Using Neutrophil Gelatinase-Associated Lipocalin, a Marker of Acute Kidney Injury, for Therapeutic Decision Making with Neurohormonal Blockade Use in Acute Decompensated Heart Failure, a Pilot Study.

Dipika Gopal, B.S.
Anna Mcdivit, M.D.
Denise Barnard M.D.
Alan Maisel, M.D.

ABSTRACT

Introduction:
Acute kidney injury (AKI) is commonly observed during the treatment of acute decompensated heart failure. During aggressive diuresis in hospitalized patients, neurohormonal antagonists are often reduced or withheld when a decrement in renal function as indicated by rising creatinine is observed. Neutrophil gelatinase-associated lipocalin (NGAL) is released by tubular epithelial cells of the nephron upon injury and has been detected in the serum much earlier than creatinine. We sought to reproduce previous data supporting the fact that NGAL is an early biomarker of AKI hypothesizing that it would be predictive of the onset or worsening of acute kidney injury. Additionally, we identified specific patient scenarios in which the idea of NGAL-guided changes in neurohormonal blockade may be instituted.

Methods:
Serial serum and urine NGAL levels were measured from 53 patients with acute decompensated heart failure admitted through the emergency department at the Veterans Affairs San Diego Healthcare System (VASDHS). Serum and urine NGAL levels were obtained on admission and daily up to five days of hospitalization. Patient demographics, laboratory values, medication regimens, physical exam information, and other tests or imaging results were recorded on case report forms. Primary and secondary diagnoses given during hospitalization were also recorded. Study protocols did not influence clinical care decision-making.

Results:
Of the 53 patients in our study cohort, 24 developed AKI as defined by the AKIN criteria. Serum NGAL was significantly elevated on time point 2 in those who developed AKI versus those who did not (201.9ng/ml, IQR 122.6-287.8 versus 141.2ng/ml, IQR 98.55-168.9, p=0.039). Additionally, the maximum serum NGAL across all five time points for each patient was significantly elevated in the AKI group (507.5ng/ml) versus the non AKI group (242.1ng/ml), p=0.007. Per logistic regression the log-odds of having AKI increases by 0.003997 (p=0.024) for each unit of increase in maximum NGAL. Increasing maximum NGAL was significantly associated with having AKI (OR = 1.004, 95% CI: 1.0005,1.0075). In patients who developed AKI, serum NGAL levels reached a maximum level 0.25 days earlier than creatinine. Mean time to maximum serum NGAL was 2.42 days compared to 2.67 days for creatinine in the AKI group (p=0.58).

Conclusions:
Serum NGAL is a potential biomarker of AKI in patients who have acute decompensated heart failure. Importantly NGAL may be a more sensitive biomarker of AKI capable of detecting renal tubular injury earlier than serum creatinine. Further studies are required to elucidate NGAL's role in guiding neurohormonal antagonist dose adjustments in the hospitalized patient.

Keywords: heart failure, acute kidney injury, neutrophil-gelatinase associated lipocalin

INTRODUCTION

Acute decompensated heart failure (ADHF) continues to be a pressing public health concern. Close to 2% of the adult population suffers from chronic heart failure (HF) resulting in over 1 million hospital admissions annually. In fact, HF is the leading cause of hospitalization of adults over the age of 65 in the United States. Up to a third of these admissions are also associated with complications including acute kidney injury (AKI), resulting in a 300% increase in length of stay, greater incidence of readmission, and a 22% increase in mortality rate.[1] HF is a complex progressive syndrome that frequently results from an inciting event, such as myocardial infarction, valvular heart disease, uncontrolled hypertension, or other myocardial injury. The common pathway from these etiologies to chronic HF is the activation of the renin-angiotensin-aldosterone system (RAAS) neurohormonal pathway, which hemodynamically and biologically affects the myocardium and systemic vasculature. Mild vasodilatory neurohormones such as B-type natriuretic peptide (BNP) are overwhelmed by the RAAS pathway and are unable to counteract the associated fluid retention.[1] High left ventricular diastolic pressure resulting in pulmonary and/or systemic congestion is commonly associated with chronic HF. These hemodynamic perturbations may induce a compromise in renal perfusion/function in what is known as the cardiorenal syndrome.[2] The course of chronic HF typically is one of periods of stability interspersed with acute decompensations. It is during these times that AKI is more likely to occur. It is estimated that three out of four cases of HF will develop acute decompensation, which carries with it a poor prognosis.[3]

Interestingly, 25% of patients with HF also have reduced glomerular filtration rate (GFR). Whether this is due to low cardiac output, congestion, or another comorbid disease such as hypertension, diabetes, or hyperlipidemia is difficult to elucidate at times. A prospective study of a cohort of 754 HF patients found only 17% of patients with a GFR > 90ml/min.[4] Additionally, the ADHF National Registry (AHDERE) notes that 33% of the 107,362 patients in the database have reduced GFR. Serum creatinine, a widely used marker of renal failure was also found to be elevated (>2mg/dl) in 30% of the ADHF population.[1] Clearly, reduction in kidney function is a marker of increased morbidity and mortality in these patients. Because the heart and kidney share reciprocal communication, it can be said that cardiorenal syndrome (CRS) develops in these situations. One of the most common cardiorenal syndromes, Type I (acute cardiorenal syndrome), describes a rapidly progressing cardiac debilitation, such as ADHF, resulting in AKI. In CRS, patients are often unresponsive to diuretics due to sodium retention as part of the 'diuretic braking' system.[5] Angiotensin Converting Enzyme inhibitors (ACE-I) and Angiotensin Receptor Blockers (ARB) have a robust history of use in treating chronic HF with reduced ejection fraction as they are the foundation of guideline based therapy unless contraindicated. They have been shown to prevent cardiac remodeling and possibly have protective effects on the kidney. However, these medications can also result in reduced GFR (due to efferent arteriolar vasoconstriction / intrarenal hemodynamic effects), and are therefore often withheld in many patients with ADHF who are being aggressively diuresed. It is therefore unclear how to best proceed with management of ACE inhibitors and ARBs when aggressively diuresing a patient. Identification of an early marker of AKI such as Neutrophil-gelatinase-associated lipocalin (NGAL) may help guide the use of these medications.

NGAL, purified and identified in 1993 by Kjeldsen et al, is a 25-kDa protein with 178 amino acids in the lipocalin family of proteins.[6] Although varying in sequence and function, this family shares a structural beta-barrel motif consisting of 8 antiparallel hydrogen bonded strands which

protect a central binding pore specific for a variety of hydrophobic molecules and cell-surface receptors. This central binding pore allows for macromolecule complexing and expression in immature neutrophils and epithelial cells giving its role in immune modulation, inflammation, and neoplastic transformation.[7-11] NGAL is physiologically released from the lung, kidney, trachea, stomach, and colon.[12] The ARCHITECT assay was evaluated by Grenier et al and was deemed to be an accurate way to measure NGAL levels.[13] Several studies have investigated the role of NGAL in AKI. Specifically, in human and animal models, serum and urine NGAL levels were 7.3 to 25-fold increased in kidneys with acute tubular necrosis compared to control kidneys. 50% of cortical tubules with acute tubular necrosis were positive for NGAL by immunofluorescence, confirming its ability to indicate lesional injury.[14] Post-transplantation, NGAL levels correlate positively with maximum creatinine and eventual dialysis requirement suggesting the potential use of NGAL as a predictor of AKI in routine cadaveric kidney allografts.[15] Mishra et al identified a cut off of 50 ug/L for urine NGAL in predicting AKI in children post cardiopulmonary bypass with extremely high sensitivity and specificity (1 and 0.98 respectively, ROC = 0.998).[16] Similarly in adult patients undergoing cardiac surgery who developed AKI, urine and serum NGAL levels peaked at 3 hours post-operatively versus creatinine, which peaked on post-operative day 4.[17] Ling et al and Hirsch et al introduced the potential of urinary NGAL as a marker for contrast-induced nephropathy (CIN) in adults and children respectively.[18,19] The role of NGAL in heart failure requires further investigation.

Our hypothesis was that high serum and urine NGAL levels at the time of admission will be predictive of the onset or worsening of acute kidney injury. We also hypothesized that NGAL guided therapy will ultimately aid in deciding to continue or withhold ACE inhibitors and ARBs in the treatment plan for acute decompensated heart failure.

METHODS

Study Population:

54 patients admitted to the VASDHS between July 2011 and September 2013 were prospectively studied. Inclusion criteria consisted of having acute decompensated HF and being of 18 years of age or older. Patients on chronic renal replacement therapy were excluded from the study. This single center prospective cohort study was obtained with written consent, approved by the VA San Diego Institutional Review Board. All study participants received standard of care therapy for heart failure independent of the study.

Study Design:

Serum and urine NGAL levels were obtained on admission and daily up to five days of hospitalization. Patient demographics, laboratory values (creatinine, brain natriuretic peptide, blood urea nitrogen, glucose, bicarbonate, hemoglobin, hematocrit, urine protein), medication regimens, physical exam information, and other tests or imaging results were recorded on case report forms. Primary and secondary diagnoses given during hospitalization were also recorded. Study protocols did not influence clinical care decision-making.

NGAL measurements:

Serum and urine samples for NGAL assay were obtained on admission and daily for up to five days of hospitalization. Samples were immediately processed, aliquotted, and stored at -80C

until the time of analysis. Urine NGAL levels were not considered in the analysis as the power was low due to limited samples collected. Urine and serum samples were analyzed for NGAL at Astute Medical using the Enzo Life Sciences kit manufactured by BioPorto Diagnostics (Catalog number: BPD-KIT-036).

Statistical Analysis:
Statistical analyses were performed on R. Figures were created in R and MS Excel. Continuous variables were analyzed using a standard t-test and categorical variables were analyzed using chi-squared tests. A non-parametric test was performed for NGAL time point 1 as it proved to have unequal variances by Levene's test of homogeneity of variances. To be consistent and because the number of samples decreased for the remainder of time points, all NGAL values were analyzed by non-parametric tests. Samples from 54 patients were analyzed for NGAL levels. Only 34 subjects were included in the statistical analysis. Five subjects were not included because they were discharged from the Emergency Department and samples were not collected from them (Patients 6, 11, 22, 28, 34). Fifteen patients were excluded because they only had two time points or had missing time points of serum NGAL (Patients 1, 3, 8, 10, 12, 13, 21, 24, 25, 26, 27, 30, 42, 45, 49). Urine NGAL levels were also not included in the analysis due to incomplete data.

RESULTS

Study Population:
Of the 34 patients analyzed, 20 patients developed AKI, 18 with Stage I AKI and 2 with Stage II (Figure 1). Stage I was defined as an increase in serum creatinine of 0.3 mg/dL or > 50% or a decrease in urine output to < 0.5ml/kg/h for six to twelve hours. Stage II was defined as an increase in serum creatinine of >100% or a decrease in urine output to < 0.5ml/kg/h for more than 12 hours. Stage III was defined as an increase in serum creatinine of > 200% or a decrease in urine output to < 0.3ml/kg/h for more than 24 hours or anuric for 12 hours or more.[20] Both admission creatinine and serum NGAL were elevated in the AKI group compared to the non AKI group, however this did not reach statistical significance. There were no significant differences between the two groups based on demographics (Table 1).

Acute Kidney Injury and NGAL:
Of the 34 patients analyzed, 20 developed AKI. Serum NGAL is represented in Figure 3 as median with IQR. Serum NGAL was significantly elevated only on time point 2 in the AKI group with a median of 201.9 ng/ml (IQR 122.6-287.8) versus the non AKI group with a median of 141.2 ng/ml (IQR 98.55-168.9, p=0.039). The maximum serum NGAL for each patient was significantly elevated in the AKI group as well (p=0.007). The median maximum serum NGAL over all five time points in the AKI group was 507.5 ng/ml versus 242.1 ng/ml in the non AKI group. Logistic regression was performed to determine the odds of having AKI based on maximum serum NGAL (equation: log odds of having AKI = -1.275824+.003997*maxNGAL). Thus for each unit of increase in maximum NGAL level, the log-odds of having AKI increases by 0.003997 (p=0.024). Increasing maximum NGAL was significantly associated with having AKI (OR = 1.004, 95% CI: 1.0005,1.0075). Using the equation (probability = Odds/(1+Odds)) a patient with a maximum NGAL level of 500ng/ml would have a 67% chance of having an AKI.

NGAL versus Creatinine:
In the AKI group, the mean time to maximum NGAL was 2.42 days while the mean time to maximum creatinine was 2.67 days, showing that NGAL peaked approximately 0.25 days before creatinine. This, however, was not statistically significant (p=0.58).

DISCUSSION

Cardiorenal syndrome is not uncommon in patients with acute decompensated heart failure. It is this connection and the compounded morbidity and mortality of end stage heart failure and renal complications that hastens this issue to the forefront of medical advancement.[21, 22] Currently, patients with ADHF who are being aggressively diuresed may or may not be taken off ACE inhibitors and ARBs due to possible development of cardiorenal syndrome clinically indicated by rising serum creatinine. However, several aspects of creatinine prove that it is an unreliable indicator of kidney injury. Independent of renal function many factors affect measured serum creatinine including age, gender, muscle mass and metabolism, medications, and hemodynamic status. Additionally, serum creatinine often rises only after steady state equilibrium is reached, which in cases of acute lesional injury could be well after the inciting event. Given the delayed rise and decreased specificity of the marker, restorative treatments that could have aided renal function at time of injury no longer become an option.[23] In order to accurately discriminate lesional acute renal injury, a marker that is specific, sensitive, and accessible is necessary. We believe this marker is NGAL.

Measuring serum NGAL upon admission has been shown to be an effective indicator of developing AKI and eventual prognosis. This was especially noticed in patients who were admitted to the hospital without prior renal complications. NGAL has been shown to be an accurate predictor of worsening renal function as it corresponds with an increase in creatinine.[24] Additionally, NGAL is more immediate as shown by the rise in serum NGAL two to eight hours before a rise in creatinine.[25] The appropriate use of ACE inhibitors and ARBs in patients with HF who acutely decompensate is poorly described and warrants further investigation. In order to effectively manage medical treatment, it is essential to accurately assess patients' kidney function as early as possible. Using NGAL as a marker of tubular damage may allow for more prompt and effective treatment of ADHF while appropriately reacting to renal complications. Certainly, NGAL may have the potential for drastically increasing survival of patients with ADHF.

It was previously shown by Aghel et al that serum NGAL levels were significantly elevated in patients who developed worsening renal function (defined as creatinine rise > 0.3 mg/dL) during hospitalization for acute decompensated heart failure (194 ng/ml versus 128 ng/ml, p = 0.001). An admission NGAL > 140 ng/ml was found to place the patient at a 7.4-fold increased risk of developing worsening kidney function during hospitalization (sensitivity = 86%, specificity = 54%).[24] Turning to AKI, Damman et al corroborated that in heart failure patients with AKI compared to controls, elevated urinary NGAL levels correlated well with elevated creatinine and reduced GFR.[12] A meta-analysis of 19 studies found that a cut off of 150 ng/ml had an AUC of 0.815 (95% CI, 0.732 – 0.892) and the power to predict necessity for renal replacement therapy.[26]

Our study shows that serum NGAL is associated with the development of AKI in patients with ADHF. Previous studies have demonstrated that serum and urine NGAL is a robust marker of AKI in patients with heart failure, post cardiac bypass surgery, transplant, and contrast induced nephropathy to name a few. This study was performed in order to fill the gap that remains in determining the strength of serum NGAL in indicating or predicting kidney injury in patients admitted to the hospital with decompensated heart failure. The most prominent finding was the difference in maximum serum NGAL between the AKI and non AKI groups. Just as we look at the maximum creatinine to define AKI status, perhaps using the peak NGAL to identify cases of acute kidney injury may be beneficial.

Examining the trends in creatinine and NGAL in relation to neurohormonal blockade regimen adjustments provides some insight to the potential use of NGAL. Figures 5 and 6 demonstrate the creatinine and serum NGAL values, respectively, prior to a change in neurohormonal blockade, specifically stopping or reducing the dosage of ACE-I/ARB/AA. The overall trend appears to be an increase in creatinine to a maximum point prior to stopping an ACE-I/ARB/AA. Interestingly serum NGAL levels appear to peak approximately one day before the time of medication regimen change or downtrend prior to medication change. We hypothesize that these patients who are developing AKI based on creatinine values, are specifically experiencing actual tubular cell damage indicated by the rise in NGAL. Firstly, this hastens the need for creating guidelines for AKI that include serum NGAL. Secondly, we hypothesize that perhaps NGAL is a useful biomarker in guiding medication regimen changes in place of creatinine with the hope of preventing further kidney injury.

Figures 7 and 8 highlight specific patient scenarios in which serum NGAL perhaps could help guide management as an indicator if acute kidney injury. In the case of Patient 23, there appears to be a steady increase in creatinine over time points 2 through 4. On time point 3 the ACE-I was stopped, presumably in response to the rising creatinine. In this example, the serum NGAL level peaked one day prior. We hypothesize that if the ACE-I was stopped one day prior in response to the NGAL level, further kidney injury could be avoided. Similarly, Patient 39 demonstrates this concept. Serum NGAL level peaked one day prior to creatinine, therefore giving the opportunity to stop or reduce the dose of the ACE-I one day earlier. These are two case scenarios among many patients in this study, limiting the validity of the hypothesis. However, recognizing these trends in further studies and eventually in the clinical setting may prove to highlight the role of NGAL. Of course, there should not be a time at which medication changes are made based solely on a biomarker such as NGAL, but the hope is that it provides another indicator to consider along with clinical exam and laboratory values creating a more complete clinical picture.

STUDY LIMITATIONS

This study is limited in power, as it is an analysis of half of the target recruitment for the completed study. Additionally, data from many subjects could not be used due to missing values. It is important to recognize that the serum creatinine values between the AKI and non AKI group were not significantly different. We believe this is due to the confounding factor of using creatinine and/or urine output as criteria for development of AKI by the latest AKIN guidelines. However, in this effort, it was still recognized that the maximum NGAL value across all time points was significantly elevated in the AKI group versus the non AKI group. This raises the

question that perhaps considering NGAL in the definition of AKI could identify cases of AKI that would not have been identified based on creatinine alone or at least earlier than if creatinine was used as the sole marker. Additionally, the low percentage of patients on outpatient ACE-I/ARB is likely reflective of the population's relatively preserved ejection fraction, making it difficult to draw meaningful conclusions about medication regimen changes.

CONCLUSION

Serum NGAL is a potential biomarker of AKI in patients who have acute decompensated heart failure. Further studies should be done to elucidate its role in guiding neurohormonal blockade regimen adjustments in the hospital.

GRAPHS

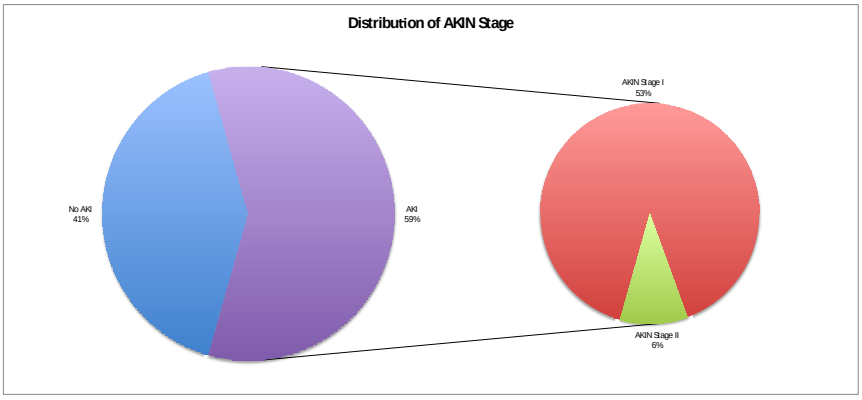

Figure 1. Of the 34 patients analyzed, 20 patients developed AKI while 14 patients did not develop AKI. Of the 20 patients that developed AKI, 18 developed Stage I and 2 developed Stage II.

	AKI (n=20)	**No AKI (n=14)**	**p value**
Age	66.2±11.4	66.71±13.1	0.904
Male Gender	19 (95%)	13 (93%)	1.000
Race			0.144
- white	13 (65%)	12 (86%)	
- black	5 (35%)	0	
- Hispanic	1 (5%)	1 (7%)	
- other	0	1 (7%)	
Admission Weight (lb)	230.24±70.24	217.66±78.61	0.643
Discharge Weight (lb)	222.72±70.64	213.76±79.68	0.758
Blood Pressure			
- Admission Systolic	131±24	140±30	0.352
- Admission Diastolic	76±19	79±17	0.625
- Discharge Systolic	125±20	121±17	0.590
- Discharge Diastolic	74±13	73±12	0.764
Physical Exam			
- rales	13 (65%)	8 (57%)	0.278
- S3	1 (5%)	3 (21%)	0.283

- S4 - Murmur - JVD - Extremity Edema	0 4 (20%) 11 (55%) 18 (90%)	0 6 (42%) 9 64%) 13 (93%)	NA 0.252 1.000 1.000
6-month Prior Creatinine (mg/dl)	1.67±1.06	1.33±0.47	0.268
Admission Creatinine (mg/dl)	1.88±1.06	1.54±0.64	0.294
Creatinine >1.5mg/dl, n	11 (55%)	6 (43%)	0.728
Admission blood urea nitrogen (mg/dl)	36.9±16.1	32.83±19.4	0.509
Log of Admission Serum NGAL (ng/ml)	2.31	2.09	0.227
Ejection Fraction (%)	42.43±23.7	43.28±19.44	0.917
Outpatient ACE Inhibitor	11 (55%)	6 (43%)	0.728
Outpatient Angiotensin Receptor Blocker	5 (35%)	3 (21%)	1.000
Outpatient Aldosterone Antagonist	2 (10%)	3 (21%)	0.627
Intravenous Contrast Administration	2 (10%)	3 (21%)	0.627
Chronic Kidney Disease	10 (50%)	3 (21%)	0.153

Table 1: Demographics of subjects in the AKI groups versus the non AKI group. Continuous variables represented as mean ± standard deviation.

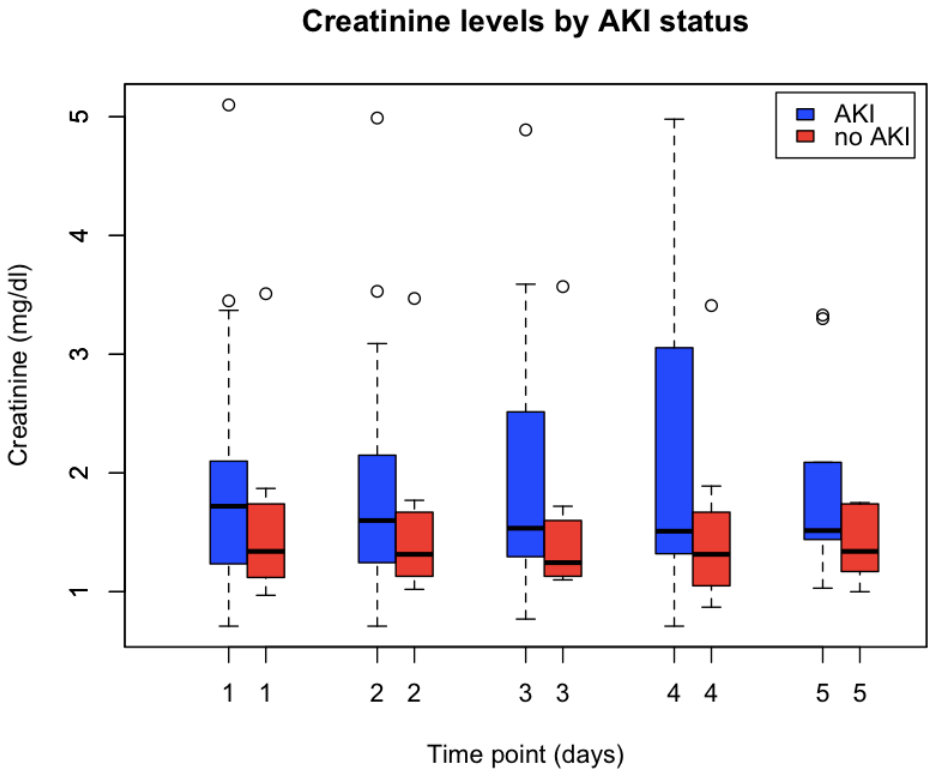

Figure 2. Creatinine levels by AKI status. At no time point was the creatinine level significantly elevated in the AKI group versus the non AKI group.

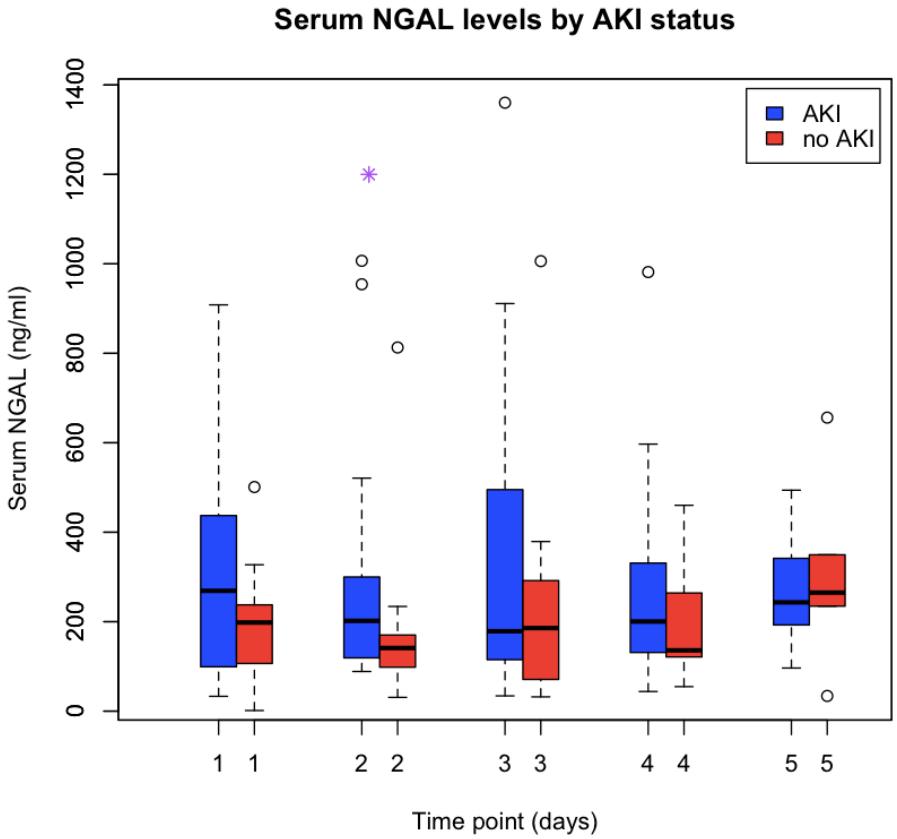

Figure 3. Serum NGAL levels by AKI status. At time point 2, serum NGAL was significantly elevated in those who developed AKI versus those who did not (201.9ng/ml, IQR 122.6-287.8 versus 141.2ng/ml, IQR 98.55-168.9, p=0.039).

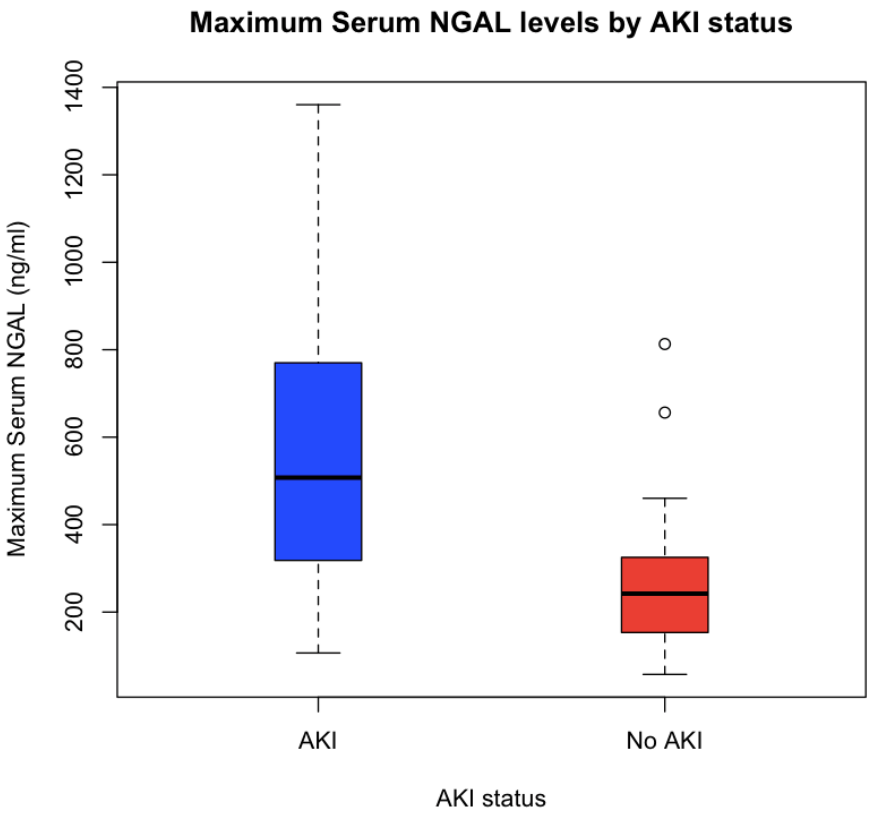

Figure 4. Maximum Serum NGAL levels by AKI status. Maximum NGAL across all five time points was tabulated for each patient in the AKI and non AKI groups. Maximum NGAL was significantly elevated in the AKI group, median 507.5ng/ml (IQR 342.8-750.3) versus the non AKI group, median 242.1ng/ml (IQR 164.3-312.3) with a p-value of 0.007.

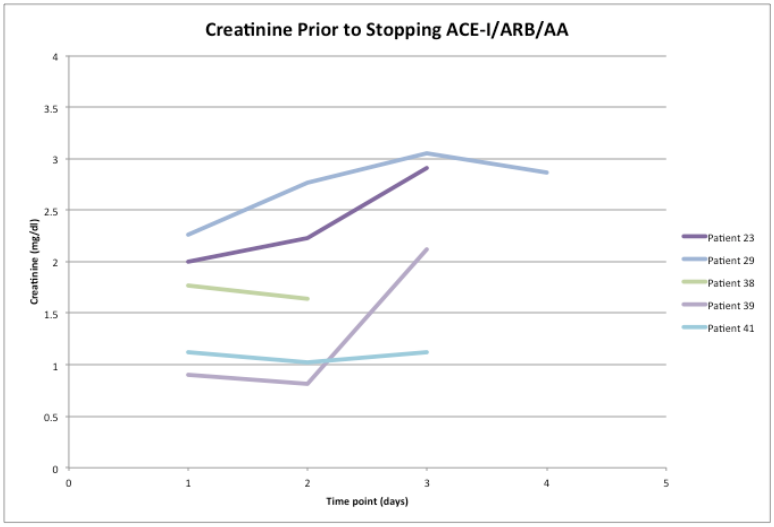

Figure 5. Creatinine Prior to Stopping ACE-I/ARB/AA.

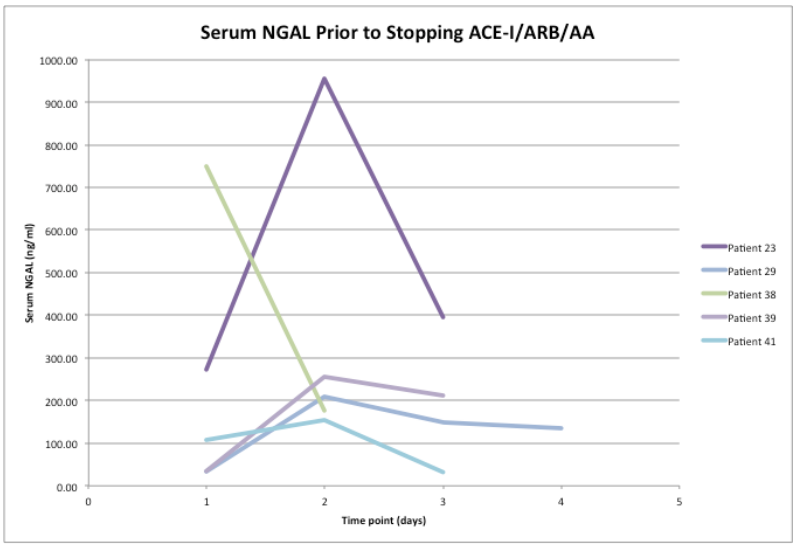

Figure 6. Serum NGAL Prior to Stopping ACE-I/ARB/AA

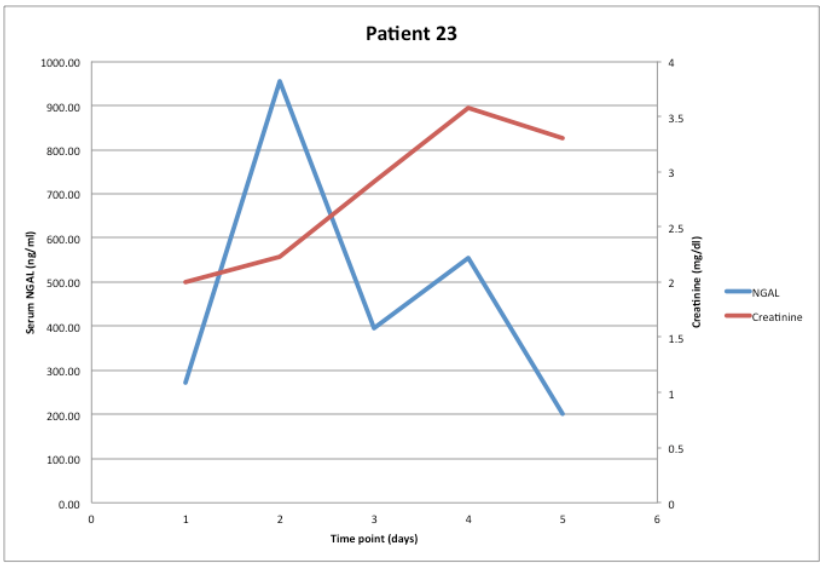

Figure 7. Patient 23. In this patient, the ACE inhibitor was stopped at time point 3.

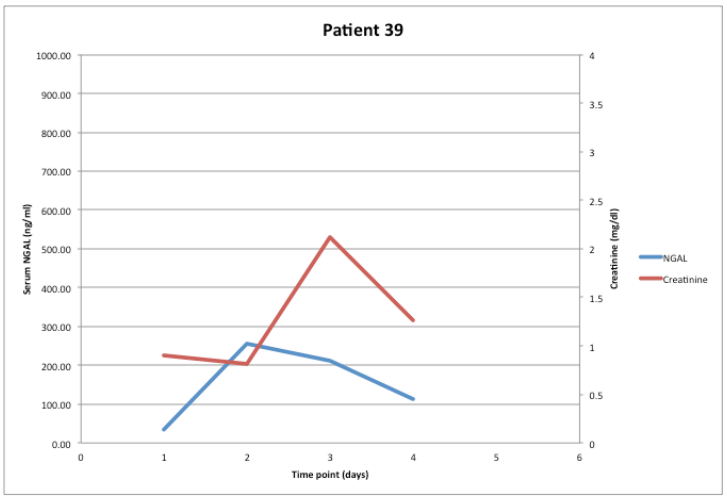

Figure 8. Patient 39. In this patient, the ACE inhibitor was stopped on tine point 3.

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