BMC Urology

RESEARCH

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Landscape of peripheral immunity in patients with upper urinary tract urolithiasis and the underlying correlations with renal function

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Abstract

Introduction Inflammatory and immunological responses are reported involved in the pathogenesis and progression of obstructive nephropathy (ON). This study was designed to investigate the characteristics of peripheral immunity in patients with upper urinary tract urolithiasis and analyze the underlying associations with renal function.

Methods Patients with unilateral upper urinary tract urolithiasis meeting the operation indications were prospectively enrolled. Preoperative circulating immune cells and inflammatory cytokines were detected in our clinical laboratory, and the indicators of renal function and calculi related parameters were particularly recorded. Patients were sectionalized into subgroups on the basis of the lesion of calculi. Characteristics of peripheral immunity in each subgroup were investigated by statistical approaches, and the underlying correlations with the degree of hydronephrosis (HN) and renal function were discussed in corresponding group.

Results Patients with ureteral calculi presented severer HN compared with renal calculi, especial middle ureteral calculi, acting as the chief culprit of ON, exhibiting the highest serum creatine and blood urea nitrogen, most impaired estimated glomerular filtration rate, and severest HN. In addition, serum interleukin-8 (IL-8) and IL-6 were demonstrated presenting statistical differences between ureteral calculi and renal calculi patients, exhibiting underlying values in comprehending ON. However, circulating immune cells were demonstrated no obvious differences among groups.

Conclusions Circulating inflammatory cytokines, referred in particular to serum IL-8 and IL-6 were partially associated with kidney injury in patients with upper urinary tract urolithiasis. But the specific influences and mechanisms between them needed to be investigated furthermore.

Keywords Obstructive nephropathy, Kidney injury, Urolithiasis, Unilateral ureteral obstruction, Peripheral immune cell, Inflammatory cytokine

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Introduction

Obstructive nephropathy (ON) is a relatively common entity in clinical settings, which could occur at all ages [1]. In adult patients, urolithiasis is a dominating etiology, and would contribute to impairing the function of the affected kidney if the obstruction couldn't be relieved in time, presenting as an increase in pressure in the proximal tube, hydronephrosis (HN), decrease of glomerular filtration rate (GFR), and ultimately progressing to renal fibrosis [1].

Inflammatory and immunological responses are widely reported involved in the pathogenesis and progression of ON as well as interstitial fibrosis [2]. Several studies verified that inflammatory cytokines infiltrated in renal tissues, for instance monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1), interleukin-6 (IL-6) and IL-1β, etc. were closely associated with the severity of injury of the affected kidney and even the degree of renal fibrosis in unilateral ureteral obstruction (UUO) animal models [3–6]. Others reported immune cells activation and infiltration in the impaired kidney of UUO mice were also common pathogenic mechanisms of kidney injury [7–9]. However, whether circulating immune cells and inflammatory cytokines were correlated with kidney injury in ON in clinical settings is indistinct. Thus, this study was designed to investigate the underlying associations between peripheral immunity and kidney injury in patients with unilateral upper urinary tract urolithiasis.

Methods

Study design and patient selection

This was a prospective cohort study conducted on patients who were diagnosed with unilateral upper urinary tract urolithiasis and receiving ureteroscopic lithotripsy (URL) surgery between Jan. 2022 and May. 2023 at our institution. Inclusion criteria were (1) newly diagnosed unilateral single ureteral (study group) or renal calculi (<2 cm, control group); (2) without contralateral urolithiasis; (3) no concomitant with ipsilateral renal or ureteral calculus; (4) no medical history of urolithiasis; (5) no pretreatments for urolithiasis, like DJ stent dwelling; (6) adult patients (age≥18 years). Exclusion criteria were (1) functional or anatomic solitary kidney; (2) abnormal preoperative eGFR (<60 mL/min/1.73 m^2); (3) with medical history of kidney insufficiency or renal atrophy or HN; (4) with uncontrolled hypertension, diabetes mellitus (DM), heart failure, intrinsic kidney disease, etc. that could deteriorate the residual renal function; (5) with urinary tract infection (UTI), inflammatory disease, immune-mediated disease, medical history of malignancy, or receiving immunotherapy, etc [10]. The flow chart of the study design was shown in Fig. 1. Patients with ureterostenosis or pyonephrosis of the affected side that were found during the operation were excluded ultimately.

All procedures performed in this study were in accordance with the ethical standards of the Ethics Committee of our hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

HN and renal function evaluation

Patients were requested to have available preoperative imaging to assess the presence and degree of ipsilateral HN. Imaging was conducted within 24 h before URL surgery using computer tomography (CT) with or without contrast, or renal ultrasound. If more than 1 imaging modality was available for a patient, preference was given to CT [11]. HN was defined as the dilation of calyx or renal pelvis (in the posterior-anterior plane), with or without renal parenchyma atrophy, and was assigned and assessed as a continuous variable [12]. Renal function was systematically evaluated by serum creatine (sCr), blood urea nitrogen (BUN), estimated GFR (eGFR) and urine neutrophil gelatinase-associated lipocalin (uNGAL) 24 h before surgery. In order to avoid iatrogenic hurt to patients, radionuclide scintigraphy was not routinely conducted, unless explicit indications were presented.

Peripheral immunity assessment

Peripheral immunity was routinely detected in our clinical laboratory. The items included and were limited to circulating immune cells, i.e., $CD3^+$T cell, $CD4^+$T cell, $CD8^+$T cell, natural killer (NK) cell, B cell and T regulatory cell (Treg), and inflammatory cytokines, like IL-1β, IL-6, IL-8, IL-10, IL-2R and TNF-α. Otherwise, other indicators, for instance, macrophage, TGF-β, IL-18 and MCP-1, etc., could not be detected. In brief, inflammatory cytokines were detected by using enzyme-linked immunosorbent assay (ELISA) after serum was extracted, and flow cytometry (FCM) was utilized to analyze circulating immune cell [13]. All procedures were conducted according to the manufacturer's instructions.

Data acquisition and grouping

Specific medical information of age, sex, body mass index (BMI), urolithiasis related parameters (size, lesion, CT value, etc.), preoperative renal function, circulating immune cells and peripheral inflammatory cytokines etc. were tested and recorded. Generally, patients were classified into ureteral calculi group (study group) and renal calculi group (control group). And for more finely, patients were furthermore grouped to calices, pelvis, upper ureter, middle ureter and lower ureter subgroup on the basis of the lesion of calculi.

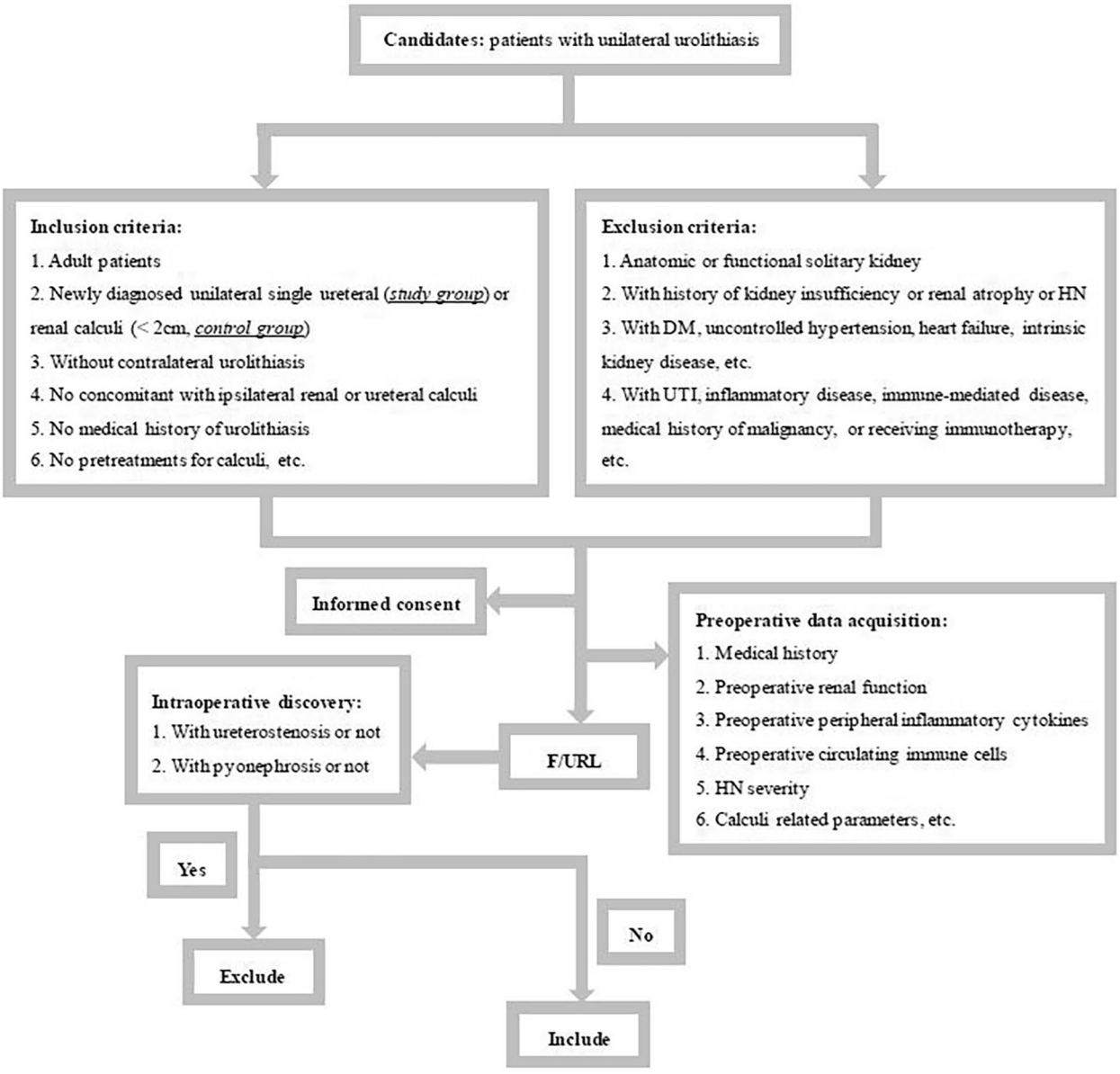

Fig. 1 Flow chart of the study design. *Abbreviations* DM diabetes mellitus, HN hydronephrosis, URL ureteroscopic lithotripsy, UTI urinary tract infection

Statistical analysis

Statistical comparisons were performed using the statistical software GraphPad Prism 10 (GraphPad Software, CA, USA). Continuous variables were expressed as mean±standard deviation (SD). Intergroup differences were tested using the Student's t test or analysis of variance (ANOVA). Tukey's multiple comparison test was further chosen for post-test of ANOVA. Categorical variables were expressed as frequency (percentage), and Chi-square test was used to test the intergroup differences. Pearson correlation analysis was applied to analyze the interdependency between variables. Statistical significance was defined as a $p<0.05$.

Results

Demographics and clinical characteristics

172 patients were ultimately enrolled and systematically reviewed. Patients were divided into two groups on the basis of the lesion of calculi, i.e., renal calculi group (control group, 29 patients) and ureteral calculi group (study group, 143 patients). Recorded clinical and demographic characteristics of all patients as well as the integrated data were shown in Table 1. Patients with renal calculi tended to larger calculi size (12.9±5.9 mm vs. 7.3±3.0 mm, $p<0.0001$), longer disease course (defined as the duration from the time of the diagnosis of urolithiasis to enrollment, 156.7±365.3 d vs. 47.6±157.0 d, $p=0.0103$), higher uNGAL level (33.8±46.8 ng/mL vs. 22.0±20.9 ng/

Table 1 Characteristics of the study population (preoperative data, $n=172$)

Characteristic	Total (172)	Patients with ureteral calculi (143)	Patients with renal calculi (29)	*p* value
Mean (SD) age (years)	53.4 (14.4)	52.8 (14.8)	56.5 (12.3)	0.2006†
Sex	-	-	-	0.8731‡
Male (%)	109 (63.4)	91 (63.6)	18 (62.1)	-
Female (%)	63 (36.6)	52 (36.4)	11 (37.9)	-
Mean (SD) BMI (kg/m^2)	24.4 (3.7)	24.2 (3.8)	25.1 (3.0)	0.2545†
Location of calculi	-	-	-	-
Upper ureter (%)	93 (54.1)	93 (65.0)	-	-
Middle ureter (%)	18 (10.5)	18 (12.6)	-	-
Lower ureter (%)	32 (18.6)	32 (22.4)	-	-
Pelvis (%)	12 (7.0)	-	12 (41.4)	-
Calices (%)	17 (9.9)	-	17 (58.6)	-
Mean (SD) calculi diameter (mm)	8.2 (4.2)	7.3 (3.0)	12.9 (5.9)	<0.0001†***
Mean (SD) calculi CT value (HU)	772.6 (335.2)	754.8 (316.6)	860.3 (410.1)	0.1227†
Mean (SD) HN (mm)	14.8 (12.8)	16.9 (12.9)	4.5 (5.5)	<0.0001†***
Mean (SD) sCr (μmol/L)	70.2 (16.2)	70.1 (15.9)	71.1 (17.8)	0.7469†
Mean (SD) BUN (mmol/L)	5.8 (1.5)	5.7 (1.4)	6.3 (1.8)	0.0648†
Mean (SD) eGFR (mL/min/1.75m^2)	97.3 (21.7)	97.8 (21.3)	94.8 (24.2)	0.5092†
Mean (SD) uNGAL (ng/mL)	24.0 (27.2)	22.0 (20.9)	33.8 (46.8)	0.0333†*
Mean (SD) disease course (d)	66.0 (209.7)	47.6 (157.0)	156.7 (365.3)	0.0103†*

Values were in mean (SD) or n (%)

Abbreviations BMI body mass index, *BUN* blood urea nitrogen, *CT* computer tomography, *eGFR* estimated glomerular filtration rate, *HN* hydronephrosis, *sCr* serum creatinine, *SD* standard deviation, *uNGAL* urine neutrophil gelatinase-associated lipocalin

† Student's t-test, ‡ Chi-square test. *** meant $p<0.001$, * meant $p<0.05$, were assumed as statistically significant

mL, $p=0.0333$), but lighter HN severity (4.5±5.5 mm vs. 16.9±12.9 mm, $p<0.0001$) when compared with ureteral calculi group, implying that patients with ureteral calculi were more tending to choose surgical treatment.

Preoperative renal function

Preoperative renal function of all patients were within normal limits according to the enrollment criteria (eGFR>60 mL/min/1.73 m^2). No statistical differences were found in sCr, BUN and eGFR between groups, though patients with ureteral calculi presented severer HN (Table 1). Regretfully, radionuclide scintigraphy was not routinely conducted in this study, it was out of the question to assess the differential renal function of the affected kidney.

In addition, patients with renal calculi presented higher preoperative uNGAL level than ureteral calculi (Table 1), which was not as expected. But when the obstruction was relieved, uNGAL dramatically elevated in patients with ureteral calculi (Fig. 2).

Landscape of peripheral immunity in upper urinary tract urolithiasis

Circulating immune cells showed no statistical differences between renal calculi group and ureteral calculi group, and even in the further splitted subgroups according to the detailed anatomic lesion of calculi, i.e., calices, pelvis, upper ureter, middle ureter and lower ureter (Tables 2 and 3). But inflammatory cytokines were mined with latent values and presented differences. 86 patients (60.1%) in ureteral calculi group exhibited elevated serum IL-6 (<2 pg/mL was defined as normal), while only 10 (34.5%) patients showed elevated IL-6 in renal calculi group ($p<0.05$, Table 2). Though no significant differences were recorded in comparison between renal calculi and ureteral calculi, IL-8 also rendered a similar tendency as IL-6 (51.7±153.8 pg/mL in ureteral calculi and 26.7±34.9 pg/mL in renal calculi group, $p=0.3858$, Table 2).

Middle ureteral calculi was more easily to elicit ON

As mentioned before and generally accepted, ureteral calculi was more likely to cause HN and kidney injury when compared with renal calculi. Most noteworthy was the middle ureteral calculi. Results from Table 3 indicated that middle ureteral calculi was the biggest culprit of ON, exhibiting the highest sCr (80.8±16.1 μmol/L), BUN (6.6±1.9 mmol/L), HN (24.7±19.9 mm), and the most impaired eGFR (82.5±16.1 mL/min/1.73 m^2, all $p<0.05$).

IL-8 and IL-6 were closely associated with kidney injury

Circulating immune cells were demonstrated no obvious correlations with HN and impaired renal function. However, inflammatory cytokines, referred in particular to serum IL-8 and IL-6, presented underlying values in comprehending ON. The highest serum concentration of IL-8 (147.1±278.7 pg/mL, $p=0.0306$) was found in the subgroup of middle ureteral calculi when compared

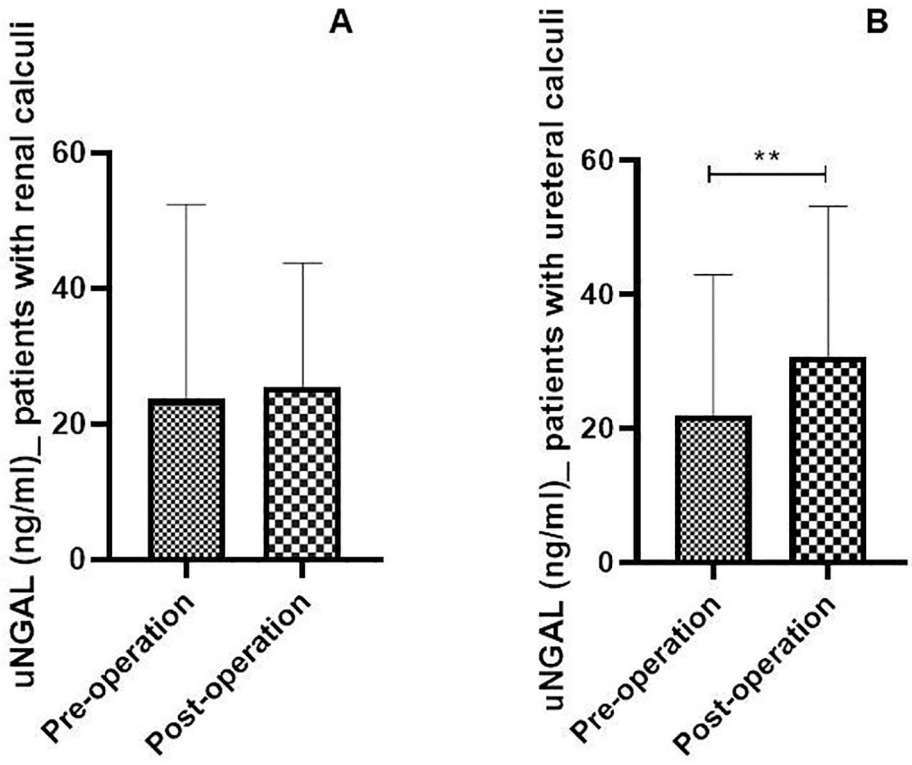

Fig. 2 Variation of uNGAL after URL surgery. 91 patients in ureteral calculi group and 17 in renal calculi group had complete and comparable data of preoperative and postoperative uNGAL. (**A**) Variation of uNGAL in renal calculi group, with the preoperative value of 23.69 ± 28.67 ng/mL and postoperative value of 25.52 ± 18.20 ng/mL, p=0.8197; (**B**) Variation of uNGAL in ureteral calculi group, with the preoperative value of 21.88 ± 21.02 ng/mL and postoperative value of 30.65 ± 22.49 ng/mL, p=0.0051. ** Meant $p < 0.01$, were assumed as statistically significant. *Abbreviations* uNGAL urine neutrophil gelatinase-associated lipocalin, URL ureteroscopic lithotripsy

with others, which was in positive accordance with the degree of HN and impaired renal function. However, no statistical linear correlations were demonstrated (Table 3; Fig. 3A and D).

In addition, as mentioned above, a higher proportion of patients was seen with elevated serum IL-6 level in the group of ureteral calculi, but it seemed that it was negatively correlated with HN and impaired renal function in the subgroup analysis of ureteral calculi based on the detailed lesion of calculi, that was patients in the subgroup of middle ureteral calculi presented the lowest proportion of patients with elevated IL-6 when compared with upper and lower ureteral calculi subgroup (Table 3; Fig. 3E).

Discussion

It has been widely recognized that inflammation plays a pivotal role in the pathophysiological processes of various forms of kidney injury, and kidney injury should be considered to have an immunological component. TGF-β1, TNF-α, MCP-1, IL-10, IL-8, IL-6, et al [14, 15] were the mainly focused inflammatory cytokines. Among all these cytokines, some have been investigated as sensitive biomarkers of kidney injury, for instance IL-6, IL-8 and IL-18, especial for the predicting of contrast-induced nephropathy, renal ischemia/reperfusion injury (RIRI), and kidney injury after cardiac or liver surgery, etc [16–22]. Jeffrey et al. [23]. reported that serum IL-8, IL-6 and urine IL-18, IL-6, IL-8 were higher in acute kidney injury (AKI) patients within the first 24 h following liver transplantation. Han et al. [24]. found AKI patients had significantly elevated serum contents of IL-6, IL-8 and IL-10, relative to the control group in older people (≥65 years) in the intensive care unit. Especial for IL-6 and IL-8, a strong correlation was observed between them with AKI (odds ratio (OR)=36.4 and 18.2, respectively). For each increase in the natural log unit in the levels of IL-6 and IL-8, the risk of developing AKI elevated by 36.4% and 18.2%, respectively [24]. However, whether serum inflammatory cytokines could be utilized for predicting kidney injury in patients with ON was still ambiguous, though Liao et al [4] proved the expression of inflammatory cytokines in blood of UUO model was also an indicator of kidney injury. The results of our study demonstrated that serum IL-6 and IL-8 could also be used as biomarkers of kidney injury in ON, which filled a gap in this field.

Table 2 Landscape of peripheral immunity (preoperative data, $n=172$)

Inflammatory cytokines / immune cells	Total (172)	Patients with ureteral calculi (143)	Patients with renal calculi (29)	*p* value
Mean (SD) IL-8 (pg/mL)	47.5 (141.2)	51.7 (153.8)	26.7 (34.9)	0.3858[†]
IL-1B (< 5 pg/mL as normal)	-	-	-	0.1711[‡]
Elevated (%)	37 (21.5)	28 (19.6)	9 (31.0)	-
Normal (%)	135 (78.5)	115 (80.4)	20 (69.0)	-
IL-6 (< 2 pg/mL as normal)	-	-	-	0.0112[‡*]
Elevated (%)	96 (55.8)	86 (60.1)	10 (34.5)	-
Normal (%)	76 (44.2)	57 (39.9)	19 (65.5)	-
IL-10 (< 9.1 pg/mL as normal)	-	-	-	> 0.9999[‡]
Elevated (%)	0 (0.0)	0 (0.0)	0 (0.0)	-
Normal (%)	172 (100.0)	143 (100.0)	29 (100.0)	-
Mean (SD) TNF-α (pg/mL)	10.6 (10.6)	10.6 (10.9)	10.8 (9.7)	0.9415[†]
Mean (SD) IL-2R (U/mL)	377.3 (117.1)	377.9 (119.0)	374.4 (109.4)	0.8831[†]
Mean (SD) CRP (mg/L)	5.4 (10.6)	5.8 (11.2)	3.1 (6.4)	0.2096[†]
Mean (SD) WBC (*10^9/L)	6.39 (1.77)	6.45 (1.73)	6.09 (1.93)	0.3113[†]
Mean (SD) $CD3^+$T (%)	72.01 (8.00)	71.53 (7.90)	74.38 (8.22)	0.0803[†]
Mean (SD) $CD4^+$ T (%)	43.37 (7.71)	43.13 (7.65)	44.52 (8.03)	0.3767[†]
Mean (SD) $CD8^+$T (%)	24.68 (7.15)	24.36 (7.09)	26.23 (7.39)	0.1989[†]
Mean (SD) NK (%)	13.32 (7.54)	13.60 (7.55)	11.98 (7.48)	0.2951[†]
Mean (SD) B (%)	14.06 (5.24)	14.26 (5.19)	13.06 (5.45)	0.2640[†]
Mean (SD) Treg (%)	5.80 (1.91)	5.87 (1.89)	5.44 (2.00)	0.2799[†]

Values were in mean (SD) or n (%)

Abbreviations CRP c-reactive protein, *IL* interleukin, *NK* natural killer cell, *SD* standard deviation, *TNF-a* tumor necrosis factor-α, *Treg* T regulatory cell, *WBC* white blood cell

[†] Student's t-test, [‡] Chi-square test. [*] meant $p<0.05$ was assumed as statistically significant

IL-6 is a pro-inflammatory cytokine that has been shown to be elevated in both serum and urine in both patients and animal models of AKI [16, 17, 19, 20]. IL-8 is an endothelial-derived chemokine and has also been demonstrated to be elevated in the setting of renal allograft dysfunction [25] and AKI diseases [18, 21, 22]. As to the specific mechanisms of how serum IL-6 and IL-8 mediated kidney injury, literature reports vary. Broadly speaking, systemic inflammation affects the kidney via pro-inflammatory cytokines and infiltration of inflammatory cells [26]. For IL-6, IL-6/ERK signaling pathway [5], IL-6/STAT3 signaling pathway [27], and IL-6/IL-6R axis [28], et al. participate in the pathological process of kidney injury and subsequent renal fibrosis. For IL-8, the underlying mechanism is that it acts as chemo-attractive action toward neutrophils by binding to IL-8 receptor and stimulating their response [29]. However, the specific mechanisms of how they involved in the pathophysiological processes of kidney injury in ON/UUO still needed to be verified and investigated by both animal and clinical researches.

NGAL is a 25-kDa protein, usually reabsorbed at the site of proximal tubule and secreted in the thick ascending limb. The increased levels in urine/serum can be detected, reflecting renal tubular injury and utilized as a potential biomarker of several pathologic conditions (e.g., AKI, diabetic nephropathy, IgA nephropathy, contrast nephropathy and obstructive hydronephrosis (UPJO)) [30–35]. Suchiang et al. [35] demonstrated that children with obstructive HN in whom surgery opted had higher values of uNGAL when compared with patients with non-obstructive HN who were treated conservatively (18.62±13.75 ng/mg Cr vs. 10.92±5.63 ng/mg Cr, $p<0.001$), and after the obstruction was relieved, the value presented a mild decline. But the results of our study draw a distinct-different conclusion. The possible cause was that we did not normalize uNGAL concentration to contemporaneous urine creatinine (uCr) concentration because urinary creatinine excretion in the setting of AKI is dynamic and its inclusion in the assessment of urinary biomarkers has been challenged [36]. Normalized levels of a biomarker reflecting tubular injury can be influenced by dynamic variations in uCr excretion rate when GFR changes. Actual timed urine collections from hospitalized patients with changing GFR and/or critical illness exhibited variability in uCr excretion rates across and within individuals [36]. Thus, whether uNGAL could be used as a biomarker for predicting renal insufficiency in UUO/ON still needs to be verified.

In addition to the inflammatory factors mentioned above, immune cells and their various subtypes also play significant roles in the physiological and pathological processes of renal injury. For instance, immune cells such as neutrophils, lymphocytes, and monocytes are involved in the inflammatory response following renal injury, contributing to the damage and repair processes of the

Table 3 Subgroup analysis according to the anatomic lesion of calculi (preoperative data, $n = 172$)

Parameters	Calices (17)	Pelvis (12)	Upper ureter (93)	Middle ureter (18)	Lower ureter (32)	*p* value
Mean (SD) HN (mm)	1.8 (3.0)	8.5 (6.0)	17.0 (11.1)[a]	24.7 (19.9)[ab]	12.5 (10.9)[ad]	< 0.0001[†***]
Mean (SD) sCr (μmol/L)	74.9 (17.5)	65.8 (17.5)	68.8 (14.7)	80.8 (16.1)[c]	67.9 (17.2)[d]	0.0208[†*]
Mean (SD) BUN (mmol/L)	6.4 (1.0)	6.2 (2.6)	5.4 (1.1)	6.6 (1.9)[c]	6.1 (1.6)	0.0033[†**]
Mean (SD) eGFR (mL/min/1.75m^2)	88.7 (17.1)	103.5 (30.3)	100.9 (19.8)	82.5 (16.1)[c]	97.3 (24.6)	0.0048[†**]
Mean (SD) uNGAL (ng/mL)	42.2 (58.6)	21.8 (17.9)	21.4 (21.1)	25.8 (22.2)	21.5 (20.0)	0.0627[†]
Mean (SD) CD3$^+$T (%)	72.76 (8.26)	76.68 (7.92)	71.53 (7.70)	71.96 (9.08)	71.30 (8.05)	0.3074[†]
Mean (SD) CD4$^+$T (%)	42.11 (8.06)	47.94 (6.93)	42.77 (7.77)	44.65 (7.46)	43.31 (7.54)	0.2222[†]
Mean (SD) CD8$^+$T (%)	26.83 (8.76)	25.39 (5.11)	24.90 (6.79)	22.72 (9.14)	23.72 (6.69)	0.4562[†]
Mean (SD) NK (%)	13.53 (8.78)	9.78 (4.61)	13.11 (6.67)	15.75 (10.67)	13.79 (7.96)	0.3209[†]
Mean (SD) B (%)	13.08 (6.10)	13.04 (4.63)	14.75 (5.00)	11.70 (5.48)	14.25 (5.32)	0.1748[†]
Mean (SD) Treg (%)	6.14 (2.11)	4.47 (1.39)	5.90 (2.07)	5.74 (1.80)	5.85 (1.35)	0.1542[†]
Mean (SD) IL-8 (pg/mL)	29.7 (41.5)	22.3 (23.8)	33.6 (96.4)	147.1 (278.7)[c]	50.5 (178.7)	0.0306[†*]
IL-1B (< 5 pg/mL as normal)	-	-	-	-	-	0.7306[‡]
Elevated (%)	5 (29.4)	4 (33.3)	18 (19.4)	4 (22.2)	6 (18.8)	-
Normal (%)	12 (70.6)	8 (66.7)	75 (80.6)	14 (77.8)	26 (81.2)	-
IL-6 (< 2 pg/mL as normal)	-	-	-	-	-[b]	0.0425[‡*]
Elevated (%)	7 (41.2)	3 (25.0)	54 (58.1)	9 (50.0)	23 (71.9)	-
Normal (%)	10 (58.8)	9 (75.0)	39 (41.9)	9 (50.0)	9 (28.2)	-
IL-10 (< 9.1 pg/mL as normal)	-	-	-	-	-	> 0.9999[‡]
Elevated	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Normal	17 (100)	12 (100)	93 (100)	18 (100)	32 (100)	
Mean (SD) TNF-α (pg/mL)	12.8 (12.2)	7.9 (3.1)	11.0 (12.7)	9.8 (4.7)	10.0 (7.0)	0.7823[†]
Mean (SD) IL-2R (U/mL)	385.7 (123.1)	358.3 (89.0)	367.8 (118.7)	442.9 (100.4)	370.6 (121.2)	0.1481[†]

Values were in mean (SD) or n (%)

Abbreviations BUN blood urea nitrogen, *eGFR* estimated glomerular filtration rate, *HN* hydronephrosis, *IL* interleukin, *NK* natural killer cell, *sCr* serum creatinine, *SD* standard deviation, *TNF-α* tumor necrosis factor-α, *Treg* T regulatory cell, *uNGAL* urine neutrophil gelatinase-associated lipocalin

[†] ANOVA, [‡] Chi-square test. [***] meant $p < 0.001$, [**] meant $p < 0.01$, [*] meant $p < 0.05$, were assumed as statistically significant

[a, b, c, d] statistical significance comparing to group of calices, pelvis, upper ureter and middle ureter, respectively

kidney through the release of inflammatory mediators and cytokines [37, 38]. In models of ON and UUO, the infiltration of immune cells has been observed and is correlated with the severity of renal fibrosis and inflammatory responses [39]. Although no significant differences in the number of circulating immune cells were observed among the groups of stone patients in our study, this does not exclude the role of immune cells within the local renal tissue. Local immune responses may differ from those in the circulation due to the influence of the local microenvironment, such as ischemia, oxidative stress, and concentrations of inflammatory mediators [40, 41]. Therefore, to gain a more comprehensive understanding of the role of immune cells in renal injury, future research may need to focus on the infiltration and activation status of immune cells within local renal tissues, as well as their relationship with the levels of biomarkers in urine/serum. Additionally, investigating the specific roles and interactions of immune cell subtypes (such as T cells, B cells, natural killer cells, etc.) in renal injury may help elucidate the complex mechanisms of renal damage and provide clues for the development of new therapeutic strategies.

A potential limitation of this study is that though it was a prospective study, it conducted in a single institution that might undergo selection bias. Secondly, the overall sample size of patients with renal calculi was small, leading to a small pool of patients for analysis. This blemish might diminish our statistical power and preclude a more robust calculation of our biomarker's predictive abilities. Thirdly, the immune items that detected in this study were not comprehensive, contributing to the relatively influenced potency of our conclusions. It is important to note that the markers used in this study for peripheral immunity assessment were all derived from serum. In some cases, inflammation may remain localized without becoming a systemic response. Therefore, urinary markers might more accurately reflect local kidney inflammation. This represents a limitation of our study, and future research should consider including the evaluation of urinary markers. Fourthly, it was widely recognized UUO won't significantly influence total renal function. However, we didn't assess the split renal function (SRF), which would also diminish our statistical power and reduce the comparability with other studies on the associations of inflammation and AKI. Finally, as reported, serum IL-6

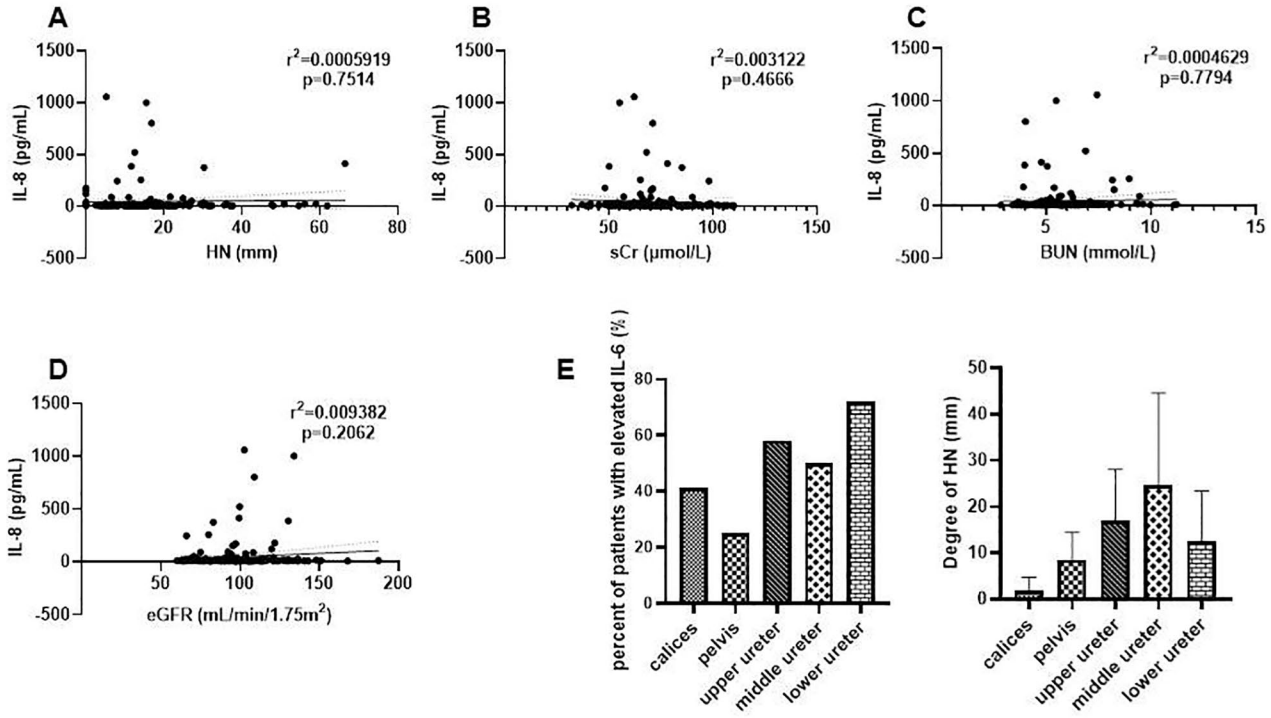

Fig. 3 Correlation of IL-8/IL-6 and renal function. (**A-D**) Pearson correlation analysis was applied to analyze the interdependency between IL-8 and indicators of renal function, i.e., HN (A), sCr (B), BUN (C), and eGFR (D). No statistical linear correlations were demonstrated. (**E**) Descriptive presentation of the percent of patients with elevated IL-6, and the degree of HN in each subgroup. Abbreviations BUN blood urea nitrogen, eGFR estimated glomerular filtration rate, HN hydronephrosis, IL-6 interleukin-6, IL-8 interleukin-8, sCr serum creatinine

and IL-8 are sensitive biomarkers of kidney injury, which rise quickly in response to AKI may have already peaked and began normalizing by the time we assessed them. For the disease course of urolithiasis in our study varied from 7 h to 1800d, the degree of elevation may underestimate the peak value and thereby diminish our diagnostic power. Consequently, further multi-centric, larger-scale and better-designed clinical studies as well as animal studies to investigate the mechanisms will be expectant.

Conclusion

In conclusion, our study demonstrated that circulating inflammatory cytokines, referred in particular to serum IL-8 and IL-6 were partially associated with kidney injury in patients with upper urinary tract urolithiasis, and might serve as biomarkers for kidney injury in patients with ON, providing a potential avenue for early diagnosis and treatment. However, more extensive researches are needed to fully understand the underlying mechanisms and to validate these findings in larger and more diverse patient populations.

Acknowledgements
The authors thank the colleagues in the department of clinical laboratory in Xinhua Hospital for their work and contributions.

Author contributions
SB Qian: study design, data collection and management, data analysis, manuscript writing and editing. YD Pan: study design, data collection, data analysis, manuscript editing. Q Li: study design, data collection, data analysis, manuscript editing. LY Zhang: study design, data collection. LJ Duan: study design, manuscript editing. Y Xu: study design, data collection. JW Cao: data collection, manuscript editing. XG Cui: study design, data management, data analysis, manuscript editing, supervision. YT Huang: study design, data management, data analysis, manuscript editing, supervision.

Funding
This work was supported by the National Natural Science Foundation of China (No. 82000638). The sponsor played no role in the study design, methods, data collection, analysis, and preparation of this manuscript.

Data availability
The data that support the findings of this study are available from the corresponding author, Yunteng Huang, upon reasonable request.

Declarations

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
All procedures performed in this study were in accordance with the ethical standards of the Ethics Committee of Xinhua Hospital and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. This study was approved by Ethics Committee of Xinhua Hospital.

Received: 26 February 2024 / Accepted: 29 July 2024
Published online: 08 August 2024

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