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Tubulointerstitial lesions of patients with lupus nephritis classified by the 2003 International Society of Nephrology and Renal Pathology Society system

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The 2003 International Society of Nephrology/Renal Pathology Society (ISN/RPS) system for classifying patients with lupus nephritis was based on glomerular lesions exclusively, despite the fact that lupus nephritis affects all compartments of the kidney. Hence, we analyzed the tubulointerstitial lesions in patients with lupus nephritis within the different classes and subclasses of the 2003 ISN/RPS system. Among 313 patients from five centers in northern China with lupus nephritis, interstitial inflammatory cell infiltration, tubular atrophy, and interstitial fibrosis were severe in 170 patients with class IV, moderate in 55 with class III, and mild in 19 with class II and in 69 with class V disease, each with significance. The severity of tubulointerstitial lesions in classes IV-segmental and III was similar, whereas the score of interstitial inflammatory cell infiltration in patients with subclass IV-global was significantly higher than that in those with subclass IV-segmental. Interstitial fibrosis and tubular atrophy were each significantly more prominent in patients with both active and chronic lesions than in those with active lesions alone. The correlation coefficient ranged from 0.222 to 0.811 comparing glomerular and tubulointerstitial indices. In multivariate Cox hazard analysis of tubulointerstitial lesions, indices of interstitial infiltration, tubular atrophy, and interstitial fibrosis were confirmed as significant independent risk factors for renal outcome. Thus, we found that the 2003 ISN/RPS classification system of lupus nephritis, based on glomerular lesions, could also reflect related tubulointerstitial lesions. Hence, we suggest that the extent of tubulointerstitial lesions may be helpful in predicting renal outcome in patients with lupus nephritis.

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KEYWORDS: classification; lupus nephritis; tubulointerstitial lesions

Lupus nephritis is common in patients with systemic lupus erythematosus (SLE). The clinical and pathological manifestations are diverse and are associated with different therapeutic response and prognosis in patients with lupus nephritis.[1–2] A precise description of renal histopathological lesions and an appropriate classification of lupus nephritis are both essential for nephrologists to guide treatment and predict prognosis in patients. Many distinguished rheumatologists, nephrologists, and pathologists have dedicated themselves to improving and refining the pathological classifications of lupus nephritis over the past four decades. Since the first classification of lupus nephritis issued by the WHO (World Health Organization) in 1974, it had been revised three times in 1982, 1995, and 2003, respectively.[3–6] The 2003 International Society of Nephrology and Renal Pathology Society (ISN/RPS) classification has been widely used internationally till now. This new classification was based on glomerular lesions exclusively, despite the fact that lupus nephritis could involve all renal components, including the glomeruli, tubules, interstitium, and blood vessels. It is a well-known fact that tubulointerstitial lesions are independent risk factors in the progression of some glomerular diseases, such as immunoglobulin (Ig)A nephropathy. Whether the new classification of lupus nephritis, based on glomerular lesions, could also reflect tubulointerstitial lesions has not yet been investigated.

The current study evaluated the tubulointerstitial lesions of 313 patients with lupus nephritis from five renal centers in

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North China, and comparisons were made among patients with different classes and subclasses on the basis of the 2003 ISN/RPS classification.

RESULTS

Baseline data of patients with lupus nephritis

Patient profiles in this study are listed in Table 1a and b. The age of onset of the 313 patients with lupus nephritis ranged from 13 to 71 years, and the average age was 31.0 years. The male-to-female ratio was 1:5.4.

According to the 2003 classification of lupus nephritis, 19 patients were classified as class II (6.1%), 55 cases as class III (17.6%, including 18 cases of class V + III), 170 cases as class IV (54.3%, 20 cases of class IV-segmental (IV-S) group and 150 cases of class IV-global (IV-G) group, including 22 cases of class V + IV), and 69 cases as class V (22.0%). There were no cases of classes I and VI in this study. Classes III and IV were further subdivided into the active (A) group, the active/chronic (A/C) group, and the chronic (C) group. Within class III, the number of III(A) was 26, III(A/C) was 29, and III(C) was 0. Within class IV-S, the number of IV-S (A) was 16, IV-S (A/C) was 4, and IV-S (C) was 0. Within class IV-G, the number of IV-G (A) was 107, IV-G (A/C) was 43, and IV-G (C) was 0.

All patients received oral prednisone therapy. The majority of patients completed treatment with oral cyclophosphamide (62/313) or monthly intravenous cyclophosphamide (600–800 mg per month) (166/313). The other patients received mycophenolate mofetil (21/313), leflunomide (35/313), and azathioprine (16/313). A total of 13 patients received prednisone alone. Most of patients achieved clinical remission, 196 with complete remission and 96 with partial remission. Overall, 21 patients presented with treatment failure.

During follow-up of 61.5 ± 34.9 months, three patients died due to severe infection (2/313) or cerebral hemorrhage (1/313). With regard to long-term renal outcome, 37 patients reached the secondary end point including 6 with doubling of serum creatinine and 31 with end-stage renal disease.

Comparison of tubulointerstitial lesions in different classes of lupus nephritis

The scores of interstitial inflammatory cell infiltration, tubular atrophy, and interstitial fibrosis among the four classes were significantly different ($P<0.001$, $P<0.001$, $P<0.001$, respectively). They were found to be the most severe in class IV, moderate in class III, and mild in classes II and V (details are provided in Figure 1a–c).

When we focused on the comparison between classes III and IV, a further analysis of Mann–Whitney *U*-test showed that the severity of interstitial inflammatory cell infiltration, tubular atrophy, and interstitial fibrosis in class IV-S and class III was similar ($P=0.669$, $P=0.378$, and $P=0.267$, respectively) and that the three indices were significantly higher in the class IV-G group than in the class III group ($P<0.001$, $P<0.001$, $P<0.001$, respectively). The score of interstitial

Table 1 | (a) General clinical profiles of patients at initial renal biopsies; (b) renal pathological profiles of patients at initial renal biopsies

(a)	
Number of patients	313
Gender (male/female)	49/264
Age (mean ± s.d.) (years)	31.0 ± 7.6
The time between presentation of lupus nephritis and biopsy (mean ± s.d. and range) (months)	11.7 ± 24.2 (0.5–96)
Number of hypertension (blood pressure ⩾ 140/90 mm Hg) (%)	160 (51.1)
Systolic blood pressure (mean and range) (mm Hg)	140 (105–175)
Diastolic blood pressure (mean and range) (mm Hg)	82 (75–92)
Number with fever (noninfectious) (%)	152 (48.6)
Number with malar rash (%)	176 (56.2)
Number with photosensitivity (%)	87 (27.8)
Number with oral ulcer (%)	101 (32.3)
Number with alopecia (%)	128 (40.9)
Number with arthralgia (%)	263 (84.0)
Number with serositis (%)	90 (28.8)
Number with neurological disorder (%)	21 (6.7)
Number with anemia (%)	196 (62.6)
Number with leukocytopenia(%)	154 (49.2)
Number with thrombocytopenia (%)	167 (53.4)
Number with hematuria (%)	223 (71.2)
Number with leukocyturia (noninfection) (%)	157 (50.2)
Number with acute renal failure (%)	78 (24.9)
Hemoglobin (mean ± s.d.) (g/l)	101.5 ± 24.2
Urine protein (mean ± s.d.) (g per 24 h)	4.76 ± 3.28
Serum creatinine (mean ± s.d.) (mg/dl)	1.32 ± 0.97
Number with positive ANA (%)	153 (100)
Number with positive anti-ds-DNA antibodies (%)	128 (40.9)
Number with positive anti-Sm antibodies (%)	91 (29.1)
Number with positive anti-SSA antibodies (%)	134 (42.8)
Number with positive anti-SSB antibodies (%)	37 (11.8)
Number with positive anti-RNP antibodies (%)	87 (27.8)
Number with anti-cardiolipin antibodies (%)	25/190(13.2)
C3 (mean ± s.d.) (g/l)	0.37 ± 0.14
SLEDAI (mean ± s.d.)	18.9 ± 6.8
Duration of follow-up (mean ± s.d.) (months)	61.5 ± 34.9
(b)	
Number of biopsies	313
Number of glomeruli (mean ± s.d.)	23.2 ± 6.7
Activity indices (AIs) score (mean ± s.d.)	8.49 ± 3.98
Endocapillary hypercellularity (mean ± s.d.)	2.58 ± 1.12
Cellular crescents (mean ± s.d.)	1.49 ± 1.75
Karyorrhexis/fibrinoid necrosis (mean ± s.d.)	1.12 ± 0.77
Subendothelial hyaline deposits (mean ± s.d.)	1.67 ± 1.45
Interstitial inflammatory cell infiltration (mean ± s.d.)	1.92 ± 0.83
Glomerular leukocyte infiltration (mean ± s.d.)	0.78 ± 0.43
Chronicity indices (CIs) score (mean ± s.d.)	2.85 ± 2.12
Glomerular sclerosis (mean ± s.d.)	0.56 ± 0.25
Fibrous crescents (mean ± s.d.)	0.23 ± 0.42
Tubular atrophy (mean ± s.d.)	1.27 ± 0.68
Interstitial fibrosis (mean ± s.d.)	1.17 ± 0.54
Vascular lesions	147/313 (47%)
Vascular deposits	76/147 (51.7%)
Thrombi	8/147 (5.4%)
Vasculitis	16/147 (10.9%)
Sclerosis	89/147 (60.5%)
Mild	21/147 (14.3%)
Moderate	112/147 (76.2%)
Severe	14/147 (9.5%)

Abbreviations: ANA, antinuclear antibody; RNP, ribonucleoprotein; SLEDAI, SLE Disease Activity Index; SSA, Sjögren's syndrome A antigen; SSB, Sjögren's syndrome B antigen.

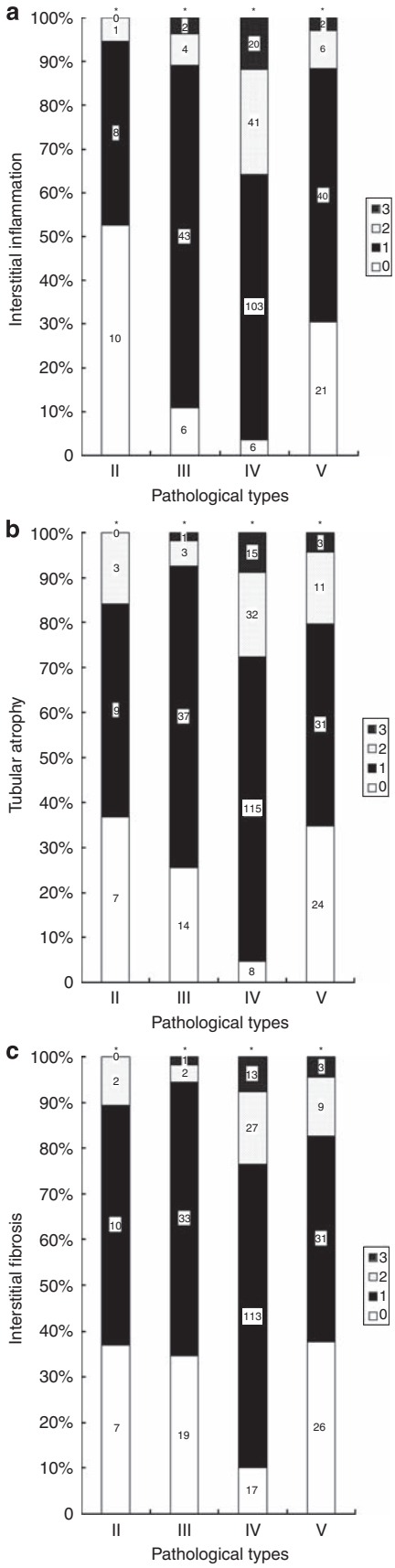

Figure 1 | Comparison of the tubulointerstitial lesions among different classes of lupus nephritis. (**a**) Interstitial inflammation; (**b**) tubular atrophy; (**c**) interstitial fibrosis.

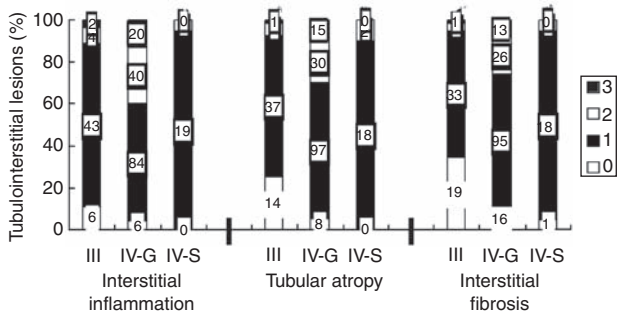

Figure 2 | Comparison of the tubulointerstitial lesions among classes III, IV-S, and IV-G of lupus nephritis.

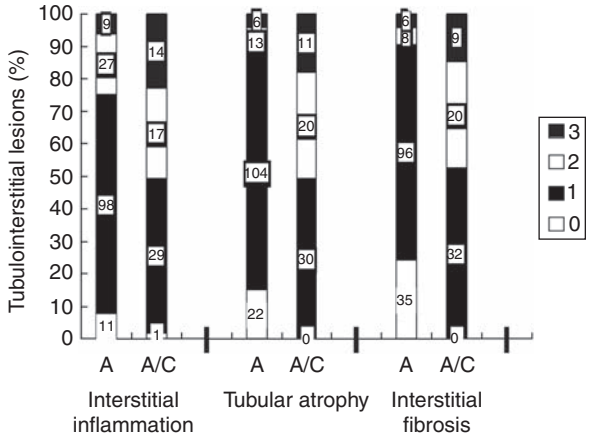

Figure 3 | Comparison of the tubulointerstitial lesions between patients with class III and class IV lupus nephritis with active lesions (A group) and those with both active and chronic lesions (A/C group).

inflammatory cell infiltration in patients with subclass IV-G was significantly higher than that in those with subclass IV-S ($P = 0.008$). There was no significant difference of tubular atrophy and interstitial fibrosis between IV-G and IV-S ($P = 0.155$ and $P = 0.194$, respectively) (details in Figure 2).

Comparison of tubulointerstitial lesions between patients with active lesions (A group) and those with both active and chronic lesions (A/C group) in class III and class IV

Scores of interstitial fibrosis and tubular atrophy in patients in the A/C group were significantly higher than those in patients in the A group ($P < 0.001$ and $P < 0.001$, respectively). There was no significant difference of interstitial inflammatory cell infiltration between the A/C and A groups ($P = 0.074$) (details in Figure 3).

Correlation between scores of glomerular and tubulointerstitial lesions

The score of interstitial inflammatory cell infiltration positively correlated with the scores of endocapillary hypercellularity ($r = 0.288$), cellular crescents ($r = 0.601$), karyorrhexis/fibrinoid necrosis ($r = 0.609$), subendothelial hyaline deposits ($r = 0.449$), and glomerular leukocyte infiltration ($r = 0.222$).

Table 2 | Comparison of clinical characteristics between the four groups of patients with different severity of glomerular and tubulointerstitial lesions

Group	Total	1	2	3	4
Number of patients	313	12	145	141	15
Gender (male/female)	49/264	2/10	28/117	16/125	3/12
Age (mean ± s.d.) (years)	31.0 ± 7.6	25.0 ± 5.3	33.0 ± 8.1	28.0 ± 4.3	32.0 ± 1.7
The time between presentation of lupus nephritis and biopsy (mean ± s.d. and range) (months)	11.7 ± 24.2 (0.5–96)	10.2 ± 14.3 (0.5–48)	12.5 ± 16.7 (2–96)	**5.4 ± 3.8 (0.5–12)**	9.8 ± 11.6 (1.5–77)
Number with hypertension (blood pressure ⩾ 140/90 mm Hg) (%)	160 (51.1)	3 (25)	102 (71)	51 (37)	4 (27)
Number with anemia (%)	196 (62.6)	4 (33)	102 (71)	76 (54)	14 (94)
Number with hematuria (%)	223 (71.2)	5 (42)	111 (77)	104 (74)	3 (20)
Number with leukocyturia (noninfection) (%)	157 (50.2)	4 (33)	87 (60)	61 (44)	5 (33)
Number with acute renal failure (%)	78 (24.9)	**0 (0)**	40 (28)	32 (23)	6 (40)
Urine protein (mean ± s.d.) (g per 24 h)	4.76 ± 3.28	**1.12 ± 0.31**	5.68 ± 2.14	4.92 ± 1.19	2.81 ± 0.44
Serum creatinine (mean ± s.d.) (mg/dl)	1.32 ± 0.97	0.72 ± 0.11	1.49 ± 0.82	1.38 ± 0.61	1.17 ± 0.24
Duration of follow-up (mean ± s.d.) (months)	61.5 ± 34.9	52.3 ± 12.7	65.1 ± 22.1	67.8 ± 31.2	60.6 ± 26.7
Number with doubling of serum creatinine or end-stage renal disease (%)	37/313 (12)	**0 (0)**	22 (16)	13 (10)	2 (14)

Numbers in bold were shown as $P<0.05$ compared with other groups.

Table 3 | Profile of patients with lupus nephritis with repeat renal biopsy

			Renal pathology type		AI		CI		I-i		T-a		I-f	
No.	Reason for re-biopsy	Int	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	Relapse	56	IV-G(A)	III(A/C)+V	8	2	2	3	2	1	1	1	1	1
2	Relapse	7	IV-G(A)	IV-G(A/C)	11	14	3	4	1	1	1	2	1	2
3	Relapse	23	IV-G(A)	IV-G(A/C)	7	17	6	9	2	3	2	3	2	3
4	Relapse	18	III(A)	IV-G(A/C)	8	12	4	3	2	1	2	1	2	1
5	Relapse	19	IV-G(A)	IV-G(A/C)	9	8	2	4	1	1	1	2	1	2
6	Relapse	46	IV-G(A)	IV-G(A/C)	10	8	4	5	2	3	1	3	1	2
7	Relapse	21	III(A)	III(A/C)	12	15	3	4	2	2	1	2	1	2
8	Re-evaluation	12	IV-S(A)	III(A/C)+V	13	7	1	6	1	2	1	2	0	2
9	Re-evaluation	9	IV-G(A)	III(A/C)+V	12	7	2	6	3	2	1	2	1	2
10	Re-evaluation	8	IV-G(A)	III(A/C)	14	3	1	3	1	2	1	2	1	2
11	Re-evaluation	11	IV-S(A)	III(A/C)	11	4	1	5	1	2	1	2	0	1

Abbreviations: AI, activity index; CI, chronicity index; I-f, interstitial fibrosis; I-i, interstitial inflammatory cell infiltration; Int, interval between biopsies (months); T-a, tubular atrophy.

The score of tubular atrophy positively correlated with the scores of glomerular sclerosis ($r=0.761$) and fibrous crescents ($r=0.811$). The score of interstitial fibrosis also positively correlated with the scores of glomerular sclerosis ($r=0.391$) and fibrous crescents ($r=0.298$).

For further evaluating the correlations between glomerular and tubulointerstitial injuries, the patients were further divided into four groups according to the severity of glomerular and tubulointerstitial lesions: group 1: nil or mild glomerular lesions with nil or mild tubulointerstitial lesions; group 2: moderate or severe glomerular lesions with moderate or severe tubulointerstitial lesions; group 3: moderate or severe glomerular lesions with nil or mild tubulointerstitial lesions; and group 4: nil or mild glomerular lesions with moderate or severe tubulointerstitial lesions. Clinical and laboratory characteristics were compared between the four groups (details in Table 2). In group 1, patients presented with the lowest ratio of acute renal failure (0%), lowest amount of proteinuria (1.12 ± 0.31 g per day), and the best renal outcome ($P<0.01$, $P<0.01$, $P<0.01$, respectively). In group 3, the time between presentation of lupus nephritis and biopsy was the shortest (5.4 ± 3.8 months, $P<0.01$). In group 4, the ratio of anemia was the highest and the ratio of hematuria was the lowest ($P<0.01$, $P<0.01$, respectively). There did not exist any statistical difference in other indices between the four groups.

Data of patients with lupus nephritis with repeat biopsies

Repeat kidney biopsy was carried out in 11 patients (Table 3). Among them, seven were in relapse (No. 1–7) and four underwent reevaluation after clinical remission (No. 8–11). Most indices of tubulointerstitial lesions, especially interstitial fibrosis and tubular atrophy, increased, although patients achieved clinical remission.

Tubulointerstitial lesions as predictors for patients' renal survival

Using the log-rank test and Kaplan–Meier curves for univariate survival analysis of renal prognosis of patients, we especially focused on the indices of tubulointerstitial lesions, including interstitial inflammatory cell infiltration, interstitial fibrosis, and tubular atrophy, and found that they were risk factors for renal outcome in lupus nephritis, that is, the higher the score of the indices, the poorer the

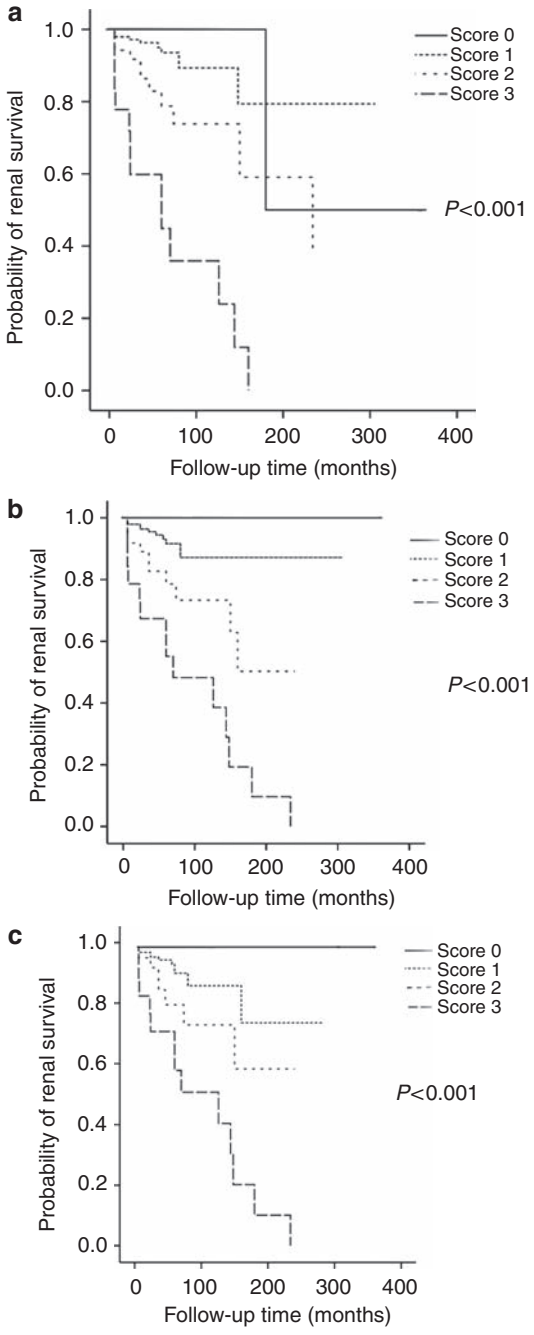

Figure 4 | Comparison of renal outcome between patients with different scores of tubulointerstitial indices in lupus nephritis. (**a**) Interstitial inflammatory cell infiltration; (**b**) tubular atrophy; (**c**) interstitial fibrosis.

renal prognosis ($P<0.001$, $P<0.001$, $P<0.001$, respectively, Figure 4a–c). Other univariate risk factors included sex (male), serum creatinine, renal pathological types, active indices, cellular crescents, chronic indices, and fibrous crescents (details in Table 4). Multicenter selection and immunosuppressants did not affect renal prognosis in the study. In further multivariate Cox hazard analysis of the three indices, the three tubulointerstitial indices were confirmed as independent risk factors for renal outcome by adjustment

Table 4 | Univariate survival analysis of patients' renal prognosis with lupus nephritis

	HR	95% CI		*P*-value
Age	1.028	0.496	2.131	0.941
Sex	0.378	0.177	0.808	**0.012**
C3	1.339	0.321	5.581	0.689
Proteinuria	1.408	0.695	2.854	0.342
Serum creatinine	24.480	8.647	63.308	**<0.001**
ANA	0.413	0.213	1.568	0.765
Anti-ds-DNA antibody	1.448	0.708	2.956	0.310
Anti-Sm antibody	0.855	0.390	1.876	0.696
Anti-SSA antibody	0.811	0.412	1.596	0.545
Anti-SSB antibody	3.177	0.436	7.032	0.369
Anti-RNP antibody	0.568	0.259	1.249	0.160
Anti-cardiolipin antibody	0.978	0.431	1.592	0.116
SLEDAI	1.212	0.317	1.698	0.079
Multicenter effect	0.371	0.212	1.541	0.875
Using or not cyclophosphamide	1.675	0.421	1.976	0.087
Renal pathological types	3.278	1.112	3.461	**0.037**
Activity indices (AIs) score	1.688	1.296	2.198	**<0.001**
Endocapillary hypercellularity	1.358	0.937	1.969	0.106
Cellular crescents	1.479	1.287	1.7	**<0.001**
Karyorrhexis/fibrinoid necrosis	1.168	0.900	1.514	0.243
Subendothelial hyaline deposits	0.988	0.736	1.327	0.938
Interstitial inflammatory cell infiltration	3.808	3.748	62.312	**<0.001**
Glomerular leukocyte infiltration	0.883	0.682	1.144	0.346
Chronicity indices (CIs) score	2.105	1.629	2.720	**<0.001**
Glomerular sclerosis	1.499	0.977	2.301	0.064
Fibrous crescents	2.634	1.772	3.916	**<0.001**
Tubular atrophy	3.069	2.115	4.456	**<0.001**
Interstitial fibrosis	2.583	1.819	3.669	**<0.001**

Abbreviations: ANA, antinuclear antibody; CI, confidence interval; HR, hazard ratio; SLEDAI, SLE Disease Activity Index.
Numbers in bold were shown as $P<0.05$.

using a stepwise model (interstitial infiltration: hazard ratio 1.847, 95% confidence interval: 1.229–2.777, $P=0.003$; tubular atrophy: hazard ratio 2.350, 95% confidence interval: 1.019–2.932, $P=0.023$; interstitial fibrosis: hazard ratio 1.956, 95% confidence interval: 1.237–2.235, $P=0.037$, respectively; Table 5a–c). We also found that the value of serum creatinine, cellular crescents, endocapillary hypercellularity, and fibrous crescents could independently indicate renal prognosis.

DISCUSSION

SLE is a prototypic autoimmune disease, which comprises a range of multisystem disorders. Renal involvement is common in SLE, and the total incidence of renal involvement among patients with SLE is ~40% with variation depending on geographic area.[7,8] There are a number of different types of renal diseases in SLE, glomerulonephritis being the most common. In addition to glomerulonephritis, physicians including nephrologists, pathologists, and rheumatologists should pay more attention to renal tubulointerstitial lesions in patients with lupus nephritis, because its presence might adversely affect the prognosis of renal disease.[9–11] In fact, tubulointerstitial renal disease is found frequently in patients with lupus nephritis. O'Dell *et al.*[10] found that tubulointerstitial abnormalities were present in 51% of the patients with lupus nephritis. Park *et al.*[11] showed that the prevalence of tubulointerstitial immune deposits was

Table 5 | (a) Interstitial inflammatory cell infiltration as the independent risk factor for renal outcome in lupus nephritis; (b) tubular atrophy as the independent risk factor for renal outcome in lupus nephritis; (c) interstitial fibrosis as the independent risk factor for renal outcome in lupus nephritis

		95% CI		
	P-value	Lower	Upper	Hazard ratio
(a)				
Multivariate Cox hazard analysis				
Sex	0.435	0.250	1.815	0.674
Age	0.704	0.494	2.840	1.185
C3	0.311	0.062	2.430	0.387
Serum creatinine	<0.001	3.895	40.004	12.483
Proteinuria	0.532	0.351	1.718	0.776
Anti-ds-DNA antibody	0.825	0.456	2.677	1.105
Anti-RNP antibody	0.607	0.254	2.227	0.752
Anti-SSB antibody	0.796	0.808	11.727	4.604
Endocapillary hypercellularity	0.176	0.443	1.161	0.717
Cellular crescents	0.076	0.980	1.497	1.211
Glomerular sclerosis	0.345	0.774	2.082	1.269
Fibrous crescents	0.285	0.776	2.371	1.356
Interstitial inflammatory cell infiltration	0.003	1.267	3.147	1.997
Multivariate stepwise Cox hazard analysis				
Serum creatinine	<**0.001**	4.709	44.281	14.440
Cellular crescents	**0.011**	1.052	1.479	1.247
Endocapillary hypercellularity	**0.024**	0.440	0.944	0.644
Interstitial inflammatory cell infiltration	**0.003**	1.229	2.777	1.847
(b)				
Multivariate Cox hazard analysis				
Sex	0.385	0.249	1.710	0.652
Age	0.820	0.378	2.160	0.904
C3	0.333	0.058	2.634	0.389
Serum creatinine	<0.001	3.871	42.554	12.835
Proteinuria	0.987	0.454	2.175	0.993
Anti-ds-DNA antibody	0.327	0.652	3.600	1.533
Anti-RNP antibody	0.496	0.240	1.997	0.692
Anti-SSB antibody	0.381	0.685	12.587	4.998
Endocapillary hypercellularity	0.178	0.440	1.165	0.716
Cellular crescents	0.060	0.991	1.513	1.225
Glomerular sclerosis	0.620	0.676	1.927	1.142
Fibrous crescents	0.235	0.802	2.460	1.404
Tubular atrophy	0.036	1.355	2.532	1.555
Multivariate stepwise Cox hazard analysis				
Serum creatinine	<**0.001**	4.554	43.974	14.152
Fibrous crescents	**0.003**	1.245	2.928	1.909
Tubular atrophy	**0.023**	1.019	2.932	2.350
(c)				
Multivariate Cox hazard analysis				
Sex	0.392	0.252	1.716	0.658
Age	0.795	0.375	2.119	0.892
C3	0.422	0.068	3.083	0.458
Serum creatinine	<0.001	4.719	48.841	15.181
Proteinuria	0.934	0.469	2.280	1.034
Anti-ds-DNA antibody	0.185	0.861	2.171	1.367
Anti-RNP antibody	0.510	0.245	2.011	0.702
Anti-SSB antibody	0.772	0.077	12.917	5.179
Endocapillary hypercellularity	0.119	0.422	1.104	0.682
Cellular crescents	0.059	0.992	1.512	1.225
Glomerular sclerosis	0.619	0.668	1.971	1.147
Fibrous crescents	0.312	0.760	2.357	1.339
Interstitial fibrosis	0.018	1.631	3.568	2.501
Multivariate stepwise Cox hazard analysis				
Serum creatinine	<**0.001**	7.772	66.116	22.669
Cellular crescents	**0.002**	1.099	1.532	1.298
Endocapillary hypercellularity	**0.040**	0.445	0.981	0.661
Interstitial fibrosis	**0.037**	1.237	2.235	1.956

Abbreviation: CI, confidence interval.
Numbers in bold were shown as $P<0.05$.

33% by immunofluorescence and 23% by electron microscopy in a cohort of 93 patients with lupus nephritis. Therefore, a comprehensive classification of lupus nephritis, which should also cover tubulointerstitial parameters, is very important for physicians to guide treatment and predict prognosis in patients with lupus nephritis.

The 2003 ISN/RPS classification of lupus nephritis has been widely used all over the world,[6,12–14] although the significance and rationality of the classification need to be examined in many centers in different parts of the world.[12,13] Is it rational for the new classification based on glomerular lesions to represent the full characteristics of lupus nephritis, and do tubulointerstitial lesions also have an important role in the progression of lupus nephritis? To provide answers for these questions, a systemic pathological analysis of tubulointerstitial lesions in a large cohort of Chinese patients with lupus nephritis, from five renal centers in North China, with different classes based on the new classification was carried out.

Importantly, we should indicate that the NIH (National Institutes of Health) scoring system, used to evaluate tubulointerstitial lesions, has gained widespread acceptance.[9,15–17] A more detailed and complex biopsy index proposed by Hill *et al.*[18] has an even stronger predictive value, but is too laborious for routine use and remains a valuable research tool.[19]

Our study showed that there exist different severity of tubulointerstitial lesions in different classes of lupus nephritis, which are graded as class IV > class III > class II and class V, and it seems that tubulointerstitial lesions are consistent with glomerular injury under the new classification. Further correlation test in the current study also illustrated that tubulointerstitial lesions correlated with some glomerular indices in lupus nephritis. Glomerular features of activity, including cellular crescents, karyorrhexis/fibrinoid necrosis, and subendothelial hyaline deposits, were highly consistent with the degree of interstitial inflammatory cell infiltration ($r > 0.4$). Similarly, the glomerular features of chronicity, such as glomerular sclerosis and fibrotic crescents, were consistent with the range of tubular atrophy and interstitial fibrosis as well. However, the *r*-value was not always strong enough (in some indices, $r < 0.4$), and we really found that some cases presented with severe glomerular injury but with mild tubulointerstitial change and *vice versa*. For patients with more severe glomerular involvement but milder tubulointerstitial injury, they might be at the early stage of lupus nephritis because they were in the shortest interval between presentation of lupus nephritis and biopsy in this study. This implies that interstitial lesions might be the consequence of glomerular injury in these patients. However, for those patients with milder glomerular injury and more prominent tubulointerstitial change, as well as for those with nonparallel changes in repeat renal biopsy (glomerular indices meliorated but tubulointerstitial scores deteriorated in the four patients with clinical remission), we proposed that tubulointerstitial lesions may be not only a consequence of glomerular injury but also an important player in lupus nephritis. For some patients, the interstitial changes may be the major renal lesions as reported previously.[20–25] Interestingly, our further multivariate Cox hazard analysis showed that the risk of doubling creatinine or end-stage renal disease increases in proportion to increasing tubulointerstitial lesions, and that the degree of tubulointerstitial lesions, including interstitial inflammatory cell infiltration, interstitial fibrosis, and tubular atrophy could have independent prognostic value in predicting renal outcome, although some classical risk factors, including sex (male), serum creatinine, renal pathological types, active indices, cellular crescents, chronic indices, and fibrous crescents, were also confirmed as predictors of renal prognosis in our study. The reasons why patients with more severe tubulointerstitial lesions were associated with a worse renal prognosis might be attributed to irreversible chronic and sclerotic lesions, which are refractory to therapy. As for 'active' interstitial inflammatory cell infiltration, some studies showed that proteinuria is a risk factor for the deterioration of renal function, which is in part a result of interstitial inflammation.[17] In fact, Yamamoto *et al.*[26] already compared interstitial inflammatory and chronic tubulointerstitial lesions between patients with lupus nephritis and those with IgA nephropathy. They found that no IgA nephropathy patient with nil or mild glomerular lesions had moderate or severe interstitial inflammatory and/or chronic tubulointerstitial lesions, but predominantly severe interstitial inflammatory lesions were found in 36% of lupus nephritis patients with nil or mild glomerular lesions. Many previous studies also reported cases with significant tubulointerstitial involvement in lupus nephritis.[20–25] Some investigators proposed different pathogenic mechanisms inducing tubulointerstitial injury in experimental and human lupus nephritis. These factors include immune complex deposition,[23,26,27] albuminuria macrophage chemokines,[28–33] some autoantibodies,[34,35] and others.[36] Recent studies suggested that activation of tubular Toll-like receptor-9 had a pathogenic role in tubulointerstitial inflammation and damage in lupus nephritis.[37–39] In some patients who underwent repeat renal biopsy, it was shown that tubulointerstitial scores deteriorated, although the patients achieved clinical remission after immunosuppressive treatment. Therefore, further therapies aimed directly at tubulointerstitial injury based on above studies should be investigated in lupus nephritis.

The most controversial aspect of the ISN/RPS 2003 classification is the introduction of subclass segmental 'IV-S' and global 'IV-G' within diffuse lupus nephritis (class IV). This stratification raises two fundamental questions: (1) Are there differences in therapeutic response and prognosis between these two subclasses? (2) Is there any pathogenic difference between the two subclasses or just a continuum of one disease? Many studies have examined the distinction between the two groups.[17,40–43] Collectively, these studies suggest that lupus nephritis class IV-S and IV-G probably represent different stages or morphological expressions within a single disease continuum,[13,44] although there might

exist pathogenic differences between the two groups.[42,45] The recent study from our center showed that the frequency of serum anti-neutrophil cytoplasmic antibodies was significantly higher in IV-S than in IV-G (20 vs 4.6%, $P=0.008$), whereas frequencies of anti-C1q IgG1 and IgG3 subclasses were significantly higher in IV-G ($P=0.006$, $P=0.011$, respectively). These data indicate that 'immune complex' or 'pauci-immune' might be prominent in the pathogenesis of subclass IV-G or IV-S, respectively.[46] In the current study, we found that the score of interstitial inflammatory cell infiltration in patients with subclass IV-G was significantly higher than in those with subclass IV-S, but the scores of tubulointerstitial indices in class IV-S and class III were similar. On the basis of our own findings, we speculated that class III and subclass IV-S might be mild or early stage to subclass IV-G. However, this observation requires more studies for clarification, including the analysis of treatment response and long-term prognosis of patients with class III, IV-S, and IV-G in the future.

Another important change in the new classification is that in classes III and IV, the proportion of glomeruli affected by active (A) and chronic (C) lesions should be indicated. We found that there was a tendency of more tubular atrophy and interstitial fibrosis in patients with both active and chronic lesions than in those with pure active lesions in class III and class IV. This finding might provide an additional explanation for the recent observation made by Hiramatsu *et al.*[17] that renal function declined only in patients with IV-G (A/C) in spite of intensified therapies.

There were some limitations in this study. First, it is not a prospective study with well-controlled treatment schedules; there was some heterogeneity in the treatment that may have influenced the associations between histological features and outcome, although most patients were treated with cyclophosphamide. Second, the sample size of patients with nonparalleled glomerular and tubulointerstitial lesions is still not large enough to draw the valid conclusion. Third, the current study lacks investigation on pathogenic mechanism on tubulointerstitial injury in lupus nephritis. Therefore, a further well-designed prospective study is required.

In conclusion, the 2003 ISN/RPS classification of lupus nephritis based on glomerular lesions could also reflect the related tubulointerstitial lesions, and the revised classification with a description of the degree of tubulointerstitial lesions might be helpful in predicting renal outcome in lupus nephritis.

MATERIALS AND METHODS

Patients

Renal histopathological data of 313 patients with renal biopsy-proven lupus nephritis, diagnosed between January 2003 and June 2007 from five hospitals in North China, were reviewed and reclassified according to the ISN/RPS 2003 classification by four experienced pathologists. Of the 313 patients, 208 were from the Peking University First Hospital, 37 from the Affiliated Hospital of Ningxia Medical University, 34 from the Cangzhou Central Hospital, 20 from the First Affiliated Hospital Baotou Medical College, and 14 were from the Chifeng Second Hospital. The pathologists classified and scored the biopsies separately, blinded to patients' data and scores of other observers. Patients with <10 glomeruli in their renal biopsies and patients with other known superimposed tubulointerstitial diseases were excluded. In this study, cases of III + V were classified as class III and cases of IV + V were classified as class IV. Patients who fulfilled the 1997 American College of Rheumatology revised criteria for SLE were included.[47] The disease activity was assessed by the SLEDAI (SLE Disease Activity Index).[48,49]

Renal histopathology

Renal biopsy specimens were examined by light microscopy, direct immunofluorescence, and electron microscopy techniques.

Light microscopy examination. Renal biopsy specimens were fixed in 4.5% buffered formaldehyde for light microscopy. Consecutive serial 2-μm thick sections were used for histological staining. Stains used included hematoxylin and eosin, periodic acid-Schiff, silver methenamine, and Masson's trichrome. Pathological parameter such as activity indices and chronicity indices were approached by renal pathologists using a modified previously reported system involving semi-quantitative scoring of specific biopsy features.[9,15] According to the revised Austin's semi-quantitative scoring system,[26] glomerular lesions were graded as following: 0 (nil), almost normal glomeruli by light microscopy; 1+ (mild), glomeruli having focal segmental mesangial proliferation free of segmental sclerosis and adhesion of tufts to Bowman's capsules; 2+ (moderate), proliferative changes extended moderately to peripheral capillary walls; 3+ (severe), severe and diffuse proliferative changes and/or segmental or global sclerosis. The scoring of interstitial inflammatory cell infiltration was as following: 0 (nil), normal; 1+ (mild), $<25\%$ of the acreage of interstitium affected; 2+ (moderate), 25–50% of the acreage of interstitium affected; 3+ (severe), $>50\%$ of the acreage of interstitium affected in each specimen. The scoring of interstitial fibrosis and tubular atrophy was assessed similarly. Differences in scoring between the pathologists were resolved by re-reviewing the biopsies and thus reaching a consensus.

Direct immunofluorescence examination. Results of direct immunofluorescence for IgG, IgA, IgM, C3, C1q, and fibrin deposits were semi-quantitatively graded from 0 to 4 according to the intensity of fluorescence.

Electron microscopy examination. Renal biopsy specimens were fixed in 2.5% paraformaldehyde for electron microscopy. After being embedded in epon, ultra-thin sections were mounted on metal grids and stained with uranyl acetate before being viewed under a transmission electron microscope (JEM-1230; JEOL, Tokyo, Japan).

Clinical evaluation

The detailed clinical data of patients were retrospectively analyzed. Serum antinuclear antibodies were detected using indirect immunofluorescence assay (EUROIMMUN, Lübeck, Germany) and anti-double-stranded DNA antibodies were detected using Crithidia luciliae indirect immunofluorescence test (EUROIMMUN). Anti-extractable nuclear antigen antibodies, including anti-Sm, anti-Sjögren's syndrome A antigen, anti-Sjögren's syndrome B antigen, and anti-ribonucleo protein antibodies, were detected using immunodotting assay (EUROIMMUN). Anti-cardiolipin

antibodies were detected using ELISA (enzyme-linked immunosorbent assay) (EUROIMMUN). Serum C3 was determined using rate nephelometry assay (Beckman-Coulter, IMMAGE, Fullerton, CA, USA, normal range >0.85 g/l).

Response to therapy includes complete remission, partial remission, and treatment failure detailed in previous studies.[50–53]

Patients were followed up in outpatient lupus clinics. The primary end point was defined as death, and the secondary end point was defined as end-stage renal disease or doubling of serum creatinine.

Informed consent was obtained for renal biopsy from each patient. The research was in compliance with the Declaration of Helsinki.

Statistical analysis

Statistical software SPSS 13.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Quantitative data were expressed as mean ± s.d., and median with range (minimum, maximum). Differences of semi-quantitative data were tested with the Kruskal–Wallis test and the Mann–Whitney *U*-test. The Spearman rank-order correlation test was performed between various lesions (*r*-value: 0.2–0.4 as mild correlation; 0.4–0.8 as moderate correlation; >0.8 as high correlation). Kaplan–Meier curves were used to analyze patients' prognosis. Univariate survival analysis was carried out using the log-rank test. Multivariate analysis of patient survival was performed using the Cox regression model. The following variables were assessed as potential predictors of renal outcomes: age, sex, proteinuria, serum creatinine, complement 3, antinuclear antibodies, anti-ds-DNA antibodies, anti-extractable nuclear antigen antibodies, anti-cardiolipin antibodies, SLEDAI, renal pathological types, activity indices, including endocapillary hypercellularity, cellular crescents, karyorrhexis/fibrinoid necrosis, subendothelial hyaline deposits, interstitial inflammation and leukocyte glomerular infiltration, chronicity indices, including glomerular sclerosis, fibrous crescents, tubular atrophy, and interstitial fibrosis, the influence of using cyclophosphamide and multi-centers effect. Variables that did not affect survival significantly were removed by a stepwise procedure according to a likelihood ratio. Results were expressed as hazard ratio with 95% confidence intervals. Statistical significance was considered as $P<0.05$.

DISCLOSURE

All the authors declared no competing interests.

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