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Urinary Biomarkers And Association With Recovery From Severe Aki

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URINARY BIOMARKERS AND ASSOCIATION WITH RECOVERY FROM SEVERE AKI

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ABSTRACT

Urinary Biomarkers and Association with Recovery from Severe AKI

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Few studies have examined the ability of urinary biomarkers to associate with recovery from acute kidney injury (AKI), a common complication for hospitalized patients that is independently associated with severe morbidity and mortality. We hypothesized that urinary biomarkers of kidney injury (NGAL, IL-18) or repair (YKL-40) would be associated with renal recovery in patients with severe AKI.

We prospectively enrolled 48 patients admitted at Yale New Haven Hospital with severe AKI (AKIN Stage 3). Daily morning urine samples were collected (until recovery up to max of 9 days). Levels of urinary YKL-40, NGAL, and IL-18 were measured. The primary exposures were the concentrations of the biomarkers on the first, last, and average daily values of the biomarker during enrollment. The primary outcome was renal recovery at discharge and was classified as "complete" if SCr $\leq$1.4 mg/dL, "partial" if SCr >1.4 and lower than peak SCr reached, and "non-recovery" if SCr > 4mg/dL or death.

Fifteen patients recovered completely, 17 recovered partially, and 16 did not recover (11 due to death). Mean time to partial recovery from the first sample collection was 2.47 ± 1.92 days and mean time to complete recovery was 6.60 ± 4.03 days. Median values of the day 1, average, last NGAL ($p= 0.01$, $p=0.01$, and $p <0.01$) and last YKL-40 ($p=0.02$) were significantly higher in patients that did not recover from AKI, compared to those that experienced partial or complete recovery. The AUCs for non-recovery for day 1, average, and last NGAL were 0.76, 0.77, and 0.81, for YKL-40 0.58, 0.66, and 0.75, and for IL-18 0.54, 0.55, and 0.59 respectively.

In conclusion, high values of YKL-40 and NGAL appear to be associated with non-recovery from severe while IL-18 did not discriminate between recovery and non-recovery in this cohort of patients. Larger studies will need to confirm these findings.

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INTRODUCTION:

Acute Kidney Injury: Definition, Diagnosis, and Treatment:

Acute kidney injury (AKI), previously known as acute renal failure, is characterized by a rapid (< 48 hours) and sustained (> 6 hours) loss of renal function. The etiologies of AKI are generally classified as pre-renal, intra-renal, or post-renal and these can be differentiated based on evidence on history of recent procedures or medications, volume status, signs or symptoms of obstruction, and knowledge of vascular or systemic disease leading to subsequent ischemic injury. Additionally, urine evaluation, fractional excretion of sodium or urea (FE_{Na}, FE_{Urea}), and renal ultrasound are diagnostic.

- Pre-renal causes of AKI are related to decreased effective blood flow to the kidney which can include states of hypovolemia, poor cardiac output, or systemic vasodilation from sepsis, renal vasoconstriction secondary to NSAIDs, ACEi/ARB, contrast agents, or hepatorenal syndrome, and large vessel disease including renal artery stenosis, renal vein thrombosis, embolism etc. ultimately leading to renal ischemia and subsequent decline in GFR.
    - Key diagnostic findings include FE_{Na} < 1%, BUN/Cr > 20, Urinary osmolality (U_{osm}) >500, Urine sodium (U_{Na}) < 20, and FE_{Urea} < 35 (especially useful when there is concurrent diuretic use and thus FE_{Na} is non-diagnostic). Urine sediment is notable for transparent hyaline casts.
- Intra-renal or intrinsic AKI is defined has damage to the kidney itself which may be at the level of the glomeruli, renal tubules, or interstitium. The causes here include glomerulonephritis (GN), acute tubular necrosis (ATN), and acute interstitial nephritis (AIN). ATN can be due to ischemia from progression of prerenal disease, toxins such as drugs (aminoglycosides, amphotericin, cisplatin),

pigments (myoglobin from rhabdomyolysis), crystal or light chain deposits, and contrast agents. AIN is typically due to drug allergies, infection (pyelonephritis), and infiltrative or autoimmune disease. Lastly small vessel disease such as cholesterol emboli or thrombotic microangiopathy can also precipitate intra-renal AKI.

  - ATN: FE_{Na} >2%, U_{osm} < 350, U_{Na} > 20, BUN/Cr < 20, pigmented granular muddy brown casts
  - AIN: WBC, WBC casts, urine eosinophils, lymphocytes, possible RBCs
  - GN: dysmorphic RBCs and RBC casts

- Post-renal causes of AKI are due to urinary tract obstruction at the bladder neck or ureteral (bilateral). Common etiologies include benign prostatic hyperplasia, nephrolithiasis, upper or lower urinary tract malignancy, obstructed urinary catheter, or urinary obstruction from anticholinergic medications.
  - Hydronephrosis on renal ultrasound, bland urine sediment with possible RBCs

Although there are several diagnostic tests and clues from history and physical exam that can help differentiate between the types of AKI, the initial suspicion for kidney injury is primarily based on rise in serum creatinine (SCr) or decline in urine output. There are two predominant classification schemes that are used to define AKI – RIFLE and AKIN criteria.

RIFLE: Acute Dialysis Quality Initiative (ADQI) 2005 (1)

	Glomerular Filtration Rate (GFR) and SCr Criteria	**Urine Output (UO) Criteria**
Risk	SCr rise by 1.5 times above baseline or GFR decline > 25%	UO < 0.5mL/kg/h for ≥ 6h
Injury	SCr rise 2x or GFR decline > 50%	UO < 0.5mL/kg/h for ≥12h
Failure	SCr rise 3x or GFR decline >75% or absolute SCr value > 4 mg/dL with an increase ≥ 0.5 mg/dL	UO < 0.3 mL/kg/h for ≥ 24h or anuria ≥ 12 h
Loss	Complete loss of kidney function or need for renal replacement therapy (RRT) > 4 weeks	
End Stage	need RRT >3 months	

AKIN: Acute Kidney Injury Network 2007 (2)

	SCr Criteria	**UO Criteria**
Stage 1	SCr > 50% from baseline or > 0.3 mg/dL from baseline	UO < 0.5mL/kg/h for ≥ 6h
Stage 2	SCr ≥ 100%	UO < 0.5mL/kg/h for ≥12 h
Stage 3	SCr ≥ 200% or SCr ≥ 4.0 mg/dL with an increase ≥ 0.5 mg/dL or initiation of RRT	UO < 0.3 mL/kg/h for ≥ 24 h or anuria ≥ 12 h

Some of the refinements in the AKIN criteria for classifying AKI over the RIFLE criteria include: lower cut-off for AKI (rise in SCr >=0.3 mg/dl), no longer using ‘estimated

glomerular filtration rate' as a criterion but instead using only SCr and urine output, and inclusion of a 48-hour window for the diagnosis of AKI and classifying patients on RRT automatically as having AKI-III.(3)

The treatment for AKI is generally multifactorial and starts with identifying the underlying disorder and type of AKI. Some common therapies including holding nephrotoxic drugs (or adjusting dosing for renally cleared drugs), optimizing hemodynamics (forward flow), correcting volume overload, electrolyte imbalances, achieving normal acid-base status, and relieving obstruction and providing intravenous hydration as needed. As a last resort, urgent dialysis may be initiated if the condition is refractory to the above treatments. Indications for RRT include metabolic acidosis, electrolyte imbalance, intoxication (methanol, ethylene glycol), severe volume overload, and uremia.

Significance & Outcomes

AKI is a common complication in hospitalized patients and its incidence has risen substantially over the past two decades.(4-6) causing a major burden on health care costs (3) and prolonging hospital stay.(7, 8) Greater than 17 million admissions annually in the US are complicated by AKI resulting in over $10 billion in costs to the health care system.(9) The incidence of AKI is estimated between 5-7% of all hospitalized patients at any given time (10) while those in the critical care setting are at 5-25% risk (11, 12) and there is an overall mortality rate of 50-80% from this complication. (12-14) With regards to severity, the population incidence of less severe AKI (AKIN stage 1 or 2) and AKI

treated with RRT (AKIN stage 3) is approximately 2000-3000 and 200-300 per million population annually, respectively.(15)

Despite significant technical advances in therapeutics, the mortality and morbidity rates associated with AKI remain dismally high and have not appreciably improved during the last four decades. (11, 16-21) AKI has been shown to be independently associated with mortality (22-25) both in-hospital and post-discharge across various clinical situations including administration of radiocontrast dye (26), cardiopulmonary bypass (21, 22), mechanical ventilation (27), and sepsis (28).

A multinational study of nearly 30,000 critically ill patients showed that approximately 5.7% individuals developed AKI during their ICU stay and nearly 73% of these patients required RRT (15). In this study, the overall hospital mortality was 60.3% and dialysis dependence at time of discharge was 13.8% for survivors. In a retrospective study of 2,973 patients with no history of chronic kidney disease (CKD) undergoing cardiothoracic surgery, the development of AKI post-operatively was linked with increased long-term mortality. (29) At 10 years, the adjusted hazard ratios were 1.23 (95% CI 1.06-1.42) for the least severe RIFLE risk class and 2.14 (95% CI 1.73-2.66) for the RIFLE failure class compared to patients without AKI. The 10-year survival rate for patients with AKI and complete or partial recovery of renal function at discharge was 44% compared to 63% for patients with no AKI. Moreover, patients with complete renal recovery after AKI had an increased adjusted hazard ratio of death of 1.28 (95% CI 1.11-1.48) compared to patients who never developed AKI.

More recently, Ostermann et al.(30) used the Riyadh Intensive Care Program database to determine the relationship between AKI severity and ICU outcome or mortality. Out of 22,303 patients admitted to 22 ICUs in UK and Germany between 1989 and 1999, a total of 7,898 (35.4%) developed AKI. The AKIN criteria was used to further stratify these patients by AKI severity. Of this subgroup, 19.1% had AKI Stage I, 3.8% had AKI Stage II and 12.5% had AKI Stage III. Mortality in the ICU was 10.7% in patients without AKI, 20.1% in AKI-I, 25.9% in AKI-II, and 49.6% in AKI-III. Additionally, increasing AKI severity was associated with increased length of ICU admission. Progression from AKI-I or AKI-II to AKI-III was associated with worse outcomes that patients who had AKI-III on admission.

Besides higher in-hospital mortality, increasing severity of AKI also associates with increased long-term mortality after hospital discharge in patients not requiring dialysis. (31) In a retrospective study examining 82,711 patients with and without AKI at least 90 days after hospitalization, 17.4% of the surviving patients died during follow-up – 29.8% in the AKI subgroup and 16.1% in the non-AKI subgroup. The adjusted mortality risk associated with AKI was 1.41 (95% CI 1.39-1.43) and increased with worsening AKI Stage I, II, III: 1.36 (1.34-1.38), 1.46 (1.42-1.50) and 1.59 (1.54-1.65), respectively.

Overall, all proposed classifications for AKI have demonstrated that increased severity of AKI is associated with higher risk of death. (32-35)

Besides hospital and immediate post-discharge mortality risk, the other major concern for patients with AKI is development of CKD and later progression to end state renal disease (ESRD). A possible mechanism explaining this phenomenon is that renal blood flow and glomerular filtration may remain impaired for a prolonged period of time despite normalization of SCr. (36) Several studies in experimental animals have also identified a process called rarefaction which is a decrease in the capillary density of peritubular capillaries due to progressive damage after AKI. (37) Genomic signature analysis in the repair stage after AKI has suggested that persistent inflammation and immune responses late after AKI could contribute to the pathogenesis of CKD. (38) In the same study, histologic scoring revealed progressive tubular fibrosis during the repair phase after AKI eventually contributing to CKD.

A retrospective cohort study of 233,803 patients hospitalized in 2000, aged ≥67 at time of discharge, with no previous history of ESRD or AKI examined the link between AKI and subsequent ESRD. (39) After adjusting for age, gender, race, diabetes, and hypertension, the hazard ratio for developing ESRD was 41.2 (95% CI 34.6-49.1) for patients with AKI and CKD relative to those without kidney disease; 13.0 (10.6-16.0) for patients with AKI and no CKD; and 8.4 (7.4-9.6) for patients CKD and no AKI. Clearly, AKI increases the risk for ESRD and associated repercussions from dialysis or immunosuppressive treatment in the case of kidney transplant.

A systematic review consisting of 13 cohort studies (11 retrospectively following 3,000 patients) comparing the risk for CKD, ESRD, and death in patients with and without

AKI, found that patients with AKI had higher risks for developing CKD (pooled adjusted HR 8.8, 95% CI 3.1-25.5), ESRD (pooled adjusted HR 3.1, 95% CI 1.9-5.0) and mortality (pooled adjusted HR 2.0, 95% CI 1.3-3.1) compared with patients without AKI. (40) This relationship was graded with greater risk associated with increasing severity of AKI. The risk for CKD or ESRD following AKI is likely due to renal fibrosis which can persist despite resolution of AKI as has been shown in experimental animal models. (37, 41, 42) Since prevalence of patients surviving after AKI has been increasing, a relationship between AKI and CKD or ESRD would pose a tremendous health care burden.(6, 8, 43)

Current Clinical Dilemma

As described earlier, the classification criterions for AKI depend primarily on rise in SCr and/or detection of oliguria to diagnose acute kidney injury. However, SCr is not a sensitive or specific marker of renal function. SCr is a poor marker of acute fluctuations in kidney function because it is influenced by non-renal factors (44, 45) such as body weight, race, age, gender, drugs, muscle metabolism, protein intake, and tubular secretion.(46) In fact, the SCr rise may not be witnessed until 48-72 hours after the initial insult to the kidney since the patients are not in steady state thus SCr lags behind renal injury.(23, 44) Furthermore, significant renal disease (*e.g.*, fibrosis) can exist with minimal or no change in SCr because of renal reserve, enhanced tubular secretion of creatinine, or other factors. (24, 46-49) During the early phases of AKI, SCr alone also cannot distinguish clinically among pre-renal, post-renal, and intrinsic renal causes. For patients who develop severe AKI and require dialysis, SCr is not helpful in detecting

recovery because the SCr value can be artificially lowered by the dialysis treatments. Other conventional biomarkers such as urinary casts and fractional excretion of sodium have also proven to be insensitive and non-specific for the early recognition of AKI. (50, 51)

In the spring of 2004, the Board of Advisors and the Council of the American Society of Nephrology conducted research retreats to steer priorities appropriately in an era of limited resources. (52) In the area of AKI, where late recognition of AKI and delayed treatment was a major predicament, the group recommended that the highest priority is to standardize and/or discover biomarkers to a) diagnose AKI before the rise in SCr; b) stratify patients with respect to severity of injury; c) provide prognostic indicators.

Just briefly, NIH investigators have defined biomarkers as "quantitative measures of biologic effects that can be objectively measured and evaluated and provide informative links between mechanism of action, pathogenic processes, and clinical effectiveness." It is also important to note the need to evaluate multiple potential biomarkers, since no single marker will provide sensitivity and specificity across a spectrum of acute kidney injury.

Specifically for the purposes of our study, an ideal AKI biomarker is one that is a responsive element in injury, highly sensitive to facilitate early detection and allow risk stratification, localized to kidney cells to minimize outside influence on the kidney stress response, and the change in the biomarker level is actionable. A troponin-like biomarker

of acute kidney injury and recovery from acute kidney injury that is easily measured, unaffected by other biological variables, and capable of risk stratification would be a tremendous advance for clinical medicine.

The lack of significant progress in the prevention and management of AKI has been attributed, in part, to the failure to identify suitable physiologic biomarkers for use in research studies testing the efficacy of new interventions. For example, the standardized use of serum cardiac enzyme concentrations has facilitated rapid progress in the management of coronary insufficiency, markedly decreasing the morbidity and mortality of acute myocardial infarction. By contrast, AKI prevention and therapy studies using variables such as urine output and serum and urine chemistries have not yielded interventions proven to decrease the morbidity (requirement for dialysis) and mortality. (53-55)

Current State of Biomarkers

What exactly happens during ischemic renal injury? Although the answer to this question continues to evolve, it appears that the proximal tubule cells undergo a host of reactions including loss of cell polarity, cell death from apoptosis and necrosis, de-differentiation and proliferation of viable cells, and re-establishment of the epithelial phenotype. (56, 57)

Several urinary and serum biomarkers have been studied in the last decade in efforts to improve early diagnosis and determine prognosis for patients presenting with AKI. These biomarkers have been studied in multiple settings including post-cardiopulmonary

bypass, post radiocontrast, post-transplant, and also to predict AKI in sepsis/ICU patients. NGAL and IL-18 are among the most extensively studied biomarkers to date which we will review in detail here since these were the biomarkers of choice for the purposes of our study for logistical reasons. Other biomarkers that have been studied with AKI include Cystatin C, LFABP (liver-fatty acid binding protein), and KIM-1 (Kidney Injury Molecule-1). Finally, we will also examine the new found association between YKL-40 and acute kidney injury. .

Interleukin-18:

Interleukin-18 (IL-18) is a proinflamatory cytokine that is induced and cleaved in the proximal tubule that is activated by cleavage by the proinflammatory caspase-1. In a mouse model of ischemic AKI, deficiency of caspase 1 and thus deficiency of IL-18 activation was found to be protective from AKI. (58) At the molecular level, during ischemic AKI,caspase-1–mediated conversion of pro–IL-18 to active IL-18 occurs. The active IL-18 is released from the tubular cell and mediates neutrophil infiltration during ischemic AKI. Administration of neutralizing anti–IL-18 antibodies affords protection against ischemic AKI. Thus, IL-18 likely a deleterious role in ischemic AKI, perhaps in part due to increasing neutrophil infiltration into the renal parenchyma. The detection of IL-18 in the urine (the active form of IL-18 exits the cells and may enter the urine after being activated in proximal tubules) and the potential therapeutic effect of neutralizing IL-18 may have future clinical implications for AKI.

In one of the earliest studies of human subjects with AKI, Parikh et al. showed that patients with acute tubular necrosis (ATN), the most common form of clinical AKI, had significantly higher median urinary IL-18 concentrations than all other condition. (59) ROC curve analysis demonstrated that the discriminatory power of the urine IL-18 test for ATN was 95% compared to those with prerenal azotemia, UTI, or CKD. They also found median urinary IL-18 concentration, measured in the first 24 hr. after kidney transplant, to be nearly 5-fold higher in patients who received a cadaveric kidney that developed delayed graft function compared to patients who received either cadaveric or living donor kidneys with prompt graft function. In kidney transplant patients, lower levels of urinary IL-18 were associated with a steeper decline in SCr concentration over post-operative days 0-4 (P=0.009)

The superiority of IL-18 for predicting AKI compared to increases in SCr was confirmed in critically ill patients using a nested case-control study within the acute respiratory distress syndrome (ARDS) network trial. (60) The median urine IL-18 levels were significantly different at 24 and 48 hours before AKI in cases as compared to controls. On multivariable analysis, urine IL-18 values predicted development of AKI twenty-four and forty-eight hours later after adjusting for demographics, sepsis, APACHE III score, SCr and urine output. On diagnostic performance testing, urine IL-18 demonstrated an area under the ROC curve of 73% to detect AKI in the next 24 hours.

The role of urine IL-18 as a predictor for AKI has also been studied in other patient populations. In cardiac surgery patients, urine IL-18 has also been an early predictor of

AKI with AUCs ranging from 0.54 to 0.90. (60-64) In subjects undergoing kidney transplantation, IL-18 was an excellent predictor of delayed graft function in with an AUC of 0.90. (65)

Analysis of the Translational Research Investigating Biomarker Endpoints in AKI (TRIBE AKI) study, a large prospective, multicenter international cohort of adult patients undergoing cardiac surgery, confirmed that the highest quintiles of urine IL-18 samples collected after cardiac surgery were associated with nearly sevenfold higher odds of AKI compared with the lowest quintiles, and also associated with longer length of hospital stay, longer ICU stay, and higher risk of dialysis or death. Moreover, urine IL-18 significantly improved the AUC to 0.76 vs. 0.69 for the clinical prediction model of AKI.(66)

Besides aiding in predicting AKI, urine IL-18 has also been studied for predicting AKI severity and predicting mortality. In early studies, urine IL-18 was found to be a weak predictor of AKI severity.(61, 67) In cardiac surgery patients, urine IL-18 at 4 hours after cardiac surgery weakly correlated with number of days with AKI. In the ARDS network trial mentioned above, the urine IL-18 values were also different between survivors and non-survivors ($p<0.05$) and in multivariable analysis, the urine IL-18 value on day 0 was an independent predictor of mortality. (60) Similar results have been found in pediatric critically ill patients. (67) The TRIBE AKI study also confirmed that urinary IL-18 levels at the time of creatinine-based diagnosis of AKI forecasted a threefold risk of progression to more severe AKI after cardiac surgery. (68)

NGAL:

Neutrophil gelatinase-associated lipocalin (NGAL) is upregulated systemically and in renal tubular cells in response to inflammation and cell injury. NGAL is expressed in low levels in several human tissues, including kidney, trachea, lungs, stomach, and colon and its expression is induced in stimulated epithelial. (69)

The exact mechanism of action for NGAL in the kidney is convoluted. Early studies had found NGAL to be upregulated in tubular epithelial cells that were undergoing proliferation while others suggested that NGAL can itself enhance the epithelial phenotyte. (70) In some epithelial cells, NGAL has been shown to possess a pro-apoptotic property allowing these epithelial cells to regulate their own demise. (71, 72) Other data has also suggested that NGAL may have a therapeutic role in ischemic AKI via its ability to ameliorate tubule cell apoptosis and enhance tubule cell proliferation by inducing re-epithelialization, (70) a role that may be related to NGAL's expression during nephrogenesis stimulating conversion of mesenchymal cells into kidney epithelia. The exact mechanism may involve recycling iron into viable cells stimulating regeneration of renal epithelial cells or serving as a reservoir to remove iron from the site of injury thus limiting iron-mediated cytotoxicity. Overall, NGAL appears to tilt the balance of proximal tubule cell fate toward cell survival after ischemic injury.

The gene for NGAL was first found to be upregulated more than 10 fold in the early post-ischemic mouse kidney in a genome-wide interrogation survey. (73) Subsequent studies have confirmed the significance of NGAL in detecting and predicting AKI. The gene for

NGAL was found to be up-regulated with detectable protein in proliferating cell nuclear antigen-positive tubule cells during ischemic reperfusion injury. (70, 74) NGAL expression in proximal tubule cells was also increased in cadaveric kidney transplantation patients with ischemic kidney injury. (75)

One of the earliest studies looked at NGAL measurements in children undergoing cardiopulmonary bypass (CPB). (76) Here, urine NGAL in patients with AKI rose more than 10-fold at 2 hours after CPB, whereas SCr did not elevate until 48-72 hours after CPB. Serum NGAL similarly increased 6-fold at 2 hours after CPB. Univariate analysis showed a significant correlation between AKI and the following: 2 hour urine NGAL, 2 hour serum NGAL, and CPB time. By multivariate analysis, the urine NGAL at 2 hours post CPB emerged as the most powerful independent predictor of AKI. A ROC curve for the 2 hour urine NGAL revealed an area under the curve of 0.998, and a sensitivity of 1.00 and specificity of 0.98 for a cutoff value of 50 ng/ml.

Urine NGAL has since emerged as a pre-eminent standalone urinary biomarker for the early prediction of AKI. In adults undergoing percutaneous coronary intervention or cardiopulmonary bypass surgery, the AUC has ranged widely from 0.61 to 0.96. (63, 64, 77-79) For patients undergoing cardiac surgery or developing contrast nephropathy, the AUCs range from 0.80-0.96. (76, 79-82)

Plasma NGAL also has been shown to predict AKI about two days earlier than clinically significant increases in SCr.(82) In children undergoing cardiac surgery, however, the

predictive value of urine NGAL appears far better with AUCs ranging from 0.78-1.00. (76, 77, 81, 83) Additionally, urine NGAL is highly predictive of delayed graft function in subjects undergoing kidney transplantation with an AUC of .90. (65)

The TRIBE AKI study, the largest AKI biomarker study performed to date, confirmed that post-cardiac surgery plasma NGAL elevation was associated with a nearly 5-fold risk of AKI, longer hospital and ICU stay, higher risk of dialysis or death, and an AUC of 0.75 for risk prediction of AKI compared to an AUC of 0.69 in the clinical prediction model of AKI. (66)

Both urinary and serum NGAL have also been found to be highly correlated with the degree of established AKI when compared to controls or subjects with CKD. (84, 85) Urine NGAL has also proved to be a valuable initial screening tool for subsequent development of AKI in adults admitted to the emergency department with a high sensitivity (0.90), high specificity (0.995) and an AUC of 0.95. (86) Additionally, in patients undergoing cardiac surgery, urine NGAL at 4 hours correlated weakly with duration of AKI ($r2 = 0.22$; $p=0.005$), and early urine NGAL levels were predictive of the need for dialysis and mortality.(61, 83) In a more heterogeneous cohort of critically patients, urine NGAL was weakly predictive of AKI persistence (AUC 0.63) or for worsening of AKI (AUC .61). (87) In genomic analysis studies, NGAL was also identified as a potential surrogate marker for progressive renal injury following AKI and may serve as a mediator of AKI-to-CKD transition. (38)

In animal models, NGAL has been used therapeutically, aiding in renal survival and recovery (88) and blockade of NGAL resulted in reduced renal regeneration. (89) NGAL's potential role in AKI prognosis has been studied in few large trials. A recent meta-analysis (19 publications, n=2539, 487 developed AKI) revealed a diagnostic odds ratio of 18.6 (95% CI 9.0-38.1)/AUC of .815 (0.73-.89) of NGAL (similar numbers for both urine and serum) to predict AKI in all clinical settings. (90) The diagnostic accuracy of serum NGAL was 17.9 (6.0-53.7)/ .775(.679-.869). NGAL level was also a useful prognostic tool with regard to the prediction of RRT initiation (12.9; 4.9-33.9) / (.782; .648-.917) and in-hospital mortality (8.8; 1.9-40.8) / (.706; .530-.747). This was confirmed in a prospective, multi-center, observational cohort study of deceased-donor kidney transplant patients which found that both NGAL and IL-18 levels in serial urine samples collected for 3 days after transplant predicted the need for dialysis within one week of transplantation and predicted graft recovery up to 3 months later. (91)
With regards to NGAL's role in predicting progression to more severe AKI, analysis of the TRIBE AKI study mentioned earlier found that plasma NGAL as associated with a sevenfold risk of AKI progression whereas urinary NGAL did not reveal a significant association with AKI progression after adjusting for clinical variables.(68)

YKL-40: YKL-40 (also known as BRP-39 or Chi3l1) is a chitinase/chitinase-like protein (C/CLPs), a class of proteins that have been shown to play a role in both innate and adaptive type 2 immune responses serving as important inhibitors of oxidant-induced lung injury, vascular permeability, and structural cell apoptosis. (92, 93) C/CLPs are dysregulated in allergic disorders and diseases characterized by acute or chronic

inflammation and tissue remodeling. (92, 94) Murine experiments have demonstrated that YKL-40 inhibits inflammatory cell apoptosis and induces alternative macrophage differentiation leading up to proliferation of Th2 cytokines that induce chemokines and transforming growth factor (TGF-B) to contribute to inflammation and tissue healing, fibrosis and remodeling. (94-96)

YKL-40 is expressed in a variety of cells, including macrophages, neutrophils, chondrocytes, fibroblasts, vascular smooth muscles cells, endothelial cells, hepatic stellate cells, and colonic, ductal, and airway epithelial cells (94, 97) . It has also been shown to be a predictor of all-cause mortality in the elderly (98) and have a significant association with rates of overall and cardiovascular mortality. (99)

The association between YKL-40 and AKI has recently been identified. (100) In urine proteomic screen in mice, BRP-39 (the murine protein product of Chi3l1) was identified as a critical component of kidney repair (up-regulated in kidney macrophages in an injury dependent fashion) that served to limit tubular cell apoptotic death in response to oxygen radical exposure by stimulating the intracellular activation of the PI3K/Akt pathway in renal tubular cells, the time course of BRP-39 expression correlated to the that of macrophage infiltration in the ischemic injured kidney. There was a direct correlation between the degree of kidney injury and the level of expression of Chi3l1/BRP-39 in the kidney and the urine. The investigators also found that in patients undergoing deceased donor kidney transplantYKL-40 (orthologous human protein to Chi3l1) was more highly expressed in urine and blood from allografts with delayed graft function (DGF) compared

to slow or immediate graft function. The first urine sample collected after transplantation demonstrated a high YKL-40 concentration in patients exhibiting DGF even when serum YKL-40 levels were indistinguishable suggesting that YKL-40 was being expressed within the injured kidney rather than being filtered from the serum. Urinary Ykl-40 obtained within hours of transplant was highly predictive for the subsequent need of dialysis. Using Brp-39 null mice, the group discovered that upregulation of BRP-39 in response to ischemic injury inhibited tubular cell apoptosis and this pathway served to limit the severity of tubular injury and promoted proliferation of viable tubular cells to affect subsequent kidney repair.

Recently, Maddens et. al. demonstrated that YKL-40 (Chi3l1) was detectable only in septic mice with severe AKI compared to those without AKI. (101) Increased urinary excretion of YKL-40 successfully discriminated sepsis from sepsis-induced AKI.

Biomarkers and Renal Recovery

Although great progress has been made in identifying novel biomarkers for detecting AKI early, identifying established AKI, and predicting the need for RRT, there is limited data presently regarding the ability for these biomarkers to prognosticate risk for clinical outcomes, include recovery from AKI.

Srisawat et al. recently published their findings on the prognostic capability of urinary biomarkers for recovery after AKI. (102) They collected urine samples on days 1, 7, and 14 in patients who developed AKI and received RRT in the ICU. Renal recovery was

defined as a living patient free of dialysis at 60 days from start of RRT. They found that patients who recovered had higher urine Cystatin C on day 1 and lower urine hepatocyte growth factor (HGF) on days 7 and 14. With regards to predictive ability for renal recovery, uHGF on day 14 and the fall of uHGF over the first 14 days were moderately predictive of renal recovery (AUC = 0.74). Additionally, decreasing urinary NGAL was predictive with AUC = 0.70. The authors also studied the ability of plasma NGAL to predict recovery from AKI following community-acquired pneumonia and found that median plasma concentrations were significantly power in patients who recovered and plasma NGAL predicted failure to recover with AUC of 0.74. (103)

HGF is a marker of renal tubular epithelial cell regeneration. (104) HGF therapy has previously shown to accelerate renal recovery in animal models of toxic or ischemic ATN. (105) Another earlier study had also demonstrated uHGF up-regulation during the acute phase of AKI with a gradual decline during recovery. (106) As stated earlier, intravenous NGAL administration has proven to be beneficial in reducing damage to tubular cells in murine models of renal injury. (78) Cystatin C over-expression suppresses Cathepsin S activity, which normally suppresses CD4(+)T cell-mediated immune response. (107) Thus, increased Cystatin C expression may be beneficial in early AKI by increasing response to injury.

STATEMENT OF PURPOSE

Several biomarkers of kidney injury have been investigated. Most studies have examined their utility at the onset of renal injury to improve differential diagnosis or detect AKI

earlier than clinical diagnosis alone. However, few studies have examined AKI biomarkers for predicting recovery from AKI.

The primary objective of this pilot study is to determine if there is an association between previously identified urinary biomarkers of acute kidney injury (AKI) with recovery from severe AKI (AKIN Stage 3). Since the biomarkers are very sensitive for kidney tubular cell injury and death, we hypothesize that measureable changes in levels of urinary biomarkers for kidney injury or repair may be associated with renal recovery in patients diagnosed with severe AKI.

The present study should pave the way for future larger studies of urinary biomarkers in patients with severe AKI and successful clinical trials by providing improved measures of ongoing kidney injury and impending kidney recovery. If this study shows consistency in the test characteristics (sensitivity, specificity, receiver operating characteristic (ROC) curve) of the urinary biomarkers for predicting renal recovery, then a larger, multicenter study can be designed to fully and definitively evaluate the use of urinary biomarkers in this setting. The ultimate goal is to improve morbidity and mortality associated with AKI.

METHODS

Study Population: 48 adult patients admitted at Yale New Haven Hospital (both intensive care units and non-intensive care) with severe AKI (AKIN Stage 3) were prospectively enrolled between July 2010 and April 2011.

Inclusion Criteria:

1. Severe AKI as defined by the AKIN Criteria - Stage 3: SCr ≥ 200% or SCr ≥ 4.0 mg/dL with an increase ≥ 0.5 mg/dL or initiation of RRT and/or UO < 0.3 mL/kg/h for ≥24 h or anuria ≥ 12 h.

Exclusion Criteria:

1. Severe AKI requiring continuous forms of hemodialysis (CVVH, CVVHD, CVVHDF)
2. Patients requiring acute peritoneal dialysis (rarely used in the United States for patients with AKI)
3. Urine output < 100 cc/day (because will be difficult to collect urinary specimens)
4. Age < 18
5. Patient or next-of-kin unable or unwilling to provide informed consent
6. Prior renal transplantation

Study Processes: Nearly all patients who develop severe AKI are seen by the nephrology team (attending and fellows). Eligible participants were identified from the nephrology consult list on a daily basis.

Primary Exposures: Biomarkers concentrations on Day 1 (date of enrollment), Last day of collection, and Average daily values (on the first 3 days) of enrollment

Primary Outcomes: Renal recovery, according to the Liano definition of AKI recovery, at discharge or 30 days defined as the following:

- "Complete" if SCr ≤1.4 mg/dL
- "Partial" if SCr > 1.4 and lower than peak SCr reached
- "Non-recovery" if SCr > 4mg/dL or death [according to the Liano definition] (108)

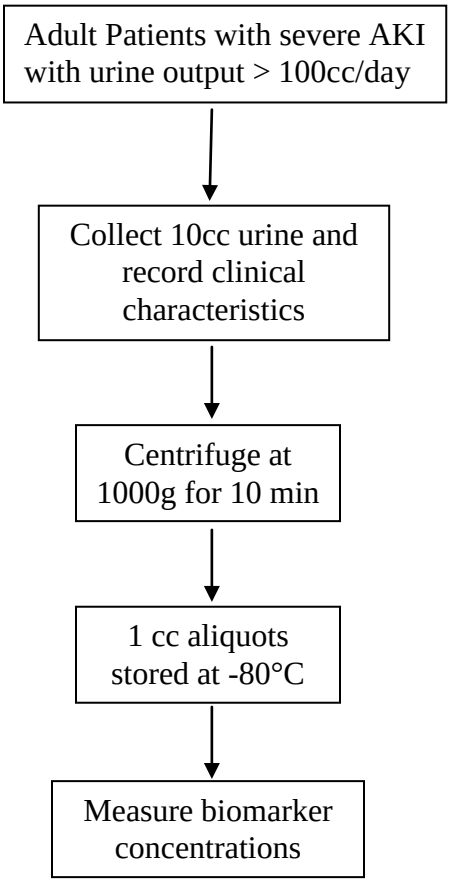
Specimen Collection:

- Daily morning urine samples (10 ml) were collected into a specimen cup from the Foley catheter or clean-catch technique by the patient until recovery (up to max of 9 days).
- Samples were hand-transported to our dedicated lab space in the 5^{th} floor of the TAC building on Cedar Street where the specimens were centrifuged at 1000g for 10 minutes, separated into 1cc aliquots into cryovials, labeled with a unique identifier number, and stored at -80^0 C for future batch measurement of AKI biomarkers.
- The person(s) performing the lab assays were blinded to the patient's identity. The assays were done at the University of Cincinnati in the lab of Dr. Prasad Devarajan.
- NGAL and IL-18 were measured with the ARCHITECT® assay (Abbott Diagnostics, Abbott Park, IL), which has a coefficient of variation (CV) of 5% and 8%, respectively
- YKL-40 was measured by a commercially available ELISA (Quidel Corporation, San Diego, CA) with intra- and inter-assay CVs of 6 and 6.5% respectively

Data Collection: Primary data was collected from the medical charts and computer system at YNHH. Key variables included age, gender, ethnic, height and weight, date of admission, date of enrollment, medical and surgical co-morbidities (acute and chronic), historical baseline SCr, admission SCr, peak SCr, ICU stay, need for mechanical ventilation, in-hospital death and cause of death, duration of hemodialysis if needed, and

SCr at discharge. Additionally, each SCr value during the entire hospital admission was recorded to identify dates when patient recovered partially or fully.

Figure 1. Specimen Collection



Data Analysis:

- For each subject, a recovery category was assigned based on the Liano definitions described above
- Mean and standard deviation and median biomarker values and IQR (inter-quartile range) were calculated for the three biomarker time-points and each of the renal outcomes. The IQR is also known as the midspread and has a breakdown point (proportion of incorrect observations) of 25%

- Since the biomarkers were rightward-skewed, we compared median values across the three recovery categories via non-parametric tests (Kruksal-Wallis). Non-parametric tests are used to study populations that take on a ranked order (ordinal data) and considered robust since these methods incorporate fewer assumptions. Kruskal-Wallis is equivalent to the one-way analysis of variance, but used for comparing groups of unequal size.
- We then analyzed the association between the first, last, and average daily biomarker concentrations and renal recovery using logistic regression models. Since we wanted higher biomarker values to reflect a positive association, we chose the dichotomous outcome to be "non-recovery" from AKI, and grouped partial and complete recovery. In addition, we adjusted for peak delta change in SCr and age.
- We used Kaplan-Meier survival analysis for assessing time to partial recovery from day 1.
- We also performed receiver operator characteristic tests to calculate the AUC for each biomarker to discriminate between non-recovery vs. partial/complete recovery.
- We also performed sensitivity analyses using an alternate definition for renal recovery in which defined renal recovery as recovery of SCr to baseline: within 25% was full recovery, 25-75% was partial recovery, and greater than 75% or death was non-recovery.

<u>Statement on methods performed by each member of the team:</u>

- Rahul Agarwal: wrote the IRB, amendment to the IRB (to include stage 3 AKI patients not on hemodialysis), and developed study methods in collaboration with Steve Coca, completed the patient identification, informed consent, sample collection/processing/labeling/storage, and primary data collection for 28 out of the 48 patients. Once all biomarker data was available for all patients, also created the necessary data tables that were eventually used by Bita Fakhri and Adam Hong to run the statistical analysis. Also, created the boxplots using median biomarker values.
- Divakar Jammalamadaka (Post-Doc fellow): patient identification, informed consent, sample collection and processing, and primary data collection for 20 out of the 48 patients
- Bita Fakhri and Kwangik (Adam) Hong (statisticians): data analysis specifically median biomarker values, tertile analysis, AUC values, and Kaplan Meier survival curves
- Steven Coca: sponsor, advisor, and principle investigator. Conceptualized study design and data analysis.

RESULTS

Based on the Liano Recovery definitions, 15 subjects recovered completely, 17 recovered partially and 16 did not recover. Eleven out of the 16 non-recovery patients died during the index hospitalization. The demographics of the patient population and the SCr values at baseline (historical or admission SCr), peak SCr during hospital admission, and terminal (discharge) SCr are listed in Table 1. Age, gender, race, and history of diabetes

were not significantly different across the three recovery definitions. Additionally, there was no significant difference in the mean baseline SCr for patients in the three recovery categories – 0.98 mg/dL for complete recovery group, 1.26 mg/dL for the partial recovery group, and 0.98 mg/dL for the non-recovery group. The mean peak SCr was above 4.0 mg/dL for all recovery categories. The terminal SCr was expectedly lowest for the complete recovery group (1.13 mg/dL) and highest for the non-recovery group (3.38 mg/dL) and furthest from baseline in the non-recovery group. The mean change from terminal SCr to baseline SCr was 0.15 mg/dL for the complete recovery group, 0.88 mg/dL for the partial recovery group, and 2.39 mg/dL for the non-recovery group. On average, partial recovery was achieved in 2.47 days from the date of enrollment while complete recovery needed 6.60 days.

Table 1: Patient Characteristics

Characteristic	Total (N=48)	Complete Recovery (N=15)	Partial Recovery (N=17)	Non-Recovery (N=16)	P Value
Age in years (mean ± SD)	58.98 (14.67)	56.33 (17.82)	59.94 (13.97)	60.44 (12.65)	0.68
Male sex – n (%)	25 (52%)	6 (40%)	10 (59%)	9 (56%)	0.52
Race – n (%)					
- White	35 (73%)	9 (60%)	13 (76%)	13 (81%)	0.5
- Black	9 (19%)	5 (33%)	2 (12%)	2 (13%)	
- Other	4 (8%)	1 (7%)	2 (12%)	1 (6%)	
Diabetes – n (%)	12 (25%)	2 (13%)	5 (29%)	5 (31%)	0.45
Intubation	25 (53%)	9 (60%)	4 (25%)	12 (75%)	0.01
Baseline Creatinine (SCr)	1.08 (0.43)	0.98 (0.22)	1.26 (0.55)	0.98 (0.37)	0.17
Peak SCr	5.14 (1.81)	4.79 (2.02)	5.39 (1.9)	5.21 (1.57)	0.64
Terminal SCr	2.24 (1.54)	1.13 (0.24)	2.14 (0.64)	3.38 (2.07)	<.0001
Δ Peak from Baseline SCr	4.06 (1.7)	3.81 (1.85)	4.14 (1.77)	4.23 (1.57)	0.71
Δ Terminal from Peak SCr	2.91 (2)	3.65 (1.92)	3.26 (1.96)	1.83 (1.74)	0.03
Δ Terminal from Baseline SCr	1.16 (1.52)	0.15 (0.28)	0.88 (0.44)	2.39 (2.07)	<.0001
Days To Recovery					
- Partial (mean ± SD)		2.47 (1.92)	2.47 (1.28)	-	
- Complete (mean ± SD)		6.60 (4.03)	-	-	

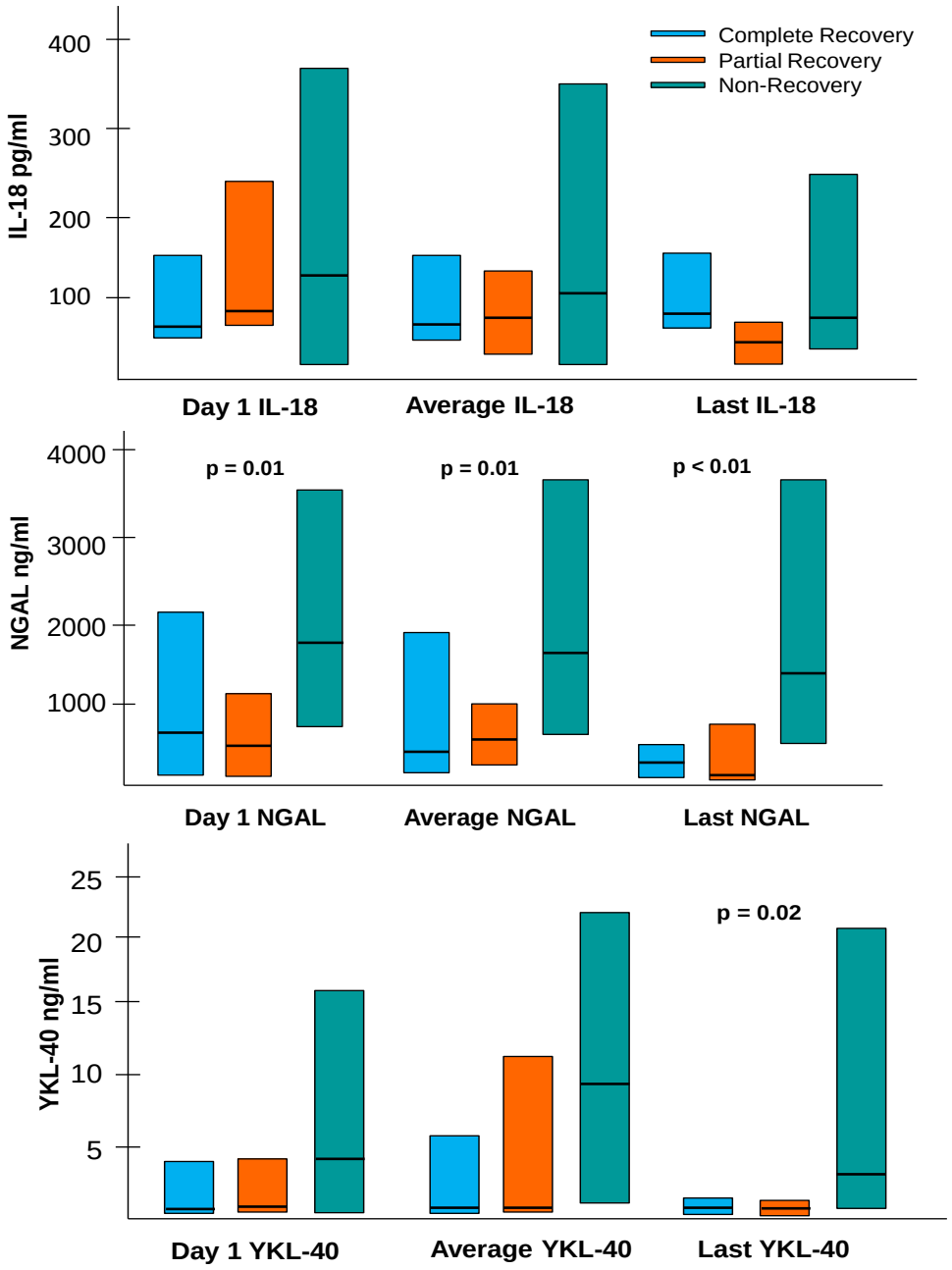
Median NGAL and YKL-40 values were generally highest in patients who did not recover from AKI compared to those who experienced partial or complete recovery. Table 2 lists the median values for each biomarker at each time point and Figure 2 shows the trend in the form of boxplots. Notably, median NGAL values at all time-points were

significantly higher (p <= 0.01) for the non-recovery group and the median YKL-40 value for the last YKL-40 collection (p = 0.02) was significantly higher in the non-recovery group compared to those that experienced partial or complete recovery. IL-18 did not discriminate between recovery and non-recovery in this pilot study.

Table 2: Median values of biomarkers by time points - Median, (1st tertile, 3rd tertile)

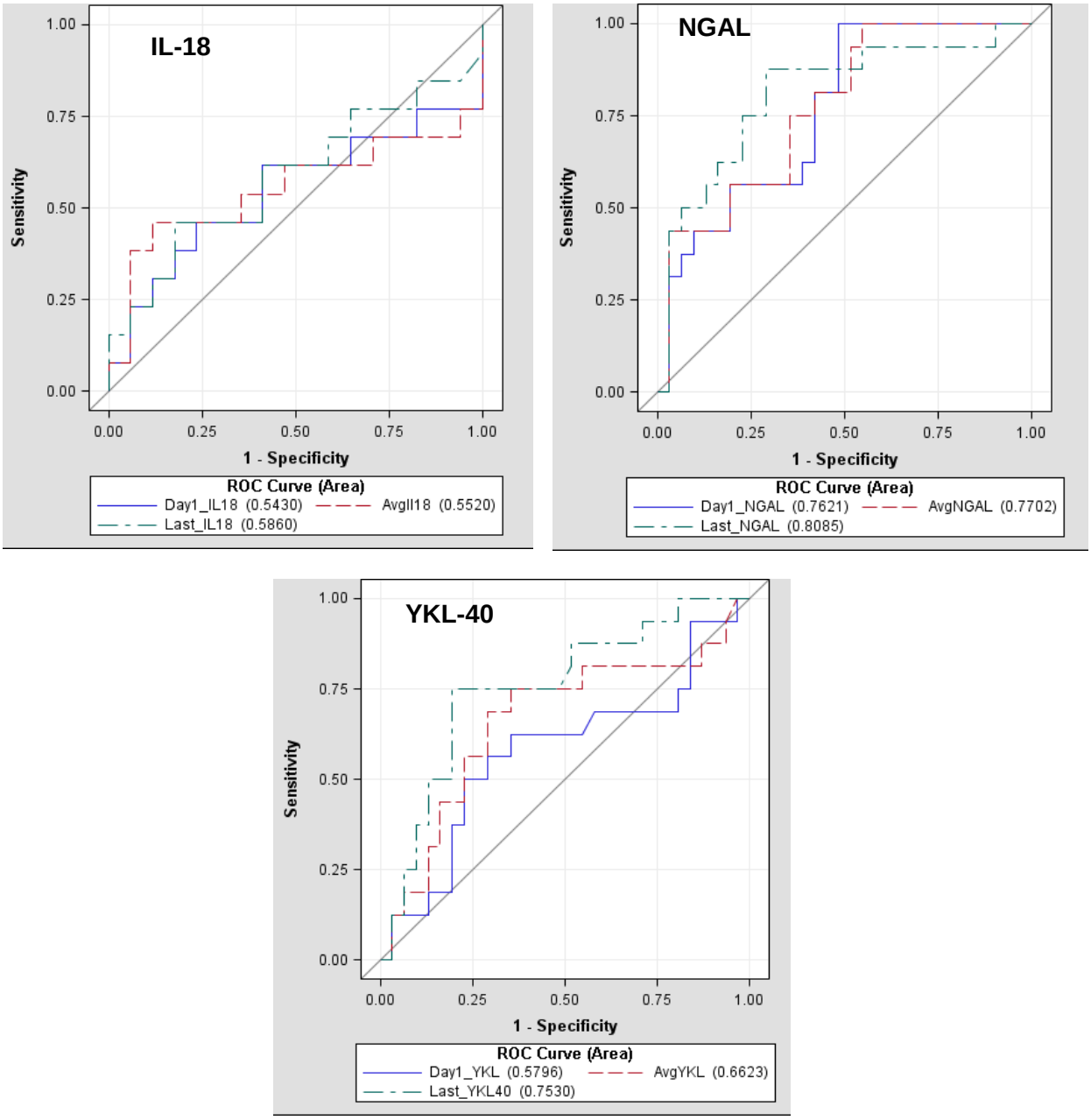
Biomarker	ALL (N=48)	Complete Recovery (N=15)	Partial Recovery (N=17)	Non-Recovery (N=16)	P Value
Day1 IL18	81(45.98, 254.1)	55.89 (45.98, 152.23)	79.14 (60.3, 254.1)	141.6 (34.28, 373.11)	0.56
Avg. Il18	79.14 (43.17, 234.99)	61.31 (51.8, 152.23)	73.37 (47.39, 143.48)	101.09 (33.43, 349.82)	0.9
Last IL18	66.5 (36.42, 179.85)	86.08 (55.89, 152.23)	49.99 (31.8, 67.6)	84.15 (59.64, 286.01)	0.29
Day1 NGAL	667.53 (138.48, 1882.16)	588.06 (84.65, 2150.77	381.47 (81.69, 1189.49)	1616.64 (635.42, 3501.71)	0.01
Avg. NGAL	713.23 (180.06, 1773.47)	353.96 (95.08, 1679.82)	553.1 (94.06, 1010.17)	1614.36 (686.33, 3681.53)	0.01
Last NGAL	261.18 (84.65, 1219.49)	168.19 (84.65, 341.91)	107.89 (65.54, 719.68)	1431 (408.78, 3689.99)	0
Day1 YKL	0.88 (0.24, 9.08)	0.45 (0.11, 4)	0.67 (0.36, 4.31)	4.24 (0.17, 16.38)	0.46
Avg. YKL	2.54 (0.32, 13.89)	0.63 (0.25, 6.53)	0.58 (0.28, 11.65)	9.47 (1.83, 23.58)	0.16
Last YKL40	0.61(0.2, 4.71)	0.34 (0.09, 1.27)	0.48 (0.22, 0.9)	3.26 (0.91, 21.59)	0.02

Figure 2: Boxplots for each biomarker by recovery category



The AUCs for non-recovery for day 1, average, and last NGAL were 0.76, 0.77, and 0.81, for YKL-40 0.58, 0.66, and 0.75, and for IL-18 0.54, 0.55, and 0.59 respectively. Figure 3 displays the ROC curves for non-recovery for each biomarker.

Figure 3: AUCs of Day 1, Last, and Average Biomarker Values for Non-Recovery



Looking specifically at the outcome of non-recovery of renal function, tertile analysis revealed that the highest tertile of NGAL for each NGAL measurement (day 1, average, and last) was strongly associated with the risk for non-recovery (more than 10-fold) compared with the lowest tertile after adjusting for change in SCr from baseline and age.

In fact, the association was even stronger for the third tertile at each time point compared to the second tertile. There were no patients in the lowest tertile for day 1 NGAL hence the second and third tertiles could not be calculated (due to division by zero in the denominator). The second tertile for the average NGAL value had a relative risk of 8.03 (95% CI 1.37-46.96) for non-recovery while the last NGAL value had a RR of 13.19 (95% CI 2.17-80.09). For the last NGAL value, the second and third tertiles were RR 5.21 (0.71-38.03) and RR 11.06, 95% CI 1.63-75.16 respectively. With regards to YKL-40, only the last YKL-40 value had second and third tertiles associated with greatest risk of non-recovery with RR 1.80 (0.38-8.47) and RR 4.83(95% CI 1.24-18.71) respectively. IL-18 did not associate with non-recovery in any significant fashion. Table 3 lists the measures of association between biomarker values at each time point divided into three tertiles for non-recovery of renal function.

Table 3: Measures of association for non-recovery of renal function

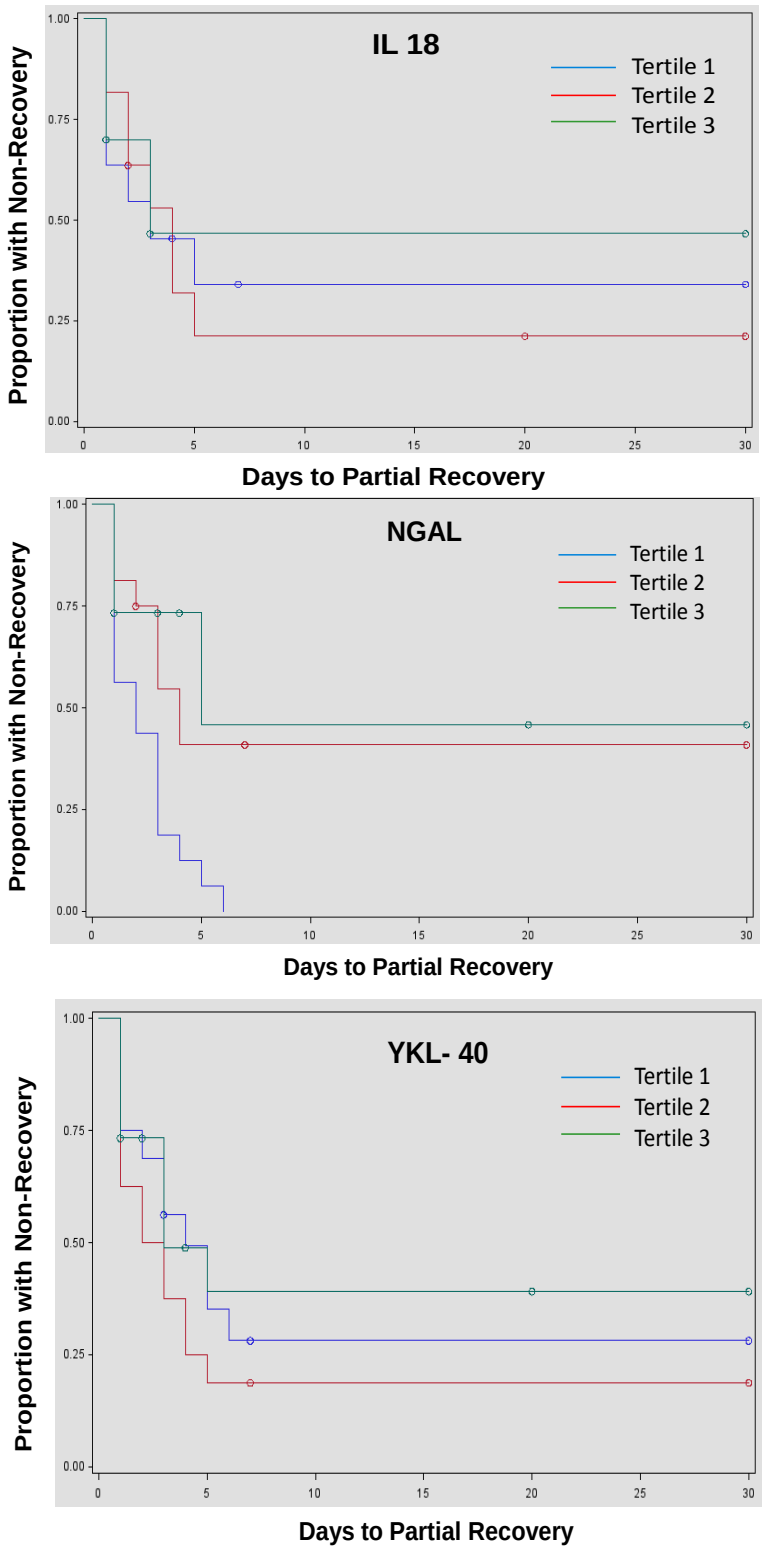
Biomarkers	**Tertiles**	**Cut-offs**	**Outcome: Non-recovery of renal function**		
			N (%)	**P value for trend**	**Adjusted RR (95% CI)***
Day 1 IL-18	1	27.03 - 51.8	4(36.36%)	0.383	Ref
	2	55.89 - 174.46	3(27.27%)		0.73 [0.2, 2.72]
	3	186.4 - 714.07	6(54.55%)		1.77 [0.58, 5.46]
Average IL-18	1	27.03 - 51.8	5(45.45%)	0.798	Ref
	2	53.66 - 112.88	3(25%)		0.53 [0.16, 1.82]
	3	143.48 - 714.07	6(50%)		1.47 [0.58, 3.74]
Last IL-18	1	27.03 - 44.03	3(30%)	0.249	Ref
	2	55.89 - 103.16	4(36.36%)		1.14 [0.33, 4.01]

	3	131.25 - 714.07	6(54.55%)		2.19 [0.68, 7.07]
Day 1 NGAL	1	13.82 - 381.47	0(0%)	0.001	-
	2	480.35 - 1402.66	7(43.75%)		Unable to calculate
	3	1521.1 - 17911.9	9(56.25%)		Unable to calculate
Average NGAL	1	13.58 - 356.13	1(6.25%)	0.003	Ref
	2	404 - 1150.38	6(37.5%)		8.03 [1.37, 46.96]
	3	1370.3 - 17911.9	9(56.25%)		13.19 [2.17, 80.09]
Last NGAL	1	13.33 - 95.35	1(6.67%)	0.001	Ref
	2	114.38 - 934.07	5(31.25%)		5.21 [0.71, 38.03]
	3	987.51 - 17911.9	10(62.5%)		11.06 [1.63, 75.16]
Day 1 YKL-40	1	0.01 - 0.36	5(31.25%)	0.261	Ref
	2	0.4 - 4	3(18.75%)		0.94 [0.28, 3.23]
	3	4.31 - 628.71	8(50%)		1.93 [0.71, 5.27]
Average YKL-40	1	0.05 - 0.42	3(18.75%)	0.024	Ref
	2	0.46 – 7.01	4(25%)		1.28 [0.34, 4.88]
	3	7.45 - 566.37	9(56.25%)		2.91 [0.95, 8.85]
Last YKL-40	1	0.02 - 0.27	2(13.33%)	0.004	Ref
	2	0.3 - 1.41	4(25%)		1.80 [0.38, 8.47]
	3	1.57 - 504.03	10(62.5%)		4.83 [1.24, 18.71]

* Adjusted Variables: Delta Peak Serum Creatinine and Age

Examination of the Kaplan-Meier survival curves revealed that nearly all patients in the lowest NGAL tertile on day 1 recovered partial kidney function in a rapid fashion (~5 days).

Figure 4: Kaplan Meier Estimates of Time to Partial Recovery by Tertiles of Day 1 of Biomarker



In our sensitivity analysis looking at the alternate definition of recovery, in which full recovery was defined as terminal SCr within 25% of baseline SCr, partial recovery as terminal SCr within 25-75% of baseline SCr, and non-recovery as terminal SCr greater than 75% of baseline SCr or death, we obtained relatively similar results to the Liano definition which defined complete recover as SCr ≤1.4 mg/dL, partial recovery as SCr > 1.4 and lower than peak SCr reached, and non-recovery as SCr > 4mg/dL or death. Per the alternate definition, 12 subjects recovered completely, 11 recovered partially, and 25 did not recover (11 deaths). The sensitivity analysis confirmed that the median values of NGAL were significantly higher at all time-points for the non-recovery group ($p<=0.01$). The median of the last YKL-40 value was also significantly higher for the non-recovery group ($p=0.01$). Again, IL-18 did not discriminate between recovery and non-recovery. Based on this alternate definition of recovery, the AUC was greater than 0.75 only for the last NGAL value and the last YKL-40 value.

DISCUSSION

AKI is a common complication in hospitalized patients posing a huge burden on health care both financially and clinically due to prolonged hospital stay and increased morbidity and mortality both in the hospital and after discharge. Until the advent of novel urinary and serum biomarkers in the past decade, detection of AKI and prognosis was largely dependent on urine output and serum creatinine values, the latter of which may not be affected until 48-72 hours after the initial insult to the kidney. However, thus far biomarkers have mostly been examined at the onset of renal injury to detect AKI early while few studies have examined AKI biomarkers for predicting recovery from AKI.

In this pilot study, we sought to determine the association between two prominent biomarkers of injury (IL-18 and NGAL) and a biomarker for repair (YKL-40) with recovery from severe AKI (stage 3 as identified using the AKIN criteria). We found that median NGAL values were highest in patients who did not recover, high levels of NGAL at all time-points (day 1 collection, average collection of first three days of enrollment, and last collection) were significantly associated with non-recovery from AKI with an AUC > 0.75, and the second and third tertile of NGAL was strongly associated with risk for non-recovery compared with the lowest tertile after adjusting for changes in SCr from baseline and age. With regards to the biomarker of repair, YKL-40, there was also a signal for non-recovery with higher values. While YKL-40 trended towards being higher in those that did not recover from the earlier time points, patients with YKL-40 values in the highest tertile from the last sample collected had five times the risk of non-recovery compared to those in the lowest teritle. The AUC for non-recovery was 0.75 for the last sample of YKL-40. In our cohort of patients, IL-18 values did not associate with recovery at any of the time points.

Urine NGAL has been shown to be up-regulated in early post-ischemic kidney and has become pre-eminent marker for detecting and predicting AKI. Previously, urine NGAL has been shown to predict graft recovery up to 3 months later in deceased-donor kidney transplant patients.(91) More recently, the work by Srisawat et al found that decreasing NGAL was predictive for recovery with AUC = 0.70 (102) and high levels of NGAL were associated with non-recovery with an AUC of 0.74 in patients with AKI following community-acquired pneumonia. (103) Our results demonstrated high NGAL levels to be

associated with non-recovery with AUC > 0.75, which is consistent with the previous work by Srisawat et al. Although the mechanism is not yet clear, a high NGAL level may associate with worse kidney function at the outset therefore reducing chances of recovery if the NGAL levels remain persistently elevated. However, it has also been shown that NGAL expression may serve a therapeutic role for recovery from AKI by inducing re-epithelialization. NGAL has the potential to be a leading marker for recovery especially since murine models have proven that IV NGAL administration can reduce damage to tubular cells.(88) An alternative theory for NGAL's action is potentially that this biomarker may first provide information on cellular injury and then the strength of the signal may determine whether or not the injury is actually repairable. Data from the TRIBE-AKI study recently showed that plasma NGAL can be used to predict progression to more severe AKI. Although AKI progression and recovery are different outcomes, the crucial point to note is that NGAL (and potentially other biomarkers in the future) can be used dynamically when AKI is already present. Besides simply predicting AKI early, one can extrapolate regarding progression and potentially towards recovery as well as seen in our pilot study. The key finding of our pilot study is that NGAL does indeed associate with recovery from AKI even though the exact mechanism may not yet be clear.

YKL-40, a chitinase/chitinase-like protein involved in tissue healing, fibrosis and remodeling, has recently been identified to be associated with AKI. (100) The investigators found that YKL-40 correlated significantly with the extent of AKI in the deceased donor population and YKL-40 obtained within hours of transplant was highly predictive for the subsequent need of dialysis. Predominant source of urinary YKL-40

after ischemic injury appears to be macrophages with increased expression within the injured kidney rather than filtration from the serum. Our results indicate that the levels of YKL-40 in the last urine sample collected were associated with non-recovery. This may be explained by the magnitude of kidney injury such that if a marker for tissue repair i.e. YKL-40 is elevated, the kidney may not yet have recovered sufficient function. The fact that early YKL-40 values did not associate significantly with recovery could suggest that the recovery process may vary significantly for each patient and early values of a "repair" biomarker cannot be predictive for eventual recovery.YKL-40 is an especially appealing biomarker since it has been studied extensively in a variety of cells, found to be a predictor for all-cause mortality, and its role is directly related to healing/repair offering a different mechanism of action than NGAL or IL-18. Again, the exact mechanism for YKL-40 and AKI is still under investigation, but in our pilot study we did find some association between this biomarker and AKI. It is unclear why only the last YKL-40 value was significant and further investigation is warranted. One possibility is that the amount of time for expression of YKL-40 is greater than 3 days thereby making the day 1 YKL 40 value and the average YKL 40 value (avg of first three days) insignificant.

Unlike previous studies such as the TRIBE-AKI study (68) where urinary IL-18 levels at the time of creatinine-based diagnosis of AKI forecasted a threefold risk of progression to more severe AKI after cardiac surgery and the observational cohort study of decease donor kidney transplant patients (91) where IL-18 was predictive of need for dialysis and eventual graft recovery, in our cohort of patients IL-18 did not associate with recovery at any time points. Since the mechanism of action of IL-18 is likely renal damage by

increasing neutrophil proliferation in the renal parenchyma, its role in predicting recovery from AKI may be limited since the neutrophils may not clear out of the parenchyma in a rapid fashion to have any predicting value. Alternatively, the persistence of high level of IL-18 (and high neutrophil load) could significantly suggest AKI progression as seen in the TRIBE-AKI study.

Overall, the kidney stress response would ideally trigger an injury signal involving many different cell types within the kidney and then the transition to healing or repair would trigger another signal that is more specific to the underlying injury process and this second signal may be integral to initiating repair.

One of the significant limitations of this pilot study was the sample size. The power was not calculated since the purpose of this pilot study was to test a robust study design and prove the concept of the potential for biomarkers to associate with recovery from AKI which has not yet been studied extensively. The high rate of death (11 out of 48) resulted in many patients falling into the non-recovery category, resulting in competing events and the potential for informative censoring. However, it is impossible to have a cohort of patients with severe AKI with a high rate of survival. Thus, it is challenging to study these biomarkers in this setting. Yet, a death rate of 11 out of 48 or approximately 23% is actually in the right ballpark when looking at a mixed critical-care and non-critical care patient population as these rates range from 20-50% during the ICU stay and up to 30% after discharge. Another concern was that some patients were enrolled when they were already down trending their SCr values, but still satisfied the inclusion criteria from

AKIN Stage 3 AKI. Consequently, the early predictive ability of the biomarker value and subsequent recovery may have been missed.

In the future, larger studies will need to confirm these pilot study findings and determine clinical utility. Larger studies would also allow for studying biomarkers in combination to determine whether association with recovery is improved when looking at various different biomarkers rather than just independently. Additionally, one could attempt to study how the biomarkers correlate with each other thus adding to their clinical utility. Finally, if future larger studies confirm that these biomarkers are associated with non-recovery, then it could help with the design and enrollment of randomized controlled trials of interventions to aide recovery from severe AKI.

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