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# Acute Kidney Injury Biomarkers: A Prospective Cohort Study In Urological Patients

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# Abstract

Several recent studies have assessed the use of biomarkers of Acute Kidney Injury (AKI), but the information among patients with stone disease and those with obstructive uropathy is limited. For this reason, we conducted a prospective cohort study to determine the urinary levels of KIM-1, Total and Monomeric NGAL in patients with hydronephrosis secondary to renal stone disease, congenital ureteropelvic junction obstruction or ureteral stricture, and in a group of healthy controls in our health care center. Urinary biomarker concentrations were evaluated before and after surgical treatment. Patients with hydronephrosis showed significantly higher baseline levels of KIM-1 compared to those patients without hydronephrosis. KIM-1 was the only urinary biomarker significantly affected by the presence of hydronephrosis. Total and Monomeric NGAL correlated with the presence of leukocyturia. Our results show that KIM-1 is a promising biomarker of subclinical AKI associated with hydronephrosis in urological patients.

# Keywords

# Dedication

This work is dedicated to my family, my reason to exist.

To my beloved wife Yelile, who has always been by my side for over 10 years.

My kids, Lucia and Elias, who inspire me to give my best in everything I do.

To my parents and siblings for supporting me and encouraging me to accept this adventure, essential to my academic training.

Above all, I dedicate this writing to God for placing all these people in my path, I owe Him everything.

# Acknowledgments

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# Table of Contents

# List of Tables

# List of Figures

# List of Appendices

# List of Abbreviations

AKI: acute kidney injury

CKD: chronic kidney disease

eGFR: estimated Glomerular Filtration Rate

ESRD: end-stage renal disease

GFR: Glomerular Filtration Rate

KIM-1: Kidney Injury Molecule-1

NGAL: Neutrophil gelatinase-associated lipocalin

PCNL: percutaneous nephrolithotomy

RBF: renal blood flow

SWL: shockwave lithotripsy

UPJO: ureteropelvic junction obstruction

URS: ureteroscopy

UTO: urinary tract obstruction

UUO: unilateral ureteral obstruction

# 1   Background

Partial or complete obstruction of the urinary tract is a common and challenging urological condition that may occur in patients of any age. Urinary tract obstruction can be classified as congenital or acquired, depending on the cause; acute or chronic, according to the time of evolution, and benign or malignant. Alternatively, it can be catalogued as upper or lower urinary tract obstruction, depending on the location as well as unilateral or bilateral. Obstruction of the urinary tract may be silent, can cause mild and longstanding symptoms or it may be the reason for an emergency treatment, such as patients with renal colic or acute urinary retention. The main concerns of urinary obstruction are pain, renal function loss, and the increased possibility of an infectious process. Several factors play an important role in the pathophysiology of urinary tract obstruction and these will be reviewed in more detail in the next sections.

Until recently, serum creatinine was considered an accurate marker to assess global renal function. Many diagnostic and therapeutic decisions have been based on the levels of this compound in blood that does not always precisely reflect the current status of kidney function. Lately, several studies have been dedicated to the scrutiny of newly discovered kidney proteins released through blood and urine that may be used as acute kidney injury (AKI) markers (Nickolas, 2008; Zappitelli, 2007). Numerous markers have been described in different patient populations and some have been proposed as potential substitutes for creatinine as an objective measure of AKI.

The most commonly investigated biomarkers are Kidney Injury Molecule-1 (KIM-1), Neutrophil Gelatinase-associated Lipocalin (NGAL), cystatin C, B2-microglobulin, interleukin-18, osteopontin and liver-type fatty acid-binding protein (L-FABP) (Koyner, 2010; Wasilewska, 2011). Unfortunately, few studies have addressed the diagnostic value of these new markers in patients with urological comorbidities, or they lack of a longitudinal follow-up to evaluate their real significance over time. For this reason, and in order to provide a thoughtful understanding of the impact of these urinary AKI markers, we designed and conducted this prospective cohort study in urological patients.

Throughout this chapter, we will explore the pathophysiology of unilateral and bilateral urinary tract obstruction and how it affects the markers used to assess the function of the kidney. Special attention will be paid to AKI, with emphasis on postrenal causes, particularly to ureteric obstruction due to stricture or stone disease. Information about the most commonly used urinary markers will be presented.  A detailed description of hydronephrosis and the grading scales are also included. Finally, the hypotheses of our research project will be discussed and the possible clinical implications will be underlined.

## 1.1  Defining urinary tract obstruction

Urinary tract obstruction (UTO) is the term used to define the blockage to the flow of urine that causes elevated pressures within the collecting system of the urinary tract and affects the normal function of the renal unit. It is usually a mechanical problem that can cause renal dysfunction and occur at any level of the urinary tract, from the renal calyces (*i*.e. infundibular stenosis) to the urethral meatus (*i*.e. urethral stenosis). The impact of the

obstruction in patients´ health status is related to the location of the obstruction, the time of onset, the baseline renal function and the presence of risk factors for electrolyte imbalances or septic processes. It is useful to classify UTO according to the etiology, location, bilateralism and time of onset, because this information will help to determine the diagnosis and proper management. **Figure 1** shows different ways of classifying UTO, and **Table 1** lists common examples associated with each type.
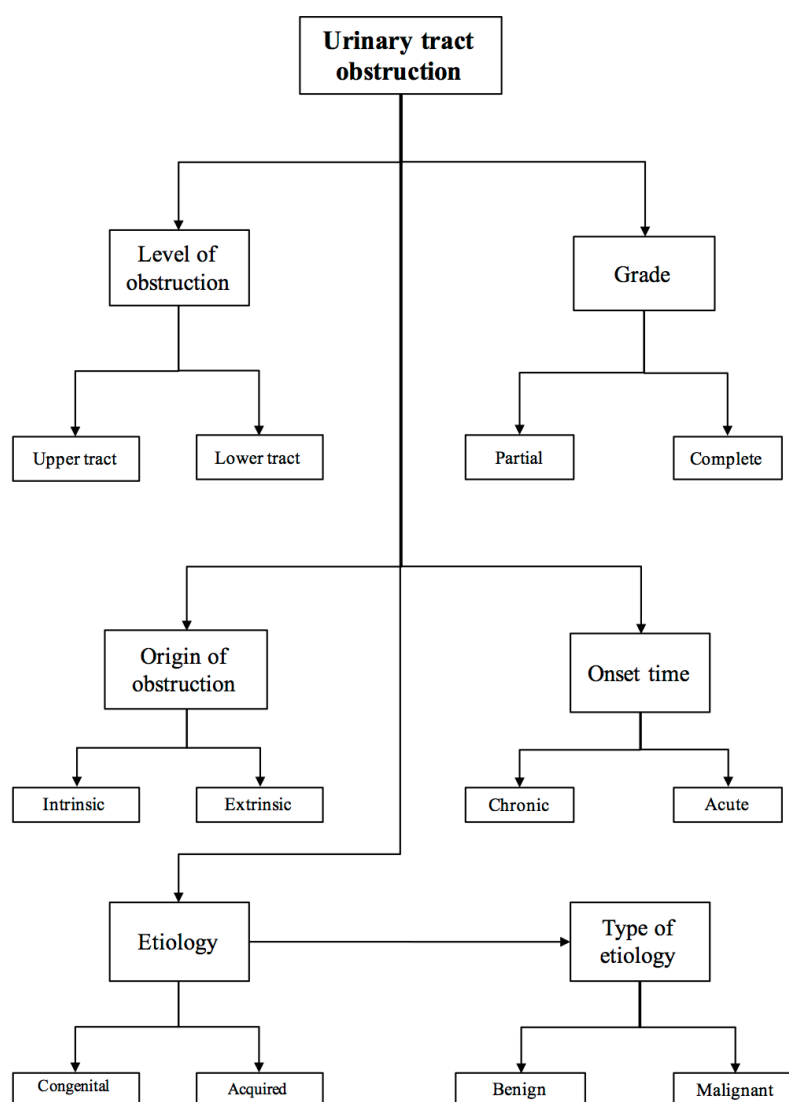


**Figure 1: Classification of urinary tract obstruction according to the location and level, etiology and time of onset (Campbell-Walsh, 2012; Loo, 1988).**

| Table 1. Possible causes of urinary tract obstruction categorized by its type | |
|---|---|
| **Upper urinary tract obstruction** | **Lower urinary tract** |
| <u>Congenital</u> | <u>Congenital</u> |
| *Polycistic kidney disease* | *Posterior urethral valves* |
| *Ureteropelvic junction obstruction* | |
| *Congenital renal cysts* | <u>Acquired</u> |
| *Ureterocele* | Neoplasic |
| *Retrocaval ureter* | *Prostate cancer* |
| | *Bladder carcinoma* |
| <u>Acquired</u> | *Carcinoma of the urethra* |
| Inflammatory | Miscellaneous |
| *Tuberculosis* | *Benign Prostatic Hyperplasia* |
| *Retroperitoneal fibrosis* | *Hypocontractile Bladder* |
| *Papillary necrosis* | *Urethral stricture* |
| Metabolic | *Phimosis* |
| *Stone disease* | |
| Miscellaneous | |
| *Ureteral stricture* | |
| *Trauma* | |
| *Iatrogenic during surgery* | |
| *Pregnancy* | |
| Neoplastic | |
| Malignant | |
| Intrinsic | |
| Upper urothelial carcinoma | |
| Extrinsic | |
| Cervical/Colorrectal cancer, Lymphoma | |

**Table 1. Most common causes of urinary tract obstruction categorized by type. (Adapted from Campbell-Walsh, 2012).**

UTO is characterized by the presence of hydronephrosis on imaging studies and most commonly detected by either renal ultrasound or abdominal Computerized Tomography imaging (CT scan). According to these studies, the presence of unilateral or bilateral obstruction may also give important clues about the cause. Hydronephrosis is not always associated with mechanical obstruction, instead, it can be related to a functional disorder

which leads to an impairment in urine flow, such as a hypocontractile bladder, vesicoureteral reflux or a non-peristaltic ureter during a pyelonephritis episode. These unique conditions where hydronephrosis is not related to obstruction were not included in our study.

## 1.2 Functional changes associated with urinary tract obstruction

Renal obstruction is associated with a myriad of hemodynamic and humoral responses that lastly lead to a reduction in the Glomerular Filtration rate (GFR) of the renal unit. GFR is a dynamic process determined by 3 different intimately related elements:

1. Glomerular ultrafiltration coefficient, which is the surface area of the glomerular membrane

2. Glomerular capillary pressure influenced by afferent and efferent resistances in glomerular vasculature.

3. Tubular regulation

UTO directly affects tubular regulation and the pathophysiology is different between unilateral and bilateral obstruction; the ultrafiltration coefficient and the capillary pressures may be affected in late phases of obstruction. Our knowledge comes from animal experiments where an increased hydrostatic pressure in the tubular system activates the cascade of events leading to renal fibrosis, although the complete kidney response to these dynamic changes is not fully understood (Vaughan, 2004). Specific and detailed description of the molecular biology of the kidney physiology is beyond the

scope of this research, and we will focus on the hemodynamic and humoral changes associated with urinary obstruction.

## 1.2.1 Pathophysiology of unilateral ureteral obstruction

The first study to evaluate kidney injury after complete unilateral ureteral occlusion was carried out in the late 1960s, with a canine model measuring vascular and ureteral pressures (Gillenwater, 1970). In fact, this was the foundation for further research in renin-angiotensine-aldosterone system (RAAS), thromboxane A2 (TA2), and other prostaglandins. Renal blood flow and ureteral pressures associated with unilateral obstruction have a relationship that is described as a "triphasic response", that proceeds as follows: Phase 1) an increase in both the renal blood flow (RBF) and ureteral pressure (UP) is followed by phase 2) with a drop in blood flow and elevated UP, and finally phase 3) a decrease in both flow and pressure.

This response was depicted after an 18-hour evaluation of unilateral ureteral obstruction (UUO) in a canine model and it is currently valid to understand the pathophysiology of complete UUO. **Figure 2** shows the 3 phases initially described by Moody *et al* in 1975 and several new findings have augmented our knowledge about this topic, where biochemical and hormonal interactions modulate renal responses.
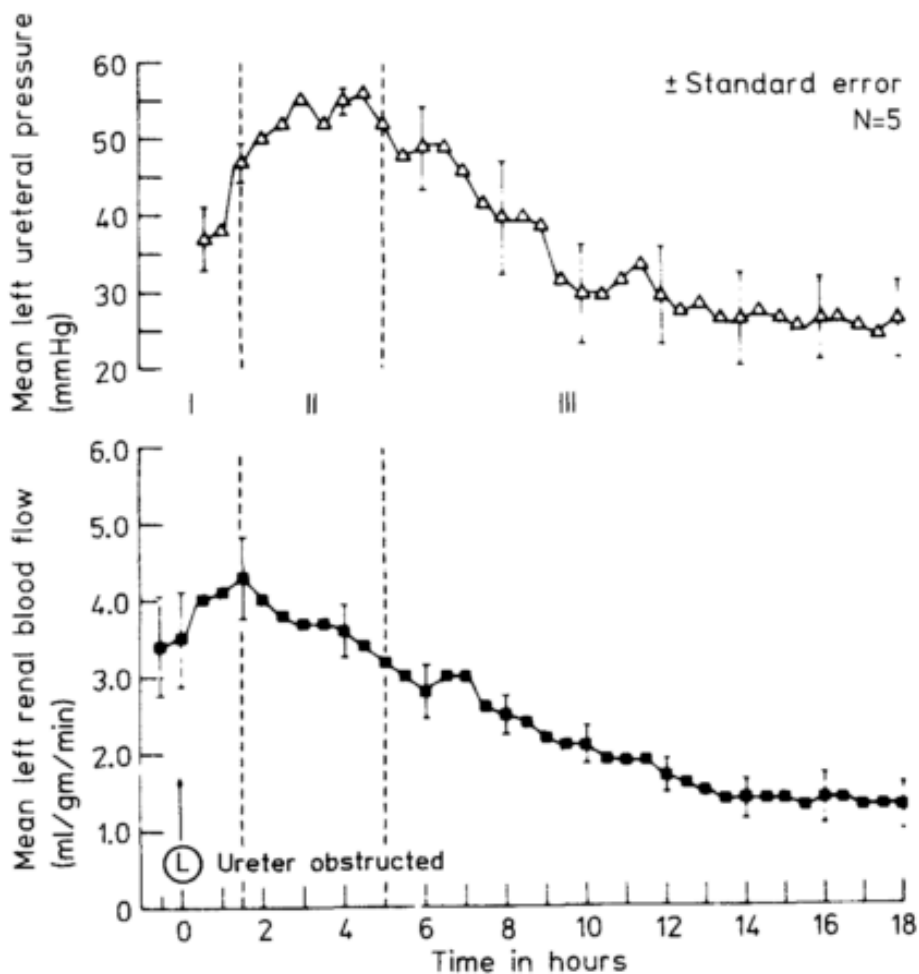
**Figure 2: Triphasic relationship between the renal blood flow and ureteral pressure after unilateral obstruction (From Loo, 1988; with permission from Springer).**

During the first two hours, the increased UP causes an immediate response regulated by

Prostaglandin E2 (PGE2) and Nitrous Oxide to increase the RBF and maintain the normal

GFR (Salvamini, 1994; Vaughan, 2004). This increment in UP depends on the GFR

which affects the urine production, the tubular fluid reabsorption and the compliance of

collecting system, especially of the renal pelvis. The second phase is characterized by a

decrease in the RBF due to contraction of the afferent arteriole at the glomerulus by the activation of the RAAS and TA2. The tubular and UP remain elevated in this phase as consequence of the initial increase in RBF, but as time passes, the pressure in tubular and collecting systems decreases because the GF ceases as the RBF diminishes.

All the aforementioned changes occur in the acute setting, and patients with a contralateral healthy kidney usually do not exhibit clinical or classic biochemical signs of AKI. Unfortunately, animal studies have documented pathological changes including fibrosis in renal tissue during the first days and weeks of obstruction, despite a normal contralateral kidney (Guerin, 2008; Nagle, 1978). Hou *et al*, after analyzing the efficacy of micro-CT scans to detect structural changes during complete UUO in a murine model, found histological fibrotic changes in renal tissue after 7 days of obstruction. Evidence of tubular atrophy and interstitial presence of inflammatory cells were evident after two weeks of obstruction (Hou, 2015). No human studies have evaluated the mechanisms of renal atrophy after acute urinary obstruction, but it is accepted that these changes also occur in humans.

In clinical practice, chronic hydronephrosis results from a more dynamic process, where the obstruction is gradual and usually incomplete, a key factor that may decrease the ureteral pressure and the subsequent hemodynamic response. Although, the evidence suggests that obstruction of the upper urinary tract leads the cascade of inflammatory and fibrotic mechanisms in renal parenchyma. Some models have been studied and will be discussed later in this chapter.

# 1.2.2 Pathophysiology of bilateral ureteral obstruction or obstruction in a solitary kidney model

The model of bilateral obstruction or complete obstruction in solitary kidneys differs from the unilateral model by the absence of a contralateral unit that filtrates and eliminates the vasoactive substances that may affect the "triphasic response". The accumulation of biochemical mediators causes a transient increment in renal blood flow with prolonged elevation in UP decreasing the eGFR. The absence of a normal contralateral kidney precludes the elimination of inflammatory mediators, increasing the inflammatory response, the cellular damage and the accumulation of waste products such as urea and creatinine. These changes are acute and their clinical implications can be catastrophic, requiring urgent management to relieve obstruction and avoid metabolic complications related to renal failure. This topic is beyond the scope of this research project, as we focused in those cases with subclinical AKI not requiring urgent management.

The following figure is intended to integrate the functional, hemodynamic and biochemical changes in unilateral and bilateral ureteral obstruction, which finally lead to a cellular fibrotic response. We also show the specific time when the most common markers of AKI are detected to demonstrate their relationship with the pathophysiologic process of renal injury. The new AKI markers are released in the early phase of obstruction, making them potential indicators of early renal injury.

| Phase | Unilateral Obstruction | Bilateral Obstruction | Time of expression of AKI marker |
|---|---|---|---|
| Acute phase (1-2 hours) | ↑RBF to compensate ↑ UP keeping ≈ GFR | ↑RBF to compensate ↑ UP but GFR ↓ | NGAL, KIM-1, IL-18, L-FABP |
| Mid phase (2-5 hours) | ↓ RBF ↓ GFR ↑ UP | ↓RBF↓ GFR | |
| Later phase (>5-24 hours) | ↓ RBF ↓ GFR ↑/≈ UP | | Creatinine, Urea, Cys C |
| Chronic phase (>24 hours) | ↓ RBF ↓ GFR | | |
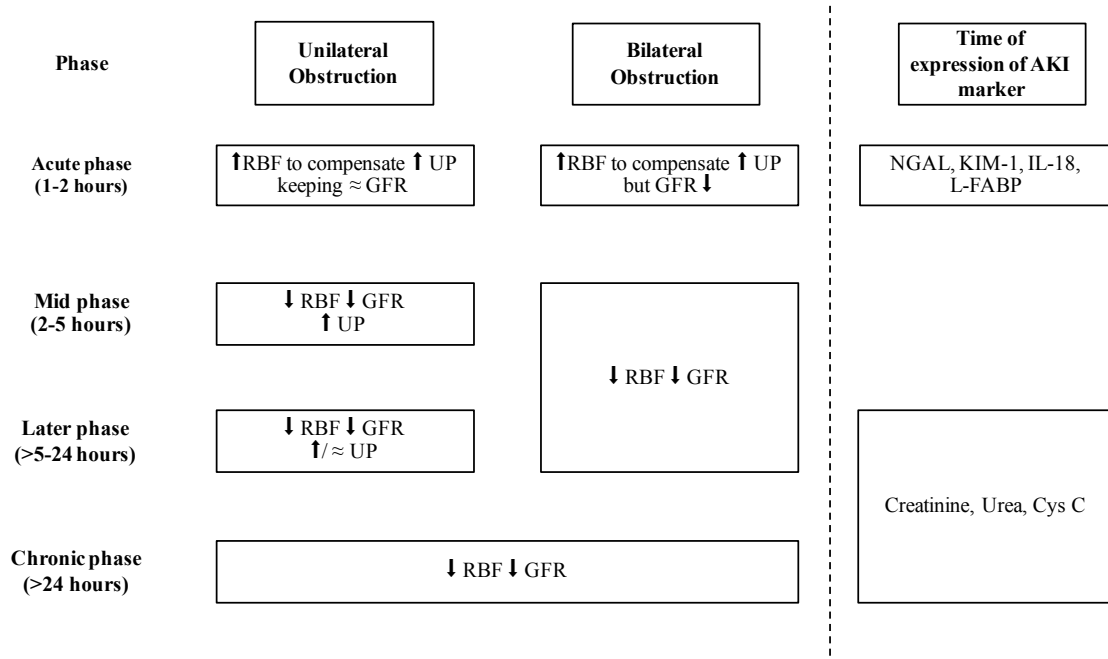
*Abbreviations:* Cys C, Cystatine C; GFR, glomerular filtration rate; IL-18, Interleukin 18; KIM-1, Kidney Injury Molecule-1; L-FABP, Liver-type Fatty Acid-Binding Protein; NGAL, Neutrophil Gelatinase-Associated Lipocalin; RBF, renal blood flow; UP, ureteral pressure.

**Figure 3: Functional changes following unilateral and bilateral obstruction and markers of kidney injury (Adapted with information from Bellomo, 2012; Campbell-Walsh, 2012; Loo 1988).**

## 1.2.3 Partial ureteral obstruction

Some researchers investigating the deleterious effects of partial ureteral occlusion have used neonatal animal models to evaluate the changes in cellular differentiation and development (Sugandhi, 2014; Wen, 1998). Glomerular and tubular structures are compromised and the overall renal function is affected. This process is likely similar to what happens in newborn patients with prenatal hydronephrosis and posterior urethral valves who have chronic kidney disease related to renal dysplasia.

Animal models creating partial ureteral obstruction have found evidence of parenchymal loss, peripelvic and interstitial fibrosis in the early phase (2-4 weeks) of unilateral obstruction (Botto, 2011; Guerin, 2008). These findings show the deleterious impact on long-term renal obstruction, even in models with incomplete obstruction. Interestingly the degree of tissue damage did not always correlate with the grade of dilatation of the upper tract. For these reasons, we believe that we should not rely only on the presence or absence of hydronephrosis in the imaging studies of patients with stone disease to rule out the existence of subclinical kidney injury.

Many research studies have focused on analyzing mechanisms to avoid apoptosis and fibrosis in animal models with renal obstruction. Nitric oxide (NO), transforming growth factor (TGF-β1), Tumoral Necrosis Factor-α (TNF-α) and other new biomarkers have been implicated in renal remodeling. Some of these will be later discussed because their expression during episodes of ischemia/obstruction has been used to identify patients with a higher risk of poor outcomes, and are the current basis for timely AKI diagnosis.

## 1.3  Mechanisms of renal injury

Urinary obstruction causes a significant derangement in the mechanisms of urinary concentration, and continuous obstruction causes cellular injury leading to tubular and interstitial atrophy, fibrosis and cellular proliferation (Ito, 2004). Apoptosis is the main pathway that leads to renal atrophy and chronic kidney disease (CKD), which is principally established by glomerular sclerosis and interstitial fibrosis.

Apoptosis is orchestrated by several intracellular and transmembrane signals involving caspase enzymes and TNF-α pathway resulting in the release of intracellular proteins with subsequent tubular cell death (Chevalier, 2009). Renal architecture is also affected when acute inflammation augments the synthesis of metalloproteinases leading to fibrotic changes.

The angiotensin pathway is a promoter of fibrotic changes by upregulating the expression of cytokines that stimulate extracellular inflammation, such as TNF-α and TGF-β1. The synthesis of extracellular matrix, mostly collagen fibers, and the signaling process to recruit inflammatory cells contributes to the histological changes that decrease the amount of parenchyma by destruction of the nephrons (Hewitson, 2009). Tubular atrophy and interstitial remodeling also cause obliteration of postglomerular peritubular capillaries, with a subsequent decrease in the GFR (Ito, 2004).

A similar biological pathway of renal fibrosis linked to ischemic injury is also exhibited in cases of chronic obstruction. The interactions between inflammatory cells and interstitial cells, besides causing fibrosis in tubular area, can cause de-differentiation into mesenchymal cells or apoptosis of endothelial cells leading to renal ischemia (Chevalier, 2009). This process may be slow, but may explain the progressive kidney damage seen in patients with chronic UUO.

As urologists we are interested in the grade and time course of obstruction because these factors will impact the histological injury of the renal parenchyma and the long term global renal function. Kerr in 1956 was the first to correlate the degree of injury with the duration of obstruction. Vaughan evaluated the radiological and pathological appearance

of unilateral obstructed renal units in dogs and found a remarkable recovery 4 weeks after relieving the obstruction. In contrast, animals that had an obstruction longer than 7 weeks however, did not have complete recovery (Kerr, 1956; Vaughan, 1971).

The extent and magnitude of the obstructive process and the degree of cell loss during the inflammatory and fibrotic post-obstructive process play important roles in the recovery of renal function. The duration of unilateral ureteral obstruction and the animal species analyzed have been found to be determinants of renal recovery (Bander, 1985; Leahy, 1989; Vaughan 1973). Other factors such as level of obstruction, compliance of collecting system, presence of infection, and integrity of lymphatic channels may affect the final pathway for histological injury (Vaughan, 2004).

Upon relief of obstruction, not all of the mechanisms behind kidney injury will halt. Progression of renal damage has been demonstrated in histological analysis in a rat study after only 3 days of complete ureteral obstruction, despite normal renal blood flow or GFR (Ito, 2004). Cochrane and colleagues developed a mouse model where UUO was carried out for 10 days, and then were allowed to recover for a period of 1, 2, 4 or 6 weeks. Results showed a significant increase in the number of macrophages and collagen fibers during the first weeks after obstruction relief with a decrease in the unilateral GFR, however, animals that were sacrificed after 6 weeks had a decreased grade of fibrosis and inflammation, but the eGFR of the affected unit was between 50-84% of the contralateral side (Cochrane, 2005). This definitely may compromise the long-term renal function, especially if we translate these findings to patients with medical conditions that chronically affect renal function, like diabetes, hypertension or recurrent stone disease.

The process of renal obstruction, either unilateral or bilateral, involves vascular, hemodynamic, and humoral pathways which are intended to respond to the insult, however these same complex mechanisms of renal restoration may contribute to architectural alterations that accelerate nephron loss and subsequent CKD (Harrison, 2015).

## 1.4   Acute Kidney Injury

Acute kidney injury (AKI) is a broad term used to define a sudden decrease in the GFR of both kidneys causing retention of waste products, such as urea and creatinine and dysregulation of extracellular volume and electrolytes (Bellomo, 2012; Palevsky, 2014). While this condition might be subclinical and may not be recognized by a medical practitioner in asymptomatic patients with normal baseline renal function, alternatively it might be life-threatening and considered as a risk factor for patient mortality in an acute illness, like septic shock. The duration, severity, and baseline renal function will determine if other metabolic anomalies will accompany the scenario. Recently, the term AKI replaced the expression "acute kidney failure", with the latter now reserved only for severe kidney injury associated with the need for renal replacement therapy, such as hemodialysis. AKI can be caused by three different mechanisms, which will be explained below, and **Figure 4** shows the most common causes of each.

- **Prerenal**, refers to the clinical situation where the glomerular filtration mechanisms are preserved and the dysfunction arises from a hypovolemic state, or renal blood flow is reduced. Clinical examples include patients with AKI related to extensive bleeding or septic patients where decreased vascular resistances diminish the renal blood flow, and patients with cardiogenic shock. The physiological response to renal

hypoperfusion involves vasodilation of the afferent arteriole and vasoconstriction of the efferent arteriole in an attempt to keep an adequate blood flow for GF. If the renal hypoperfusion is not reversed, ischemic insult to the tubules will occur which is called Acute Tubular Necrosis (ATN).

- **Renal** causes of AKI are the most common and involve a primary dysfunction within the nephron. ATN is by far the most frequent cause of AKI in the emergency department, being intimately related to prerenal disturbances, but some intrinsic and extrinsic toxins and exogenous agents may cause direct tubular damage (i.e. cisplatin, non steroidal anti-inflammatory drugs)

- **Postrenal** causes are of particular interest to urologists because an appropriate and timely treatment can prevent serious complications. These are related to acute obstruction of the urinary system; and as previously explained the severity is associated to the time of onset and bilateralism.
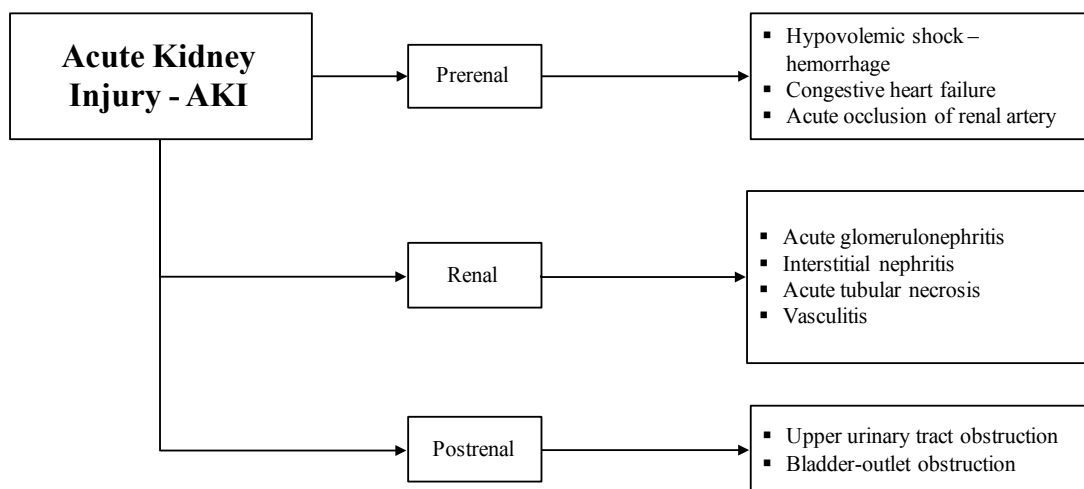


**Figure 4: Common causes of AKI according to the specific cause.**

## 1.4.1 Definition and diagnosis

Different groups have defined AKI, and distinct classifications have been developed to improve the threshold of detection and the quality of health care. The most common schemes are the Risk, Injury, Failure, Loss, and End-stage Kidney (RIFLE) and Acute Kidney Injury Network (AKIN) criteria. Definitions are similar and are based on creatinine levels, urine output and preferably require knowledge of baseline serum creatinine of the patients (Bellomo, 2004; Mehta, 2007).

AKI is a diagnosis commonly encountered in the emergency department and the Intensive Care Unit, with prevalence rates as high as 50-60% (Bouchard, 2015). Patients with AKI usually present clinical manifestations from other systems associated with an underlying disease. Diagnosis of AKI is made after biochemical test are ordered, such as serum blood nitrogen urea and creatinine, or clinically by measuring the urine output. Both parameters are functional and do not reflect the histological renal injury.

The RIFLE criteria, proposed by the ADQI group (Acute Dialysis Quality Initiative), graded renal injury in three levels and determined two different outcomes accordingly. It uses the serum levels of creatinine or the urine output of patients over time to grade the level of injury (Bellomo, 2004). The AKIN criteria is also commonly used in the acute setting and employs similar parameters as the RIFLE classification, but addresses only one outcome, the need for renal replacement therapy (Mehta, 2007). As **Figure 5** demonstrates, RIFLE definitions depend on a proportional increase of serum creatinine or oliguria for more than 6 hours. AKIN also defines Stage 1 injury as an increase creatinine

of ≥ 0.3 mg/dl. These definitions obviously do not fit all cases and reveal the need for more specific and practical markers of kidney injury.

| RIFLE Criteria | | | AKIN Criteria | | |
|---|---|---|---|---|---|
| Risk | ↑Cr x 1.5, eGFR decreases > 25% | UO < 0.5 ml/kg/hr (6 hrs) | ↑Cr x 1.5, ↑ ≥ 0.3 mg/dl in Cr | UO < 0.5 ml/kg/hr (6 hrs) | Stage 1 |
| Injury | ↑Cr x 2, eGFR decreases > 50% | UO < 0.5 ml/kg/hr (12 hrs) | ↑Cr x 2 | UO < 0.5 ml/kg/hr (12 hrs) | Stage 2 |
| Failure | ↑Cr x 3, eGFR decreases > 75%, Cr ≥ 4 mg/dl | UO < 0.3 ml/kg/hr (24 hrs) Anuria x 12 hrs | ↑Cr x 3, Cr ≥ 4 mg/dl with ↑ ≥ 0.5 mg/dl | UO < 0.3 ml/kg/hr (24 hrs) Anuria x 12 hrs | Stage 3 |
| Loss | Complete loss of renal function for > 4 weeks | | Patients requiring Renal Replacement Therapy are considered to have met Stage 3 | | Loss |
| End-Stage | End Stage Renal Disease > 3 months | | End Stage Renal Disease > 3 months | | End-Stage |

*Abbreviations:* Cr, Creatinine; eGFR, estimated glomerular filtration rate; UO, urine output.

**Figure 5: RIFLE and AKIN criteria of acute kidney injury (Adapted from Bellomo, 2004 and Mehta, 2007).**

**Serum Creatinine**

Creatinine (Cr) has been considered the gold standard for global kidney function evaluation. The absolute value might be affected by the rate of production, the hydration status of the patient and the type of creatinine assay. Moreover, abnormal serum levels are only observed when more than 50% of the glomerular filtration rate is affected. In unilateral kidney injury in the presence of a normal contralateral unit, creatinine may not be elevated at all. One other important drawback of serum creatinine measurement is the

need for a baseline value to compare with and to define AKI according to the appropriate classification. Because it can take more than 24-48 hours to increase in the setting of AKI it loses the diagnostic performance when there is a sudden change in the eGFR. In summary, by using serum creatinine as a marker of AKI we are delaying the diagnosis by identifying the functional consequence of the kidney damage, and not the injury by itself.

**Cystatin C**

Cystatin is a protease inhibitor produced by almost all human cells in a constant rate, it is filtered by the glomerular membrane and seems to be unaffected by factors other than the GFR. It has been proposed as an alternative option from serum creatinine to assess renal function and predict mortality and cardiovascular events. Several population-based studies have evaluated its role in the detection and prognosis of CKD finding a good association with the eGFR and better predictive rates for ESRD and survival than serum creatinine (Ingelfinger, 2013; Shlipak, 2013). Cystatin C has also been useful to detect subclinical AKI in different cohorts of ICU patients (Gaygisiz, 2016) but its role in urological patients has been inconsistent, and it has not been associated with hydronephrosis (Karakus, 2016; Madsen, 2012). Moreover, it may reflect a functional consequence of the renal damage, instead of a true injury.

**Urine output**

Low urine output, also called oliguria (< 0.5 ml/kg/hr) is considered a diagnostic parameter for AKI diagnosis (**Figure 5**), and can also be monitored to evaluate the patient's response to therapeutic maneuvers. Change in urine output is one of the first signs of kidney injury and it appears even before biochemical changes. Prerenal causes of

AKI are characterized by anuria or oliguria, while urine output in renal and postrenal situations vary from anuria to polyuria.

Several observations have been raised about the clinical utility of these definitions and the need of individualization in specific situations. The current recommendation is to use the first available serum creatinine and avoid using a historical value (Fliser, 2012). Other authors suggest the use of urine output by ideal body weight. These limitations come from the absence of evidence regarding the ideal cut-off value to determine the different stages of AKI, independently of the criteria used.

New evidence suggests that, these criteria may be replaced by biomarkers of renal tubular injury, such as KIM-1 and NGAL (Siew, 2012). Until further evidence proves the positive clinical impact of these new methods, we will continue to use the aforementioned criteria to detect patients with kidney injury.

**Urine protein and urine protein-creatinine ratio**

Proteinuria is a well-defined effect of glomerular disease and has been used to determine and monitor the grade of injury in different clinical situations. It may reflect a decreased capacity of reabsorption at the level of renal tubules, implicating tubular or interstitial damage, but an increased protein production by systemic disease (*e.g.* multiple myeloma) can also cause proteinuria by an overflow mechanism. This parameter is recommended for detecting and monitoring proteinuria in patients with CKD, but it not the best strategy to evaluate AKI because it directly relies on creatinine excretion, which is usually diminished in patients with AKI (Nguyen, 2009).

## 1.4.2 Post renal causes of AKI

Urological patients with AKI are seen in less than 10% of the hospitalized cases, but the prompt diagnosis with early relief of obstruction in these patients can prevent lifelong kidney damage (Caddeo, 2013). **Table 1** already described the most common causes associated with upper urinary tract obstruction. As urologists we face AKI in patients with bilateral hydronephrosis, most commonly caused by bladder outlet obstruction or bilateral obstructive stone disease. On the other hand, unilateral hydronephrosis is usually not accompanied by AKI, except in patients with chronic kidney disease or with a non-functioning contralateral renal unit. Despite this, the obstructive process may initiate the cascade of metabolic mechanisms that could lead to renal fibrosis.

As previously discussed, UUO is also associated with the initiation of injury mechanisms that lead to fibrosis, cellular apoptosis and chronic kidney damage. The usual clinical parameters of kidney function are insufficient to detect unilateral kidney damage in patients with a contralateral normal kidney.

We focused our research on the evaluation of AKI markers in patients with stone disease and hydronephrosis. We planned to also include patients with UPJO, which is a specific condition of upper urinary tract obstruction that is usually not associated with other inflammatory conditions.

## 1.4.2.1 Renal stone disease

Nephrolithiasis is one of the most common urological causes of emergency department visits; patients usually seek medical evaluation after an episode of renal colic. Initial evaluation is performed to determine the diagnosis, and imaging studies are completed to define the appropriate management, which depends on the clinical manifestations, association with infectious processes, and comorbid conditions. Several studies have shown an increased risk of CKD and ESRD in patients with nephrolithiasis (Keddis, 2013).

Patients requiring urgent management usually have a double J stent or nephrostomy placed after being evaluated in the emergency department. Those without an urgent indication for treatment, despite the grade of obstruction, are seen in an outpatient basis to determine the need for further management. Patients with small ureteric stones (<7mm) may be offered medical expulsive therapy to promote the passage of the calculus avoiding an invasive procedure (Miller, 2007; Nakada, 2015). Approximately 15-20% of the patients require an invasive management, either emergent decompression of the urinary tract or definitive therapy: shockwave lithotripsy (SWL), ureteroscopy (URS) and percutaneous nephrolithotomy (PCNL) (Nakada, 2007).

Specific indications for stone management are described in the literature (Preminger 2007; Türk, 2015), and are beyond the scope of this study, but to summarize, large kidney stones (usually > 2cm) are treated with PCNL, smaller kidney stones and ureteric calculi required SWL or URS. For the purpose of this study, we did not evaluate patients who had SWL because several studies have found an increase expression of AKI markers

during this treatment, which is related to injury of the renal parenchyma and might affect the interpretation of our results (Nader, 2013; Nikoobakht, 2006).

## 1.4.2.2 Ureteropelvic junction obstruction and ureteric stricture

Ureteropelvic junction obstruction (UPJO) is a urological disease encountered in the pediatric and adult populations characterized by a congenital anatomical anomaly that impairs the flow of urine from the renal pelvis to the proximal ureter. The obstruction results from an aperistaltic segment of ureter or the presence of abnormal blood vessels that cause extrinsic compression of the upper ureter. Imaging studies usually reveal a severely dilated renal pelvis with an abrupt transition to a normal size upper ureter. Some patients may have secondary renal calculi associated with long-standing obstruction, although these stones are typically not the cause of obstruction.

Regardless of the cause and the age at the time of diagnosis, the clinical assessment should determine if the mechanical obstruction affects the dynamic flow of urine to the lower urinary tract, which means there is a functional repercussion to the renal unit. A diuretic renal scan is considered the gold standard to determine the presence of functional obstruction. A flat curve and a clearance half-life of the radiotracer > 20 minutes in the renography scan is considered diagnostic of a functional UPJO (Khan, 2014). Treatment of UPJO is warranted to relief symptomatic episodes of pain, infection but mostly to avoid further deterioration of the renal function. The best treatment option is a minimally invasive pyeloplasty, performed by either a laparoscopic or robotic approach and it is associated with a success rates greater than 90% (Khan, 2014). Equivocal cases with

radiotracer half-life of 10-19 minutes were not historically considered to have functional obstruction, and clinical observation was warranted. Recent literature suggests that pyeloplasty provides adequate functional results (Ozayar, 2015). Postoperative follow-up includes a diuretic renal scan to evaluate the split renal function and rule out restenosis of the recently created ureteropelvic anastomosis.

Patients with hydronephrosis caused by nephrolithiasis or UPJO obstruction are ideal candidates to evaluate the impact of urinary tract obstruction on urinary AKI markers levels. They have preoperative radiological evaluation, which is used to plan the intervention and to determine the presence and grade of hydronephrosis. Renal function assessment is always made during the initial evaluation at the initial urological consultation. An invasive procedure is performed (URS/PCNL/pyeloplasty) to remove the cause of obstruction and follow-up studies determine the success of the intervention. The standard of care will allow the collection of urine samples and correlate clinical, radiological and biochemical findings with the levels of these markers in this set of urological patients.

## 1.5   Diuretic renography

Diuretic renography is a nuclear non-invasive study which provides a measurement of the renal function in each of the renal units, while detecting the presence of obstruction in the upper urinary tract. This test allows discrimination of the urinary tract system dilation without obstruction, from true physiological obstruction. It is based on the principle that the injected radiotracer in freely filtered and accumulated in the renal collecting system, and in the absence of urinary tract obstruction the diuretic increases the urine flow

clearing the tracer from the renal silhouette. Tracer activity along with time are plotted in a X-Y graph (Time-activity curve), which is visually evaluated with the aid of computerized software to determine the clearance half-time of the radiotracer, less ten minutes is considered normal. Several factors may play an important role in obtaining and interpreting the results, like hydration status, administrated diuretic dose and time, previously determined region of interest (ROI) and the method of evaluation (computerized vs visual) (Karam, 2003; Sarkar, 1992).

In clinical practice, a half-life clearance > 20 minutes after the diuretic dose is considered diagnostic for obstruction. Depending of the site and cause of obstruction, appropriate management should be tailored according to patient's conditions. However, some patients show clinical signs and symptoms of chronic obstruction with a diuretic renogram that fails to show obstruction (1/2 life clearance 10-20 minutes). There is no agreement about the most appropriate treatment, being observation, temporal stent placement or surgery accepted management strategies. Despite that current treatment indications are based on the renogram findings of obstruction, there is recent evidence that patients with equivocal UPJO may benefit from minimally-invasive treatment, by preserving long term renal function (Ozayar, 2015). This is also another argument that reflects that our current methods for urinary tract obstruction diagnosis are not 100% accurate, and need to be revised to improve patients' health care.

## 1.6   Markers of Acute Kidney Injury

Since it was first introduced, over 80 years ago, creatinine has been considered as an accurate parameter to determine global renal function. Despite its widespread use,

creatinine is considered a poorly sensitive marker in the acute setting, mainly because it may take 48-72 hours after the renal injury for serum levels to rise. It is usually measured in conjunction with serum urea, which accumulates in the blood stream due to ineffective glomerular function.

Both serum parameters do not reflect the renal damage in "real time". Glomerular filtration needs to decrease to more than 40-50% to show increments in baseline levels, making them insensitive for detection of acute kidney injury (Siew, 2011). Moreover, slight injury to renal parenchyma, which may be presented in cases of incomplete or unilateral obstruction, may not be detected by these standard tests.

Despite the improvements in the management of acutely ill patients, the mortality and morbidity associated with AKI has not decreased during the last two decades (Chertow, 2005). One of the main reasons is that serum creatinine reflects renal function but not real-time damage (Nguyen, 2007: McWilliams, 2014). Contrary to what has happened in myocardial infarction, where troponins are very sensitive indicators of abnormal myocardial perfusion making earlier interventions effective to improve patients' outcomes, the diagnosis of AKI is delayed by the use of serum creatinine. This shortcoming with serum creatinine has stimulated research into identifying more accurate markers of renal function (Siew, 2011).

Recently, some translational research reports suggested the promising role of proteins that are upregulated after kidney injury (Bonventre, 2014). Initially, animal experiments revealed good specificity and sensitivity of these molecules to detect renal damage after

ischemic or nephrotoxic insult (Siew, 2011), and human cohort studies have evaluated the diagnostic performance of several biomarkers in AKI and CKD (Hsu, 2015).

In fact, the American Society of Nephrology designated AKI biomarkers research as a top priority, leading to the identification of at least 20 different potential markers. A biomarker is a specific feature that can be measured and indicates a biological process (Atkinson, 2001). According to several studies and expert opinion, the most promising are NGAL, KIM-1, Interleukin-18 (IL-18), cystatin-C, liver-type fatty acid-binding protein (L-FABP), glutation S-transferase (GST) and N-acetyl-B-D-glucosaminidase (NAG). Clinical studies have analyzed their particular role in predicting outcomes of critically ill patients (Zappitelli, 2007), risk of AKI in patients after cardiac surgery (Perrotti, 2015), chronic renal injury in diabetic patients (Sabbisetti, 2014), contrast-induced nephropathy (Tong, 2015), and renal transplant patients with graft dysfunction (Alachkar, 2011; Malyszko, 2010).

These proteins are now considered as biomarkers of AKI; they can be measured in blood and urine and have shown a better correlation with the effects of AKI than serum creatinine (Siew, 2011). The detection of these molecules relies in three different mechanisms that may provide signs of renal injury in an opportune fashion, showing more sensitivity to AKI than serum creatinine:

- Structural proteins excreted during initial tubular damage
- Molecules not reabsorbed after tubular dysfunction
- Protein production after specific genes are upregulated during acute tubular injury

The third mechanism has been studied the most, with KIM-1 and NGAL as the more commonly evaluated biomarkers in basic and clinical studies (Cost, 2013; Karakus, 2016, Urbschat, 2014).

Multiple questions are still remaining, specifically about the clinical usefulness of these proteins, especially in urological patients. For example, KIM-1 has been catalogued as a marker of injury, but also some evidence exists in animal studies about a possible anti-inflammatory effect (Yang, 2015). On the other hand, NGAL can also be secreted by activated neutrophils, which may reduce the sensibility for AKI detection in patients with inflammatory conditions, other questions include:

- Is the renal obstruction associated with subclinical acute kidney injury?
- Does the hydronephrosis grade correlate with the extent of renal injury measurable by AKI markers?
- Are any of these proteins a reliable marker of hydronephrosis?
- Can we use these biomarkers in urological patients to assess other systemic conditions that may cause renal injury?

McWilliam and colleagues reported the reference intervals for KIM-1 and NGAL in healthy children analyzing more than 250 samples from UK and USA being an essential step for further use of these biomarkers in clinical settings, such investigations have not been performed in adult populations (McWilliam, 2014). In order to control the variations in hydration status and urine production, urinary biomarkers are usually normalized to the urinary levels of creatinine.

Our research was centered in the evaluation of KIM-1 and NGAL, which can be measured in urine, and are mostly expressed in renal tissue. We will present a summary of the most important information related to these two biomarkers.

## 1.6.1 KIM-1

Initially named TIM-1(Tissue Injury Molecule-1), this marker was identified using mRNA analysis after renal ischemic injury in rats. It is a transmembrane protein expressed in small amounts in normal kidney, but highly replicated in the proximal tubular epithelial cells after an ischemic insult, having a potential role in the regeneration of the normal function of the tubular cells (Ichimura, 1998). Recently, Yang and colleagues proposed an antiinflammatory effect by enhancing the phagocytic process and protecting the kidney from AKI (Yang, 2016).

KIM-1 has a immunoglobulin-like domain, specifically located in the apical membrane of proximal tubular cells. Histological analysis has revealed a predisposition for expression in the proximal renal tubule. The ectodomain is stable and appers in the urine after renal insult. It has been qualified by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for preclinical assessment of nephrotoxicity (Bonventre, 2014; Dieterle, 2010).

Several theories have been evaluated in recent years to elucidate the overexpression of KIM-1 after renal injury. Because it is highly expressed in tubular epithelium after cell injury, KIM-1 may play a role in the regeneration of epithelial cells by facilitating their restoration, but other studies have shown that KIM-1 has a renoprotective effect, by

facilitating the apoptotic response of injuried cells (Bonventre, 2014). Despite the evidence regarding the possible protective effect of KIM-1, chronically injured cells also express KIM-1 on their surface augmenting the interstitial fibrotic process, Whether the expression is mainly affected by acute or chronic injury, a consensus exists regarding the limited expression in healthy kidney tissue.

Animal studies have demonstrated an increased expression of urinary KIM-1 after renal insult. A rat model experiment found an increased expression of tissue and urinary KIM-1 in animals after cisplatin-induced nephrotoxicity; it was the most sensitive marker, correlated with the progression of kidney lesion and was observed before histological injury (Vinken, 2012). Vaidya and colleagues found a significant increment in urinary KIM-1 levels after bilateral renal ischemic injury in rats, while serum creatinine remained within normal limits (Vaidya, 2009).

Several clinical studies have demonstrated the relationship between KIM-1 expression and chronic and acute kidney injury. It has been found to be a good predictor of postoperative AKI in patients undergoing cardiac surgery, even before serum creatinine elevation (Han, 2009). Sabbisetti and collegues found that elevated serum KIM-1 levels predicted the rate of eGFR and the risk of developing ESRD in Type 1 diabetic patients (Sabbisetti, 2014).

Our Urology department investigated the expression of KIM-1 after shockwave lithotripsy in kidney stone patients. We demonstrated higher urinary KIM-1 levels in stone disease patients compared to healthy subjects, and we noted a significant increment

in the urinary levels hours after the lithotripsy suggesting transient renal tissue injury caused by the SWL (Nader, 2013).

Some case-control studies have found elevated urinary levels of KIM-1 in patients with obstructive nephropathy compared to controls. Wasilewska and collegues compared pediatric patients with UPJO to patients with mild hydronephrosis and healthy children and found higher preoperative KIM-1 levels in those with UPJO, decreasing up to 75% three months after the pyeloplasty (Wasilewska, 2011). Another study from China, evaluate the expression of KIM-1 and NGAL in patients with AKI and obstructive nephropathy and found a good predictive value to diagnose AKI, furthermore the same group demonstrated that postoperative levels of these markers were predictors of renal function at one year measured by serum cretininea, albeit radiological information was missing and patients had severely impared renal function (Xie, 2014; Xue, 2014).

## 1.6.2 NGAL

Neutrophil gelatinase-associated lipocalin (NGAL, lipocalin 2) is a 178-aminoacid polypeptide chain which belongs to the lipocalin superfamily, and was originally isolated from neutrophils (Kjeldse, 1993). Different molecular forms have been described and will be discussed later, but several clinical studies have concluded it is a sensitive biomarker of AKI (Mårtensson, 2014). Most of the information regarding the origin and biochemical role comes from animal studies, and several theories have been created to explain its function after renal injury.

Zappitelli and collegues found urinary NGAL to be useful marker of AKI severity in critically-ill children (Zappitelli, 2007). Kuwabara investigated urinary and serum expression of NGAL in mice with obstructive nephropathy. Unilateral ureteral obstruction causes a 100-fold increment in NGAL synthesis in distal nephrons, which suggests damage to tubular epithelia (Kuwabara, 2009). Other AKI studies have confirmed the increased synthesis in tubular epithelia, but models of chronic injury have found that urinary NGAL also correlates with the eGFR.

NGAL is not only secreted in renal tissue, it has also been isolated in hepatocytes and immune cells, being involved as part of the innate response against gram-negative bacteria. In fact, it was initially discovered in the immune response during bacterial infections, because it binds to siderophores for iron transport and prevents the growth of bacteria dependant on iron supply. Xu and collegues compared NGAL levels in patients during an acute bacterial or viral infection and found a positive correlation of its levels with a bacterial infection (Xu, 1995). Genetically modified mice lacking both NGAL gene copies have been found to have an increase risk in *Escherichia coli* infections (Berger, 2006), and a recent small clinical study demonstrated that pediatric patients with recurrent UTI have decreased urinary NGAL levels compared to patients without recurrent UTI (Forster, 2014). Recent studies have address the prognostic value of plasma NGAL (Total NGAL) as a marker of systemic inflammatory response symptoms (SIRS) and sepsis, it was found as a predictor of mortality and multiple organ dysfunction (Wang, 2015). NGAL has been evaluated after renal ischemic injury: van den Akker *et al* found that serum NGAL was a good predctor of delay graft function after kidney transplantation. A small prospective cohort study patients undergoing partial

nephrectomy failed to show postoperative urinary NGAL as a predictor factor of renal injury (Sprenkle, 2013; van den Akker, 2015). Whether NGAL is most specific as a marker of systemic inflammatoion or renal injury remains unanswered (Martensson, 2014).

Differences in the expression of NGAL in several clinical situations are explained by the presence of different molecular forms of NGAL in urine and plasma. Cai *et al* analyzed urine samples of patients after cardiac surgery, patients with UTI and in incubated HK-2 (human kidney epithelial) cells by using Western blot analysis they determined the origin of the 3 molecular NGAL forms (Cai, 2009). The 21-25-kD monomer was primary secreted by HK-2 cells and to some extent by the activated neutrophils, and may reflect the severity of tubulointestitial damage (Nickolas, 2012). The 45-kD disulfide-linked homodimer was secreted by neuthrophils and the 135-kD heterodimeric, conjugated with gelatinase, also secreted by ephitelial cells, found in small quantities only (Glassford, 2013; Nickolas, 2012).

Their findings were supported by the discovery of elevated levels of urinary Monomeric NGAL in patients with AKI after cardiac surgery, while patients with UTI, exhibited an increased level of the dimeric form. Authors also found differences in the specificity of the antibody epitope of both forms, which was used to develop specific essays for each molecular form.

The vast majority of published studies about NGAL include the use of essays with some kind of cross-reaction between the monomeric and homodimeric form. This could explain the differences in clinical performance of the test, and the contradictory results.

Moreover, multiple studies about AKI excluded patients with conditions that may interfere with the expression of this marker, such as stone disease. For example, Holzscheiter confirmed the role of NGAL as a promising tool to detect tubular injury but it was influenced by the grade of leukocyturia (Holzscheiter, 2014).

Nickolas and collegues evaluated the relationship between urinary Monomeric NGAL measured by immunoblot and the histological evidence of tubulo-intestitial damage in patients with CKD. Their findings support the theory that Monomeric NGAL reflects tubular damage. They found a higher correlation with tubular athrophy and intestitial fibrosis, although Monomeric NGAL did not correalted with leukocyte infiltrate or mesangial proliferation, suggesting a better association with chronic rather than acute processes (Nickolas, 2012).

Mårtensson and colleagues were able to characterize the expression of Monomeric NGAL by renal epithelial cells from the homodimeric form secreted by neutrophils using two different ELISA assays (Mårtensson, 2012). Recently, a human monomeric-specific NGAL ELISA kit was made commercially available (Bioporto, Hellerup, Denmark), and showed <1% cross-reactivity with the homodimer form. This test improves the ability to detect Monomeric NGAL, which in the absence of inflammatory conditions could be mainly released by renal tubular cells (Bangert, 2012).

## 1.6.3 Other biomarkers

Some other molecules have been investigated in basic and translation research as potential markers of kidney injury. Animal and human studies have shown contradictory

results regarding the expression of cystatin, B2-microglobulin, interleukin-18, osteopontin and liver-type fatty acid-binding protein (L-FABP). We therefore elected to study the two markers showing most promise, KIM-1 and NGAL.

Despite the recent investigations, there was a need to prospectively evaluate the role of these markers in urological patients with urinary tract obstruction and analyze their expression considering the grade of obstruction and the presence of inflammatory conditions, such as stone disease or ureteric stents. Similarly, the use of a novel assay to determine the specific expression of Monomeric NGAL might aid in the evaluation of AKI in urological patients who usually suffer inflammatory conditions in the urinary tract that may affect the levels of NGAL. We believe our study will provide sufficient information to determine if these molecules are useful in the evaluation of urological patients.

## 1.7   Hydronephrosis

The term hydronephrosis is an anatomical denomination commonly used to describe dilatation of the renal pelvis and/or calyces (Cho, 2007; Ito, 2010). Hydronephrosis is not a synonym of obstruction, but is usually associated with it, except in cases of vesicoureteral reflux, congenital megaureter or in patients with acute pyelonephritis accompanied by a non-peristaltic ureter, as we previously discussed. It is a radiological diagnosis, usually made after a renal ultrasound or a CT scan. In clinical practice it is commonly accepted that the grade of hydronephrosis correlates with the grade of obstruction, although this is just a supposition, because diuretic renogram is required to determine the presence and grade of obstruction.

The most acknowledged classification for hydronephrosis comes from the Society of

Fetal Ultrasound (SFU), which uses coronal and axial views of abdominal ultrasound to

assess the aspect of the renal parenchyma and the collecting systems (Fernbach, 1993).

This classification has been generalized and used as standard for adult population studies.

Cho and Ito described a similar hydronephrosis scale using CT findings in patients with

upper urothelial carcinoma to predict aggressiveness of the disease (Cho, 2007; Ito,

2010). The following table correlates both classifications:
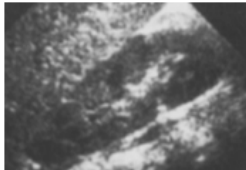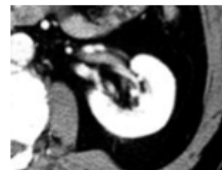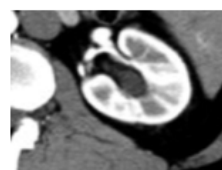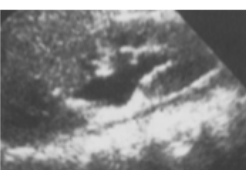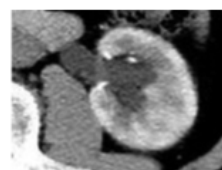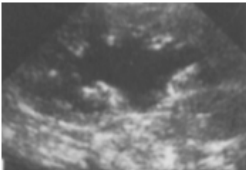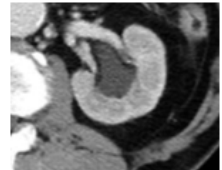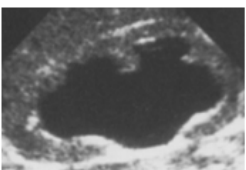


**Table 2. Grading of hydronephrosis according to SFU and CT criteria (Adapted with permission from Fernbach, 1993, and Ito, 2011).**

In clinical practice, and according to the treatment guidelines for stone disease, only the cases associated with septic processes and severe renal impairment are treated in an urgent fashion (Preminger, 2007; Türk, 2014). Most of the patients with stone disease and hydronephrosis have to wait weeks to months to receive definitive management. These patients have an increased risk to suffer "subclinical" renal injury, because creatinine levels are within normal limits, but the deleterious pathophysiologic process of obstruction lasts until the obstruction is mitigated.

Another important factor that is difficult to extrapolate to clinical practice is the possibility of having a partial or a complete ureteral obstruction. The grade of hydronephrosis does not always correlate with the grade of obstruction. Several other factors play an important role, such as hydration status, split renal function, and time of onset. Complete obstruction of urine flow through the ureter might be accompanied by urine flow into venous or lymphatic systems. Whether this affects the pathophysiology of obstruction is not yet determined, but will probably be negligible.

## 1.8  Charlson comorbidity index (CCI)

The Charlson Comorbidity Index is a commonly used categorization that was described in 1987 to grade comorbid conditions, which may affect the mortality of the patients in longitudinal studies. It is widely used in the medical literature to evaluate the most significant comorbidities that may affect the expectancy of life of the patients. Initially included patient's age and 19 different conditions, it was recently updated to include 23 items that received a score, to predict the 10-year survival. CCI has been validated and accepted to describe patients' comorbidities (Charlson, 1987; Charlson, 2008). We

planned using this index to determine and compare the baseline comorbidities of the patients.

## 1.9  Purpose of the study

This study will analyze the role of 3 AKI markers in urological patients: KIM-1, Total and Monomeric NGAL. To the best of our knowledge, there are no published studies describing the different isoforms of NGAL in urological patients. The main objective of our research is to determine the urinary levels of KIM-1, Total and Monomeric NGAL, before and after treatment in patients with urinary tract obstruction caused by intrinsic ureteral obstruction or stone disease. The results of this research may help design a larger multicenter study to assess the cost and time for diagnosis and follow-up of patients with hydronephrosis related to UPJO or stone disease. Additionally, we will be able to detect if patients with hydronephrosis due to obstruction and normal kidney function, according to serum creatinine, also have subclinical AKI as shown by an increase expression of urine markers.

If we prove our hypotheses to be correct, urinary AKI biomarkers may aid in the diagnosis and discrimination of patients with obstruction (caused by stone or by UPJO) and kidney injury with the ease of a simple urine test. Our results may help reduce the reliance on imaging that is costly and may be associated with ionizing radiation exposure. Furthermore, overall results will provide information about the safety of delaying some interventions in patients with hydronephrosis without urinary tract obstruction. Moreover, a urine-based test that could be employed at the point of care might permit more timely intervention. These results may offer evidence regarding the

impact of stone disease on the urinary levels of AKI and might give useful information about the inclusion of urologic patients in large trials for AKI evaluation through urinary markers analysis.

Our research project is intended and designed to evaluate the levels of the most commonly investigated urinary markers in patients with unilateral upper urinary tract obstruction. We expect to assess the following central hypothesis:

a. Baseline urinary levels of KIM-1 and Monomeric NGAL will be statistically significantly higher in patients with unilateral renal obstruction caused by either the presence of a urinary stone or by an ureteric stricture compared to urinary levels after the treatment of obstruction.

Secondary hypotheses to be considered are:

a. KIM-1 and Monomeric NGAL will be statistically significantly different between patients with and without obstruction

b. KIM-1 and Monomeric NGAL levels will be positively correlated with the the grade of hydronephrosis and the overall kidney function measured by the eGFR.

c. Monomeric NGAL will have a better correlation with obstruction and renal function than Total NGAL

We expect that patients with hydronephrosis due to obstruction will have higher levels of KIM-1 and Monomeric NGAL compared to healthy subjects and to patients with non-obstructing stone disease.

Additionally, we plan to determine if patients with hydronephrosis and normal kidney function have subclinical AKI as shown by an increased expression of urinary biomarkers. If we prove our hypotheses to be correct, urinary AKI biomarkers may aid in the diagnosis and discrimination of patients with hydronephrosis as to the presence or absence of physiological obstruction with the ease of a simple urine test. These results may also provide evidence regarding the impact of stone disease in the urinary levels of AKI.

## 2  Study design

This prospective cohort study was planned to observe the clinical course of patients with unilateral renal obstruction causing hydronephrosis due to stone disease or intrinsic ureteral disease, and to explore the expression of KIM-1, Total and Monomeric NGAL within the usual standard of care. We followed the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines (von Elm, 2008; **Appendix 1**) and the clinical study was approved by the Western University Health Science Research Ethics Board (**Appendix 2**).

Our main objective was to determine the urinary levels of KIM-1 and NGAL (Total and Monomeric isoform), before and after treatment, in patients with hydronephrosis caused by intrinsic ureteral obstruction or stone disease. We chose these sets of patients for two reasons: Stone disease is the most common cause of upper urinary tract obstruction and affects many patients. Patients with intrinsic ureteral obstruction comprise a special situation where diagnosis and follow-up may be challenging with conventional imaging, and require the use of renography for diagnosis and follow-up.  The potential impact of our results could therefore be very relevant.

Additionally, by including patients with obstruction without kidney stones and patients without obstruction and kidney stones we would know whether there are differences in the expression of Monomeric NGAL. Inflammatory conditions are thought to cause an increment in the Total NGAL concentration, but theoretically will not significantly affect Monomeric NGAL levels. We also aim to assess the expression of these markers in patients with stone disease and no hydronephrosis and in a healthy control group because

the medical literature does not have clear evidence about the pattern of expression of these proteins in these populations.

All this information will allow a comprehensive evaluation about the clinical utility of these markers in urological patients. The study design will facilitate the evaluation of urinary marker expression in a longitudinal approach: when obstruction is present, and after the obstruction is relieved. Appropriate clinical and radiological follow-up will strengthen the results and several analyses will be performed to explore the role and the factors affecting the expression of these markers in urological patients.

We have already stated that our primary focus will be to assess the expression of KIM-1 and both Total and Monomeric NGAL in patients with hydronephrosis, but we also plan to analyze the differences in the expression of these urinary markers in patients with inflammatory stone disease and indwelling ureteric stents, where an inflammatory response may be present. We hypothesize that KIM-1 and Monomeric NGAL will be elevated in patients with hydronephrosis. We also hypothesize that these two markers would correlate to the grade of hydronephrosis and overall renal function. In order to complete the planned analyses, this cohort study investigated 3 different groups:

1. Patients with hydronephrosis
2. Patients with stone disease and no hydronephrosis
3. Control group of subjects without stone disease or hydronephrosis

Based on the standard of care in our urological practice, we were able to prospectively evaluate the concentration of urine markers in patients during their treatment and follow-up. This gave us different patient populations that were evaluated independently and

compared among them longitudinally. Stone disease diagnosis and follow-up require imaging studies, such as Computed Tomography scan (CT scan) or a renal ultrasound which are usually performed as part of the standard of care by the treating urologists. This information was used to determine the presence or absence of hydronephrosis to include them in one of our 2 studied groups of patients and evaluate the stone characteristics.

A preoperative urine sample was collected, along with two postoperative urine samples. Patients who required a double J stent placement during the surgical procedure, were followed during the two succeeding appointments to evaluate clinical and radiological parameters and collect urine samples. During the third visit, patients would no longer have a ureteric stent, and the levels of the urine markers should not be affected by the stent, but might be affected by the presence of residual stones.

We anticipated that some patients with ureteral stones, while waiting for the surgical procedure could have spontaneously passed the stone. These patients were asked to give a second urine sample during the next clinic visit, after stone passage in order to complete the study. Likewise, in very selected cases, patients did not require ureteric stent placement after the surgical management of their stones and they completed the follow-up after only one postoperative visit.

The design of this study allowed a comparison of urine marker levels during the initial urological evaluation between two groups of patients and the control group. As there is some evidence suggesting a possible influence of inflammatory conditions on AKI markers expression, we decided to evaluate biomarker concentration using the third urine

sample, when patients do not have stents and the burden of residual stones is lower. Furthermore, we were able to explore if urinary markers expression correlates with the grade of leukocyturia. The following diagram illustrates the recruitment and flow of patients' assessment along the study:
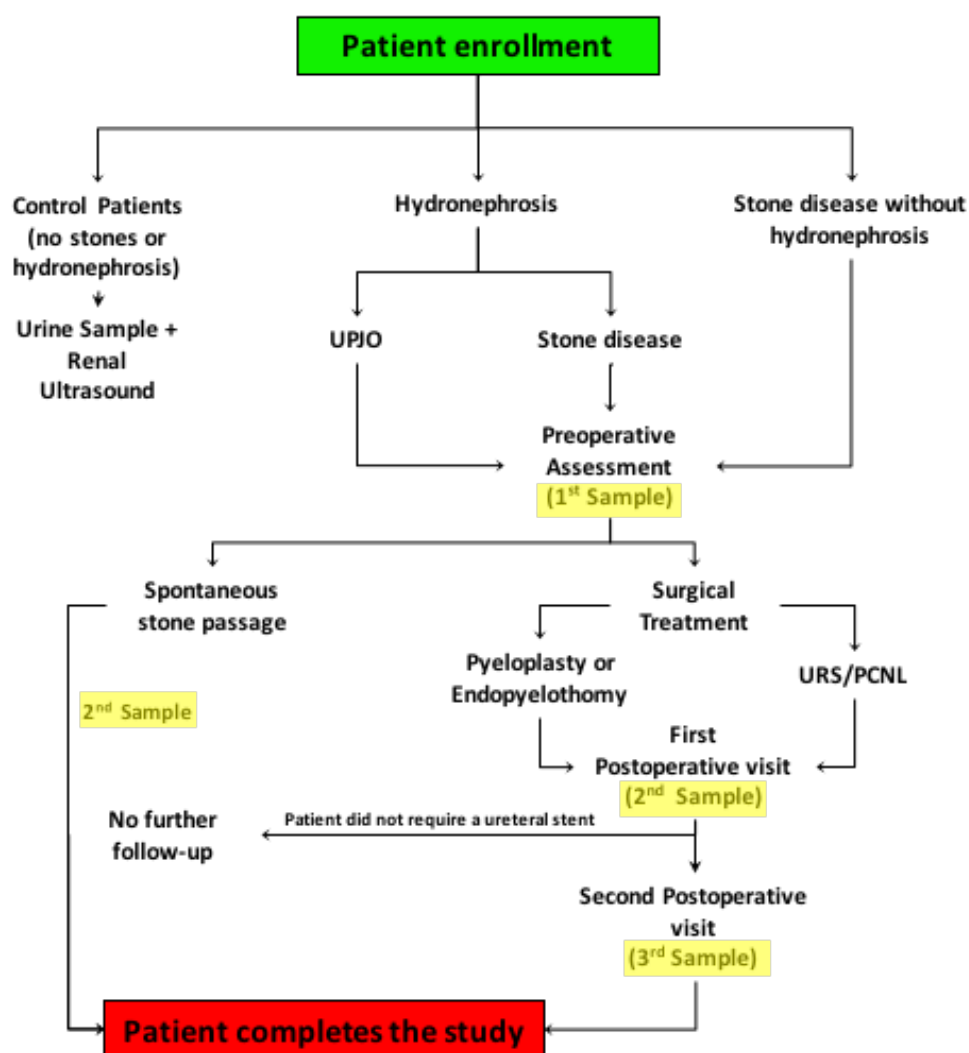


**Figure 6: Patient recruitment algorithm.**

## 2.1  Sample size calculation

No studies exist in the literature about the role and urine levels of Monomeric

NGAL in urological patients. The information available came from trials performed

in ICU (Intensive Care Unit) patients with sepsis and AKI. They used two different

assays and reported median urinary concentrations of monomeric NGAL between

145-350 ng/mL (Martesson, 2012; Bangert, 2013). This data cannot be used to

calculate an appropriate sample size because our study involves patients in a

different clinical scenario and who might have subclinical AKI, expecting lower

biomarker levels. We propose an exploratory analysis for the Monomeric NGAL

isoform, therefore the calculation was based on previous urological literature on

KIM-1.

As we previously discussed, literature about these biomarkers in urological patients

is sparse, and only few studies longitudinally analyze KIM-1 urinary concentration

in pediatric patients. According to previous data about the adjusted KIM-1

concentration in urine from our centre (Fahmy, 2013), we were able to estimate a

sample size (Wan, 2014). Using an $\alpha$ value of 0.05, with a power of 0.90 and a

calculated effect size of 0.7, we needed 24 patients with hydronephrosis to detect a

2-sided difference in KIM-1 levels  before and after the intervention. On the other

hand, if we included 24 patients in each of the groups (hydronephrosis and no

hydronephrosis) we will be able to detect a significant difference in KIM-1 levels

between the two groups, with a power of 0.77, keeping an $\alpha$ value of 0.05.

A 1:2 ratio of control to study subjects will allow an adequate statistical analysis to compared the findings all groups of patients. Clinical follow-up included 2 postoperative visit, and we expected a possible 20-25% lost of follow-up, which is considered acceptable for this type of study (Kristman, 2004). We planned to recruit 30 patients in each of the study groups to complete the calculated sample size.

## 2.2  Recruitment and follow-up

Exclusion and inclusion criteria were assessed after patients were clinically evaluated and the surgical treatment was decided during their Urology Clinic visit. The study was explained to potential candidates, which were invited to participate in our trial. Patients willing to participate signed appropriate documents according to out Ethics Board Committee guidelines (Consent Form/Letter of Information) and were asked to give a mid-stream urine sample (**Appendix 3**).

Briefly, to be included in the "hydronephrosis group" patients needed to have unilateral ureteric stricture or unilateral stone disease, both associated with unilateral hydronephrosis, amenable to treatment by ureteroscopy, percutaneous nephrolithotomy or pyeloplasty. Patients recruited in the "no hydronephrosis group" needed to have imaging studies confirming the presence of unilateral stone disease in the absence of hydronephrosis, and requiring an endourological procedure as definitive stone treatment.

All patients with active urinary tract infection, evidence of bilateral stone disease, preoperative presence of ureteric stent, use of indwelling bladder catheter or recent

history of sepsis were excluded from the study. **Appendix 4** describes the specific details for each inclusion and exclusion group criteria. This study was conducted at St. Joseph Health Care (SJHC) London, and all recruited patients were required to have postoperative follow-up in our Urology Clinic.

After patients agreed to participate, demographical, clinical, and radiological variables were evaluated from patients' office charts and hospital electronic records to complete the data collection. It is the standard of practice that patients have a complete medical assessment the first time they visit the Urology Clinic, and these variables are evaluated by the treating urologist when discussing with the patient the best treatment option. The following table describes the variables evaluated at the first visit.

| Table 3: Baseline variables | | | | |
|---|---|---|---|---|
| **Demographics** | **Medical history** | **Clinical** | **Radiological** | **Laboratory** |
| Age<br>BMI<br>Gender | CCI<br>    Diabetes, HTN<br>History of stone disease<br>Previous stone disease<br>treatment | Planned procedure<br>Recent visit to ER<br>Episode related to renal<br>colic | Type of imaging study<br>Presence of stone<br>Presence of ureteric stricture<br>Presence of hydronephrosis<br>Grade of hydronephrosis<br>Single/multiple stone disease<br>Location of the stone<br>Largest diameter of stone<br>Incidental findings in imaging | Preoperative SCr<br>eGFR (CKD-EPI)<br>Presence of leukocyturia<br>Urinary creatinine<br>Urinary AKI markers<br>    KIM-1, Monomeric<br>    & Total NGAL |

*\* BMI, body mass index; CCI: Charlson Comorbidity Index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; ER: Emergency Room; HTN: hypertension; SCr, serum creatinine; UA: urinalysis; UPJ: ureteropelvic*

**Table 3. Evaluated baseline characteristics.**

Patients then went on to have their surgeries at SJHC, with regular postoperative follow-up visits in the urologists' office. We kept track of these visits and according to our follow-up algorithm urine samples were collected and postoperative

information was recorded. The following table describes the clinical and radiological variables evaluated after the surgical management of all patients.

| Table 4: Follow up information collected during first and second postoperative visits |
|---|
| Presence of ureteric stent |
| Imaging study for follow up |
|     CT-scan, KUB, US |
| Presence of residual stones |
|      Single/Multiple, Location |
| Presence and grade of hydronephrosis |
| Urinalysis results |
| Urinary creatinine |
| Urinary AKI markers |
| Presence of incidental findings on imaging studies |

*\* KUB: kidney-ureter-bladder simple plain film; US: ultrasound*

**Table 4. Information collected during follow-up visits.**

Radiological information was assessed by the treating urologist and was included in the main database after a careful evaluation of the images in our Centricity, One View electronic imaging system (GE Healthcare, Barrington, IL, USA). Axial and coronal reconstruction images from the CT scans were evaluated to determine the presence and dimensions of the stones. Patients without CT scan, had a KUB and a renal ultrasound to assess stone characteristics and determine the presence of hydronephrosis.

The grade of hydronephrosis was determined by evaluating CT scan axial images at the level of the renal pelvis and calices or transversal renal ultrasound images, according to the hydronephrosis classification previously discussed (**Table 2, Chapter 1**). Incidental findings on imaging studies, such as presence of renal cysts, tumors, or anatomical anomalies were detailed.

Serum creatinine is routinely measured in patients before the surgical procedure. The value was recorded and the eGFR calculated using the CKD-EPI formula (Levey, 2009). Presence and total leukocyte count per μL of urine and normalized values of AKI biomarkers (according to urinary creatinine) were recorded.

Healthy subjects from hospital staff were invited to participate as the control group. A medical interview was completed to evaluate previous medical conditions and to exclude family and personal history of kidney stones. A bilateral renal ultrasound was performed by a Urologist, transverse and axial views were evaluated to rule out the presence of hydronephrosis and kidney stones (Hitachi-Aloka, ProSound SSD 3500, CT, USA; 3.5 Mhz convex transducer). A negative urinalysis was the last inclusion criteria for controls. Subjects with any abnormality in the renal ultrasound or an abnormal urinalysis were contacted to have an appointment with a general practitioner, and were excluded from the analysis.

The recruitment period lasted 10 months, from March 2015 to January 2016. All follow-up visits were completed by February 2016. All patients and controls were assessed in the Urology Clinic at St. Joseph Health Care, London, Ontario.

## 2.3  Urine sample collection

Mid-stream urine samples from all participants were collected in a 100 mL sterile plastic urine container. A 8 mL conical urinalysis tube was used to collect a portion of the sample and sent for urinary creatinine measurement and routine urinalysis to the main laboratory facilities.

We centrifuged 10-15 mL of urine for 5 minutes at 1000 rpm at room temperature. The urine supernatant was aliquoted in 1.5 ml Eppendorf tubes and stored at -80C at our facilities for further AKI markers analysis. All initial samples were labeled with a unique study number and a research number to cross match and de-identify the results. Sample handling followed published recommendations to avoid denaturalization of the markers and were stored within 2 hours of initial collection (Parikh, 2014).

## 2.4  Urine sample analysis

Urinary creatinine was measured by enzymatic colorimetric method using the Roche Modular P Chemistry analyzer, and results were reported as mmol/L (reference ranges: male, 3.5-25mmol/L; female, 2.6-20mmol/L).  A conversion factor of 88.42 was used to express the creatinine concentration in mg/dl from μmol/L. Urinalysis was initially performed using dipstick (Multistix SG, Siemens, Germany) which includes chemical examination for protein, blood, glucose, ketones, gravity, nitrite, leukocytes and blood. A microscopic analysis was done only if the dipstick revealed abnormal results, and the total number of red and white blood cells (cells/high power field) was recorded. The normal range for microhematuria and leukocyturia in the microscopic examination is < 5 cells/hpf (Lab Test Information Guideline, SJHC).

## 2.5  AKI biomarker levels

AKI marker analysis was comprised in 3 different assays; one each for KIM-1,

Monomeric and Total NGAL. After finishing the recruitment phase the samples

were thawed at room temperature and were analyzed according to the

manufactures specifications of each of the ELISA kits. All urine samples were

diluted to adjust the effective pH for each antibody reaction and the detection limit

of each ELISA experiment.

## 2.5.1  KIM-1

A commercially available ELISA kit (Human TIM-1/KIM/HAV, R&D Systems,

Minneapolis, MN, USA) was used to determine urinary KIM-1 concentration in all urine

samples. The kit came with a microtiter plate coated with a KIM-1 antibody which binds

to the KIM-1 molecules in the sample, HRP streptavidin which binds to KIM-1 antibody,

TMB substrate which detects HRP streptavidin, KIM-1 standards and buffers. Either

standards or 1:4 dilution of each urine sample followed by biotinylated antibody which

interacts with the KIM-1 antibody followed by Streptavidin/Horseradish peroxidase was

added to each well of the microtiter plate. In between each addition plate was incubated

for 30min at room temperature with mixing at 200rpm. After final 30min TMB substrate

was added.  A blue color was generated in the solution and the intensity of blue color was

correlated to the number of KIM-1 molecules in the urine sample. Plate was incubated

exactly 20min at room temperature and the reaction was stopped by changing the pH of

the reaction mixture with stop solution.  Absorbance readings of each well at 590nm and

260nm were recorded using the EON BIOTEK plate reader supplied with Gen5 software.

Background absorbance of each sample was corrected by subtracting the 260nm reading from that of relevant 590nm one. This whole process was conducted in duplicates. Standard curve for each plate was created using the absorbance readings of standards and KIM-1 concentration of each sample (40samples / plate) were calculated.

## 2.5.2 Total NGAL

The total NGAL ELISA kit (Bioporto Diagnostics, Hellerup, Denmark) was used to determine the concentration of total NGAL (the three different isoforms of NGAL: monomeric, homodimeric and MMP-9/NGAL) in urine samples (Pedersen, 2010). Analysis method was same as KIM-1 but with the plates were coated with antibodies which were specific for total NGAL and the incubation period after each addition was 1 hour instead of 30mins. Urine samples used in this analysis were diluted in 1:500 according to the manufacturer's instructions.

## 2.5.3 Monomeric NGAL

Monomeric NGAL was measured using a commercially available Monomeric NGAL ELISA kit (Bioporto Diagnostics, Hellerup, Denmark) which contains Monomeric NGAL specific antibody coated microtiter plate. Analysis procedure was exactly same as that of total NGAL

Absorbance values that were lower or higher than the detection limit of each analysis were re-analyzed using either lower or higher sample dilutions. Each of the obtained values for all three biomarkers was normalized against creatinine levels of the relevant urine sample by simple division, using the following formula:

$$\frac{[\text{AKI marker}]\ \text{ng/mL}}{[\text{Urine Creatinine}]\ \text{mg/mL}} = [\text{AKI marker}]\ \text{ng/mg Creatinine}$$

## 2.6  Statistical analysis

Our main hypothesis stated that urinary biomarkers would be significantly different before and after the relief of the obstruction. We have formulated the following hypothesis for the statistical analysis, allowing an $\alpha=0.05$:

$H_O$: Median preoperative biomarker levels = Median postoperative biomarker levels

$H_A$: Median preoperative levels $\neq$ Median postoperative levels

Demographic, clinical and biochemical characteristics were analyzed depending on the type of variable. **Appendix 5** describes the coding of all the analyzed variables during the recruitment and follow-up of participants. Correlation between hydronephrosis grade and urine marker levels was evaluated with Spearman coefficient. Continuous variables were analyzed with Shapiro-Wilk test to determine the normality of the data distribution. Dichotomous variables were analyzed using Chi-square and Fisher's exact test and continuous variables were compared with T-student or Mann-Whitney U test according to the type of distribution.

The non-parametric Levene's test was used to evaluate the variances between the the distribution of the biomarkers levels in the entire cohort. Normalized urine marker levels were assessed among the different groups using Mann-Whitney U test. Median

preoperative and postoperative levels were compared with the Wilcoxon signed rank test. We evaluated the correlation between urinary biomarkers levels and presence of leukocyturia, stone disease, grade of hydronephrosis and eGFR. De-identified biomarker results, clinical and radiological data were stored in an electronic database and all information was analyzed with SPSS v.20 (Armonk, NY, IBM), P-values were derived from 2-tailed test, and a p<0.05 was considered statistically significant.

## 2.7  Funding

# 3 Results

We were able to complete the recruitment and follow-up of patients according to the previously calculated sample size. We decided to initially analyze the urinary biomarkers' concentration of only the first and third urine sample, to avoid the possibility of highly abnormal values related to the presence of leukocytosis in patients with ureteric stent. These initial results would provide some light about the practicality of evaluating the second urine sample and save cost of this research project, because high values would require repeating ELISA analysis, which may increase the initial planned budget.

A total of 66 patients and 12 controls were initially included in our study. **Figure 7** is the flow diagram of the cohort, which shows that 9 patients were initially excluded from further follow-up due to incomplete baseline information or changes in their management strategy. A total of 44 patients completed the follow-up; 24 from the hydronephrosis group and 20 from the no hydronephrosis group. Some patients spontaneously passed the stone and some others decided to have their clinical follow-up in another city after the surgical intervention. Only one control subject had an abnormal urinalysis, and was excluded from the study. Postoperative assessment was conducted with a median of 68 days (IQR 47).

The main hypothesis involved the comparison of AKI markers in patients with hydronephrosis, and the number of patients recruited fulfilled the sample size calculation. The statistical analysis for the secondary hypotheses comprised the information from all patients included in the no hydronephrosis group and the 24 patients who completed the follow-up in the hydronephrosis group.

**78 Subjects**
Total Recruited

**66 patients**

**12 Controls**

9 patients
Excluded

1 Control
Excluded

33 patients
Hydronephrosis
Group 1

24 patients
No Hydronephrosis
Group 2

Group 3

9 patients
Lost to follow up

4 patients
Lost to follow up

**24 patients**
**Completed Study**

**20 patients**
**Completed Study**

**11 Controls**
**Completed Study**

*Excluded patients: 4 patients did not have follow-up at our center; 3 patients had not finished the follow up at the end of the recruitment phase and the surgical plan changed in 2 patients after initial enrollment. Excluded controls: one with abnormal urinalysis. All patients described as "Lost to follow up" were missed in the postoperative visits or had incomplete radiological studies.*

**Figure 7: Flow chart of patients recruited and patients included in the final analysis.**

## 3.1  Expression of biomarkers in the hydronephrosis group

A total of 24 patients recruited in the hydronephrosis group completed the follow-up.

Stone disease was the most common cause of obstruction in our hydronephrosis cohort

(22/24 patients), while one patient had congenital UPJO obstruction and one had an

acquired ureteric stricture in the mid-ureter. In 63% of the cases the diagnosis was made

after an episode of acute renal colic, and most of the patients showed moderate

hydronephrosis (grade 2,3) at the time of enrollment. According to the serum creatinine

values, only 4 patients had abnormal preoperative levels, and the median eGFR of the

group was 85 ml/min/1.73m$^2$.

Twenty patients had a ureteroscopy performed, two patients passed the stone while

waiting for the surgical treatment, one had a PCNL to deal with a stone located in the

renal pelvis, and one patient had a robotic pyeloplasty. All procedures were performed

without complications, and one patient with an impacted ureteric stone required

placement of a nephrostomy tube to relieve the obstruction. After completing follow-up,

all patients were stent free and 21 patients did not have hydronephrosis in the

postoperative studies, and 7 showed residual stone fragments. Only three patients had

hydronephrosis at the last study evaluation, two of them had had an impacted ureteric

stone associated with grade 4 hydronephrosis and the patient with UPJO had mild

hydronephrosis during the last follow-up visit.

Demographic characteristics were similar between patients and controls. **Table 5** shows

the comparison in the demographic, clinical and radiological characteristics. The

distribution of the absolute and normalized values of Total NGAL, Monomeric NGAL

and KIM-1 were statistically significantly different between hydronephrotic patients and

controls. **Figure 8** shows the boxplot graphs of the normalized levels of the three urine

biomarkers in the hydronephrosis and control groups.

| Table 5. Comparison between baseline characteristics of patients with hydronephrosis and control group | | | | |
|---|---|---|---|---|
| | | **Hydronephrosis Group** | **Controls** | ***p value*** ✻ |
| | ***n=*** | 24 | 11 | |
| **Age** | *Median (years), IQR* | 58.5, 15 | 50, 21 | 0.09 |
| **Gender** | *Female/ Male* | 9/15 | 4/6 | 0.66 |
| **BMI** | *Median (kg/m2), IQR* | 30.7, 11.6 | 27.9, 9 | 0.14 |
| **CCI** | *Median (IQR)* | 1 (3) | 1(1) | 0.16 |
| **Diabetes/Hypertension** | | 6/9 | | |
| **Previous history of stone disease** | | 13 | | |
| **Diagnosis related to acute renal colic** | | 15 | | |
| **Imaging study** | *CT scan/Renal ultrasound* | 16/8 | | |
| **Cause of obstruction** | *Stone* | 22 | | |
| | *UPJO/Stricture* | 2 | | |
| **Site of obstruction** | *Renal pelvis/UPJO* | 1/1 | | |
| | *Ureter* | 22 | | |
| **Planned procedure** | *Ureteroscopy* | 22 | | |
| | *Pyeloplasty* | 1 | | |
| | *Endopyelothomy* | 1 | | |
| **Side** | *L/R* | 12/12 | | |
| **Largest diameter of stone** | *Median (mm), IQR* | 7 (4) | | |
| **Hydronephrosis** | *Grade 1 / 2 / 3 / 4* | 4 / 12 / 6/ 2 | | |
| **Preoperative S Cr** | *Median (mmol/L)* | 85.5 (32) | | |
| **Preop eGFR** | *Mean (ml/min)* | 78.25 (29) | | |
| **Patients with eGFR < 90 ml/min/1.73m2** | | 16 | | |
| **Patients with eGFR < 60 ml/min/1.73m2** | | 5 | | |
| **Leukocyturia** | | 7 | 0 | **0.045** |
| **ABS KIM-1** | *Median ng/dl, (IQR)* | 0.782 (2.57) | 0.376 (0.43) | **0.017** |
| **NORM KIM-1** | *Median, (IQR)* | 1.19 (1.87) | 0.36 (0.8) | **0.020** |
| **ABS Total NGAL** | *Median ng/dl, (IQR)* | 15.84 (28.69) | 8.72 (11.80) | **0.030** |
| **NORM Total NGAL** | *Median, (IQR)* | 21.84 (64.46) | 6.32 (8.45) | **0.009** |
| **ABS mNGAL** | *Median ng/dl, (IQR)* | 12.96 (16.01) | 8.33 (10.60) | **0.017** |
| **NORM mNGAL** | *Median, (IQR)* | 17.54 (25.85) | 5.14 (8.44) | **0.016** |

*ƒ ABS: absolute; BMI, body mass index; CCI, Charlson Comorbidity Index; mNGAL, Monomeric NGAL; NORM: normalized values expressed in ng/mg creatinine; S Cr: serum creatinine. ✻ Chi-square test for categorical variables and Student-T test or Mann Whitney-U test for continuous data.*

**Table 5. Comparison between preoperative characteristics of hydronephrosis group patients and controls.**

**Figure 8. Boxplot graph showing the distribution of normalized values of biomarkers in patients with hydronephrosis and controls.**

Except for three patients, all others in the hydronephrosis group showed a decreased urinary level of KIM-1 in the postoperative visit compared to the preoperative concentration. One of those three patients had pre-existing CKD and residual hydronephrosis, the other two only showed leukocyturia and residual stone disease. **Figure 9** is a waterfall plot that shows the change in the postoperative levels of KIM-1 after surgical management of the 24 patients in the hydronephrosis group.
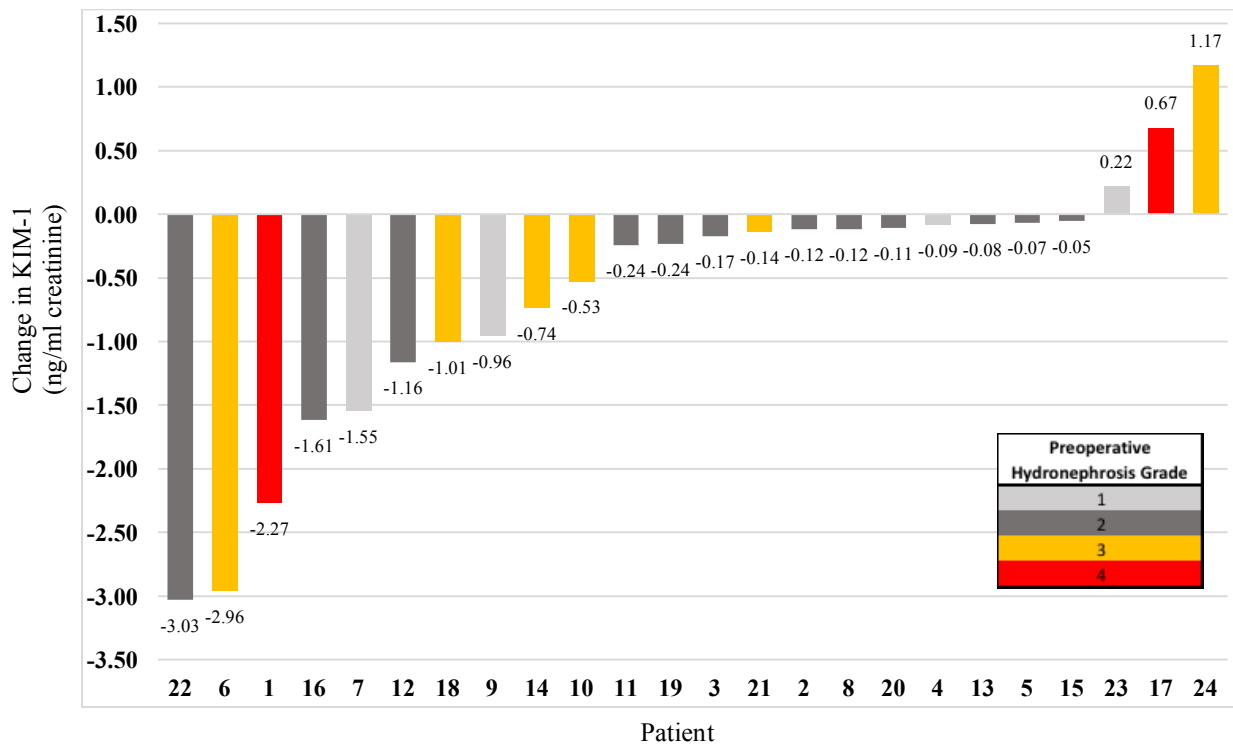


**Figure 9. Waterfall plot showing the change in KIM-1 levels before and after treatment in all patients from the Hydronephrosis Group.**

Based on previous research, we designed this study to evaluate if the normalized urinary levels of the KIM-1 and Monomeric NGAL were different after treatment and relief of the unilateral obstruction *(alternative hypothesis -$H_A$-)*, the null hypothesis ($H_0$) stated that the median levels of the biomarkers were similar before and after treatment. Wilcoxon Signed Rank test showed no significant differences in the Total and Monomeric NGAL levels, while on the contrary KIM-1 was statistically significantly lower after treatment (**Table 6, Figure 10**). Regarding Total and Monomeric NGAL the $H_0$ was accepted, although it has to be rejected for KIM-1, accepting the fact that KIM-1 levels significantly changed after relieving the unilateral obstruction.

| Table 6. Median concentration of biomarkers in patients with hydronephrosis before and after relieving the obstruction | | | | |
|---|---|---|---|---|
| | | **Preoperative** | **Postoperative** | ***p value \**** |
| **Normalized KIM-1** | *Median, IQR* | 1.19 (1.87) | 0.76 (1.51) | 0.002 |
| **Normalized Total NGAL** | *Median, IQR* | 21.84 (64.46) | 25.11 (32.79) | 0.53 |
| **Normalized mNGAL** | *Median, IQR* | 17.54 (25.85) | 17.97 (18.60) | 0.50 |

*Normalized levels are expressed in ng/mg creatinine. \*Wilcoxon Signed Rank Test*

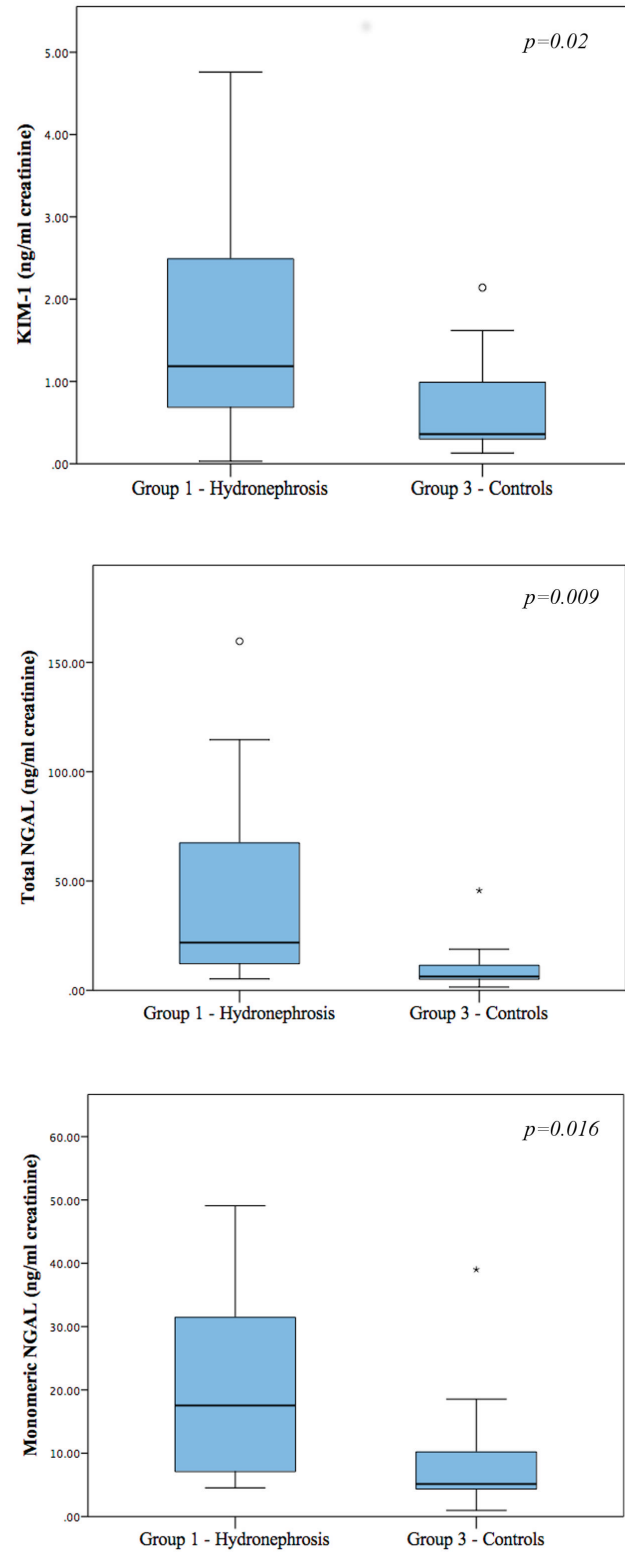**Table 6. Comparison of preoperative and postoperative urinary biomarkers' concentration.**
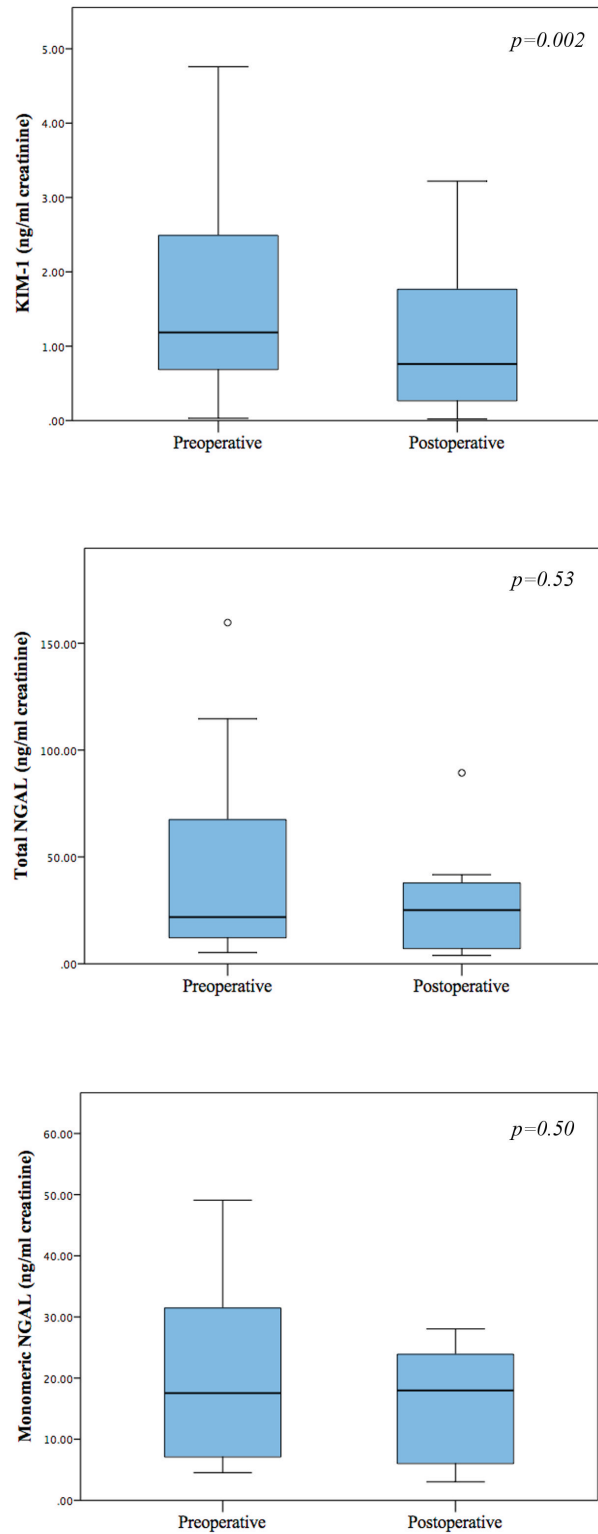
**Figure 10. Boxplot graph showing the distribution of normalized values of biomarkers in patients with hydronephrosis before and after treatment**

Considering that 3 patients had residual hydronephrosis after the treatment and 7 patients had residual stone fragments, we performed a *post hoc* analysis excluding these postoperative samples. Results were similar, showing only significant differences in KIM-1 levels, after comparing 24 vs 21 patients (residual hydronephrosis) and 24 vs 15 (residual hydronephrosis and/or stones) (**Table 7**).

| Table 7. Median urinary concentration of biomarkers excluding patients with residual hydronephrosis or stone | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Preoperative (24 patients) | vs | Postoperative (21 patients) | p value * | vs | Postoperative (15 patients) | p value * |
| Normalized KIM-1 | *Median, IQR* | 1.19 (1.87) | | 0.75 (1.48) | 0.001 | | 0.75 (1.55) | 0.003 |
| Normalized Total NGAL | *Median, IQR* | 21.84 (64.46) | | 29.47 (58.76) | 0.37 | | 26.74 (54.86) | 0.82 |
| Normalized mNGAL | *Median, IQR* | 17.54 (25.85) | | 18.67 (20.12) | 0.55 | | 18.67 (18.82) | 0.15 |

*Normalized levels are expressed in ng/mg creatinine. *Wilcoxon Signed Rank Test*

**Table 7. *Post hoc* analysis including patients without residual hydronephrosis and residual stone after treatment.**

After resolution of the hydronephrosis, KIM-1 concentration decreased to levels that were statistically similar to of the control group (0.76 vs 0.36 ng/ml creatinine, p=0.36).

## 3.2 Factors associated with biomarkers expression

The results of the following sections are based on the inclusion of the baseline characteristics of all recruited patients with complete information and the urinary biomarkers' concentrations of the first collected urine samples (preoperative). Some statistical analyses also integrated the results obtained from the control group, in this case, a note is made.

## 3.2.1 Comparison of biomarker levels in patients with and without hydronephrosis

Demographic, radiological and biochemical characteristics of the two groups of patients are shown in **Table 8**. Both groups were comparable in terms of age, gender, comorbidities, and previous history of stone disease. Besides the obvious difference in the hydronephrosis status, we also found significant differences in the number of patients diagnosed after an episode of renal colic, the size of the treated stone and the preoperative serum creatinine (p<0.05). Despite the disparities in the preoperative creatinine levels, the calculated eGFR was similar between both groups. The number of patients with CKD stage ≥ 3 was significantly higher in the hydronephrosis group and no differences were found in the presence of leukocyturia.

The distribution of the preoperative levels of the biomarkers is also shown in **Table 8**. Interestingly, patients with hydronephrosis had significantly higher levels of absolute and normalized KIM-1 compared to those patients without hydronephrosis. (p=0.035, p=0.006). Monomeric and Total NGAL failed to show a significant difference between the group of patients with hydronephrosis and those without it.  After comparing the postoperative levels in the two groups, we did not find any significant difference in the median values of KIM-1 (p=0.81), Total NGAL (p=0.89) or Monomeric NGAL (p=0.37).

| Table 8. Comparison between baseline characteristics of hydronephrosis and no hydronephrosis groups. | | | |
|---|---|---|---|
| | | **Patients with hydronephrosis** | **Patients without hydronephrosis** | ***p value**** |
| **n=** | | 24 | 24 | |
| **Age** | *Median (years), IQR* | 58.5, 15 | 57, 19 | 0.74 |
| **Gender** | *Female/ Male* | 9/15 | 12/12 | 0.56 |
| **BMI** | *Median (kg/m2), IQR* | 30.7, 11.6 | 29.5, 6.55 | 0.65 |
| **CCI** | *Median (IQR)* | 1 (3) | 2 | 0.61 |
| **Diabetes** | | 6 | 3 | 0.46 |
| **Hypertension** | | 9 | 11 | 0.77 |
| **Previous history of stone disease** | | 10 | 9 | 1 |
| **Side** | *L/R* | 16/17 | 15/10 | 0.38 |
| **Acute episode** | | 15 | 4 | **0.0001** |
| **Largest diameter (axial)** | *Median (mm)* | 7 | 8.5 | **0.021** |
| **Hydronephrosis** | *Grade 0* | 0 | 24 | **0.0001** |
| | *Grade 1* | 4 | 0 | |
| | *Grade 2* | 7 | 0 | |
| | *Grade 3* | 11 | 0 | |
| | *Grade 4* | 2 | 0 | |
| **Preoperative S Cr** | *Median (mmol/L)* | 85 | 75 | **0.043** |
| **Preoperative eGFR ml/min/1.73m2** | *Mean (ml/min)* | 76.9 | 84.5 | 0.21 |
| **Patients with eGFR < 90 ml/min/1.73m2** | | 16 | 13 | 0.17 |
| **Patients with eGFR < 60 ml/min/1.73m2** | | 7 | 0 | **0.016** |
| **Leukocyturia in** | | 7 | 6 | 1 |
| **NORM KIM-1** | *Median, IQR* | 1.19 (1.87) | 0.64 (0.88) | **0.006** |
| **NORM Total NGAL** | *Median, IQR* | 21.84 (64.46) | 25.26 (40.7) | 0.88 |
| **NORM mNGAL** | *Median, IQR* | 17.54 (25.85) | 22.76 (25.14) | 0.35 |
| **Additional findings** | *Cyst* | 2 | 3 | 1 |
| | *Renal mass* | 2 | 1 | |

*categorical variables were compared using Chi-square test; continuous variables were compared with Student's T-test or Mann-Whitney U test. ∫ mNGAL: Monomeric NGAL; NORM: normalized values expressed in ng/mg creatinine; S Cr: serum creatinine.*

**Table 8. Comparison between hydronephrosis and no hydronephrosis groups.**

## 3.2.2 Correlations between biomakers and grade of hydronephrosis

For the evaluation of these possible correlations, we included the preoperative information of all patients and controls. We used Spearman's test to correlate the urinary levels of the normalized biomarkers and the grade of hydronephrosis. KIM-1 was the only biomarker showing a significant correlation with the grade of hydronephrosis, and a moderate correlation ($r_s$=0.39, p=0.002). Total and Monomeric NGAL did not correlate with the grade of hydronephrosis.

## 3.2.3 Correlations between biomarkers and renal function

Serum creatinine levels were available for all recruited patients. Biomarkers' levels from preoperative urine samples were used to evaluate a possible correlation with the renal function measured by serum creatinine, estimated GFR, and by CKD stage according to the Kidney Disease Improving Global Outcomes classification (KDIGO). We did not find any significant correlation for any biomarker (**Table 9**). Our hypothesis that KIM-1 and Monomeric NGAL would correlate with the overall renal function was not proven.

| Table 9. Spearman's correlation coefficients of normalized urinary biomarkerslevels and renal function. | | | | | | |
|---|---|---|---|---|---|---|
| | Preoperative serum creatinine (mmol/L) | p-value | eGFR (ml/min/1.73m2) | p-value | KDIGO stage | p-value |
| Normalized KIM-1 | -0.004 | 0.98 | -0.162 | 0.27 | 0.128 | 0.39 |
| Normalized Total NGAL | -0.103 | 0.53 | -0.147 | 0.37 | 0.014 | 0.93 |
| Normalized monomeric NGAL | -0.122 | 0.46 | -0.123 | 0.45 | -0.005 | 0.98 |

**Table 9. Correlation between normalized biomarkers' values and different ways to assess renal function.**

## 3.2.4 Other correlation analyses with biomarker levels

We explored the association of preoperative continuous variables with the concentrations of the biomarkers. The preoperative information from all patients (48) was used. The normalized values of all three biomarkers positively correlated with the Charlson Comorbidity Index, but after adjusting for the age, these correlations were no longer significant (p>0.05). No correlations were found between the mean largest diameter of the stone and the level of any biomarker.

After analyzing the preoperative urine samples, the grade of leukocyturia showed a moderate correlation with the normalized levels of Total and Monomeric NGAL ($r_s$=0.43, p=0.007, $r_s$=0.45, p=0.001) respectively, similar correlations were found evaluating the postoperative urine sample, and these remain significant after adjusting for age and CCI. Patients showing leukocyturia in the preoperative urine sample had significantly higher levels of Total and Monomeric NGAL (p=0.016, p=0.010) than those patients without leukocyturia, independently of the group they belonged to. None

of those patients had significant findings in the urinalyses suggesting a urinary tract infection, and all postoperative urine samples were from stent free patients. KIM-1 was not associated with the presence of leukocyturia. KIM-1 levels were similar between patients with and without preoperative leukocyturia (p=0.01).

To evaluate any possible correlation between the three urinary biomarkers, we analyzed the preoperative urine samples of all patients and controls. We found significant correlations between all the markers, from weak to strong, being higher between Total NGAL and Monomeric NGAL as it would be expected (**Table 10**). Interestingly, the strength of the correlations was different among patients without hydronephrosis compared to the coefficients of patients with obstruction (**Table 11**).

| Table 10. Spearman's correlation coefficients of normalized biomakers in first urine sample of patients and control group | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Normalized KIM-1 | p-value | Normalized Total NGAL | p-value | Normalized monomeric NGAL | p-value |
| Normalized KIM-1 | | | 0.561 | <0.0001 | 0.487 | <0.0001 |
| Normalized Total NGAL | 0.561 | <0.0001 | | | 0.971 | <0.0001 |
| Normalized monomeric NGAL | 0.487 | <0.0001 | 0.971 | <0.0001 | | |

**Table 10. Correlation coefficients between normalized biomarker levels in the first urine sample.**

| Table 11. Spearman's correlation coefficients of normalized biomakers according to the group (Hydronephrosis vs No hydronephrosis) | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | Hydronephrosis | | | | | |
| | | Normalized KIM-1 | p-value | Normalized Total NGAL | p-value | Normalized monomeric NGAL | p-value |
| No Hydronephrosis | Normalized KIM-1 | | | 0.639 | 0.010 | 0.504 | 0.056 |
| | Normalized Total NGAL | 0.774 | <0.0001 | | | 0.971 | <0.0001 |
| | Normalized monomeric NGAL | 0.792 | <0.0001 | 0.987 | <0.0001 | | |

**Table 11. Correlation coefficients between preoperative biomarkers in both groups.**

The initial sample size calculation was formulated in line with previously reported KIM-1 values. The inclusion of Monomeric NGAL analysis in our study, made it the first clinical trial evaluating this marker which previous reports suggesting it was more specific for AKI. In view of our results, we decided not to analyze the second urine sample of the patients, because results from Total and Monomeric NGAL would be affected by leukocyturia, which is expected with the presence of a ureteric stent.

## 3.3   Biomarker expression in the no hydronephrosis group

Demographic characteristics between the no hydronephrosis group and controls showed no differences, except for the presence of comorbidities measured by the Charlson Comorbidity Index (CCI): patients had a median CCI of 2, while controls had a median of 1. After comparing the normalized values of the biomarkers we found that Total and Monomeric NGAL were significantly different both in the preoperative and postoperative urine samples. KIM-1 measurements did not significantly differ at any

point of the follow-up between controls and patients in the no hydronephrosis group

(**Table 12**).

We also compared the preoperative and postoperative levels and we found that values

were not statistically significantly different despite the intervention for the stone disease.

(KIM-1 p=0.30, Total NGAL p=0.33, Monomeric NGAL p=0.25). **Figure 11** is a before-

after graph of the KIM-1 in this group which shows comparable results despite the

treatment.
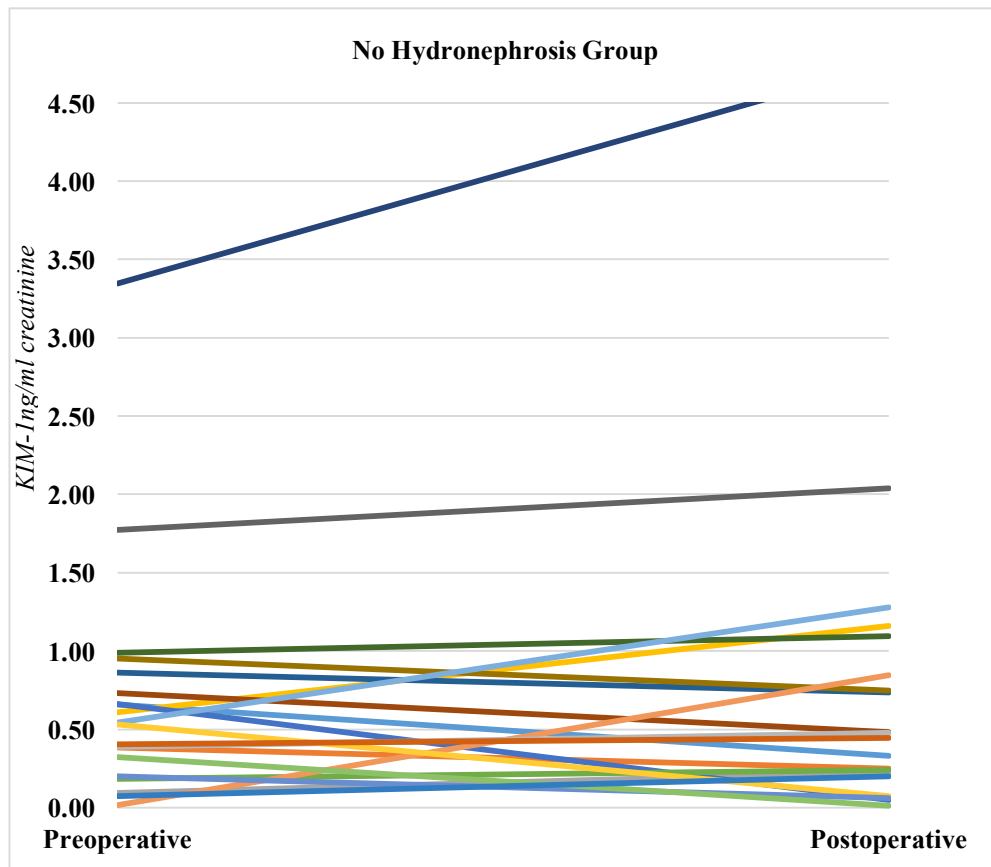


**Figure 11. Before-after graph of KIM-1 levels in no hydronephrosis group.**

| Table 12. Median concentration of biomarkers in patients without hydronephrosis compared to control group | | | | | | |
|---|---|---|---|---|---|---|
| | | Preoperative | Control Group | *p value* * | Postoperative | *p value* *ϕ* |
| **Normalized** | *Median, IQR* | 0.64 (0.88) | 0.36 (0.8) | 0.36 | 0.75 (1.00) | 0.903 |
| **Total NGAL** | *Median, IQR* | 25.26 (40.7) | 6.32 (8.45) | <0.0001 | 34.58 (39.27) | <0.0001 |
| **Monomeric NGAL** | *Median, IQR* | 22.76 (25.14) | 5.14 (8.44) | <0.0001 | 28.73 (40.57) | <0.0001 |

*Normalized levels are expressed in ng/mg creatinine. *Mann-Whitney U Test. ϕ Compared to controls.*

**Table 12. Biomarkers' concentration between the no hydronephrosis group and controls.**

## 3.4  KIM-1 as a diagnostic test for hydronephrosis

Using ROC Curve analysis with the preoperative urine sample of all patients, we were able to calculate the best cut-off point for normalized KIM-1 to predict the presence of hydronephrosis either in the preoperative CT scan or renal ultrasound. The area under the curve (AUC) was 0.73 (95% CI 0.58-0.87, p=0.006), and a KIM-1 value of 0.735 ng/mg creatinine was the balance point to achieve the highest sensitivity (75%) and specificity (67%) (**Figure 12**). The Positive Predictive Value (PPV) is 59% and the Negative Predictive Value (NPV) 77%.

None of the other analyzed biomarkers showed a significant AUC to detect hydronephrosis. If we use the preoperative and postoperative urine samples with their imaging studies as independent events, the AUC to detect hydronephrosis for KIM-1 was 0.69 (95% CI 0.58 - 0.81, p=0.003).
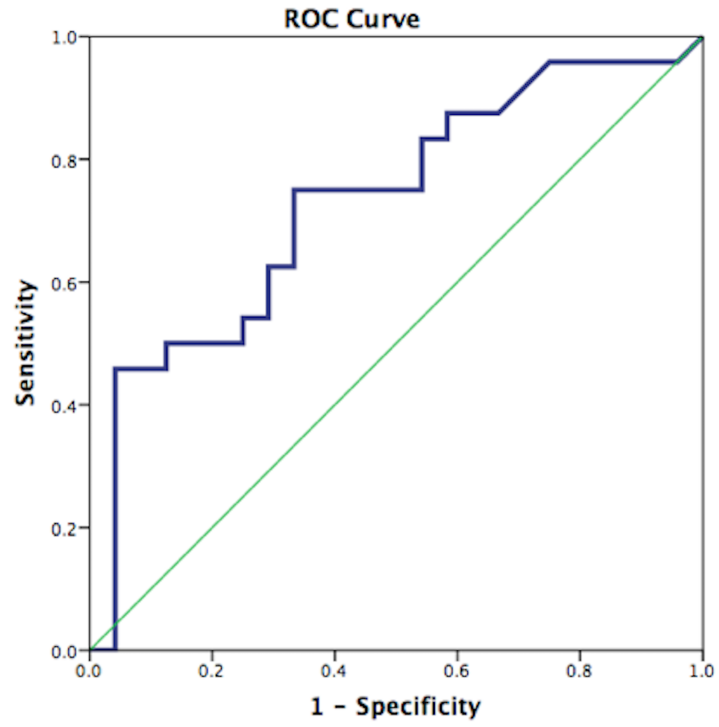
**Figure 12. ROC curve for hydronephrosis detection using KIM-1.**

# 4  Discussion and Conclusions

We were able to complete the recruitment and follow-up of patients according to the previously calculated sample size. After these initial results we decided to analyze only the first and third urine sample to avoid the possibility of highly skewed values related to the presence of a ureteric stent in the second sample. We will see that because KIM-1 was not affected by the presence of leukocyturia, further analysis of KIM-1 concentration in the second urine sample will be performed. The sample size calculation was based on the previously reported urinary levels of KIM-1, but we also had planned to explore the expression of Total and Monomeric NGAL. In order to correct for individual urinary protein production and hydration status, the normalized biomarkers levels were used to perform the statistical analysis, as all the recent literature recommends (Hsu, 2015). The urinary levels of the three biomarkers in patients with hydronephrosis were statistical significantly different compared to the control group. This information has been proven by several urological studies, demonstrating that biomarkers are able to discriminate between healthy subjects and patients with the specific renal disease, such as obstruction or infection (Cost, 2013; Xie, 2014; Yilmaz, 2009).

We believe our results provide a comprehensive overview of the expression of the studied biomarkers in these groups of urological patients. According to our design, the findings are statistically strong enough to prove differences and similarities in the expression of KIM-1 in the three studied groups. The information was prospectively collected, and the statistical tests should be considered adequate for non-normally

distributed data. Specific assumptions for each test were taken into consideration to properly analyze all the variables in the different hypotheses involving the three biomarkers. We should be cautious in the interpretation of the results regarding the expression of Monomeric NGAL however, because of the exploratory nature of the study in this regard. We acknowledge that the negative results about Total and Monomeric NGAL could result from a small sample size, but the correlation noted with the levels of leukocyturia may affect their usefulness in urological patients. This observation should be taken into account if further studies are planned to assess these particular biomarkers.

## 4.1 Biomarkers expression in hydronephrosis group

Patients with hydronephrosis had significantly higher levels of urinary KIM-1 at baseline compared to at the end of the follow-up period, after the obstruction was relieved. Likewise, this group of patients demonstrated an increased level of urinary KIM-1 compared to both patients with stone disease without obstruction and healthy control subjects. These results may corroborate the fact that hydronephrosis due to obstruction causes kidney injury measured by the increased expression of the biomarker. It could be designated as subclinical, because serum creatinine was within the normal limits, but slightly higher in the obstructed patients, compared to the other studied groups. If treatment decisions were to be based on serum creatinine levels, treatment might be delayed especially in patients with unilateral obstruction and normal contralateral kidney function.

From our perspective, the most important finding is the fact that KIM-1 elevation appears to be related to the presence of obstruction. As a reflection of the presence of

AKI injury, KIM-1 could potentially be used as a hydronephrosis biomarker in several clinical situations. More large-scale studies are needed to validate the feasibility of its widespread use as an accurate marker of obstruction.

Most of the longitudinal studies about biomarkers expression in patients with hydronephrosis have evaluated pediatric patients. Karakus studied 4 urinary biomarkers, including Total NGAL and KIM-1, in patients with obstructive antenatal hydronephrosis. He found that all biomarkers were higher compared to the control group, and NGAL and KIM-1 decreased to the levels showed by control group 6 months after the surgery. Interestingly, urinary KIM-1 concentration of the control group was very similar to what Wasilewska and collegues found in another set of pediatric patients with UPJO, and to our findings (0.60 vs 0.58 vs 0.36 ng/mg creatinine) (Karakus, 2015; Wasilewska, 2010). We found that KIM-1 decreased after relieving the obstruction, but not as low as the control group (0.75 vs 0.36 ng/ml), and this could be explained by the time lapse between surgery and the collection of the second postoperative urine sample. In our case it was only 2 months, compared to 6 in Karakus' study. The other reason might be the fact that patients in both studied groups already had some sort of CKD (29 out of 48 patients had an eGFR < 90 ml/min), which could be associated with higher baseline levels of KIM-1. A recently published case-control study about urinary biomarkers of and risk for ESRD (End-Stage Renal Disease), revealed that patients with ESRD had increased levels of urinary biomarkers, specifically, patients who developed ESRD had a mean baseline KIM-1 level of 914 pg/mg creatinine (0.914 ng/mg creatinine) compared to controls who showed a median concentration of 665 pg/mg creatinine (0.665 ng/mg creatinine) (Foster MC, 2016).

Another objective of this study was to also evaluate a group of patients with obstruction who had undergone diuretic renography. These are usually patients with congenital UPJO who required this investigation for diagnosis. We wanted to explore the expression of these markers before and after treatment and compare it to the actual "gold standard" and the radiological appearance of hydronephrosis. Unfortunately, the actual recruitment of UPJO patients was quite low; only 4 patients were initially recruited and one finished the study. This patient showed a decreased urinary concentration of KIM-1 in the postoperative urine sample, despite showing residual hydronephrosis in the imaging studies. No definitive conclusion can be made, but the recruitment of patients with UPJO will continue in order to explore the relationship between urinary marker expression and obstruction evaluation by renography. Although studies in the pediatric population suggested the potential usefulness of these biomarkers, results cannot be extrapolated to adult patients because most of the pediatric patients with UPJO obstruction have antenatal hydronephrosis which may affect the development of the renal parenchyma and the subsequent expression of biomarkers (Karakus, 2016; Madsen, 2013).

Urbschat and collegues in a cross-sectional study evaluated the expression of serum and urinary NGAL and KIM-1 in patients with "acute obstructive nephropathy". Their main results were different from our findings: KIM-1 was similar between controls and patients, and serum and urinary NGAL was statistically significantly different between both groups; they concluded that NGAL could be a potential marker of postrenal AKI. An important drawback of this study is the fact that all included patients with acute renal colic were considered to have postrenal AKI, despite the presence of hydronephrosis or the serum creatinine level. Additionally, patients with renal colic without hydronephrosis

were included in the studied group. The authors concluded that NGAL and a "negative urinary KIM-1" may facilitate the diagnosis of postrenal AKI (Urbschat, 2014). On the contrary, Xie *et al*, found a correlation between the preoperative and early postoperative urinary levels of KIM-1 in patients with obstructive nephropathy with the recovery of renal function 1 year after obstruction management. This conclusion raises the possibility of using KIM-1 not only as a diagnostic test, but also as an objective prognostic tool in patients with obstructive nephropathy (Xie, 2014). Sabbisetti also found the prognostic value of serum KIM-1 to predict the loss of GFR in diabetic patients with CKD (Sabbisetti, 2014).

Whether KIM-1 has a protective role in cases of acute injury, or promotes a fibrotic process by its chronic expression, remains to be determined (Humphreys, 2013; Yang, 2015). Further details about its expression in the acute and chronic setting must be addressed in future basic and clinical research.

The urinary levels of Total and Monomeric NGAL were similar in patients with hydronephrosis before and after the relief of the obstruction, corroborating the absence of hydronephrosis with imaging studies in 21 of the 24 patients. All postoperative analyzed urine samples came from stent free patients. We determined if the presence of residual hydronephrosis affected the NGAL levels using a *post hoc* analysis. By excluding cases with residual hydronephrosis or stone fragments after the procedure, we did not find significant differences in Total and Monomeric NGAL concentrations.

We believe that by including patients without urgent indications for active management, we automatically excluded patients with systemic inflammatory response symptoms

(SIRS) from our trial. This allowed a more objective measurement of the impact of hydronephrosis in the expression of Total and Monomeric NGAL by removing inflammation as a potential cause from NGAL release.

Despite many research studies concluding that urinary and serum NGAL are reliable markers of AKI in other populations, Martensson and Bellomo discussed some reasons that may explain the differences in the diagnostic performance of NGAL for AKI in the published literature: 1) the use of creatinine as a "gold standard" to compare the global renal function may affect the sensitivity of the biomarker in "serum creatinine false-negative AKI", 2) chronic comorbidities that may affect the baseline expression of NGAL (i.e. CKD) 3) variations in the time lapse of biomarker measurement, and 4) the absence of specific essays to differentiate between the different NGAL isoforms (Martensson, 2014). In our study, we tried to account for one of those factors by measuring the Monomeric NGAL isoform.

We are the first group to explore the urinary expression of Monomeric NGAL using a new ELISA kit claiming to have < 1% of cross reaction with Homodimeric NGAL. We hypothesized that by measuring the monomeric isoform, which is more specific for tubular epithelial cells, the diagnostic performance for kidney injury would increase (Bangert, 2012; Cai, 2010; Nickolas, 2012). Total NGAL and Monomeric NGAL showed similar expression in the studied groups, and no associations were related to the presence of hydronephrosis. Furthermore, the median values in the postoperative evaluation were higher than the baseline levels, but differences were not significantly different.

After analyzing our results, and comparing them with the previous literature, we believe that urinary KIM-1 has shown a promising future as an AKI marker in urological patients. If future studies corroborate our findings, and they find a correlation between the biomarker levels and the baseline renal function, we may have an opportunity to evaluate the prognostic significance of a urinary marker in urological patients.

We have proven that despite the presence of normal median values of serum creatinine, patients with unilateral ureteral obstruction have increased levels of urinary KIM-1 which has been recognized as an accurate marker of early kidney injury. Given that serum creatinine has failed to accurately stratify our patients with UTO because it cannot detect "subclinical injury" and a substantial decrease in the glomerular filtration is needed to affect the serum levels of creatinine, KIM-1 emerges as a potential biomarker in this set of patients.

Further research should be conducted to elucidate the physiology behind the expression of KIM-1 during acute and chronic renal injury. In addition, further work is required to assess the effect of unilateral obstruction in the biomarkers levels in the affected unit compared to the bladder.

## 4.2 Biomarkers expression in studied groups: hydronephrosis vs no hydronephrosis

Our results suggest that urinary KIM-1 has the potential to become a useful marker for subclinical AKI associated with the presence of unilateral urinary obstruction. The design of the study allowed the evaluation of KIM-1 expression in a longitudinal (before

and after treatment) and cross-sectional fashion: KIM-1 was significantly higher in patients with hydronephrosis compared to patients with stone disease and no hydronephrosis, with similar demographic and medical characteristics. While we found significant differences in the serum creatinine levels, the median eGFR between the two groups was similar, and the number of patients with eGFR < 90 ml/min was comparable, although the median serum creatinine value of both groups was within the normal limits.

Several important findings need to be underlined to better understand our results: 1)KIM-1 levels were higher in patients with hydronephrosis, 2) the median postoperative level of KIM-1 in the hydronephrosis group after treatment was similar to what patients without hydronephrosis showed after surgery (0.75 ng/ml creatinine), 3) median KIM-1 level of the control group was significantly different only in the hydronephrosis group, and 4) despite the significant differences in Total and Monomeric NGAL levels between the hydronephrosis and control groups, the expression of these two markers was similar among the two groups of patients before and after relieving obstruction.

Our study is the first to analyze the impact of biomarkers' expression before and after treatment in such detail. As briefly mentioned, there have been some studies that evaluated KIM-1 expression in hydronephrotic adult patients: Ursbchat *et al* did not find differences in the expression of urinary KIM-1, but they compared patients with acute renal colic without differentiating the presence or absence of hydronephrosis. Their results regarding NGAL expression could be affected also by the presence of SIRS commonly associated with renal colic. We mentioned that Xie and colleagues found that KIM-1 was a good predictor of AKI and a prognostic tool to determine the recoverability of renal function after an episode of obstructive nephropathy. These results should be

cautiously evaluated because the mean eGFR of the included patients was 11.5

mL/min/1.73m$^2$, and those with better function recovery had a mean eGFR 53.5

mL/min/1.73m$^2$ showing a large proportion of patients with late stages of CKD, and the

results might not be reproduced in patients with normal eGRF (Xie, 2014; Xue, 2014).

We also compared the levels of the three biomarkers in patients without hydronephrosis

to the control group. **Table 12** showed that both Total and Monomeric NGAL were

different between preoperative and postoperative samples in the no hydronephrosis

group in comparison with the controls. If these markers were good parameters of AKI in

our population we would expect to be similar in the absence of hydronephrosis. Only

KIM-1 was comparable in those groups, irrespectively of the presence of urolithiasis,

suggesting that urinary KIM-1 is only affected by the presence of hydronephrosis.

## 4.3   KIM-1 correlated with the grade of hydronephrosis

In our study, KIM-1 was the only biomarker associated with the grade of

hydronephrosis. After evaluating the performance of Total and Monomeric NGAL in

both groups of patients, the correlation analysis confirms that these urinary biomarkers

are not accurate enough to detect hydronephrosis in patients with co-existing conditions

associated with urinary tract inflammation. In fact, the difference in the preoperative and

postoperative levels was not statistically significant, although numerically both

biomarkers were elevated. This previous finding was not expected, based on the

published information as we hypothesized that at least Monomeric NGAL would have

better correlation than Total NGAL with the occurrence of hydronephrosis. The grade of

hydronephrosis positively correlated with the expression of KIM-1. Despite the fact that

the 24 patients with hydronephrosis were divided by 4 grades, which may have decreased the chances of finding an association, we found a moderate correlation between both variables. We envision a large scale multicentric study which may be able to corroborate our findings, especially about the the impact of baseline renal function in the expression of KIM-1. Such a study may evaluate the likelihood of using KIM-1 as a potential tool to stratify the management of patients with hydronephrosis according to the baseline renal function.

Postoperative KIM-1 levels in patients with hydronephrosis were lower than before obstruction management, and almost all patients without hydronephrosis in the follow-up studies had decreased levels. While measuring the concentration of biomarkers in voided urine specimens may reflect the status of both renal units, and possibly "dilute" the biomarkers concentration of the affected unit, we aimed to assess an easy to perform and non-invasive test. The idea of evaluating the unilateral expression of the biomarkers would require an invasive procedure such as percutaneous nephrostomy insertion or retrograde catheter placement to collect urine from the affected unit. A ROC analysis showed that KIM-1 had a sensitivity of 75% and specificity of 67% to detect hydronephrosis. We believe this outcome deserves further evaluation in a large prospective cohort study to increase the number of patients with the different grades of hydronephrosis. The small number of patients in each of the hydronephrosis grade categories precluded a more specific analysis by each individual category.

## 4.4   Biomakers and overall renal function

Several studies have shown a correlation between the AKI markers expression and renal function. Malyszko and collegues evaluated KIM-1 and NGAL in blood of patients after successful kidney transplantation, and the later had a strong correlation with serum creatinine levels (Malyszko, 2010). In our previous study, we found a higher level of urinary KIM-1 in patients with CKD compared to those with normal eGFR (Fahmy, 2013). Based on this and other findings previously discussed, we expect a certain grade of correlation between renal function and the biomarkers' baseline levels. None of the three evaluated biomarkers showed a significant correlation with the renal function measured by serum creatinine or the calculated eGFR. Furthermore, the CKD categories did not affect the baseline levels of any marker.

One important fact that should be addressed, is that serum creatinine might not be the best reference standard, despite being the most commonly used test to measure global kidney function (Siew, 2011). The time between the acute episode of obstruction and the inclusion of the patients varied in this study. All patients were recruited in the Urology Clinic without needing an urgent intervention, which may affect the acute expression of the markers. This needs further evaluation, because the increment of creatinine levels depends on the individual renal reserve and the length of time between the renal insult and the evaluation.

McIlroy prospectively evaluated the relationship between urinary NGAL and baseline renal function in patients undergoing cardiac surgery. They found a positive relationship between these two variables only in patients with an eGFR > 60 ml/min (McIlroy, 2009). This study included more than 400 patients and showed no differences in the perioperative NGAL levels despite the development of AKI in patients with CKD stage 4

and 5, which underlies the importance of baseline renal function on the biomarkers expression. Besides the fact that NGAL might be affected by the presence of leukocyturia, and these patients usually have a bladder catheter, they showed an association only in patients with normal baseline function. The small sample size of our study precluded the possibility of finding a positive relationship between the renal function and the eGFR.

Although it was found that KIM-1 levels decreased after the treatment of the hydronephrosis, and that the perioperative values of KIM-1 in patients without hydronephrotic were similar to the control group, they were numerically higher. This finding might be explained by the fact that patients had some degree of CKD compared to the controls, assuming that subjects included in the control group had normal renal function. One limitation of this study is the fact that serum creatinine was not measured in the control group, and despite enrolling patients without significant comorbidities and assuming a normal renal function, the actual serum creatinine and eGFR were not available for analysis.

## 4.5   Other factors related to biomarkers' expression

Initially, we found a moderate correlation analysis between age, Charlson Comorbidity Index, eGFR and the biomarkers' expression. After adjusting for other clinical variables however, this correlation was not found to be significant. No other demographic or medical variables evaluated were correlated with the biomarkers' levels.

Ours is not the first study showing an association between leukocyturia and the expression of any of the biomarkers. As we previously discussed, a case-control study found that urinary levels of NGAL were higher in patients with UTI than in controls; the same group in another study found a correlation between NGAL and the grade of leukocyturia (Urbschat, 2014). Decavele *et al* studied the urinary expression of NGAL and the presence of white cells in urine and concluded that high count of leukocytes contributes to the expression of urinary NGAL, and proposed a correction factor to increase the accuracy of the test (Decavele, 2011). The authors used a different assay to analyze the levels of NGAL precluding the use of their formula in our study, nevertheless our results showed a significant correlation between both Total and Monomeric NGAL and the presence of leukocyturia.

We recognize that leukocyturia is a very common finding in urological conditions, and despite the use of a more specific isoform to detect subclinical AKI such as Monomeric NGAL, the nature and origin of the NGAL molecule may preclude its use in this set of patients. Leukocyturia may be caused by the presence of residual stone disease, urinary infection, or by the presence of indwelling catheters or ureteric stents.

KIM-1 seems to be expressed in the voided urine specimen without any significant correlation with the presence of leukocyturia. In a previous study, assessing the impact of endourological management in the expression of KIM-1, we found that ureteroscopy did not significantly affect the urinary levels of KIM-1 (Nader, 2013). This is of particular importance because in this cohort study we analyzed postoperative samples at a median follow-up of two months after the surgical procedure. Both studies support the fact that

inflammatory conditions of the urinary tract, specifically stone disease or endourological manipulations, do not affect the levels of KIM-1.

Our study answered some questions regarding the expression of these markers in urological patients. Most of the large scale studies evaluating AKI biomarkers in the emergency department excluded patients with urological conditions. We have found that KIM-1 appears to be unaffected by the presence of kidney stones, and we corroborated the fact that leukocyturia could potentially affect the urinary levels of Total and Monomeric NGAL. Researchers may consider these facts when designing future studies about the impact of different renal conditions on the expression of AKI biomarkers.

## 4.6   Limitations of the study

Despite the prospective design of this cohort study and the meticulous criteria for patient inclusion and exclusion, our study is not without limitations. Due to the fact than we did not want to interfere with the standard of treatment, some patients had to be excluded from the final analysis. After the enrollment in the study, some patients had their follow-up in another city or did not return for clinical and urinary biomarker evaluation due to missing their appointments. Furthermore, due to the nature of this observational study, the follow-up was not completely standardized and in some cases the clinical evolution of the patient changed the usual postoperative evaluation and the subsequent exclusion of the patients from the final analysis. Despite this, 48 out of 66 patients were included in the final analysis which allowed us to perform the planned analysis. It is accepted that cohort studies have the potential to experience information bias, meaning that the exclusion of subjects lost to follow-up may affect the results. For these reasons, and

specially after this type of exploratory study, our results need to be replicated in a larger group.

We assumed that the renal function of the healthy subjects was normal, and this may be a limitation while trying to correlate the expression of the biomarkers with the eGFR, because these subjects were not included in that analysis. In order to facilitate the recruitment of controls, we decided not to evaluate this parameter. Along with this limitation, we did not measure the serum creatinine at the follow-up visits in the included patients because it is usually not within the standard of care. However, it may have helped determine if the changes in the evaluated biomarkers had a correlation with the evolution of this parameter after the resolution of the obstructive process.

Another limitation is the fact that not all preoperative imaging studies were performed the same day patients gave the urine sample for biomarker analysis. The evaluated preoperative imaging study might not have reflected the dynamic evolution of the urinary tract obstruction. As we previously mentioned, this was due to the observational design of the study. All postoperative urine samples however, were collected the same day the imaging study was performed, reassuring the relationship between the absence of hydronephrosis and the decreased levels of KIM-1.

The fact of including patients with different causes of hydronephrosis in group 1 (stone disease, UPJO, ureteric stricture) could be interpreted as a limitation, because the outcomes from a renal function perspective could be different. Nevertheless, the design was made to evaluate the impact of hydronephrosis in the levels of the biomarkers, independently of the cause. For this reason we determine that the exposure variable

would be the obstruction detected by imaging studies, and the main objective the urinary levels of the three biomarkers before and after the surgical management.

We acknowledge that hydronephrosis is not a synonym of obstruction, and there are some conditions, as those discussed in Chapter 1, that are not associated with urinary tract obstruction. Nevertheless, the standard clinical practice in urology assumes that in the presence of a stone the associated hydronephrosis reflects an obstructive process. It is not clinically useful or ethical to perform a renogram to determine if these patients are truly obstructed in order to define the most appropriate management. Equivocal cases, where hydronephrosis is not always a sign of obstruction, such as in patients with UPJO, require a renogram with diuretic for correct diagnosis (Ozayar, 2015). We planned to include these patients, however, the number of adult patients presenting UPJO was low, and only one patient completed the study.

## 4.7 Future directions

These results have identified an association with hydronephrosis and the urinary expression of KIM-1. While Total and Monomeric NGAL did not show a correlation with the presence of hydronephrosis, our results support a possible relationship with leukocyturia, hindering its use in this population.

The recruitment of patients with UPJO will continue in order to evaluate the correlation of these biomarkers with the renogram results, a study that is considered the "gold standard" for the diagnosis of urinary tract obstruction. As we previously discussed, some studies have evaluated the expression of these biomarkers in the pediatric

population, but there are no published studies with adult patients. We believe our results should be further evaluated in a large multicentric scale study including clinical and biomarker assessment in the acute renal colic event. KIM-1 analysis will be performed for the second urinary sample, and it will help to determine if the presence of double J stent affects the urinary concentration. As we previously mentioned, the analysis of the second urine sample for Total and Monomeric NGAL will not be performed in order to save economical resources.

Although, several questions regarding the actual function and significance of these molecules that are expressed in early renal injury remain unanswered, this study adds important information to the existing literature and raises the interest of finding a reliable marker of obstruction, even in cases with subclinical AKI. On the other hand, KIM-1 should be evaluated in future studies as a potential point-of-care test to rule out the presence of obstruction, where normal values may predict the absence of AKI and may save further imaging in patients with urinary tract obstruction.

Future research is needed to corroborate if KIM-1 would be a sensitive and specific urinary test to detect AKI in urological patients. The promising implications in clinical practice include the possibility of prioritizing non-urgent management in patients with urinary tract obstruction, by having an objective measurement to detect AKI, but also the fact that KIM-1 seems to be not affected by inflammatory conditions may allow the inclusion of this set of patients in further research about these biomarkers.

# Bibliography

Bangert K, Baer A, Hjortrup PB, Perner A, Otto L, Bioporto U. The Monomer is the Major Form of NGAL in Urine and Plasma in Severe Sepsis. *J Am Soc Nephrol*. 2013;24(2010):2235.

Bangert K, Rosenkilde N, Jørgensen JP, Baer A, Otto L, Bioporto U. Immunochemical Determination of Monomer , Homodimer and Total Neutrophil Gelatinase-Associated Lipocalin. *J Am Soc Nephrol*. 2012;23:2012.

Bellomo R, Ronco C, Kellum J a, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care*. 2004;8(4):R204-R212.

Bonventre J V. Kidney injury molecule-1: A translational Journey. *TransAm Clin Climatol Assoc*. 2014;125:293-299.

Botto N, Azoulay R, Peuchmaur M, El Ghoneimi A. Renal parenchymal fibrosis and atrophy are not correlated with upper tract dilatation: Long-term study of partial unilateral ureteral obstruction in neonatal mice. *J Pediatr Urol*. 2011;7(3):310-316.

Bouchard J, Acharya A, Cerda J, et al. A Prospective International Multicenter Study of AKI in the Intensive Care Unit. *Clin J Am Soc Nephrol*. 2015;10(8):1324-1331.

Caddeo G, Williams ST, McIntyre CW, Selby NM. Acute kidney injury in urology patients: incidence, causes and outcomes. *Nephrourol Mon*. 2013;5(5):955-961.

Cai L, Rubin J, Han W, Venge P, Xu S. The origin of multiple molecular forms in urine of HNL/NGAL. *Clin J Am Soc Nephrol*. 2010;5(12):2229-2235.

Chertow GM. Acute Kidney Injury, Mortality, Length of Stay, and Costs in Hospitalized Patients. *J Am Soc Nephrol*. 2005;16(11):3365-3370.

Chevalier RL, Forbes MS, Thornhill B a. Ureteral obstruction as a model of renal interstitial fibrosis and obstructive nephropathy. *Kidney Int*. 2009;75(11):1145-1152.

Cho KS, Hong SJ, Cho NH, Choi YD. Grade of Hydronephrosis and Tumor Diameter as Preoperative Prognostic Factors in Ureteral Transitional Cell Carcinoma. *Urology*. 2007;70(4):662-666.

Cochrane  a. L. Renal Structural and Functional Repair in a Mouse Model of Reversal of Ureteral Obstruction. *J Am Soc Nephrol*. 2005;16(12):3623-3630. doi:10.1681/ASN.2004090771.

Cost NG, Noh PH, Devarajan P, et al. Urinary NGAL levels correlate with differential renal function in patients with ureteropelvic junction obstruction undergoing pyeloplasty. *J Urol*. 2013;190(4 Suppl):1462-1467.

Decavele A-SC, Dhondt L, De Buyzere ML, Delanghe JR. Increased urinary neutrophil gelatinase associated lipocalin in urinary tract infections and leukocyturia. *Clin Chem Lab Med*. 2011;49(6):999-1003. doi:10.1515/CCLM.2011.156.

Devarajan P. Biomarkers for the early detection of acute kidney injury. *Curr Opin Pediatr*. 2011;23(2):194-200.

Dieterle F, Sistare F, Goodsaid F, et al. Renal biomarker qualification submission: a dialog between the FDA-EMEA and Predictive Safety Testing Consortium. *Nat Biotechnol*. 2010;28(5):455-462.

Fahmy N, Sener A, Sabbisetti V, et al. Urinary expression of novel tissue markers of kidney injury after ureteroscopy, shockwave lithotripsy, and in normal healthy controls. *J Endourol*. 2013;27(12):1455-1462.

Fernbach SK, Maizels M, Conway JJ. Ultrasound grading of hydronephrosis: Introduction to the system used by the society for fetal urology. *Pediatr Radiol*. 1993;23(6):478-480.

Gaygısız Ü, Aydoğdu M, Badoğlu M, Boyacı N, Güllü Z, Gürsel G. Can admission serum cystatin C level be an early marker subclinical acute kidney injury in critical care patients? *Scand J Clin Lab Invest*. 2016;76(2):143-150.

Glassford NJ, Schneider AG, Xu S, et al. The nature and discriminatory value of urinary neutrophil gelatinase-associated lipocalin in critically ill patients at risk of acute kidney injury. *Intensive Care Med*. 2013;39:1714-1724. doi:10.1007/s00134-013-3040-7.

Guerin F, Azoulay R, Berrebi D, et al. Partial Unilateral Ureteral Obstruction in Newborn Mice: Magnetic Resonance Imaging and Pathology Studies. *J Urol*. 2008;179(4):1553-1563.

Holzscheiter L, Beck C, Rutz S, et al. NGAL, L-FABP, and KIM-1 in comparison to established markers of renal dysfunction. *Clin Chem Lab Med*. 2014;52(4):537-546.

Hou J, Fujino M, Cai S, Ding Q, Li X-K. Noninvasive Monitoring and Evaluation of the Renal Structure and Function in a Mouse Model of Unilateral Ureteral Occlusion Using Microcomputed Tomography. *Int Surg*. 2015;100(7-8):1237-1243.

Hsu C -y., Ballard S, Batlle D, et al. Cross-Disciplinary Biomarkers Research: Lessons Learned by the CKD Biomarkers Consortium. *Clin J Am Soc Nephrol*. March 2015:1-9.

Ichimura T, Bonventre J V., Bailly V, et al. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. *J Biol Chem*. 1998;273(7):4135-4142.

Ingelfinger JR, Marsden P a. Estimated GFR and risk of death--is cystatin C useful? *N Engl J Med*. 2013;369(10):974-975.

Irkilata L, Aydin HR, Demirel HC, Aydin M, Daggullu M. Is urinary kidney injury molecule-1 a noninvasive marker for renal injury in patients with ureteral stones? *Arch Clin Exp Surg*. 2015.

Ito Y, Kikuchi E, Tanaka N, et al. Preoperative hydronephrosis grade independently predicts worse pathological outcomes in patients undergoing nephroureterectomy for upper tract urothelial carcinoma. *J Urol*. 2011;185(5):1621-1626.

Karakuş S, Oktar T, Kucukgergin C, et al. Urinary IP-10, MCP-1, NGAL, Cystatin-C and KIM-1 Levels in Prenatally-Diagnosed Unilateral Hydronephrosis: the Search for an Ideal Biomarker. *Urology*. 2015;87:185-192.

Keddis MT, Rule AD. Nephrolithiasis and loss of kidney function. *Curr Opin Nephrol Hypertens*. 2013;22(4):390-396. doi:10.1097/MNH.0b013e32836214b9.

Kerr WS. Effects of complete ureteral obstruction in dogs on kidney function. *Am J Physiol*. 1956;184(3):521-526.

Khan F, Ahmed K, Lee N, Challacombe B, Khan MS, Dasgupta P. Management of ureteropelvic junction obstruction in adults. *Nat Publ Gr*. 2014;11(11):629-638.

Kjeldsen L, Johnsen  a H, Sengeløv H, Borregaard N. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. *J Biol Chem*. 1993;268(14):10425-10432.

Koyner JL, Vaidya VS, Bennett MR, et al. Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. *Clin J Am Soc Nephrol*. 2010;5(12):2154-2165.

Kristman V, Côté P. Loss to follow-up in cohort studies: How much is too much? *Eur J Epidemiol*. 2004;19(8):751-760.

Lameire N, Van Biesen W, Vanholder R. Acute renal failure. *Lancet*. 2005;365(9457):417-430.

Loo MH, Felsen D, Weisman S, Marion DN, Vaughan ED. Pathophysiology of obstructive nephropathy. *World J Urol*. 1988;6:53-60.

Madsen MG. Urinary biomarkers in hydronephrosis. *Dan Med J*. 2013;60:1-20.

Malyszko J, Koc-Zorawska E, Malyszko JS, Mysliwiec M. Kidney injury molecule-1 correlates with kidney function in renal allograft recipients. In: *Transplantation Proceedings*. 42. 2010:3957-3959.

Mårtensson J, Bellomo R. The Rise and Fall of NGAL in Acute Kidney Injury. *Blood Purif*. 2014;37(4):304-310.

Mårtensson J, Xu S, Bell M, Martling C-R, Venge P. Immunoassays distinguishing between HNL/NGAL released in urine from kidney epithelial cells and neutrophils. *Clin Chim Acta*. 2012;413(19-20):1661-1667.

McIlroy DR, Wagener G, Lee HT. Neutrophil gelatinase-associated lipocalin and acute kidney injury after cardiac surgery: the effect of baseline renal function on diagnostic performance. *Clin J Am Soc Nephrol*. 2010;5(2):211-219.

McWilliam SJ, Antoine DJ, Sabbisetti V, et al. Reference intervals for urinary renal injury biomarkers KIM-1 and NGAL in healthy children. *Biomark Med*. 2014;8(10):1189-1197.

Perrotti A, Miltgen G, Chevet-Noel A, et al. Neutrophil Gelatinase-Associated Lipocalin as Early Predictor of Acute Kidney Injury After Cardiac Surgery in Adults With Chronic Kidney Failure. *Ann Thorac Surg*. 2015;99(3):1-6.

Miller NL, Lingeman JE. Management of kidney stones. *BMJ*. 2007;334(7591):468-472.

Nakada SY, Hsu THS. *Campbell-Walsh Urology*. Vol Tenth Edit. Elsevier; 2012.

Nickolas TL, Forster CS, Sise ME, et al. NGAL (Lcn2) monomer is associated with

tubulointerstitial damage in chronic kidney disease. *Kidney Int*. 2012;82(6):718-722.

Nickolas TL, O'Rourke MJ, Yang J, et al. Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. *Ann Intern Med*. 2008;148(11):810-819.

Ozayar A, Friedlander JI, Shakir N a., Gahan JC, Cadeddu J a., Morgan MSC. Equivocal Ureteropelvic Junction Obstruction on Diuretic Renogram—Should Minimally Invasive Pyeloplasty be Offered to Symptomatic Patients? *J Urol*. 2015;193(4):1278-1282.

Parikh CR, Butrymowicz I, Yu A, et al. Urine stability studies for novel biomarkers of acute kidney injury. *Am J Kidney Dis*. 2014;63(4):567-572.

Pedersen KR, Ravn HB, Hjortdal VE, Nørregaard R, Povlsen J V. Neutrophil gelatinase-associated lipocalin (NGAL): validation of commercially available ELISA. *Scand J Clin Lab Invest*. 2010;70(5):374-382.

Preminger GM, Tiselius HG, Assimos DG, et al. 2007 Guideline for the Management of Ureteral Calculi. *J Urol*. 2007;178(6):2418-2434.

Shalabi A, Abassi Z, Awad H, et al. Urinary NGAL and KIM-1: potential association with histopathologic features in patients with renal cell carcinoma. *World J Urol*. 2013;31(6):1541-1545.

Shlipak MG, Sarnak MJ, Katz R, et al. Cystatin C and the risk of death and cardiovascular events among elderly persons. *N Engl J Med*. 2005;352(20):2049-2060.

Siew ED, Ware LB, Ikizler T a. Biological Markers of Acute Kidney Injury. *J Am Soc Nephrol*. 2011;22(5):810-820.

Singh I, Strandhoy JW. *Pathophysiology of Urinary Tract Obstruction*. Vol Tenth Edit. Elsevier Inc.; 2012. doi:10.1016/B978-1-4160-6911-9.00040-2.

Sivalingam S, Stormont I, Nakada SY. Contemporary practice patterns in the management of acute obstructing ureteral stones. *J Endourol*. 2015; Ahead of Print.

Sprenkle PC, Wren J, Maschino AC, et al. Urine Neutrophil Gelatinase-Associated Lipocalin as a Marker of Acute Kidney Injury After Kidney Surgery. *J Urol*. 2013;190(1):159-164.

Sugandhi N, Srinivas M, Agarwala S, et al. Effect of stem cells on renal recovery in rat model of partial unilateral upper ureteric obstruction. *Pediatr Surg Int*. 2014;30(2):233-238.

Türk C, Knoll T, Petrik A, et al. Pocket Guidelines on urolithiasis. *Eur Urol*. 2014;40(4):362-371.

Urbschat A, Gauer S, Paulus P, et al. Serum and urinary NGAL but not KIM-1 raises in human postrenal AKI. *Eur J Clin Invest*. 2014;44(7):652-659.

Urbschat A, Obermüller N, Paulus P, et al. Upper and lower urinary tract infections can be detected early but not be discriminated by urinary NGAL in adults. *Int Urol Nephrol*. September 2014.

Vaidya VS, Waikar SS, Ferguson M a, et al. Urinary Biomarkers for Sensitive and Specific Detection of Acute Kindey Injury in Humans. *Clin Transl Sci*. 2008;1(3):200-

208.

Van De Vrie M, Deegens JK, Van Der Vlag J, Hilbrands LB. Effect of long-term storage of urine samples on measurement of kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL). *Am J Kidney Dis*. 2014;63(4):573-576.

van den Akker EK, Hesselink DA, Manintveld OC, IJzermans JNM, de Bruijn RWF, Dor FJMF. Neutrophil Gelatinase-Associated Lipocalin, but Not Kidney Injury Marker 1, Correlates with Duration of Delayed Graft Function. *Eur Surg Res*. 2015;55(4):319-327.

Vinken P, Starckx S, Barale-Thomas E, et al. Tissue Kim-1 and urinary clusterin as early indicators of cisplatin-induced acute kidney injury in rats. *Toxicol Pathol*. 2012;40(7):1049-1062.

Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol*. 2014;14(1):135.

Wang B, Chen G, Zhang J, Xue J, Cao Y, Wu Y. Increased Neutrophil Gelatinase-Associated Lipocalin is Associated with Mortality and Multiple Organ Dysfunction Syndrome in Severe Sepsis and Septic Shock. *Shock*. 2015;44(3):234-238.

Wen JG, Chen Y, Djurhuus JC. Experimental partial unilateral ureter obstruction. I. Pressure flow relationship in a rat model with mild and severe acute ureter obstruction. *J Urol*. 1998;160(4):1567-1571.

Xie Y, Xue W, Shao X, et al. Analysis of a urinary biomarker panel for obstructive nephropathy and clinical outcomes. *PLoS One*. 2014;9(11):e112865.

Xu SY, Pauksen K, Venge P. Serum measurements of human neutrophil lipocalin (HNL) discriminate between acute bacterial and viral infections. *Scand J Clin Lab Invest*. 1995;55(2):125-131.

Xue W, Xie Y, Wang Q, Xu W, Mou S, Ni Z. Diagnostic performance of urinary kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin for acute kidney injury in an obstructive nephropathy patient. *Nephrology (Carlton)*. 2014;19(4):186-194.

Yang L, Brooks CR, Xiao S, et al. KIM-1-mediated phagocytosis reduces acute injury to the kidney. *J Clin Invest*. 2015;125(4):1620-1636.

Yilmaz A, Sevketoglu E, Gedikbasi A, et al. Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin. *Pediatr Nephrol*. 2009;24(12):2387-2392. 79. Zappitelli M, Washburn KK, Arikan A a, et al. Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: a prospective cohort study. *Crit Care*. 2007;11(4):R84.

# Appendix 1 - STROBE Statement

| | Item No | Recommendation | Section (Page) |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | ✓ i |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | ✓ ii |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | ✓ Chapter 1 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | ✓ 37-39 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | ✓ 40-43 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | ✓ 45-48 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | ✓ |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed | ✓ 99 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. | ✓ Chapter 2 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | ✓ 46-47, 101-102 |
| Bias | 9 | Describe any efforts to address potential sources of bias | ✓ 45-48 |
| Study size | 10 | Explain how the study size was arrived at | ✓ 44 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | ✓ 52-53 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | ✓ 52-53 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | ✓ |
| | | (*c*) Explain how missing data were addressed | ✓ 54 |
| | | (*d*) Explain how loss to follow-up was addressed | |
| | | (*e*) Describe any sensitivity analyses | Not performed |

**Results**

| | | | |
|---|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | ✓ 54-55 |
| | | (b) Give reasons for non-participation | ✓ |
| | | (c) Consider use of a flow diagram | ✓ 55 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | ✓ 57 |
| | | (b) Indicate number of participants with missing data for each variable of interest | ✓ |
| | | (c) Summarise follow-up time (eg, average and total amount) | ✓ 54 |
| Outcome data | 15* | Report numbers of outcome events or summary measures over time | ✓ 59 |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). | |
| | | (*b*) Report category boundaries when continuous variables were categorized | ✓ |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | ✓ 62 |

**Discussion**

| | | | |
|---|---|---|---|
| Key results | 18 | Summarise key results with reference to study objectives | ✓ 72 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | ✓ 85-86 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | ✓ 84-85 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | ✓ 78-80 |

**Other information**

| | | | |
|---|---|---|---|
| Funding | 22 | Give the source of funding and the role of the funders for the present study | ✓ 53 |

# Appendix 2 – Western University Health Science Research Ethics Board Approval Notice

**Western** **Research Ethics**

**Research**

**Western University Health Science Research Ethics Board**
**HSREB Delegated Initial Approval Notice**

**Principal Investigator**: Dr. Hassan Razvi
**Department & Institution**: Schulich School of Medicine and Dentistry\Surgery, Western University

**HSREB File Number**: 106204
**Study Title**: Biomarkers of Kidney Injury in a Urological Population
**Sponsor**: Division of Urology Internal funding

**HSREB Initial Approval Date**: February 27, 2015
**HSREB Expiry Date**: February 27, 2016

**Documents Approved and/or Received for Information**:

| Document Name | Comments | Version Date |
|---|---|---|
| Advertisement | Poster | 2015/02/04 |
| Letter of Information & Consent | Study Group | 2015/02/04 |
| Western University Protocol | | 2015/02/04 |
| Letter of Information & Consent | Control Group | 2015/02/04 |
| Data Collection Form/Case Report Form | | 2014/12/09 |

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review. If an Updated Approval Notice is required prior to the HSREB Expiry Date, the Principal Investigator is responsible for completing and submitting an HSREB Updated Approval Form in a timely fashion.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940

Ethics Officer to Contact for Further Information

*This is an official document. Please retain the original in your files.*

Western University, Research, Support Services Bldg., Rm. 5150

# Appendix 3 – Consent and Letter of Information

**St Joseph's HEALTH CARE LONDON**

**Biomarkers of Kidney Injury in a Urological Population**

**Letter of Information**

Principal Investigator:     Dr. Hassan Razvi
Co-Investigators:     Dr. Stephen Pautler & Dr. John Denstedt

**INTRODUCTION**
You have been asked to voluntarily participate in this research study because you have been scheduled for treatment/surgery for your kidney stone(s).

**PURPOSE**
The purpose of this study is to analyse your urine for the presence of some proteins that your kidney may release due to stone disease and/or an obstructed kidney. These protein biomarkers may be helpful in the future diagnosis and management of patients with these urological conditions.

**STUDY SELECTION**
It is expected that 150 patients, both men and women, will be enrolled, at St. Joseph's Hospital, St. Joseph's Health Care London.

**PROCEDURES**
If you agree to participate, information about you (age and gender) and about your medical and urological history as well as information about your current urinary condition and treatment will be collected.  You will be asked to provide a voided urine sample before your surgery and again at each of your follow-up appointments at 3 to 6 weeks and again at 3 to 6 months, here at St. Joseph's Hospital.

A portion of your each of your urine samples will be sent to the hospital laboratory for routine microscopy/chemistry and to test the level of creatinine (how well your kidneys filter waste). The remainder of each sample will be shipped to the Laboratory at Harvard University for biomarker analysis.  The data from all participants will be compiled and the analysis will be done here at St. Joseph's Hospital.

**RISKS**
The specific risks associated with your surgical procedure will be explained to you by your urologist. There are no known additional risks associated with your participation in this study.

**BENEFITS**
You will not receive any benefit from participating in this study. However, your participation in this research study may help future patients.

**PARTICIPATION**
Participation in this study is voluntary.  You may refuse to participate, refuse to answer any question or withdraw from the study at any time with no effect on your future care.  To protect the integrity of the study, you will not be able to withdraw your data from the study after your urine samples have been

collected and sent for analysis. You may also be withdrawn without your permission, if, in the opinion of the investigators, further participation would not be in your best interest.

You do not waive any legal right by signing the consent form. Every precaution will be taken to prevent any injury to you during the study. However, if an injury does occur, you will obtain medical care in the same way that you normally would obtain your medical care.

**ALTERNATIVES**
As an alternative to participation in this study, you may choose not to participate and no urine will be collected for the study.

**COMPENSATION**
You will not be paid for your time for taking part in this study.

**CONFIDENTIALITY**
All information obtained during the course of this study is strictly confidential. You have a right to privacy and as permitted by applicable law, all reasonable measures will be taken to protect the confidentiality of your records. Your urine samples will be sent to the laboratory at Harvard University and will be identified only with a unique study number. Information resulting from this study and from your medical record may be used for research purposes and may be published. However, you will not be identified personally in such publications. All research records will be stored in secure research offices. While we will do our best to protect your information there is no guarantee that we will be able to do so.

Information gathered from the study will be made available to the investigators. Representatives of The University of Western Ontario Health Sciences Research Ethics Board may require access to your study-related records or may contact you to monitor the conduct of the research. Representatives from Lawson Health Research Institute may require access your study record for quality assurance purposes.

**QUESTIONS / FURTHER INFORMATION**
Please keep this Letter of Information and if you have any questions about this study, please do not hesitate to contact your urologist or the study coordinator. If you no longer wish to participate in the study, you must let your urologist or the study coordinator know.
> Investigators/Urologists
> > Dr. Razvi
> > Dr. Pautler
> > Dr. Denstedt
> The study Coordinato

A copy of your signed consent form will be made available to you.

If you have any questions about your rights as a research participant or the conduct of the study you may contact Dr. David Hill, Scientific Director, Lawson Health Research Institute at

**St JOSEPH's**
HEALTH CARE
LONDON

**Biomarkers of Kidney Injury in a Urological Population**

**CONSENT FORM**

I, _____ have read

the Letter of Information, have had the nature of the study explained to me and I agree to

participate.  All questions have been answered to my satisfaction.

_____          _____
Date                                       Signature of Participant

 

 

_____
Name of person responsible for
obtaining this consent

 

_____          _____
Date                                       Signature of person responsible for
obtaining this consent

# Appendix 4 – Inclusion and Exclusion Criteria Checklists

### A) Patients

**Biomarkers of Kidney Injury in a Urological Population**

Patient Name:_____ JNumber:_____

**Patients….**
- ☐ can only be enrolled once in this study;
- ☐ must be planning on returning here for both follow-up appointments.

**INCLUSION & EXCLUSION CRITERIA**

☒= Yes, **INCLUSION CRITERIA**
**Patients with ureteropelvic junction obstruction (UPJO) or ureteral stricture causing unilateral hydronephrosis:**
- ☐ Patients at least 18 years old;
- ☐ Patients willing to provide informed consent;
- ☐ Patients with diagnosis of UPJO or ureteral stricture associated with unilateral hydronephrosis;
- ☐ Patients with UPJO may have a renal stone, but it should not be the cause of the obstruction;
- ☐ Patients with a diuretic renogram performed before the surgical intervention/procedure.

☒= No, **EXCLUSION CRITERIA**
**Patients with ureteropelvic junction obstruction (UPJO) or ureteral stricture causing unilateral hydronephrosis:**
- ☐ Active urinary tract infection;
- ☐ Patients with an indwelling ureteric stent;
- ☐ Use of indwelling bladder catheter;
- ☐ Recent history of sepsis or septic shock (< 3 months);
- ☐ If it is in the treating urologist's opinion that participation in this study is not in the patient's best interest.

☒= Yes, **INCLUSION CRITERIA**
**Patients with stone disease without hydronephrosis:**
- ☐ Patients at least 18 years old;
- ☐ Patients willing to provide informed consent;
- ☐ Patients scheduled for PCNL or ureteroscopy to treat kidney stone(s);
- ☐ Patients with CT KUB scan or a KUB and a renal ultrasound;
- ☐ CT-KUB or renal ultrasound negative for hydronephrosis;
- ☐ Patients with at least one determination of serum creatinine in the last 6 months.

☒= No, **EXCLUSION CRITERIA**
**Patients with stone disease without hydronephrosis:**
- ☐ Presence of a ureteric stent;
- ☐ Active urinary tract infection;
- ☐ Patients with hydronephrosis defined by dilation of renal pelvis or calyces seen on CT scan or renal ultrasound at least 3 months before the surgical procedure;
- ☐ Use of indwelling bladder catheter;
- ☐ Recent history of sepsis or septic shock (< 3 months);
- ☐ If it is in the treating urologist's opinion that participation in this study is not in the patient's best interest.

☒= Yes, **INCLUSION CRITERIA**
**Patients with unilateral hydronephrosis due to stone disease without need for urgent management:**
- ☐ Patients at least 18 years old;
- ☐ Patients with unilateral stone disease located in ureter or at ureteropelvic junction:
- ☐ Patients with a CT KUB or KUB and renal ultrasound performed before the surgical intervention/procedure;
- ☐ Presence of hydronephrosis on the affected side;
- ☐ Patients with a serum creatinine measured no more than 6 months before the surgical intervention/procedure;
- ☐ Patients scheduled for PCNL or ureteroscopy to relieve the obstruction.

☒= No, **EXCLUSION CRITERIA**
**Patients with unilateral hydronephrosis due to stone disease without need for urgent management:**
- ☐ Contralateral hydronephrosis from any cause;
- ☐ Active urinary tract infection;
- ☐ Use of indwelling bladder catheter;
- ☐ Recent history of sepsis or septic shock (< 3 months);
- ☐ If it is in the treating urologist's opinion that participation in this study is not in the patient's best interest.

Comments:

**B) Controls**

<div style="border:1px solid black; padding:20px;">

**Biomarkers of Kidney Injury in a Urological Population**

**Name:**_____

❏ Subjects can only be enrolled once in this study.

**INCLUSION CRITERIA**

☒= Yes
❏ **Healthy subjects (Control group**):
   ❏ At least 18 years of age;
   ❏ Willing to provide informed consent;
   ❏ Able to provide a voided urine simple;
   ❏ Willing to have a renal ultrasound.

**EXCLUSION CRITERIA**

☒= No
❏ **Healthy subjects (Control group)**:
   ❏ Previous personal or family history of stone disease by patient history;
   ❏ Abnormal renal ultrasound;
   ❏ Abnormal dip-stick urinalysis:
     Blood, leukocytes, nitrites, proteins or glucose is positive.

</div>

# Appendix 5 – Coding of the analyzed variables

| Baseline variables | | | |
|---|---|---|---|
| **Demographics** | **Type of Variable** | **Values** | **Units** |
| Age | Continuous | 0-99 | years |
| BMI | Continuous | 15-50 | kg/m2 |
| Gender | Categorical, dichotomous | Male, Female | |
| **Past medical hisotry** | | | |
| CCI | Categorical, ordinal | 0->8 | |
| Diabetes | Categorical, dichotomous | Yes, No | |
| Hypertension | Categorical, dichotomous | Yes, No | |
| Previous stone disease | Categorical, dichotomous | Yes, No | |
| **Clinical** | | | |
| Episode related to renal colic | Categorical, dichotomous | Yes, No | |
| Length of time from colic to enrollment | Continuous | 1-99 | weeks |
| Planned procedure | Categorical, nominal | URS, PCNL, Pyeloplasty | |
| Recent visit to ER | Categorical, dichotomous | Yes, No | |
| **Radiological** | | | |
| Imaging study (CT scan/Renal US) | Categorical, nominal | CT Scan, Renal US | |
| Presence and grade of hydronephrosis | Categorical, dichotomous | Yes, No | |
| Presence of stone/ureteric stricture | Categorical, dichotomous | Yes, No | |
| Grade of hydronephrosis (Modified Fernbach & Ito classification) | Categorical, ordinal | 0-4 | |
| Single or multiple stone disease | Categorical, dichotomous | Single, Multiple | |
| Location of the stone (kidney, UPJ, ureter) | Categorical, nominal | Kidney, UPJ, Ureter | |
| Largest diameter of stone | Continuous | 0-50 | mm |
| Additional incidental findings in imaging | Qualitative | | |
| **Laboratory findings** | | | |
| Preoperative serum creatinine | Continuous | 50-500 | mg/mL |
| eGFR according CKD-EPI formula | Continuous | 0-120 | ml/min |
| Presence of leukocyturia in urinalysis | Categorical, dichotomous | Yes, No | |
| Number of leukocytes in urine | Continuous | 0->500 | cells/uL |
| Urinary creatinine | Continuous | 0-50 | mg/mL |
| Absolute urinary levels of KIM-1, monomeric & homodimeric NGAL | Continuous | 0-2000 | ng/mL |
| Normalized urinary levels of KIM-1, monomeric & homodimeric NGAL | Continuous | 10-1000 | ng/mg creatinine |

| Follow-up | | | |
|---|---|---|---|
| **Clinical** | | | |
|     Presence of ureteric stent | Categorical, dichotomous | Yes, No | |
| **Radiological** | | | |
|     Imaging study for follow up | Categorical, nominal | CT Scan, Renal US, KUB | |
|     Presence of residual stones | Categorical, dichotomous | Yes, No | |
|     Number of residual stones | Categorical, dichotomous | Single, mutiple | |
|     Presence and grade of hydronephrosis | Categorical, dichotomous | Yes, No | |
|     Grade of hydronephrosis (Modified Fernbach & Ito classification) | Categorical, ordinal | 0-4 | |
| **Biochemical** | | | |
|     Presence of leukocyturia in urinalysis | Categorical, dichotomous | Yes, No | |
|     Number of leukocytes in urine | Continuous | 0->500 | cells/uL |
|     Urinary creatinine | Continuous | 0-50 | mg/mL |
|     Absolute urinary levels of KIM-1, monomeric & homodimeric NGAL | Continuous | 0-2000 | ng/mL |
|     Normalized urinary levels of KIM-1, monomeric & homodimeric NGAL | Continuous | 0-100 | ng/mg creatinine |
|     Presence of incidental findings on imaging studies | Qualitative | | |

*\* CCI: Charlson Comorbidity Index; ER: Emergency Room; HTN: hypertension; KUB: kidney-ureter-bladder x-rays; UA: urinalysis; UPJ: ureteropelvic junction; US: ultrasound.*

*Units abbreviations: mg, miligram; mL, mililiter; ng, nanogram.*

# Appendix 6 – Permission to use figures

*Figure 2*

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*Figures in Table 2*

**Vita**

| | |
|---|---|
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| **Publications:** | **Olvera-Posada** D, Alenezi H, Tailly T, Dion M, Denstedt JD, Razvi H. Assessing the magnitude of effect of bone structures on shockwave lithotripsy fragmentation: Results from an in vitro study. J Endourol. 2016 Jan 6. |

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| | **Olvera-Posada D**, Dion M, Alenezi H, Razvi H, and Pautler SE. Robotic Proximal Ureteropyelostomy After Unsuccessful Endourologic Management of Complicated Proximal Ureteral Stone Disease. Journal of Endourology Case Reports. October 2015, 1(1): 30-32. |
| | **Olvera-Posada** D, Tailly T, Alenezi H, Violette PD, Nott L, Denstedt JD, Razvi H. Risk factors for postoperative complications after percutaneous nephrolithotomy (PCNL) in a tertiary referral centre. J Urol. 2015 Dec;194(6):1646-51. |
| | **Olvera-Posada D**, Armengod-Fischer G, Vázquez-Lavista  LG, Maldonado-Ávila M, Rosas- Nava E, Manzanilla-García H, Castillejos-Molina RA, Méndez-Probst CE, Sotomayor M, Feria- Bernal G, Rodríguez-Covarrubias F. Emphysematous pyelonephritis: multicenter clinical and therapeutic experience in Mexico. Urology. 2014Jun;83(6):1280-4. |
| | **Olvera-Posada D**, Suarez M, Castillejos-Molina RA, Gabilondo-Navarro F, Mendez-Probst CE., Validation of the Spanish version of Ureteral Stent Symptom Questionnaire (USSQ): Prevalence of symptoms in a tertiary care center in Mexico. J Endourol. 2014:3 (28): 377- 382. |
| | **Olvera-Posada D**, Villeda-Sandoval C, Ramírez-Bonilla M, Sotomayor M, Rodriguez-Covarrubias F, Feria-Bernal G, Méndez-Probst CE, Castillejos-Molina R. Natural history of pyuria and microhematuria after prostate surgery. Actas Urol Esp. 2013;3 (10):625-9. |
| | **Olvera-Posada D**, Garcia-Mora A, Culebro C, Castillejos-Molina R, Sotomayor M, Feria-Bernal G, Rodriguez-Covarrubias FT. Prognostic factors in emphysematous pyelonephritis. Actas Urol Esp 2012. Apr;37(4):228-32. |