J Rheum Dis 2022;29(4):232-242
https://doi.org/10.4078/jrd.22.0011
pISSN: 2093-940X, eISSN: 2233-4718

Original Article

Interstitial Inflammation in the ISN/RPS 2018 Classification of Lupus Nephritis Predicts Renal Outcomes and is Associated With Bcl-2 Expression

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Objective: To investigate the histopathological characteristics of patients with lupus nephritis in the 2018 revised International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification and assess the prognostic factors.

Methods: This study enrolled 92 patients with lupus nephritis, who had conventional treatment and renal biopsy. Each renal tissue was evaluated according to 2018 ISN/RPS classification, and quantified apoptotic regulator protein, the B-cell lymphoma-2 protein (Bcl-2), expressions in selected lymphocyte subsets were measured using novel computational approaches using multicolor confocal images. Histopathological characteristics and prognostic factors of end-stage renal disease (ESRD) and chronic kidney disease (CKD) were compared. Follow-up data were obtained, and survival analysis was conducted.

Results: During follow-up period (average: 74.3 months), 16 and 18 patients progressed ESRD and CKD, respectively. Multivariable analysis of age, sex, disease activity and pathological features in ISN/RPS demonstrated the extent of interstitial inflammation (grade 0~3) was significantly associated with both ESRD and CKD. When interstitial inflammation was divided into mild (grade 0, 1) and severe (grade 2, 3), Cox regression analysis showed that patients with severe interstitial inflammation were significantly increased risk of both ESRD and CKD (hazard ratio: 4.67 and 3.8, respectively). Bcl-2 expression in CD4+ and CD20 cells was significantly higher in the severe interstitial inflammation group compared to in mild interstitial inflammation patients (p=0.006 and 0.010, respectively).

Conclusion: The extent of interstitial inflammation can predict clinical renal outcomes. Significantly elevated Bcl-2 expression in both CD4+ and CD20 cells was found in severe interstitial inflammation compared with mild interstitial inflammation.

Keywords: Lupus nephritis, Inflammation, Prognosis

INTRODUCTION

The most common manifestation of systemic lupus erythematosus (SLE) is lupus nephritis [1]. Approximately 40% of patients with SLE develop lupus nephritis in the disease course and thus have been associated with poor prognosis [2]. Lupus nephritis–associated mortality and morbidity remain stable for a 10-year period [3]. Predictive markers and optimal treatment strategies are required to improve long-term outcomes of lupus nephritis.

Although glomerular lesions adopted by International Society of Nephrology/Renal Pathology Society (ISN/RPS) classifica-

Received March 8, 2022; **Revised** May 30, 2022; **Accepted** June 28, 2022, **Published online** July 26, 2022

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tion in 2003 are the most common cause of lupus nephritis, measuring histologic activity of glomerular lesions, which does not identify patients for subsequent renal failure [4]. On the contrast, it was reported that tubulointerstitial lesions including tubulointerstitial inflammation and interstitial fibrosis/tubular atrophy were associated with predictors of poor renal outcomes independent of glomerular lesions [5-9].

A working group for lupus nephritis proposed a revision of ISN/RPS classification in 2018 to clarify the definitions of lupus nephritis [10]. This revision incorporated the evaluation of the above-mentioned lesions in terms of activity index (AI) for interstitial inflammation and chronicity index (CI) for interstitial fibrosis/tubular atrophy by semi-quantitative scoring system to underscore the significance of tubulointerstitium.

B-cell lymphoma-2 (Bcl-2) is one of the anti-apoptotic proteins, which are overexpressed in peripheral lymphocytes of humans and play an important role in regulating autoreactive lymphocytes [11]. Treatment of a murine model of SLE with Bcl-2 antagonist prolonged survival and prevented the development of interstitial inflammation, suggesting decreased in situ immunity in lupus nephritis [12]. Treatment with Bcl-2 antagonist in patients with SLE was found to be associated with reductions in lymphocytes, especially in B lymphocytes [13]. These results support Bcl-2 inhibitors as one of the potential therapeutic agents for the treatment of SLE.

This study aimed to characterise pathological features according to 2018 revised ISN/RPS classification and then compare those with prognosis in patients with lupus nephritis. Subsequently, the expression of Bcl-2 in T and B lymphocytes were investigated using multicolor confocal images infiltrating kidney tissues.

MATERIALS AND METHODS

Patients and study design

This study was a retrospective cohort. A total of 110 adult patients with lupus nephritis were identified fulfilling the SLE classification criteria of the American College of Rheumatology in 1997 [14]. They underwent renal biopsy at Kyungpook National University Hospital (KNUH) between May 2005 and January 2020. Of these, 17 patients had inadequate biopsy specimens and 1 patient had normal renal tissue. Thus, 92 patients were enrolled in this study. Their pathological features according to 2018 revised ISN/RPS classification and baseline clinical characteristics, including renal function deterioration during observational periods were assessed. This study was approved by the Institutional Review Board (IRB) and Ethics Committee at confirmed KNUH (approval number 2020-07-059). Since the study involved a minimum risk to the enrolled patients, and no identifiable information was used, the requirement for informed consent was waived by the IRB. Relevant guidelines and regulations were followed for the procedures.

Histological assessments

All renal specimens (n=92) included more than 10 glomeruli and were evaluated under light microscopy in accordance with 2018 revised ISN/RPS classification. Periodic acid–Schiff, periodic acid methenamine silver, and Masson's trichrome stains were routinely used. The AI and CI were assessed according to the modified National Institute of Health (mNIH) system. The components of AI included endocapillary hypercellularity, neutrophils/karyorrhexis, fibrinoid necrosis, hyaline deposits, cellular/fibrocellular crescents, and interstitial inflammation. The components of CI included global/segmental sclerosis, fibrous crescents, interstitial fibrosis, and tubular atrophy. The scores of fibrinoid necrosis and cellular/fibrocellular crescents were doubled and ranged from 0 to 3 with respect to the involved areas (0%, 0; <25%, +1; 25%~50%, 2+; >50%, 3+).

Multiplex immunofluorescence staining

Specimens of 4-μm sections were cut from formalin-fixed paraffin-embedded blocks. Slides were heated for at least 1 hour in a dry oven at 60˚C followed by multiplex immunofluorescence staining with a Leica Bond Rx Automated Stainer (Leica Biosystems, Newcastle, UK). Briefly, the slides were baked for 30 minutes and dewaxed with Leica Bond Dewax solution (#AR9222; Leica Biosystems) followed by antigen retrieval with Bond Epitope Retrieval 2 (#AR9640; Leica Biosystems) in a pH 9.0-solution for 30 minutes. The slide was then incubated with primary antibodies for Bcl-2 (226R-16, dilution 1:50; Cell Marque, Rocklin, CA, USA) for 30 minutes, followed by detection using Polymer HRP Ms+Rb (ARH1001EA; Akoya Biosciences, Marlborough, MA, USA). Visualisation of Bcl-2 was accomplished using Opal 570 TSA (dilution 1:150) for 10 minutes after which the slide was treated with Bond Epitope Retrieval 1 (#AR9961; Leica Biosystems) for 20 minutes to remove bound antibodies before the next step in the sequence. The slide was then incubated with primary antibodies for CD4 (ab133616,

dilution 1:200; Abcam, Cambridge, UK) for 30 minutes followed by detection using Polymer HRP Ms+Rb (ARH1001EA; Akoya Biosciences). Visualisation of CD4 was carried out using Opal 520 TSA (dilution 1:150) for 10 minutes after which the slide was treated with Bond Epitope Retrieval 1 (#AR9961; Leica Biosystems) for 20 minutes to remove bound antibodies before the next step in the sequence. The slide was then incubated with primary antibodies for CD20 (ab9475, dilution 1:100; Abcam) for 30 minutes followed by detection using Polymer HRP Ms+Rb (ARH1001EA; Akoya Biosciences). Visualisation of CD20 was accomplished using Opal 480 TSA (dilution 1:150) for 10 minutes after which the slide was treated with Bond Epitope Retrieval 1 (#AR9961; Leica Biosystems) for 20 minutes to remove bound antibodies before the next step in the sequence. Nuclei were subsequently visualised with 4',6-diamidino-2-phenylIndole (DAPI), and the section was coverslipped using ProLong Gold antifade reagent (P36934; Invitrogen, Carlsbad, CA, USA).

Image acquisition and quantitative data analysis

Slides were scanned using the Vectra Polaris Automated Quantitative Pathology Imaging System (Akoya Biosciences), and images were analysed using the InForm 2.4 software and TIBCO Spotfire (Akoya Biosciences). Representative slides of each emission spectrum and unstained tissue slide were used to acquire reliable unmixed images. Each individually stained section was used to establish a spectral library of fluorophores for multispectral analysis. This spectral library formed the reference for target quantitation as the intensity of each fluorescent target was extracted from the multispectral data using linear unmixing. Each cell was identified by detecting nuclear spectral elements (DAPI). All the immune cell populations from each panel were characterised and quantified using the cell segmentation tool by the InForm image analysis software. The data obtained from InForm were sent to Spotfire software, and positivity threshold of each factor was determined. All cells in each slide were designated as positive or negative for each antibody, and the data were categorised and exported to an XLS file for analysis. The numbers of positive cells were counted in each slide.

Selection of region of interest

Among the areas of the scanned renal interstitial images (Supplementary Figure 1), the region of interest (ROI) was marked with a rectangular shape as high-power field area (0.24 mm^2) in the area of having the most inflammatory cells. Identified nuclei (stained with DAPI) and specific membrane stains were counted on these ROI. Then, the total number of DAPI and specific stains in each ROI was assessed.

Clinical evaluation and renal outcomes

The clinical characteristics were analysed for demographic data, SLE disease activity index 2000 (SLEDAI-2K) [15], medication history, and glomerular filtration rate (GFR) at the time of the renal biopsy and at the latest clinic visit (or to the date of renal failure ensued). GFR was estimated using the isotope dilution mass spectroscopy—traceable Modification of Diet in Renal Disease study equation [16]. The primary outcome was end-stage renal disease (ESRD), which is defined as the initiation of renal replacement therapy or renal transplantation or death because of renal problem. The secondary outcome was chronic renal failure (CKD), which was defined as GFR<60 mL/min/1.73 m^2 during 3 months. Patients generally visited hospital once in every few months with blood tests.

Statistical analysis

Continuous variables were expressed as the mean±standard deviation (SD) and the median±interquartile range, and categorical variables were expressed as numbers and percentages. Comparisons were performed using either Student's t-test or Fisher's exact test. Baseline clinical and pathological data were assessed using univariate and multivariate Cox proportional hazards model to investigate predictors of renal outcomes. Hazard ratios (HR) and 95% confidence intervals (95% CI) for renal outcomes in both univariate and multivariate models (i.e., model 1 and model 2) were calculated. Model 1 was adjusted by baseline clinical characteristics, AI, and CI. Model 2 was adjusted by baseline clinical characteristics and each pathological component of AI and CI. Survival analysis was done using Kaplan–Meier method to determine predictive value according to each score of interstitial inflammation. p-values of ≤0.05 were considered statistically significant. All statistical analyses were performed using IBM SPSS version 19 (IBM Co., Armonk, NY, USA), and graphics were generated using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Baseline characteristics of the study participants with lupus nephritis

Baseline clinical and pathological characteristics of the study participants (n=92) at the time of the biopsy are presented in Table 1. The mean (±SD) age of patients was 34.9±14.6 years, and 83.7% (n=77) were female. We followed these patients for 74.3±55.3 months. The induction treatment was given to 90 patients, predominantly cyclophosphamide. The proportion of patients with class III (±Class V) and class IV (±Class V) was 17.4% (16/92) and 56.5% (52/92), respectively. The median for AI and CI of the mNIH was 6 (3, 8) and 1 (0, 3), respectively, revealing broad ranges. The frequency distribution of AI and CI is demonstrated in Supplementary Figure 2. Eighteen and 16 of enrolled patients progressed CKD and ESRD, respectively and their characteristics were shown in Supplementary Tables 1 and 2.

Identification of pathological and clinical predictors of ESRD

Baseline disease activities (i.e., proteinuria, serum C3 levels, and SLEDAI) were not associated with subsequent ESRD. However, lower baseline GFR was found to be significantly associated with future ESRD, which remained significant after adjustment for age, sex, and mNIH index (HR: 0.97, 95% CI: 0.96~0.99, p=0.003 in model 1; HR: 0.98, 95% CI: 0.96~0.99, p=0.018 in model 2). Although higher CI in mNIH index was significantly associated with an increased risk of ESRD in univariable analysis, which was not significant after adjustment for age, sex, and GFR (model 1 in Table 2). Although interstitial inflammation, glomerulosclerosis, and interstitial fibrosis were significantly associated with subsequent ESRD in univariate analysis, higher interstitial inflammation was identified only as independent predictors after adjustment (HR: 2.23, 95% CI: 1.25~3.98, p=0.007) (model 2 in Table 2).

Identification of pathological and clinical predictors of CKD

Lower baseline GFR and SLEDAI were significantly associated with CKD, which remained significant after adjustment for age, sex, and mNIH index (HR: 0.97, 95% CI: 0.95~0.99, p=0.007; HR: 0.90, 95% CI: 0.83~0.99, p=0.026 in model 1; HR: 0.96, 95% CI: 0.94~0.99, p=0.006; HR: 0.90, 95% CI: 0.81~0.99,

Table 1. Baseline characteristics of the study participants with lupus nephritis

Baseline characteristics	Values
Clinical characteristics (n=92)	
Age at biopsy (yr)	34.9±14.6
Duration of disease (mo)	106.3±64.2
Follow-up duration (mo)	74.3±55.3
Sex, female	77 (83.7)
Glomerular filtration rate (mL/min/1.73 m^2)	82.6±43.0
Proteinuria (gm/24 hr) (n=87)	3,699±3,053
Anti-nuclear antibody titer (n=91)	1,383±4,464
Anti ds-DNA positivie	72 (78.3)
Anti ds-DNA titer	283.5±471.6
C3 (mg/dL) (n=90)	45.1±25.3
C4 (mg/dL) (n=90)	8.6±7.3
SLEDAI-2K (n=70)	17.7±7.6
Induction immunosuppression	
Corticosteroid only	2 (2.2)
Corticosteroid+CYC	61 (66.3)
Corticosteroid+MMF	17 (18.5)
Corticosteroid+AZP or CsA	12 (13.0)
Pathological characteristics	
ISN/RPS lupus nephritis class	
Class I	2 (2.2)
Clsss II	7 (7.6)
Class III (±Class V)	16 (17.4)
Class IV (±Class V)	52 (56.5)
Class V	15 (16.3)
Activity and chronicity	
Activity	6 (3, 8)
Endocapillary hypercellularity	1 (0, 3)
Neutrophils/Karyorrhexis	2 (1, 3)
Fibrinoid necrosis	0 (0, 0)
Hyaline deposits	0 (0, 1)
Cellular/fibrocellular crescents	0 (0, 0)
Interstitial inflammation	1 (0, 1)
Chronicity	1 (0, 3)
Glomerulosclerosis	0 (0, 1)
Fibrous crescents	0 (0, 0)
Tubular atrophy	0 (0, 1)
Interstitial fibrosis	0 (0, 1)

Values are presented as mean±standard deviation, number (%), or median (interquartile range). C3: complement 3, SLEDAI-2K: systemic lupus erythematosus disease activity index 2000, CYC: cyclophosphamide, MMF: mycophenolate mofetil, AZP: azathioprine, CsA: cyclosporin, ISN/RPS: International Society of Nephrology/Renal Pathology Society.

Table 2. Multivariable prediction factors for ESRD

Factors	Univariate analysis		Multivariate analysis			
			Model 1		Model 2	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p -value
Clinical features						
Age at biopsy (yr)	1.02 (0.99~1.06)	0.171				
Sex, male	1.27 (0.36~4.46)	0.708				
Glomerular filtration rate	0.97 (0.96~0.99)	0.003	0.97 (0.96~0.99)	0.003	0.98 (0.96~0.99)	0.018
Proteinuria at biopsy	1.00 (1.00~1.00)	0.614				
Anti-dsDNA positivity	0.87 (0.27~2.76)	0.818				
C3 (mg/dL)	1.00 (0.98~1.02)	0.896				
SLEDAI	0.91 (0.83~1.00)	0.053				
Pathological features						
ISN/RPS class						
Class III vs Class IV	1.31 (0.36~4.73)	0.677				
2018 mNIH index						
Activity index	1.10 (0.95~1.27)	0.219				
Chronicity index	1.20 (1.02~1.42)	0.028				
Every component						
Active lesion						
Endocapillary hypercellularity	1.16 (0.78~1.72)	0.474				
Neutrophils/Karyorrhexis	1.29 (0.80~2.07)	0.297				
Fibrinoid necrosis	0.05 (0.00~431)	0.663				
Hyaline deposits	0.63 (0.29~1.37)	0.241				
C/F crescents	1.01 (0.44~2.34)	0.979				
Interstitial inflammation	2.52 (1.50~4.22)	<0.001			2.23 (1.25~3.98)	0.007
Chronic lesion						
Glomerulosclerosis	1.70 (1.06~2.71)	0.027				
Fibrous crescents	0.05 (0.00~547)	0.668				
Tubular atrophy	1.56 (0.97~2.49)	0.066				
Interstitial fibrosis	1.61 (1.02~2.53)	0.042				

Model 1 was adjusted by baseline clinical characteristics, activity index (AI), and chronicity index (CI). Model 2 was adjusted by baseline clinical characteristics and each pathological component of AI and CI. ESRD: end stage renal disease, HR: harzard ratio, 95% CI: 95% confidence intervals, C3: complement 3, SLEDAI: systemic lupus erythematosus disease activity index, ISN/RPS: International Society of Nephrology/Renal Pathology Society, mNIH: modified National Institute of Health, C/F: cellular/fibrocellular.

p=0.037 in model 2, respectively) (Table 3). Although higher AI and CI in mNIH index were significantly associated with an increased risk of CKD, those were not significant after adjustment for age, sex, GFR, and SLEDAI (model 1 in Table 3). Although interstitial inflammation, glomerulosclerosis, tubular atrophy, and interstitial fibrosis were significantly associated with subsequent CKD, higher interstitial inflammation was only significantly associated after adjustment (HR: 3.26, 95% CI: 1.42~7.50, p=0.005) (model 2 in Table 3).

Renal survival by the extent of interstitial inflammation

Survival curves were analysed using Kaplan–Meier method for renal outcomes in patients with lupus nephritis with respect to the extent of interstitial inflammation of AI index in mNIH. Thirty-seven percent (n=34) of patients were graded as 0, 39.1% (n=36) as 1, 17.4% (n=16) as 2, and 6.5% (n=6) as 3. The survival curve for ESRD (Figure 1A) and CKD (Figure 1C) demonstrated that patients with no interstitial inflammation on renal biopsy (score of 0) had consistent flap curve with excellent

Table 3. Multivariable prediction factors for CKD

Factors	Univariate analysis		Multivariate analysis			
			Model 1		Model 2	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Clinical features						
Age at biopsy (yr)	1.02 (0.99~1.05)	0.120				
Sex, male	1.36 (0.46~4.15)	0.560			0.13 (0.02~1.16)	0.068
Glomerular filtration rate	0.97 (0.95~0.98)	<0.001	0.97 (0.95~0.99)	0.007	0.96 (0.94~0.99)	0.006
Proteinuria at biopsy	1.00 (1.00~1.00)	0.632				
Anti-dsDNA positivity	0.53 (0.21~1.34)	0.179				
C3 (mg/dL)	1.00 (0.99~1.02)	0.712				
SLEDAI	0.91 (0.83~0.99)	0.028	0.90 (0.83~0.99)	0.026	0.90 (0.81~0.99)	0.037
Pathological features						
ISN/RPS class						
Class III vs Class IV	1.83 (0.53~6.34)	0.341				
2018 mNIH index						
Activity index	1.16 (1.02~1.33)	0.024				
Chronicity index	1.23 (1.07~1.42)	0.005				
Every component						
Active lesion						
Endocapillary hypercellularity	1.40 (0.97~2.01)	0.072				
Neutrophils/Karyorrhexis	1.32 (0.86~2.03)	0.205				
Fibrinoid necrosis	0.05 (0.00~108)	0.628				
Hyaline deposits	0.58 (0.28~1.21)	0.146				
C/F crescents	1.54 (0.89~2.67)	0.122				
Interstitial inflammation	2.45 (1.54~3.88)	<0.001			3.26 (1.42~7.50)	0.005
Chronic lesion						
Glomerulosclerosis	1.72 (1.13~2.61)	0.011				
Fibrous crescents	0.05 (0.00~109)	0.628				
Tubular atrophy	1.73 (1.16~2.60)	0.008			0.50 (0.21~1.20)	0.120
Interstitial fibrosis	1.69 (1.14~2.53)	0.010				

Model 1 was adjusted by baseline clinical characteristics, activity index (AI), and chronicity index (CI). Model 2 was adjusted by baseline clinical characteristics and each pathological component of AI and CI. CKD: chronic kidney disease, HR: harzard ratio, 95% CI: 95% confidence intervals, C3: complement 3, SLEDAI: systemic lupus erythematosus disease activity index, ISN/RPS: International Society of Nephrology/Renal Pathology Society, mNIH: modified National Institute of Health, C/F: Cellular/fibrocellular.

prognosis during follow-up period, while those with interstitial inflammation score of 1 had intermediate prognosis. Patients with interstitial scores of 3 and 4 showed poor prognosis. These results show the relationship between interstitial inflammation and subsequent renal outcomes (ESRD in Figure 1A, CKD in Figure 1C) is significant with respect to the extent of interstitial inflammation (p=0.001 in both). Interstitial inflammation was divided into mild (grade 0~1) versus severe (grade 2~3) for further analysis. Patients with severe interstitial inflammation were at greater risk of ESRD and CKD compared to those with mild interstitial inflammation (Figure 1B and 1D). After multivariate logistic analysis including age, sex, and GRF, severe interstitial inflammation predicted poor prognosis for ESRD (HR: 4.67, 95% CI: 1.56~13.82; p=0.005) and CKD (HR: 3.80, 95% CI: 1.45~9.98; p=0.007).

Quantifying Bcl-2 expression according to the extent of interstitial inflammation

As interstitial inflammation of the mNIH was the most important predictor for renal outcomes, Bcl-2, which is one of the anti-apoptotic proteins, expression was compared with respect to the extent of interstitial inflammation. Distributions of Bcl-

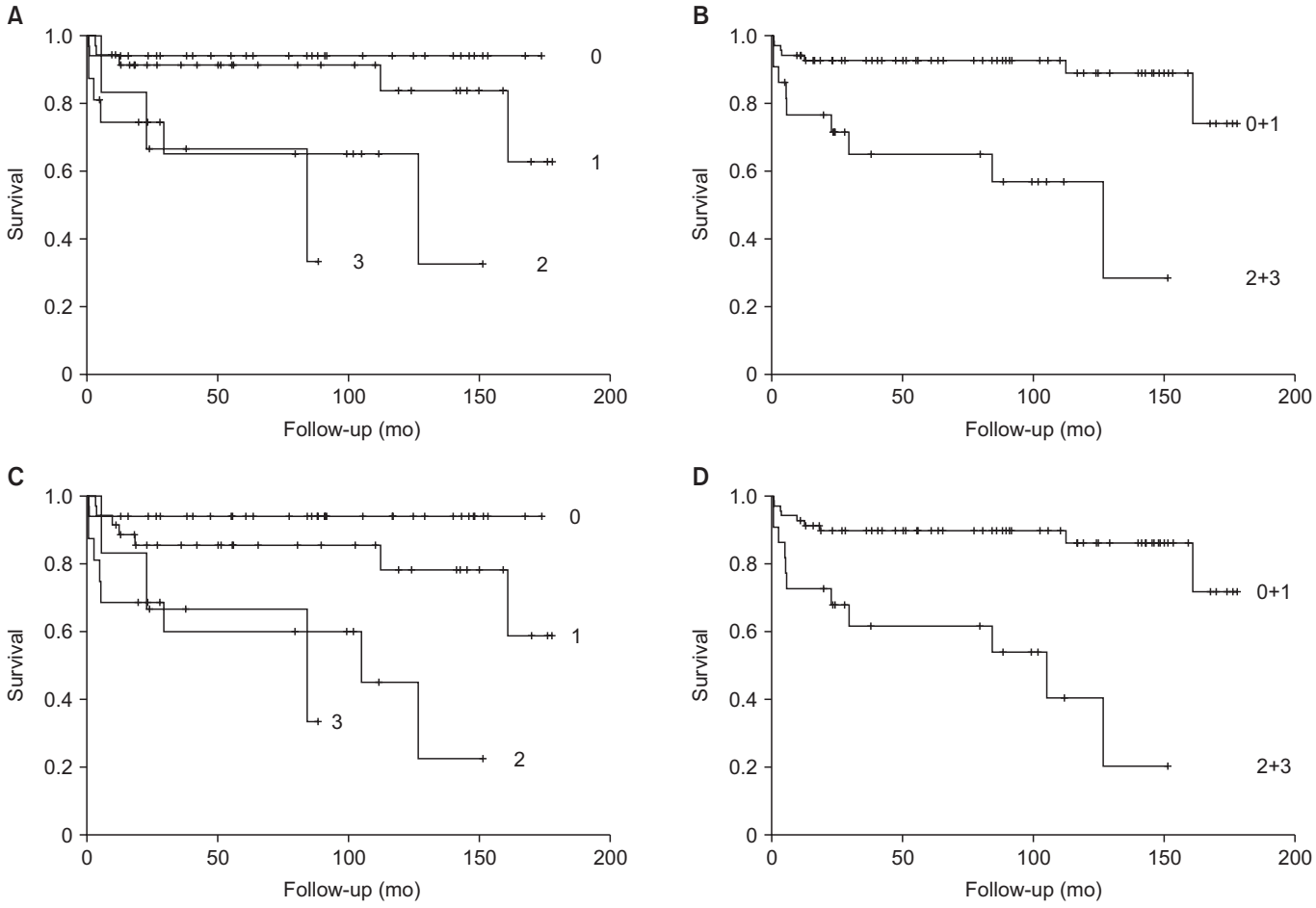

Figure 1. Prediction of renal survival by interstitial inflammation severity. Kaplan–Meier curves are described according to the interstitial inflammation (A, C) in ISN/RPS classification and mild (grades 0 and 1) versus severe (grades 2 and 3) interstitial inflammation (B, D). Kaplan–Meier survival curves with ESRD (A, B) and CKD (C, D) in patients with lupus nephritis. ISN/RPS: International Society of Nephrology/Renal Pathology Society, ESRD: end-stage renal disease, CKD: chronic kidney disease.

2 expression in renal tissues were calculated in 28 enrolled patients (mild [n=20] vs. severe [n=8]). Multicolor confocal imaging revealed that the frequency of Bcl-2 expression was significantly increased in renal tissues with severe interstitial inflammation compared to those with mild inflammation (p=0.005), whereas there was no difference in total cells between severe and mild interstitial inflammation of renal samples. Frequency of Bcl-2-positive cells among CD4+ and CD20+ cells in renal tissues with severe interstitial inflammation was found to be even higher than those with mild interstitial inflammation (p=0.006 and 0.010, respectively) (Figure 2).

Correlations between interstitial inflammation, each components of AI and CI and Bcl-2 positivity

Interstitial inflammation was significantly correlated with scores of AI and CI (r=0.487, p<0.001; r=0.653, p<0.001, respectively). This was also significantly correlated with each component of AI and CI, including endocapillary hypercellularity, cellular/fibrocellular crescents, glomerulosclerosis, tubular atrophy, and interstitial fibrosis. Interestingly, Bcl-2 positivity showed significant correlations with interstitial inflammation (Table 4) but not AI and CI (data not shown).

DISCUSSION

The extent of interstitial inflammation in mNIH AI from 2018 revised classification and GFR were found to be significantly associated with clinical renal outcomes in both ESRD and CKD based on multivariate model approach, and significantly elevated Bcl-2 expression was found in severe interstitial inflammation compared to mild interstitial inflammation of lupus nephritis. Although the renal outcomes were significantly

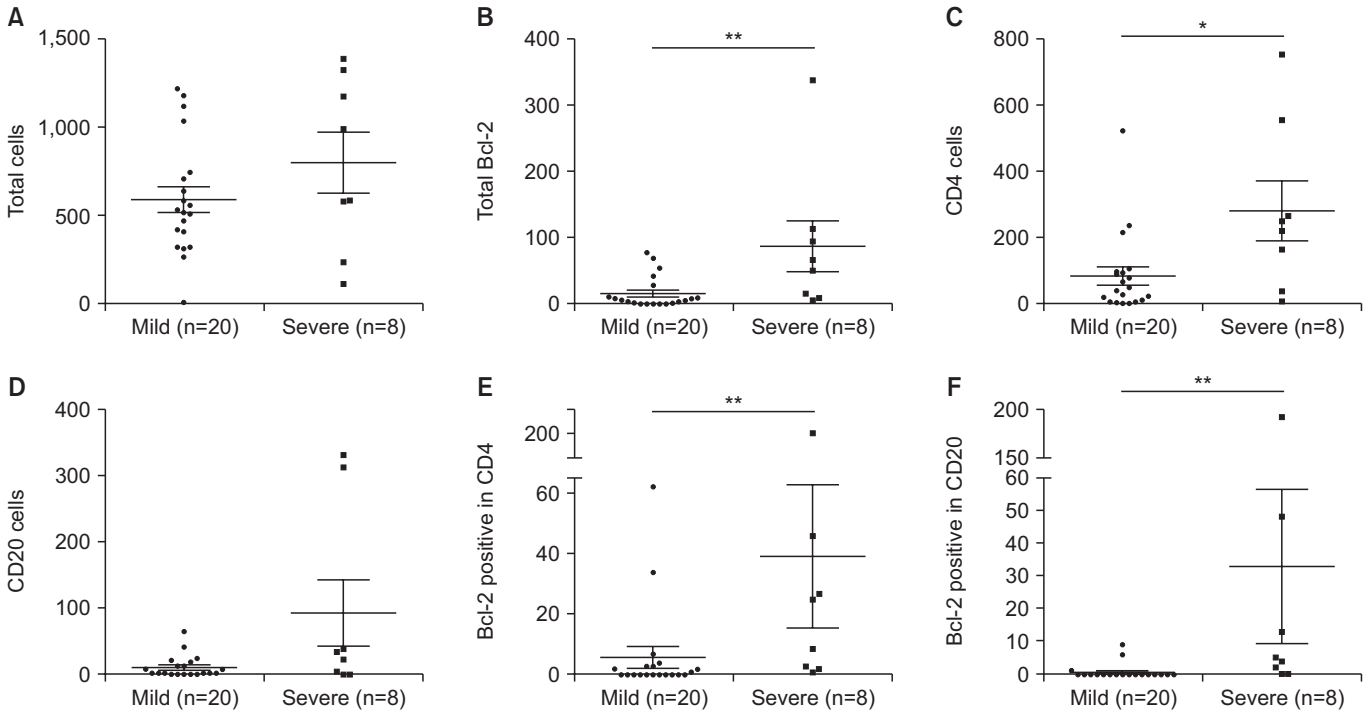

Figure 2. B-cell lymphoma-2 (Bcl-2) expression in patients with lupus nephritis by interstitial inflammation severity. The frequency of Bcl-2 expression was calculated on interstitium in renal tissues from 28 enrolled patients with mild (n=20) versus severe (n=8) interstitial inflammation. Total cells (A), frequency of Bcl-2 (B), CD4+ cells (C), CD20+ cells (D), Bcl-2 expressing CD4+ cells (E), and Bcl-2 expressing CD20+ cells (F) were compared between patients with mild and severe interstitial inflammation of renal samples. Mild=score (0~1); severe=scores (2~3) in activity index of interstitial inflammation. *p<0.05; **p<0.01.

Table 4. Correlation between interstitial inflammation, each component of activity index and chronicity index and Bcl-2 positivity in the patients with lupus nephritis

Pathological characteristics	Coefficient	p-value
Activity index	0.487	<0.001
Endocapillary hypercellularity	0.350	0.001
Neutrophils/Karyorrhexis	0.178	0.090
Fibrinoid necrosis	−0.042	0.693
Hyaline deposits	−0.112	0.288
Cellular/fibrocellular crescents	0.268	0.010
Interstitial inflammation		
Chronicity index	0.653	<0.001
Glomerulosclerosis	0.476	<0.001
Fibrous crescents	0.049	0.643
Tubular atrophy	0.622	<0.001
Interstitial fibrosis	0.642	<0.001
Total Bcl-2	0.538	0.004
Bcl-2 positive in CD4	0.533	0.005
Bcl-2 positive in CD20	0.471	0.015

Correlations between interstitial inflammation, each components of AI and CI and Bcl-2 positivity. Correlations are expressed by using Spearman correlation coefficients. Bcl-2: B-cell lymphoma-2 protein, AI: activity index, CI: chronicity index.

associated with scores of CI and each of its component (i.e., glomerulosclerosis, tubular atrophy, and interstitial fibrosis) in univariate analysis, those significances were disappeared after adjustment. Scores of interstitial inflammation were found to be correlated with all components of CI except fibrous crescents. Therefore, it is important to evaluate the extent of interstitial inflammation to predict renal prognosis in patients with lupus nephritis.

Approximately 40% of SLE patients develop lupus nephritis in the disease course, and this is associated with poor prognosis [2]. However, the associated mortality and morbidity of lupus nephritis remain the same for a 10-year period [3]. A new approach will be required to improve prognosis for patient with lupus nephritis. There is a pressing need for predictive markers to identify those patients at risk of renal failure who had conventional treatment. The results of this study may help to find poor prognostic patients with lupus nephritis and plan better treatment strategies.

Scores of CI were reported to be more important prognostic factors compared to those of AI for renal failure [17]. Since agents, such as cyclophosphamide or mycophenolate, for the

remission induction would sufficiently show therapeutic effects on some components of the AI, the scores of AI may be less important in patients with conventional treatment [18]. The results of this study also revealed that scores of CI, not AI, were significantly associated with renal failure in univariate analysis. However, when each component of AI and CI with multivariable analysis was analysed, interstitial inflammation was identified as an independent risk factor that can predict renal outcomes.

The importance of tubulointerstitial inflammation in determining prognosis in lupus nephritis was previously demonstrated by quantitatively assessing the severity using CD45 staining [9]. Increasing severity of interstitial nephritis was associated with a higher risk of progression to renal failure [9]. Our study not only highlighted the importance of interstitial inflammation as a predictor of renal outcomes but also suggested that it can be assessed based on the mNIH index from 2018 revised ISN/RPS classification. Umeda et al. [17] reported that CI and its component were associated with subsequent renal decline using 2018 revised ISN/RPS classification. However, they did not analyse each of the pathological components such as interstitial inflammation in AI [16]. This study evaluated multivariable associated factors for renal outcomes after adjustment using AI and CI (model 1) and each pathological component (model 2).

The concept of inflammation that leads to fibrosis was reported in previous studies that demonstrated the link between interstitial inflammation and scarring [19,20]. These reports indicated that the severity of interstitial inflammation was independently associated with advanced interstitial fibrosis/tubular atrophy. This study finding was similar to the results of our study that reported interstitial inflammation was significantly correlated with CI including components, such as glomerular sclerosis and interstitial fibrosis/tubular atrophy. Therefore, early recognition of lupus nephritis with severe interstitial inflammation and optimal therapeutic strategies for these patients are required to improve prognosis.

Bcl-2 expression in the interstitium of renal tissues was investigated because they represent not only a regulator of survival of immune cells, such as dendritic cells and T and B lymphocytes, but also in situ immunity in lupus nephritis [12,21]. Our results showed that the frequency of total Bcl-2, Bcl-2-positive CD4, and CD20+ cells was significantly increased in severe interstitial nephritis compared to mild interstitial nephritis. Furthermore, the frequency of Bcl-2 positivity significantly correlated with scores of interstitial inflammation. These findings are consistent with previous studies [22-24] and indicate that severe interstitial inflammation is independently associated with in situ adaptive immune responses. Particularly, our data showed that increased interstitial inflammation correlated with dysregulation of apoptosis proteins in lupus nephritis which results in the survival of autoreactive immune cells and this maintain persistent disease activity [25].

There are certain limitations of this study. First, this was a retrospective observational study, and a relatively small sample size might have skewed the results obtained. However, we analysed two renal outcomes based on ESRD and CKD, and similar results were obtained in both. Second, because patients were enrolled in a single centre, multicenter studies of patients with lupus nephritis are warranted to investigate the generalizability of this study findings.

CONCLUSION

In conclusion, the results of this study indicated that the severity of interstitial inflammation from a revision of 2018 ISN/RPS classification provided useful information for predicting the renal outcomes in patients with lupus nephritis who had conventional treatment. Furthermore, Bcl-2 expressing lymphocytes were significantly increased in severe interstitial nephritis of renal tissues.

SUPPLEMENTARY DATA

Supplementary data can be found with this article online at https://doi.org/10.4078/jrd.22.0011.

FUNDING

This work was supported by Biomedical Research Institute grant, Kyungpook National University Hospital (2018) and by the National Research Foundation of Korea Grants funded by the Korean Government (NRF-2019R1F1A1062038).

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

SJL and YJK conceived the study concept and designed the study. SJL, EJN, MHH, and YJK participated in data acquisition. SJL, MHH, and YJK participated in data analysis and interpretation. All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Lee had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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