Urinary sediments could differentiate the endocapillary proliferative lupus nephritis and endocapillary proliferative IgA nephrology

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Abstract

Background: The role of manual urine sediment examination in the diagnosis and prognostication of endocapillary proliferative glomerulonephritis remains to be elucidated. This study aims to investigated the differences of urinary sediment findings between lupus nephritis and IgA nephropathy with endocapillary proliferative glomerulonephritis and further evaluated associations of leukocyturia with disease activity, pathological features and prognosis.

Methods: The urinary sediment of 126 patients, including 92 patients with lupus nephritis and 34 patients with IgA nephropathy, with a renal biopsy-proven endocapillary proliferative glomerulonephritis were examined in the morning before renal biopsy according to a standardized method. The urinary elements investigated including various cells, casts and crystals. The associations of the level of leukocyturia and disease activity, pathological features and prognosis were further analyzed.

Results: In the patients with endocapillary proliferative glomerulonephritis, normal to mild leukocyturia (≤12/HPF), and moderate to severe leukocyturia (>12/HPF) were found in 52(41.27%) and 74 (58.73%) patients, respectively. The proportion of moderate to severe leukocyturia, the frequency of urinary white blood cells casts and waxy casts were significantly higher in endocapillary proliferative lupus nephritis patients compared with endocapillary proliferative IgA nephropathy patients (P<0.001, P=0.020, P=0.010, respectively). In the proliferative lupus nephritis group, the levels of leukocyturia was significantly correlated with serum creatinine (r=0.288, P=0.005), eGFR (r=-0.284, P= 0.006), serum C3 (r=-0.275, P= 0.009) , SLEDAI scores (r=0.383, P=0.001) and glomerular leukocyte infiltration (r=0.285, P= 0.002). A multivariate analysis showed that leukocyturia was identified as an independent risk factor for renal outcome in proliferative lupus nephritis (HR: 1.456, 95% CI: 1.083-1.957, P=0.013) but not in IgA nephropathy (HR: 1.069, 95% CI: 0.494-2.312, P=0.866).

Conclusions: Urinary sediments of the endocapillary proliferative lupus nephritis and endocapillary proliferative IgA nephrology differed in many aspects. Leukocyturia could reflect the disease activity and prognosis of endocapillary proliferative glomerulonephritis, especially in lupus nephritis.

Background

The examination of urine sediment to diagnose kidney disease and guide therapy is a time-honored practice that provides critical information about the patients' underlying kidney injury. Manual urine sediment examination could also guide therapy and assist in prognostication[1–3].

Endocapillary proliferative glomerulonephritis is featured of diffuse endocapillary and mesangial cell proliferate glomerulonephritis [4, 5]. It could be caused by Group A *β-hemolytic streptococcus*, also other infections by bacteria such as *staphylococcus aureus* and *streptococcus viridans*. Moreover, immune-complex mediated endocapillary proliferative glomerulonephritis in systemic diseases accounts for a large group, especially IgA nephropathy and lupus nephritis.

The urine sediments of this disease entity are characterized by a large number of erythrocytes and red blood cells (RBC) casts [3, 6]. Patients with proliferative lupus nephritis (classes III or IV ± V) were reported to show a higher number of urinary acanthocytes and erythrocytes compared to other classes of lupus nephritis (classes I, II, or V) and urinary acanthocytes can be used as an easy tool for early diagnosis of proliferative lupus nephritis[7]. Sterile pyuria and/or white blood cells (WBC) casts reflect endocapillary proliferative glomerulonephritis in patients with dysmorphic erythrocytes and/or RBC casts [3, 8]. However, no detailed descriptions of urinary sediments in different etiology of endocapillary proliferative glomerulonephritis was reported.

Herein, we investigated the differences of urinary sediment findings between lupus nephritis and IgA nephropathy with endocapillary proliferative glomerulonephritis, and further evaluated associations of leukocyturia with disease activity, pathological features and prognosis.

Patients And Methods

Patients

Complete data of 126 patients with biopsy-proven endocapillary proliferative glomerulonephritis between March 2011 and July 2019 at Peking University First Hospital were enrolled in the study. These included 92 patients with endocapillary proliferative lupus nephritis,

and 34 patients with endocapillary proliferative IgA nephropathy. Patients with lupus nephritis were diagnosed according to the 1997 American College of Rheumatology revised criteria for systemic lupus erythematosus (SLE) [9]. Diagnosis of IgA nephropathy was based upon the Oxford classification [10, 11], and patients with Henoch-Schonlein purpura, liver cirrhosis, and other secondary etiologies of IgA nephropathy were excluded by detailed clinical and laboratory examinations. Both endocapillary proliferative lupus nephritis and endocapillary proliferative IgA nephropathy were defined as the presence of lesions with diffuse and global endocapillary hypercellularity (involving ≥ 50% of all glomeruli, glomerular lesion involves more than half of the glomerular tuft)[10, 12, 13]. Notably, endocapillary hypercellularity due to increased number of cells within glomerular capillaries, causing narrowing of the lumina and the hypercellularity might reflect proliferation, inflammatory cell infiltration or endothelial cell swelling[14].

Clinical Assessment

The following clinical data were systematically recorded: gender, age at kidney biopsy, acute kidney injury (AKI), hemoglobin, 24-hour urine protein excretion, serum creatinine and eGFR. AKI was defined using the Kidney Disease: Improving Global Outcomes (KDIGO) criteria and consensus report of the Acute Disease Quality Initiative (ADQI) 16 Workgroup [15, 16]. The clinical disease activity for patients with lupus nephritis was measured using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [17, 18] .

The patients were followed up in outpatient clinics. The primary endpoint was defined as death, and the secondary endpoints were defined as end-stage renal disease (ESRD) or doubling of serum creatinine levels or 50% eGFR decline.

Urine Microscopic Examination

Morning urine samples were obtained on the day of renal biopsy. Urine sediment examination was manually performed by specialized two skilled nephrologists in our renal testing laboratory according to the following standardized method, which was shown to provide reproducible inter sample quantitative results [19]. Briefly, 10 mL aliquot of urine were centrifuged for five minutes at 450 g. The supernatant was removed and the sediment was resuspended into solution with 0.2 mL of supernatant urine. Transfer by a precision pipette of 20 ul of resuspended sediment to a standardized glass slides and covering the sample with a 18 × 18 mm coverslip. Urine sediment sample was examined under a phase contrast microscope and a polarized light microscope (Nikon, Japan) within 3 hours of urine collection.

Each sample was first screened at low magnification (× 100). Erythrocytes, leukocytes, renal tubular epithelial cells and lipid droplets were examined with high power magnification (× 400) and 10 high power fields (HPFs) were randomly selected for quantification. According to national existent criterion, microscopic hematuria and leukocyturia were defined as > 3 erythrocyte /HPF or > 5 leukocyte/HPF, respectively. In addition, the severity of hematuria and leukocyturia, based on the interquartile range (IQR) of the number of RBC/HPF and the number of WBC/HPF were defined, respectively. Renal tubular epithelial cells and lipid droplets were recorded as present or absent. Urinary casts, including hyaline casts, granular casts, RBC casts, WBC casts, epithelial cells casts, mixed cellular casts, lipid laden casts, waxy casts were examined with low power magnification (× 100) and 20 low power fields (LPFs) were randomly selected for quantification. The presence of casts was scored as 1 and its absence as 0.

The research was carried out in compliance with the principles of the Declaration of Helsinki and was approved by the ethics committee of Peking University First Hospital (No. 2014(749)). Informed consent was obtained from each patient for blood sampling, urine sampling and renal biopsy.

Laboratory Assessment

Serum antinuclear antibodies (ANA) were detected using an indirect immunofluorescence assay (EUROIMMUN, Lübeck, Germany). Anti-double-stranded DNA antibodies were detected using a Crithidia luciliae indirect immunofluorescence test (EUROIMMUN, Lübeck, Germany). Anti-cardiolipin antibodies were detected using an enzyme-linked immunosorbent assay (ELISA) (EUROIMMUN, Lübeck, Germany). Circulating IgA and C3 levels were determined using the rate nephelometry assay (IMMAGE; Beckman-Coulter, USA).

Renal Histopathology

The renal biopsy specimens were inspected routinely for light microscopy, direct immunofluorescence and electron microscopy techniques.

Lupus nephritis was classified according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification system [12]. Pathological parameters such as activity indices (AI) and chronicity indices (CI) were determined by experienced renal pathologists using a previously reported system involving semi-quantitative scoring of specific biopsy features [20, 21]. The AI contain the following parameters: endocapillary hypercellularity, cellular crescents, karyorrhexis/fibrinoid necrosis, subendothelial hyaline deposits, interstitial inflammation and leucocyte infiltration, whereas the CI include glomerular sclerosis, fibrous crescents, tubular atrophy and interstitial fibrosis.

IgA nephropathy were graded according to the updated Oxford classification [10, 11]. The Oxford MESTC grades (M, mesangial hypercellularity; E, endocapillary hypercellularity; S, glomerulosclerosis; T, tubular atrophy and interstitial fibrosis; and C, cellular or fibrocellular crescents) was used to evaluate the pathologic lesions of biopsy specimens in our study.

Statistical analysis

Statistical software SPSS 20.0 (SPSS, Chicago, IL) was performed for statistical analysis. Quantitative data were expressed as mean ± SD or median (IQR) and categorical data were expressed as ratio. Associations between continuous variables were tested by Pearson's correlation coefficient test and Spearman Rank Correlation. The significance of differences between groups was dependent on the distribution of data (normal or non-normal) and was determined using independent sample t test or Mann Whitney U test as appropriate for comparison of continuous scores between 2 groups. Categorical variables were compared using χ^2 test between groups. Univariate and multivariable Cox regression model was applied to identify prognostic factors associated with renal outcomes. All *P*-values below 0.05 were considered statistically significant.

Results

General data of patients

The detailed general clinic-pathological data of the patients were shown in Table 1 and Supplementary Table 1. Of the 126 patients included in the analysis, 92 patients with proliferative lupus nephritis, and 34 patients with endocapillary proliferative IgA nephropathy.

Table 1
Clinical data of patients with endocapillary proliferative glomerulonephritis

	All patients n = 126	Patients with LN n = 92	Patients with IgAN n = 34
Gender (male/female)	41/85	24/68	17/17
Age (mean ± s.d.) (years)	35.84 ± 16.00	33.92 ± 14.13	41.03 ± 19.50
Acute kidney injury no. (%)	37(29.37%)	28(30.43%)	9(26.47%)
Urine protein (median, IQR) (g/24 h)	4.42;2.79–7.71	4.53;2.88–7.42	4.21;2.50–9.01
Serum creatinine (median, IQR) (umol/l)	99.1;75.5-176.3	107.1;78.9-179.3	80.1;69.5-129.7
eGFR (mean ± s.d.) (ml/min per 1.73 m^2)	67.33 ± 37.78	62.70 ± 35.18	79.85 ± 42.08
Serum C3 (mean ± s.d.) (g/l)	0.49 ± 0.31	0.35 ± 0.14	0.85 ± 0.33
Serum IgA (median, IQR) (g/l)			2.60;1.71–4.44
SLEDAI (median, IQR)		21;19–24	
Antinuclear antibody (+) no. (%)		91(98.91%)	
Anti-dsDNA antibody (+) no. (%)		80(86.96%)	
Anti-cardiolipin antibody (+) no. (%)		4(4.35%)	

LN lupus nephritis, *IgAN* IgA nephropathy, *IQR* interquartile range, *SLEDAI* Systemic Lupus Erythematosus Disease Activity Index, *dsDNA* double-stranded DNA

In the proliferative lupus nephritis group, 24 (26.1%) were male and 68 (73.9%) female, with a mean age of 33.92 ± 14.13 years. At the time of renal biopsy, the median proteinuria was 4.53 (IQR 2.88–7.42) g/24 hours, and the average estimated glomerular filtration rate (eGFR) was 62.70 ± 35.18 ml/min per 1.73 m^2. According to the 2003 ISN/RPS classification system of lupus nephritis, all patients were classified as class IV (100%, including 21 as class IV + V).

The mean age of the patients with endocapillary proliferative IgA nephropathy was 41.03 ± 19.50 years and the male-to-female ratio was 1:1. At the time of renal biopsy, the median proteinuria was 4.21 (IQR 2.50–9.01) g/24 hours, and the mean eGFR was 79.85 ± 42.08 ml/min per 1.73 m^2. The pathological lesions of IgA nephropathy patients were graded using the Oxford classification (MESTC scores). Mesangial hypercellularity (M1), endocapillary hypercellularity (E1), and segmental glomerulosclerosis (S1) were found in 34 (100%), 34 (100%), and 9 (26.5%) patients, respectively. For tubular atrophy and interstitial fibrosis (Oxford-T) and crescent (Oxford-C) lesions, T0, T1, and T2 were found in 17(50.0%), 15 (44.1%), and 2 (5.9%) patients, while C0, C1, and C2 were found in 4 (11.8%), 12(35.3%), and 18(52.9%) patients.

Comparisons of urine sediment between patients with proliferative lupus nephritis and patients with endocapillary proliferative IgA nephropathy

The severity of microscopic hematuria and leukocyturia were defined according to the IQR of all the patients. In the patients with endocapillary proliferative glomerulonephritis, normal to mild hematuria (≤ 35/HPF), and moderate to severe hematuria (> 35/HPF) were found in 31(24.60%) and 95 (75.40%) patients, respectively; normal to mild leukocyturia (≤ 12/HPF), and moderate to severe leukocyturia (> 12/HPF) were found in 52(41.27%) and 74 (58.73%) patients, respectively. The frequency of different types of urinary casts were shown in Table 2.

Table 2
Comparisons of urine sediment between patients with proliferative lupus nephritis and patients with endocapillary proliferative IgA nephropathy

	All enrolled patients n = 126	Patients with lupus nephritis n = 92	Patients with IgA nephropathy n = 34	*P*-value
Erythrocytes, HPF				0.525
Normal to mild, ≤ 35/HPF No. (%)	31(24.60%)	24(26.09%)	7(20.59%)	
Moderate to severe, > 35/HPF No. (%)	95(75.40%)	68(73.91%)	27(79.41%)	
Leukocytes, HPF				**< 0.001**
Normal to mild, ≤ 12/HPF No. (%)	52(41.27%)	29(31.52%)	23(67.65%)	
Moderate to severe, > 12 /HPF No. (%)	74(58.73%)	63(68.48%)	11(32.35%)	
Renal tubular epithelial cells, HPF No. (%)	33(26.19%)	21(22.82%)	12(35.29%)	0.158
Lipid droplets, HPF No. (%)	32(25.40%)	18(19.57%)	14(41.18%)	**0.013**
Hyaline casts, LPF No. (%)	101(80.16%)	73(79.35%)	28(82.35%)	0.707
Granular casts, LPF No. (%)	64(50.79%)	50(54.35%)	14(41.18%)	0.189
Red blood cells casts, LPF No. (%)	60(47.62%)	45(48.91%)	15(44.12%)	0.632
White blood cells casts, LPF No. (%)	66(52.38%)	54(58.70%)	12(35.29%)	**0.020**
Epithelial cells casts, LPF No. (%)	38(30.16%)	26(28.26%)	12(35.29%)	0.445
Mixed cellular casts, LPF No. (%)	57(45.24%)	45(48.91%)	12(35.29%)	0.173
Lipid laden casts, LPF No. (%)	23(18.25%)	12(13.04%)	11(32.35%)	**0.013**
Waxy casts, LPF No. (%)	45(35.71%)	39(42.39%)	6(17.65%)	**0.010**

HPF high-power field, *LPF* low-power field

P value was used to indicate the difference between the patients with lupus nephritis and the patients with IgA nephropathy. A two-tailed P < 0.05 was considered statistically significant

There was no significant difference of the microscopic hematuria between patients with proliferative lupus nephritis and patients with endocapillary proliferative IgA nephropathy. The proportion of normal to mild leukocyturia (≤ 12/HPF) in patients with proliferative lupus nephritis were significantly lower than that in the patients with endocapillary proliferative IgA nephropathy (29(31.52%) versus 23(67.65%), $P < 0.001$), whereas the proportion of moderate to severe (> 12/HPF) leukocyturia in lupus nephritis group were significantly higher than that in IgA nephropathy group (63(68.48%) versus 11(32.35%), $P < 0.001$) (shown in Fig. 1A, 1B). Urinary leukocytes were classified in 19 patients with proliferative lupus nephritis, polymorphonuclear leukocytes > 50%, and mononuclear leukocytes > 50% were found in 14 (73.78%), and 5 (26.32%) patients, respectively. In addition, the frequency of urinary WBC casts (shown in Fig. 1C) and waxy casts (shown in Fig. 1D) were significantly higher in proliferative lupus nephritis patients compared with endocapillary proliferative IgA nephropathy patients (54(58.70%) versus 12(35.29%), $P = 0.020$; 39(42.39%) versus 6(17.65%), $P = 0.010$, respectively), whereas the frequency of urinary lipid droplets and lipid laden casts were significantly lower in proliferative lupus nephritis patients compared with endocapillary proliferative IgA nephropathy patients (18(19.57%) versus 14(41.18%), $P = 0.013$; 12(13.04%) versus 11(32.35%), $P = 0.013$, respectively). No significant differences were found of the other types of urinary casts (hyaline casts, granular casts, RBC casts, epithelial cells casts, and mixed cellular casts) between the two groups (Table 2).

Correlation analysis of leukocyturia with clinicopathological data in endocapillary proliferative glomerulonephritis

Of the patients with endocapillary proliferative glomerulonephritis, the levels of leukocyturia were significantly correlated with serum albumin (r=-0.206, *P* = 0.021), serum creatinine (r = 0.275, *P* = 0.002), eGFR (r=-0.233, *P* = 0.009) and serum C3 (r=-0.351, *P* < 0.001). In terms of renal pathological data, it was found that leukocyturia was positively correlated with glomerular leukocyte infiltration (r = 0.294, *P* = 0.001) (Table 3).

Table 3
Correlation analysis of leukocyturia with clinicopathological data in endocapillary proliferative glomerulonephritis

	All patients **n = 126** **r value *P* value**	**Patients with LN** **n = 92** **r value *P* value**	**Patients with IgAN** **n = 34** **r value *P* value**
Clinical data			
Age (years)	**-0.298 0.001**	-0.168 0.109	**-0.458 0.007**
Albumin (g/l)	**-0.206 0.021**	-0.015 0.886	-0.292 0.094
Urine protein (g/24 h)	0.072 0.425	0.044 0.684	0.208 0.237
Serum creatinine (μmol/l)	**0.275 0.002**	**0.288 0.005**	0.070 0.694
eGFR (ml/min per 1.73 m^2)	**-0.233 0.009**	**-0.284 0.006**	0.123 0.488
C3 (g/l)	**-0.351 0.001**	**-0.275 0.009**	0.036 0.840
SLEDAI		**0.383 0.001**	
Renal histopathology indices			
Crescents /Cellular crescents/ Oxford-C	0.102 0.724	0.177 0.470	0.204 0.829
Interstitial inflammatory cell infiltration	0.114 0.652	0.188 0.374	0.291 0.411
Glomerular leukocyte infiltration	**0.294 0.001**	**0.285 0.002**	0.266 0.568
Activity indices (AIs) score		0.171 0.114	
Chronicity indices (CIs) score		-0.097 0.369	
Oxford-S			0.304 0.398
Oxford-T			0.301 0.447
LN lupus nephritis, *IgAN* IgA nephropathy, *SLEDAI* Systemic Lupus Erythematosus Disease Activity Index			
S glomerulosclerosis, T tubular atrophy and interstitial fibrosis, C cellular or fibrocellular crescents			
A two-tailed P < 0.05 was considered statistically significant			

In the proliferative lupus nephritis group, the levels of leukocyturia was significantly correlated with serum creatinine (r = 0.288, *P* = 0.005), eGFR (r=-0.284, *P* = 0.006), serum C3 (r=-0.275, *P* = 0.009) and SLEDAI scores (r = 0.383, *P* < 0.001). In terms of renal pathological data, leukocyturia was positively associated with glomerular leukocyte infiltration (r = 0.285, *P* = 0.002) (Table 3).

There was a negative correlation between leukocyturia and age at kidney biopsy (r=-0.458, *P* = 0.007) in endocapillary proliferative IgA nephropathy. No correlations were found between the leukocyturia and other clinicopathological parameters (Table 3).

Comparisons of clinicopathological data between patients with leukocyturia and patients without leukocyturia

Patients with leukocyturia presented with significantly higher frequency of granular casts, WBC casts and waxy casts compared with those without (59(59.60) versus 5(18.52), *P* < 0.001; 61(61.62) versus 5(18.52), *P* < 0.001; 40(40.40) versus 5(18.52), *P* = 0.04, respectively).The serum levels of albumin and C3 was significantly lower in the patients with leukocyturia than that in the patients

without leukocyturia (25.1 ± 5.9 versus 28.3 ± 6.1 g/l, $P = 0.02$; 0.46 ± 0.28 versus 0.61 ± 0.36 g/l, $P = 0.02$, respectively). The prevalence of glomerular leukocyte infiltration was significantly higher in the patients with leukocyturia compared with those without (88(88.89%) versus 17(62.96%), $P = 0.004$) (Table 4).

Table 4
Comparisons of clinicopathological data between patients with leukocyturia and patients without leukocyturia

	All patients (n = 126)			Patients with LN (n = 92)			Patients with IgAN (n = 34)		
	Lkc (-)	Lkc (+)	*P* value	Lkc (-)	Lkc (+)	*P* value	Lkc (-)	Lkc (+)	*P* value
Urine sediment no. (%)									
Erythrocytes (moderate to severe)	18(66.67)	77(77.78)	0.24	9(64.29)	59(75.64)	0.58	9(69.23)	18(85.71)	0.47
Renal tubular epithelial cells	6(22.22)	27(27.27)	0.60	2(14.29)	19(24.36)	0.63	4(30.77)	8(38.10)	0.95
Lipid droplets	3(11.11)	29(29.29)	0.05	0(0.00)	18(23.08)	0.10	3(23.08)	11(52.38)	0.09
Hyaline casts	21(77.78)	80(80.81)	0.73	9(64.29)	64(82.05)	0.25	12(92.31)	16(76.19)	0.46
Granular casts	5(18.52)	59(59.60)	**0.001**	2(14.29)	48(61.54)	**0.001**	3(23.08)	11(52.38)	0.09
Red blood cells casts	9(33.33)	51(51.52)	0.09	5(35.71)	40(51.28)	0.28	4(30.77)	11(52.38)	0.22
White blood cells casts	5(18.52)	61(61.62)	**0.001**	3(21.43)	51(65.38)	**0.002**	2(15.38)	10(47.62)	0.12
Epithelial cells casts	8(29.63)	30(30.30)	0.95	5(35.71)	21(26.92)	0.73	3(23.08)	9(42.86)	0.42
Mixed cellular casts	10(37.04)	47(47.47)	0.33	6(42.86)	39(50.00)	0.62	4(30.77)	8(38.10)	0.95
Fatty casts	3(11.11)	20(20.20)	0.42	0(0.00)	12(15.38)	0.25	3(23.08)	8(38.10)	0.59
Waxy casts	5(18.52)	40(40.40)	**0.04**	3(21.43)	36(46.15)	0.09	2(15.38)	4(19.05)	0.79
Clinical data									
Anemia no. (%)	20(74.07)	78(78.79)	0.60	12(85.71)	66(84.62)	0.92	8(61.54)	12(57.14)	0.80
Albumin (g/l) (mean ± s.d.)	28.3 ± 6.1	25.1 ± 5.9	**0.02**	26.4 ± 5.7	24.3 ± 5.4	0.18	30.3 ± 6.4	28.0 ± 6.8	0.26
Urine protein (g/24 h) (median, IQR)	3.7(2.6,8.0)	4.6(2.9,7.8)	0.41	3.6(3.0,7.3)	4.6(2.6,7.5)	0.86	3.8(2.1,9.5)	5.8(3.5,8.9)	0.26
Serum creatinine (μmol/l) (median, IQR)	82(70,124)	104(77,180)	0.11	102(75,128)	113(81,189)	0.22	73(66,225)	82(71,137)	0.55
eGFR (ml/min per 1.73 m^2) (mean ± s.d.)	74.3 ± 38.8	65.4 ± 37.5	0.28	73.1 ± 34.0	60.8 ± 35.3	0.23	75.7 ± 44.8	82.4 ± 41.2	0.68
C3 (g/l) (mean ± s.d.)	0.61 ± 0.36	0.46 ± 0.28	**0.02**	0.43 ± 0.14	0.34 ± 0.13	**0.03**	0.79 ± 0.43	0.89 ± 0.26	0.38
Acute kidney injury no. (%)	5(18.52)	32(32.32)	0.16	1(7.14)	27(34.62)	0.08	4(30.77)	5(23.81)	0.96

LN lupus nephritis, *IgAN* IgA nephropathy, *Lkc* leukocyturia, *IQR* interquartile range, *SLEDAI* Systemic Lupus Erythematosus Disease Activity Index

S1 present segmental glomerulosclerosis, T1-T2 severity of tubular atrophy/interstitial fibrosis (T1 = 26–50%; T2 > 50%), C1-C2 present crescent (C1 = 1–25%; C2 = 26–100%)

A two-tailed P < 0.05 was considered statistically significant

	All patients (n = 126)			Patients with LN (n = 92)			Patients with IgAN (n = 34)		
	Lkc (-)	Lkc (+)	*P* value	Lkc (-)	Lkc (+)	*P* value	Lkc (-)	Lkc (+)	*P* value
SLEDAI (median, IQR)				14(11,23)	22(20,24)	**0.003**			
Renal histopathology indices no. (%)									
Crescents /Cellular crescents/C1-C2	22(81.48)	81(81.82)	0.97	2(0,2)	2(2,4)	**0.04**	11(84.62)	19(90.48)	0.60
Interstitial inflammatory cell infiltration	26(96.30)	95(95.96)	0.94	1(1,1)	1(1,2)	0.87	12(92.31)	20(95.24)	0.72
Glomerular leukocyte infiltration	17(62.96)	88(88.89)	**0.004**	2(1,2)	2(1,2)	**0.04**	4(30.77)	11(52.38)	0.22
Activity indices (AIs) score				8(6,8)	9(7,10)	**0.04**			
Chronicity indices (CIs) score				2(1,3)	2(1,2)	0.48			
S1							5(38.46)	4(19.05)	0.40
T1-T2							7(53.85)	10(47.62)	0.72
LN lupus nephritis, *IgAN* IgA nephropathy, *Lkc* leukocyturia, *IQR* interquartile range, *SLEDAI* Systemic Lupus Erythematosus Disease Activity Index									
S1 present segmental glomerulosclerosis, T1-T2 severity of tubular atrophy/interstitial fibrosis (T1 = 26–50%; T2 > 50%), C1-C2 present crescent (C1 = 1–25%; C2 = 26–100%)									
A two-tailed P < 0.05 was considered statistically significant									

In the proliferative lupus nephritis group, the frequency of urinary granular casts and WBC casts were significantly higher in the patients with leukocyturia compared with those without (48(61.54%) versus 2(14.29%), $P = 0.001$; 51(65.38%) versus 3(21.43%), $P = 0.002$, respectively). Moreover, the serum C3 levels were significantly lower in the patients with leukocyturia than that in the patients without leukocyturia (0.34 ± 0.13 versus 0.43 ± 0.14 g/l, $P = 0.03$). SLEDAI scores was significantly higher in the patients with leukocyturia than that in the patients without leukocyturia (22(20,24) versus 14(11,23), $P = 0.003$). The scores of cellular crescents, glomerular leukocyte infiltration, activity indices were significantly higher in the patients with leukocyturia compared with those without (2(2,4) versus 2(0,2), $P = 0.04$; 2(1,2) versus 2(1,2), $P = 0.04$; 9(7,10) versus 8(6,8), $P = 0.04$, respectively). (Table 4).

In the endocapillary proliferative IgA nephropathy group, no significant clinicopathological difference was found between the patients with leukocyturia and those without leukocyturia.

Association of leukocyturia and renal outcomes in patients with endocapillary proliferative glomerulonephritis

In our study, 56 patients with endocapillary proliferative glomerulonephritis were regularly followed up, with an average follow-up time of 35.7 ± 24.1months. With regard to long-term renal outcomes, 9 patients (9/56, 16.07%) reached the composite end point, defined as ESRD or doubling of serum creatinine levels or 50% eGFR decline. Using the log-rank test for univariate survival analysis, we found that leukocyturia was a risk factor for renal outcome (HR: 1.233, 95% CI: 1.042–1.460, $P = 0.015$). In the further multivariate Cox hazard analysis, leukocyturia remained as an independent risk factor for renal outcome (HR: 1.519, 95% CI: 1.103–2.091, $P = 0.010$) (Table 5).

Table 5
Univariate and multivariate analysis of independent prognostic factors for renal survival

All patients	**Univariable**		**Multivariable**	
Risk Factor	**HR (95% CI)**	P **value**	**HR (95% CI)**	P **value**
Age	1.033 (0.994 to 1.074)	0.101		
Gender	0.134 (0.028 to 0.648)	**0.012**		
Proteinuria	1.086 (0.959 to 1.230)	1.092		
Serum creatinine	1.004 (1.002 to 1.006)	**< 0.001**	1.003 (1.000 to 1.006)	**0.030**
Leukocyturia (non-infection)	1.233 (1.042 to 1.460)	**0.015**	1.519 (1.103 to 2.091)	**0.010**
Crescents	1.162 (0.691to 1.954)	0.572		
Glomerular leukocyte infiltration	0.297 (0.047 to 1.189)	0.086		
Patients with lupus nephritis	**Univariable**		**Multivariable**	
Risk Factor	**HR (95% CI)**	P **value**	**HR (95% CI)**	P **value**
Age	0.999 (0.937 to 1.067)	0.988		
Gender	0.182 (0.033 to 0.993)	**0.049**		
SLEDAI	0.987 (0.791 to 1.233)	0.910		
Acute kidney injury	1.525 (0.992 to 2.342)	0.054		
Leukocyturia (non-infection)	1.463 (1.085 to 1.973)	**0.013**	1.456(1.083 to 1.957)	**0.013**
Activity indices score	1.402 (1.022 to 1.923)	**0.036**		
Chronicity indices score	1.440 (1.036 to 2.000)	**0.030**		
Patients with IgA nephropathy	**Univariable**		**Multivariable**	
Risk Factor	**HR (95% CI)**	P **value**	**HR (95% CI)**	P **value**
Age	1.062 (0.984 to 1.146)	0.120		
Gender	0.460 (0.069 to 3.069)	0.423		
Urine protein	1.056 (0.839 to 1.330)	0.641		
Serum creatinine	1.020 (0.977 to 1.065)	0.366		
Leukocyturia (non-infection)	0.957 (0.823 to 1.112)	0.562	1.069 (0.494 to 2.312)	0.866
S1	0.510 (0.046 to 5.611)	0.582		
T1-T2	0.631 (0.056 to 7.060)	0.709		
C1-C2	1.882 (0.492 to 7.198)	0.356		

HR hazard ratio, *95% CI* 95% confidence interval, *SLEDAI* Systemic Lupus Erythematosus Disease Activity Index

S1 present segmental glomerulosclerosis, T1-T2 severity of tubular atrophy/interstitial fibrosis (T1 = 26–50%; T2 > 50%), C1-C2 present crescent (C1 = 1–25%; C2 = 26–100%)

A two-tailed P < 0.05 was considered statistically significant

In subgroup analysis, 45 patients with proliferative lupus nephritis were followed up for an average duration of 36.5 ± 26.11months. During follow-up, 6 (6/45, 13.33%) patients reached ESRD. A univariate analysis revealed that gender, leukocyturia, AI and CI were risk factors for renal outcome (HR: 0.182, 95% CI: 0.033–0.993, $P = 0.049$; HR: 1.463, 95% CI: 1.085–1.973, $P = 0.013$; HR: 1.402, 95% CI: 1.022–1.923, $P = 0.036$; HR: 1.440, 95% CI: 1.036-2.000, $P = 0.030$, respectively) and a subsequent multivariate analysis showed that leukocyturia was identified as an independent risk factor for renal outcome (HR: 1.456, 95% CI: 1.083–1.957, $P = 0.013$). However, we

found that the leukocyturia was not an independent risk factor for renal outcome in endocapillary proliferative IgA nephropathy using the Cox hazard model for multivariate survival analysis (HR: 1.069, 95% CI: 0.494–2.312, *P* = 0.866) (Table 5).

Discussion

In this study, we investigated the differences of urinary sediment findings between lupus nephritis and IgA nephropathy with endocapillary proliferative glomerulonephritis and further evaluated associations of leukocyturia with disease activity, pathological features and prognosis.

We found predominant leukocyturia in a series of 126 patients with endocapillary proliferative glomerulonephritis by urinary sediment analysis. Leukocyturia (> 5 leukocyte/HPF) was seen in 99(78.6%) patients, 74 of whom had moderate to severe leukocyturia (> 12/HPF). Meanwhile, microscopic hematuria and a group of urinary casts were also prevalent, like hyaline casts, granular casts, RBC casts and WBC casts, et al. Endocapillary proliferative glomerulonephritis were more prevalent in acute postinfectious glomerulonephritis. Nasr SH et al analyzed the urine sediment in 86 patients (56 ± 16 years) with acute postinfectious glomerulonephritis, among which 72.1% were diffuse endocapillary proliferative glomerulonephritis. In this group, 54.7% had leukocyturia [5]. In another study of 109 elderly patients (≥ 65 years) with postinfectious glomerulonephritis, leukocyturia was present in 65% of patients [22]. Therefore, the frequency of leukocyturia seems to be more prevalent in non-infectious endocapillary proliferative glomerulonephritis.

Next, we compared the urine sediment findings in two subgroups of non-infectious endocapillary proliferative glomerulonephritis. We found that the prevalence of moderate to severe leukocyturia, urinary WBC casts and waxy casts were significantly higher in proliferative lupus nephritis than those in endocapillary proliferative IgA nephropathy. Taken together, it indicated that there was significant difference in urinary sediment in different clinicopathologic types of endocapillary proliferative glomerulonephritis, especially leukocyturia. Thus, urine sediment findings may provide diagnostic information that often identifies the potential renal injury.

More interestingly, levels of leukocyturia was closely associated with clinical or pathological renal injury indices such as the serum creatinine level, eGFR, serum C3 level, SLEDAI scores and glomerular leukocyte infiltration in proliferative lupus nephritis; In addition, the patients with leukocyturia presented with more severe renal injury, like lower serum C3 value, higher SLEDAI scores, and higher renal histopathological scores, including cellular crescents, glomerular leukocyte infiltration, and total activity indices compared with those without leukocyturia. Our study suggested that leukocyturia was closely associated with the disease activity and clinicopathology severity of proliferative lupus nephritis. Previous study showed that the appearance of isolated pyuria was correlated with renal activity in patients with lupus nephritis [23]. It indicated that leukocyturia can be regard as a marker that reflect renal disease activity in lupus nephritis. More importantly, leukocyturia could not only reflect disease activity, but also the renal histopathological changes. A recent study showed that one-third of lupus nephritis patients who achieved a complete clinical response (defined as 24-hour proteinuria < 500 mg/day and improvement or maintenance of serum creatinine) after induction therapy had persistently high histologic activity confirmed by repeat kidney biopsy, while 62% of lupus nephritis patients with a complete histologic remission on repeat biopsy were still clinically active [24]. Urine sediment findings of our study (particularly leukocyturia) may reduce the discordance between clinical activity and histologic activity and prevent patients from repeat renal biopsy.

Accordingly, urine sediment analysis might be a noninvasive “liquid biopsy”. This examination may help initiate intervention treatment without delay before a renal biopsy or for patients who have a contraindication for this procedure.

More importantly, based on a multivariate survival analysis in our study, leukocyturia was identified as an independent risk factor for renal outcome in endocapillary proliferative glomerulonephritis, as well as in proliferative lupus nephritis. These results indicated that leukocyturia may be a useful biomarker in predict prognosis in lupus nephritis. In contrast, previous research, including our center, failed to show that leukocyturia was correlated with poorer outcomes in lupus nephritis [25], which because the levels of leukocyturia was quantified from urine routine test.

In the classification of leukocyturia, the proportion of mononuclear cell was not predominant in endocapillary proliferative lupus nephritis. Previous studies by Chan RW, et al suggested that urinary mononuclear cell was markedly elevated in patients with active lupus, and CD3 + and CD20 + cells are the major component of urinary mononuclear cell in SLE patients [26]. The different findings might result from the following reasons: firstly, polymorphonuclear neutrophils are the most common component of pyuria but can also be seen with inflammatory kidney lesions and glomerular neutrophil infiltration was indeed an active index and observed in our study by

renal biopsy; secondly, pathological classification of lupus nephritis may affect the classification of leukocyturia, all patients in our study were classified as endocapillary proliferative lupus nephritis, while in Chan RW' study, both proliferative and non-proliferative lupus nephritis were included; finally, immunocytochemical staining of urinary cell or flowcytometry are more reliable methods to differentiate mononuclear cell from other cell types in urinary sediment.

However, no significant correlations were found between the leukocyturia and renal injury characteristics in endocapillary proliferative IgA nephropathy in our study. Previous studies indicated that a significant increase of urinary leukocytes was observed in patients with active-crescentic IgA nephropathy as compared with other patients without active-crescentic. Moreover, the extent of active crescents in biopsies was significantly correlated with the number of macrophages and NK cells in urine [27]. Thus, we speculate that the identification of these urinary leukocytes by immunohistochemistry stain or flow cytometry examination might be better in monitoring disease activity in IgA nephropathy. In addition, no associations were found between leukocyturia and renal outcomes in endocapillary proliferative IgA nephropathy. Nakayama K, et al found that simultaneous appearance of lipid laden casts, oval fat bodies and granular casts in urinary sediments is an important sign of poor prognosis in IgA nephropathy [28]. Thus, urinary casts might be more important to exactly estimate the prognosis of IgA nephropathy.

Though we obtained the urine sample the same day of the kidney biopsy and manually analyzed urinary sediment examination by two well-trained nephrologists, this study still has some limitations. Firstly, it was a retrospective study with small sample size. Secondly, only two clinicopathologic diagnoses types of endocapillary proliferative glomerulonephritis were included. More types like post-streptococcai glomerulonephritis and other infectious related glomerulonephritis need to be included in the further study.

In conclusion, urinary sediments of the endocapillary proliferative lupus nephritis and endocapillary proliferative IgA nephrology differed in many aspects. Leukocyturia reflects the disease activity and prognosis of endocapillary proliferative glomerulonephritis, especially in lupus nephritis.

Abbreviations

RBC
Red blood cells; WBC:White blood cells; SLE:Systemic lupus erythematosus; AKI:Acute kidney injury; KDIGO:Kidney Disease Improving Global Outcomes; ADQI:Acute Disease Quality Initiative; SLEDAI:Systemic Lupus Erythematosus Disease Activity Index; ESRD:End-stage renal disease; HPFs:High power fields; IQR:Interquartile range; LPFs:Low power fields; ANA:Antinuclear antibodies; ELISA:Enzyme-linked immunosorbent assay; ISN/RPS:International Society of Nephrology/Renal Pathology Society; AI:Activity indices; CI:Chronicity indices; eGFR:Estimated glomerular filtration rate

Declarations

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YT and JZL: research idea and study design; MY and JZL: data acquisition; XJY: pathology review; MY, JZL and YT: data analysis/interpretation; MY: statistical analysis; MY: writing manuscript; YT, JZL and HZ: supervision or mentorship. All authors read and approved the final version of the manuscript.

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Availability of data and materials

All data generated and analyzed in this study are disclosed.

Ethics approval and consent to participate

The research was carried out in compliance with the principles of the Declaration of Helsinki. The study was approved by the ethics committee of Peking University First Hospital (approval number: 2014[749]).

Consent for publication

Informed consent was obtained from all patients.

Competing interests

The authors have declared no conflicts of interest.

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Supplementary Information

Additional file 1: Supplementary Table 1. Renal pathological data of patients with endocapillary proliferative glomerulonephritis.

Figures

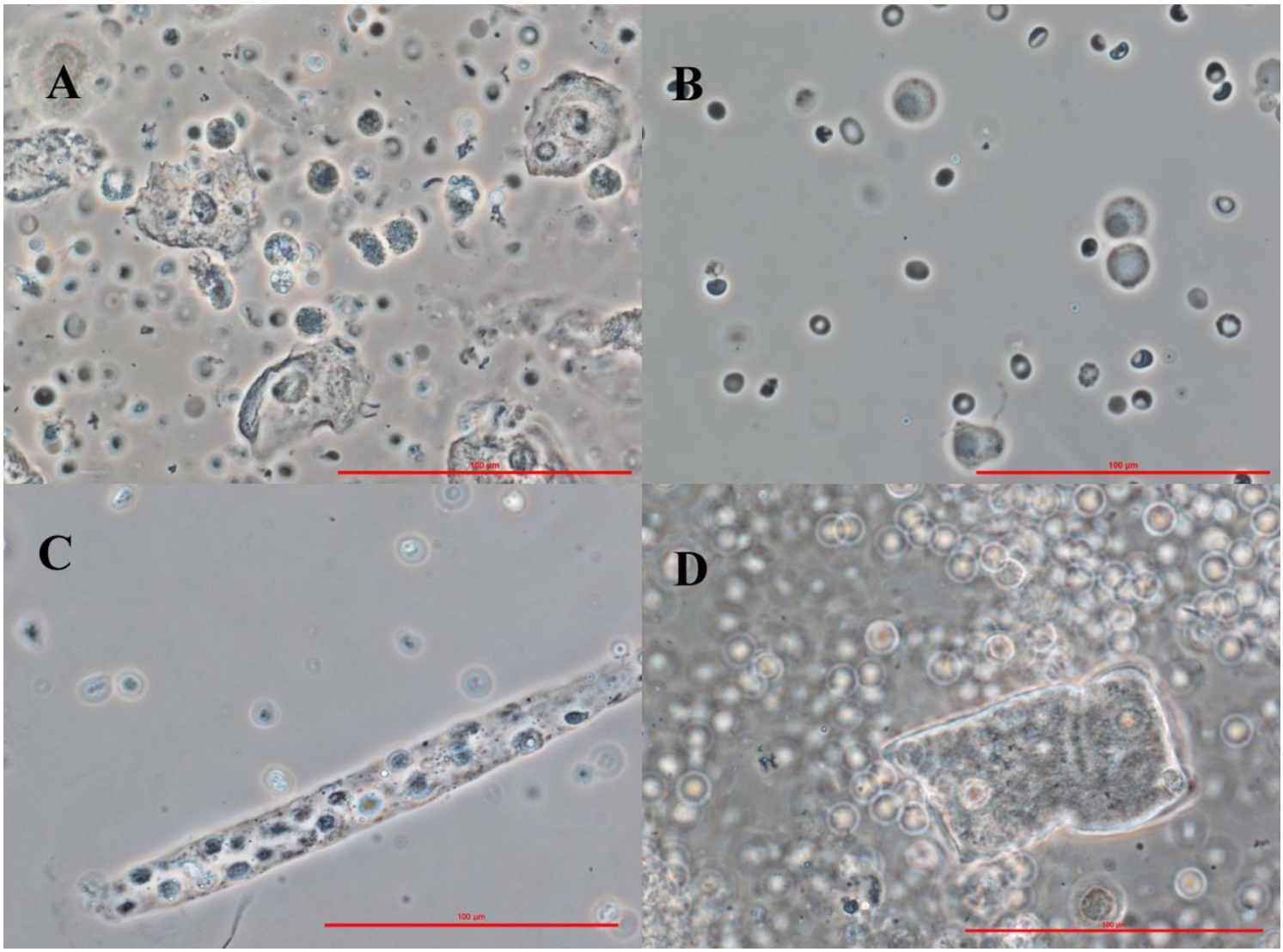

Figure 1

Urinary sediment findings seen in the patients with endocapillary proliferative glomerulonephritis. (A) High-powered view of urinary leukocytes in patients with endocapillary proliferative lupus nephritis (x 400). (B) High-powered view of urinary leukocytes in patients with endocapillary proliferative IgA nephropathy (x 400). (C) High-powered view of urinary white blood cell cast (x 400). (D) High-powered view of urinary waxy cast (x 400). Scale bars 100μm. (All images by phase contrast microscopy).

Supplementary Files

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- SupplementaryTable1.docx
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