 (0, 2)	0 (0, 2)	2 (0, 2)	0.046
Interstitial inflammation	1 (0, 1)	1 (0, 1)	1 (1, 2)	0.004
Glomerular leukocyte infiltration	1 (0, 2)	1 (0, 2)	1 (0, 1)	0.345
CI score	2 (0, 3)	2 (0, 3)	2 (1, 4)	0.051
Glomerular sclerosis	0 (0, 1)	0 (0, 1)	0 (0, 1)	0.573
Fibrous crescent	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.249
Tubular atrophy	1 (0, 1)	1 (0, 1)	1 (0, 1)	0.007
Tubular atrophy (≥2), n (%)	55 (11.5)	41 (10.0)	14 (20.9)	0.009
Interstitial fibrosis	1 (0, 1)	1 (0, 1)	1 (0, 1)	0.055
Direct immunofluorescence				
IgA	1 (0, 2)	1 (0, 2)	1 (0, 2)	0.207
IgG	2 (1, 3)	2 (1, 3)	2 (1, 2)	0.005
IgM	1 (0, 2)	1 (0, 2)	1 (0, 2)	0.870
C1q	2 (1, 2)	2 (1, 2)	2 (1, 2)	0.180
C3	2 (1, 2)	2 (1, 2)	2 (1, 3)	0.252
Fibrin	0 (0, 1)	0 (0, 1)	0 (0, 1)	0.390

Notes: Data are presented as number (%) and/or median (25th, 75th); *comparison of Class IV; AI, activity indices; CI, chronicity indices.

terol (LDL-C), and hemoglobin when compared with the ANCA-negative LN patients. Additionally, ANCA-positive patients were more likely to present with Class IV lupus nephritis (42.3% versus 61.2%, $P = 0.004$), and had higher scores for karyorrhexis/fibrinoid necrosis, cellular glomerular crescents, interstitial inflammation, and tubular atrophy ($P = 0.041$, $P = 0.046$, $P = 0.004$, and $P = 0.007$, respectively). The two groups showed no significant differences regarding other pathological features as determined by light microscopic examinations. Additionally, with the exception of slightly less intense IgG staining in the ANCA-positive group ($P = 0.005$), there was no significant difference between the two groups regarding the majority of their immune deposits. The renal histopathological characteristics of ANCA positive and negative patients are shown in **Table 2**.

Treatment and clinical outcomes

There was no significant difference in the therapeutic regimens used for treating the two groups of patients (ANCA-negative and ANCA-positive) (**Table 1**). In our cohort study, the patients were followed-up for a median time of 56 months (range, 3-162 months). The overall cumulative event-free survival rates at 1, 3, 5, and 10 years as determined by Kaplan-Meier analysis were 94.6%, 91.6%, 88.4%, and 79.2% for ANCA-negative patients, and 93.9%, 87.5%, 83.7%, and 60.1% for ANCA-positive patients. A survival analysis showed that patients in the ANCA-positive group had significantly worse outcomes than patients in the ANCA-negative group ($P = 0.015$, **Figure 2**). In the ANCA-negative group, 33 patients died; among whom, 2 died due to CVD events, 14 died of infection, and 17 died of unknown reasons; while 15 patients reached ESRD and 3 patients reached D-SCr. In the ANCA-positive group, 10 patients died; among whom, 4 died of CVD events, and 6 died of unknown reasons; while 6 reached ESRD and 2 reached D-SCr.

Univariate survival analysis was used to evaluate factors that may affect composite outcome in all the patients with lupus nephritis. We found that ANCA was a risk factor of reaching the composite outcomes in lupus nephritis (HR, 1.93; 95% CI, 1.13-3.31; $P = 0.017$), other univariate risk factors included male gender (HR, 1.84; 95% CI, 1.09-3.13; $P = 0.024$), systolic blood pressure (HR, 1.01; 95% CI, 1.00-1.02; $P = 0.021$), 24-h urine protein (HR, 1.09; 95% CI,

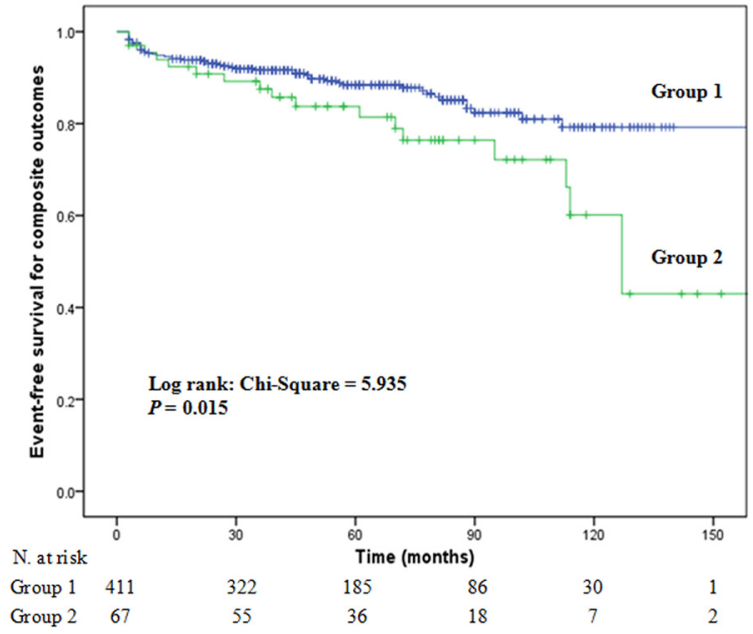

Figure 2. Kaplan-Meier plots for composite outcomes of LN patients with (group 2) and without (group 1) ANCAs.

1.02-1.17; P = 0.013), baseline serum creatinine concentration (HR, 1.65; 95% CI, 1.41-1.93; P < 0.001), AI score (HR, 1.07; 95% CI, 1.01-1.13; P = 0.019), cellular crescent (HR, 1.19; 95% CI, 1.05-1.36; P = 0.006), interstitial inflammation (HR, 1.78; 95% CI, 1.34-2.37; P < 0.001), CI score (HR, 1.24; 95% CI, 1.13-1.36; P < 0.001), glomerular sclerosis (HR, 1.60; 95% CI, 1.24-2.07; P < 0.001), tubular atrophy (HR, 1.77; 95% CI, 1.34-2.35; P < 0.001), and interstitial fibrosis (HR, 1.74; 95% CI, 1.33-2.27; P < 0.001). Higher hemoglobin was a protective factor (HR, 0.98; 95% CI, 0.97-0.99; P < 0.001). However, on multivariate regression, only baseline serum creatinine concentration (HR, 1.37; 95% CI, 1.04-1.81; P = 0.028) and CI score (HR, 1.18; 95% CI, 1.04-1.34; P = 0.010) were independently associated with primary outcome events. ANCA was not found to be a risk factor for composite outcome on multivariate Cox regression (**Table 3**).

Discussion

In this present study, we reported the prevalence of ANCA-positivity in one Chinese LN cohort and identified certain characteristics in LN patients that were associated with ANCA seropositivity. Furthermore, the ANCA-positive LN patients were shown to have a poor prognosis; e.g., ~60.1% of ANCA seropositive patients reached the primary outcome within 10 years. Additionally, a patient's initial serum creatinine concentration and CI score were the independent risk factors for the primary outcome. To the best of our knowledge, this current study is the largest ever conducted to investigate the clinical outcomes of ANCA-positive LN patients.

The overall prevalence of ANCA-positivity in our cohort of lupus nephritis patients was 14.0% (67/478). This was similar to the 16.4% (93/566) prevalence found in a large European cohort of SLE patients diagnosed by IIF methods [11], but lower that the 37.3% (19/51) prevalence reported in a Korean study [15]. These discrepancies in the rates of ANCA-positivity might result from the following factors: (1) we excluded patients who were treated by chronic dialysis upon their first admission; (2) we excluded patients who did not have any follow-up data; (3) the different sample sizes and patient populations in the studies may have affected their results. Various studies have provided conflicting ideas concerning the clinical implications of ANCAs being detected in SLE patients. While the results of some investigations have suggested that ANCAs, disease activity, and organ involvement are all associated [11, 15, 27, 28], other studies have failed to show such associations [16, 29]. Manolova *et al.* [27] found a positive correlation between the presence of BPI-, LF-, and PR3-ANCAs and disease activity, as well as an association between PR3-ANCAs and arthritis. A study by Chin *et al.* [15] of 59 patients with SLE in South Korea revealed an association between ANCA seropositivity and the presence of both nephritis and anti-ds-DNA antibodies. Additionally, a study by Lee *et al.* [28] of 79 SLE patients in Hong Kong found that LF-ANCA positivity was correlated with disease activity, lymphadenopathy, and crescentic glomerulonephritis. Meanwhile, Galeazzi *et al.* [11] studied a large cohort of SLE patients from 11 European centers and found a positive correlation between the presence of ANCAs detected by IIF and various disorders including serositis, live do reticu-

Table 3. Results of Cox proportional analyses for composite outcomes of patients with lupus nephritis

	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
ANCA	1.93	1.13-3.31	0.017	1.44	0.79-2.62	0.238
Male	1.84	1.09-3.13	0.024	1.76	0.95-3.29	0.074
Age (years)	1.01	0.99-1.03	0.275	1.01	0.98-1.03	0.682
SLEDAI	1.04	0.99-1.09	0.097	1.03	0.97-1.09	0.329
Systolic BP (mmHg)	1.01	1.00-1.02	0.021	1.01	0.99-1.02	0.456
Diastolic BP (mmHg)	1.01	1.00-1.03	0.121			
Urine protein (g/24 hours)	1.09	1.02-1.17	0.013	1.09	1.00-1.19	0.051
Hemoglobin (g/L)	0.98	0.97-0.99	< 0.001	0.99	0.97-1.00	0.110
Creatinine (mg/dL)	1.65	1.41-1.93	< 0.001	1.37	1.04-1.81	0.028
HDL-C (mmol/L)	0.59	0.33-1.04	0.067	0.74	0.38-1.46	0.391
LDL-C (mmol/L)	1.04	0.91-1.20	0.544			
Albumin (g/L)	0.98	0.94-1.01	0.158			
C-reactive protein (mg/L)	1.01	1.00-1.03	0.091	1.00	0.99-1.02	0.805
AI score	1.07	1.01-1.13	0.019	0.94	0.87-1.02	0.112
Endocapillary hypercellularity	1.16	0.84-1.60	0.368			
Subendothelial hyaline deposits	1.10	0.78-1.55	0.580			
Karyorrhexis/fibrinoid necrosis	1.03	0.88-1.20	0.736			
Cellular crescent	1.19	1.05-1.36	0.006			
Interstitial inflammation	1.78	1.34-2.37	< 0.001			
Glomerular leukocyte infiltration	1.15	0.87-1.51	0.329			
CI score	1.24	1.13-1.36	< 0.001	1.18	1.04-1.34	0.010
Glomerular sclerosis	1.60	1.24-2.07	< 0.001			
Fibrous crescent	1.09	0.59-2.02	0.776			
Tubular atrophy	1.77	1.34-2.35	< 0.001			
Interstitial fibrosis	1.74	1.33-2.27	< 0.001			
Immunosuppressive therapy	1.11	0.66-1.86	0.694	1.29	0.71-2.35	0.407

Notes: SLEDAI: systemic lupus erythematosus disease activity index; BP: blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; AI: activity indices; CI: chronicity indices; creatinine: 1 mg/dL = 88.41 μmol/L.

laris, venous thrombosis, and arthritis. However, other autoantibodies such as aCL and anti-SSA/Ro showed stronger correlations than ANCAs with the same clinical disorders. Moreover, other studies have failed to identify any correlation between ANCAs and either disease activity or organ involvement [16, 29]. In our current study, we failed to identify a significant difference between ANCA-positive and ANCA-negative LN patients regarding the majority of their clinical manifestations and laboratory test results. However, we did find that ANCA-positive LN patients had higher rates of seropositivity for aCL antibodies, as well as higher ESR values and levels of CRP; however, they had lower values for eGFR and lower levels of hemoglobin. These findings suggest that the presence of ANCAs might be associated with the active phases of lupus nephritis and higher degrees of kidney damage. A previous study showed an association between ANCAs and the presence of diffuse proliferative LN (WHO class IV) [15], as well as crescent formation in patients with WHO class IV glomerulonephritis [28]. In agreement with those results, our current study also found a significantly higher proportion of 2003 ISN/RPS class IV LN in an ANCA-positive group, as well as higher scores for karyorrhexis/fibrinoid necrosis, cellular crescents, interstitial inflammation, and fewer IgG deposits in that group. These findings suggest that ANCA seropositivity in LN patients may represent more than the coincidence. Nasr *et al.* [30] proposed that either one of the two conditions (lupus nephritis or ANCA seropositivity) may facilitate development of the second. They also pro-

posed that ANCA-associated necrotizing and crescentic glomerulonephritis may occur superimposed on lupus nephritis [30].

While there are no established guidelines for treating ANCA-positive lupus nephritis, the current regimen used for treating AAV is similar to that used for treating proliferative lupus nephritis, and has components of both initial and maintenance therapy [31]. In cases with disease relapse, maintenance immunosuppressive therapy may be extended to ≥ 18-24 months [32, 33]. At our center, similar therapeutic regimens are used for treating LN patients with and without ANCAs. There is minimal information available concerning the long-term outcomes of ANCA-positive LN patients. A Korean study of 51 LN patients found that patients with ANCAs, and especially p-ANCAs, more frequently showed deterioration of renal function than LN patients without ANCAs after 32.5 months (range, 0.2-178 months) of follow-up [15]. However, due to the small sample size and the fact that only a few patients had renal deterioration, the investigator did not assess the association of ANCA and the clinical outcomes. Our current results obtained from a large cohort study showed that LN patients who were ANCA-positive had significantly worse long-term clinical outcomes compared with ANCA-negative patients. Nevertheless, the presence of ANCAs was not independently associated with occurrence of the primary outcome, and a patient's initial serum creatinine concentration was the only independent risk factor. This finding is consistent with previous reports indicating that serum creatinine concentrations are highly predictive of long-term outcomes for patients with lupus nephritis or AAV [34, 35].

Strength of this study is that to our best knowledge, it is the largest study ever conducted to investigate long-term outcomes in patients with ANCA-positive lupus nephritis. Additionally, the studied patients displayed had a wide range of clinical manifestations and were followed up for a median time of 56 months (range, 3-162 months). However, this study also has some limitations that should be mentioned. First, although it was the largest LN cohort study conducted to date, it had a retrospective rather than a prospective design. Second, not all patients were tested for ANCAs by both an IIF assay and ELISA. Some patients were tested only by IIF and others only by ELISA. Third, there was only limited data available concerning patient response to treatment and causes of death.

In conclusion, the prevalence of ANCA seropositivity among LN patients at our center was 14.0%. Patients with ANCA-positive lupus nephritis had worse renal function and more active renal pathological changes when compared to ANCA-negative patients. Lupus nephritis patients with ANCAs had less favorable clinical outcomes compared to lupus nephritis patients without ANCAs. The initial serum creatinine concentration and CI score were the strongest predictors of primary outcome events for a lupus nephritis patient; whereas ANCAs were not independently associated with those events. In this regard, our data will be of importance to physicians developing strategies for treating lupus nephritis patients.

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Disclosure of conflict of interest

None.

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