Original Article

# Urine neutrophil gelatinase-associated lipocalin (NGAL) measurement is not predictive for ureteral patency in pediatric patients following pyeloplasty: a pilot study

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Biomarker, neutrophil gelatinase associated lipocalin, pyeloplasty, ureteropelvic junction obstruction

## Abstract

**Objective:** To evaluate the benefit of urine neutrophil gelatinase-associated lipocalin (NGAL) measurement to predict the ureteral patency in pediatric patients undergoing pyeloplasty.

**Materials and Methods:** Ureteropelvic junction obstruction patients who underwent unilateral dismembered pyeloplasty had urine NGAL measurements taken intraoperatively during pyeloplasty and postoperatively at six months following surgery. All patients were evaluated preoperatively and postoperatively with renal scans. Pairwise comparisons and correlation analyses were performed to determine the dynamics and benefits of urine NGAL measurement.

**Results:** Thirteen patients were included in this pilot study with a mean age of 3.2 years at surgery. Mean intraoperative bladder urine level was 4.43 ng/mL, and median intraoperative renal pelvic urine NGAL level was 3.70 ng/mL. There was no significant difference between these two levels (p-value = 0.76). Six months after pyeloplasty, 9/13 patients demonstrated significant reduction in the bladder urine NGAL level (at least 50% reduction), and 5/13 patients showed ureteral patency based on postoperative renal scan (more than 5% improvement in differential renal function or the conversion of diuretic half time. However, the finding of significant reduction of urine NGAL level did not correlate with ureteral patency (r = -0.50, p-value = 0.08).

**Conclusion:** Although bladder urine NGAL level reduces in most pediatric patients following pyeloplasty, this decline is not reflective of the finding of ureteral patency from renal scanning. The benefits of urine NGAL measurement in this context remain unclear and require further large-scale investigation.

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## Introduction

Prenatal hydronephrosis is one of the most common abnormalities found using ultrasound[1]. Although spontaneous resolution occurs in most cases, in some instances various degrees of permanent kidney injury can occur and it may lead to renal deterioration if left untreated[2]. Out of the different etiologies of prenatal hydronephrosis, ureteropelvic junction obstruction (UPJO) accounts for 19-25% and is usually identified in clinical practice[3]. Currently, two management pathways for UPJO are generally advocated: watchful waiting and surgical correction known as pyeloplasty[4]. In most situations, the latter is usually selected for patients who demonstrate one or more of the indications for surgery which include: the presence of symptoms associated with obstruction, impairment of overall function, progressive impairment of ipsilateral function, development of stones or infection and rarely, casual hypertension[5]. Of the various surgical techniques used in UPJO correction, dismembered pyeloplasty has been most frequently adopted by urologists and pediatric surgeons because of its simplicity and adaptability to almost all scenarios[6,7].

Although most patients are symptom-free following pyeloplasty, one question is paramount – how to determine whether the obstruction is corrected and the urine drainage from renal pelvis to ureter is no longer restricted. In general, these outcomes are evaluated using a combination of renal ultrasonography and renal scan[8]. Nevertheless, these imaging techniques are far from perfect. Resolution of hydronephrosis visualized from renal ultrasound is not always predictive of successful pyeloplasty, since most patients will demonstrate residual hydronephrosis. Renal scan images may be more reflective of an improvement in differential renal function (DRF), but it inevitably exposes pediatric patients to a significant amount of ionizing radiation, requires intravenous contrast injection, and sometimes necessitates sedation during the imaging procedure[9].

Recently, researchers have found several plasma and urine biomarkers potentially associated with obstructive uropathy, which are potentially useful in the evaluation of patients with urinary tract obstruction[10]. Neutrophil gelatinase-associated lipocalin (NGAL) is a promising one that has been widely investigated. Earlier studies have reported that urine NGAL levels were significantly higher in children with UPJO than in control groups[11,12]. This increase was also followed by a decrease to the same levels as the controls after surgical correction of UPJO[12]. However, whether this pattern of urine NGAL reduction reflects an actual "ureteral patency" remains unknown, and this was the exploratory aim of this study.

## Materials and Methods

This prospective pilot study was carried out at King Chulalongkorn Memorial Hospital, Bangkok, Thailand. After approval was obtained from the institutional review board (IRB# 310/57), guardians of all consecutive eligible UPJO patients were informed about this study. If there was agreement to participate, written consent was acquired. Inclusion criteria were patients below 18 years of age who were diagnosed with unilateral UPJO requiring treatment with dismembered pyeloplasty. Exclusion criteria were bilateral UPJO patients, those diagnosed with posterior urethral valve (PUV), single functioning kidney patients, or those who had prior surgery on the ipsilateral kidney. We also excluded patients who had had a prior urinary diversion either with ureteral stent or percutaneous nephrostomy tube and those with complications caused by urinary tract infection within the last three months. Based on a previous finding that bladder urine NGAL level decreased by approximately 50% following UPJO correction, a sample size of 13 patients was required for this study[12].

Patient demographic parameters were prospectively collected including patient age at surgery, gender, associated urinary tract anomalies, and history of renal surgery. Within the month prior to surgery, an MAG-3 renal scan was performed to determine the differential renal function (DRF) and diuretic half-time. At the time of surgery, two urologists (C.B. and M.U.) conducted the open dismembered pyeloplasty surgical technique proposed by Anderson-Hynes[13]. Initially, a urethral catheter was inserted, and a 10 mL bladder urine specimen (BU1) was collected for research purposes. Then, the patient was turned into the lateral decubitus position, and a small flank incision was made to enter the retroperitoneal space. Once the renal pelvis and proximal ureter were dissected free from the surrounding tissue, another 10 ml of renal pelvic urine (PU)

was aspirated from the affected kidney via an 18-gauge needle. Resection of the stenotic part was carried out, and pyeloureterostomy anastomosis was performed over an indwelling 4.6 Fr ureteral stent which was subsequently removed after 6 weeks.

At six months, a voided midstream urine specimen (BU2) was collected at the clinic, and MAG-3 renal scan was performed to measure the postoperative DRF and the diuretic half-time clearance. In this study, ureteral patency was defined by the improvement of more than 5% of DRF[14] or the conversion of diuretic half time to less than 20 minutes.

All three urine specimens (BU1, PU, and BU2) were delivered to the laboratory facility within one hour of collection. They were centrifuged at 3000 cycles per minute at room temperature. Subsequently, the supernatant parts were drawn, divided into three tubes, and stored at -80°C. At the time of analysis, these specimens were defrosted, and NGAL level was measured using the NGAL Test™ Reagent Kit which uses enzyme-linked immunosorbent assay (BioPorto Diagnostics A/S, Hellerup, Denmark). The different assays were strictly quality controlled by the manufacturer with an inter-assay variation of well below 5%. All urine NGAL levels were expressed as an absolute value.

Comparison of NGAL levels was made using a paired t-test and a Mann-Whitney test. Significant NGAL reduction was noted if the NGAL level decreased by at least 50% following pyeloplasty. This outcome was further correlated against postoperative ureteral patency using Spearman's correlation. Statistical analyses were performed using Stata/SE version 14.1 (StataCorp, College Station, TX, USA). Data are expressed as mean± tandard deviation or percentage with statistical significance being assumed at $p < 0.05$.

## Results

Sixteen pediatric patients were newly diagnosed with UPJO at our institution during the study period. However, three were excluded due to bilateral obstruction, single functioning kidney, and persistent urinary tract infection requiring preoperative ureteral stent placement. The remaining 13 patients were included in the analysis. The mean age at surgery was 3.2 ± 3.4 years (range 0.6-10.4 years), males predominated (n=8), females (n=5), and the majority of obstructions were on the left side (69.2%). Preoperative renal scans revealed a mean DRF of 43.1 ± 9.5% in the affected kidney, and the diuretic half-time was prolonged (> 20 minutes) in all patients (Table 1).

The mean intraoperative bladder urine (BU1) NGAL level was 4.43 ± 3.93 ng/mL, and the median intraoperative renal pelvic urine (PU) NGAL level was 3.70 ng/mL (Table 1). There was no statistically significant difference between bladder urine and renal pelvic urine NGAL levels (p-value = 0.76). All patients underwent dismembered pyeloplasty without any major perioperative complication and recovered uneventfully.

At six months after surgery, the mean postoperative bladder urine (BU2) NGAL level was 1.00±0.90 ng/mL, which was significantly lower than the intraoperative BU1 NGAL level (p-value = 0.01). Overall, 11/13 patients demonstrated some degree of NGAL reduction, and 9/13 patients demonstrated significant reduction (at least a 50% decrease from baseline level). Five out of 13 patients showed ureteral patency from the renal scan profiles specifically: 1) at least 5% elevation in DRF (3 patients), or 2) conversion of half-time to less than 20 minutes (3 patients) (Table 2).

Despite no clinical deterioration being found after surgery, the dynamics of urine NGAL did not show a significant correlation with postoperative radiologic outcome assessment. Following pyeloplasty, the demonstration of significant NGAL reduction (n = 9) was not significantly correlated

**Table 1.** Demographics and perioperative biochemical and radiological parameters.

| Parameters | Value |
|---|---|
| Age at surgery, mean±SD (years) | 3.2 ± 3.4 |
| Gender, n (%) | |
| - Male | 8 (61.5) |
| - Female | 5 (38.5) |
| Laterality, n (%) | |
| - Left | 9 (69.2) |
| - Right | 4 (30.8) |
| Preoperative DRF, mean±SD (%) | 43.1 ± 9.5 |
| Preoperative half time, n (%) | |
| - > 20 minutes | 13 (100) |
| Intraoperative bladder urine NGAL level, mean±SD (ng/mL) | 4.43 ± 3.93 |
| Intraoperative renal pelvic urine NGAL level, median (ng/mL) | 3.70 |

**Table 2.** Postoperative biochemical and radiological parameters.

| Parameters | Value |
|---|---|
| Postoperative DRF, mean±SD (%) | 43.3 ± 11.7 |
| At least 5% elevation in postoperative DRF, n (%) | |
| - Presence | 3 (23.1) |
| - Absence | 10 (76.9) |
| Postoperative half time, n (%) | |
| - 10-20 minutes | 3 (23.1) |
| - < 10 minutes | 0 |
| - > 20 minutes | 10 (76.9) |
| Postoperative bladder urine NGAL level, mean±SD (ng/mL) | 1.00 ± 0.90 |
| Reduction in postoperative bladder urine NGAL level, n (%) | |
| - Presence | 11 (84.6) |
| - Absence | 2 (15.4) |
| Significant reduction (at least 50%) in postoperative bladder urine NGAL level, n (%) | |
| - Presence | 9 (69.2) |
| - Absence | 4 (30.8) |

to the finding of ureteral patency based on postoperative renal scan (r = -0.50, p-value = 0.08). Even looking at any degree of NGAL reduction (n = 11), this factor did not correlate well with postoperative ureteral patency (r = -0.37, p-value = 0.22).

## Discussion

The goal of follow-up in UPJO patients after pyeloplasty is to identify those who are in danger of losing renal function from recurrent or unresolved obstruction[15]. To date, there has been no consensus regarding an appropriate surveillance strategy in this group of patients[16]. The majority of urologists commonly utilize a combination of clinical well-being, renal ultrasonography, and renal scan. Nevertheless, these tools are yet to be perfected and have certain drawbacks. Recurrent symptoms after surgery are usually detectable at a late phase of renal deterioration, and persistent symptoms such as pain may not correlate to anatomical obstruction[17]. In the case of ultrasonography, long-term evolution of residual hydronephrosis is relatively common[16]. A significant number of patients demonstrate stable hydronephrosis on ultrasound, while renal scanning shows an improved DRF[15]. In general, the renal scan is accepted as a gold standard for evaluating improvement in renal function after pyeloplasty, with a change of 5% considered as significant[14]. However, this imaging modality requires a significant exposure of ionizing radiation to the patients[18] which may negatively affect organ maturation in the long-term. Thus, when selecting a surveillance imaging modality following pyeloplasty, the radiation risks versus the diagnostic benefits should be weighed properly.

To overcome the complexity of imaging procedures and the associated radiation issue, several urine biomarkers have been investigated in the setting of obstructive uropathy[19]. They include Epidermal Growth Factor (EGF), Normal T- cell Expressed and Secreted (RANTES), Osteopontin (OPN), Neutrophil Gelatinase-Associated Lipocalin (NGAL), Cystatin C (CyC), β2-Microglobulin (β2-M), Kidney Injury Molecule-1 (KIM-1), and Carbohydrate Antigen 19-9 (CA-19-9), with various sensitivity, specificity and receiver operating characteristic (ROC) curves being reported in determining their clinical diagnostic and prognostic values[19]. Among these biomarkers, urine NGAL has demonstrated a promising diagnostic profile in numerous studies[11,12,20]. It is a 25-kDa protein secreted into urine via the thick ascending limb and collecting duct[21]. An increase in urine NGAL level is a well-established biomarker of kidney injury[22,23], and its role has also been examined in various kidney diseases such as diabetic nephropathy and nephritic syndrome[22,24].

Cost et al. have demonstrated that median bladder urine NGAL level was significantly higher in children with UPJO compared to controls (18.6 versus 8.3 ng/mg, p-value = 0.004). Within the subset of UPJO patients, renal pelvic urine NGAL was significantly higher than bladder urine NGAL, a finding that differed to ours. That study group also found that NGAL levels correlated well with their corresponding DRF (r = -0.359, p-value = 0.004). However, there was no published data regarding surgical outcomes or comparison to postoperative urine NGAL level[20]. Wasilewska et al., on the other hand, investigated the effect of pyeloplasty on urine NGAL levels of 20 children with unilateral UPJO. Similar to our findings, they found no difference between bladder urine and renal pelvic urine NGAL levels. A statistically significant correlation between preoperative urine NGAL level and differential renal function was also found when evaluating by MAG-3 renal

scan (r = -0.422, p-value <0.05). Three months after surgery, the bladder urine NGAL level had significantly decreased; however, there was no data published with regards to postoperative DRF, clinical outcomes, or other radiologic parameters of obstructive uropathy[11].

The major strength of our study is the comparison of NGAL dynamics to postoperative radiologic outcome assessment. Despite the low rates of significant improvement in DRF and half-time conversion, our surgical outcomes were comparable to others[14,25]. This may correspond to the fact that, although widely utilized, follow-up with a renal scan is still controversial, and its clinical benefits are considerably uncertain. Similarly to findings in previous studies, we found that bladder urine NGAL level reduced in most patients following pyeloplasty[11,12]. Nevertheless, this change was not associated to any of the ureteral patency parameters including improved DRF and diuretic half-time conversion.

Several limitations of this study should be addressed in future research. First, although prospectively conducted, the subject number was relatively small, and all findings need to be interpreted carefully. Second, we did not assign a group of matched controls to enable comparison of urine NGAL level. Third, one study has shown a daily biological variation of 27% for urine NGAL level in healthy young subjects[26] and we measured urine NGAL only once for every specimen. The intermediate variation levels would not be accounted for if there were changes. Finally, we did not standardize urine NGAL with urine creatinine level (urine NGAL/Creatinine ratio) a useful parameter previously described in other published literature[11,20]. However, this practice is still controversial. For example, one study indicated that urine NGAL/Creatinine ratio had a higher daily variation compared to absolute NGAL level[26]. This should be minimized in our study as we compared NGAL level from various specimens in a pairwise fashion, therefore interpersonal variability should be limited.

## Conclusion

Bladder urine NGAL levels reduced in the majority of pediatric UPJO patients after pyeloplasty. However, our study did not demonstrate that the NGAL decline showed a significant correlation to the finding of ureteral patency obtained from renal scan. Whether urine NGAL measurement has a clinical benefit in the evaluation of postoperative ureteral patency remains unclear and requires further large-scale investigation.

## Conflict of Interest

The authors declare no conflict of interest.

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