Title
Sensitivity and Specificity of Neutrophil Gelatinase-Associated Lipocalin (NGAL) in an Emergency Room Population

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IRB Protocol

A. Study Purpose and Rationale

Urinary Neutrophil Gelatinase Associated Lipocalin (NGAL) is a protein produced in the distal tubule of the nephron. It was discovered in the laboratory of Dr. Jonathon Barasch at Columbia University Medical Center. NGAL's function is to aid in the conversion of metanephric mesenchyme into epithelia during the formation of the kidney. Furthermore, through gene expression and microarray studies, it was found to be massively upregulated during early phases of acute tubular injury (ATN) in a mouse model. Human studies have confirmed that NGAL is expressed in the setting of both acute and chronic kidney damage and is excreted into the urine where it can be measured. In addition the level of NGAL expressed in the various forms of kidney damage varies. Because urine NGAL concentrations in the various forms of kidney damage are different, we believe that NGAL may be able to discriminate these forms of kidney dysfunction. A diagnostic test that can classify a patient with an abnormal serum creatinine as having stable chronic kidney disease, acute tubular necrosis, urinary obstruction or pre-renal azotemia has broad clinical applications in multiple arenas, in particular an inpatient hospital setting or in the emergency room. We therefore propose a study evaluating the ability of urine NGAL to discriminate these various types of kidney dysfunction presenting to an emergency room in an urban setting. We will use the following hypothesis and specific aim:

Hypothesis 1: In patients that present to an urban emergency room, a single urine NGAL measurement can classify their kidney disease as stable CKD, ATN, urinary outlet obstruction or Pre-Renal azotemia.
Specific Aim 1: In patients with an abnormal serum creatinine presenting to the CUMC ER, we will evaluate the association between urine NGAL levels and kidney dysfunction diagnosis.

B Background

B1. NGAL in Urine and Serum is a Quantitative Marker of Renal Disease

Initial studies were done in mice. These studies showed that the intensity and duration of NGAL protein measured in the urine and serum correlated with the extent of ischemia in a mouse model. Furthermore, reversal of the kidney damage by pharmacological intervention was shown to reduce NGAL expression (Stec, Watkins). In a first study in humans, researchers at Columbia University found that patients had different levels of NGAL in the serum and urine depending on whether the kidney disease was due to ATN, ATN with sepsis or CKD (See Table 1).

Diagnosis	Serum (ng/ml)	Urine (ng/ml)
Normal	21	22
ATN	146	557
ATN Sepsis	331	2786
CKD	49	119

These data were from a small cross-sectional study but they indicate that a single measurement of NGAL levels may distinguish types of renal disease that otherwise require time consuming repetitive observations of the patient or invasive testing.

B2. Preliminary Human Studies

Several human studies have produced results that confirm those from animal models. In addition, levels of urinary NGAL, in contrast to serum creatinine, have not been found to fluctuate with patient age, gender, muscle mass, or nutritional status. We believe these preliminary data suggest that urinary levels of NGAL are a potential, robust marker of advancing tubular damage in the setting of KD.

B2a. In a prospective study of surgery-associated acute kidney injury (AKI which includes ATN) in children, those who developed AKI, defined as a >50% increase in serum creatinine, demonstrated a 15-fold increase in serum and urine NGAL. Urine NGAL was sensitive (100%) and specific (98%) for the delayed rise in creatinine. Further, NGAL levels rose within 2 hours of cardiopulmonary bypass, whereas the gold standard, which is the serum creatinine, became diagnostic over the subsequent 24-48 hours.

B2b. In a prospective study of surgery-associated acute kidney injury (AKI) in adults, those who developed AKI, defined as a >50% increase in serum creatinine, demonstrated a 4-fold increase in urine NGAL levels. Urine NGAL was a sensitive (80%) and specific (70%) for the delayed rise in creatinine. Furthermore, the difference between acute renal failure and no renal disease was evident within 3 hours of cardiopulmonary bypass, whereas serum creatinine, the gold standard, became diagnostic only over the subsequent 24-48 hours.

B2c. Preliminary data from an ongoing prospective study undertaken to determine whether urinary NGAL can identify renal impairment in very low birth weight infants with PDA have produced similar results. Infants without PDA had exp(mean(log.ngal)) = 42.2; infants with PDA but not AKI had exp(mean(log.ngal)) = 94.3; infants with PDA and AKI had exp(mean(log.ngal)) = 243.6 which differed significantly from these other values. Together, these data suggest that NGAL is a sensitive and rapidly expressed biomarker of AKI.

B2d. Urine NGAL levels also correlate with chronic kidney disease (CKD) severity and possibly predict its progression. Urine NGAL, along with serum creatinine and urine protein, was measured in forty-four patients with CKD. A follow-up serum creatinine was then obtained at the next office visit. NGAL levels were then evaluated for their association with changes in kidney function as defined by both the serum creatinine and estimated glomerular filtration rate (eGFR). Urine NGAL levels were significantly correlated with both the baseline serum creatinine and eGFR. Furthermore, there was an association with increasing NGAL levels and worsening kidney function at the follow-up visit. Indeed, NGAL levels were associated with the severity of chronic pathological changes on kidney biopsy.

B3. Community Acquired Renal Failure: Scope of the Problem

Previous studies have found renal impairment to be a prevalent and significant medical problem. In a study of 4,622 consecutive patients admitted to the medical and surgical service at an urban

tertiary care hospital, 7.2% were found to display some degree of renal insufficiency. The overall mortality rate was 19.4% for these patients; this rate increased to 37.8 % in those with creatinine greater than 3.0mg/dl. A similar study conducted in Madrid found an acute renal failure incidence rate of 209 cases per million population and a mortality rate of 26.7%. Together, these studies suggest that significant proportion of all hospital admissions will be complicated by some form of renal impairment.

B4. Comparison of NGAL and Current Methods of Diagnosis

AKI is poorly characterized and difficult to diagnose early. Additional challenges include the inability to predict severity, measure progression, or measure response to therapy. The standard measure of AKI, serum creatinine, has poor specificity and sensitivity in these situations: patients are not in steady state and, hence serum creatinine lags behind both renal injury and recovery. Other markers of disease such as urine casts and fractional excretion of sodium are nonspecific and insensitive. Reliance on these traditional markers slows recognition of AKI and therefore delays treatment.

Because of these shortcomings, a number of biomarkers of AKI have recently emerged. First, Cystatin C is a 13kD cysteine protease inhibitor that is released at a constant rate from all nucleated cells into the plasma. This marker does not require age, gender, or muscle mass adjustments. Cystatin C appears to predict renal function at least as well as creatinine in CKD and better than creatinine in AKI. Second, Kidney Injury Molecule-1 (KIM-1) is an orphan transmembrane receptor of unknown function that is induced to very high levels in the proximal tubule after ischemic and nephrotoxic injury. This molecule is cleaved and appears in the urine. In a small study involving 40 patients, only 9 of whom demonstrated AKI, urinary KIM-1 distinguished ischemic AKI from prerenal azotemia and CKD. Third, Interleukin-18 (IL-18) is a proinflammatory cytokine that is cleaved to the mature form by caspase-1 in injured proximal tubules and released into the urine after ischemia. Urine IL-18 increased significantly in 14 patients with AKI compared to 36 normal subjects and patients with prerenal azotemia, UTI, chronic renal insufficiency, and nephritic syndrome. Elevation of urinary IL-18 could predict AKI one day before creatinine in 138 patients with adult respiratory distress syndrome with an area under (AUC) the ROC of 0.73.

NGAL compares favorably to these molecules and may even be superior as a marker of AKI. Unlike Cystatin C, NGAL is not merely a marker of filtration status. In preliminary studies of AKI, NGAL demonstrated a greater AUC than KIM-1 (0.998 to 0.879). In comparison to urinary IL-18, which in studies peaked 12 hours following cardiopulmonary bypass, NGAL peaked at 2 hours.

NGAL is produced by the nephron in response to tubular epithelial damage and is a marker of TI injury. It has been well established that in ATN from ischemia or nephrotoxicity that NGAL levels rise, even after mild “subclinical” renal ischemia or exposure to nephrotoxins, in spite of normal serum creatinine levels. From our preliminary data we know that NGAL is expressed by the ATN and CKD kidney of various etiologies, and that elevated urinary NGAL levels may distinguish these entities and be predictive of progressive kidney failure.

B5. Summary of the Study

The purpose of our study is to determine whether urinary NGAL levels are able to distinguish the classical categories of renal disease. Previous studies have strongly suggested that this protein marks those with fulminant renal dysfunction with greater sensitivity and time resolution than

currently used markers. Studies to date have been in highly selected populations: children and adults following cardiac surgery, infants with cardiovascular anomalies, and patients with known CKD. Demonstration of similarly robust sensitivity and specificity in a broad Emergency Room population would strengthen the conception of NGAL as a marker of early or advancing kidney dysfunction. Most importantly, if NGAL can distinguish between types of renal disease at presentation in the ER, it might have important implications regarding ER management of these common presentations. For example, it could reduce diagnostic ambiguity and lag time from hours or days to seconds.

C. Study Design and Statistical Analysis:

This study will assess the sensitivity and specificity of urinary NGAL to distinguish classical categories of renal disease. Toward this end, patients will be recruited from the Columbia University Medical Center Emergency Department.

Inclusion criteria stipulate that the patient:

A) must be greater than 18 years of age

B) not have clinical symptoms or evidence of a urinary tract infection by standard urinary dip stick.

C) must satisfy the following age and sex stratified serum creatinine levels:

1. men between ages 18 and 50 with serum creatinine greater than 1.2mg/dl
2. women between ages 18 and 50 with serum creatinine greater than 1.2mg/dl
3. men older than 50 with serum creatinine greater than 1.0mg/dl
4. women older than 50 with serum creatinine greater than 0.8mg/dl

Control Patients

Age and sex matched patients who satisfy A, B criteria, but do not satisfy C criteria will be recruited.

Number of Patients A minimum of 348 patients who satisfy A-C inclusion criteria will be recruited for this study. We plan to enroll 350 patients. This number will provide the power to show that admission urinary NGAL levels are greater than 80% sensitive for diagnosis of ATN or acute on chronic kidney disease assuming the actual sensitivity is 95%. A minimum of 350 patients who satisfy A, B criteria, but do not satisfy C criteria will be recruited as controls.

D. Study Procedure:

Collection of Samples and Data Eligible patients will be identified in collaboration with Columbia University Emergency Department physicians. At study entry, demographic data including gender, age, current medications, medical history and previous diagnosis of kidney disease will be recorded. Patients will next provide up to ten milliliters of urine; a similar amount of urine will be collected from catheterized patients. This urine would otherwise have been discarded. These samples will be spun in centrifuge equipment located in the laboratory of Dr. Jonathan Barasch and stored at -80C. At a later date, the amount of NGAL present in these urine samples will be quantified by both Western Blot and ELISA assays. In addition, urine Na and urine urea, urine specific gravity, urine cells will be measured by standard lab techniques.

After patients provide urine, their subsequent hospital course will be followed using WebCis. All patients enrolled in the study will have their inpatient history followed from the date of admission to the date of discharge. The following information will be collected during their inpatient admission: laboratory studies including electrolyte panels, urinalysis, and urine microscopy, medication history, procedure history, imaging history, and complication history (see table 2).

Category:	Studies of Interest:	Source:
Laboratory Studies	Daily serum chemistry panels, complete blood counts	WebCis
Urine Microscopy	Presence of urinary casts	WebCis
Urinalysis	Fractional excretion of sodium, urine sodium, urine osmolality, urine urea, urine specific gravity, urine cells	WebCis and Dr. Jonathan Barasch's lab
Experimental Studies	Urinary NGAL, urinary IL-18, KIM -1, serum cystatin-C	Dr. Jonathan Barasch's lab

Outcome of the Analysis The diagnosis of the renal disease in the enrolled patients will be made using both retrospective and prospective data obtained from WebCis. Specifically, we will monitor for the development of any one of the following conditions:

A) ATN, defined as loss of renal function, measured by a decline in kidney function developing over a period of hours to days. In accordance with the Second Consensus Conference of the Acute Dialysis Quality Initiative, ATN will be stratified based on the severity and duration of injury into stages of Risk, Injury, Failure, and Loss.

1. Risk denotes a 1.5X increase in serum creatinine or 25% decrease in GFR
2. Injury denotes a 2X increase in serum creatinine or 50% decrease in GFR
3. Failure denotes a 3X increase in serum creatinine or 75% decrease in GFR
4. Loss denotes elevated serum creatinine for greater than 4 weeks.

Corroborating evidence for ATN will include the presence of muddy brown granular casts or epithelial casts on urinary microscopy, or $FE_{NA}^{+} > 1\%$, or consistently depressed GFR despite adequate hydration, or urine sodium greater than 40meq/L, or urinary osmolality less than or equal to 350mosmol

B) Pre-renal disease, defined as $FE_{NA}^{+} < 1\%$, or urine sodium < 20meq/L, or the presence of hyaline casts on urinalysis, or BUN:Cr ratio > 20:1, or urinary osmolality greater than or equal to 500mosmol

C) CKD, defined as either:

1. kidney dysfunction for greater than or equal to three months as evidenced by structural or functional abnormalities, with or without decrease in GFR, manifest by either
    a) pathological abnormalities
    b) the presence of kidney damage markers, including abnormalities in the serum creatinine, the presence of urine protein or abnormalities in imaging
2. GFR less than 60 ml/minl1.73m2 for greater than or equal to three months with or without kidney damage.

3. All of these patients will display no decrease in GFR during their hospital course.

D) CKD with superimposed ARF, defined as either:

1. kidney dysfunction for greater than or equal to three months as evidenced by structural or functional abnormalities, with or without decrease in GFR, manifest by either
    a) pathological abnormalities
    b) the presence of kidney damage markers, including abnormalities in the serum creatinine, the presence of urine protein or abnormalities in imaging
2. GFR less than 60 ml/minl1.73m2 for greater than or equal to three months with or without kidney damage.
3. All of these patients will display a new-onset, sustained greater than or equal to 25% decrease in GFR immediately prior to or during the hospital.

E) Urinary obstruction as defined by urinary tract imaging studies.

Upon WebCis review, it is likely that certain patients might fit into more than one of these five diagnoses. First, in these situations, establishing the presence or absence of CKD, according to previously listed criteria, will become the primary priority. Onset of ATN, pre-renal disease, or urinary obstruction within the setting of CKD will be categorized as CKD with superimposed ARF. Second, certain patients might demonstrate a number of these conditions over the course of their hospital courses. For example, it is plausible that a patient who presents to the ER with pre-renal disease might advance to ATN. In these instances, renal diagnoses will be assigned according to laboratory measures closest to the time of NGAL sampling. In addition, ultimate outcomes will be recorded. For, while the urinary NGAL level at the time of admission might correctly indicate which patients are in pre-renal failure, it might also predict which of these patients are at high risk to progress to acute tubular necrosis. This analysis, though secondary, must be accounted for. Third, all laboratory evidence might not coincide with a particular diagnosis. In these situations, the diagnosis most supported by the combination of inpatient course and body of laboratory data will be assigned.

One of these diagnoses will be assigned to all patients before NGAL levels are quantified. Admission NGAL levels will be paired with the subsequent diagnosis of renal dysfunction, and sensitivities and specificities of NGAL in marking these categories will then be computed.

E. Study Drugs:
None

F. Medical Devices:
No medical devices will be analyzed in this study.

G. Study Questionnaires:
None

H. Study Subjects:
Inclusion criteria stipulate that the patient
A) must be greater than 18 years of age

B) must not have clinical symptoms or evidence of a urinary tract infection by standard urinary dip stick.
C) must satisfy the following age and sex stratified serum creatinine levels:

1. men between ages 18 and 50 with serum creatinine greater than 1.2mg/dl
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I. Recruitment of Subjects:
Review of initial standard clinical laboratory measures will identify potential subjects. Assuming theses patients satisfy previously stated inclusion criteria and with the assent of their Emergency Room physician, these patients will be approached regarding potential inclusion in this study. All subjects will only be recruited in this manner.

J. Confidentiality of Subjects:
Confidentiality will be protected via the assignment of study identification numbers, which will be used for data processing. A list of patient identification numbers will be kept in a separate location. All data will be stored in encrypted files on computer. All patient identifying information will be kept separate from these files.

K. Potential Conflict of Interest:
None

L. Location of the Study:
All urine samples will be collected in the Columbia University Medical Center Emergency Room. These samples will subsequently be processed in the laboratory of Dr. Jonathan Barasch in the Black Building on the Columbia University Medical Center Campus.

M. Potential Risks:
None

N. Potential Benefits:
Because the study does not involve active treatment and seeks only to assess the sensitivity and specificity of admission urinary NGAL levels, this study does not promise benefit to the patients who agree to participate in the study. Future patients might benefit from kidney preserving

treatments instituted as a result of NGAL-afforded early detection and diagnosis of renal diseases.

O. Alternative Therapies:
Not applicable

P. Compensation to Subjects:
None

Q. Cost to Subjects:
Subjects will not incur any additional cost as a result of participating in this study.

R. Minors as Research Subjects:
In accordance with this study's inclusion criteria, no participants less than 18 years of age will be recruited.

S. Radiation or Radioactive Substances:
This study will not involve radioactive substances.

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