ABSTRACT

Acute Kidney Injury in the Setting of Cirrhosis

Justin Miles Belcher

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Acute kidney injury (AKI) is a common complication in patients with cirrhosis and is associated with significant mortality. Despite the overall poor outcomes, there exists hope for such patients as, unlike in the majority of setting of AKI, specific treatments are available which have been shown to improve renal function and mortality. However, historically intransient difficulties in differential diagnosis and prognosis have limited the extent to which such treatments can be appropriately utilized. In addition, though AKI has long been appreciated as a feared complication, the definitions of AKI employed in studies involving patients with cirrhosis have not been standardized, lack sensitivity, and have often been limited to narrow clinical settings. We conducted a multicenter, prospective observational cohort study of patients with cirrhosis and AKI, drawn from multiple hospital wards, utilizing the modern acute kidney injury network (AKIN) definition and assessed the association between AKI severity and progression with in-hospital mortality. Following this we investigated whether early changes in serum cystatin C levels were more closely associated with subsequent outcomes than similarly early changes in serum creatinine. We subsequently assessed whether novel biomarkers of kidney structural injury, measured on the day of fulfilling AKI criteria, can predict progression of AKI and mortality. Finally, we investigated the ability of biomarkers to assist with differential diagnosis and potentially change the way in which causes of AKI in cirrhosis are conceptualized.

192 patients were enrolled and included in the study. In the first phase, 85 (44%) of these were found to progress to a higher AKIN stage after initially fulfilling AKI criteria. Patients

achieved a peak severity of AKIN stage 1, 26%, stage 2, 24%, and stage 3, 49%. Progression was significantly more common and peak AKI stage higher in non-survivors than survivors ($p < 0.0001$). After adjusting for baseline renal function, demographics and critical hospital and cirrhosis-associated variables, progression of AKI was independently associated with mortality (adjusted odds ratio = 3.8, 95% confidence interval (CI) 1.3-11.1). We conclude that AKI, as defined by AKIN criteria, in patients with cirrhosis is frequently progressive and severe and is independently associated with mortality in a stage-dependent fashion.

Unfortunately, accurately predicting which patients will experience the worst outcomes is challenging as serum creatinine correlates poorly with glomerular filtration in patients with cirrhosis and fluctuations may mask progression early in the course of AKI. Cystatin C, a low-molecular-weight cysteine proteinase inhibitor, is a potentially more accurate marker of glomerular filtration. In the second phase of our study we evaluated whether early changes in serum cystatin C would associate more strongly with a composite endpoint of dialysis or mortality than early changes in creatinine. Of 106 patients studied with at least 2 blood samples, 37 (35%) met the endpoint. Cystatin demonstrated less variability between samples than creatinine. Patients were stratified into four groups reflecting changes in creatinine and cystatin: both unchanged or decreased 38 (36%) (Scr-/CysC-); only cystatin increased 25 (24%) (Scr-/CysC+); only creatinine increased 15 (14%) (Scr+/CysC-); and both increased 28 (26%) (Scr+/CysC+). With Scr-/CysC- as the reference, in both instances where cystatin rose, Scr-/CysC+ and Scr+/CysC+, the primary outcome was significantly more frequent in multivariate analysis, $P = 0.02$ and 0.03, respectively. However, when only creatinine rose, outcomes were similar to the reference group. We therefore concluded that changes in cystatin levels early in AKI are more closely associated with eventual dialysis or mortality than creatinine and may allow more rapid identification of patients at risk for adverse outcomes.

The next aspect of the study evaluated urinary biomarkers, including neutrophil gelatinase-associated lipocalin (NGAL), IL-18, kidney injury molecule-1 (KIM-1), liver-type fatty

acid-binding protein (L-FABP), albuminuria and the fractional excretion of sodium (FENa) as predictors of AKI progression and in-hospital mortality. Of 188 patients with available urine samples, 44 (23%) experienced AKI progression alone and 39 (21%) suffered both progression and death during their hospitalization. NGAL, IL-18, KIM-1, L-FABP and albuminuria were significantly higher in patients with AKI progression and death. These biomarkers were independently associated with this outcome after adjusting for key clinical variables including model of end stage liver disease score, IL-18 (relative risk [RR], 4.09; 95% CI, 1.56 to 10.70), KIM-1 (RR, 3.13; 95% CI, 1.20 to 8.17), L-FABP (RR, 3.43; 95% CI, 1.54 to 7.64), and albuminuria (RR, 2.07; 95% CI, 1.05-4.10) per log change. No biomarkers were independently associated with progression without mortality. FENa demonstrated no association with worsening of AKI. When added to a robust clinical model, only IL-18 independently improved risk stratification on a net reclassification index. This phase of the study demonstrated that multiple structural biomarkers of kidney injury, but not FENa, are independently associated with progression of AKI and mortality in patients with cirrhosis. However, injury marker levels were similar between those without progression and those with progression alone.

Knowledge of which patients are at the highest risk of adverse outcomes may allow for earlier targeting of treatments but only if clinicians can may objective, accurate diagnoses as to the cause of AKI. The most common etiologies of AKI in this cirrhosis are prerenal azotemia (PRA), acute tubular necrosis (ATN) and hepatorenal syndrome (HRS). However, establishing an accurate differential diagnosis is extremely challenging. Urinary biomarkers of kidney injury distinguish structural from functional causes of AKI and we hypothesized that they may facilitate more accurate and rapid diagnoses. In the next phase of our study we therefore assessed multiple biomarkers for differential diagnosis in clinically adjudicated AKI. Patients (n = 36) whose creatinine returned to within 25% of their baseline within 48 hours were diagnosed with PRA. In addition, 76 patients with progressive AKI were diagnosed by way of blinded retrospective adjudication. Of these progressors, 39 (53%) patients were diagnosed with ATN,

19 (26%) with PRA, and 16 (22%) with HRS. Median values for neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), liver-type fatty acid binding protein (L-FABP), and albumin differed between etiologies and were significantly higher in patients adjudicated with ATN. The fractional excretion of sodium (FENa) was lowest in patients with HRS, 0.10%, but did not differ between those with PRA, 0.27%, or ATN, 0.31%, P = 0.54. The likelihood of being diagnosed with ATN increased step-wise with the number of biomarkers above optimal diagnostic cutoffs. From these results we concluded that urinary biomarkers of kidney injury are in fact elevated in patients with cirrhosis and AKI due to ATN and that incorporating biomarkers into clinical decision making has the potential to more accurately guide treatment by establishing which patients have structural injury underlying their AKI.

Unfortunately, despite these promising results, it is likely that, as long as the focus is on assigning patients one of three distinct diagnoses, there will always be overlap in biomarkers values between groups such that, on the individual rather than population level, their utility will not be fully optimized. In the final phase of our study we evaluated a diagnostic algorithm utilizing optimal cutoffs for FENa and NGAL and the current diagnostic categories of PRA, ATN and HRS. In conclusion, we suggest moving beyond current diagnoses by instead attempting to physiologically phenotype patients using both function (FENa, urinary cystatin C) and structural (NGAL) urinary biomarkers. Figures are presented demonstrating that patients fall into distinct physiologic clusters which may allow more precise targeting of therapies.

Acute Kidney Injury in the Setting of Cirrhosis

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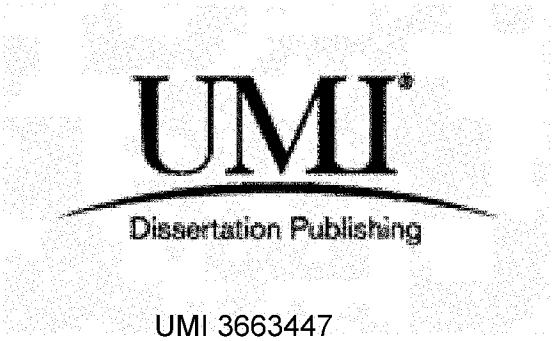

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Table of Contents

List of Tables & Figures

Chapter 1. Introduction **Page**

Chapter 2. Association of AKI with Mortality and Complications in Hospitalized Patients with Cirrhosis

Chapter 3. Early Trends in Cystatin C and Outcomes in Patients with Cirrhosis and Acute Kidney Injury

Chapter 4. Urinary Biomarkers and Progression of Acute Kidney Injury in Patients with Cirrhosis

Introduction

Overview of Clinical Problem

Acute kidney injury (AKI) is a common and devastating complication in patients with cirrhosis, occurring in an estimated 19% of hospitalizations[1], and is associated with significant mortality, 55-91%[2-4]. In recognition of the primacy of renal dysfunction in affecting outcomes in patients with cirrhosis, the model of end-stage liver disease (MELD) score incorporates creatinine as a critical determinant of short-term mortality and is the main criterion used to prioritize organs for liver transplantation. The clinical impact of this infelicitous confluence of illnesses will continue to worsen as the incidence of both AKI and cirrhosis are increasing[5]. Clinicians called to manage patients with AKI in the setting of cirrhosis must answer several key questions. What is the etiology of the patient's AKI? What is the prognosis regarding the course of the renal dysfunction? Should the patient receive specific therapy? If so, how early should treatment be initiated and what are the appropriate agents? To answer these questions one must be able to accurately determine the underlying cause and severity of AKI and stratify the patient based on their likelihood of suffering progressive AKI and/or mortality. Unfortunately, clinical care of patients with cirrhosis and AKI is severely hindered by often underappreciated flaws in our current tools for diagnosis and prognosis and by limitations in the current conceptualization of AKI in this unique setting. Devising a research approach to overcome these challenges and address the myriad uncertainties confounding the management of AKI in cirrhosis requires understanding the means by which AKI develops in this setting, grasping the reasons behind the challenging differential diagnosis and appreciating the process whereby novel tests can untangle this Gordian knot.

Patients with Cirrhosis are at High Risk for Acute Kidney Injury

The increased propensity for AKI in patients with cirrhosis stems from hemodynamic abnormalities typical of patients with cirrhosis and ascites (**Figure 1**)[6]. These abnormalities occur after the development of portal hypertension and portosystemic collaterals and consist of splanchnic and systemic vasodilatation. Vasodilatation results in a decrease in effective arterial blood volume, which in turn stimulates neurohumoral systems, specifically the renin-angiotensin-aldosterone system (RAAS), sympathetic nervous system, and nonosmotic release of antidiuretic hormone. Activation of RAAS and the sympathetic nervous system result in sodium retention, increased intravascular volume, and a hyperdynamic circulatory state characterized by low systemic vascular resistance and increased cardiac output.[7] An increased production of nitric oxide is considered the main cause of vasodilatation in cirrhosis. In experimental cirrhosis, nitric oxide blockade increases systemic blood pressure and sodium excretion and decreases ascites while down-regulating activation of the RAAS.[8,9] Recent studies indicate that increased angiogenic factors also appear to contribute importantly to vasodilatation.[10]

Although these compensatory mechanisms initially are able to maintain a reasonable arterial pressure, as cirrhosis progresses and vasodilatation worsens, such mechanisms are no longer adequate and patients experience a further decrease in effective blood volume with enhanced activation of vasoconstrictive systems.[7] This activation leads to preferential vasoconstriction in several vascular beds, most prominently the renal and central nervous systems.[10,12] The predilection toward renal vasoconstriction cannot be countered by the usual intrarenal release of vasodilatory substances such as prostaglandins and prostacyclin owing to decreased production in the renal vasculature in advanced cirrhosis and vasoconstriction is exacerbated further by local release of vasoconstrictors such as endothelin and thromboxane.[13] Although the majority of these patients have a high cardiac output, a relatively lower output (<6 L/min) with a lack of response to further arterial vasodilatation and physiological stressors also

can compound the deceased renal blood flow and has been shown to be a strong predictor of hepatorenal syndrome (HRS).[14-16]

The renal vasculature typically is able to autoregulate renal perfusion in the setting of decreased flow via tubuloglomerular feedback and the myenteric stretch reflex, ensuring an essentially constant blood flow to the kidneys irrespective of fluctuations in systemic blood pressure. However, when mean arterial pressure reaches a decisive threshold around 65 mm Hg, autoregulatory mechanisms are overwhelmed and renal blood flow begins to decrease in proportion to renal perfusion pressure.[17] That is, for any given perfusion pressure, the amount of blood the kidney actually receives will decrease progressively.[18,19] Patients with advanced cirrhosis are therefore both predisposed to renal hypoperfusion and ill-equipped to respond to it. As discussed later, such hypoperfusion can lead to a decrease in renal filtration owing simply to low blood flow. Strong evidence is lacking that such chronic hypoperfusion itself leads to ischemic injury. However, hypoperfusion clearly predisposes cirrhotic patients to structural kidney injury when coupled with a second hit such as abrupt shifts in intravascular volume or exposure to nephrotoxic medications. Once structural injury is established in patients with cirrhosis, recovery may be retarded because of an inability to reconstitute optimal renal perfusion even after resolution of the precipitating insult.

Precipitants of AKI are Common

Patients with cirrhosis, already inclined to renal dysfunction, also frequently are exposed to precipitants that serve to tip what is in essence chronic low-grade renal hypoperfusion into frank filtration failure and AKI (**Figure 1**)[6]. Among hospitalized patients, bacterial infections, most commonly spontaneous bacterial peritonitis, potentiate splanchnic arterial vasodilatation via endotoxemia and cytokine overproduction.[20,21] Gastrointestinal hemorrhage, common in decompensated cirrhosis, leads to AKI in 26% of cases via hypotension and further diminution of effective circulating volume.[22] Large-volume paracentesis involving the removal of more than

4 to 5 L of fluid also may precipitate AKI through intravascular depletion and worsening vasodilatation.[23,24]

Although such dramatic insults frequently are associated with severe AKI in hospitalized patients, the majority of AKI in cirrhotic patients develops in outpatients[25,26] who experience frequent fluctuations in intravascular volume owing to use of diuretics and lactulose-associated diarrhea. The resulting decrement in renal blood flow exacerbates existing hypoperfusion and cannot be compensated for by patients' compromised autoregulation mechanisms.

Challenges with Acute Kidney Injury Diagnosis

Despite improved understanding of the above precipitants of and physiology underlying AKI in cirrhosis, considerable confusion continues to surround its diagnosis. One of the primary reasons for such struggles is that creatinine, the chief arbiter of renal filtration, is unsuited for this role in patients with cirrhosis. In this setting, low protein intake, loss of muscle mass, diminished hepatic synthesis of creatine, and an enlarged volume of distribution decrease serum creatinine levels irrespective of renal function and thus lead to overestimation of the glomerular filtration rate (GFR).[27] Major extrarenal influences on serum creatinine level are shown in **Figure 2**. In a steady state, the 2 primary equations used to estimate GFR, Cockcroft–Gault and Modification of Diet in Renal Disease (MDRD), perform equally poorly, estimating GFR within 50% of the measured value in only 9% and 7% of patients, respectively[28]. Although superior in some settings, a newer equation, the Chronic Kidney Disease Epidemiology Collaboration[29], does not appear superior to MDRD in cirrhosis.[30] Estimating equations utilizing cystatin C may be superior to those with creatinine but are not widely available and still lack accuracy and precision[31-33]. Creatinine is untethered even further from GFR in the setting of AKI, in which changes in levels can lag decreases in GFR by several days. The traditional definition of AKI in cirrhosis involves an increase to greater than 1.5 mg/dL.[34] However, with the earlier-described factors frequently resulting in a baseline creatinine level as low as 0.5 or 0.6 mg/dL,

adherence to such an increased threshold delays diagnosis for patients with severe AKI and fails to detect many cases of mild to moderate disease. This problem is compounded when creatinine at the time of admission is considered the baseline level in patients with cirrhosis. When compared with the use of accurate outpatient baseline values, such an approach obscures the very presence of approximately 60% of AKI cases[25]. The traditional creatinine-based definitions of AKI in cirrhosis therefore are not only unhelpful for the critical distinction of etiology but also are insensitive for identification of AKI itself.

Seeking to remedy this failing and bring the approach to acute renal dysfunction in cirrhosis in line with evolving conceptions of AKI, a working group composed of members of the International Ascites Club (IAC) and the Acute Dialysis Quality Initiative recently published diagnostic criteria for what they term "hepatorenal disorders," covering AKI, CKD, and HRS.[35] The group recommended adaptation of the Acute Kidney Injury Network (AKIN) criteria to define AKI instead of the traditional definition in cirrhosis using a fixed creatinine cut-off value of greater than 1.5 mg/dL, although the threshold of 2.5 mg/dL for the specific diagnosis of type 1 HRS was left unchanged. Even more recently, the IAC recommended adopting the Kidney Disease Improving Global Outcome (KIDIGO) criteria and abandoning the requirement of a creatinine > 2.5 mg/dL for the diagnosis of HRS[36] (**Table 1**)[37]. The benefits of adopting the new, more sensitive definition are 2-fold. First, although necessarily losing specificity, lowering the threshold for a diagnosis of AKI will increase sensitivity and the association between even mild acute increases in creatinine and adverse outcomes has been well established.[38] Second, and perhaps more importantly, the lower threshold of AKIN will identify those more severe cases who ultimately would have qualified for the 1.5-mg/dL threshold as having AKI significantly earlier, thus facilitating earlier interventions and potentially improving outcomes.[39]

Despite these theoretical benefits, at the time of the adaptation of the new consensus criteria, several crucial questions remained unresolved. What will be the outcomes of this newly classified AKI? Should it be treated? If so, what level of creatinine increase should trigger

intervention? Is it harmful to wait and treat only patients whose AKI progresses or can aggressive treatment be withheld only for those patients who fail conservative management? The prospective, multi-center cohort study detailed in this dissertation was designed to address multiple issues regarding AKI in the setting of cirrhosis including differential diagnosis and prognosis. However the most fundamental goal was to situate these questions of management in a solid clinical context so as to determine the actual importance of accurate diagnosis and prognosis. To do so we sought to clarify the natural history and epidemiology of AKI in hospitalized patients with cirrhosis utilizing the AKIN criteria. To this end we investigated the mortality of patients with cirrhosis and AKI diagnosed via AKIN, the degree to which this mortality increased step-wise with peak AKIN stage and, critically, whether and to what degree progressing to a higher AKIN stage than that at initial presentation potentiated the risk for mortality. The results for this phase of the study are presented in **Chapter 2**.

Despite the promise of these revised criteria, the logical outcome of the new definition will be an increase in patients with cirrhosis fulfilling criteria for AKI because of the definition's increased sensitivity but with a resulting decrease in specificity. With frequent fluid shifts and altered splanchnic, systemic, and renal hemodynamics, patients with cirrhosis may experience fluctuations in creatinine levels. In this setting, the decreased specificity of the new definition is potentially problematic because patients with cirrhosis often are subjected to numerous blood draws, providing atypically numerous opportunities to capture hypercreatininemia attributable merely to benign oscillations inherent to cirrhotic physiology. In a subset of 53 hospitalized patients with AKI from the study detailed in this dissertation, there was a median of 10 creatinine measurements over the year preceding admission (unpublished data). This differs from a typical surgery or intensive care unit (ICU) patient who may have had only 1 to 2 measurements of serum creatinine level, if any, and carries a risk of ascertainment bias. Given the significant challenges inherent to the differential diagnosis of AKI in cirrhosis (see below), clinicians frequently employ the kitchen sink approach to treatment, throwing multiple therapies at patients

simultaneously before arriving at a diagnosis. In patients with cirrhosis, such treatments are frequently both scarce (albumin) and expensive (albumin and terlipressin), and not without risk (excessive volume in patients with volume in ATN, ischemic complications of vasoconstrictors). With vastly more patients now qualifying for a diagnosis of AKI and in light of recent data that patients whose creatinine peaks at less than 1.5 mg/dL fare much better than those who experience a higher rise[40,41], there is concern that in some patients the risks of treatment may outweigh the rewards. Unfortunately, while MELD performs well in predicting mortality, neither it nor any other scoring system or tests has been shown to predict progression of AKI. Until approaches to differential diagnosis can be refined, objective tests are urgently needed that allow for the prediction for which patients are at highest risk for progression of their AKI.

Cystatin C is a low-molecular-weight cysteine proteinase inhibitor synthesized at a constant rate by all nucleated cells. Cystatin C is freely filtered by the glomerulus, nearly completely reabsorbed and catabolized by the proximal tubule, and does not undergo secretion. Cystatin C levels are less influenced by nonrenal factors than creatinine and it has thus been proposed as a superior marker of glomerular filtration (factors affecting cystatin C levels are shown in **Figure 3**). In AKI, cystatin C rises more rapidly than creatinine in some settings and has been shown to associate more strongly with outcomes[42-45]. We hypothesized that early changes in serum cystatin C, in the days immediately after the diagnosis of AKI under the new criteria, may more accurately reflect changes in renal function than early changes in creatinine and thus may better predict progression of AKI. This aspect of the study is detailed in **Chapter 3**.

Etiologies of AKI

Etiologies of AKI in patients with cirrhosis traditionally are divided into functional vs structural causes but more appropriately can be thought of as existing on a continuous spectrum. Patients may and often do show features consistent with both functional and

structural etiologies and these conditions overlap (**see Chapter 6**). Very few patients manifest pure functional or structural disease but under current practice patients' diagnoses typically have been dichotomized into these categories. Only about a third of cases of AKI in cirrhosis are caused by intrinsic renal disease, mostly acute tubular necrosis (ATN), although systemic conditions such as glomerulonephritis also may be present (**Figure 4**)[1]. In contrast, the most common etiology of AKI in cirrhosis is renal hypoperfusion, accounting for 68% of cases.[1] Approximately two-thirds of these hypoperfusion-associated AKI cases, or 45% of all AKIs, show improvement with volume expansion and are considered prerenal azotemia (PRA). The remaining one-third, or 23% of total AKIs, are not volume-responsive and receive a diagnosis of HRS. HRS represents the endpoint of the pathophysiologic cascade, detailed above, that is triggered by the development of portal hypertension. The resulting splanchnic vasodilatation, corresponding fall in systemic vascular resistance and subsequent activation of the renin-angiotensin system, sympathetic nervous system and arginine vasopressin result in profound renal vasoconstriction and hypoperfusion. When vasoconstriction is sufficiently advanced, renal hypoperfusion is no longer reversible with volume resuscitation and patients experience the progressive and unrelenting decline in renal function characteristic of HRS. AKI in such patients is therefore primarily functional in nature.

HRS is divided into 2 types (types 1 and 2). Type 1 typically occurs in the inpatient setting, develops in less than 2 weeks, and often is associated with a precipitating event such as spontaneous bacterial peritonitis or volume depletion (gastrointestinal hemorrhage, over-diuresis). Survival for Type 1 was historically < 2 weeks and still may exceed 80% at 3 months absent a liver transplant[53]. Type 2 is more insidious, slowly progressive, occurs primarily in outpatients with refractory ascites, and can be considered a unique form of chronic kidney disease (CKD) in patients with cirrhosis. For the remainder of this dissertation, HRS refers to type 1 HRS.

Etiology Affects Prognosis and Guides Management

Although the development of AKI in cirrhosis is associated universally with increased mortality, the magnitude of this effect is strongly contingent on the specific etiology of AKI, with HRS portending the worst prognosis. Martin-Llahi et al. prospectively evaluated 562 patients with cirrhosis and AKI and adjudicated patients into the somewhat unconventional diagnoses of "renal failure because of parenchymal nephropathy," "hypovolemia-related renal failure," "renal failure associated with infection," and "hepatorenal syndrome."[53] Outcomes differed significantly by etiology with 3-month mortality rates for patients with AKI as a result of parenchymal injury, hypovolemia, infection, and HRS of 27%, 54%, 69%, and 85%, respectively, and etiology was associated independently with mortality. In multiple studies of cirrhosis and AKI, the diagnosis of HRS as opposed to other etiologies has been associated independently with mortality.[53,54] Critically, despite the extent to which accurate determination of etiology therefore dictates clinicians' ability to formulate a prognosis, cause of AKI is not factored into the MELD score.

In addition to facilitating improved prognostication regarding risk of mortality, establishing the etiology of AKI in patients with cirrhosis is critical for guiding therapy as disease-specific treatments with the potential to improve outcomes, if correctly and judiciously applied, are available. Arriving at an accurate diagnosis is imperative because these treatments vary greatly, entail significant expense, use scarce resources and have potentially significant toxicity. In spite of the severity of renal dysfunction, kidneys in patients with HRS are primarily structurally intact. Kidney function in this setting therefore can be markedly improved if renal blood flow is restored. The combination of volume expansion and vasoconstrictors, particularly terlipressin, a V_1-vasoconstrictor that acts to augment systemic circulating volume and thereby improve renal perfusion, shows significant promise.[55] In addition, in patients with advanced cirrhosis, liver transplantation can restore systemic vascular resistance, mitigate systemic and renal

vasoconstriction and restore normal renal hemodynamics. Patients with HRS at the time of liver transplantation can thus experience rapid improvement in renal function post-transplant[13]. Patients with cirrhosis and PRA require fluids, but the deleterious consequences of overzealous fluid administration, as occurs when ATN is misdiagnosed, are increasingly recognized.[56] Patients with ATN should be dialyzed if clinically indicated but in such patients with frank structural injury, interventions to restore renal perfusion do not result in resolution of AKI and application of vasoconstrictors or liver transplantation are therefore inappropriate. Finally, patients with ATN must be differentiated from patients with HRS when considering a combined liver/kidney transplant.

Current Diagnostic Strategies in Cirrhosis are Inadequate

Unfortunately, current diagnostic strategies are inadequate and confidently differentiating between PRA, ATN, and HRS is notoriously difficult. The strong potential therefore exists for misallocation of scarce resources and potentially harmful unnecessary treatments. As a marker of filtration, creatinine detects declines in kidney function but cannot determine whether such a decline is due to hypoperfusion or to structural injury. The fractional excretion of sodium (FENa), though ubiquitously applied by nephrologists evaluating AKI, has historically been difficult to interpret in the setting of cirrhosis. Cirrhotic patients frequently present with low urine sodium irrespective of AKI[57] due to hemodynamically induced sodium avidity and even ATN can present with a FENa <1%[58-59]. The traditional dichotomy wherein a FENa <1% indicates hypoperfusion and >1-2% signifies tubular dysfunction and ATN is therefore inapplicable and the test is not typically utilized in cirrhotic patients. Similarly, urine microscopy is potentially helpful in the differential diagnosis of AKI[60] but can be complicated in cirrhosis by biliary staining of sediment, can fail to correlate with urine electrolytes[61] and has not been rigorously evaluated in this setting. Patients often present with a mixed picture with both low FENa and granular casts. In

addition, a lack of response to a volume challenge cannot always rule out PRA given the extent to which patients with advanced cirrhosis third space fluids.

The International Ascites Club (IAC) attempted to standardize the diagnosis of HRS by establishing 6 clinical criteria[39]. There is considerable evidence however that these criteria lack specificity. Patients with ATN often 1) present with ascites 2) have creatinine > 1.5 mg/dL 3) do not respond to volume resuscitation 4) lack significant proteinuria or hematuria and 5) have no gross structural changes to the kidney. Ischemic ATN can develop in the absence of shock (6) and indeed frequently occurs in the setting of ostensibly normal blood pressure[62]. In addition, the degree of creatinine elevation does not distinguish ATN from HRS[59].

Evidence and Hazards of Misclassification

With clinicians hamstrung by inadequate diagnostic criteria, there is considerable evidence that etiologies of AKI in cirrhosis are frequently misdiagnosed and that such misclassification leads to worse outcomes and wasted resources. In recognition of the impact of renal function on mortality in patients with cirrhosis, the MELD scoring system, which heavily weighs serum creatinine in formulating a score for the severity of cirrhosis, was adopted to rank the priority of potential recipients of liver transplants. As a result, the frequency of combined liver-kidney transplants has increased steadily, with nearly 400 (7% of all liver transplants) now performed annually in the US (UNOS 2013). While AKI due to HRS is expected to resolve with liver transplant, ATN, when severe, may transition directly to chronic kidney disease or end-stage renal disease, necessitating a concomitant liver-kidney transplant. However, of 1041 solitary liver recipients on dialysis for AKI at the time of transplant, 334 (32%) remained dialysis dependent post-transplant[64]. Those patients whose renal function fails to improve following liver transplant are forced to be subsequently listed for kidney transplant. Because kidney allografts received in combination with a liver suffer from lower rates of acute rejection[65,66] and enjoy longer half-lives than those transplanted sequentially[65], this misdiagnosis likely results in clinical

harm. At the same time, renal graft survival in combined transplants is far shorter than for sister kidneys used for solitary renal transplants due to a higher rate of combined transplant recipients dying with functioning kidneys[67]. This is critical as 27% of patients who receive a combined liver-kidney transplant have a measured native kidney GFR of > 30ml/min 1 year post-operatively and thus were inappropriately transplanted[68]. With a critical shortage of donor kidneys and expanding transplant wait list, both the failure to transplant the organs concurrently in patient with significant structural kidney injury and the provision of a combined transplant to patients whose functional AKI required only a liver constitute a misallocation of tremendously scarce resources.

As noted above, terlipressin, a V_1-vasoconstrictor, has been found promising in the treatment of HRS. However, meta-analysis of terlipressin trials has shown reversal of HRS to occur in only 50% of patients[55]. A significant percentage of non-responders may actually have been suffering from ATN rather than HRS and were thus unlikely to significantly benefit from alterations in renal perfusion. Such misclassification is significant because it results in the unnecessary exposure of patients to the risk of adverse events. As a potent vasoconstrictor, treatment with terlipressin may result in up to 40% of recipients experiencing adverse events including myocardial or intestinal ischemia, circulatory overload and arrhythmias[69].

Although the clinical consequences of misdiagnosis are readily apparent, more insidious but no less deleterious are effects on research. In a seminal study intending to lead to the approval of terlipressin in the United States, Sanyal et al[70] randomized 56 patients with presumptive HRS to treatment with terlipressin and albumin and 56 to albumin plus placebo. Although the terlipressin group showed a greater rate of renal recovery, this difference did not quite achieve statistical significance ($P = .056$). The response rate to placebo, in a disease typically universally progressive if untreated, was 12%. It is likely that some of these responders to placebo in fact did not have HRS but rather ATN, which resolved. Misclassification in this setting attenuates studies' statistical power, biases effect estimates towards the null and may

result in falsely negative trials[71] and delayed appreciation of the benefit of and approval for future HRS specific therapies.

Novel Biomarkers Can Improve Diagnostic Accuracy and Treatment Allocation

The critical diagnostic shortcoming in diagnosis and prognosis is that serum creatinine is a marker of kidney filtration, not injury, and thus cannot distinguish functional from structural etiologies of AKI. An objective, physiologically based test administered prior to terlipressin initiation or liver transplant capable of distinguishing functional from structural AKI would revolutionize our ability to predict renal response to specific therapies and ultimately improve patient outcomes. Investigation of biomarkers for this purpose was identified as a key area for future research at a recent summit on combined liver-kidney transplant[72]. The study of AKI has been revolutionized by investigation into multiple novel urinary biomarkers of kidney injury. Nearly 30 biomarkers of AKI (primarily tubular) have recently been investigated for early detection, differential diagnosis and prognosis of AKI[27]. Biomarkers investigated in at least 2 human studies are shown in **Figure 5**[73]. Among the most promising are interleukin 18 (IL-18), kidney injury molecule 1 (KIM-1), liver-type fatty acid–binding protein (L-FABP) and neutrophil gelatinase-associated lipocalin (NGAL). Critically, increased levels of such biomarkers are specific evidence of structural injury rather surrogate markers for deceased filtration. Biomarkers of injury therefore should allow for differentiation of functional (HRS) from structural (ATN) AKI and indeed have shown the ability to distinguish ATN from PRA, urinary tract infection, and CKD (IL-18,[74] KIM-1,[75] and NGAL[76]), as well as from interstitial nephritis, allograft rejection, and obstructive nephropathy (KIM-1[75]). Biomarkers reflecting tubular injury also have been associated successfully with outcomes, including both worsening of AKI and mortality, in several settings including cardiac surgery,[77] heart failure,[78] ICU,[79] and transplant settings.[80] Given the disparate etiologies of AKI in cirrhosis, it was unclear whether biomarkers, either conventional ones such as FENa and proteinuria or novel injury markers would associate with outcomes in

this setting. We therefore investigated the association of multiple biomarkers with AKI progression and mortality (**Chapter 4**).

Given the tremendous physiologic difference between functional and structural AKI in cirrhosis and the impact this distinction has on the potential for successful treatment, injury biomarkers would seem particularly well suited for differential diagnosis in this setting. The critical need for research in this area was recognized by the study group on HRS at the recent 8th international consensus conference of the Acute Dialysis Quality Initiative (ADQI) group which listed the evaluation of injury biomarkers for the study of AKI in cirrhosis as one of their primary targeted areas for further research[81]. We hypothesized that urinary injury biomarkers would be higher in patients with cirrhosis and structural (ATN) as opposed to functional (HRS) AKI and thus could facilitate an objective differential diagnosis. The results of this aspect of the study are presented in **Chapter 5**.

Future Directions

While the current diagnostic utility of biomarkers will be to assist clinicians in assigning a diagnosis of PRA, ATN or HRS, their true potential may outstrip even this laudable contribution. Clinicians evaluating cirrhotic patients with AKI currently remain compelled to assign a unifying diagnosis, fixating on what the patient "has" rather than focusing on the status of their renal physiology/pathology to which treatment must be tailored. The critical task is to distinguish where a patient falls on the spectrum of functional and structural disease and, once this is established, identify early in AKI which patients are likely to progress and initiate prompt therapy in these cases. The root problem is that creatinine is a marker of renal filtration but does not reflect the presence or absence of frank structural injury and thus provides no guidance on AKI etiology or the likelihood of response to various targeted therapies. The AKIN criteria allow earlier diagnosis but do not absolve the flaws in creatinine's insensitivity to changes in GFR and, more importantly, still do not distinguish functional from structural disease or predict which

patients will progress. In **Chapter 6** we appraised an algorithm utilizing FENa and NGAL with optimal cutoffs for its diagnostic utility within the current trichotomous diagnostic framework. Finally, given the overlap in biomarker values on the individual level, we evaluated moving beyond current diagnoses by instead attempting to physiologically phenotype patients using both function (FENa, urinary cystatin C) and structural (NGAL) urinary biomarkers. Such an approach would attempt to establish whether or not a patient's renal function would improve with restoration of renal blood flow by locating them in a spectrum of physiologic clusters rather than attempting to confirm on them a single, specific diagnosis.

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Figure 1. Precipitants, mechanisms and clinical correlates of HRS and ATN in cirrhosis.

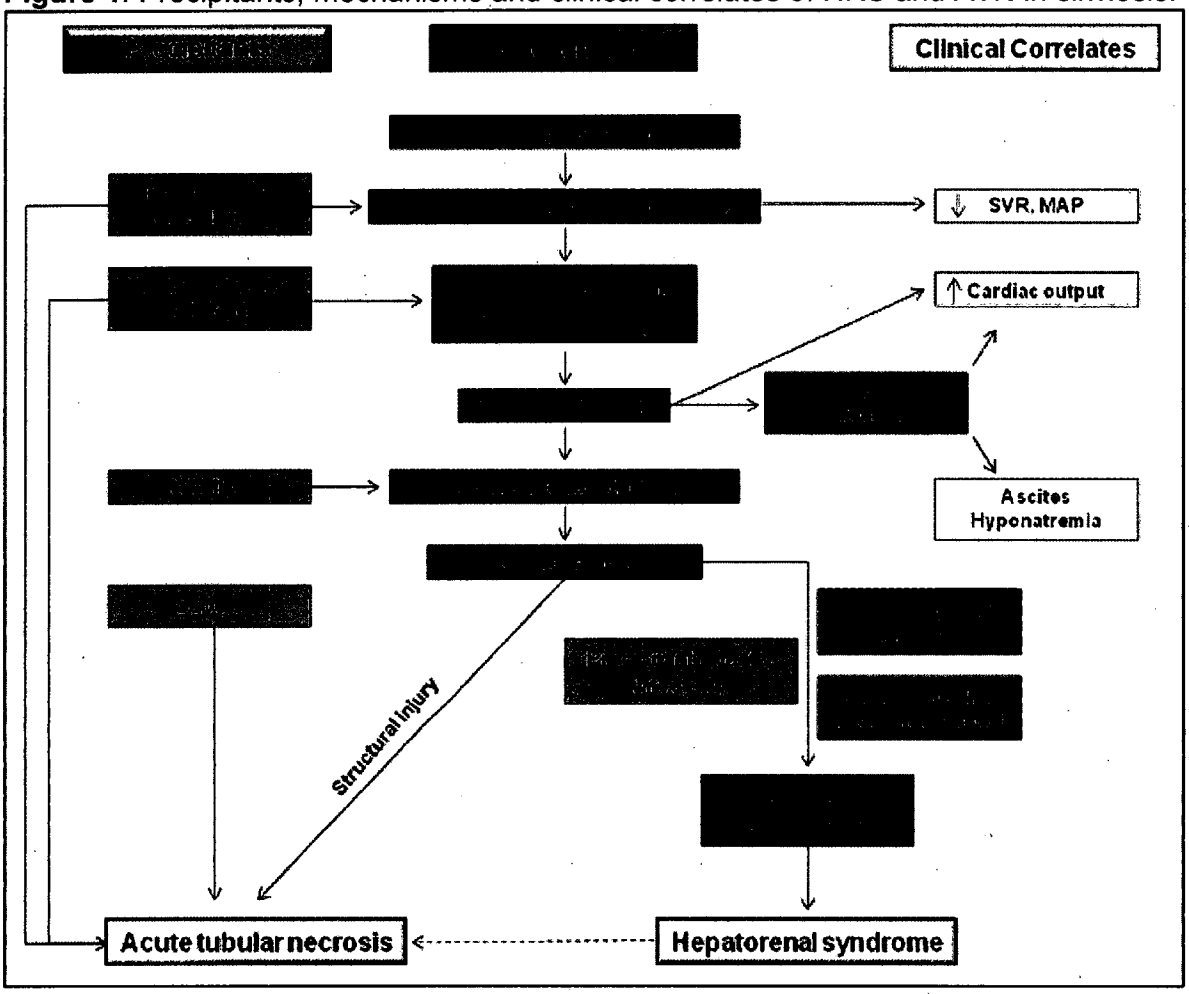


Figure 1. Portal hypertension leads to splanchnic and systemic vasodilatation, decreasing effective arterial blood volume. This decrease stimulates activation of SNS, RAAS and ADH with resulting retention of sodium and water, increasing cardiac output, ascites and hyponatremia. The increased activity of vasoconstrictor systems also causes renal vasoconstriction and chronically decreased renal perfusion. Any factor that worsens vasodilatation (infection, LVP, vasodilators) or decreases blood volume (diarrhea, overdiuresis, bleeding) can decrease renal perfusion further and lead to AKI. In advanced cirrhosis, splanchnic vasodilatation and renal vasoconstriction can become refractory to volume expansion and, compounded by decreased cardiac function (akin to high output heart failure), lead to severe renal hypoperfusion and development of hepatorenal syndrome. Alternatively, precipitants may be severe enough to produce structural tubular injury (e.g. septic or hypovolemic shock) and acute kidney injury. The extent to which prolonged, severe hepatorenal syndrome can progress to acute tubular necrosis remains unclear and is thus depicted with a dashed line.

Abbreviations: LVP, large volume paracentesis; SVR, systemic vascular resistance; MAP, mean arterial pressure; GI, gastrointestinal; SNS, sympathetic nervous system; RAAS, renin-angiotensin-aldosterone system; ADH, anti-diuretic hormone; NSAIDS, non-steroidal anti-inflammatory drugs; GFR, glomerular filtration rate

Figure 2. Extra-renal influences on serum creatinine levels.

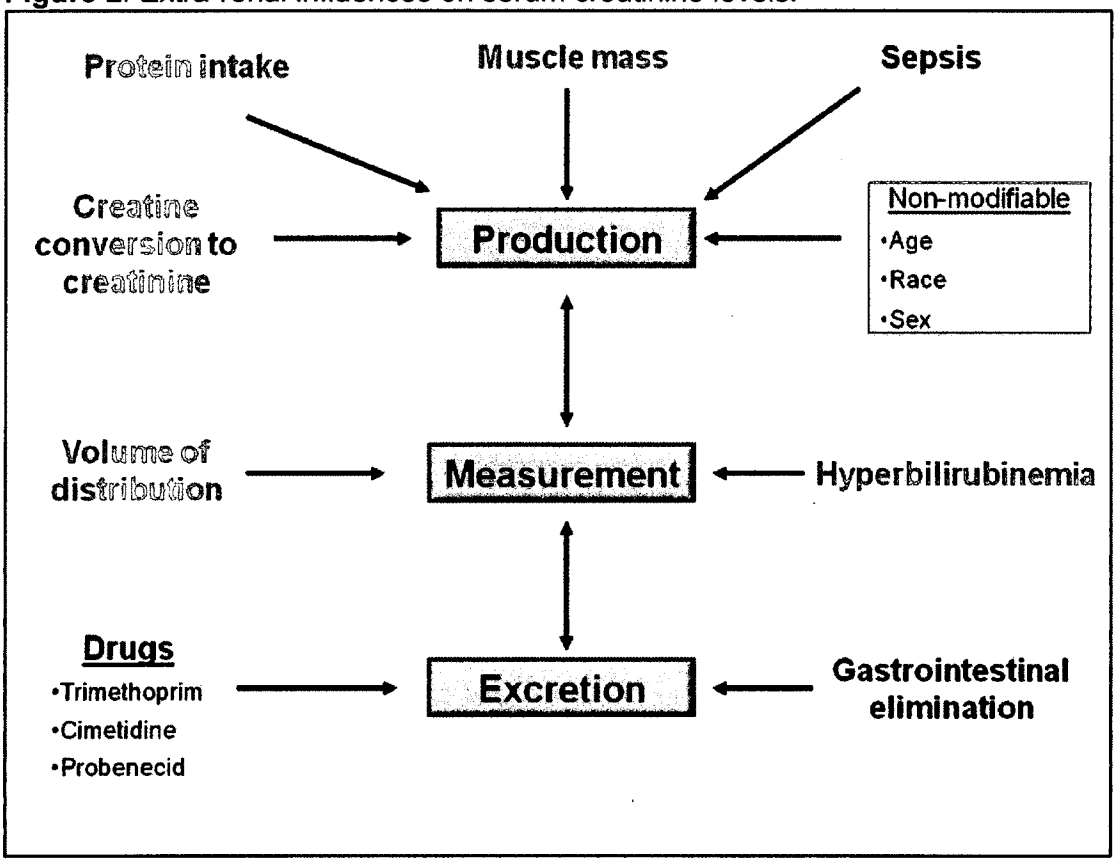


Figure 2. Primary extra-renal influences on serum creatinine are depicted. In addition to the status of renal filtration, creatinine levels are affected by factors that influencing its production and excretion as well as those that impact its measurement. Factors especially relevant to patients with cirrhosis are depicted in red.

Figure 3. Non-renal influences on cystatin C levels

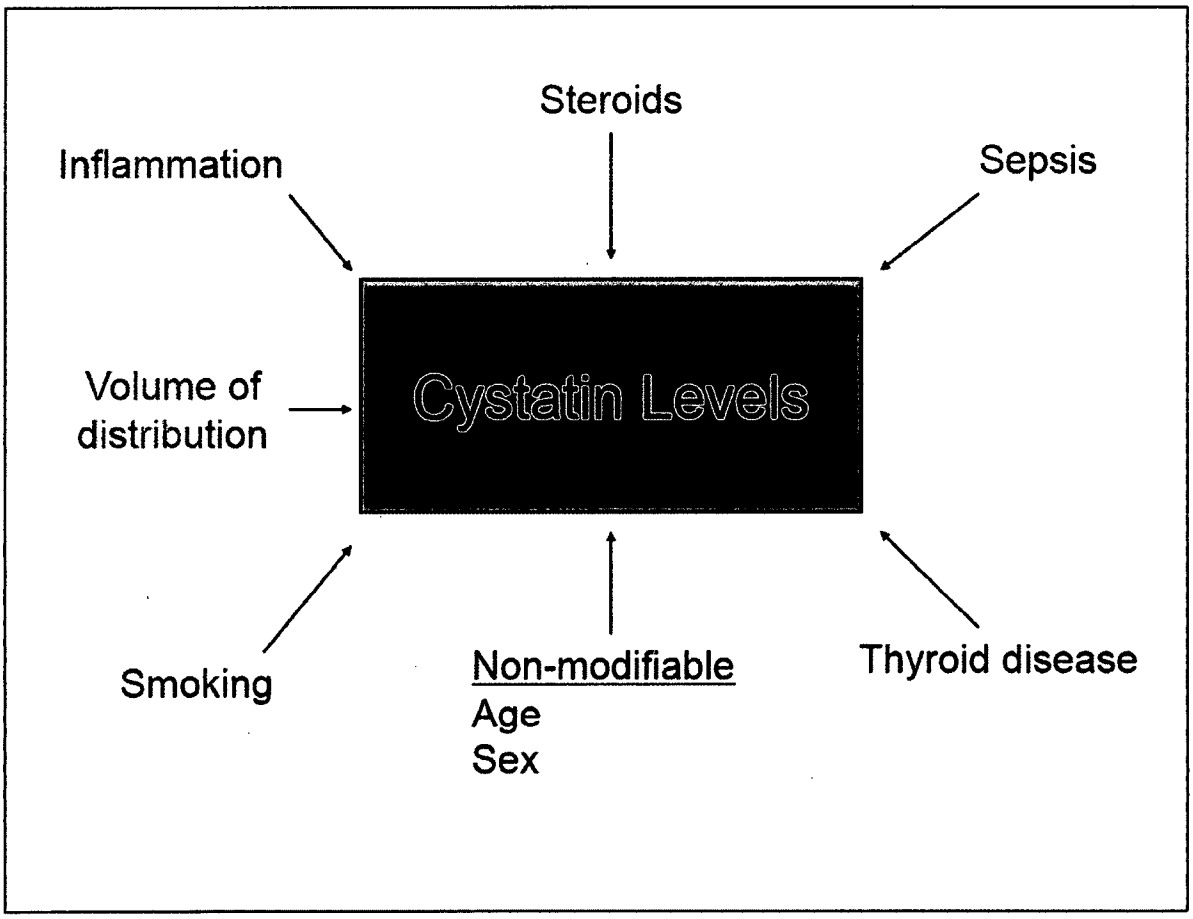


Figure 3. Non-renal influences on serum cystatin C levels. Those factors that are particularly problematic in patients with cirrhosis are highlighted in red. Unlike creatinine, non-renal determinants only impact cystatin C levels via alteration in production and volume of distribution.

Table 1. Classification/Staging System for Acute Kidney Injury According to KDIGO

AKI Stage	Serum Creatinine Criteria	Urine Output Criteria
AKI Stage 1	Increase in serum creatinine ≥ 0.3 mg/dL within 48 hours or increase to ≥ 150-200% from baseline within 7 days	Urine output < 0.5 ml/kg/hr for > 6 hr
AKI Stage 2	Increase of serum creatinine to > 200-300% from baseline	Urine output < 0.5 ml/kg/hr for ≥ 12hr
AKI Stage 3	Increase of serum creatinine to > 300% from baseline or serum creatinine ≥ 4.0 mg/dL or treatment with renal replacement therapy	

Abbreviations: KDIGO, Kidney Disease: Improving Global Outcomes; AKI, acute kidney injury

Figure 4. Prevalence and types of acute kidney injury in hospitalized patients with cirrhosis.

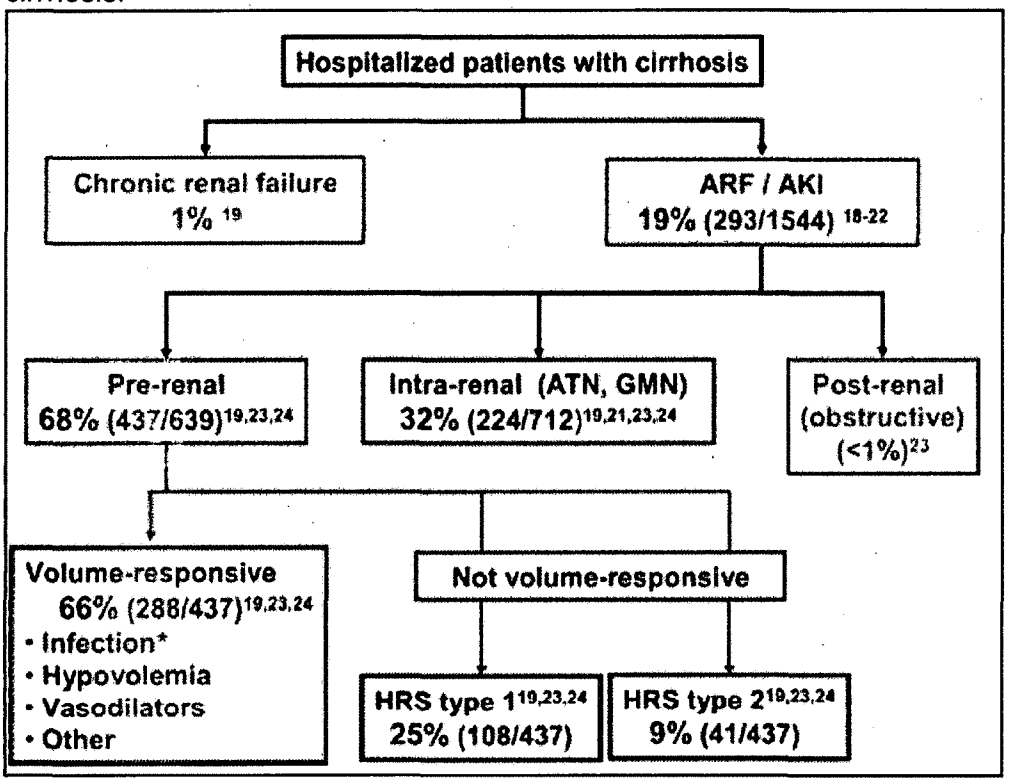
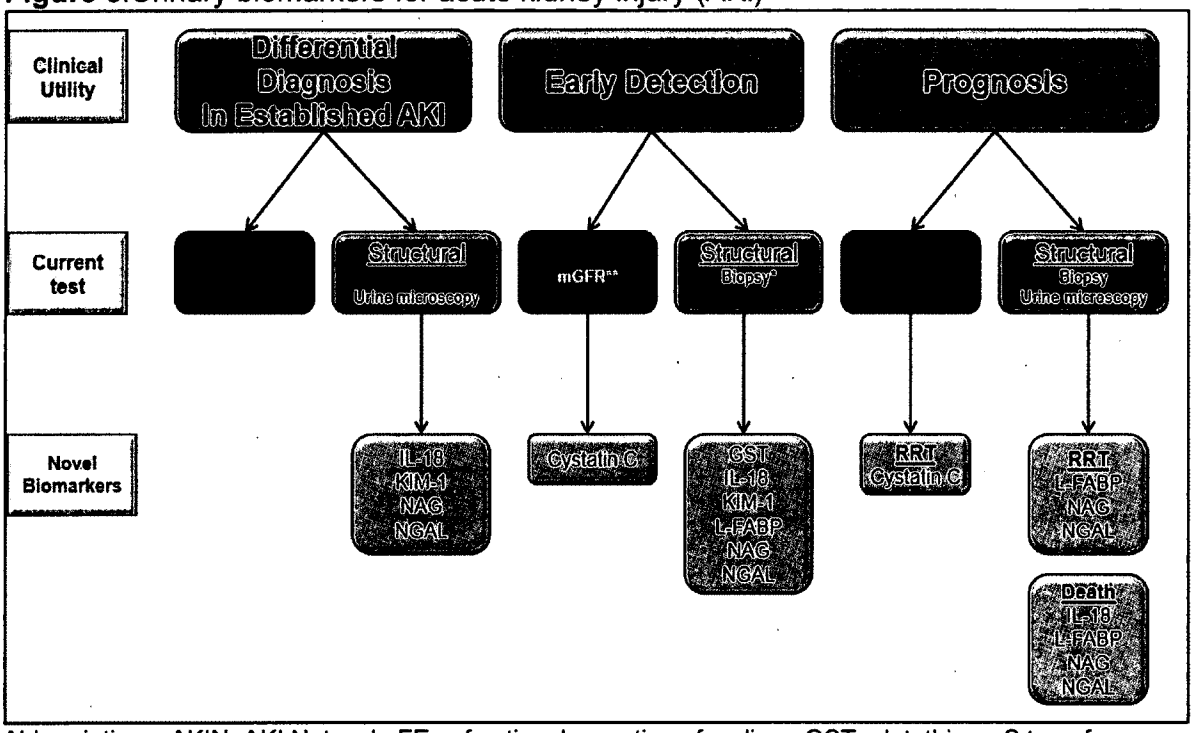


Figure 3. Percentages and numbers were obtained by adding up patients in the references cited. References 18-24 in the figure refer to references 46-52 in this chapter.
Abbreviations: ATN, acute tubular necrosis; GMN, glomerulonephritis; HRS, hepatorenal syndrome

Figure 5.Urinary biomarkers for acute kidney injury (AKI)



Abbreviations: AKIN, AKI Network; FE_{Na}, fractional excretion of sodium; GST, glutathione *S*-transferase; IL, interleukin; KIM-1, kidney injury molecule 1; L-FABP, liver-type fatty acid–binding protein; mGFR, measured glomerular filtration rate; NAG, *N*-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; RIFLE, risk, injury, failure, loss, end-stage disease; SCr, serum creatinine; RRT, renal replacement therapy.

*Established gold standards.

**Early detection possible only through biopsy in setting of protocol transplant biopsies.

Chapter 2: Association of AKI with Mortality and Complications in Hospitalized Patients with Cirrhosis

Introduction

Acute kidney injury (AKI) is one of the most severe complications of cirrhosis and portends an ominous prognosis[1]. The development of AKI is often linked with the onset of other complications of cirrhosis such as variceal bleeding and spontaneous bacterial peritonitis (SBP) and occurs in up to 19% of hospitalized patients with cirrhosis[2]. While the hepatorenal syndrome (HRS) has long been associated with prodigious mortality[3], more recent recognition of the general hazard associated with AKI in cirrhosis has led to the incorporation of serum creatinine as one of the three variables comprising the model of end-stage liver disease (MELD) score. This model has met with marked success in predicting short term mortality and is used to determine allocation priority for orthotropic liver transplantation[4].

Though recognition of the primacy of AKI in determining outcomes in hospitalized patients with cirrhosis has been a welcome advance, studies attempting to quantify and expand upon AKI's impact have been limited by several flaws. Most significantly, studies of AKI in cirrhosis have suffered from a lack of standardization in AKI definitions. Moreover, they have often utilized elevated creatinine thresholds which are outdated and lack sensitivity[5-8]. Creatinine, a suboptimal marker for renal function under any circumstance, is especially insensitive to a decline in glomerular filtration rate (GFR) in the setting of cirrhosis[9]. The reliance on elevated thresholds leads to an over selection of the most severe cases, limiting the ability to evaluate factors associated with disease progression and the bearing of AKI severity on outcomes. Many studies examining the impact of AKI in cirrhosis have treated the presence of AKI as a dichotomous variable and assessed outcomes of these patients relative to those with stable kidney function. While much attention has been devoted in these investigations to

eliciting risk factors for the development of AKI, few studies have explored variables associating with disease progression and outcomes only in the subset of patients with AKI[10]. Those few studies that have employed more current definitions such as the acute kidney injury network (AKIN) or the risk, injury, failure, loss and end-stage kidney disease (RIFLE) criteria have primarily focused on patients admitted to an intensive care unit and are thus not generalizable to all hospitalized patients[11,12]. In addition, rigorous attempts to establish an accurate baseline creatinine based on outpatient values were not performed. Finally, due to enrollment difficulties inherent to this extremely ill population, studies of AKI in cirrhosis have often been retrospective or restricted to small numbers of patients.

The aim of this phase of my study was to prospectively assess a large cohort of hospitalized cirrhotic patients with AKI to understand the natural history, trajectory and recovery patterns of the disease. Specifically, we sought to evaluate the impact of AKI severity and disease progression on in-hospital death. The AKIN criteria for the diagnosis of AKI were utilized to detect a decline in renal function at the earliest possible instance[11].

Patients and Methods

Study design

This prospective, multi-center observational cohort study was carried out at four tertiary care academic centers in the US. Potential participants were identified by a daily screening of patients on medical intensive care units, transplant floors and on each hospital's hepatology service. Laboratory tests of all patients with cirrhosis were reviewed daily for the presence of AKI (see "Definitions"). Patients were eligible for the study if they presented for admission with AKI or developed it during the course of the hospitalization. Inclusion criteria included a known diagnosis of cirrhosis (see "Definitions"), age ≥ 18 years, presence of AKI and the availability of a documented serum creatinine within 1 year prior to AKI. Exclusion criteria included prior kidney or liver transplant, advanced chronic kidney disease (baseline creatinine > 4.0 mg/dL),

acute or chronic renal replacement therapy at the time of enrollment, estimated life expectancy of less than 3 days, confirmed pregnancy, other known causes of renal insufficiency such as glomerulonephritis or hydronephrosis and previous participation in the study. If a patient was unable to provide consent, a surrogate decision maker was sought. All patients were enrolled within 5 days of meeting AKI criteria. The study was approved by the institutional review board or human investigations committee at each institution.

Variables

Independent Variables

Cirrhosis- Patients were eligible who had an existing diagnosis of cirrhosis obtained from medical records and which was based on liver biopsy, when available, or on a combination of clinical, biochemical, ultrasonographic and endoscopic findings.

AKI- The AKIN criteria **(Table 1)** were applied for the diagnosis of AKI. As urine collection and output documentation can be inconsistent, only the definition of an increase in serum creatinine of 0.3 mg/dL or a 50% rise from baseline was utilized. Renal failure in the setting of cirrhosis has previously been defined as a serum creatinine greater than 1.5 mg/dL[6-8]. However, in light of recent evidence that much smaller decrements in renal function are associated with adverse outcomes[14], a working group composed of members of the International Ascites Club (IAC) and the Acute Dialysis Quality Initiative (ADQI) have proposed employing the AKIN definition in the setting of cirrhosis[15]. Patients were considered to have worsening of AKI if they progressed to a higher AKIN stage or, if they presented in stage 3, if they subsequently required renal replacement therapy. Death was not considered to represent progression of AKI.

Baseline creatinine- Baseline creatinine was defined as the most recent stable measurement prior to admission for the index hospitalization. When possible, outpatient measurements were selected though values were also used from previous admissions not complicated by AKI. In rare cases, patients without an outpatient measurement were included in the analytic cohort if,

prior to a rise in creatinine fulfilling the above definition of AKI, they manifested at least 5 days of stable values within the normal creatinine range following admission. In these instances, the creatinine at admission was considered the baseline.

Other variables- Chronic kidney disease (CKD) was defined as a GFR < 90 ml/min as calculated with the CKD-EPI equation using the baseline creatinine value[16]. While the term "CKD" classically implies structural damage, many patients with cirrhosis have a chronically depressed GFR due instead to persistent hypoperfusion and their renal function may thus be partially reconstituted with restitution of perfusion. However, the granular data required to distinguish true kidney injury, such as chronically active urine sediment or proteinuria, from pre-existing decreases in GFR due to hypoperfusion was not consistently available for patients coinciding with the outpatient creatinine used to establish their baseline function. We therefore use the term CKD with the understanding that chronically depressed GFR need not imply irreversible structural injury in patients with cirrhosis. When controlling for baseline CKD in our model predicting in-hospital mortality, GFR > 90 ml/min was utilized as the reference range; CKD stages are defined in **Table 3**. Baseline proteinuria was defined as an outpatient value of 1+ or greater on dipstick or 30mg/dL when quantitated by clinical laboratory. HRS therapy refers to the use of midodrine and octreotide. While such therapy is often paired with albumin, albumin use was nearly ubiquitous in our cohort and thus not considered indicative of dedicated therapy for HRS. Urinary tract infections (UTI) and bacteremia were defined by positive cultures. The diagnosis of pneumonia required either a positive sputum culture or findings on radiography. SBP was defined by a fluid polymorphonuclear leukocyte count ≥ 250 cells/mm^3. The presence of hepatic encephalopathy (HE) was determined by clinical diagnosis reflected in the patient's medical chart. MELD and Child-Pugh scores were calculated on the day of first sample collection.

Outcomes- Our primary outcome was in-hospital mortality during the index hospitalization.

Statistics

Categorical variables were expressed as proportions and compared using Chi-square and Fisher's Exact test, as appropriate. Normally or near-normally distributed variables were reported as means with standard deviations (SD) and compared by Student's *t*-test. Non-normally distributed continuous variables were reported as medians with inter-quartile ranges (IQR) and compared by the Kruskal-Wallis test. Normality was assessed using the Kolmogorov-Smirnov test. Variables thought likely to associate with the primary outcomes were assessed by univariate analysis. Those found to have a p-value of less than 0.2 on univariate analysis were included in a multiple logistic regression model evaluating the impact of worsening of AKI on mortality. Stepwise selection of variables was performed to build the model. Model accuracy was assessed by calculating the area under the receiver operating characteristic curve (AUC) and goodness-of-fit verified with the Hosmer-Lemeshow test. A 2-sided $p<0.05$ was considered significant for all analysis. Statistical analysis was performed using SAS, version 9.2 (SAS Institute, Cary, NC).

Results

Cohort characteristics

A total of 219 patients with cirrhosis and AKI were enrolled into the study over a period of 29 months. Twenty-seven patients were subsequently excluded, with reasons being an excessive interval between the onset of AKI and time of first sample collection (n=15), lack of a documented baseline creatinine level (n=4), recent treatment with nephrotoxins (n=3), a diagnosis of acute hepatitis rather than cirrhosis (n=2) and other causes (n=3). Baseline demographic, clinical and laboratory data of the 192 patients included in the study are shown in **Table 2**. The mean patient age was 55.1 ± 9.3 and 136 (71%) were male. Fifty (26%) patients died during their hospitalization. The primary etiologies of cirrhosis were alcohol (29%), alcohol and HCV (27%) and HCV alone (17%). There was no difference in etiologies between survivors

and non-survivors. The majority of patients had previously suffered complications of cirrhosis including ascites, 76%, hepatic encephalopathy, 67%, variceal bleeding, 23% and SBP, 16%. Reasons for admission were similar between the two groups. The median Child-Pugh score was 10.5 and MELD 26.3 at the time of enrollment, reflecting the severity of cirrhosis. Not unexpectedly, both Child-Pugh (12 vs. 10, p <0.0001) and MELD scores (34.1 vs. 23.6, p <0.0001) were higher in non-survivors than in survivors. However, there was no difference in median serum sodium levels or presence of hyponatremia at the time of enrollment between the two groups.

Kidney Variables and Mortality

The impact of renal variables on survival is shown in **Table 3**. A majority of patients, 91%, had a documented out-patient creatinine while seventeen (9%) had creatinine values from their admission used as a baseline level. Overall, 119 (62%) patients had CKD with 53 (28%) having a baseline GFR < 60ml/min. CKD more prevalent and median GFR was lower (73ml/min vs. 91ml/min, p = 0.048) in survivors than non-survivors. Proteinuria was present at baseline in 12% of patients and did not differ between the two groups. Remarkably, AKI was present in 116 (60%) patients at admission, while an additional 17 (9%) developed AKI within 48 hours of hospitalization. The remaining 59 (31%) patients experienced AKI later in the course of their hospital stay at a median of 7 days post admission, IQR 4-10 days. Mortality was significantly higher in those patients who developed AKI subsequent to admission than in those who presented with AKI, 36 vs. 21%, respectively (p = 0.01). At the time of first fulfilling AKIN criteria, 48% of patients had stage 1 AKI, 29% stage 2 and 23% stage 3. A decrease in serum creatinine occurred in 70 (37%) patients within 48 hours of first meeting AKIN criteria. Such early evidence of improvement was significantly more common in survivors than in non-survivors, 59 (42%) vs.11 (22%), respectively (p = 0.01). Conversely, the severity of AKI worsened following the initial fulfillment of AKIN criteria in 85 (44%) of patients. Progression of AKI was significantly more common among those patients who developed AKI in the hospital, 59%, than in those who

presented already experiencing AKI, 35% ($p = 0.001$). Critically, worsening of AKI was markedly more common among non-survivors, 80%, than among survivors, 32% ($p < 0.0001$). A strong step-wise association was noted between degree of progression and mortality **(Figure 1)**.

AKI in the setting of cirrhosis was ultimately severe with peak AKIN stages 1, 2 and 3 attained in 26%, 24% and 49% of patients, respectively. Mortality increased in a stage-response manner with severity of AKI. The likelihood and degree of progression, along with subsequent mortality, is presented by initial AKIN stage in **Figure 2**. For patients with peak stages of 2 and 3, those who progressed to that degree had higher mortality than those presented with that level of dysfunction but did not progress **(Figure 3)**. Remarkably, patients with a peak severity of AKIN stage 1 did extremely well, with only 1 (2%) death. Non-survivors ultimately experienced significantly more severe AKI, with 84% reaching a peak of stage 3 vs. 38% of survivors, ($p < 0.0001$). Dialysis was required for 46 (24%) patients and was utilized more frequently among non-survivors, 58%, than among survivors, 12%, ($p < 0.0001$). Of those patients requiring dialysis, 57% died during the index hospitalization while still requiring renal replacement therapy, 17% were discharged on dialysis and 26% recovered renal function by the time of discharge.

Multivariate logistic regression was employed to evaluate the independent association between worsening of AKI and death. On univariate analysis, progression of AKI was associated with death with an odds ratio (OR) of 8.62 (95% CI 3.96-18.77, $p < 0.0001$). After adjustment for baseline CKD, demographics, hospital events and variables related to severity of cirrhosis, the adjusted OR was attenuated but remained strongly significant, OR 3.8 (95% CI 1.31-11.08) (**Table 4**). On ROC curve analysis, worsening of AKI alone was able to predict death with an AUC of 0.74.

Other Complications

The associations between severity of AKI and general medical and cirrhosis specific hospital complications are listed in **Table 5**. The rate of both general medical and cirrhosis

specific complications was higher with worsening severity of AKI. HRS specific therapy was often employed, with 45% of patients receiving midodrine and 46% octreotide while use of albumin, 82%, was nearly ubiquitous. The use of midodrine, octreotide and albumin increased significantly with severity of AKI. Patients with higher stage AKI were more likely to be admitted to the intensive care unit (ICU) and less likely to be transferred out alive. The use of mechanical ventilation and vasopressors was higher with worsening peak AKIN stage. Of those patients who survived to discharge, the median length of hospital stay increased by AKIN stage from 9 to 10 to 14 days, respectively ($p = 0.01$).

Discussion

The development of AKI in the setting of cirrhosis has long been recognized to confer a grim prognosis and is known to be independently predictive of death in patients with SBP and variceal hemorrhage[8,17]. Unfortunately, estimates of the incidence of AKI in cirrhosis and attempts to quantify the impact of AKI on mortality have suffered from a lack of standardization in the definition of AKI. Utilizing markedly elevated creatinine thresholds ranging from 1.5[6-8] to 3.5[18] mg/dL, AKI in the setting of cirrhosis has been associated with a striking mortality of 55-91%. However, the use of such stringent cutoffs ensures selection bias wherein only the most severe cases of AKI would qualify. The lack of sensitivity inherent in these AKI definitions is particularly problematic in patients with cirrhosis where significant muscle atrophy and reduced hepatic conversion of creatine to creatinine results in potentially significant renal dysfunction being masked by an ostensibly normal creatinine value. This danger is compounded by cirrhotic patients' unique vulnerability to AKI. In addition to inducing what is functionally a state of constant diminished renal bloodflow[19], progression of cirrhosis is associated with a loss of ability to maintain renal perfusion via tubuloglomerular feedback[20,21]. In this setting, the frequent volume shifts that accompany titration of lactulose and altered oral intake due to encephalopathy will precipitate numerous episodes of AKI not captured by such rigid definitions.

Several recent studies have attempted to rectify this shortcoming by investigating the impact of AKI on mortality in the setting of cirrhosis utilizing the modern RIFLE criteria, whose stages of "R", "I" and "F" are analogous to AKIN stages 1, 2 and 3[22]. Jenq et al. studied 134 patients with cirrhosis admitted to the ICU and found a mortality of 32.1% in those without AKI, 68.8% for RIFLE-R, 71.4% for RIFLE-I and 94.8% for RIFLE-F[11]. AKI was diagnosed based on creatinine at the time of ICU admission and the association of mortality with peak RIFLE stage or AKI progression was not assessed. Cholongitas et al. followed a large cohort of 412 cirrhotic patients also admitted to the ICU, evaluating the impact of AKI on mortality during ICU stay or within 6 weeks of unit discharge[12]. The authors noted a similar stage-dependent association between AKI and mortality with rates increasing from 42.5% in those without AKI to 71% for RIFLE-R and 88% for RIFLE-I/F. The significant increase in mortality in those patients with only mild AKI (RIFLE-R) speaks to the value of these sensitive criteria for ICU prognosis. However, both studies only included patients in the ICU, where AKI is often associated with multi-system organ failure and severe sepsis. Ribeiro de Carvalho et al. studied 91 patients with cirrhosis and AKI by AKIN criteria at hospital admission including 83 with stage 1, 5 stage 2 and 3 with stage 3[23]. Patients were staged by comparing creatinine values drawn within 48 hours of admission. Any patient seen to have a change of 0.3 mg/dl or greater, in either direction, was classified as having AKI. The magnitude of this change determined the AKIN stage as no baseline values were considered and no assessment was made of progression or peak stage. Presence of AKI conferred an overall OR of 2.6 for hospital mortality but quantifying the risk by stage was limited by the small number of patients with more advanced disease.

In our study, we have investigated the impact of AKI, using the AKIN definition, on the mortality of hospitalized patients with cirrhosis, independent of admission ward. The overall mortality was 26%, significantly lower than in those studies utilizing less sensitive definitions or confined to the ICU. However, a pronounced stage-dependent response was again seen, with mortality for peak AKIN stages 1, 2 and 3 of 2%, 15% and 44%, respectively. Similarly,

advancing stages of AKI were associated with a higher incidence of medical complications, including bacteremia, pneumonia and UTI, and with cirrhosis-specific complications such as ascites, encephalopathy and SBP. Paradoxically, the presence of a lower baseline GFR conferred a survival advantage. Though the CKD-EPI equation has been shown to correlate best with measured GFR[24], it can significantly overestimate renal function in patients with cirrhosis[25]. It may be that more advanced cirrhotics with lower muscle mass and decreased hepatic creatinine production were falsely estimated to have higher GFR's. Additionally, CKD is a strong risk factor for AKI and may be underappreciated in cirrhosis[26,7]. It is possible that the development of AKI without pre-existing renal dysfunction requires a stronger renal insult and greater systemic illness, thus placing this group at a higher risk for death from non-renal causes.

While AKI is typically thought of as an inpatient syndrome, the majority (60%) of our patients presented to the hospital already in AKI. This speaks to the tenuous chronic perfusion status of cirrhotic patients and their vulnerability to mild or moderate outpatient insults. However, nearly half of the patients (48%) were still in stage 1 AKI at the time of first meeting AKIN criteria. A critical result then of our study is the identification of progression of AKI as a powerful independent risk factor for mortality. Indeed, as seen in **Figures 2** and **3**, assessment of AKI progression has the potential to add granularity to the association between AKI severity and mortality. The accuracy of AKI progression for predicting death, evidenced by an AUC of 0.74, is remarkable given the high rate of mortality in the cohort. The striking, nearly four-fold, increase in mortality in those patients whose AKI progressed, and contrasting decrease in mortality in those who showed early improvement, is vital due of the presence, rare in AKI, of disease specific therapies in the setting of cirrhosis. The etiology of AKI in cirrhosis has been estimated to be 68% hypoperfusion and 32% intra-renal, primarily ATN[2]. Rapid and aggressive intervention early in the course of AKI in fluid responsive patients to optimize volume status and restore renal perfusion may prevent progression of AKI and the subsequent development of ATN. Those patients who do not respond to volume and do not have evidence of frank kidney

injury have classically been diagnosed with HRS, long the most dreaded of cirrhosis complications. However, HRS is undergoing a revolution as improved understanding of its physiology has facilitated targeted treatments[28]. Terlipressin, a nonselective V1 vasopressin agonist, has been successfully employed along with albumin to mitigate splanchnic and systemic vasodilatation and restore effective circulating volume and renal perfusion in patients with HRS[29-31]. Critically, application of terlipressin has been shown to improve renal hemodynamics and GFR even in those patients who do not yet meet HRS diagnostic criteria[32]. However, the use of such early interventions has until recently been hampered by a consensus that the diagnosis of AKI in cirrhosis requires a serum creatinine of at least 1.5 mg/dL[5,33]. The risk inherent in such stringent criteria is evidenced by recent data demonstrating that response to terlipressin declines with increasing creatinine at treatment initiation[34].

Seeking to modernize this perception, a recent working group comprised of members of the Acute Dialysis Quality Initiative and the International Ascites Club proposed adopting the AKIN criteria within the spectrum of what they term "hepatorenal dysfunction" for all acute deteriorations in renal function in cirrhotic patients, irrespective of a structural or functional nature[15]. However, for HRS Type 1, distinguished as a specific form of AKI, the new proposal retains a diagnostic creatinine threshold of 2.5 mg/dL. Given the well-known limitations of serum creatinine as an accurate marker of renal function in patients with cirrhosis, due in part to low muscle mass and decreased production[35-38], patients must suffer a marked decrease in GFR before their creatinine rises to this level. With the availability of effective interventions and our demonstration of poor outcomes associated with worsening, we believe that this threshold should be lowered. When there is not clear evidence of ATN or other intrinsic disease, vasoconstrictor therapy should be initiated in all patients when they progress to a higher stage of AKI. The potential impact of this approach is apparent in our study where 56 patients had a creatinine < 2.5 mg/dL upon first meeting AKIN criteria but ultimately rose to >2.5. Of these, 31 (55%) progressed to a higher AKIN stage prior to reaching 2.5 and thus would have been

treated sooner under this strategy. The effectiveness of such an approach could be studied in a trial enrolling patients with AKI and fulfilling the IAC criteria of ascites, lack of response to 48 hours of volume resuscitation and absence of shock, nephrotoxic exposure or evidence of structural injury but without a creatinine cutoff. Patients would be randomized to receive vasoconstrictors either upon first stage progression or when creatinine reaches 2.5.

More fundamentally, there is no reason to think that, even under the threshold of 2.5, higher creatinine at the time of initiation of vasoconstrictor therapy will not associate with decreased response rates. HRS must be conceptualized as the terminal end of a physiologic spectrum; guidelines for treatment should hinge upon phenotyping patients on this spectrum and need not invoke a given degree of renal dysfunction. Ideally, patients at high risk of progression would be treated immediately upon meeting criteria for AKI. We have shown development of AKI as an inpatient to be a risk factor for progression but prognosis could be enhanced further through novel biomarkers capable of distinguishing structural from functional AKI etiologies and, if functional, quantifying the intensity of renal vasoconstriction. Ultimately, trials of vasoconstrictors could be optimized by using such biomarkers as entrance criteria, thereby selecting only those patients with predominately functional disease. Our study has several important strengths. We prospectively enrolled one of the largest cohorts of patients with cirrhosis and AKI in the literature. Baseline creatinine levels were rigorously assessed with >90% of patients' determined by stable values drawn within a year prior to admission. The critical importance of this approach towards ascertaining baseline is underscored by >60% of our patients presenting with AKI, where the use of admission creatinine as the baseline value would have obscured the severity or even the presence of AKI. In using the sensitive AKIN definition, we have included patients with a broad spectrum of AKI severity. Patients were enrolled throughout the hospital, including those who presented with AKI and those who subsequently developed it, rendering our findings regarding the impact of AKI severity and progression on mortality broadly generalizable. However our study is not without limitations. As

an observational study, we were unable to assess the impact of volume expansion and HRS specific therapy on progression of AKI. Evidenced by the large number of patients presenting to the hospital in AKI, the onset of AKI in cirrhotics is frequently in the outpatient setting but we were unable to evaluate patterns of progression and recovery prior to admission.

Conclusions

The results of this phase of the study confirmed that AKI, as defined by AKIN criteria, is associated with in-hospital mortality in the setting of cirrhosis in a stage-dependent manner. While those patients who exhibit early recovery from AKI do well, worsening of AKI is independently associated with mortality. Further studies are required to investigate the implementation of more sensitive criteria for AKI and the development of earlier and more discriminating diagnostic tests. It is possible that early recognition of AKI and prompt, aggressive treatment to mitigate disease progression may improve outcomes in this complex clinical setting.

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Table 1. Classification/Staging system for acute kidney injury according to AKIN[13]

AKI Stage	Serum Creatinine Criteria	Urine Output Criteria
AKI Stage 1	Increase in serum creatinine ≥ 0.3 mg/dL or increase to ≥ 150-200% from baseline	Urine output < 0.5 ml/kg/hr for > 6 hr
AKI Stage 2	Increase of serum creatinine to > 200-300% from baseline	Urine output < 0.5 ml/kg/hr for > 12hr
AKI Stage 3	Increase of serum creatinine to > 300% from baseline or serum creatinine ≥ 4.0 mg/dL after a rise of at least 0.5 mg/dL or treatment with renal replacement therapy	Urine output < 0.3 mL/kg/hr for 24hr or anuria for 12 hr

Abbreviations: AKIN, acute kidney injury network; AKI, acute kidney injury

Table 2. Baseline and clinical characteristics of all patients and those for non-survivors and survivors

	Total N=192	Non-Survivors N=50	Survivors N=142	p
Age in years - mean ± SD	55.1 ± 9.3	54 ± 8.9	55.5 ± 9.5	0.35
Male sex – *n* (%)	136 (71)	35 (70)	101 (71)	0.88
BMI – median (IQR)	31.3 ± 8.9	32 (26.5-34.6)	31 (25-35.2)	0.28
Race – *n* (%)				
White	137 (71)	27 (54)	110 (77)	0.002
Black	27 (14)	10 (20)	17 (12)	0.16
Hispanic	24 (13)	11 (22)	13 (9)	0.02
Diabetes – *n* (%)	51 (27)	11 (22)	40 (28)	0.40
Active Cancer – *n* (%)	21 (11)	6 (12)	15 (11)	0.78
Cirrhosis etiology – *n* (%)				
Alcohol	56 (29)	16 (32)	40 (28)	0.61
Alcohol and HCV	52 (27)	13 (26)	39 (27)	0.84
HCV	33 (17)	9 (18)	24 (17)	0.86
NASH	17 (9)	2 (4)	15 (11)	0.25
Cryptogenic	12 (6)	2 (4)	10 (7)	0.73
Autoimmune	11 (6)	5 (10)	6 (4)	0.16
Other	11 (6)	4 (8)	7 (5)	0.48
Previous complications of cirrhosis – *n* (%)				
Ascites	146 (76)	37 (74)	109 (77)	0.63
Hepatic encephalopathy	129 (67)	35 (70)	94 (66)	0.62
Variceal bleed	45 (23)	9 (18)	36 (25)	0.29
SBP	31 (16)	10 (20)	21 (15)	0.33
Reason for admission – *n* (%)				
Hepatic encephalopathy	52 (27)	13 (26)	39 (27)	0.84
Refractory ascites/edema	23 (12)	5 (10)	18 (13)	0.62
AKI	22 (11)	3 (6)	19 (13)	0.95
GI bleed	15 (8)	4 (8)	11 (8)	0.42
Abdominal pain	14 (7)	4 (8)	10 (7)	0.76
Jaundice	10 (5)	4 (8)	6 (4)	0.29
Transplant work-up	6 (3)	1 (2)	5 (4)	1
SBP	6 (3)	3 (6)	3 (2)	0.18
Infection other than SBP	6 (3)	5 (10)	1 (1)	0.005
Other	38 (20)	10 (20)	28 (20)	1
Child-Pugh Class[a] - *n* (%)				
A	4 (2)	0	4 (3)	<0.0001[b]
B	60 (31)	4 (8)	56 (39)	
C	125 (65)	46 (92)	79 (56)	
Child-Pugh score – median (IQR)	10.5 (9-12)	12 (11-13)	10 (8-11)	<0.0001
MELD score – mean ± SD	26.3 ± 9.5	34.1 ± 8.6	23.6 ± 8.2	<0.0001
Bilirubin – median (IQR)	4.1 (1.8-10.6)	12.7 (6.3-23)	3 (1.6-5.8)	<0.0001
INR – median (IQR)	1.7 (1.3-2.2)	2.2 (1.7-2.6)	1.5 (1.3-1.9)	<0.0001
Sodium – mean ± SD	133 ± 6.4	134 ± 8	133 ± 6	0.57
Hyponatremia at enrollment[c] – *n* (%)	64 (33)	16 (32)	48 (34)	0.82
Length of hospitalization – median (IQR)	12 (6-19)	15 (8-40)	10 (6-17)	0.008

Abbreviations: N, number; SD, standard deviation; BMI, body mass index; IQR, inter-quartile range; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis; SBP, spontaneous bacterial peritonitis; AKI, acute kidney injury; GI, gastrointestinal; MELD, model for end-stage liver disease;

[a]Child-Pugh Class is at time of enrollment

[b]Jonckheere-Terpstra trend test

[c]Serum sodium <130 mEq/

Table 3. Renal variables and associations with survival

	Total N=192	Non-Survivors N=50	Survivors N=142	p
Baseline eGFR – median (IQR)[a]	76 (58-101)	91 (60-110)	73 (56-98)	0.048
CKI stages				
GFR 60-89 ml/min/m ^{2}	66 (35)	12 (24)	54 (39)	*0.04
GFR 30-59 ml/min/m ^{2}	46 (24)	11 (22)	35 (25)	
GFR 29-15 ml/min/m ^{2}	7 (4)	1 (2)	6 (4)	
Proteinuria[b] – *n* (%)	23 (12)	5 (10)	18 (13)	0.62
Creatinine on admission – median (IQR)	1.8 (1.2-2.55)	1.6 (1-2.5)	1.9 (1.3-2.6)	0.13
Creatinine at enrollment – median (IQR)	2.2 (1.6-3.4)	3 (2.1-3.7)	2 (1.4-3.1)	0.0008
Peak creatinine – median (IQR)	2.7 (1.9-4.2)	3.8 (2.7-5.2)	2.4 (1.8-3.9)	<0.0001
Timing of AKI relative to admission				
Outpatient	116 (60)	23 (46)	93 (66)	0.01
Inpatient	76 (40)	27 (54)	49 (34)	
Any creatinine decrease within 48 hrs				
Yes	70 (37)	11 (22)	59 (42)	0.01
No	120 (63)	39 (78)	81 (58)	
AKIN stage at first meeting criteria				
1	91 (48)	20 (40)	71 (51)	0.06*
2	56 (29)	13 (26)	43 (31)	
3	43 (23)	17 (34)	26 (19)	
AKIN stage progressed				
Yes	85 (44)	40 (80)	45 (32)	<0.0001
No	107 (56)	10 (20)	97 (68)	
Peak AKIN stage				
1	50 (26)	1 (2)	49 (35)	<0.0001*
2	47 (24)	7 (14)	40 (28)	
3	95 (49)	42 (84)	53 (37)	
Dialysis – n (%)	46 (24)	29 (58)	17 (12)	<0.0001
CVVH	21 (46)	18 (36)	3 (2)	<0.0001
HD	12 (26)	5 (10)	7 (5)	0.2
Both	13 (28)	6 (12)	7 (5)	0.09

Abbreviations: N, number; eGFR, estimated glomerular filtration rate; IQR, inter-quartile range; CKI, chronic kidney impairment; CVVH, continuous venovenous hemofiltration; HD, hemodialysis; AKIN, acute kidney injury network; AKI, acute kidney injury; CKI, chronic kidney impairment

[a] eGFR at baseline by CKD-EPI equation: $GFR = 141 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] or 1.159 [if black], where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

[b] Microalbuminuria (30mg/dL) or greater on dipstick or quantitative measurement prior to admission

*Jonckheere-Terpstra trend test

Figure 1. Degree of AKI progression and mortality

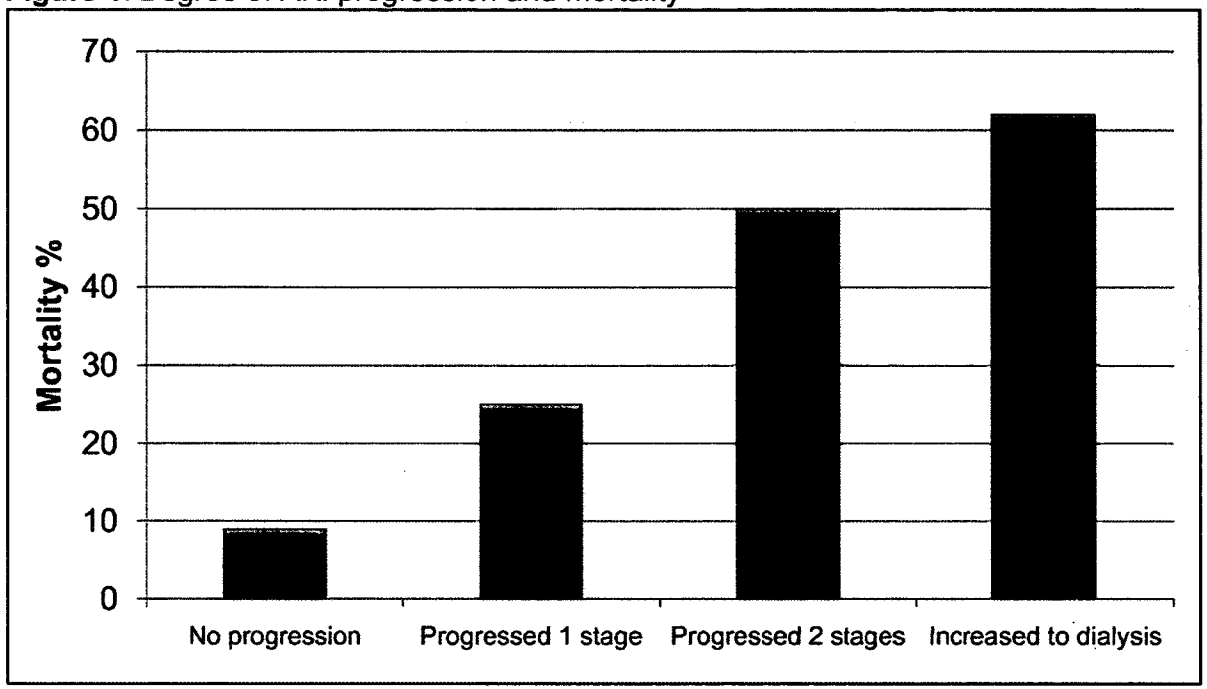

Figure 1. Progression is defined by an increase in AKIN stage after initially fulfilling AKIN criteria. Progression to dialysis refers to any patient who presented as non-dialysis dependent but subsequently developed the requirement for dialysis.

Figure 2. Incidence and extend of AKI progression and subsequent mortality by initial AKIN stage

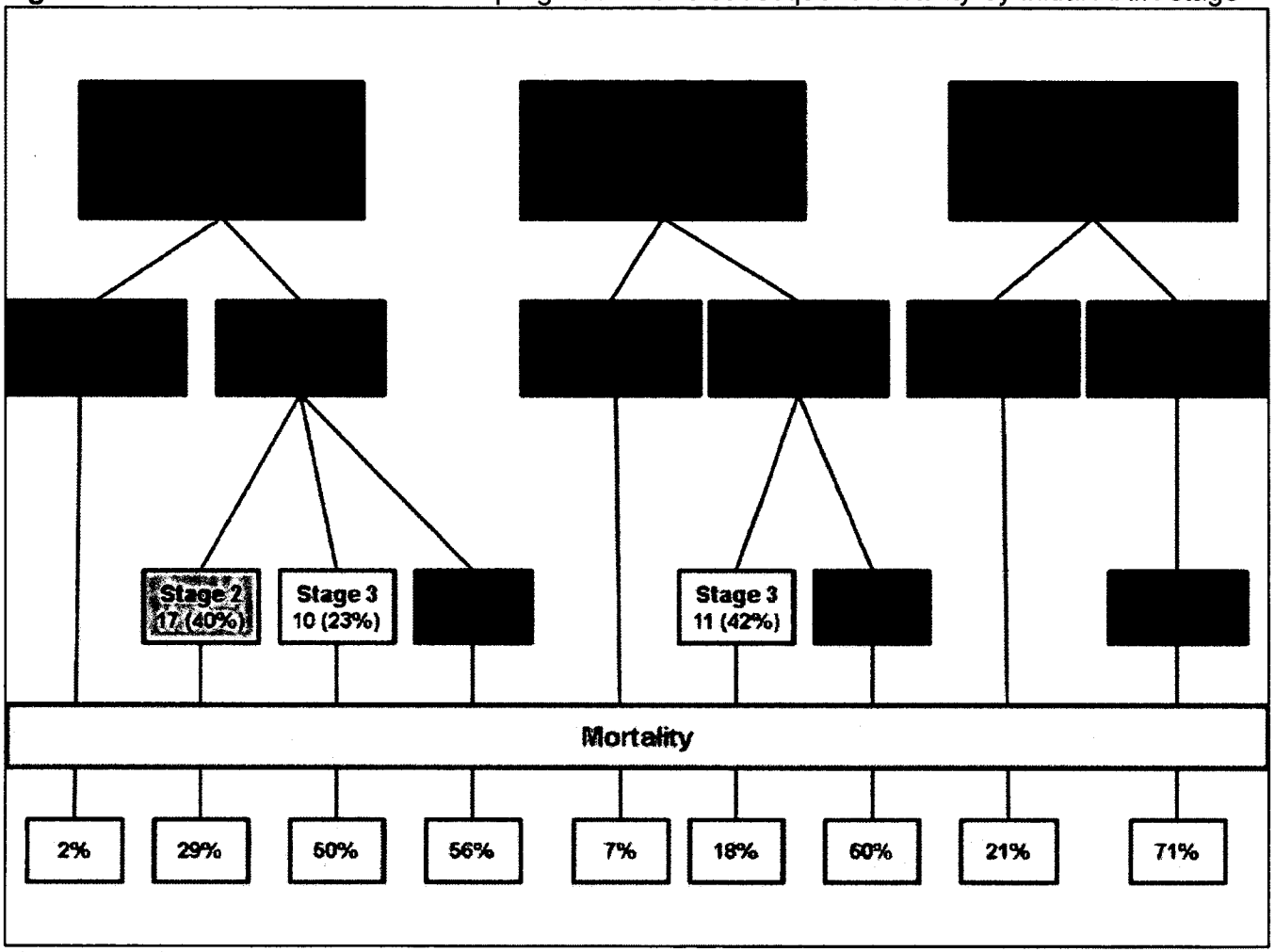

Figure 2. Patients are categorized by their stage upon first meeting AKIN criteria. Progression refers to worsening to a higher AKIN stage, with patients who are initially in stage 3 by creatinine criteria but not requiring dialysis counted as progressing if dialysis was subsequently initiated.

* 3 patients were initiated on dialysis on the day of enrollment

Figure 3. Impact of AKI progression versus peak severity on mortality

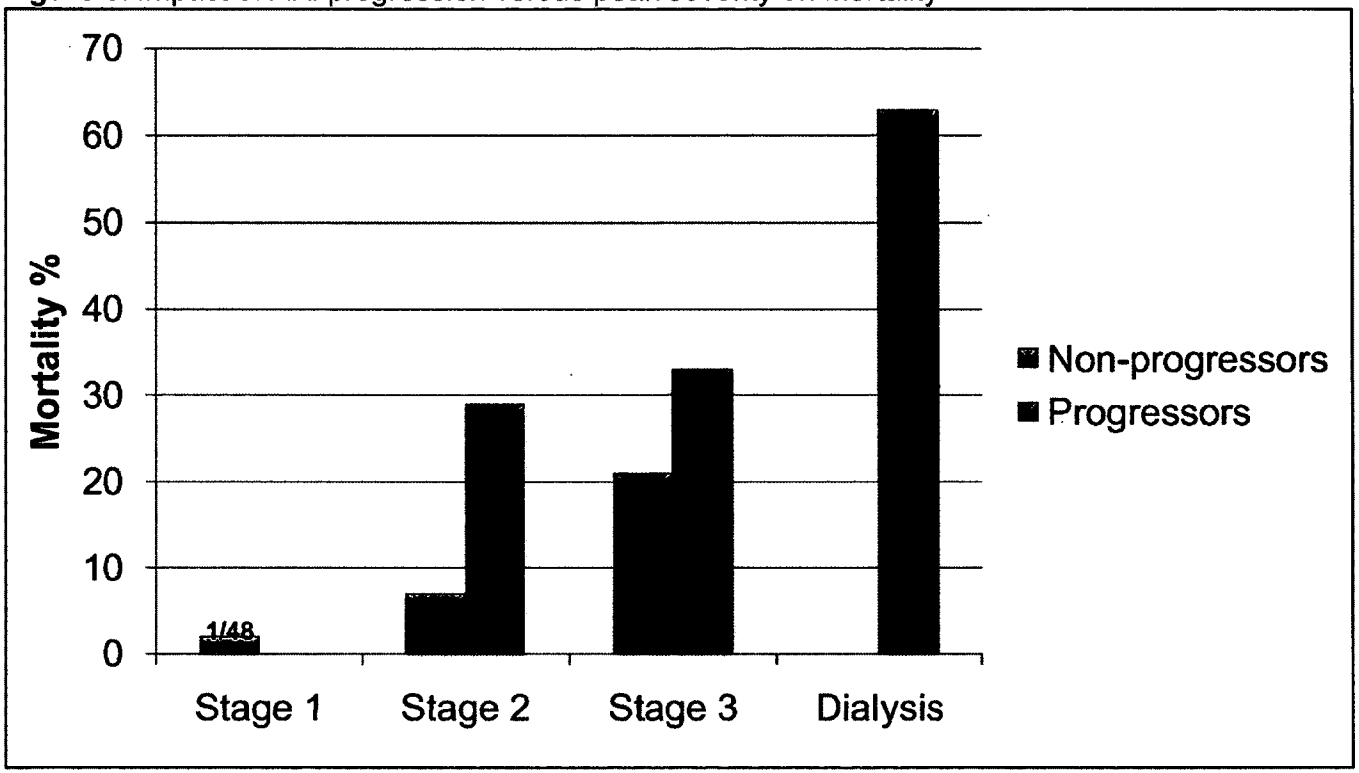

Figure 3. Patients are categorized by the peak AKIN stage they attained. "Progressors" refers to patients who worsened from their initial stage to achieve this peak while "Non-progressors" were already in their peak stage at the time of first meeting AKIN criteria.

Table 4. Association of AKIN stage progression and in-hospital mortality adjusted for multiple variables

Model	Adjusted Odds ratio	95% CI	p
AKIN stage progression	8.62	3.96-18.77	<0.0001
AKIN stage progression + CKD stage[a]	10.04	4.43-22.74	<0.0001
AKIN stage progression + CKD stage + demographics[b]	9.56	4.23-21.61	<0.0001
AKIN stage progression + CKD stage + demographics + hospital events[c]	7.13	2.42-21.06	0.0004
AKIN stage progression + CKD stage + demographics + hospital events + cirrhosis variables[d]	3.80	1.31-11.08	0.01

[a] GFR > 90 ml/min taken as no CKD and serves as reference
[b] Race, age and sex
[c] Pressor use, UTI, pneumonia, GI bleed, bacteremia
[d] Albumin therapy, HRS therapy, HE, SBP, MELD
Abbreviations: AKIN, acute kidney injury network; CI, confidence interval; CKD, chronic kidney disease; GFR, glomerular filtration rate; UTI, urinary tract infection; GI, gastrointestinal; HRS, hepatorenal syndrome; HE; hepatic encephalopathy; SBP, spontaneous bacterial peritonitis; MELD model for end-stage liver disease

Table 5. Hospital events by severity of AKI

	Total N=192	AKIN Stage 1 N=50	AKIN Stage 2 N=47	AKIN Stage 3 N=95	p
Medical complications					
Bacteremia – n (%)	34 (18)	5 (10)	4 (9)	25 (26)	0.005
GI bleed – n (%)	45 (24)	10 (20)	10 (21)	25 (27)	0.34
Pneumonia – n (%)	35 (18)	6 (12)	6 (13)	23 (24)	0.04
UTI – n (%)	55 (29)	7 (14)	8 (17)	40 (42)	<0.0001
Hepatic complications					
Ascites	160 (83)	34 (68)	39 (83)	87 (92)	0.0004
Hepatic encephalopathy	120 (63)	23 (46)	32 (68)	65 (68)	0.02
SBP	38 (20)	1 (2)	11 (23)	26 (27)	0.0008
Variceal bleed	16 (8)	4 (8)	3 (6)	9 (9)	0.66
HRS specific therapy					
Albumin	158 (82)	33 (66)	40 (85)	85 (89)	0.001
Midodrine	86 (45)	11 (22)	15 (32)	60 (63)	<0.0001
Octreotide	89 (46)	8 (16)	18 (38)	63 (66)	<0.0001
Admitted to ICU – n (%)	94 (50)	15 (30)	17 (36)	62 (65)	<0.0001
Transferred out alive – n (%)	60 (64)	14 (93)	15 (88)	31 (50)	0.002
Survived to discharge – n (%)	47 (50)	14 (93)	11 (65)	22 (35)	<0.0001
Mechanical ventilation	62 (32)	6 (12)	8 (17)	48 (51)	<0.0001
Vasopressor usage	49 (26)	3 (6)	6 (13)	40 (42)	<0.0001
Days from admission to discharge – median (IQR)	10 (6-17)	9 (6-13)	10 (6-17)	14 (8-35)	0.01

Abbreviations: N, number; ICU, intensive care unit; SICU, surgical intensive care unit; CCU, cardiac care unit; GI, gastrointestinal, UTI, urinary tract infection; SBP, spontaneous bacterial peritonitis; HRS, hepatorenal syndrome; IQR, inter-quartile range

Chapter 3. Early Trends in Cystatin C and Outcomes in Patients with Cirrhosis and Acute Kidney Injury

Introduction

Acute kidney injury (AKI) is a common complication in patients with cirrhosis and associates with higher mortality in proportion to progressive AKI severity[1,2]. However, the most common indicator of renal function, serum creatinine, may be an unreliable surrogate for glomerular filtration rate (GFR) due to the impact of non-renal determinants such as sex, race, age, body composition and medications. In the setting of an acute drop in GFR, creatinine is insensitive to small decrements in function, and its rise can lag actual kidney injury by several days. These shortcomings of creatinine are magnified in patients with cirrhosis, as they have an enlarged volume of fluid distribution and decreased creatinine production secondary to muscle atrophy and liver dysfunction, further dissociating creatinine from GFR[3]. The accuracy of creatinine in reflecting GFR declines with worsening stages of cirrhosis[4] and can be further compromised by elevated bilirubin interfering with creatinine assays[5]. We have previously shown that progression of AKI associates with mortality[6]. However, progression of AKI to a higher creatinine defined stage may be delayed in the setting of cirrhosis due to early fluctuation in creatinine levels unrelated to renal function and potentially beneficially treatments may resultantly be deferred. A more accurate means of rapidly and accurately detecting changes in renal function early in the course of AKI that associate with outcomes may allow for more prompt initiation of therapy and improved outcomes.

Cystatin C is a low-molecular-weight cysteine proteinase inhibitor synthesized at a constant rate by all nucleated cells. Cystatin C is freely filtered by the glomerulus, nearly completely reabsorbed and catabolized by the proximal tubule and does not undergo secretion. Cystatin C levels are less influenced by non-renal factors than creatinine and it has thus been proposed as a superior marker of glomerular filtration. In AKI, cystatin rises more rapidly than

creatinine in some settings and has been shown to associate more strongly with outcomes. Cystatin performs better than creatinine in early detection of AKI in the emergency room[7] intensive care unit (ICU)[8,9] and following pediatric cardiac surgery[10]. Cystatin associates with duration of AKI[11], need for renal replacement therapy[8,12] and short and long term mortality in AKI[12,13]. Patients who experience increases in both cystatin C and creatinine experience worse outcomes than those with an increase in either marker alone[14,15]. In patients with cirrhosis, cystatin C has been shown to more accurately correlate with measured GFR than creatinine or creatinine based estimation equations[16]. Cystatin C is also more sensitive than creatinine in cirrhotics for detecting mild decreases in baseline GFR[17,18] and superior in predicting AKI or 3 month mortality[19]. Despite these attributes, cystatin C has been challenging to study in patients with cirrhosis and AKI due to the typical lack of a documented baseline value. The absence of a baseline renders cystatin ineffectual in practice for diagnosing AKI prior to creatinine as there is no value to compare to for assessment of absolute or relative changes. However, due to its lesser dependence on non-renal determinants, small changes in cystatin levels early in the course of AKI may be more reflective of true trends in renal function than those of creatinine, which might continue to oscillate for several days before displaying a clear trend towards renal worsening or recovery. An alternative study design therefore is comparing trends in cystatin C and creatinine levels immediately following the onset of clinical apparent AKI to evaluate the relative utility of early fluctuations in each marker in predicting outcomes following AKI. We conducted a prospective multi-center study in patients with cirrhosis comparing changes in cystatin C and creatinine immediately following onset of AKI as predictors of dialysis and mortality during this early time period.

Subjects and Methods

Study design

The details of the cohort of patients with cirrhosis and AKI and study design have been described previously[6]. This prospective, multi-center observational cohort study was conducted between 2009 and 2011 at four tertiary care academic centers in the US. Eligible patients were admitted with AKI (see "Variables") or developed it during the course of hospitalization. Inclusion criteria included a known diagnosis of cirrhosis (see "Definitions"), age ≥ 18 years and availability of documented serum creatinine within 1 year prior to AKI. Exclusion criteria included prior kidney or liver transplant, advanced chronic kidney disease (CKD) (baseline creatinine > 4.0 mg/dL), acute or chronic renal replacement therapy at enrollment, estimated life expectancy < 3 days, confirmed pregnancy and other known causes of renal insufficiency such as glomerulonephritis or urinary obstruction. Informed consent was obtained from all participants or, if patients were unable to provide consent, from designated surrogates. All consecutive eligible patients identified during screening were approached for enrollment and all participants were enrolled within 5 days of meeting AKI criteria. The study was approved by the institutional review board at each institution.

Sample Collection and Biomarker Measurement

A fresh 10-ml blood sample was collected daily for three days following the onset of AKI. Samples were immediately refrigerated and then centrifuged at 5000 x *g* for 10 minutes at -4°C. Aliquots of 1 ml of supernatant were subsequently stored within 6 hours of collection in cryovials at -80°C for cystatin C measurement. No additives or protease inhibitors were utilized. Measurement was performed on subsequently thawed aliquots without undergoing any additional freeze-thaw cycles. Cystatin C was measured using a BN II nephelometer (Siemens AG, www.siemens.com), which has an approximate coefficient of variation of 2%[20]. Creatinine was measured from samples collected as part of routine clinical care via the modified Jaffe method. Laboratory measurements were performed by personnel blinded to patient information.

Variables

Independent Variables

Cirrhosis- Patients were eligible who carried an existing documented diagnosis of cirrhosis based on liver biopsy, when available, or a combination of clinical, biochemical, ultrasonographic and endoscopic findings.

AKI- The acute kidney injury network (AKIN) criteria were applied for diagnosis of AKI as recommended by a working group composed of members of the International Ascites Club (IAC) and the Acute Dialysis Quality Initiative (ADQI)[21]. AKIN quantifies the severity of AKI based on degree of increase in serum creatinine relative to baseline and is defined as follows: stage 1, increase in creatinine by 0.3 mg/dL or 50%; stage 2, 2 to 3-fold increase; stage 3, >3-fold increase, or creatinine >4.0 mg/dL after a rise of at least 0.5 mg/dL or acute dialysis requirement. As urine collection and output documentation can be inconsistent, only the serum creatinine component of the AKIN criteria was utilized.

Baseline serum creatinine- Baseline serum creatinine was defined as the most recent stable measurement within a year prior to admission for the index hospitalization. When possible, outpatient measurements were utilized though values were also used from previous admissions not complicated by AKI. In rare cases, patients without an outpatient measurement were included in the analytic cohort if, prior to onset of AKI, they manifested at least 5 initial days from admission of stable values within the normal creatinine range. In these instances, the creatinine at admission was considered the baseline.

Other variables- When calculating between-sample percent change in creatinine and cystatin C, the first and last available sample were utilized. GFR was estimated via the CKD-EPI equation using the baseline creatinine value[22]. CKD was defined by as GFR < 60 mL/min. MELD and Child-Pugh scores were calculated on the day of first sample collection.

Outcomes- Our primary outcome was a composite of dialysis and in-hospital mortality during the index hospitalization.

Statistics

Categorical variables were expressed as proportions and compared using Chi-square and Fisher's Exact test, as appropriate. Normally or near-normally distributed variables were reported as means with standard deviations (SD) and compared by Student's *t*-test. Non-normally distributed continuous variables were reported as medians with inter-quartile ranges (IQR) and compared by Wilcoxon rank sum test. Normality was assessed using the Kolmogorov-Smirnov test. Correlation between the percentage change between samples of creatinine and cystatin C was assessed via Pearson.

Patients were categorized into four groups based on trends between the first and last sample of two filtration biomarkers, serum creatinine and serum cystatin C. The groups one to four were, respectively, when both biomarkers were unchanged or fell, only serum creatinine exhibited any increase, only serum cystatin C increased and both increased. As our intent was to compare the association between small, early changes in filtration markers with outcomes, no threshold was utilized as to what constituted an increase. With the group with both biomarkers unchanged or falling as the reference, we determined crude and adjusted relative risks of each other group for our composite primary outcome with multivariate modified Poisson regression using SAS PROC GENMOD. Adjustment was made for critical demographics variables associated with filtration markers including race, age, and sex. Goodness-of-fit was verified with the Hosmer-Lemeshow test. A 2-sided $p<0.05$ was considered significant for all analysis. Statistical analysis was performed using SAS, version 9.2 (SAS Institute, Cary, NC).

Results

A total of 192 patients were enrolled in our cohort with cirrhosis and AKI. Of these, 106 had at least 2 blood samples collected and were included in this study. Samples were not collected in the remaining 86 patients either due to failure to consent to blood collection or

initiation of dialysis prior to obtaining consent. Baseline demographic, clinical and laboratory characteristics for the entirety of study participants and the four groups designated by trends in creatinine and cystatin C are shown in **Table 1**. There were no significant differences in any demographic variables or in those relating to the patients' liver disease between those patients who did and did not have serum samples collected. The mean patient age was 56.3 and 66% were male. Thirty-seven (35%) patients met the primary composite endpoint during their hospitalization. Of these, 28 patients died and 22 required dialysis, with 13 of these experiencing both dialysis and death. On sensitivity analysis, there was no difference in death, 28/106 (26%) vs. 22/86 (26%), or the composite of death or dialysis, 37/106 (35%) vs. 30/86 (35%), between those patients with and without blood samples obtained. The majority of patients had advanced cirrhosis evidenced by previously suffered complications including ascites, 76%, hepatic encephalopathy, 63%, variceal bleeding, 23% and SBP, 12%. Reasons for admission were similar between the four groups. The median Child-Pugh score was 10 and MELD 26.4 at the time of enrollment. There was no difference in Child-Pugh and MELD scores across groups, nor were serum sodium levels or the presence of hyponatremia at enrollment significantly different.

Biomarkers and prognosis

Three blood samples were collected in 77 (73%) patients, and two were collected in the remainder, 29 (27%). The first sample was collected a median of 2 (IQR 2-4) days after first meeting AKIN criteria. While creatinine and cystatin C levels from the first sample were moderately correlated, $r^2 = 0.55$, the relative changes in creatinine and cystatin C values between the first and last sample were less so, $r^2 = 0.3$, $p < 0.0001$. Correlations between creatinine and cystatin C levels in the initial samples and between relative and absolute changes in each filtration marker between samples are shown in **Figure 1a, 1b** and **1c**, respectively. Cystatin C exhibited less variability between samples than seen with creatinine

with the interquartile range for percent change in creatinine ranging from -17 to +11% compared with cystatin C ranging from -9 to +12%. A change of <10% was observed in 35/106 (33%) patients by creatinine and 53/106 (50%) patients based on cystatin C (p = 0.018). The median change in cystatin C values differed significantly between those patients with the primary outcome, +6% (95% CI -2 to +14%), and those without, -3% (-9 to +9%), p = 0.03. The difference in changes in creatinine for those with and without the primary outcome trended in the same direction but did not reach statistical significance, 0% (-12 to +17%) vs. -5% (-21 to +8%), p = 0.07. Patients experiencing an increase in cystatin C levels between samples were significantly more likely to meet the primary endpoint, 47%, than those without such an increase, 23%, p = 0.008. However, there was no significant difference in the incidence of dialysis or mortality among those whose creatinine increased, 40%, than among those where it did not, 32%, p = 0.41 (**Table 2**). Neither the cystatin C nor creatinine values from the first sample collected showed any association with the primary outcome.

Patients were stratified into four mutually exclusive groups based on changes in creatinine and cystatin C: both unchanged or decreased 38 (36%) (Scr-/CysC-); only cystatin C increased 25 (24%) (Scr-/CysC+); only creatinine increased 15 (14%) (Scr+/CysC-); and, both creatinine and cystatin C increased 28 (26%) (Scr+/CysC+). The incidence of dialysis or death for each group is shown in **Table 3**. Taking the Scr-/CysC- group as the reference, in both instances where cystatin C rose, Scr-/CysC+ and Scr+/CysC+, the occurrence of the primary outcome was significantly higher, p = 0.02 and 0.03, respectively. However, in the group where only creatinine rose, outcomes were similar to the reference group. Both the Scr-/CysC+ and Scr+/CysC+ but not Scr+/CysC- groups were associated with a significantly increased relative risk for the primary outcome in unadjusted analysis as well as after adjustment for age, race and sex.

Discussion

AKI in patients with cirrhosis is often severe and associated with significant mortality risk. Potentially efficacious therapies exist but must be appropriately applied to patients at highest risk for adverse outcomes[23]. We have demonstrated that progression to a more advanced stage of AKI is independently associated with mortality but the likelihood of progression can be difficult to predict early in the course of AKI. Creatinine levels are dependent on multiple demographic and clinical factors beyond renal function and thus may be susceptible to short term fluctuations early in the course of AKI unrelated to changing GFR[3,24]. Cirrhosis potentiates these shortcomings of creatinine due to associated low protein intake, reduced muscle mass, defective creatinine production and frequent large fluid shifts. In patients with cirrhosis, creatinine based estimation of GFR is within 50% of measured values in only 9% of patients[25]. Cystatin C has been proposed as a biomarker of glomerular filtration less susceptible to extra-renal variation. In patients with cirrhosis, GFR estimates are less biased and more precise with cystatin C than creatinine[25,26]. Cystatin levels, but not creatinine, are associated in cirrhotic patients with development of AKI and mortality over a 3-6 month period[19] and the onset of hepatorenal syndrome and mortality at one year[27]. The purpose of this study was to compare the association of changes in cystatin C and creatinine early in the course of AKI in patients with cirrhosis with a composite outcome of dialysis or death.

In our study, changes in cystatin C, but not creatinine, over the period of sample collection differed significantly for those with and without the primary outcome. Participants experiencing a rise in cystatin C alone (Scr-/CysC+) between samples progressed to the need for dialysis or death at the same rate as those with a rise in both biomarkers of filtration (Scr+/CysC+). However, those with a rise in creatinine alone (Scr+/CysC-) experienced the primary outcome with no greater frequency than those in whom both biomarkers fell (Scr-/CysC-). Relative to the group in which both markers fell, both groups with rising cystatin were independently associated with the primary outcome. The lack of association between rising creatinine and our primary endpoint stands in contrast to our previous demonstration of a

strong association between progression of AKI to a higher creatinine defined stage and mortality[6]. This discrepancy is again evidence of the poor sensitivity of creatinine for detecting acute falls in renal filtration function. Given its extra-renal influences and the extent to which changes in levels lag falling GFR, creatinine rising over the entire duration of an AKI episode sufficient to qualify for a higher AKI stage is indeed specific for a significant fall in renal function and resultantly associates with poor outcomes. Over the short term however, early in the course of AKI, creatinine changes need not reflect trends in renal function and thus show poor association with outcomes when not coupled with similar changes in cystatin C levels.

Cystatin C strongly associates with outcomes in multiple settings of AKI including ICU[9], emergency room[7] and transplant[28]. Intriguingly, changes in cystatin C may be more specific to outcomes than creatinine. Kwon et al. studied 274 ICU patients, of whom 84 (30.7%) developed AKI[29]. The mortality in patients with acute elevation in cystatin C but without creatinine based AKI (28.6%) was similar to patients with AKIN stage 1 AKI (33.3%) and far outstripped that of patients with no elevations in either biomarker (5.7%). This finding mirrors ours of poor outcomes in patients with Scr-/Cys+. The apparent prognostic advantage of cystatin C may be due to its ability to more accurately reflect early/small changes in GFR due to fewer non-renal influences. Early in AKI, before GFR has undergone a truly dramatic fall, creatinine may be subject to greater fluctuations than cystatin C, fluctuations untethered from changes in GFR. In our study, creatinine and cystatin C levels exhibited good correlation at time of first sample collection, $r^2 = 0.55$. However, the correlation between changes in these markers over the course of sample collection was significantly lower, $r^2 = 0.3$. Changes in cystatin C levels during the period of sample collection were more tightly bunched than those of creatinine. Cystatin C demonstrated less variability with a smaller interquartile range for changes and a significantly higher number of patients with a change of <10%.

In addition to being more specific for early changes in GFR than creatinine, cystatin C may also be more sensitive. The superiority of cystatin C over creatinine for detecting early

acute changes in renal function has been noted in multiple settings. Herget-Rosenthal et al. performed daily serum collections on 85 ICU patients deemed high risk of developing AKI [8]. In the 44 (52%) patients who developed AKI as defined by the risk, injury, failure, loss, end-stage (RIFLE) criteria, cystatin C levels detected AKI (defined by a 50% increase from baseline) 1.5 ± 0.6 days earlier than serum creatinine. Similar results have been noted in the ICU[8,9], following iodinated contrast[30] and post-operatively in pediatric[10], though not adult[15], cardiac surgery.

Our study has several significant strengths. Data were collected prospectively for what is, in this challenging study population, a large cohort of patients. Unlike many studies of cirrhosis and AKI, ours was multi-center and contained patients from both general medical floors and the ICU, enhancing its generalizability. However, the study is not without limitations. Cystatin C can be influenced by several non-renal factors including steroids and thyroid function. While we do not have data for these variables, it is reassuring that none of the baseline and demographic variables in **Table 1** predicted which of the four groups patients would assort into. This is especially true for cirrhosis etiology, where the potential use of steroids to treat acute hepatitis in alcohol related cirrhosis did not dictate the pattern of changes in cystatin C. However, we cannot definitively rule out that changes in cystatin may be reflecting some other physiologic process in addition to renal function that may be contributing to the primary outcome. We did not have data on baseline cystatin C levels and patients were enrolled based on creatinine defined AKI. This raises a concern that the results could be biased for patients whose creatinine fell due to regression to the mean. However, the lack of association between enrollment cystatin and creatinine values and the primary outcome assuages this concern.

Conclusions

Changes in serum cystatin C early in the course of AKI in patients with cirrhosis associate more strongly with the need for dialysis and mortality than do changes in serum creatinine. Prospective trials indexing interventions to changes in cystatin are required to

determine if routine monitoring of cystatin C in patients with cirrhosis and AKI may lead to improved outcomes.

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Table 1. Baseline demographic, clinical and laboratory values

	Total N = 106	Scr-/CysC- N = 38	Scr-/CysC+ N = 25	Scr+/CysC- N = 15	Scr+/CysC+ N = 28	p
Age in years - mean ± SD	56.3 ± 8.9	54.6 9.8	57.1 10.2	58.1	56.9	0.52
Male sex – *n* (%)	70 (66)	26 (68)	17 (68)	8 (53)	19 (68)	0.74
BMI – median (IQR)	30.6 (25.7-36)	32.2 (26.3-36.8)	29.2 (25.5-32.6)	33.2 (25.7-21.3)	31.5 (25-36.5)	0.41
Race – *n* (%)						
White	76 (72)	30 (79)	17 (68)	12 (80)	17 (61)	0.34
Black	16 (15)	4 (11)	4 (16)	2 (13)	6 (21)	0.67
Hispanic	12 (11)	4 (11)	3 (12)	1 (7)	4 (14)	0.90
Diabetes – *n* (%)	24 (23)	12 (32)	2 (8)	3 (20)	7 (25)	0.16
Active Cancer – *n* (%)	13 (12)	5 (13)	2 (8)	2 (13)	4 (14)	0.92
Baseline creatinine mg/dL – median (IQR)	1.02 (0.8-1.3)	1 (0.8-1.2)	0.97 (0.8-1.2)	1.1 (0.9-1.43)	1.18 (0.8-1.56)	0.12
CKD[a]	34 (32)	9 (24)	8 (32)	6 (40)	11 (39)	0.51
Cirrhosis etiology – *n* (%)						
Alcohol	32 (30)	11 (29)	12 (38)	4 (27)	5 (18)	0.13
Alcohol and HCV	27 (25)	13 (34)	3 (12)	1 (7)	10 (36)	0.04
HCV	19 (18)	6 (16)	3 (12)	2 (13)	8 (29)	0.45
NASH	10 (9)	2 (5)	2 (8)	3 (20)	3 (11)	0.40
Cryptogenic	4 (4)	1 (3)	1 (4)	2 (13)	0 (0)	0.16
Autoimmune	7 (7)	3 (8)	2 (8)	2 (13)	0 (0)	0.28
Other	8 (8)	3 (8)	2 (8)	2 (13)	1 (4)	0.65
Previous complications of cirrhosis – *n* (%)						
Ascites	81 (76)	27 (71)	21 (84)	11 (73)	22 (79)	0.67
Hepatic encephalopathy	67 (63)	22 (58)	15 (60)	11 (73)	19 (68)	0.68
Variceal bleed	24 (23)	12 (32)	5 (20)	3 (20)	4 (14)	0.42
SBP	12 (12)	3 (8)	5 (20)	2 (13)	4 (14)	0.55
Reason for admission – *n* (%)						
Hepatic encephalopathy	26 (25)	10 (26)	4 (16)	5 (33)	7 (25)	0.63
Refractory ascites/edema	16 (15)	6 (16)	4 (16)	3 (20)	3 (11)	0.86
AKI	12 (11)	3 (8)	2 (8)	2 (13)	5 (18)	0.60
GI bleed	8 (8)	3 (8)	1 (4)	0 (0)	4 (14)	0.44
Abdominal pain	7 (7)	4 (11)	1 (4)	1 (7)	1 (4)	0.78
Jaundice	5 (5)	3 (8)	1 (4)	1 (7)	0 (0)	0.50
Transplant work-up	6 (6)	1 (3)	2 (8)	1 (7)	2 (7)	0.70
SBP	4 (4)	0 (0)	2 (8)	0 (0)	2 (7)	0.20
Infection other than SBP	4 (4)	2 (5)	1 (4)	1 (7)	0 (0)	0.57
Other	20 (19)	7 (18)	7 (28)	2 (13)	4 (14)	0.63
Child-Pugh Class[b] - *n* (%)						0.17
B	37 (35)	17 (45)	6 (24)	7 (47)	7 (25)	
C	69 (65)	21 (55)	19 (76)	8 (53)	21 (75)	
Child-Pugh score – median (IQR)	10 (9-12)	10 (9-12)	11 (10-12)	10 (8-12)	10 (10-12)	0.39
MELD score – mean ± SD	26.4 ± 9.5	25 ± 9	26.8 ± 9.8	23.8 ± 7.4	29.3 ± 10.5	0.20
Bilirubin – median (IQR)	4 (1.8-9.1)	3 (1.6-6.4)	6.1 (2.6-9.6)	3.8 (1.6-5.5)	5.5 (2-16.7)	0.31
INR – median (IQR)	1.7 (1.3-2.3)	1.5 (1.2-2.3)	1.8 (1.4-2.3)	1.6 (1.2-1.8)	1.9 (1.4-2.7)	0.08
Sodium – mean ± SD	133 ± 6	133 ± 6	132 ± 7	135 ± 7	133 ± 7	0.74
Hyponatremia at enrollment[c] – *n* (%)	34 (32)	12 (32)	10 (40)	5 (33)	7 (25)	0.71

[a]CKD defined as GFR < 60ml/min calculated via CKD-EPI equation

[b]Child-Pugh Class and MELD score are at time of enrollment

[c]Serum sodium <130 mEq/L

Abbreviations: SD, standard deviation; BMI, body mass index; IQR, inter-quartile range; CKD, chronic kidney disease, HCV; hepatitis C virus; NASH, non-alcoholic steatohepatitis; SBP, spontaneous bacterial peritonitis; MELD, model of end-stage liver disease; INR, international normalized ratio

Figure 1a. Correlation between creatinine and cystatin

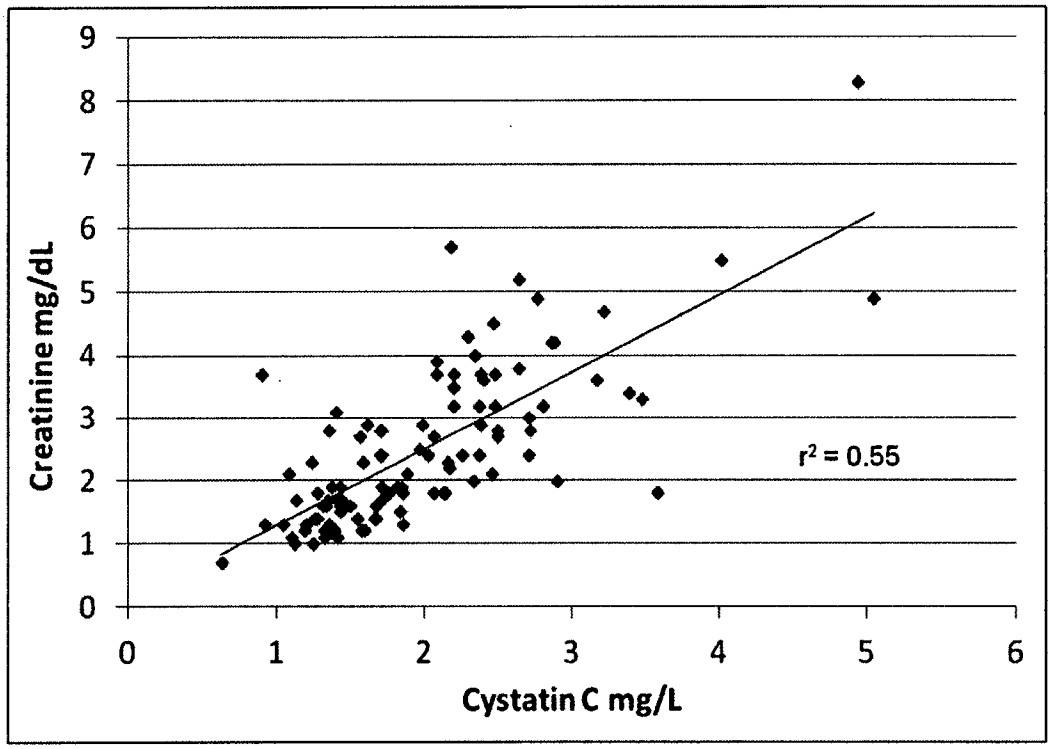

Figure 1. (A) Correlation between creatinine and cystatin C values from first sample collection. (B) Correlation between relative changes in creatinine and cystatin C values from first to last sample collection. (C) Correlation between absolute changes in creatinine and cystatin C values from first to last sample collection.

Figure 1b. Correlation between creatinine and cystatin

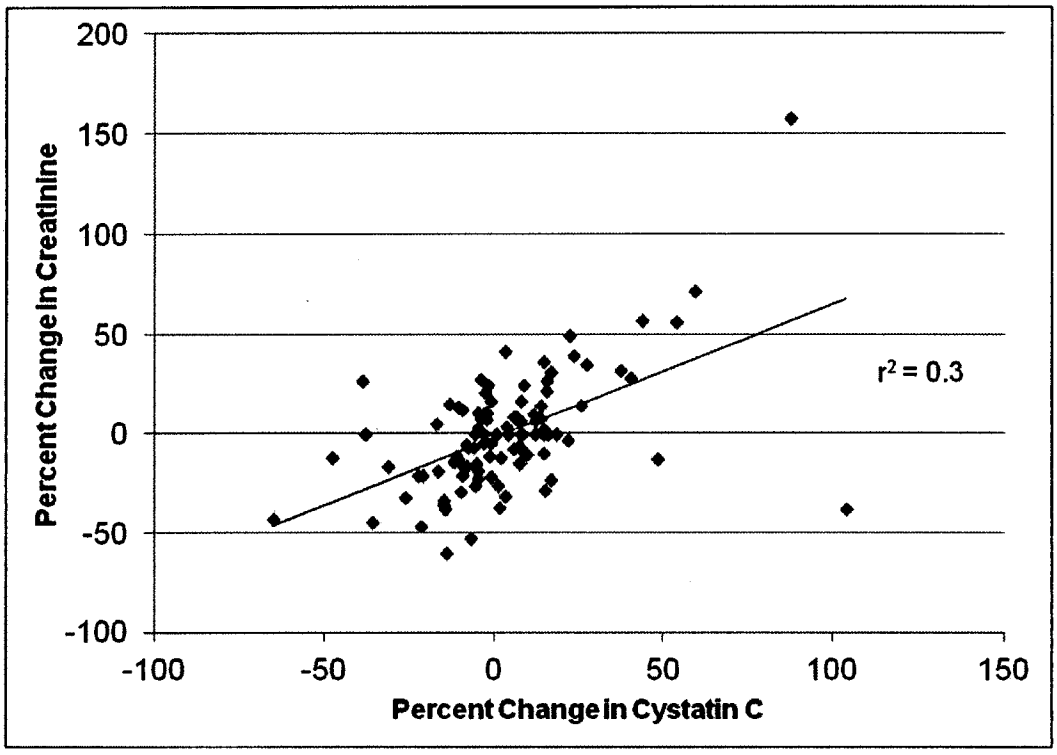

Figure 1. (A) Correlation between creatinine and cystatin C values from first sample collection. (B) Correlation between relative changes in creatinine and cystatin C values from first to last sample collection. (C) Correlation between absolute changes in creatinine and cystatin C values from first to last sample collection.

Figure 1c. Correlation between creatinine and cystatin

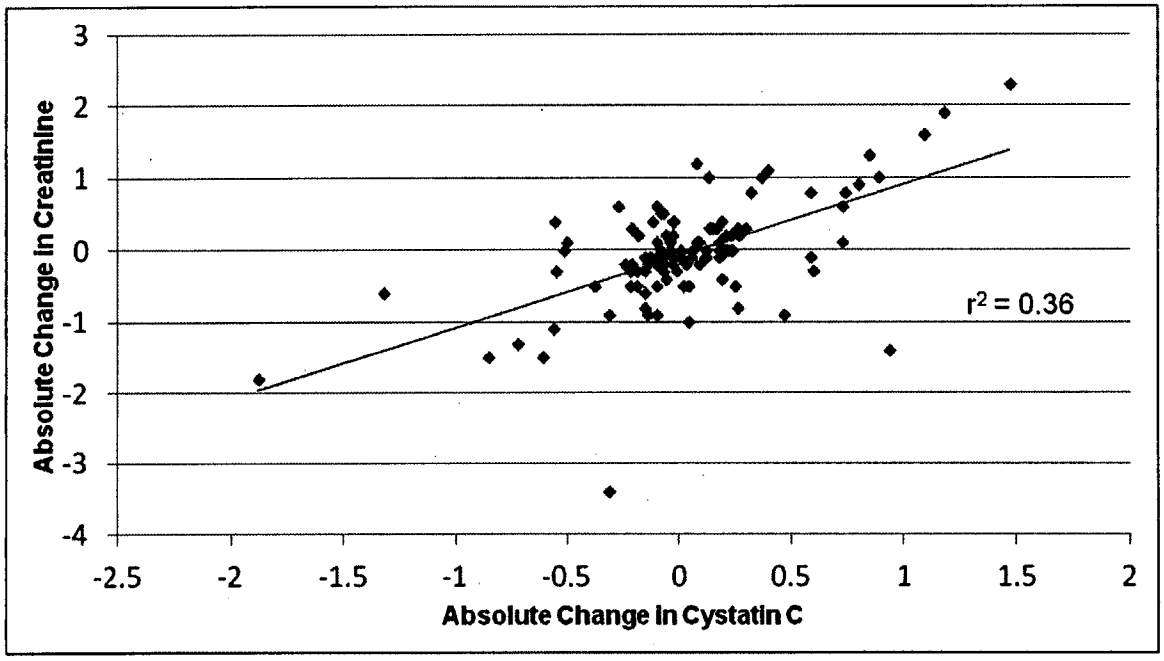

Figure 1. (A) Correlation between creatinine and cystatin C values from first sample collection. (B) Correlation between relative changes in creatinine and cystatin C values from first to last sample collection. (C) Correlation between absolute changes in creatinine and cystatin C values from first to last sample collection.

Table 2. Association between increasing filtration markers and the primary outcome

	Death/Dialysis, N (%)	Dialysis-free Survival, N (%)	p
Creatinine			
Increase (N = 43)	17 (40)	26 (60)	0.41
No increase (N = 63)	20 (32)	43 (68)	
Cystatin C			
Increase (N = 53)	25 (47)	28 (53)	0.008
No increase (N = 53)	12 (23)	41 (77)	

Table 3. Independent association of trends in filtration markers and the primary outcome

	Death or Dialysis, N (%)	Death or Dialysis	
		Unadjusted RR (95% CI)	Adjusted* RR (95% CI)
Scr-/CysC- (N = 38)	8 (21)	1.00	1.00
Scr-/CysC+ (N = 25)	12 (48)	2.28 (1.09-4.77)	2.27 (1.07-4.85)
Scr+/CysC- (N = 15)	4 (27)	1.27 (0.45-3.59)	1.32 (0.46-3.75)
Scr+/CysC+ (N = 28)	13 (46)	2.21 (1.06-4.59)	2.17 (1.03-4.61)

*Adjusted for race, age and sex

Abbreviations: RR, relative risk; CI, confidence interval; Scr, serum creatinine; CysC, cystatin C

Chapter 4. Urinary Biomarkers and Progression of Acute Kidney Injury in Patients with Cirrhosis

Introduction

Acute kidney injury (AKI) is common in patients with cirrhosis, complicating 20% of hospitalizations[1-3]. The risk of death increases with peak severity of AKI[4-8] and progression of AKI to a higher stage defined by the acute kidney injury network (AKIN) criteria. AKI progression is associated with mortality independent of the model of end-stage liver disease (MELD) score[4]. Intervening to prevent progression therefore may reduce mortality. Despite the overall grim prognosis for patients with cirrhosis and AKI, disease specific treatments carrying the potential to improve outcomes, if correctly and judiciously applied, are available for patients with hepatorenal syndrome (HRS)[9-12]. For example, patients with progressive acute tubular necrosis (ATN) may be managed with dialysis and those with severe, irreversible disease benefit from combined liver-kidney transplant[13]. For reasons of safety and equity, such aggressive therapies should ideally be offered only to those patients at greatest risk for progressive renal dysfunction and death. However, in practice, where clinically distinguishing AKI etiology is frequently challenging, patients often receive a "kitchen sink" approach of multiple aggressive therapies irrespective of whether they are at high risk for AKI progression or death. If a patient is unlikely to progress or die, aggressive management can likely be held while time is taken to clarify the etiology of AKI. However, if a patient is at high risk for adverse outcomes, early and aggressive action should be taken.

Unfortunately, predicting which patients will suffer progressive AKI, and identifying those progressors who will proceed to death, is clinically challenging. In patients with cirrhosis, an enlarged volume of fluid distribution, low protein intake and decreased creatinine production secondary to muscle atrophy and liver dysfunction significantly dissociates creatinine levels from reflecting the true presence and severity of kidney dysfunction[14]. Correspondingly,

creatinine fluctuations early in the course of AKI are difficult to interpret, taking several days to resolve into a definitive trend demonstrating progression. As a result of this delay, potentially beneficially treatments may be deferred. An accurate, objective and reproducible means of anticipating AKI progression or death at the time of AKI diagnosis is urgently needed to allocate treatments, stratify patients for inclusion in trials and prioritize liver and kidney transplantations.

Research into structural AKI has been revolutionized by investigation of multiple urinary biomarkers of kidney tubular injury that independently predict AKI progression in multiple clinical settings[15-17]. Among the most promising are neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1) and liver-type fatty acid binding protein (L-FABP). While NGAL has been studied for early detection of AKI following liver transplant[18,19] and for differential diagnosis of AKI in cirrhosis[20,21], few studies have evaluated these biomarkers for prognosis in patients with cirrhosis and AKI[20,21]. With a unique mix of functional (HRS) and structural disease (ATN, glomerulonephritis), the association in cirrhosis between tubular injury biomarker levels and outcomes is unclear. In this setting, it is possible that biomarkers of tubular function, such as the fractional excretion of sodium (FENa), and traditional markers of both glomerular and tubular injury, such as urine albumin, may also provide additional prognostic accuracy. Indeed, albuminuria is predictive of impending AKI in patients with cirrhosis[22]. We have therefore conducted a multi-center prospective study evaluating urinary biomarkers of kidney injury and tubular function for prediction of AKI progression and progression with mortality in patients with cirrhosis.

Materials and Methods

Study design

The details of the cohort and study design have been described previously[4]. This prospective, multi-center observational cohort study was conducted over 29 months between 2009 and 2011 at four tertiary care academic centers in the US. Eligible patients were admitted

with AKI (see "Definition") or developed it during the course of hospitalization. Inclusion criteria included a known diagnosis of cirrhosis (see "Definitions"), age ≥ 18 years and availability of a documented serum creatinine within 1 year prior to AKI. Major exclusion criteria included prior kidney or liver transplant and advanced chronic kidney disease or renal replacement therapy at the time of enrollment. All consecutive eligible patients were enrolled within 5 days of meeting AKI criteria. Informed consent was obtained from all patients or their proxy decision makers. The study was approved by the institutional review board at each participating institution.

Sample Collection and Biomarker Measurement

A fresh 10-ml urine sample was collected daily for three days. Samples were immediately refrigerated and centrifuged at 5000 x *g* for 10 minutes at -4°C. Aliquots of 1-ml of supernatant were stored within 6 hours of collection at -80°C. No additives or protease inhibitors were utilized. All biomarkers were measured from frozen aliquots that did not undergo any additional freeze-thaw cycles. Laboratory measurements were performed by personnel blinded to patient information. ELISA methods, coefficient of variation and detection ranges were as described previously for measurement of NGAL[23], IL-18[24], KIM-1[25] and L-FABP[25]. Urine creatinine was measured by modified Jaffe reaction.

Variables

Independent Variables

Cirrhosis- Eligible patients carried a documented diagnosis of cirrhosis based on liver biopsy, when available, or on a combination of clinical, biochemical, imaging and endoscopic findings.

AKI- AKI was defined as a rise in creatinine of 0.3 mg/dL or 50% from baseline as recommended by a working group composed of members of the International Ascites Club (IAC) and the Acute Dialysis Quality Initiative (ADQI) who based this cut-off on Stage 1 of the

AKIN criteria[26]. As documentation of urine output was incomplete, this aspect of the criteria was not utilized.

Baseline serum creatinine- Baseline serum creatinine was defined as the most recent stable measurement prior to admission as e use of outpatient values results in less misclassification of AKI incidence, severity and prognosis compared to utilizing hospital admission, hospital nadir or imputed values[27]. The median and inter-quartile range (IQR) for the interval between creatinine utilized for baseline and hospital admission in the present study was 26 (9-73) days.

Other variables- Baseline glomerular filtration rate (GFR) was estimated via the MDRD-4 equation[28]. Chronic kidney disease was defined as GFR < 60 ml/min/1.73m^2. MELD and Child-Pugh scores were calculated on the day of first sample collection. HRS was diagnosed via the 2007 IAC criteria[29].

Outcomes- Our primary outcomes consisted of progression to a higher AKIN stage and progression to a higher stage with subsequent death which were compared separately with patients who did not progress. If patients who presented with Stage 3 AKI but not requiring renal replacement therapy subsequently required dialysis, this was considered progression. Patients who died without progression were excluded from the primary analysis as for them death may have been a competing risk for progression. Biomarker values for these excluded patients did not differ from those with progression and death (**Supplemental Table 1**).

Statistics

Categorical variables were expressed as proportions and compared using Chi-square and Fisher's exact test, as appropriate. Normally or near-normally distributed variables were reported as means with standard deviations (SD) and compared by Student's *t*-test. Non-normally distributed continuous variables were reported as medians with IQR and compared by the Wilcoxon rank sum test. Normality was assessed using the Kolmogorov-Smirnov test. NGAL values were bounded at an upper limit of 1000 ng/mL with no lower bound. KIM-1 was bounded

at an upper limit of 60 ng/ml and a lower limit of 0.056 ng/ml. L-FABP was bounded at an upper limit of 400 ng/ml and a lower limit of 0.57 ng/ml. IL-18 did not have an upper limit but the lower limit of detection for the assay was 25 pg/mL. All patients below this threshold were assigned a value of 15 pg/mL. Biomarker values from Day 0 (the first day of sample collection) were used for all analyses.

Biomarkers were log transformed and analyzed as continuous variables given their non-normal distribution. We determined crude and adjusted relative risks for each biomarker for progression alone and progression with death using a Poisson logistic regression model with patients without progression of AKI as the reference group. Utilizing the clinical model we developed through our association of AKI progression with mortality[4], we adjusted for critical covariates including presence of CKD, demographics (race, age, and sex), MELD score and serum sodium. Relative risks were calculated rather than odds ratios to avoid artificial inflation of point estimates due to high prevalence of outcomes. To assess biomarkers' ability to discriminate risk, we calculated the area under the receiver operating curve (AUC) for each biomarker for each outcome. To evaluate biomarkers for improvements in risk discrimination, we calculated a category-free net reclassification index (NRI) for each biomarker for the outcome of AKI progression and death. This was performed by utilizing binary logistic regression models (for no progression vs. progression and death) constructed with the above noted clinical variables and evaluating changes in model predictions with and without each biomarker. Finally, we determined the optimal cutoff for each biomarker for predicting AKI progression and death by maximizing the Youden Index and calculated the relative risk for this outcome by number of biomarkers above these cutoffs. This was achieved using a regression model with the above clinical variables and the number of biomarkers above their cutoff as an ordinal variable (with zero markers as the reference). Biomarkers were evaluated for collinearity using Pearson's test and evaluated for consistency across days of sample collection using paired t-tests. In supplemental analysis, biomarkers levels were compared across groups in

those patients who did and did not meet IAC criteria for HRS. Goodness-of-fit was verified with the Hosmer-Lemeshow test. A 2-sided $p<0.05$ was considered significant for all analysis. Statistical analysis was performed using SAS, version 9.2 (SAS Institute, Cary, NC) and R, version 2.10.1.

Results

Cohort characteristics

A total of 219 patients with cirrhosis and AKI were prospectively enrolled. Thirty-one patients were subsequently excluded for the following: prolonged interval between onset of AKI and time of first sample collection (n=15), lack of documented baseline creatinine level (n=4), recent treatment with nephrotoxins (n=3), diagnosis of acute hepatitis rather than cirrhosis (n=2), anuria (n=2) and other causes (n=5).

AKI progression

Forty-four (23%) patients experienced AKI progression alone and 39 (21%) had AKI progression and subsequently died during their hospitalization. Ten patients (5%) died without progression and were excluded from the primary analysis. Baseline demographic, clinical and laboratory data of the entire cohort and of those patients with and without AKI progression are shown in **Tables 1 and 2**. Neither baseline GFR nor the presence of CKD varied between the three groups. The delta creatinine between baseline and admission did not differ between the three groups, 0.8 mg/dL vs. 0.8 vs 0.5, p=0.28. Baseline proteinuria was only present in 22 (12%) patients and was similar across groups. The majority of patients had decompensated cirrhosis as evidenced by the history of ascites (76%), hepatic encephalopathy (67%), variceal bleeding (23%) and SBP (16%). Reasons for admission were similar across groups but during the course of hospitalization rates of urinary tract infections and pneumonia differed. The median Child-Pugh score was 10.5 and median MELD score was 26.3. Both Child-Pugh (12 vs. 10 vs. 10, P <0.0001) and MELD scores (34.3 vs. 26.5 vs. 22.1, P <0.0001) were higher in

those who progressed and died than in those with progression alone or those without progression.

Three urine samples were collected in 134 (71%) participants, two samples in 42 (22%), and only one sample was collected in 12 (6%) participants. The first sample was collected at a median of 2 (IQR 1-3) days after first meeting AKIN criteria. Median values for biomarkers are shown in **Table 3**. Sensitivity analysis using raw biomarker values and those corrected for urine creatinine showed minimal variation (data not shown). To facilitate cross-study comparison of results, NGAL, IL-18, KIM-1 and L-FABP are therefore presented as raw values. Log-transformed biomarkers demonstrated moderate correlations between each other **(Supplemental Table 2)**.

Median values for all biomarkers varied across the three groups. Tubular injury markers were highest in patients with progression and death. While all tubular injury markers distinguished patients with progression and death from no progression, only NGAL distinguished progression alone from no progression. Microalbuminuria was higher in patients with progression and death than those with progression alone or no progression, 84 mg/dL (45-233) vs. 29 (9-164) vs. 21 (5-73), respectively, and this distinction persisted when correcting for urinary creatinine. FENa was significantly lower in patients with progression alone but did not differ between those without progression and those with progression and death. In subgroup analysis, similar trends were seen in patients who did not meet IAC criteria for HRS but not in those who did (**Supplemental Table 3**). The medians and interquartile ranges of injury markers are depicted in **Figure 1**. The biomarker levels over three days of sample collection are shown in **Supplemental Figure 1**. Median time from sample collection until death for those patients who died was 8 (5-19) days. There was no significant difference in any biomarkers between the patients who died before (n=26) vs after 8 days (n=23).

In multivariable analysis, IL-18, relative risk (RR) 4.09 (1.56-10.70), KIM-1 3.13 (1.20-8.17), L-FABP 3.43 (1.54-7.64) and albuminuria 2.07 (1.05-4.10) per log unit were

independently associated with AKI progression and death relative to no progression (**Table 4**). NGAL exhibited a strong trend but did not reach statistical significance, 2.30 (0.94-5.60), primarily due to significant collinearity with MELD. No biomarkers were independently associated with progression without death. FENa was not associated with the primary outcome on any analysis. AUC's, optimal cutoffs, sensitivities and specificities of each biomarker for AKI progression and death are shown in **Table 5**. The ability of biomarkers to improve risk discrimination as determined by NRI is presented in **Table 6**. The four urinary biomarkers with the strongest risk discrimination, NGAL, IL-18, L-FABP and albumin, were selected and unadjusted and adjusted relative risks for AKI progression and death by number of biomarkers above their optimal diagnostic cutoffs are shown in **Figure 2a.** Outcomes by number of biomarkers above the cutoff for AKI progression and death are shown in **Figure 2b**.

Discussion

In patients with the grave combination of cirrhosis and AKI, renal dysfunction is often progressive. We have recently shown that AKI progression is associated with over 3-fold odds of mortality independent of MELD score[4]. Progression of AKI strongly modifies the association between peak AKI severity and mortality. Patients who initially present with stage 1 AKI and progress to stage 2 have mortality of 29% vs. 7% in those who present in stage 2 but do not progress[4]. Similarly, those presenting in stage 1 and progressing to stage 3 have mortality of 50% vs. 21% in those who present in stage 3 and do not progress. It is therefore critical to know which patients are destined to progress so as to guide prognosis and treatment decisions. Ideally, clinicians would identify patients at highest risk of both progression and death as they would warrant the earliest and most aggressive intervention. Unfortunately, the lack of objective tests to predict AKI progression delays initiation of treatment and hinders clinical trials. The efficacy of treatment for HRS declines with increasing creatinine at treatment initiation[30]; it is

likely that more accurate identification of patients at high risk for progression of their AKI would allow earlier commencing of therapy and improved outcomes.

Quantitating the degree of injury the kidney has sustained may allow for more prescient prediction of AKI progression in patients with structural AKI. However, the standard metric of kidney function, serum creatinine, measures changes in filtration but does not directly reflect the degree, if any, of frank structural injury. Biomarkers reflecting tubular injury have been successfully associated with outcomes, including both worsening of AKI and mortality, in several settings including cardiac surgery[15], heart failure[31,32], ICU[16] and transplant[17]. Additional data indicate that increased post-AKI albuminuria, generally a hallmark of glomerular injury but also associated with tubular injury, connotes worse prognosis[15].

In the present study, there was a clear correlation between urinary injury biomarker levels and outcomes. NGAL, IL-18, KIM-1, L-FABP and albuminuria were significantly higher in patients with AKI progression and death as compared to patients with no progression and IL-18, KIM-1, L-FABP and albuminuria were independently associated with this outcome. Critically, this suggests injury biomarkers may serve to identify patients at highest risk for the worst outcomes who may derive maximal benefit from early and aggressive interventions. Indeed, the likelihood of progression and death was progressively higher with increasing number of elevated biomarkers. Assessed through the NRI, only IL-18 showed the ability to improve risk stratification for this outcome beyond our clinical model, though L-FABP and albumin demonstrated a strong trend towards such risk reclassification. However, compared to patients without progression, no biomarkers were independently associated with progression without death. In addition, biomarker values were similar in patients with death alone compared to those with progression and death. It is possible that biomarkers overall in the setting of cirrhosis may, with regards to prognosis, best serve as markers of severity of illness rather than predictors of AKI progression alone. As such their elevation may precede deterioration of patients' clinical status. While patients with the worse outcomes had a higher frequency of ICU admissions and

requirements for mechanical ventilation and vasopressor therapy, 44 (49%) patients who were admitted to the ICU had biomarkers drawn prior to ICU admission and 21 (43%) patients started on vasopressors had biomarkers drawn prior to pressor initiation. Alternatively, biomarkers of structural injury may associate with AKI progression in patients with tubular damage but not in those with a functional disease such as HRS. Since our cohort was analyzed as a whole, such a signal may have been lost.

Importantly, there was no difference in FENa between groups. The median FENa for all three groups was significantly below 1%, reflecting preserved sodium avidity in cirrhotic patients even after tubular injury. Cirrhotic patients with AKI suffer a mixture of structural (ATN) and functional (pre-renal azotemia, HRS) etiologies of renal dysfunction. Despite this diverse physiology, the association between injury biomarkers and outcomes is similar to that seen where structural AKI predominates[15-18]. There is evidence that some degree of tubular injury may be present even in those patients fulfilling criteria for HRS, albeit of a degree far milder than seen with ATN[21]. It is striking then that, along with the negative findings regarding FENa, our results suggest the primacy of structural injury in determining outcomes when generally applied to a cohort composed of patients with cirrhosis and both "functional" and "structural" AKI.

This study has several important strengths. Unlike many studies of AKI and cirrhosis, it is not restricted to ICU patients, improving the generalizability of the findings. The size of this cohort is one of the largest in the literature for this difficult to study population. The evaluation of multiple biomarkers is critical in cirrhosis where AKI is physiologically distinct from other settings such as surgery, sepsis or ICU. Finally, the prospective design allowed for robust and complete data collection on multiple critical covariates.

Our study is not without limitations. The etiology of AKI was not considered and thus patients likely suffered from a mix of pre-renal azotemia, ATN and HRS. However, accurate adjudication of AKI etiology must frequently be done retrospectively and thus would not be

available to clinicians at the time of biomarker measurement. Potentially divergent associations by AKI etiology between biomarkers and outcomes would, if anything, be expected to bias our results toward the null. The use of outpatient values for baseline creatinine results in the least misclassification of AKI incidence, severity and prognosis, but this approach does mean the exact timeframe for a rise in creatinine is unknown[27]. Kidney injury biomarkers in hospitalized cirrhosis patients without AKI are only minimally above normal ranges (unpublished data) and the significantly elevated values even in those patients who do not experience progression or death therefore suggests that AKI is indeed ongoing at hospital admission.

Conclusions

This phase of the study confirms that multiple structural biomarkers of kidney injury, but not FENa, are independently associated with progression of AKI and mortality in patients with cirrhosis. Elevated injury markers were seen in patients who ultimately progressed and died but levels were similar between those without progression and those with progression alone. Further research in a larger cohort is required to validate this finding and to determine if biomarkers may identify cirrhotic patients most likely to benefit from disease specific AKI treatments.

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Supplemental Table 1. Biomarker values in patients with progression and death *vs* death alone

	Progression and Death N=39	Death Alone N=10	P
Tubular injury markers			
NGAL (ng/ml)	366 (112-910)	233 (72-1000)	0.57
IL-18 (pg/ml)	90 (15-325)	79 (15-122)	0.40
KIM-1 (ng/ml)	8.3 (4-17.1)	6.1 (2.4-11.7)	0.16
L-FABP (ng/ml)	38 (13-73)	139 (12-334)	0.77
Tubular function markers			
FENa (%)	0.31 (0.1-0.91)	0.14 (0.05-0.85)	0.31
Glomerular injury marker			
Albumin (mg/dL)	84 (45-233)	28 (23-94)	0.06

Abbreviations: NGAL, neutrophil gelatinase associated lipocalin; IL-18, interleukin-18; KIM-1, kidney injury molecule-1;
L-FABP, liver-type fatty acid binding protein; FENa, fractional excretion of sodiu

Table 1. Baseline and demographic characteristics

	Total N=188	No Progression N=95	Progression alone N=44	Progression with death N=39	Death Alone N=10	P*
Age in years - mean ± SD	55 ± 9.3	55.7 ± 9.6	54.7 ± 8.9	54.7 ± 9	50.8 ± 7	0.10
Male sex – *n* (%)	133 (71)	66 (69)	32 (73)	29 (74)	6 (60)	0.83
BMI (kg/m^2)– median (IQR)	30.1 (25.5-35.1)	30 (24.5-35.3)	29.6 (26-33.9)	30.9 (27-34.8)	26.7 (25.7-32.7)	0.40
Race/Ethnicity – *n* (%)						
White	134 (71)	70 (74)	37 (84)	21 (54)	6 (60)	0.01
Black	27 (14)	14 (15)	3 (7)	9 (23)	1 (10)	0.26
Hispanic	23 (12)	10 (11)	3 (7)	8 (21)	2 (20)	0.06
CKD[a]	65 (35)	31 (33)	21 (48)	12 (31)	1 (10)	0.48
eGFR (ml/min/1,73m^2) – median (IQR)	70 (55-98)	70 (57-93)	64 (43-88)	81 (54-100)	101 (78-115)	0.14
Proteinuria[b] – *n* (%)	22 (12%)	14 (15)	3 (7)	5 (13)	0 (0)	0.36
Baseline Scr (mg/dL)- median (IQR)	1 (0.8-1.3)	1 (0.8-1.3)	1.1 (0.8-1.5)	1 (0.8-1.3)	0.7 (0.6-1)	0.09
Creatinine at meeting AKIN criteria (mg/dL)-median (IQR)	2.2 (1.6-2.9)	2.1 (1.6-2.7)	2.4 (1.6-3.3)	2.1 (1.5-2.8)	2.3 (1.6-4.1)	0.50
Peak creatinine – median (IQR)	2.7 (1.9-4.2)	2.2 (1.7-3.1)	3.4 (2.4-4.8)	4 (3.1-5.5)	2.5 (2.2-5.1)	<0.001
Diabetes – *n* (%)	49 (26)	29 (31)	10 (23)	9 (23)	1 (10)	0.56
Active Cancer – *n* (%)	21 (11)	10 (11)	5 (11)	4 (10)	2 (20)	0.98
Cirrhosis etiology – *n* (%)						
Alcohol	55 (29)	27 (28)	12 (27)	10 (26)	6 (60)	0.17
Alcohol and HCV	52 (28)	26 (27)	13 (30)	12 (31)	1 (10)	0.57
HCV	32 (17)	14 (15)	10 (23)	6 (15)	2 (20)	0.87
NASH	17 (9)	10 (11)	5 (11)	2 (5)	0 (0)	0.62
Cryptogenic	12 (6)	7 (7)	3 (7)	2 (5)	0 (0)	0.96
Autoimmune	11 (6)	6 (6)	0 (0)	4 (10)	1 (10)	0.26
Other	10 (5)	5 (5)	1 (2)	4 (10)	0 (0)	0.38
Previous complications – *n* (%)						
Ascites	142 (76)	71 (75)	35 (81)	30 (77)	6 (60)	0.91
Hepatic encephalopathy	126 (67)	61 (64)	31 (70)	28 (72)	6 (60)	0.77
Variceal bleed	44 (23)	23 (24)	12 (27)	7 (18)	2 (20)	0.31
SBP	30 (16)	13 (14)	7 (16)	9 (23)	1 (10)	0.53

Abbreviations: N, number; SD, standard deviation; BMI, body mass index; IQR, inter-quartile range; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; Scr, serum creatinine; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis; SBP, spontaneous bacterial peritonitis

[a]CKD is defined as estimated GFR 60 < ml/min/1.73m^2 by MDRD equation

[b]Microalbuminuria (30mg/dL) or greater on dipstick or quantitative measurement prior to admission

*Universal f-test across 4 groups

Table 2. Hospital events and complications

	Total N=188	No Progression N=95	Progression alone N=44	Progression with death N=39	Death Alone N=10	P*
Reason for admission – *n* (%)						
Hepatic encephalopathy	50 (27)	30 (32)	8 (18)	10 (26)	2 (20)	0.33
Refractory ascites/edema	23 (12)	11 (12)	7 (16)	5 (13)	0 (0)	0.53
AKI	22 (12)	12 (13)	7 (16)	1 (3)	2 (20)	0.23
GI bleed	14 (7)	8 (8)	2 (5)	3 (8)	1 (10)	0.97
Abdominal pain	14 (7)	5 (5)	5 (11)	2 (5)	2 (20)	0.67
Jaundice	10 (5)	5 (5)	1 (2)	3 (8)	1 (10)	0.58
Transplant work-up	6 (3)	5 (5)	0 (0)	0 (0)	1 (10)	0.43
SBP	6 (3)	2 (2)	1 (2)	2 (5)	1 (10)	0.21
Other	39 (21)	17 (18)	12 (27)	13 (33)	0 (0)	0.45
Child-Pugh Class[a] - *n* (%)						<0.001[b]
A	4 (2)	4 (4)	0 (0)	0 (0)	0 (0)	
B	60 (32)	42 (45)	14 (32)	4 (10)	0 (0)	
C	123 (66)	48 (51)	30 (68)	35 (90)	10 (100)	
Hospital Complications						
UTI	53 (28)	14 (15)	18 (41)	16 (41)	5 (50)	<0.001
SBP	37 (20)	12 (13)	11 (25)	12 (31)	2 (20)	0.002
Pneumonia	35 (19)	11 (12)	4 (9)	15 (38)	5 (50)	<0.001
Bacteremia	33 (18)	11 (12)	8 (18)	11 (28)	3 (30)	0.06
ICU admission	90 (48)	24 (25)	20 (45)	38 (97)	8 (80)	<0.001
Mechanical ventilation	62 (33)	9 (9)	13 (30)	34 (87)	6 (60)	<0.001
Vasopressor therapy	49 (26)	6 (6)	8 (18)	29 (74)	6 (60)	<0.001
Dialysis	44 (23)	0 (0)	16 (36)	27 (69)	1 (10)	<0.001
Child-Pugh score – median (IQR)	10.5 (9-12)	10 (8-11)	10 (9-12)	12 (11-13)	12.5 (11-14)	<0.001
MELD score[a] – mean ± SD	26.3 ± 9.5	22.1 ± 8.4	26.5 ± 6.9	34.3 ± 9.3	34.3 ± 6.1	<0.001
Serum sodium at enrollment (mmol/L)– median (IQR)	133 (130-138)	134 (130-137)	132 (128-137)	133 (130-138)	134 (124-143)	0.31
Hyponatremia at enrollment[c] – *n* (%)	62 (33)	29 (31)	17 (39)	13 (33)	3 (30)	0.30
WBC (n=170) (x1000/μL) – median (IQR)	7.4 (5-10.9)	6.1 (4.4-8.5)	7.2 (5.2-10.1)	12.4 (7.3 -10.3)	13.9 (10.4-14)	<0.001
Hbg (n=170) (g/dL) – median (IQR)	8.7 (7.9-10.1)	8.9 (8.2-10.1)	8.8 (7.3-10.3)	8.1 (7.5-9.1)	8.3 (8-11.1)	0.02
AST (n=155) (U/L) – median (IQR)	64 (39-113)	56 (39-89)	50 (32-88)	118 (49-276)	116 (69-272)	<0.001
ALT (n=155) (U/L) – median (IQR)	33 (18-51)	29 (20-46)	23 (14-47)	39 (24-106)	44 (30-79)	0.001
Midodrine – *n* (%)	80 (45)	32 (34)	25 (57)	23 (59)	6 (60)	<0.001
Octreotide – *n* (%)	81 (46)	32 (34)	25 (57)	24 (62)	7 (70)	<0.001

Length of stay (days)[d]- median (IQR)	12 (6-19)	9 (6-14)	16 (10-35)	15 (8-31)	17 (5-26)	<0.001

Abbreviations: N, number; AKI, acute kidney injury; GI, gastrointestinal; SBP, spontaneous bacterial peritonitis; UTI, urinary tract infection; IQR, inter-quartile range; MELD, model for end-stage liver disease; SD, standard deviation; WBC, white blood cell count; Hgb, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase

[a]Child-Pugh Class and MELD score are at time of enrollment

[b]Jonckheere-Terpstra trend test

[c]Serum sodium <130 mEq/L

[d]Days from admission until discharge or death

*Universal f-test across 4 groups

Table 3. Summary statistics for urine biomarkers by progression and mortality

	No progression N=95	Progression without death N=44	P-value (vs. no progression)	Progression with death N=39	P-value (vs. no progression)	Overall P*
Tubular injury markers						
NGAL (ng/ml)	76 (17-180)	100 (49-544)	0.02	366 (112-910)	<0.001	<0.001
IL-18 (pg/ml)	15 (15-62)	21 (15-67)	0.38	90 (15-325)	<0.001	<0.001
KIM-1 (ng/ml)	5 (1.8-11.7)	5.8 (2.6-9.5)	0.96	8.3 (4-17.1)	0.004	0.009
L-FABP (ng/ml)	8 (3-19)	12 (6-29)	0.06	38 (13-73)	<0.001	<0.001
Tubular function markers						
FENa (%)	0.32 (0.1-0.89)	0.15 (0.04-0.59)	0.02	0.31 (0.1-0.91)	0.97	0.04
Glomerular injury marker						
Albumin (mg/dL)	21 (5-73)	29 (9-164)	0.23	84 (45-233)	<0.001	<0.001

Abbreviations: NGAL, neutrophil gelatinase associated lipocalin; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid binding protein; FENa, fractional excretion of sodium

*P value from overall ANOVA model.

Supplemental Table 2. Correlation of biomarkers*

	NGAL	IL-18	KIM-1	L-FABP	Albumin
NGAL	1	-	-	-	-
IL-18	0.462	1	-	-	-
KIM-1	0.34	0.396	1	-	-
L-FABP	0.448	0.531	0.426	1	-
Albumin	0.363	0.463	0.526	0.398	1
FENa	-0.118	-0.045	-0.182	-0.002	-0.033

*Correlation calculated using log base 10 of biomarkers

Supplemental Table 3a. Summary statistics for urine biomarkers by progression and mortality in patients not meeting IAC HRS criteria

	No progression N=76	Progression without death N=35	Progression with death N=36	P*
Tubular injury markers				
NGAL (ng/ml)	67 (19-144)	157 (58-690)[a]	406 (117-955)	<0.001
IL-18 (pg/ml)	15 (15-55)	15 (15-85)	132 (15-366)	<0.001
KIM-1 (ng/ml)	5.4 (1.6-10.9)	5.0 (2.5-9.7)	9.4 (4.1-19.1)	0.003
L-FABP (ng/ml)	9 (3-20)	12(6-30)	39 (15-75)	0.002
Tubular function markers				
FENa (%)	0.28 (0.13-0.97)	0.27 (0.12-0.71)	0.38 (0.17-0.91)	0.15
Glomerular injury marker				
Albumin (mg/dL)	21 (4-77)	53 (7-240)	95 (52-243)	0.28

Abbreviations: HRS, hepatorenal syndrome; NGAL, neutrophil gelatinase associated lipocalin; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid binding protein; FENa, fractional excretion of sodium
*P value is for linear trend using SAS PROC Glm "Contrast" statement

Supplemental Table 3b. Summary statistics for urine biomarkers by progression and mortality in patients meeting IAC HRS criteria

	No progression N=15	Progression without death N=7	Progression with death N=3	P*
Tubular injury markers				
NGAL (ng/ml)	132 (66-260)	55 (20-64)[a]	53 (49-102)	0.28
IL-18 (pg/ml)	29 (15-91)	15 (15-65)	27 (15-90)	0.63
KIM-1 (ng/ml)	4.5 (2.7-12.8)	5.9 (0.7-11.3)	5.5 (0.7-14.9)	0.98
L-FABP (ng/ml)	9 (2-17)	11 (2-67)	5 (3-13)	0.68
Tubular function markers				
FENa (%)	0.28 (0.05-0.87)	0.02 (0.02-0.04)	0.05 (0.02-0.09)	0.47
Glomerular injury marker				
Albumin (mg/dL)	23 (15-65)	15 (11-52)	7 (6-41)	0.49

Abbreviations: HRS, hepatorenal syndrome; NGAL, neutrophil gelatinase associated lipocalin; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid binding protein; FENa, fractional excretion of sodium
*P value is for linear trend using SAS PROC Glm "Contrast" statement

Figure 1. Biomarker levels for patients with no progression, progression alone and progression with death

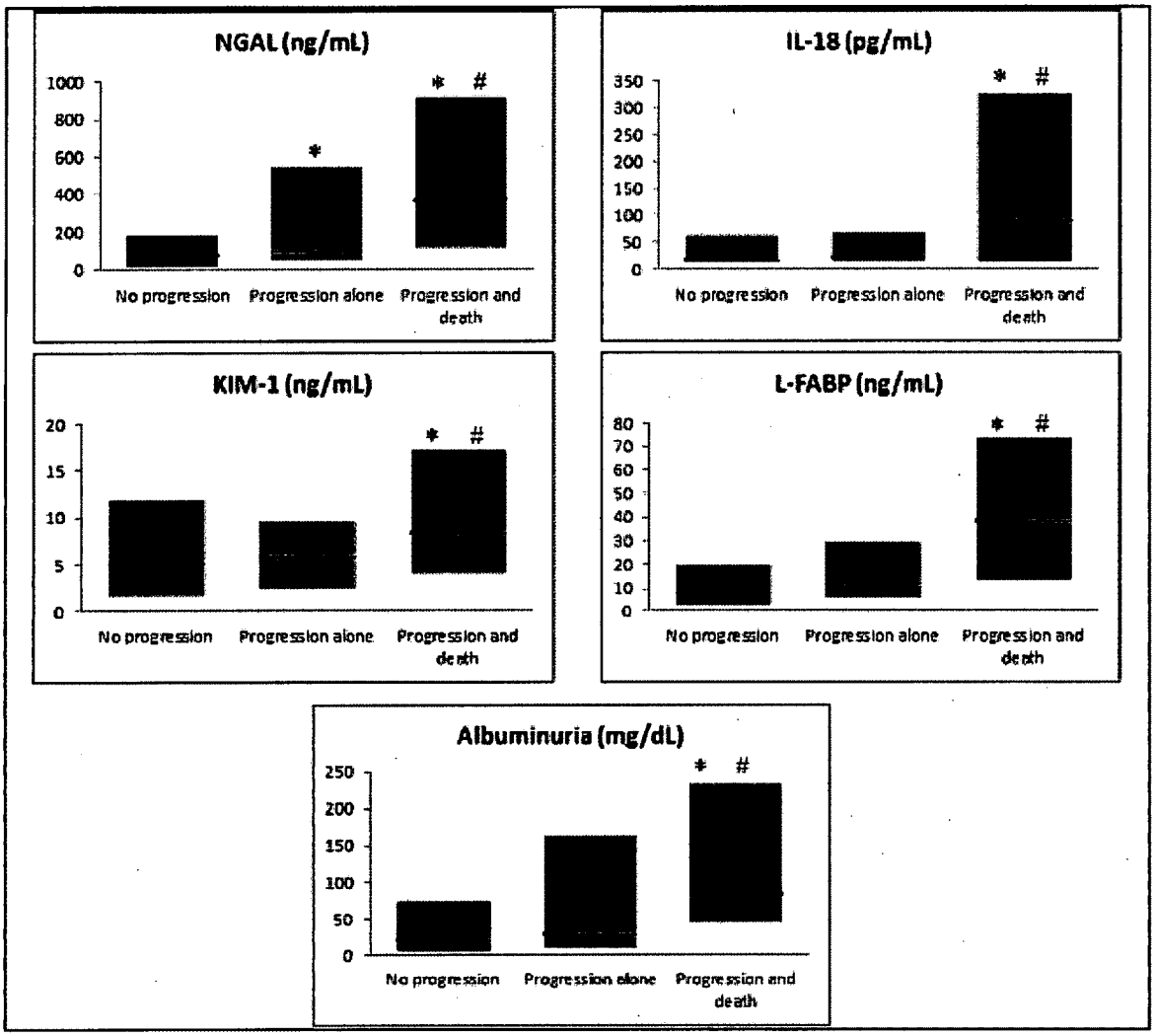
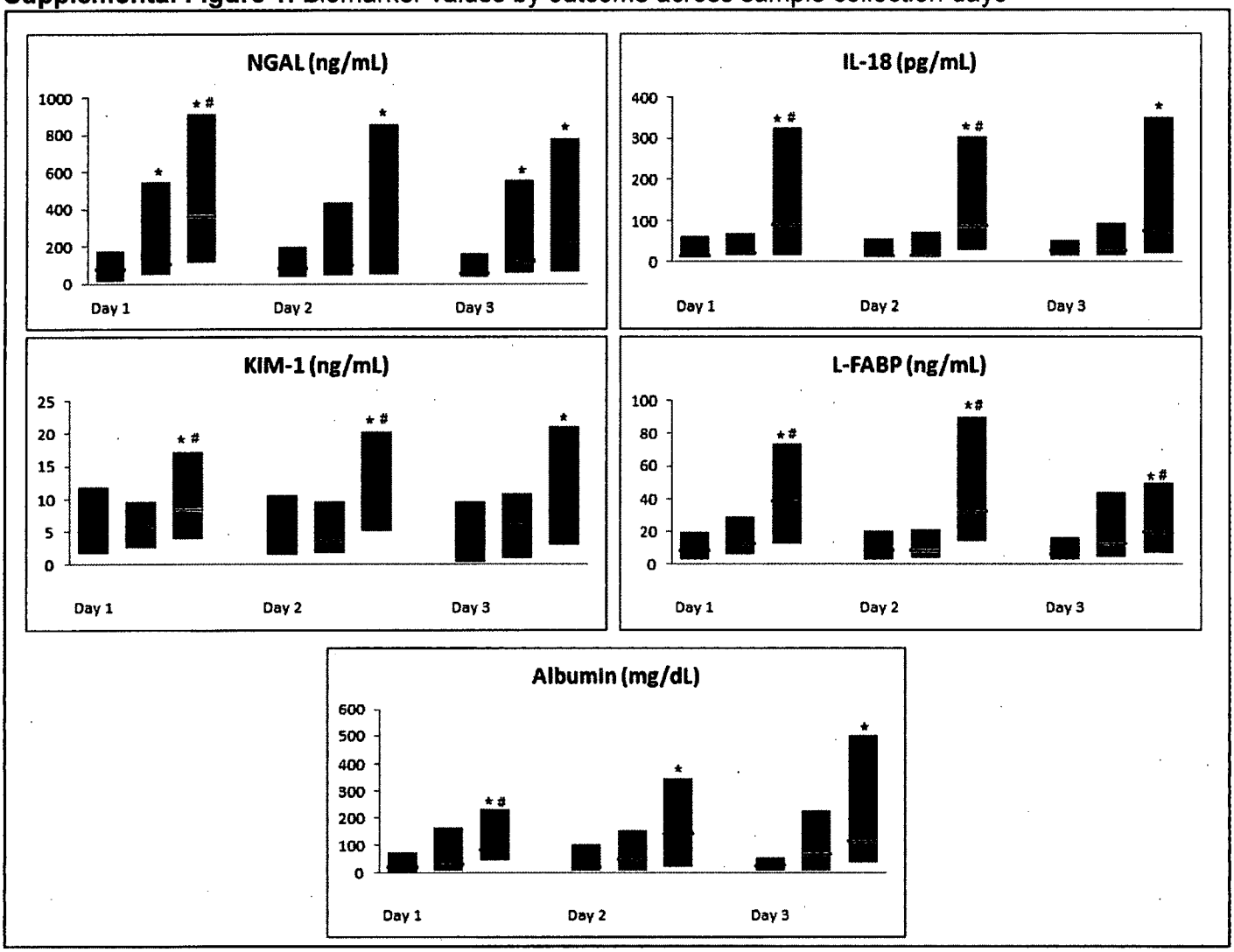


Figure 1. Biomarker values are presented for patients who did not have progression of AKI, those who had progression alone and those with progression and death. Data is presented as box plots with the horizontal black line representing the median and the shaded region the inter-quartile range. Blue bars depict patients without progression (N=95), green bars are patients with progression alone (N=44) and red bars are patients with progression and death (N=35). Groups where the biomarker level is statistically higher than in patients without progression are designed at with "*" and those significantly higher than patients with progression alone are designated with "#".

Supplemental Figure 1. Biomarker values by outcome across sample collection days



Supplemental Figure 1. Daily biomarker values among patients who did not have progression of AKI, those who had progression alone and those with progression and death are presented for the 3 days of sample collection. Data is presented as box-plots with the black line representing the median and shaded region the inter-quartile range. Blue bars depict patients without progression, green bars are patients with progression alone and red bars are patients with progression and death. Groups where the biomarker level is statistically higher that in patients without progression are designed at with "*" and those significantly higher than patients with progression alone are designated with "#".

Table 4. Association of biomarkers with the AKI progression and mortality

Urine Biomarker* (Log transformed)	No Progression vs Progression without Death		No Progression vs Progression with Death	
	Unadjusted RR (95% CI)	Adjusted RR (95% CI)	Unadjusted RR (95% CI)	Adjusted RR (95% CI)**
Tubular injury markers				
NGAL	2.24 (1.25-4.00)	1.70 (0.82-3.54)	5.18 (2.55-10.52)	2.30 (0.94-5.60)
IL-18	1.33 (0.64-2.80)	1.31 (0.55-3.14)	4.92 (2.40-10.09)	4.09 (1.56-10.70)
KIM-1	1.10 (0.66-1.81)	0.95 (0.52-1.72)	2.98 (1.42-6.24)	3.13 (1.20-8.17)
L-FABP	1.71 (0.96-3.06)	1.86 (0.94-3.67)	4.23 (2.20-8.15)	3.43 (1.54-7.64)
Tubular function marker				
FENa	0.59 (0.30-1.16)	0.57 (0.25-1.31)	1.24 (0.65-2.35)	1.25 (0.52-2.97)
Glomerular injury marker				
Albumin	1.26 (0.79-1.99)	1.14 (0.65-1.98)	2.48 (1.48-4.17)	2.07 (1.05-4.10)

Abbreviations: RR, relative risk; CI, confidence interval; NGAL, neutrophil gelatinase associated lipocalin; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid binding protein; FENa, fractional excretion of sodium

*Biomarkers are $\log_{10}$ transformed and RR are per log-unit change**Adjusted for CKD stage + demographics (race, age and sex) + MELD score + Serum Sodium

Table 5. Biomarkers risk discrimination for AKI progression and death

	AUC	Cutoff	Sensitivity	Specificity	Positive Likelihood Ratio	Negative Likelihood Ratio
Tubular injury markers						
NGAL (ng/ml)	0.77 (0.68-0.85)	287	0.62	0.85	4.18	0.45
IL-18 (pg/ml)	0.71 (0.61-0.81)	55	0.64	0.75	2.54	0.48
KIM-1 (ng/ml)	0.66 (0.56-0.76)	3.3	0.90	0.38	1.45	0.27
L-FABP (ng/ml)	0.76 (0.66-0.85)	21	0.67	0.81	3.52	0.41
Tubular function markers						
FENa (%)	0.50 (0.39-0.61)	0.10	0.92	0.20	1.16	0.40
Glomerular injury marker						
Albumin (mg/dL)	0.73 (0.64-0.82)	41	0.79	0.66	2.32	0.32

Abbreviations: NGAL, neutrophil gelatinase associated lipocalin; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid binding protein; FENa, fractional excretion of sodium

Table 6. Net reclassification indices for biomarkers and AKI progression and death*

	Non-Event NRI	Event NRI	Overall NRI (95% CI)
NGAL	0.09	0.14	0.23 (-0.12-0.58)
IL-18	0.32	0.19	0.51 (0.16-0.86)
KIM-1	-0.06	0.19	0.12 (-0.23-0.45)
L-FABP	0.28	0.03	0.31 (-0.04-0.66)
FENa	-0.01	-0.03	-0.04 (-0.41-0.33)
Albumin	0.16	0.19	0.35 (-0.02-0.72)

Abbreviations: NRI, net reclassification index; CI, confidence interval; NGAL, neutrophil gelatinase associated lipocalin; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid binding protein; FENa, fractional excretion of sodium; SE, standard error

*Clinical model includes CKD stage + Demographics (race, age and sex) + MELD score + Serum Sodium

Figure 2a. Association between biomarker panel and AKI progression and death

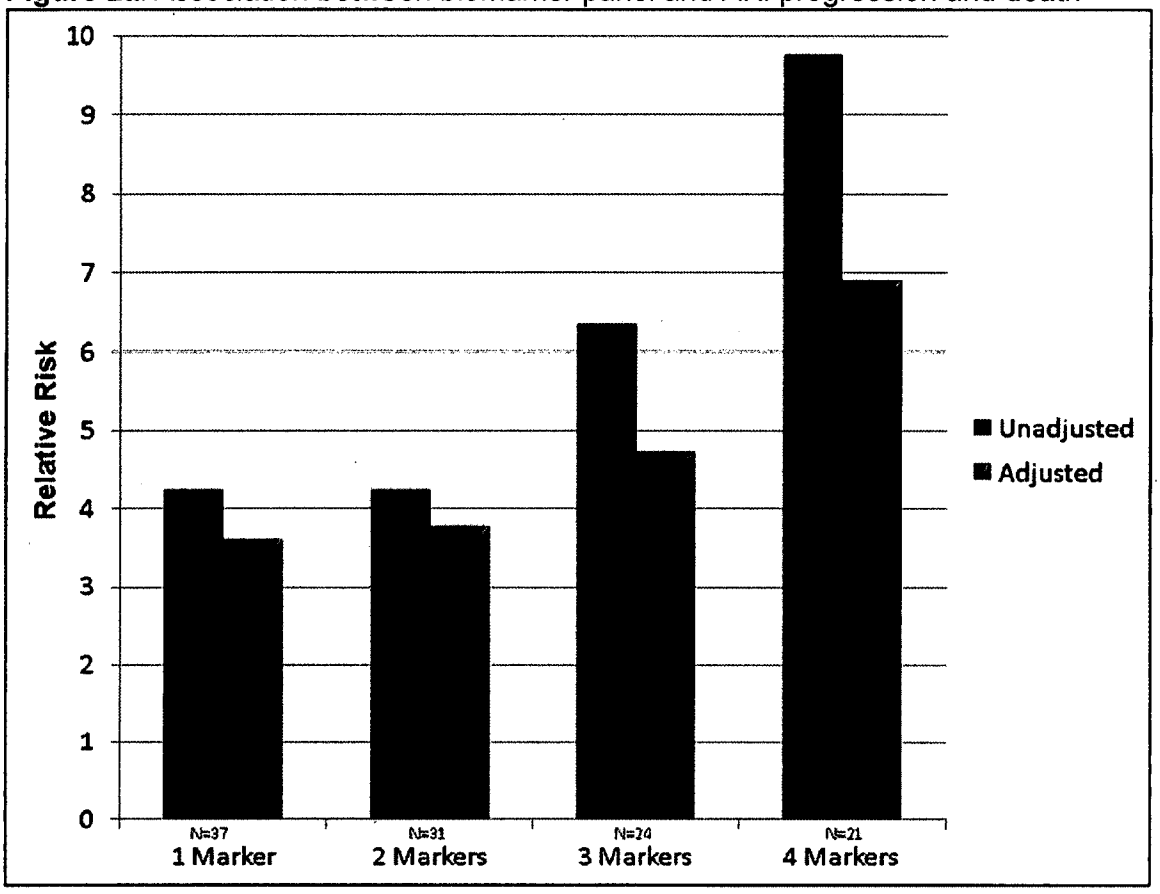


Figure 2a. Figure presents the association between the number of biomarkers above their optimal cutoff for AKI progression and death and the unadjusted and adjusted relative risk for this outcome. All values are relative to having no markers over their cutoffs (N=65). Markers used in the panel include NGAL, IL-18, L-FABP and albumin. Adjusted model is adjusted for CKD stage, demographics (race, age, and sex), MELD score and serum sodium. Confidence intervals for adjusted RR are: 1 marker 1.21-10.67; 2 markers 1.25-11.40; 3 markers 1.62-13.72; 4 markers 2.32-20.46.
Biomarker cutoffs: NGAL, 287 ng/ml; IL-18, 55 pg/mL; L-FABP, 21 ng/mL; Albumin 41 mg/dL

Figure 2b. Association between biomarker elevation and outcomes

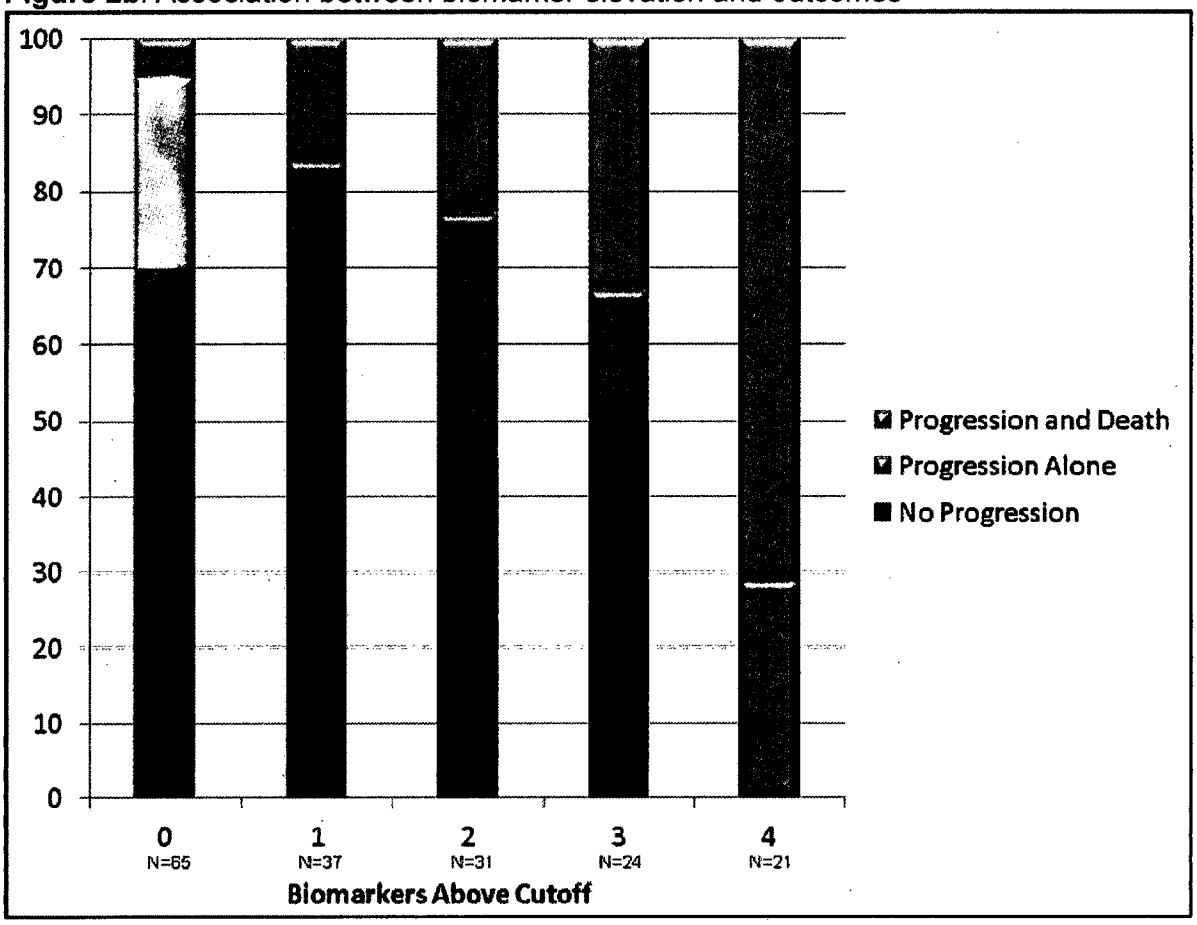


Figure 2b. The percentage of patients without AKI progression or death, progression alone and progression and death by the number of biomarkers of structural injury above their optimal cutoff for prediction of progression and death. Biomarkers included in the panel include NGAL, IL-18, L-FABP and albumin.

Chapter 5. Kidney Biomarkers and Differential Diagnosis of Patients with Cirrhosis and Acute Kidney Injury

Introduction

Acute kidney injury (AKI) is common in patients with cirrhosis, occurring in 20% of hospitalizations[1], and is associated with significant mortality[2-4]. The most common causes of AKI in this setting are pre-renal azotemia (PRA), acute tubular necrosis (ATN) and hepatorenal syndrome (HRS). Despite the overall poor prognosis for patients with cirrhosis and AKI, viable treatments do exist but differ significantly by AKI etiology. PRA should be treated with aggressive volume expansion[5] while such fluid administration is unhelpful and even potentially harmful in patients with ATN[6]. HRS may be reversed with restoration of renal perfusion, either via vasoconstrictor therapy plus intravenous albumin[7] or liver transplant[8]. Patients with severe ATN may reasonably be treated with dialysis. Unfortunately, current diagnostic strategies are often unable to make the challenging yet crucial distinction between structural and functional disease. HRS is diagnosed via the International Ascites Club (IAC) criteria, now set within a more broad classification system of AKI in cirrhosis proposed jointly by the IAC and the Acute Dialysis Quality Initiative (ADQI)[9]. However, these criteria are neither sensitive nor specific and may result in misallocation of scarce resources and potentially harmful unnecessary treatments.

It is obvious that new, objective tests to accurately facilitate the distinction of structural from functional AKI in patients with cirrhosis are urgently needed. There is currently tremendous research interest in novel urinary biomarkers of structural kidney injury for early diagnosis, differential diagnosis and prognosis in AKI[10]. Multiple biomarkers, including neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18) and kidney injury molecule-1 (KIM-1) are able to distinguish structural from functional causes of AKI in numerous clinical settings[11-13]. Such biomarkers, which are specifically reflective of frank structural injury, may be particularly well suited to untangle the frequently vexing diagnostic distinction between ATN and

HRS. However, in patients with cirrhosis, where kidney biopsies are uncommonly performed, the very lack of an effective existing diagnostic test or criteria makes the development of new tests challenging as the gold standard against which new tests are to be compared is known to be flawed. Patients whose AKI rapidly resolves can be assumed to have had PRA. However, in patients with progressive AKI, where accurately distinguishing etiology is most therapeutically critical, confidently determining the differential diagnosis it can be extremely challenging. The IAC criteria for HRS are useful for their simplicity in that they can be employed at the bedside to diagnose the etiology of AKI without requiring knowledge of the patient's entire hospital course but often lack the granularity of data required for distinguishing structural from functional disease. Alternatively, retrospective adjudication by expert clinicians with access to data on the entirety of the course of a patient's AKI, while obviously not applicable for point of care diagnosis, provides a more robust gold standard for the development of new objective tests which may then themselves be applied at the bedside.

While biomarkers hold tremendous promise to clarify the diagnostic muddle of AKI in cirrhosis, it is unlikely any will result in a clear "positive" or "negative" cut off, tests results will need to be interpreted in light of the overall clinical picture. Similarly, it may be that a combination of multiple markers is more informative than any alone. The few previous studies of AKI biomarkers in cirrhosis have only looked at one marker[14-16], used IAC[14,15] or unconventional criteria[16] as the gold standard and did not explore how results could be incorporated into clinical decision making. We have conducted a prospective, multi-center study of patients with cirrhosis and AKI that measured multiple urinary biomarkers, including NGAL, IL-18, KIM-1, liver-type fatty acid binding protein (L-FABP), albumin and fractional excretion of sodium (FENa). In this analysis, we assess the ability of these biomarkers to improve the differential diagnosis of patients with clinically adjudicated etiologies of AKI. Subsequently, we have employed likelihood ratios to demonstrate how biomarkers results, through the identification of patients with ATN, can clarify uncertain clinical diagnoses.

Materials and Methods

Study design

The details of the cohort and study design have been described previously[4]. This prospective, multi-center observational cohort study was conducted over 29 months between 2009 and 2011 at four tertiary care academic centers in the US. Eligible patients were admitted with AKI (see "Definition") or developed it during the course of the hospitalization. Inclusion criteria included a known diagnosis of cirrhosis (see "Definitions"), age ≥ 18 years, and availability of a documented baseline serum creatinine. Exclusion criteria included prior kidney or liver transplant, advanced chronic kidney disease (baseline creatinine > 4.0 mg/dL), acute or chronic renal replacement therapy at the time of enrollment, clinically estimated life expectancy < 3 days, confirmed pregnancy and other known causes of renal insufficiency such as glomerulonephritis or urinary obstruction. Consent was obtained from all patients or their surrogate decision maker. If a patient was unable to provide written consent and a surrogate was unavailable, a urine specimen was nevertheless collected. Over the following seven days, delayed consent was sought from either patient or surrogate. If consent could not be obtained during this period, the urine sample was discarded. All consecutive eligible patients identified during screening were approached for enrollment.. The study was approved by the institutional review board at each of the participating institution.

Sample Collection and Biomarker Measurement

A fresh 10-ml urine sample was collected daily for three days either via clean catch or Foley catheter tubing. Samples were immediately refrigerated and then centrifuged at 5000 x *g* for 10 minutes at -4°C. Aliquots of 1 ml of supernatant were subsequently stored within 6 hours of collection in cryovials at -80°C for NGAL, IL-18, KIM-1, L-FABP, albumin, sodium and creatinine measurements. No additives or protease inhibitors were utilized. All biomarkers were

measured from frozen aliquots that did not undergo any additional freeze-thaw cycles. Laboratory measurements were performed by personnel blinded to patient information. Sekisui Diagnostics LLC developed assays for KIM-1 and L-FABP. Capture antibodies were bound to Multi-Assay 96 well plates (MesoScale Discovery [MSD], Gaithersburg, MD) and detection antibodies were biotinlyated. Signal generation relied on strepavidin coupled Sulfo-Tag (MSD). The Sulfo-Tag includes ruthenium(II)-tris-bipyridine, which in combination with a triproplyamine read buffer generates an electrochemical signal detected by a Sector Imager 2400™ (MSD). Sekisui Diagnostics LLC also developed the rabbit anti-KIM-1 antibodies (for capture and detection) and recombinant hKIM-1 (for standards and controls). CMIC (Tokyo, Japan) supplied monoclonal antibodies and rec hL-FABP standards. The detection range for KIM-1 is .056–60 ng/mL while L-FABP is .057–400 ng/mL. The intra-assay coefficient of variation is ≤10% for both assays. ELISA methods, coefficient of variation and the detection ranges were as described previously for the measurement of NGAL[17] and IL-18[18]. Urine creatinine was measured by the modified Jaffe reaction.

Adjudication:

Adjudication of the cause of AKI was performed by a committee of two nephrologists and one hepatologist after the patient was discharged or expired. Adjudicators were selected to provide a breadth of experience and primary site of clinical practice (University *vs* Veterans Administration). Only those patients whose AKI progressed to a higher AKIN stage were adjudicated. This decision was made for reasons of practicality and because the greatest diagnostic confusion is typically seen in patients whose AKI continues to progress despite initial standard management. If patients who presented with Stage 3 AKI by creatinine criteria but not requiring renal replacement therapy subsequently required dialysis, this was considered as progression. Adjudicators were provided with a standardized data form containing key variables related to the patients' medical history, hospital presentation, general medical and cirrhosis

specific hospital events, medical therapies and renal function. Additionally, data were provided detailing vital signs and fluid balance for a period of 10 days surrounding biomarker collection. Options for diagnosis included PRA, HRS and intrinsic kidney disease, to be specified as ATN or other pathologies. Final diagnosis was contingent on the agreement of at least two adjudicators. Adjudicators were blinded to measurements of NGAL, IL-18, KIM-1, L-FABP and albumin but had access to urine sodium values if these were measured in the course of clinical care.

Variables

Independent Variables

Cirrhosis- Patients were eligible who carried an existing documented diagnosis of cirrhosis based on liver biopsy, when available, or on a combination of clinical, biochemical, imaging and endoscopic findings.

AKI- AKI was defined as arise in creatinine of 0.3 mg/dL or 50% from baseline as recommended by a working group composed of members of the IAC and the ADQI who based this cut-off on Stage 1 of the acute kidney injury network (AKIN) criteria[19].

Baseline serum creatinine- Baseline serum creatinine was defined as the most recent stable measurement within a year prior to admission for the index hospitalization. The use of outpatient values for establishing baseline creatinine has been shown to result in less misclassification of AKI incidence, severity and prognosis compared to utilizing hospital admission, hospital nadir or imputed values[20]. When possible, outpatient measurements were utilized though values were also used from previous admissions not complicated by AKI. The median and inter-quartile range (IQR) for the interval between the creatinine utilized for baseline and hospital admission was 26 (9-73) days. In rare cases, patients without an outpatient measurement were included in the analytic cohort if, prior to onset of AKI, they manifested at least 5 initial days from admission

of stable values within the normal creatinine range. In these instances, the creatinine at admission was considered the baseline.

Other variables- Glomerular filtration rate (GFR) was estimated via the CKD-EPI equation using the baseline creatinine value[21]. Chronic kidney disease was defined as estimated GFR < 60 ml/min/1.73m^2 present for at least 3 months. Model of end-stage liver disease (MELD) and Child-Pugh scores were calculated on the day of first sample collection.

Outcomes- Our primary outcome was AKI diagnosis. Patients were diagnosed with PRA either via adjudication in those patients whose AKI progressed or by the designation of PRA in patients whose AKI did not progress and whose serum creatinine returned to within 25% of baseline within 48 hours of developing AKI. HRS and ATN were diagnosed via adjudication in patients with progressive AKI.

Statistics

Categorical variables were expressed as proportions and compared using Chi-square and Fisher's exact test, as appropriate. Normally or near-normally distributed variables were reported as means with standard deviations (SD) and compared by Student's *t*-test. Non-normally distributed continuous variables were reported as medians with IQR and compared by the Wilcoxon rank sum test. Normality was assessed using the Kolmogorov-Smirnov test. NGAL values were bounded at an upper limit of 1000 ng/mL with no lower bound. All patients with values above 1000 ng/mL were assigned a value of 1000. KIM-1 was bounded at an upper limit of 60 ng/ml and a lower limit of 0.056 ng/ml. L-FABP was bounded at an upper limit of 400 ng/ml and a lower limit of 0.57 ng/ml. IL-18 did not have a bounded upper limit but the lower limit of detection for the assay was 25 pg/mL. All patients below this threshold were assigned a value of 15 pg/mL.

The primary analysis evaluated biomarkers ability to identify patients with ATN. Areas under the curve (AUC) with 95% confidence intervals (CI) were calculated to evaluate

biomarkers for risk discrimination. Optimal cutoffs were determined for diagnosing ATN versus non-ATN. Utilizing these cutoffs, biomarker performance was assessed through the calculation of sensitivity, specificity and positive and negative likelihood ratios. Likelihood ratios were then applied to examples wherein pretest probability for ATN is converted to posttest. Those biomarkers whose levels differed significantly between diagnoses were selected for a panel and relative risks for ATN were calculated based on number of markers above their optimal cutoffs. To determine internal validity of the results, a leave-10-out cross validation was performed using SAS Proc Surveyselect. In a secondary analysis, biomarkers were also evaluated for their ability to distinguish the three distinct diagnoses of PRA, HRS and ATN. A 2-sided $p<0.05$ was considered significant for all analysis. Statistical analysis was performed using SAS, version 9.2 (SAS Institute, Cary, NC). The conditional probability curves were constructed using the spreadsheet devised by MacEneaney and Malone[22].

Results

A total of 188 patients with cirrhosis and AKI with available urinary biomarkers were enrolled in the study. Of these, 83 experienced progression of their AKI. The distribution of adjudicators diagnoses is shown in **Supplemental Table 1**.Thirty-nine (53%) patients were diagnosed with ATN, 19 (26%) with PRA and 16 (22%) with HRS. 36 additional patients were assigned a diagnosis of PRA due to their creatinine returning to within 25% of baseline within 48 hours. The breakdown of patient diagnosis is shown in **Figure 1**. Baseline demographic, clinical and laboratory data for all adjudicated patients and for those with and without ATN are shown in **Table 1**. There was no difference in cirrhosis etiology or previous complications of cirrhosis between groups. The reason for admission was similar between the two groups excepting jaundice and infections other than spontaneous bacterial peritonitis which were more common in patients diagnosed with ATN. Median baseline estimated GFR was lower in patients without ATN than in those with ATN (67 vs 84ml/min/1.73m^2) though this did not reach statistical

significance (P = 0.09). Serum creatinine at the time of sample collection differed significantly between groups and was higher in patients diagnosed with ATN. Patients with ATN had more advanced cirrhosis as assessed both by the model for end-stage liver disease (MELD) score (31 *vs* 24) and Child-Pugh (11 *vs* 10). Though intravenous albumin administration was near ubiquitous in all groups, patients adjudicated with ATN were treated more frequently with midodrine and octreotide. The number of IAC criteria fulfilled for the diagnosis of HRS (5/6) was identical between the two groups.

Biomarker values for patients diagnosed with ATN and non-ATN are shown in **Table 2a** and **Figure 2a**. Values for patients with PRA, HRS and ATN are presented in **Table 2b** and, for albumin and FENa, the only widely commercially available of the biomarkers under study, in **Figure 2b**. Urine samples for biomarker analysis were collected a median of two days following onset of AKI and a median of 26 days from the establishment of patients' baseline creatinine. Biomarkers were collected over three consecutive days. The values for all biomarkers did not differ over the days of sample collection and results from the first day of collection are presented. Sensitivity analysis of results using raw biomarker values and those corrected for urine creatinine showed minimal variation (data not shown). To facilitate cross-study comparison of results with published literature, NGAL, IL-18, KIM-1, L-FABP and albumin are therefore presented as raw values. Median values for NGAL, IL-18, KIM-1, L-FABP and albumin were significantly higher in patients adjudicated with ATN *vs* non-ATN. FENa did not differ between the two groups. When assessing the three distinct diagnoses, all biomarkers except FENa were able to distinguish ATN from PRA but only NGAL, IL-18, albumin and FENa differed significantly between patients with ATN and HRS. Critically, FENa was the only biomarker to distinguish HRS from PRA, 0.1% *vs* 0.27%, p=0.01.

AUC's and optimal cutoffs of each biomarker for the diagnosis of ATN *vs* non-ATN are depicted in **Table 3**. AUCs derived from leave-10-out cross validation are presented alongside those for the entire cohort. The potential practical utility of the three biomarkers with the best

discrimination, NGAL, IL-18 and albumin, as well as FENa, when incorporated into clinical decision making is demonstrated through the application of likelihood ratios to determine post-test probabilities for ATN (**Figure 3**). The post-test probability is calculated using positive and negative likelihood ratios assuming the biomarker level is above or below the optimal cutoff, respectfully. For example, in a patient with a pre-test probability of 40% for the diagnosis of ATN, the finding of a urinary NGAL level above 365 ng/mL would raise the post-test probability to 76%. Similarly, the finding of FENA below 0.1% would lower the post-test probability for ATN to 16%. To examine the utility of biomarkers in combination, the four biomarkers which distinguished ATN from non-ATN with a p-value of < 0.01 (NGAL, IL-18, L-FABP and albumin) were selected and the relative risk for ATN was calculated for successive numbers of these biomarkers above their optimal cutoff, relative to none of the four being elevated (**Table 4**). The proportion of patients diagnosed with PRA, HRS and ATN with increasing numbers of these biomarkers above their optimal cutoff for ATN cutoff is shown in **Figure 4**.

Discussion

In this study, we have demonstrated that multiple urinary biomarkers of kidney injury have the ability to distinguish ATN from non-ATN in patients with cirrhosis and progressive AKI. Patient diagnoses were rigorously established via expert adjudicators based upon clinical data and blinded to biomarker values. While injury biomarkers were highest in patients with ATN, levels were similar between patients with PRA and HRS. FENa in patients with ATN was significantly higher than in those with HRS but did not differ from PRA. Using likelihood ratios, we have shown that injury biomarkers including urine albumin have the potential to significantly modify clinicians' post-test probability for the diagnosis of ATN in a patient with cirrhosis and AKI.

Distinguishing patients with ATN from those with HRS or PRA is often clinically challenging but carries profound significance for both patient care and research. While the

diagnostic criteria proposed by the IAC[23] are consistent with our understanding of the pathophysiology of HRS, patients with ATN can, and often do, fulfill all six criteria. Indeed, the median number of IAC criteria fulfilled was 5/6 across all three diagnoses. The inability to make this distinction is critical as for HRS, unlike much of AKI, there exists specific therapies tailored to the physiology of the renal dysfunction. Reversal of cirrhotic physiology and restitution of renal blood flow, either via vasoconstrictors[7] or liver transplantation[8], has been shown to reverse AKI. However, without objective tests, there is evidence that, despite clinicians' best efforts, significant misclassification occurs. 50% of patients treated with terlipressin do not experience renal recovery while 12% of patients receiving placebo do recover[7]. It is likely many of these may, in fact, have ATN. Patients who have suffered AKI for greater than 6-8 weeks are thought to be unlikely to spontaneously recover renal function following liver transplant and are therefore listed for a combined transplant[24,25]. However, 24% of patients with cirrhosis requiring dialysis for 8-12 weeks prior to solitary liver transplant recover renal function post-operatively[26] while 27% of patients who receive a combined liver-kidney transplant have a measured native kidney GFR of > 30ml/min 1 year post-operatively[27].

The key distinction is not so much whether a patient with cirrhosis and AKI is dichotomously labeled as "having" ATN or HRS, but rather determining if their acute drop in GFR is primarily due to frank structural injury or a functional failure of filtration. Kidney injury biomarkers, which are efficacious for differential diagnosis of renal dysfunction in multiple clinical settings[11-13], would seem to hold particular promise in patients with cirrhosis and AKI where both functional and structural diseases can manifest with severe, progressive AKI. While the performance of novel biomarkers in our cohort is indeed encouraging, the ability of albumin to identify patients with ATN and FENa to distinguish HRS from ATN and PRA is particularly significant as these point-of-care tests are currently readily available. The utility of FENa in patients with cirrhosis and AKI has often been dismissed as the majority of patients fall below 1%, regardless of whether their AKI is structural or functional. It appears however that the

intense sodium avidity characteristic of HRS may in fact be identifiable with FENa. Though further research is required to validate the specific cutoffs, reappraisal of albumin and FENa in patients with AKI and cirrhosis has the potential to immediately impact challenging diagnostic cases.

Critically, the discriminatory performance of new diagnostic tests is contingent not only on the sensitivity and specificity of the test under investigation but also those of the gold standard against which it is compared. A new biomarker that is in fact 100% sensitive and specific for a disease state can appear to function poorly when evaluated against an even modestly fallible gold standard[28]. Given the limitations of the IAC criteria, we therefore chose to use expert, retrospective clinical adjudication for our gold standard diagnoses.

Despite utilizing different diagnostic methods, other investigators examining biomarkers in patients with cirrhosis and AKI have found results similar to ours. NGAL levels in our study were similar to those seen by these investigators for patients with HRS and ATN, though we found higher levels in patients with PRA, 78 ng/ml (16-206), than those seen by Verna et al., 20 ng/ml (15-45)[14], or Fagundes et al. 30 µg/g (20-59)[15]. IL-18 has also shown promise, demonstrating an AUC of 0.88 to distinguish ATN from function AKI in 94 ICU patients with cirrhosis and AKI[16].

While HRS is classically considered as a purely functional disease, it is interesting to note that both Verna et al. and Fagundes et al. found NGAL levels in patients with HRS to be significantly higher than in those with PRA. The finding of injury biomarkers in HRS as intermediary between PRA and ATN is potentially consistent with recent speculation that HRS may in fact contain some degree of structural injury[29]. Given this spectrum of pathology, there is likely to be overlap in injury marker levels between HRS and mild ATN. While the presence of elevated injury markers in our study is consistent with ATN, their absence does not necessarily imply HRS. It appears then that the most immediate clinical application of our findings regarding biomarkers of injury will be to identify significant ATN, not to identify HRS or PRA. Injury

biomarkers can therefore serve not to identify those patients who *should* receive HRS specific therapy but, instead, to *exclude* those with significant structural injury who are unlikely to respond or benefit from treatment, sparing potential unnecessary side effects and optimizing resource utilization and organ allocation. The tantalizing potential of FENa to identify HRS will require validation in future studies.

For this reason, we sought to demonstrate the utility of using likelihood ratios for ruling in or out ATN. Likelihood ratios estimate how much clinicians should shift clinical suspicion for a disease based on a given test result and are derived from a test's sensitivity and specificity. Incorporating biomarker results through the use of cutoffs and likelihood ratios can greatly assist clinicians confronted with diagnostic and therapeutic conundrums. For example, when deciding whether to utilize vasoconstrictors in a patient with cirrhosis and AKI where the diagnosis is very unclear and there is a 50% probability of ATN, the finding of NGAL above or albumin below their cutoffs strongly re-stratifies them in favor of, 82% probability, or away from, 25%, the diagnosis of ATN, respectively. If the pre-test probability was higher or lower, the post-test probabilities would be even more definitive. Irrespective of pre-test probability, 91% patients with cirrhosis and AKI with NGAL, IL-18, L-FABP and albumin above their respective cutoffs had ATN while only 7% of those without any marker positive did so. Our findings also hold tremendous promise for research where the use of biomarkers to identify ATN would allow investigators enrolling patients for a trial of new HRS therapy to exclude patients with ATN, avoiding misclassification bias and improving study power.

Our study has several strengths. The use of rigorous clinical adjudication provides the best possible diagnostic gold standard outside of biopsy, which is rarely performed in this setting. By evaluating multiple biomarkers, we have demonstrated that a panel of markers may be most efficient for identifying ATN. Our findings will require validation in an external cohort. However, the strikingly consistent AUC point estimates seen with leave-10-out cross validation indicates robust internal validity. Finally, we chose to adjudicate only those patients with

progressive AKI. Such patients are typically the most challenging for clinicians with treatment decisions being both critical and fraught with confusion. Indeed, while at least 2 out of 3 adjudicators agreed on 74/76 (97%) patients, there was 3 out of 3 agreement in only 37/76 (49%), emphasizing again the critical need for objective diagnostic tests. Despite these strengths, our study is not without limitations. Though clinical adjudication offers the best possibility of accurately phenotyping patients, the true gold standard is a kidney biopsy. However, while studies suggest that biopsies can safely be performed in many patients with cirrhosis[30], they are rarely executed for concerns of bleeding risk. Finally, as an observational study, treatment of patients was not standardized and thus we could not assess the relationship between biomarker levels and treatment response.

Conclusions

Multiple urinary biomarkers show the ability to distinguish clinically adjudicated ATN in patients with progressive AKI and cirrhosis. Further research is required to determine if such biomarkers can improve outcomes by more accurately phenotyping the pathophysiology of AKI and thereby triaging only those patients with primarily functional disease to HRS specific treatments. Ultimately, a panel combining markers for both vasoconstriction and structural injury may provide the greatest granularity for determining where on the spectrum of functional to structural disease a patient with cirrhosis and AKI lies.

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Supplemental Table 1. Breakdown of individual adjudicator diagnoses

	PRA N=19	HRS N=16	ATN N=39
Progressors			
Adjudicator 1 (Nephrology)	16	14	44
Adjudicator 2 (Hepatology)	19	18	37
Adjudicator 3 (Nephrology)	17	17	40
Non-progressors			
Adjudicator 1 (Nephrology)	6/6	0	0
Adjudicator 2 (Hepatology)	6/6	0	0
Adjudicator 3 (Nephrology)	6/6	0	0

Abbreviations: PRA, pre-renal azotemia; HRS, hepatorenal syndrome; ATN, acute tubular necrosis; AKI, acute kidney injury

Figure 1. Process for determination of differential diagnosis

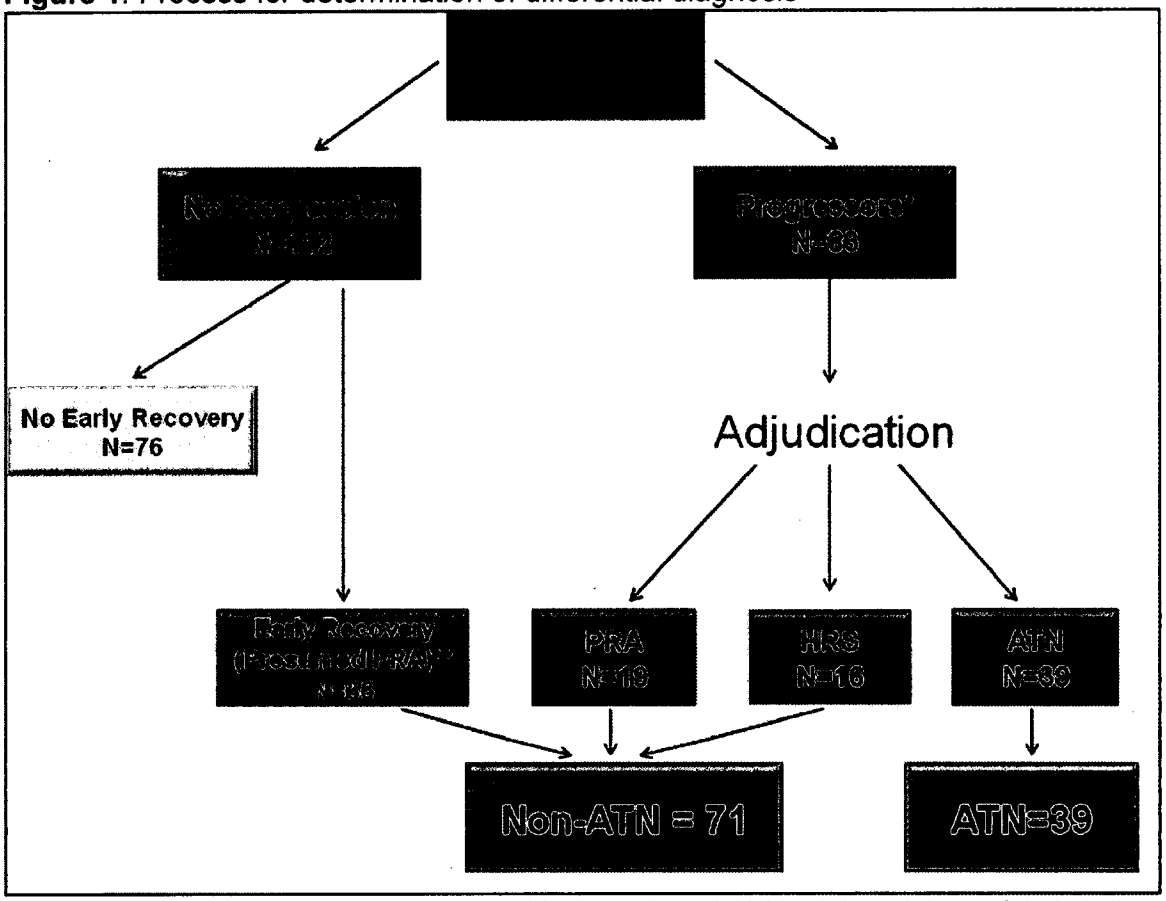

Figure 1. The process by which patients with cirrhosis and AKI had the etiology of their AKI determined.

*7 patients who progressed were enrolled during the pilot phase of the study and had incomplete data collection. These patients were excluded from adjudication to avoid information bias. In addition, 2 patients who did not have 2/3 adjudicator diagnostic agreement were excluded. Of the remaining 74, 3/3 adjudicators agreed for 37 patients and 2/3 for 37 patients.

**Of the non-progressors with rapid recovery who were assigned a diagnosis of PRA, 6 (17%) were additionally adjudicated and all 6/6 were adjudicated as having PRA.

Table 1. Baseline and clinical characteristics of all patients and those with and without ATN

	Total N = 110	Not ATN N=71	ATN N=39	P*
Age in years - mean ± SD	55.3 ± 9.8	56.4 ± 9.4	53.3 ± 10.3	0.24
Male sex – *n* (%)	76 (69)	48 (68)	28 (72)	0.65
BMI – median (IQR)	30.4 (25.6-35)	28.1 (25-33.3)	32.2 (28.6-36.1)	0.61
Race – n (%)				
White	83 (75)	56 (79)	27 (69)	0.26
Black	13 (12)	7 (10)	6 (15)	0.39
Hispanic	12 (11)	7 (10)	5 (13)	0.75
Diabetes – *n* (%)	30 (27)	18 (25)	12 (31)	0.54
Active Cancer – *n* (%)	13 (12)	8 (11)	5 (13)	0.81
Renal function				
Baseline creatinine mg/dL – median (IQR)	1 (0.8-1.3)	1.1 (0.8-1.3)	1.0 (0.7-1.2)	0.27
CKD[a]	30 (27)	21 (30)	9 (23)	0.46
Baseline GFR ml/min – median (IQR)	72 (58-98)	67 (53-95)	84 (60-102)	0.09
Proteinuria – *n* (%)[b]	11 (10)	5 (7)	6 (15)	0.16
Creatinine mg/dL – median (IQR)[c]	2.1 (1.5-3.5)	1.8 (1.4-2.5)	3.3 (2.3-4.1)	<0.001
BUN mg/dL – median (IQR)[c]	44 (29-62)	41 (27-54)	49 (33-72)	0.06
Peak creatinine mg/dL - median (IQR)	2.6 (1.8-4.2)	2.2 (1.6-3.4)	4.1 (2.9-5.2)	<0.001
Dialysis – *n* (%)	27 (25)	9 (13)	18 (46)	<0.001
Cirrhosis etiology – n (%)				
Alcohol	30 (27)	19 (27)	11 (28)	0.87
Alcohol and HCV	30 (27)	22 (31)	8 (21)	0.24
HCV	20 (18)	14 (20)	6 (15)	0.57
NASH	11 (10)	9 (13)	2 (5)	0.32
Cryptogenic	6 (5)	3 (4)	3 (8)	0.66
Autoimmune	8 (7)	4 (6)	4 (10)	0.37
Other	5 (5)	0 (0)	5 (13)	0.004
Previous complications of cirrhosis – n (%)				
Ascites	83 (76)	57 (80)	26 (68)	0.17
Hepatic encephalopathy	72 (65)	49 (69)	23 (59)	0.29
Variceal bleed	24 (22)	18 (25)	6 (15)	0.23
SBP	13 (12)	10 (14)	3 (8)	0.37
Reason for admission – n (%)				
Hepatic encephalopathy	23 (21)	17 (24)	6 (15)	0.29
Refractory ascites/edema	16 (15)	13 (18)	3 (8)	0.16
Abdominal pain	11 (10)	7 (10)	4 (10)	0.95
AKI	14 (13)	10 (14)	4 (10)	0.77
GI bleed	10 (9)	6 (8)	4 (10)	0.75
Jaundice	8 (7)	2 (3)	6 (15)	0.02
Infection other than SBP	3 (3)	0 (0)	3 (8)	0.04
SBP	3 (3)	1 (1)	2 (5)	0.27
Other	22 (20)	13 (18)	9 (23)	0.55
Cirrhosis severity[c]				
Child-Pugh Class - n (%)				0.006
A	3 (3)	3 (4)	0 (0)	
B	35 (32)	29 (41)	6 (15)	
C	72 (65)	39 (55)	33 (85)	

Child-Pugh score – median (IQR)	10 (9-12)	10 (8-12)	11 (10-12)	0.005
MELD score – mean ± SD	26.4 ± 9.5	24 ± 7.9	31 ± 10.5	<0.001
Sodium – mean ± SD	133 ± 6	133 ± 5	134 ± 6	0.78
Hyponatremia[d] – *n* (%)	33 (30)	22 (31)	11 (28)	0.76
MAP (max) - mmHg mean ± SD	85 ± 14	83 ± 12	90 ± 17	0.05
MAP (min) - mmHg mean ± SD	67 ± 12	68 ± 11	67 ± 14	0.43
IAC criteria – median (IQR)	5 (4-5)	5 (4-5)	5 (4-5)	0.43
Hospital complications – n (%)				
HEENC	58 (53)	34 (48)	24 (62)	0.17
Ascites	91 (83)	58 (82)	33 (85)	0.70
SBP	22 (20)	9 (13)	13 (33)	0.01
EVB	10 (9)	7 (10)	3 (8)	1.00
Pneumonia	19 (17)	6 (8)	13 (33)	0.001
Bacteremia	18 (16)	8 (11)	10 (26)	0.05
GI Bleed	26 (24)	13 (24)	13 (33)	0.08
UTI	27 (25)	16 (23)	11 (28)	0.50
Therapies – n (%)				
Albumin	97 (88)	61 (86)	36 (92)	0.32
Midodrine	53 (48)	29 (41)	24 (62)	0.04
Octreotide	56 (51)	28 (39)	28 (72)	0.001

Abbreviations: N, number; PRA, pre-renal azotemia; HRS, hepatorenal syndrome; ATN, acute tubular necrosis; SD, standard deviation; BMI, body mass index; IQR, inter-quartile range; CKD, chronic kidney disease; GFR, glomerular filtration rate; BUN, blood urea nitrogen; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis; AKI, acute kidney injury; GI, gastrointestinal; SBP, spontaneous bacterial peritonitis; MELD, model for end-stage liver disease; MAP, mean arterial pressure; HEENC, hepatic encephalopathy; EVB, esophageal variceal bleed; UTI, urinary tract infection

[a]CKD is defined as estimated GFR 60 < ml/min by CKD-EPI equation

[b]Microalbuminuria (30mg/dL) or greater on dipstick or quantitative measurement prior to admission

[c]BUN, creatinine and indices of cirrhosis severity are on day of sample collection

[d]Serum sodium <130 mEq/L

*Universal f-test

Table 2a. Summary statistics for urine biomarkers in patients with and without ATN

	Non-ATN N=71	ATN N=39	p
Tubular injury markers			
NGAL (ng/ml)	59 (22-203)	565 (76-1000)	<0.001
IL-18 (pg/ml)	15 (15-65)	124 (15-325)	<0.001
KIM-1 (ng/ml)	5.1 (2.1-10.7)	8.4 (4.1-18.3)	0.02
L-FABP (ng/ml)	10 (4-19)	27 (8-103)	0.001
Tubular function marker			
FENa (%)	0.24 (0.06-0.48)	0.31 (0.12-0.65)	0.29
Glomerular injury marker			
Albumin (mg/dL)	21 (4-70)	92 (44-253)	<0.001

Abbreviations: ATN, acute tubular necrosis; NGAL, neutrophil gelatinase associated lipocalin; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid binding protein; FENa, fractional excretion of sodium

Figure 2a. Biomarker values for patients with and without ATN

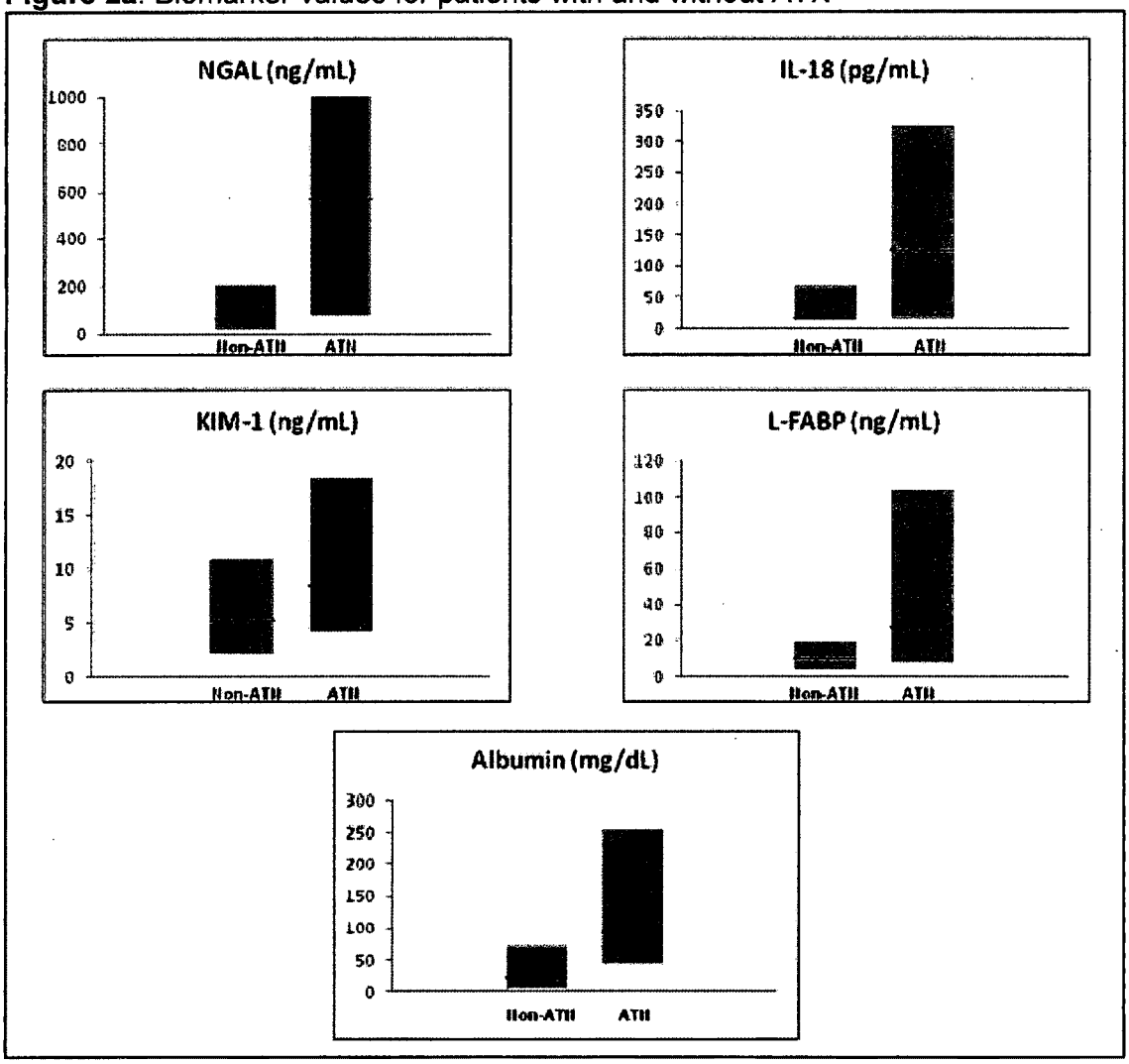

Figure 2a. Biomarker values are shown for patients with and without ATN. Dark horizontal lines represent medians while the shaded boxes represent interquartile ranges. Biomarkers values are statistically significantly higher in patients with ATN for all biomarkers.

Table 2b. Summary statistics for urine biomarkers by diagnosis

	PRA N=55	HRS N=16	ATN N=39	p
Tubular injury markers				
NGAL (ng/ml)	54 (17-180)	115 (51-373)	565 (76-1000)***, ##	<0.001
IL-18 (pg/ml)	15 (15-49)	37 (15-90)	124 (15-325)***, #	<0.001
KIM-1 (ng/ml)	4.4 (1.8-11.7)	7.6 (4.5-10.1)	8.4 (4.1-18.3)**	0.03
L-FABP (ng/ml)	9 (4-18)	14 (6-20)	27 (8-103)***	0.002
Tubular function marker				
FENa (%)	0.27 (0.13-0.58)	0.10 (0.02-0.23)**	0.31 (0.12-0.65)##	0.01
Glomerular injury marker				
Albumin (mg/dL)	21 (4-70)	24 (13-129)	92 (44-253)***, #	<0.001

Abbreviations: PRA, pre-renal azotemia; HRS, hepatorenal syndrome; ATN, acute tubular necrosis; NGAL, neutrophil gelatinase associated lipocalin; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid binding protein; FENa, fractional excretion of sodium

Values significantly different from pre-renal azotemia indicated with * $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Values significantly different from HRS indicated with # $p < 0.05$; ## $p \leq 0.01$; ### $p \leq 0.001$

Figure 2b. FENa and albumin for patients with PRA, HRS and ATN

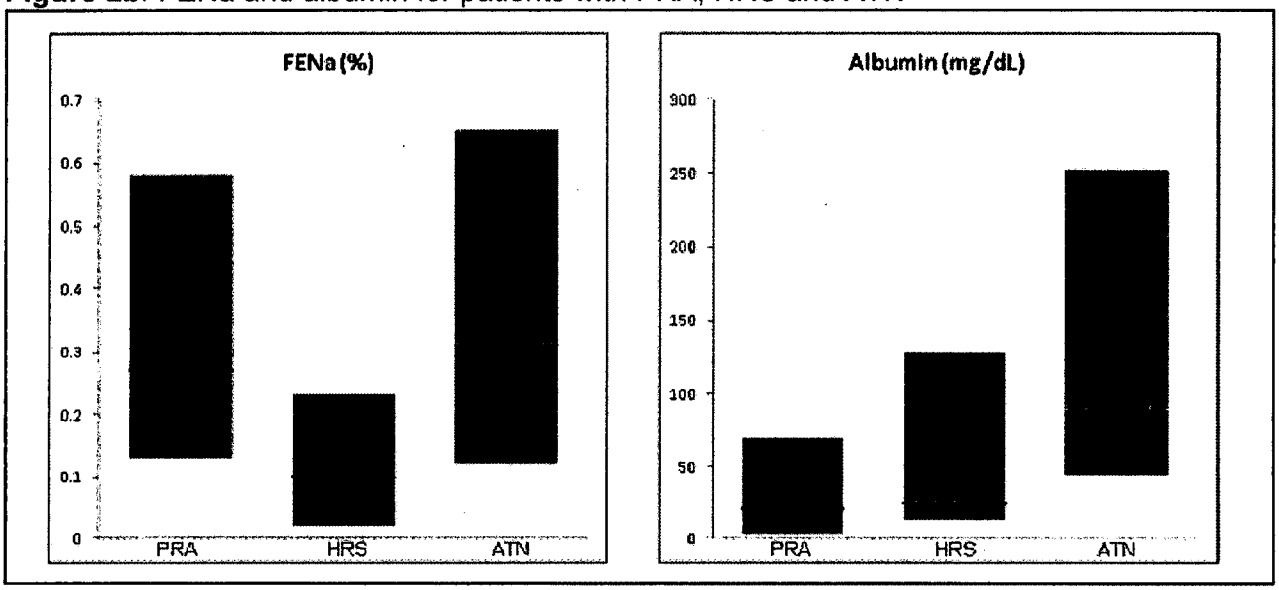

Figure 2b. FENa and albumin values are shown for patients with PRA, HRS and ATN. Dark horizontal lines represent medians while the shaded boxes represent interquartile ranges. FENa is statistically significantly lower in patients with HRS as compared to both PRA and ATN while albumin is significantly higher in patients with ATN than in those with either PRA or HRS.

Table 3. Measures of test performance characteristics

	Optimal Cut Point	Proportion Over Cut Point with ATN	AUC (95% CI)	Validation AUC*
Tubular injury markers				
NGAL (ng/ml)	365	25/35 (71%)	0.78 (0.69-0.88)	0.787
IL-18 (pg/ml)	85	21/33 (64%)	0.71 (0.61-0.81)	0.711
KIM-1 (ng/ml)	15.4	15/24 (63%)	0.64 (0.53-0.75)	0.639
L-FABP (ng/ml)	25	21/30 (70%)	0.69 (0.57-0.80)	0.688
Tubular function marker				
FENa (%)	0.1	22/62 (35%)	0.56 (0.45-0.68)	0.563
Glomerular injury marker				
Albumin (mg/dL)	44	29/52 (56%)	0.73 (0.64-0.83)	0.734

Abbreviations: ATN, acute tubular necrosis; AUC, area under the curve; CI, confidence interval; NGAL, neutrophil gelatinase associated lipocalin; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid binding protein; FENa, fractional excretion of sodium

*Validation AUCs derived from leave-10-out cross validation performed with SAS Proc Surveyselect

Figure 3. Graph of conditional probabilities for urine biomarkers

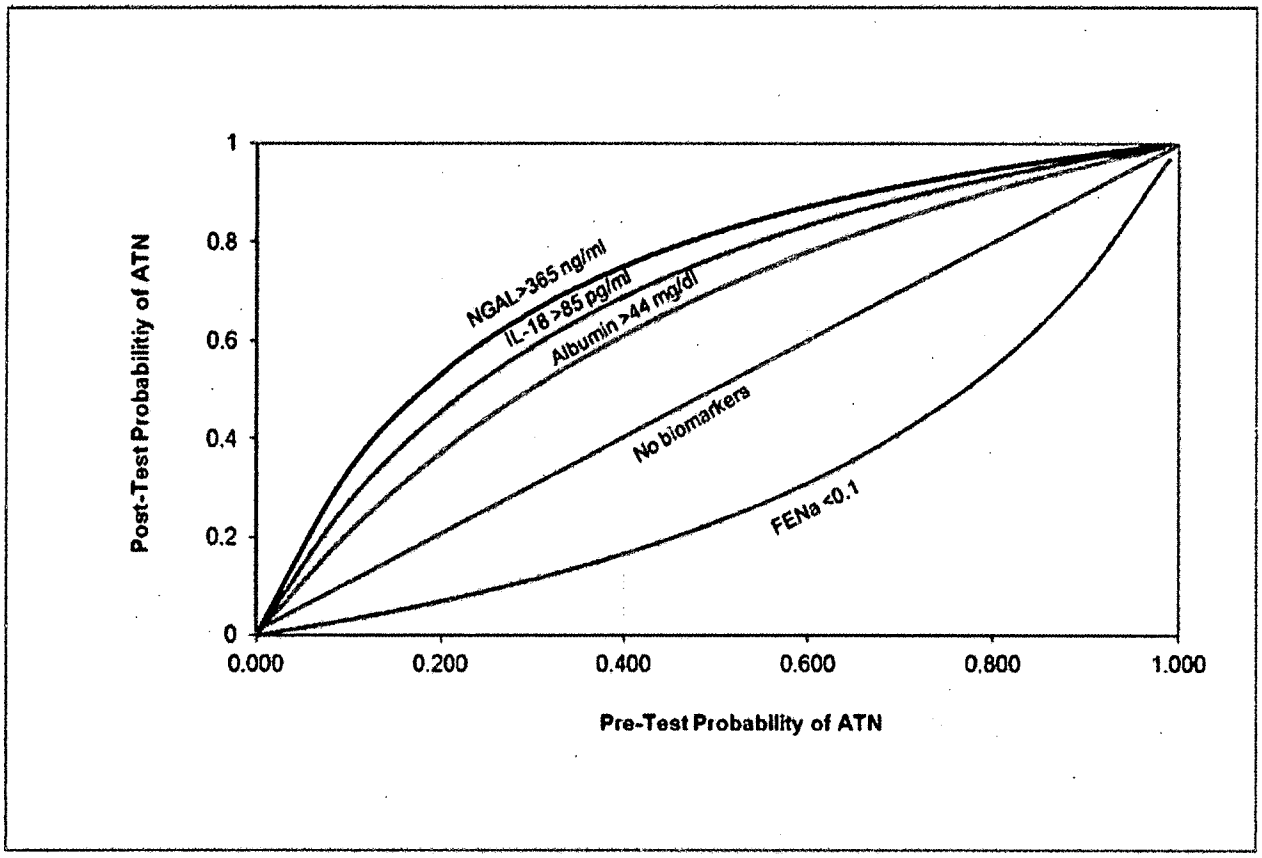

Figure 3. Figure depicts the conditional probabilities for the diagnosis of ATN utilizing biomarkers at their optimal cutoff. For each pre-test probability, a post-test probability is calculated utilizing a positive (NGAL, IL-18, albumin) or negative (FENa) likelihood ratio[23].
Formula: Likelihood ratio- = (1-sensitivity)/specificity; Likelihood ratio+ = sensitivity/(1-specificity); pretest odds = pretest probability/(1-pretest probability); posttest odds = pretest odds x LR; posttest probability = posttest odds/(posttest odds + 1)
Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; ILK-18, interleukin-18; FENa, fractional excretion of sodium; ATN, acute tubular necrosis

Table 4. Association between biomarker panel and the diagnosis of ATN

	Relative Risk*
0 Markers Positive	1.00
1 Marker Positive	4.63 (1.29-16.61)
2 Markers Positive	6.98 (2.14-22.75)
3 Markers Positive	9.78 (3.10-30.86)
4 Markers Positive	13.33 (4.40-40.39)

Abbreviations: ATN, acute tubular necrosis
Biomarker cutoffs: NGAL, 365 ng/ml; IL-18, 85 pg/mL; L-FABP, 25 ng/mL; Albumin 44 mg/dL
*Unadjusted

Figure 4. Association between biomarker elevation and diagnosis

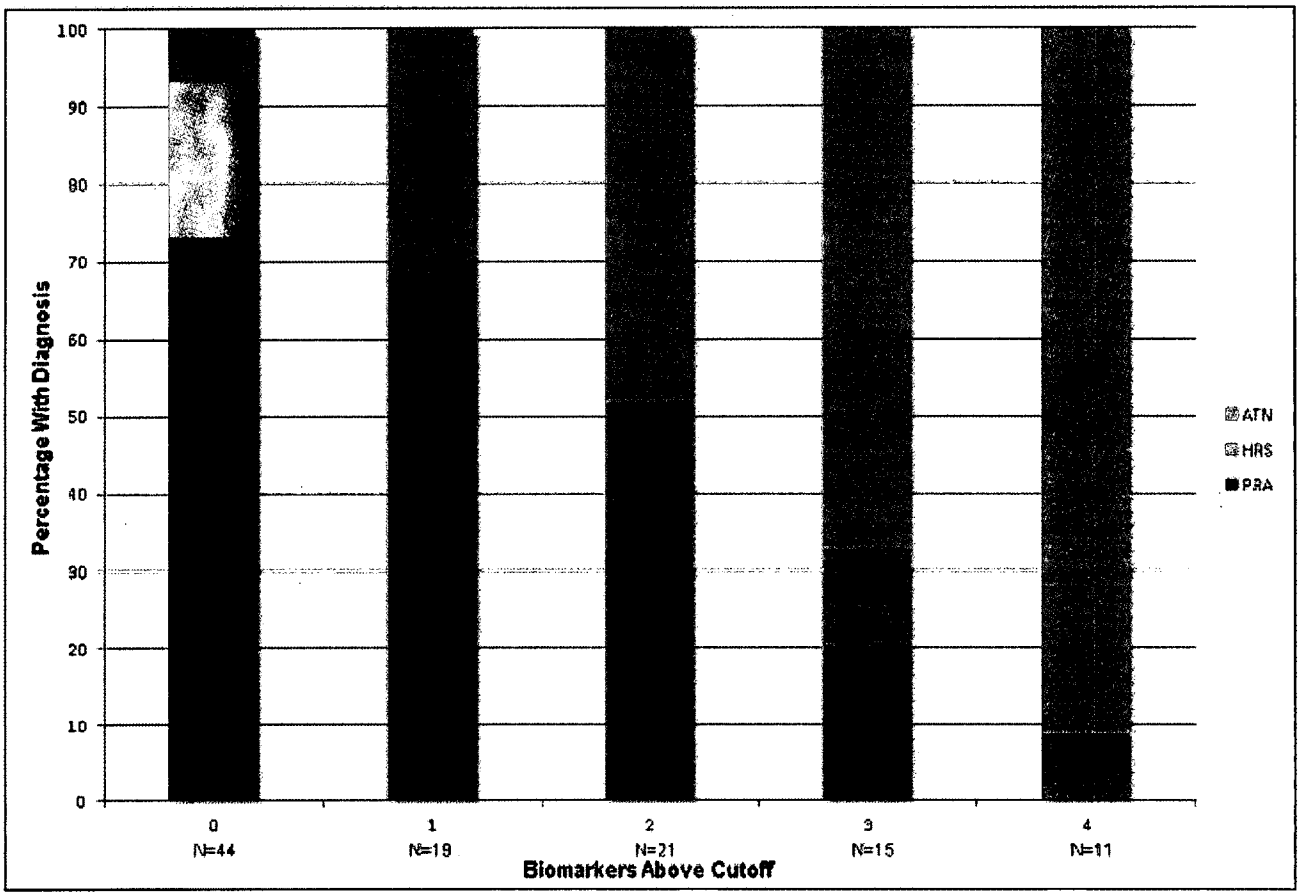

Figure 4. The percentage of patients with pre-renal azotemia (PRA), hepatorenal syndrome (HRS) and acute tubular necrosis (ATN) by the number of biomarkers of structural injury above their optimal cutoff for the diagnosis of ATN.

Chapter 6. Future Directions

As detailed above, distinguishing between etiologies in patients with cirrhosis and AKI is critical in assigning treatments. Renal function in ATN, when structural injury has led to tubule dysfunction, should not improve with restitution of renal blood flow until the injury has healed. However, if tubular functional integrity is intact, renal function should improve along with renal perfusion, as would be seen with PRA and HRS. Unfortunately making this distinction clinically has historically be vexing for physicians as many of the traditional tests, such as FENa and urine microscopy, used to determine a diagnosis for a patient with AKI are ineffectual in the setting of cirrhosis. It is likely that urinary biomarkers will provide a critical new tool in clinicians' diagnostic armamentarium. However, a more fundamental problem is physicians' ineluctable inclination to treat these diagnoses as distinctly demarcated entities where each patient must be assigned one and only one diagnosis. In reality, the pathophysiology underlying AKI in patients with cirrhosis exists along a spectrum. Patients are frequently seen who are found to have granular casts and tubular epithelial cells on microscopy but also low FENa and urine sodium levels. It is the very rare patient who can be thought of as having a purely functional or purely structural cause behind a rise in creatinine. The problem with being beholden to a system of trichotomous diagnoses can be seen in **Figure 1**. While, at the study level, patients diagnosed with ATN have significantly higher NGAL levels than those with PRA or HRS, on the individual level there remains overlap. Patients whose clinical course was consistent with HRS had levels of NGAL indicating significant structural injury and some who appeared consistent with ATN had very low NGAL, indicating a lack of structural damage. What should the diagnosis be in such patients? Ultimately, it is probably in fact futile and counterproductive to be forced to decide what a patient "has".

In deciding treatment in AKI, the fundamental question is whether a restoration of renal blood flow will lead to restitution in kidney function. The critical task then is to distinguish where

a patient falls on the spectrum of functional and structural disease and whether their tubular functional integrity is intact. To ultimately optimize outcomes and appropriately power clinical trials, treatments should be targeted not towards a patient's singular diagnosis but, instead, to their particular location along this spectrum. While it is likely that many patients' pathophysiology do in fact cluster around our traditionally conceptualized disease entities, others will exhibit overlap reflected in a clinical presentation that defies singular characterization. New diagnostic tests therefore may serve best not to refine clinicians' ability to assign a blanket diagnostic label but instead to phenotype the physiology underlying AKI.

There is evidence from this study that the use of functional and structural markers to identify physiologic phenotypes or clusters may indeed be more useful for guiding treatment decisions than the traditional paradigm of assigning a single, specific diagnosis. The details of the adjudication process are detailed in **Chapter 5** and baseline demographic, clinical and laboratory data for all adjudicated patients and for those with and without ATN are shown in **Table 1** of that chapter. Biomarker values for patients by adjudicated diagnosis are shown here in **Table 1**. As the upper quartile for FENa in patients diagnosed with HRS was 0.23%, patients were stratified as having a FENa above or below 0.25%. Biomarkers values for each diagnosis stratified by FENa levels are shown in **Table 2**. The vast majority of patients with FENa > 0.25% were adjudicated as having PRA 32 (60%) or ATN 19 (36%) with only 2 (4%) adjudicated to HRS. Patients with ATN had significantly higher biomarkers of structural injury (NGAL, IL-18, L-FABP and albumin) as well as of tubular dysfunction (cystatin C) than did those with PRA. Among those patients with FENA < 0.25%, the diagnoses were mixed with 23 (40%) diagnosed with PRA, 20 (37%) with ATN and 14 (25%) with HRS. All injury biomarkers were significantly higher in patients with ATN than in those with PRA. Patients with HRS had remarkably intact tubular function (median urinary cystatin C of 0) and injury markers similar to those seen with PRA. A potential diagnostic algorithm utilizing these findings is shown in **Figure 2**.

While obviously of some use, utilization of such an algorithm will clearly lead to a significant number of patients being "misclassified". However, on closer inspection of **Table 2** a potential way out of this diagnostic morass presents itself as several physiologic clusters are apparent. Patients diagnosed with HRS who had a FENa < 0.25%, who constituted 88% (14/16) of HRS patients, had only mild elevation in injury biomarkers (Group 1). The cystatin C level was extremely low, with a median value of 0. Cystatin C is freely filtered by the glomerulus but, with intact tubules, is nearly entirely taken up and degraded such that it should be virtually undetectable in the urine. When tubules are injured, they can no longer optimally uptake cystatin and it begins to leak into the urine, analogous to high FENa in the setting of tubular injury wherein tubules cannot optimally resorb sodium. Along with the low FENa, these extremely low cystatin C levels confirm the tubular integrity of these patients. Taken together with the low injury markers, this identifies the primarily function nature of these patients' AKI. It is highly likely that restoration of renal perfusion would result in recovery of renal function in this group. In stark contrast to these patients are those diagnosed with ATN who have a FENa >0.25% (Group 2). These patients are characterized by extremely elevated injury markers as well as a markedly high level of cystatin C, 0.17 (0.04-0.32) mg/L. The injury markers and loss of tubular functional integrity is consistent with frank injury and such patients would clearly then fall on the structural end of the AKI spectrum. It is unlikely that their renal function would be improved by measures to improve renal perfusion and, were they to be listed for transplantation, should be considered for a combined liver-kidney transplant. The tantalizing potential of grouping patients in pathophysiologic clusters as opposed to by diagnoses becomes clear when looking at patients diagnosed with ATN who have FENa <0.25% (Group 3). As with Group 2, they have very elevated levels of injury markers and indeed the groups are indistinguishable by this metric. However, patients in Group 3 appear have maintained tubular integrity as evidenced by the low FENa and only modest elevation in cystatin C. An alternative presentation of this data is seen in **Table 3**. Unlike Group 2, such patients very well may benefit from improvements

in renal perfusion and, were they to be considered for transplant, would most likely be appropriate for a solitary liver. If Groups 2 and 3 were biopsied they would both show histologic evidence of what we consider to be ATN. However, through the combination of functional and structural biomarkers, it is readily apparent that such patients fall at very different locations along the functional to structural spectrum of AKI.

Rather than trying to distinguish between the constructs of HRS and ATN, patients may instead be thought to have primarily functional dysfunction with only minimal injury, significant injury but with retained tubular integrity or structural injury resulting in tubular dysfunction. To evaluate whether patients do in fact cluster in such physiologic groupings, **Figures 3** and **4** were created. In these figures (where each circle represents a patient) NGAL levels are on the x-axis, FENa on the y-axis and urinary cystatin C is represented by the size of the circles, with larger circles reflecting higher values. **Figure 3** depicts patients who met 6/6 IAC criteria for the diagnosis of cirrhosis (yellow circles) and those who did not (green circles). The circles' colors in **Figure 4** are derived from the adjudicated diagnosis, with green representing PRA, red ATN and yellow HRS. The results are telling and illustrate the difficulties of clinical differential diagnosis. Several patients diagnosed with ATN have very low FENa, low cystatin and low NGAL, indicating a lack of structural injury and intact tubular integrity. Similarly there are patients diagnosed with HRS with elevated NGAL and FENa and with cystatin C levels indicating tubular dysfunction. In both cases visualizing the patients' location on the functional to structural spectrum of injury would likely change the way in which they were managed. Utilizing such an objective panel of markers, obtained on the first day of AKI, would make targeting of etiology specific treatment both more accurate and expeditious. Ultimately additional biomarkers, perhaps those reflecting the intensity of renal vasculature vasoconstriction, could be incorporated into the panel. Further research is required to demonstrate improved outcomes with treatment decisions guided by urinary biomarkers.

Figure 1. NGAL values for individual patients across diagnoses

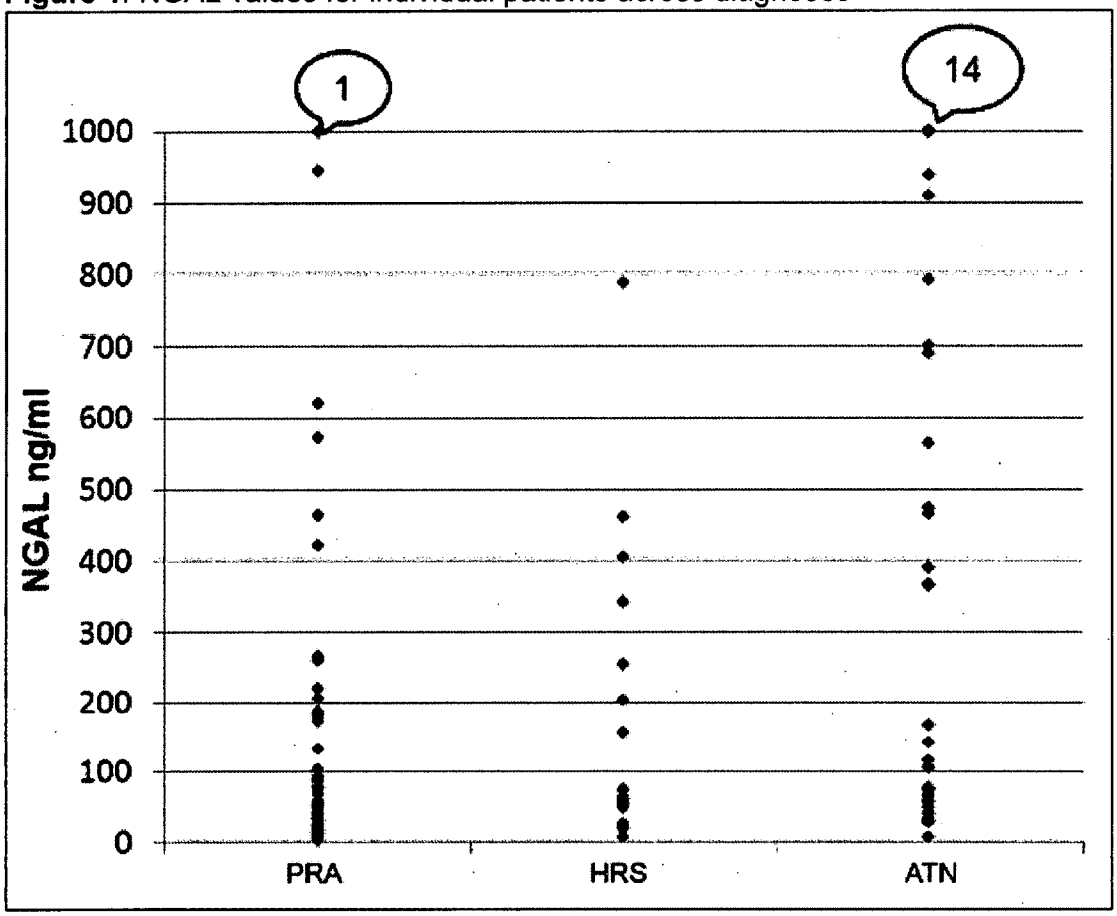

Figure 1. NGAL values for each adjudicated patient are presented. The NGAL assay was Winsorized at 1000 ng/mL. Therefore, while there appear to be a single patient with the value of 1000 for both PRA and ATN, these dots represent 1 patient for PRA but 14 patients with ATN who had a value greater than 1000.

Table 1. Summary statistics for urine biomarkers by diagnosis

	PRA N=55	HRS N=16	ATN N=39	p
Tubular injury markers				
NGAL (ng/ml)	54 (17-180)	115 (51-373)	565 (76-1000)***, ##	<0.001
IL-18 (pg/ml)	15 (15-49)	37 (15-90)	124 (15-325)***, #	<0.001
KIM-1 (ng/ml)	4.4 (1.8-11.7)	7.6 (4.5-10.1)	8.4 (4.1-18.3)**	0.03
L-FABP (ng/ml)	9 (4-18)	14 (6-20)	27 (8-103)***	0.002
Tubular function marker				
FENa (%)	0.27 (0.13-0.58)	0.10 (0.02-0.23)**	0.31 (0.12-0.65)##	0.01
Glomerular injury marker				
Albumin (mg/dL)	21 (4-70)	24 (13-129)	92 (44-253)***, #	<0.001

Abbreviations: PRA, pre-renal azotemia; HRS, hepatorenal syndrome; ATN, acute tubular necrosis; NGAL, neutrophil gelatinase associated lipocalin; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid binding protein; FENa, fractional excretion of sodium

Values significantly different from pre-renal azotemia indicated with * $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Values significantly different from HRS indicated with # $p < 0.05$; ## $p \leq 0.01$; ### $p \leq 0.001$

Table 2. Biomarker values by diagnosis stratified by FENa

	FENa ≤ 0.25%			FENa > 0.25%		
	PRA N=23	**ATN N=20**	**HRS N=14**	**PRA N=32**	**ATN N=19**	**HRS N=2**
Cystatin C	0.06 (0.03-0.12)	0.06 (0-0.15)	0 (0-0.03)	0.06 (0.02-0.13)	0.17 (0.04-0.32)	0.16 (0-0.31)
NGAL	68 (38-186)	578 (156-1000)	115 (53-342)	45 (15-118)	565 (57-1000)	235 (8-462)
IL-18	26 (15-71)	65 (15-218)	37 (15-112)	15 (15-46)	131 (27-513)	41 (15-68)
KIM-1	5.1 (2.1-11.7)	10.1 (5-18.6)	7.6 (5.5-9.7)	4.1 (1.8-11.7)	6.3 (3.9-17.6)	5.3 (0.2-10.5)
L-FABP	11 (4-19)	27 (13-62)	14 (7-19)	9 (3-17)	16 (4-162)	12 (2-22)
Albumin	21 (4-65)	69 (17-165)	24 (11-172)	19 (4-72)	233 (63-856)	45 (18-72)

Abbreviations: PRA, pre-renal azotemia; HRS, hepatorenal syndrome; ATN, acute tubular necrosis; FENa, fractional excretion of sodium; NGAL, neutrophil gelatinase associated lipocalin; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid binding protein

Table 3. Biomarker values by FENa Stratified by Diagnosis

	PRA		ATN		HRS	
	FENa ≤ 0.25% N=23	**FENa > 0.25% N=32**	**FENa ≤ 0.25% N=20**	**FENa > 0.25% N=19**	**FENa ≤ 0.25% N=14**	**FENa > 0.25% N=2**
Cystatin C	0.06 (0.03-0.12)	0.06 (0.02-0.13)	0.06 (0-0.15)	0.17 (0.04-0.32)	0 (0-0.03)	0.16 (0-0.31)
NGAL	68 (38-186)	45 (15-118)	578 (156-1000)	565 (57-1000)	115 (53-342)	235 (8-462)
IL-18	26 (15-71)	15 (15-46)	65 (15-218)	131 (27-513)	37 (15-112)	41 (15-68)
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Abbreviations: PRA, pre-renal azotemia; HRS, hepatorenal syndrome; ATN, acute tubular necrosis; FENa, fractional excretion of sodium; NGAL, neutrophil gelatinase associated lipocalin; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; L-FABP, liver-type fatty acid binding protein

Figure 2. Diagnostic algorithm using optimal cutoffs for the diagnosis of ATN

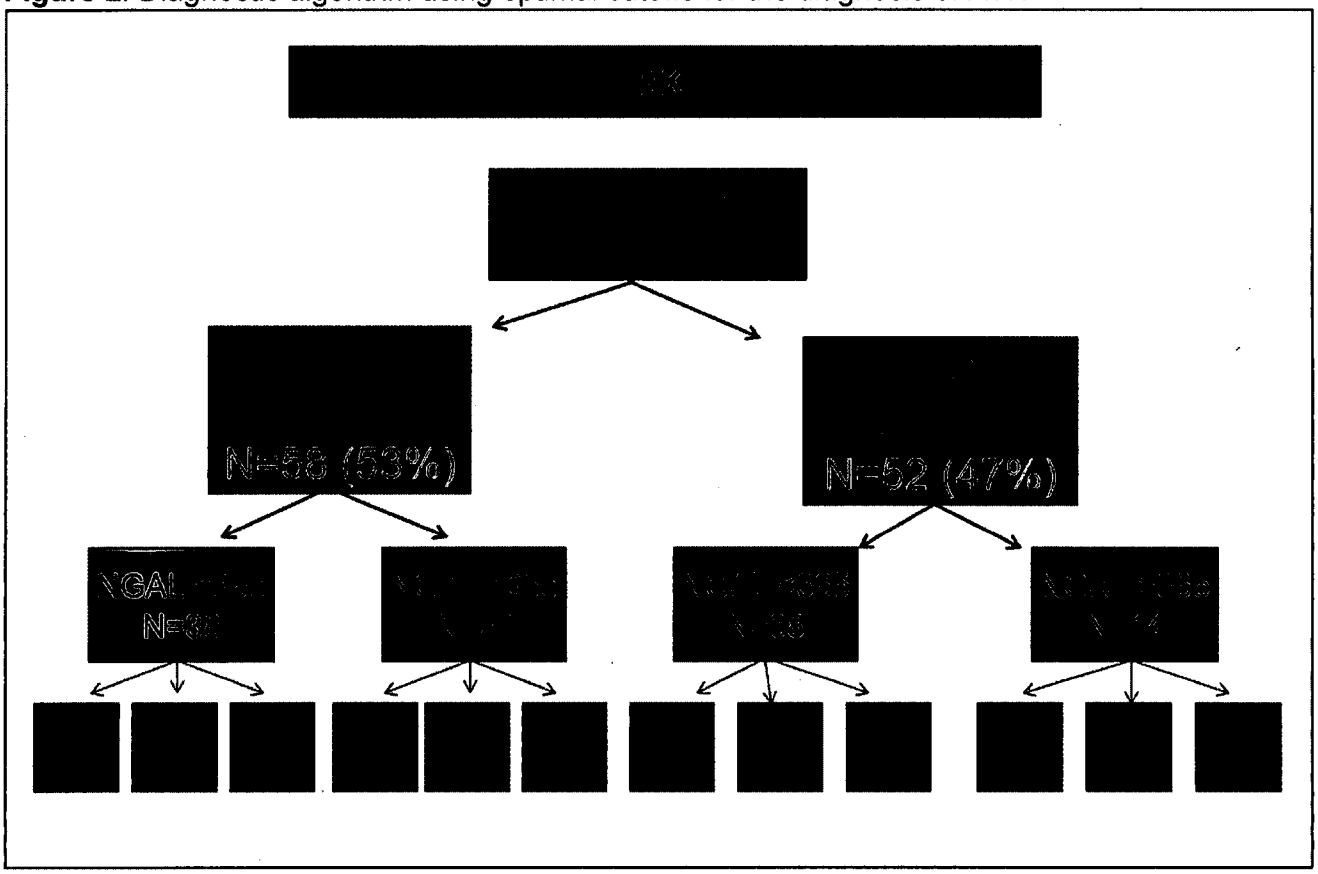

Figure 2. A diagnostic algorithm is presented using optimal cutoffs for the diagnosis of ATN. Cutoffs were derived from AUC analysis.
Abbreviations: AKI, acute kidney injury; FENa, fractional excretion of sodium; NGAL, neutrophil gelatinase-associated lipocalin; PRA, pre-renal azotemia; HRS, hepatorenal syndrome; ATN, acute tubular necrosis

Figure 3. Pathophysiologic profile of patients with and without IAC diagnosed HRS

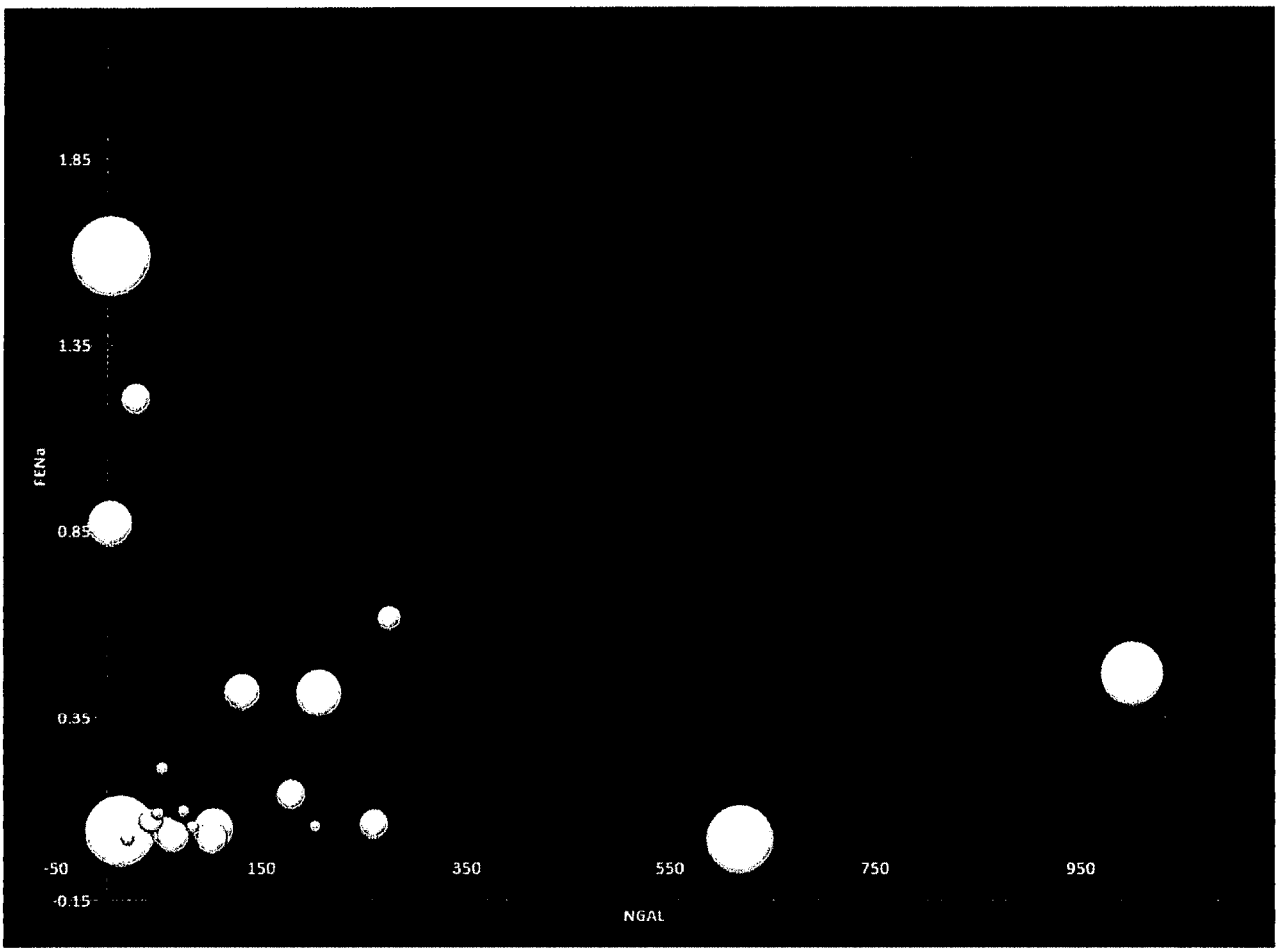

Figure 3. Pathophysiologic profile of patients with and without IAC diagnosed HRS. Bubble size reflects urinary cystatin C levels, with larger bubble representing higher levels. The yellow bubbles represent patients who met 6/6 IAC criteria for the diagnosis of ATN while the green represent patients who did not.
Abbreviations: IAC, International Ascites Club; HRS, hepatorenal syndrome; FENa, fractional excretion of sodium; NGAL, neutrophil gelatinase-associated lipocalin

Figure 4. Pathophysiologic profile of patients by adjudicated diagnosis

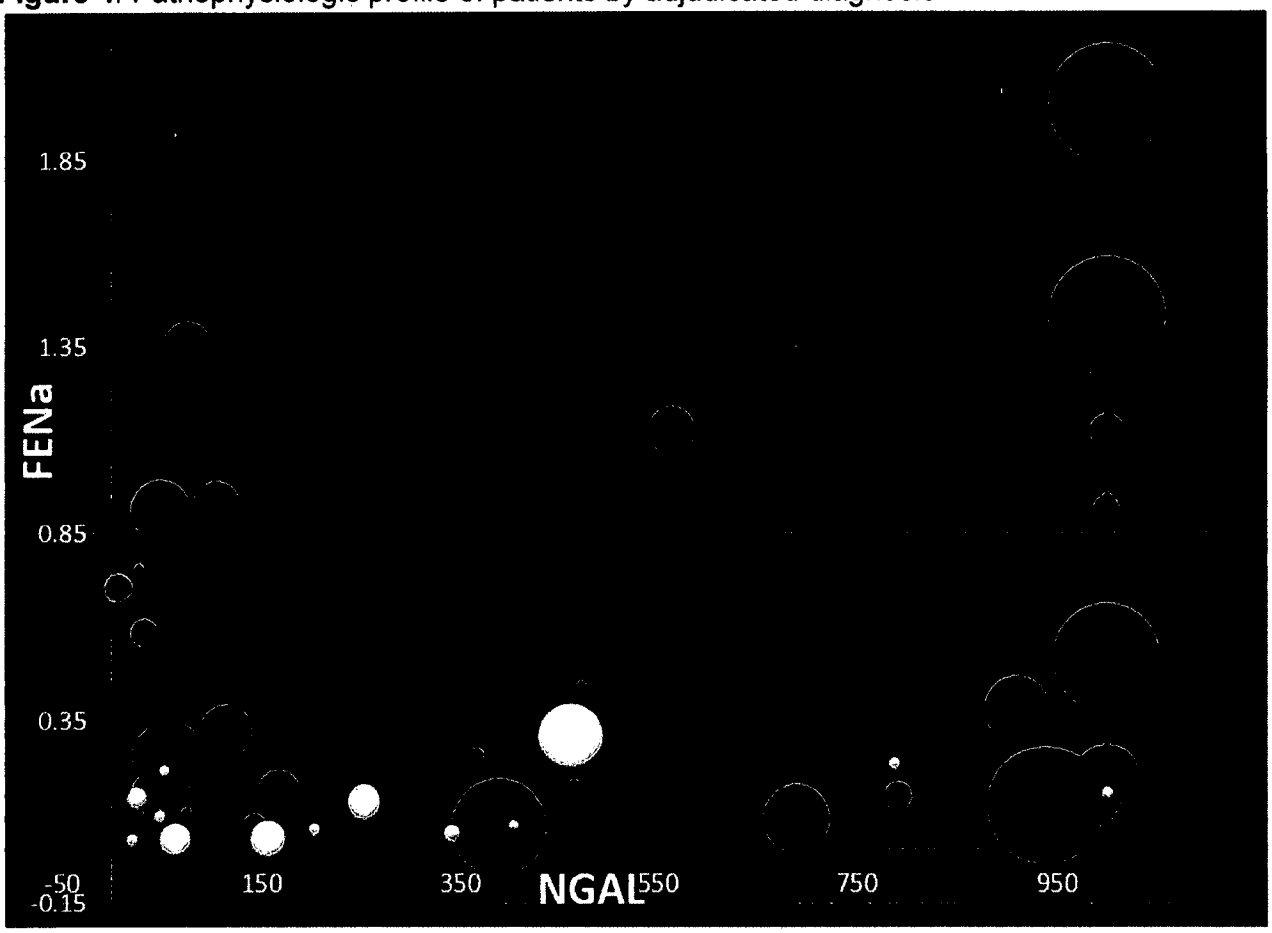

Figure 4. Pathophysiologic profile of patients by adjudicated diagnosis. Bubble size reflects urinary cystatin C levels, with larger bubble representing higher levels. The green bubbles represent patients diagnosed with PRA, red patients diagnosed with ATN and yellow those diagnosed with HRS.
Abbreviations: FENa, fractional excretion of sodium; NGAL, neutrophil gelatinase-associated lipocalin; PRA, pre-renal azotemia; ATN, acute tubular necrosis; HRS, hepatorenal syndrome

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