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Urinary NGAL-to-creatinine ratio is associated with urolithiasis in children: a cross-sectional multiparameter study

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Urinary NGAL-to-creatinine ratio is associated with urolithiasis in children: a cross-sectional multiparameter study

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Abstract

Background: Pediatric urolithiasis involves urinary supersaturation, crystal formation, and potentially crystal–tubular epithelial interactions. However, the relevance of urinary biomarkers reflecting tubular stress and injury in affected children remains incompletely characterized. This study aimed to evaluate a panel of urinary biomarkers in children with urolithiasis and healthy controls.

Methods: This cross-sectional observational study compared urinary biomarkers in children with a documented history of ultrasonographically confirmed urolithiasis and healthy controls. The study included 113 children, comprising 72 patients with urolithiasis and 41 healthy controls. Urinary concentrations of 12 biomarkers were measured using the Bio-Plex Pro RBM Human Kidney Toxicity Panels 1 and 2. Urinary biomarker-to-creatinine ratios were analyzed to account for variation in urine concentration. Between-group comparisons were performed using the Mann–Whitney U test with Bonferroni correction. Logistic regression analyses were performed using log2-transformed biomarker-to-creatinine ratios.

Results: Among the 12 creatinine-normalized biomarkers, NGAL/Cr was the only marker that remained significantly higher in children with urolithiasis after correction for multiple comparisons (median 0.48 [0.22–1.34] vs 0.16 [0.06–0.40] ng/mg Cr; adjusted $p < 0.001$). In univariate logistic regression, each twofold increase in NGAL/Cr was associated with higher odds of urolithiasis (OR 1.28, 95% CI 1.09–1.50; $p = 0.002$; adjusted $p = 0.024$). In the final multivariable model, NGAL/Cr remained independently associated with urolithiasis (OR 1.35, 95% CI 1.13–1.60; $p = 0.001$), together with age. The final model yielded an AUC of 0.80 (95% CI 0.72–0.88).

Conclusions: In this cross-sectional study, urinary NGAL/Cr was the biomarker most consistently associated with urolithiasis. This association does not establish whether NGAL reflects a causal mechanism, stone burden, obstruction, subclinical tubular injury, or related clinical characteristics. External and prospective validation in age- and sex-matched cohorts is needed before clinical use can be considered.

Keywords: urolithiasis; NGAL; urinary biomarkers; children; renal tubular biomarkers; pediatric nephrology

Introduction

Pediatric urolithiasis is an increasingly important clinical problem characterized by a multifactorial pathogenesis and a substantial risk of recurrence [1]. Stone formation in children may be associated with metabolic abnormalities, genetic susceptibility, urinary tract anomalies, infections, dietary factors, and insufficient fluid intake [2, 3]. Although urinary supersaturation and crystallization remain central mechanisms of stone formation, they do not fully explain why crystals are retained within the kidney or why urolithiasis develops only in a subset of predisposed individuals [4].

Increasing attention has therefore focused on interactions between urinary crystals and the renal tubular epithelium. Experimental observations suggest that crystal exposure may induce epithelial stress, inflammatory signaling, and tubular injury, whereas alterations of the tubular surface may facilitate crystal adhesion and retention [5]. This bidirectional interaction provides a biological rationale for investigating urinary proteins associated with tubular dysfunction, epithelial injury, and inflammation in children with urolithiasis [6].

Several urinary biomarkers have been investigated in pediatric urolithiasis, including neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), interleukin-18 (IL-18), cystatin C, osteopontin, and other proteins involved in tubular function or inflammatory responses. However, the available evidence remains limited and inconsistent. Some studies have reported higher urinary NGAL concentrations or NGAL-to-creatinine ratios in children with urolithiasis, whereas others found no significant overall differences in NGAL, KIM-1, or IL-18 between stone formers and healthy controls, apart from selected subgroups, such as children with

hydronephrosis. Urinary cystatin C has also been reported to be increased in children with urolithiasis in a small prospective study [7–9].

The variability in previous findings may be related to small sample sizes, differences in patient characteristics and clinical context, variation in biomarker selection, and differences in analytical methodology. Most pediatric studies have evaluated only a limited number of biomarkers simultaneously. A multiparameter approach may allow a broader assessment of proteins reflecting different aspects of tubular stress, inflammation, and renal protein handling within the same population.

This study aimed to evaluate a panel of 12 urinary biomarkers in children with ultrasonographically confirmed urolithiasis and healthy controls and to identify the biomarkers most strongly associated with urolithiasis.

Materials and methods

Study design and participants

This cross-sectional observational study included 113 children, comprising 72 patients with previously ultrasonographically confirmed urolithiasis and 41 healthy controls. Participants were recruited between March 2023 and April 2026 at the Department of Pediatrics and Nephrology, Medical University of Bialystok, Poland. Children were included in the urolithiasis group based on a documented history of at least one urinary stone confirmed by ultrasonography. At the time of biomarker assessment, some children no longer had a detectable urinary stone. In children with a history of renal colic, urine samples for biomarker assessment were collected at least one month after the acute episode. At the time of sampling, participants were clinically stable and had no acute symptoms related to stone disease.

For children in the urolithiasis group, available clinical and imaging characteristics included the number of urinary stones, maximum stone diameter, stone location, the presence and grade of hydronephrosis, history and number of renal colic episodes, recurrent urinary tract infections, stone composition when available, and family history of urolithiasis. Previous spontaneous stone passage or urological intervention was recorded when documented. Acute urinary tract infection and active systemic inflammation were exclusion criteria. Routine urinalysis findings, including microscopic hematuria, leukocyturia, bacteriuria, proteinuria, and crystalluria, were recorded but did not constitute exclusion criteria in the absence of clinical evidence of acute infection. Concomitant medications potentially affecting urinary composition were recorded from the medical records and were not used as exclusion criteria.

The control group consisted of 41 children considered clinically healthy, recruited as a convenience sample from children attending the hospital for non-acute reasons as well as from healthy children of hospital staff. Urine samples were collected for the purposes of the study. Eligibility required no known history of urolithiasis or kidney disease and no clinical evidence of acute urinary tract infection or active systemic inflammation at the time of sampling. Routine ultrasonographic screening of the urinary tract was not performed in the control group.

Ethics approval

The study was approved by the Bioethics Committee of the Medical University of Bialystok (approval no. APK.002.169.2023). Written informed consent for urine sample collection and analysis within the study was obtained from the parents of all participants.

Clinical assessment

Clinical data were obtained from medical records and included age, sex, anthropometric parameters, and selected laboratory variables. In children with urolithiasis, ultrasonographic findings were reviewed to confirm the diagnosis. The primary comparison was performed between children with imaging-confirmed urolithiasis and healthy controls.

Urine sample collection and laboratory measurements

Midstream urine samples were collected for routine laboratory assessment and biomarker measurement. Urinary creatinine concentration was measured using a Cobas 6000/C501 analyzer (Roche Diagnostics Corporation, Indianapolis, IN, USA) with a kinetic Jaffé method without deproteinization. Biomarker concentrations were normalized to urinary creatinine and expressed as biomarker-to-creatinine ratios.

Urine sample processing and biomarker measurements

Urine samples were stored at 4°C for no longer than 2 h after collection and were subsequently centrifuged for 10 min at 1000 rpm. The supernatant was stored at 4°C and then frozen at −80°C for no longer than 4 months. Samples were thawed immediately before analysis and were not subjected to repeated freeze–thaw cycles. Urinary concentrations of 12 biomarkers were measured using the Bio-Plex Pro RBM Human Kidney Toxicity Panels 1 and 2 (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer’s instructions. Measurements were performed using a Bio-Plex 200 system at the Department of Hygiene, Epidemiology and Ergonomics, Medical University of Bialystok, Bialystok, Poland. Prior to analysis, the Bio-Plex 200 system was validated and calibrated using the Bio-Plex Validation Kit 4.0 and Bio-Plex Calibration Kit (Bio-Rad Laboratories).

The analyzed biomarkers included calbindin, clusterin, glutathione S-transferase pi (GST-π), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), monocyte chemoattractant protein-1 (MCP-1), albumin, β2-microglobulin, cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), osteopontin, and trefoil factor 3 (TFF3).

Biomarker measurements were performed in two analytical batches. The first batch, analyzed in March 2025, included 34 children with urolithiasis and 20 controls. The second batch, analyzed in April 2026, included the remaining 38 children with urolithiasis and 21 controls. Thus, samples from both study groups were represented in each analytical batch.

Handling of out-of-range measurements

Results reported by the Bio-Plex Manager software as out of range below (OOR<) or out of range above (OOR>) were replaced with predefined analyte-specific numerical substitution values before statistical analysis to allow their inclusion in quantitative analyses. The exact substitution values applied to each biomarker, together with the number and proportion of OOR< and OOR> observations overall and separately in the urolithiasis and control groups, are provided in Supplementary Table S1. These values correspond to those used in the final dataset subjected to statistical analysis. No formal sensitivity analysis using alternative OOR substitution approaches was performed.

Statistical analysis

Statistical analyses were performed using Stata version 18.0 (StataCorp, College Station, TX, USA). The distribution of continuous variables was assessed using the Shapiro–Wilk test. As continuous variables did not follow a normal distribution, they are presented as median and interquartile range (Q1; Q3). Comparisons between

children with urolithiasis and controls were performed using the Mann–Whitney U test.

Creatinine-normalized urinary biomarker values were used in the principal analyses. Because biomarker-to-creatinine ratios showed right-skewed distributions, they were log2-transformed before logistic regression analyses. Accordingly, odds ratios derived from these models represent the change in the odds of urolithiasis associated with a twofold increase in the respective biomarker-to-creatinine ratio.

Univariate logistic regression analyses were performed with urolithiasis status as the dependent variable. To account for multiple testing, Bonferroni correction was applied separately to the 12 between-group comparisons of creatinine-normalized biomarkers and to the 12 univariate logistic regression analyses. Thus, within each family of analyses, p-values were adjusted simultaneously across all 12 biomarkers. Biomarkers that remained statistically significant after Bonferroni correction in univariate logistic regression were considered for inclusion in the multivariable model. Age and sex were additionally entered as potential confounders. Backward stepwise logistic regression was subsequently applied, with non-significant variables sequentially removed.

Model fit was assessed using the Hosmer–Lemeshow goodness-of-fit test. The discriminative ability of the final model was evaluated using receiver operating characteristic analysis and the area under the curve with a 95% confidence interval. Analyses were performed on a complete-case basis for the respective variables. A two-sided p-value < 0.05 was considered statistically significant.

Results

Study population and urinary biomarkers

The study included 113 children, of whom 72 had previously ultrasonographically confirmed urolithiasis and 41 were healthy controls. Children with urolithiasis were older than controls, with a median age of 13.5 [7.0–16.0] years compared with 7.0 [6.0–11.0] years ($p < 0.001$). Median urinary creatinine concentration was lower in children with urolithiasis than in controls (59.61 [36.97–104.57] vs 123.0 [75.4–162.0] mg/dL; $p < 0.001$). At the time of study assessment, a proportion of children with a history of urolithiasis no longer had a detectable urinary stone, whereas others had persistent stone disease of variable number and size. Detailed clinical, laboratory, and stone-related characteristics are presented in **Table 1**.

Table 1. Clinical, laboratory, and stone-related characteristics of children with urolithiasis and healthy controls.

Characteristic	Children with urolithiasis (n = 72)	Healthy controls (n = 41)	p-value
General characteristics			
Age, years	13.5 [7.0–16.0]	7.0 [6.0–11.0]	<0.001
Female sex, n (%)	38 (52.8)	21 (51.2)	1.000
Urinary creatinine, mg/dL	59.61 [36.97–104.57]	123.0 [75.4–162.0]	<0.001
Additional clinical and laboratory characteristics			
Height, cm	158.5 [131.1–168.5]	—	—
Weight, kg	48.3 [27.9–61.8]	—	—
BMI, kg/m²	18.9 [16.0–22.9]	—	—
Serum creatinine, mg/dL	0.59 [0.44–0.74]	—	—
eGFR, mL/min/1.73 m²	109.8 [92.8–125.9]	—	—
CRP, mg/L	0.60 [0.60–0.75]	—	—
Microscopic hematuria, n (%)	5 (6.9)	—	—
Leukocyturia, n (%)	16 (22.2)	—	—
Bacteriuria, n (%)	21 (29.2)	—	—
Trace proteinuria, n (%)	8 (11.1)	—	—
Crystalluria, n (%)	4 (5.6)	—	—
Positive family history of urolithiasis, n/N (%)	45/71 (63.4)	—	—
Stone-related characteristics			
No detectable stone at study assessment, n (%)	30 (41.7)	—	—
One detectable stone, n (%)	20 (27.8)	—	—
2–5 detectable stones, n (%)	21 (29.2)	—	—
≥6 detectable stones, n (%)	1 (1.4)	—	—
Maximum stone diameter, mm*	4.0 [3.0–6.0]	—	—

Characteristic	Children with urolithiasis (n = 72)	Healthy controls (n = 41)	p-value
Renal location among children with a detectable stone, n/N (%)	41/42 (97.6)	—	—
Ureteral location among children with a detectable stone, n/N (%)	1/42 (2.4)	—	—
Hydronephrosis, n/N (%)	13/70 (18.6)	—	—
Hydronephrosis grade 1, n/N (%)	12/13 (92.3)	—	—
Hydronephrosis grade 2, n/N (%)	1/13 (7.7)	—	—
Previous renal colic, n/N (%)	42/70 (60.0)	—	—
One renal colic episode, n/N (%)	32/70 (45.7)	—	—
≥2 renal colic episodes, n/N (%)	10/70 (14.3)	—	—
Recurrent urinary tract infection, n/N (%)	4/71 (5.6)	—	—
Stone composition available, n (%)	16 (22.2)	—	—
Predominant weddellite composition, n/N (%)	8/16 (50.0)	—	—
Predominant calcium phosphate composition, n/N (%)	4/16 (25.0)	—	—
Predominant struvite composition, n/N (%)	3/16 (18.8)	—	—
Predominant whewellite composition, n/N (%)	1/16 (6.3)	—	—

Data are presented as median [Q1–Q3] or n (%), unless otherwise indicated. Between-group comparisons were performed using the Mann–Whitney U test for continuous variables and Fisher’s exact test for sex. Variables not systematically available in healthy controls are indicated by an em dash. Maximum stone diameter was available for 42 children with a detectable stone at the time of assessment. Stone composition was available for 16 children; predominant composition was defined as the component with the highest reported percentage. BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

Both unadjusted urinary biomarker concentrations and creatinine-normalized biomarker-to-creatinine ratios are presented in **Table 2**.

Among the 12 creatinine-normalized urinary biomarkers, NGAL/Cr was the only biomarker that remained significantly different between children with urolithiasis and controls after Bonferroni correction. Median NGAL/Cr was 0.48 [0.22–1.34] ng/mg Cr in children with urolithiasis and 0.16 [0.06–0.40] ng/mg Cr in controls (unadjusted $p < 0.001$; Bonferroni-adjusted $p < 0.001$).

Calbindin/Cr, GST-π/Cr, and IL-18/Cr showed nominal between-group differences before correction for multiple comparisons ($p = 0.040$, $p = 0.042$, and $p = 0.046$, respectively); however, none remained statistically significant after Bonferroni correction. No statistically significant differences were observed for clusterin/Cr, KIM-1/Cr, MCP-1/Cr, albumin/Cr, β2-microglobulin/Cr, cystatin C/Cr, osteopontin/Cr, or TFF3/Cr. Detailed results for unadjusted biomarker concentrations are presented in Table 2, and creatinine-normalized biomarker-to-creatinine ratios are presented in **Table 3.**

Table 2. Unadjusted urinary biomarker concentrations in children with urolithiasis and healthy controls.

Biomarker	Children with urolithiasis (n = 72) Median [Q1–Q3], ng/mL	Healthy controls (n = 41) Median [Q1–Q3], ng/mL
Calbindin	28.03 [15.78–53.81]	36.07 [16.56–65.21]
Clusterin	15.45 [1.57–35.66]	20.84 [1.57–90.08]
GST-π	0.280 [0.235–1.648]	0.235 [0.235–3.230]
IL-18	0.005 [0.005–0.005]	0.005 [0.005–0.005]
KIM-1	0.220 [0.125–0.530]	0.440 [0.180–0.890]
MCP-1	0.145 [0.078–0.330]	0.450 [0.210–0.710]
Albumin	12,425.85 [4,551.05–35,402.88]	12,505.19 [3,843.86–32,534.25]
β2-microglobulin	50.67 [17.51–109.00]	86.68 [29.24–203.94]
Cystatin C	19.42 [10.10–64.63]	54.96 [12.59–136.16]
NGAL	25.33 [15.62–72.39]	17.72 [6.94–36.99]
Osteopontin	258.50 [136.12–722.52]	737.29 [409.88–1,428.53]
TFF3	575.97 [238.34–1,184.06]	969.66 [577.92–1,698.86]

Data are presented as median [Q1–Q3]. Unadjusted biomarker concentrations correspond to values in the final analytical dataset after handling of out-of-range measurements as described in the Methods. GST-π, glutathione S-transferase pi; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemoattractant protein-1; NGAL, neutrophil gelatinase-associated lipocalin; TFF3, trefoil factor 3.

Table 3. Comparison of urinary biomarker-to-creatinine ratios between children with urolithiasis and healthy controls.

Biomarker-to-creatinine ratio	Children with urolithiasis (n = 72) Median [Q1–Q3]	Healthy controls (n = 41) Median [Q1–Q3]	Unadjusted p-value	Bonferroni-adjusted p-value
Urinary calbindin/Cr, ng/mg Cr	0.49 [0.20–0.96]	0.29 [0.17–0.57]	0.040	0.483
Urinary clusterin/Cr, ng/mg Cr	0.20 [0.03–0.76]	0.19 [0.02–1.03]	0.903	1.000
Urinary GST-π/Cr, ng/mg Cr	0.009 [0.004–0.030]	0.003 [0.002–0.076]	0.042	0.512
Urinary IL-18/Cr, ng/mg Cr	0.0001 [0.00005–0.0002]	0.00005 [0.00003–0.0002]	0.046	0.561
Urinary KIM-1/Cr, ng/mg Cr	0.005 [0.002–0.009]	0.005 [0.001–0.008]	0.954	1.000
Urinary MCP-1/Cr, ng/mg Cr	0.002 [0.001–0.007]	0.004 [0.002–0.006]	0.081	0.968
Urinary albumin/Cr, ng/mg Cr	195.39 [59.79–672.08]	163.52 [26.18–274.76]	0.055	0.668
Urinary β2-microglobulin/Cr, ng/mg Cr	1.07 [0.24–2.20]	0.96 [0.21–2.36]	0.842	1.000
Urinary cystatin C/Cr, ng/mg Cr	0.45 [0.14–1.17]	0.47 [0.09–1.24]	0.769	1.000
Urinary NGAL/Cr, ng/mg Cr	**0.48 [0.22–1.34]**	**0.16 [0.06–0.40]**	**<0.001**	**<0.001**
Urinary osteopontin/Cr, ng/mg Cr	5.10 [1.94–11.01]	6.41 [4.10–10.09]	0.249	1.000
Urinary TFF3/Cr, ng/mg Cr	9.51 [5.16–19.99]	7.82 [5.54–13.77]	0.492	1.000

Data are presented as median [Q1–Q3]. Between-group comparisons were performed using the Mann–Whitney U test. Bonferroni adjustment was applied across the 12 creatinine-normalized biomarker comparisons. Adjusted p-values are capped at 1.000. Cr, creatinine; GST-π, glutathione S-transferase pi; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemoattractant protein-1; NGAL, neutrophil gelatinase-associated lipocalin; TFF3, trefoil factor 3.

Univariate logistic regression

In univariate logistic regression analyses, creatinine-normalized biomarker values were log2-transformed, and odds ratios therefore represent the change in the odds of urolithiasis associated with a twofold increase in the respective biomarker-to-creatinine ratio. NGAL/Cr was significantly associated with urolithiasis (OR 1.28, 95% CI 1.09–1.50; p = 0.002), and this association remained significant after Bonferroni correction (adjusted p = 0.024). In univariate logistic regression, each twofold increase in NGAL/Cr was associated with 28% higher odds of belonging to the group with ultrasonographically confirmed urolithiasis.

Although calbindin/Cr was nominally associated with urolithiasis in the unadjusted analysis (OR 1.28, 95% CI 1.03–1.60; p = 0.026), this association did not remain

statistically significant after Bonferroni correction. None of the remaining biomarkers showed a statistically significant association after correction for multiple comparisons. Detailed results are presented in **Table 4**.

Table 4. Univariate logistic regression analyses of urinary biomarker-to-creatinine ratios associated with urolithiasis.

Variable	N	Odds ratio	95% CI	P value	Bonferroni-adjusted P value
Urinary calbindin/Cr, per twofold increase	113	1.28	1.03–1.60	0.026	0.312
Urinary clusterin/Cr, per twofold increase	113	1.04	0.92–1.18	0.511	1.000
Urinary GST-π/Cr, per twofold increase	113	1.06	0.93–1.20	0.400	1.000
Urinary IL-18/Cr, per twofold increase	113	1.19	0.93–1.53	0.167	1.000
Urinary KIM-1/Cr, per twofold increase	113	1.02	0.82–1.27	0.854	1.000
Urinary MCP-1/Cr, per twofold increase	113	0.82	0.66–1.02	0.079	0.948
Urinary albumin/Cr, per twofold increase	113	1.03	0.94–1.13	0.550	1.000
Urinary β2-microglobulin/Cr, per twofold increase	113	1.02	0.91–1.13	0.772	1.000
Urinary cystatin C/Cr, per twofold increase	113	1.04	0.93–1.16	0.493	1.000
Urinary NGAL/Cr, per twofold increase	**113**	**1.28**	**1.09–1.50**	**0.002**	**0.024**
Urinary osteopontin/Cr, per twofold increase	113	0.88	0.76–1.03	0.103	1.000
Urinary TFF3/Cr, per twofold increase	113	1.07	0.85–1.35	0.548	1.000

Odds ratios are reported per twofold increase in the respective urinary biomarker-to-creatinine ratio because biomarker ratios were log2-transformed before analysis. Bonferroni adjustment was applied across the 12 univariable biomarker models. Adjusted P values were calculated as min(12 × unadjusted P value, 1.000). Cr, creatinine; CI, confidence interval; GST-π, glutathione S-transferase pi; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemoattractant protein-1; NGAL, neutrophil gelatinase-associated lipocalin; TFF3, trefoil factor 3.

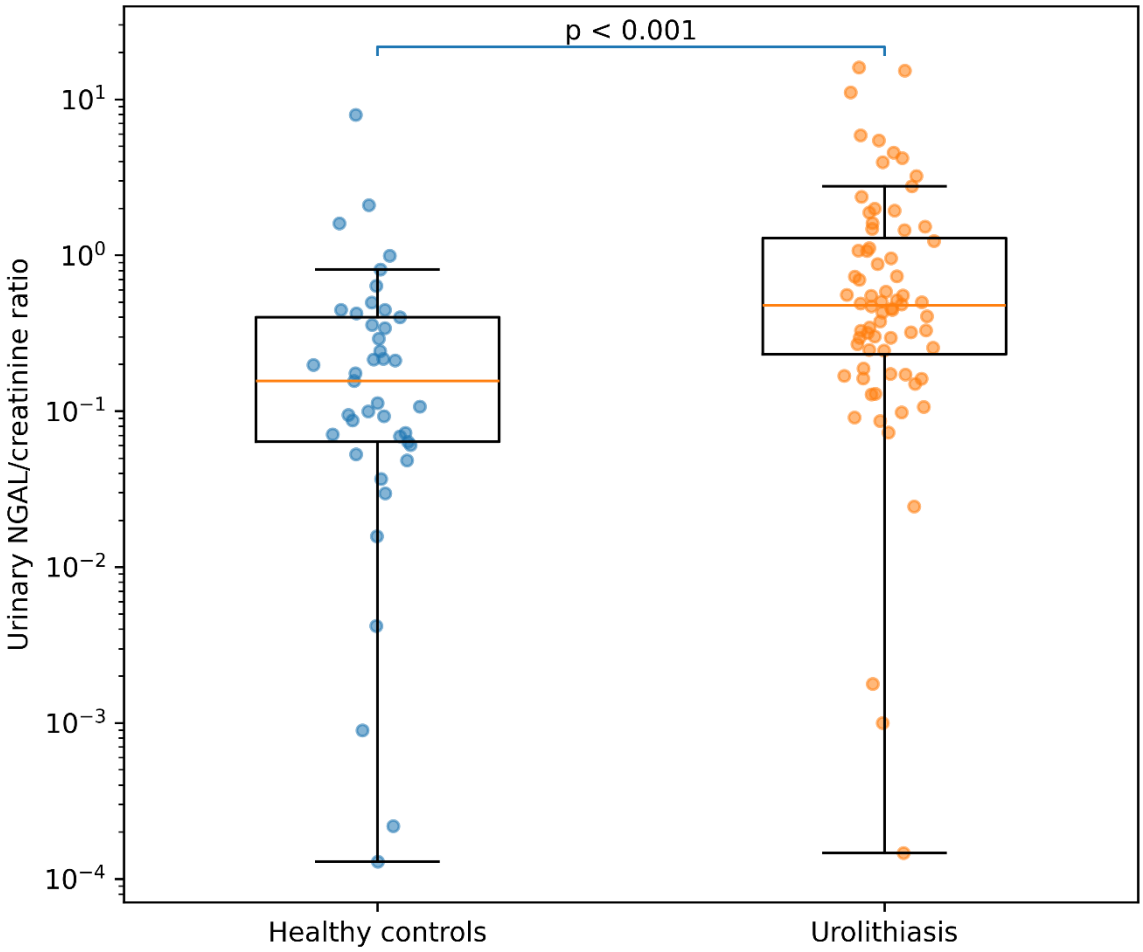


Fig. 1 Distribution of urinary NGAL-to-creatinine ratios in children with urolithiasis (n = 72) and healthy controls (n = 41). Boxplots show the median and interquartile range, and individual observations are displayed. The y-axis is shown on a logarithmic scale and displays NGAL/Cr in ng/mg creatinine.

Multivariable logistic regression

NGAL/Cr was included in the multivariable analysis together with age and sex as potential confounders. Following backward stepwise selection, NGAL/Cr and age were retained in the final model, whereas sex was not retained.

In the final model, higher NGAL/Cr remained independently associated with urolithiasis (OR 1.35, 95% CI 1.13–1.60; p = 0.001). Each twofold increase in NGAL/Cr was associated with approximately 35% higher odds of belonging to the urolithiasis group. Age was also independently associated with group membership (OR 1.22 per year, 95% CI 1.10–1.35; p < 0.001).

Table 5. Multivariable logistic regression model of variables associated with urolithiasis.

Variable	N	Odds ratio	95% CI	P value
Urinary NGAL/Cr, per twofold increase	**113**	**1.35**	**1.13–1.60**	**0.001**
Age, per 1-year increase	113	1.22	1.10–1.35	<0.001

Discriminative performance of the final model

The Hosmer–Lemeshow goodness-of-fit test showed no evidence of poor model fit (p = 0.743). The final model incorporating NGAL/Cr and age yielded an area under the receiver operating characteristic curve of 0.80 (95% CI 0.72–0.88), indicating good discrimination between children with urolithiasis and controls **(Fig. 2)**. This estimate

has not been internally or externally validated.

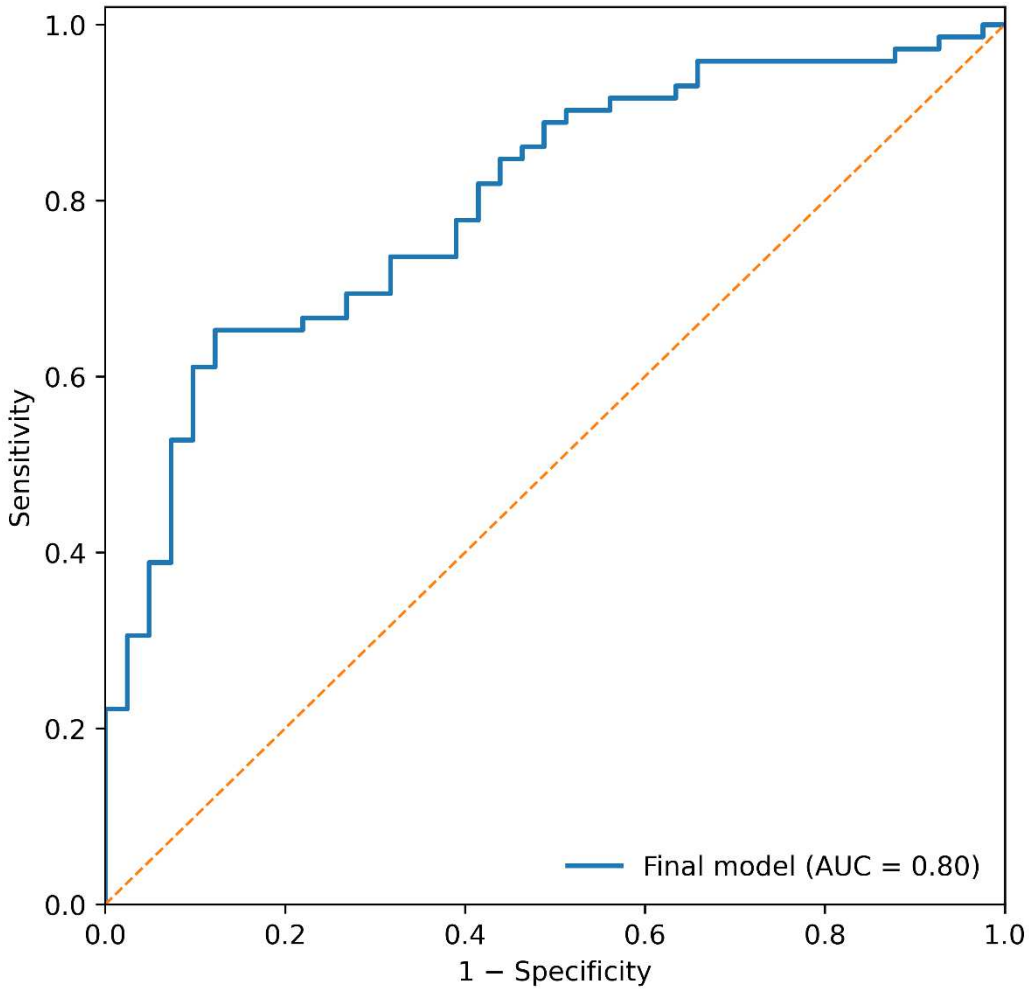


Fig. 2 Receiver operating characteristic curve for the multivariable model including log2-transformed urinary NGAL/Cr and age. The apparent AUC in the development cohort was 0.80 (95% CI 0.72–0.88). The model has not undergone internal or external validation.

Discussion

Within this single-center cross-sectional cohort, NGAL/Cr was the only creatinine-normalized urinary biomarker that remained significantly associated with urolithiasis after correction for multiple comparisons. Higher urinary NGAL/Cr was also associated with urolithiasis in the multivariable model including age. The final model showed an apparent AUC of 0.80; however, this estimate has not been internally or externally validated. These findings should be interpreted cautiously because of between-group differences in age and urinary creatinine concentration. Importantly, the cross-sectional design does not establish causality or determine whether higher NGAL/Cr precedes, results from, or merely accompanies stone disease.

Our findings are consistent with part of the previous pediatric literature. Kandur et al. reported significantly higher urinary NGAL/Cr in children with nephrolithiasis and hypercalciuria than in healthy controls, whereas KIM-1/Cr and IL-18/Cr did not differ significantly between groups [7]. Kovacevic et al. similarly found increased urinary NGAL and cystatin C in children with urolithiasis despite normal serum creatinine concentrations, supporting the concept that urinary proteins may reflect renal alterations not captured by conventional markers of kidney function [9].

However, pediatric results have not been uniform. Taşdemir et al. found no significant overall differences in urinary NGAL/Cr, KIM-1/Cr, or NAG/Cr between children with urolithiasis or microlithiasis and healthy controls. Notably, NGAL/Cr and NAG/Cr were higher in the subgroup with hydronephrosis [8]. These discrepancies may reflect differences in age distribution, stone burden, obstruction, metabolic abnormalities, clinical stage, sampling conditions, and analytical methodology.

The possible association between NGAL and urolithiasis is biologically plausible but should not be interpreted as specific evidence of stone-induced tubular injury. NGAL is responsive to a variety of renal and inflammatory stimuli. More broadly, experimental evidence indicates that calcium oxalate crystal exposure can disrupt renal tubular epithelial integrity, alter tight-junction function, increase oxidative stress, and activate intracellular signaling pathways involved in epithelial dysfunction [10]. A recent mechanistic review further emphasizes that supersaturation and crystallization alone do not fully explain crystal retention, and that epithelial stress responses, membrane injury, inflammatory signaling, and regulated cell-death pathways may contribute to lesion persistence and progression [11]. Accordingly, increased urinary NGAL in stone formers may reflect tubular stress or inflammatory responses associated with stone disease, obstruction, or crystal–epithelial interactions rather than a stone-specific molecular process.

A major consideration in pediatric urinary biomarker studies is the influence of age and sex. Recent reference data from healthy children demonstrated that all evaluated urinary biomarker-to-creatinine ratios were age dependent, while NGAL/Cr was additionally associated with sex and was generally higher in girls [12, 13]. This issue is particularly important in the present study because children with urolithiasis were older than controls. Age was therefore included in the multivariable analysis and remained in the final model, while sex was not retained. Importantly, NGAL/Cr remained associated with urolithiasis after adjustment for age. Nevertheless, because of the substantial age imbalance between groups, residual confounding cannot be excluded and the observed difference in NGAL/Cr should not be attributed solely to stone disease.

Normalization to urinary creatinine is commonly used to reduce variation related to urine concentration, but this approach also requires caution in children. Urinary creatinine excretion is influenced by age, growth, muscle mass, and body size, and recent pediatric reference data demonstrate that biomarker/Cr ratios themselves vary considerably across childhood [12]. In the present cohort, urinary creatinine concentrations also differed between the urolithiasis and control groups. Therefore, although creatinine normalization improves comparability of spot urine measurements, it does not fully eliminate developmental and anthropometric influences and may itself contribute to between-group variability. Because urinary creatinine concentrations differed between groups, an influence of this difference on the observed NGAL/Cr association cannot be excluded.

The absence of significant results for the remaining biomarkers after correction for multiple comparisons is also relevant. Calbindin/Cr, GST-π/Cr, and IL-18/Cr showed nominal between-group differences, but these did not remain significant after Bonferroni correction. KIM-1/Cr, cystatin C/Cr, osteopontin/Cr, MCP-1/Cr, and the remaining biomarkers were not significantly different between groups. This is consistent with the broader literature, in which urinary biomarkers of tubular injury have shown heterogeneous and sometimes contradictory results in kidney stone disease. A systematic review by Brewin et al. concluded that NGAL, KIM-1, NAG, and other urinary biomarkers may be elevated in kidney stone disease, but their diagnostic and prognostic roles remain uncertain because they can be influenced by obstruction, infection, and other causes of renal injury [14]. The present findings therefore suggest that, within this multiparameter panel and study population, NGAL/Cr provided the most reproducible association with urolithiasis rather than indicating that the remaining proteins have no biological relevance.

An important strength of the present study is the simultaneous assessment of 12 urinary biomarkers reflecting different aspects of tubular function, epithelial stress, inflammation, and renal protein handling within the same cohort. Correction for multiple testing was applied, and NGAL/Cr remained significant both in between-group comparisons and logistic regression. In addition, children with urolithiasis were assessed outside acute renal colic; in those with a previous colic episode, sampling was performed at least one month later. This reduces the likelihood that the observed biomarker differences reflect only an acute obstructive or inflammatory episode.

Several limitations should be acknowledged. This was a single-center study using a healthy control group, which may limit generalizability and may increase apparent between-group discrimination. The groups were not age-matched; although age was incorporated into the multivariable model, residual confounding remains possible. Healthy controls were not routinely screened by ultrasonography, and asymptomatic urolithiasis therefore cannot be completely excluded. Biomarkers were normalized to urinary creatinine despite age-related variability in creatinine excretion, and measurements were performed in two analytical batches, which may have introduced additional variability. Finally, results reported by the Bio-Plex Manager software as out of range were replaced with predefined numerical substitution values before statistical analysis to retain these observations in quantitative analyses. This procedure may have affected biomarker distributions and effect estimates, particularly for analytes with a higher proportion of out-of-range results, and should be considered when interpreting these findings. The discriminative performance of the final model was evaluated in the development cohort without internal or external validation; therefore, the reported AUC should be considered an apparent estimate and may overestimate performance in other population**s.**

In conclusion, in this cross-sectional single-center study, urinary NGAL/Cr was the biomarker most consistently associated with ultrasonographically confirmed urolithiasis after correction for multiple comparisons and adjustment for age. The apparent discrimination of the model combining NGAL/Cr and age was encouraging but requires internal and external validation. Prospective studies with age- and sex-matched controls, standardized urine collection and preanalytical procedures, and detailed assessment of stone burden, obstruction, recurrence, and metabolic abnormalities are needed before urinary NGAL can be considered for clinical application.

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