2014

An analysis of the mechanisms of acute kidney injury and novel biomarkers

BOSTON UNIVERSITY

SCHOOL OF MEDICINE

Thesis

AN ANALYSIS OF THE MECHANISMS OF ACUTE KIDNEY INJURY AND NOVEL BIOMARKERS

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B.S., University of California Berkeley, 2011

Submitted in partial fulfillment of the

requirements for the degree of

Master of Arts

2014

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ACKNOWLEDGEMENTS

First, I would like to thank my readers: Dr. Gwynneth Offner and Dr. Andrew Siedlecki. I had the privilege of being under the supervision of Dr. Offner as she was an instructor in the Biochemistry course and my personal advisor in the Graduate Medical Sciences program. I thank her for the guidance she gave me in navigating through my graduate medical education and for her flexibility in accommodating my educational path when unforeseeable life events came about. To Dr. Siedlecki, I thank him for giving me the opportunity to learn as a research trainee in the renal division at Brigham and Women's Hospital as well as imparting his wisdom and expertise in guiding and focusing my thesis on acute kidney injury. I would also like to thank all the professors and students in the GMS program for creating an exciting and collaborative atmosphere that encourages inquisitive learning and progress. Finally, I would like to thank my parents for always supporting me in my education and career goals even in the midst of obstacles and setbacks and my older sister who challenges me to think critically and purposefully in all aspects of my life.

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ABSTRACT

Acute Kidney Injury (AKI) is a prevalent systemic disorder that has an extremely high rate of mortality even after detection. Historically, the diagnosis and treatment of AKI was marred by the lack of universally accepted criteria defining AKI. Therefore, reports of incidence and mortality varied widely depending on location and the criteria used at the time, but all reports indicated a poor prognosis for the patient. Until recently, the only modes of detecting AKI were primarily through measurements of three clinical findings: serum creatinine concentration, blood urea nitrogen concentration, and urine output. While these measurements are still widely used as standard practice, they have limitations in their utility because their values can fluctuate depending on a person's age, gender, race, diet, and other comorbid conditions. Nevertheless, as these were the only universally accepted units of measurement for kidney function, the Acute Dialysis Quality Initiative (ADQI) used them to create the Risk, Injury, Failure, Loss, and End stage kidney disease (RIFLE) criteria to classify the severity of kidney injury across clinical settings. Eventually, modifications were made by the Acute Kidney Injury Network (AKIN) to increase the sensitivity of AKI diagnosis. It was not until the last

decade that new biomarkers of kidney injury began to be researched that provided earlier detection of physical kidney injury before functional manifestations would present themselves. Some of these new biomarkers include cystatin C, kidney injury molecule-1 (KIM-1), and neutrophil gelatinase associated lipocalin (NGAL). This study will investigate how the properties of these new biomarkers are superior when compared to those of serum creatinine in early detection of AKI and specification as to the local site of injury within the nephron. The conclusion is that cystatin C has the potential to indicate damage to glomerular filtration while KIM-1 and NGAL have the ability to indicate damage to the proximal tubule. Along with the ability to provide information as to the specific site of renal injury, the levels of cystatin C, KIM-1, and NGAL increase much more rapidly and to a much higher value than serum creatinine once physical renal damage has occurred. These characteristics along with future research will allow for earlier detection of AKI, more personalized treatment plans, and an overall better prognosis for the patient.

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ABBREVIATIONS

ADHF	Acute Decompensated Heart Failure
ADQI	Acute Dialysis Quality Initiative
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
ARDS	Acute Respiratory Distress Syndrome
ARF	Acute Renal Failure
ASN	American Society of Nephrology
ATN	Acute Tubular Necrosis
BUN	Blood Urea Nitrogen
BNP	N-terminal Brain Natriuretic Peptide
CA-AKI	Community-Acquired Acute Kidney Injury
CKD	Chronic Kidney Disease
ESICM	European Society of Intensive Care Medicine
ESKD	End Stage Kidney Disease
ESRD	End Stage Renal Disease
GBM	Glomerular Basement Membrane
GFR	Glomerular Filtration Rate
HA-AKI	Hospital-Acquired Acute Kidney Injury
ICU	Intensive Care Unit
IDDM	Insulin-Dependent Diabetes Mellitus

ISN	International Society of Nephrology
KIM-1	Kidney Injury Molecule-1
NAG	N-Acetyl-β-Glucosaminidase
NGAL	Neutrophil Gelatinase-Associated Lipocalin
NKF	National Kidney Foundation
RIFLE	Risk, Injury, Failure, Loss, and End-stage renal disease
RRT	Renal Replacement Therapy

INTRODUCTION

The incidence of mortality increases with the decline of kidney function because the kidneys are the functional organ responsible for maintaining many different homeostatic conditions (Murugan & Kellum, 2011). This fact along with the prevalence of hospital-acquired acute kidney injury (HA-AKI) in hospitalized patients makes early detection and proper treatment to prevent the progression of this disease a high priority. It has been estimated in the past that 5-25% of hospitalized patients will acquire HA-AKI (Pruchnicki & Dasta, 2002). With nearly 50% of cases proving to be fatal, early detection is critical to the treatment of HA-AKI because early renal failure often times has no clinical manifestations (Nally, 2002).

Acute kidney injury (AKI) is characterized by a decreased urinary excretion of nitrogenous wastes, usually in the form of urea nitrogen and creatinine, resulting from a sudden loss of renal function (Nolan & Anderson, 1998). AKI is further subcategorized into prerenal, postrenal, and intrinsic subtypes. This distinction is important because the initial evaluation and treatment management differ depending on the origin of the renal insult (Nally, 2002). Intrinsic AKI, at a frequency of 66-80%, and prerenal AKI, at a frequency of 12-25%, are the most common types of HA-AKI (Pruchnicki & Dasta, 2002). While many different diseases affecting renal parenchymal cells can lead to intrinsic AKI, the most common etiology is acute tubular necrosis (ATN) (Palevsky, 2012). ATN usually develops as a result of hypotension, renal ischemia, sepsis, or nephrotoxin exposure (Palevsky, 2012).

In order to properly diagnose AKI, a standardized set of criteria was determined to measure glomerular function as a result of changes in urine output and serum creatinine level, and together they formed the Risk, Injury, Failure, Loss, and End-stage renal disease (RIFLE) criteria (Murugan & Kellum, 2011). Figure 1 illustrates the numerical values associated with diagnosing AKI using the RIFLE criteria. While the development of the RIFLE criteria was instrumental in providing a consistent standard to measure the degree of AKI, it was further modified by the Acute Kidney Injury Network (AKIN) in 2005 to include an important subset of patients who had decreased renal function more pronounced than physiological variation but not enough to meet RIFLE criteria as a result of early or mild AKI (Murugan & Kellum, 2011).

HA-AKI is generally seen in specific settings. Patients have a high incidence of developing HA-AKI if they also present with comorbid conditions such as diabetes mellitus, congestive heart failure, or have been admitted to the Intensive Care Unit (ICU), most commonly as a result of multiple organ failure. The development of end-stage renal disease (ESRD) is as high as 40% in patients who have had insulin-dependent diabetes mellitus (IDDM) for 20 years ("Complications of Diabetes Mellitus," n.d.). A clinical epidemiologic study on the risk factors of HA-AKI showed that patients with diabetes were affected to a greater extent by volume depletion during an episode of AKI than patients without diabetes (Shusterman et al., 1987). The same study also showed that there is statistically significant evidence that congestive heart failure is a risk factor for developing AKI (Shusterman et al., 1987). While the development of AKI in hospitalized

patients is already frequent, HA-AKI is even higher for patients admitted to the ICU with reports ranging from 22-67% (Murugan & Kellum, 2011).

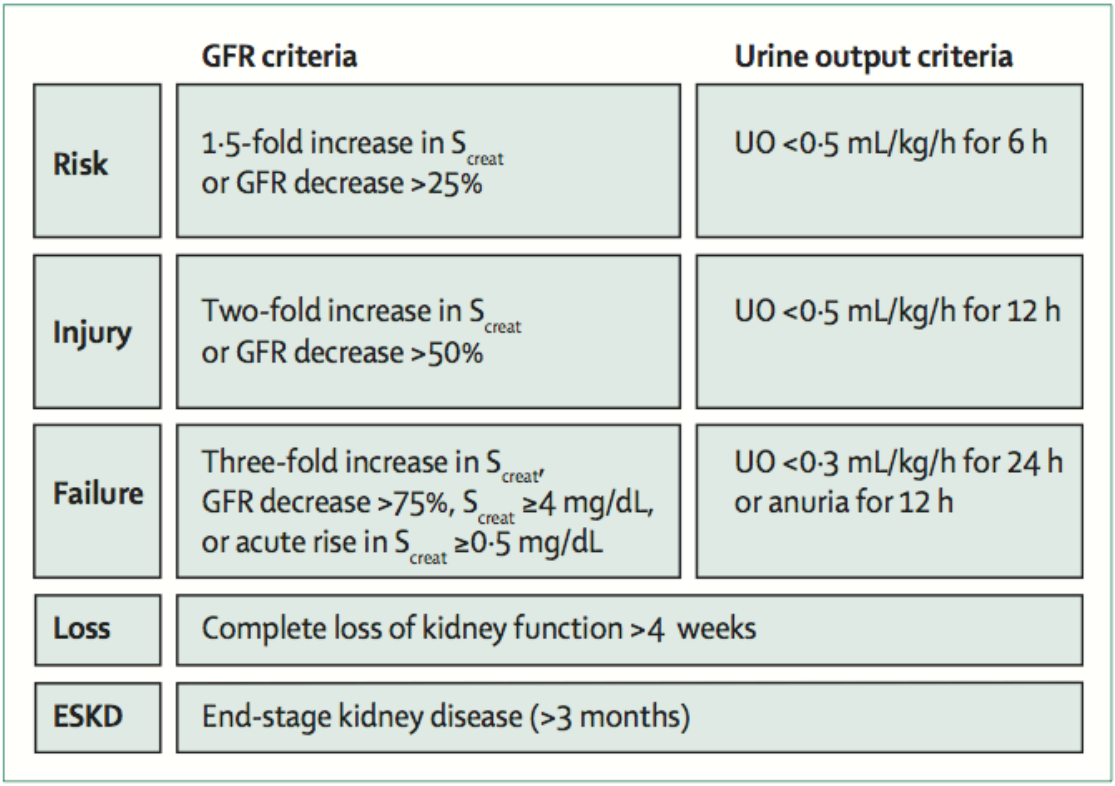


Figure 1 RIFLE Criteria For Acute Kidney Injury. A patient moves from risk (class R) to failure (class F) as GFR and UO deteriorate. GFR = glomerular filtration rate. S_{creat} = serum creatinine concentration. UO = urine output. Figure taken from (Bellomo, Kellum, & Ronco, 25).

BACKGROUND

The development of Acute Kidney Injury has been classified into two different categories: Community-Acquired Acute Kidney Injury (CA-AKI) and Hospital-Acquired Acute Kidney Injury (HA-AKI). A patient is said to have CA-AKI if upon admission, the patient's serum creatinine level is elevated enough to clinically classify as AKI under the RIFLE criteria (Schissler et al., 2013). Similarly, a patient is diagnosed with HA-AKI if

the patient presents with an increasing serum creatinine level twenty-four hours or more after being hospitalized (Schissler et al., 2013). A retrospective analysis of patient information at a Veterans Affairs hospital has shown that while different physiological causes may lead to CA-AKI versus HA-AKI, there is no significant difference in the severity of AKI between the two when comparing serum creatinine levels and using the RIFLE criteria to quantify the distribution of patients among the different categories (Schissler et al., 2013). Therefore, it is important to first understand how kidney injury occurs and what physiological changes result in the fluctuation of biomarker levels that are currently being used to diagnose and classify AKI.

The Progression of AKI

The most common cause of AKI is Acute Tubular Necrosis (ATN), and this usually follows a form of ischemia or nephrotoxic injury to the renal tubules (Tolwani, 2012). Table 1 lists out examples of different sources of nephrotoxic and ischemic injury. For patients that are already in the ICU, ATN can develop from a combination of factors including nephrotoxic medications, sepsis, and compromised renal perfusion (Schrier et al., 2004). The development of ischemic ATN can be categorized into four chronological phases: initiation, extension, maintenance, and recovery (Tolwani, 2012).

Table 1 Etiologies of Acute Tubular Necrosis. Acute Tubular Injury can arise from an array of different sources. The most common include nephrotoxic injury, ischemia, and sepsis. Table taken from (Palevsky, 2012).

NEPHROTOXIC	ISCHEMIC
Exogenous	Prolonged prerenal azotemia
Radiocontrast agents	Hypotension
Aminoglycosides	Hypovolemic shock
Amphotericin B	Cardiopulmonary arrest
Cisplatinum	Cardiopulmonary bypass
Acetaminophen	Aortic surgery
Endogenous	**SEPSIS**
Hemoglobin	
Myoglobin	

The initiation phase is set off by extended periods of renal ischemia and usually presents with injury directly to both the endothelial cells and the epithelial cells lining the renal tubules (Sharfuddin & Molitoris, 2011). Once these cells have undergone physical injury, the Glomerular Filtration Rate (GFR) usually decreases (Tolwani, 2012). Although it is not completely understood how tubular injury results in the decreased function of the glomeruli, three major mechanisms have been determined to play a role in the observed decrease in renal function (Palevsky, 2012).

1. Tubular obstruction – the renal tubule epithelial membrane ruptures following ischemia or nephrotoxic injury resulting in obstructions of the tubular lumen (Palevsky, 2012).
2. Glomerular filtrate back leak – the glomerular basement membrane (GBM) can be compromised as a result of luminal obstruction and retrograde flow across the GBM (Palevsky, 2012).

3. Intrarenal vasoconstriction – although ATN is characterized by injury to the tubular epithelium, there is still a reactive vasoconstriction in the microvasculature that directly results in decreased glomerular perfusion and GFR (Palevsky, 2012).

Injury to the kidney enters the extension phase as continued injury to the tubular epithelium and endothelium activate inflammatory mediators, which exponentially increase cellular injury (Tolwani, 2012). As with other organ failures, the progression of AKI is characterized by cytokine activation and subsequent systemic inflammation (Murugan & Kellum, 2011). This is because the injured cells release danger-associated molecules that carry on the inflammatory response to organs remote to the site of injury, leading to activated immune cells and the inflammation of these remote organs (Murugan & Kellum, 2011). The third phase, maintenance, typically lasts severl weeks, and during this time, the GFR re-stabilizes at a much lower level while the potential for other uremic complications increases (Tolwani, 2012). Physical complications include a decrease in urine output, and during this time, most patients rely on one form of renal replacement therapy, whether it be continuous hemofiltration or dialysis (Palevsky, 2012). During the fourth and final phase, recovery, the tubular epithelial cells undergo repair and regeneration with a subsequent and gradual improvement in GFR (Tolwani, 2012). Sometimes this is also called the diuretic phase because of the brisk increase in urinary output (Palevsky, 2012). Unfortunately, even if the GFR recovers to nearly normal levels, the residual injury to the renal tubules leads to an overall decrease in GFR compared to

baseline, a loss of the ability to acutely increase the GFR following certain stimuli, and other symptoms that may last for months or years (Palevsky, 2012).

Traditional Biomarkers Used To Diagnose AKI

Since the renal system is responsible for maintaining physiologic homeostasis in the body, it is essential to assess overall kidney function, and GFR has become the indicator used by doctors in the clinical setting while caring for patients. Accurately measuring GFR is important because its measurement can help doctors to assess the severity of renal dysfunction or determine how far along kidney disease has progressed. However, it is not physically possible to measure GFR directly, so instead, it is calculated by measuring the renal clearance of filtration markers (Stevens & Levey, 2005). It is important that GFR is calculated using the renal clearance of a substance whose plasma concentration is stable, is non-reactive, and is filtered freely in the glomeruli while simultaneously not being metabolized, secreted, or reabsorbed (Stevens et al., 2006). An endogenous biomarker that fits all those ideal characteristics does not exist, but serum creatinine and serum urea have been used in the clinical setting to measure the level of renal function.

The best overall measurement of renal function is GFR. Factoring in age, gender, and body size, the normal value in young men is about 130 ml per minute per 1.73 m^2, and the normal value in young women is about 120 ml per minute per 1.73 m^2 (Stevens et al., 2006). GFR can be measured by calculating the plasma or urinary clearance of a marker with ideal characteristics such as inulin or also by using exogenous markers such

as iohexol, iothalamate, diethylene triamine pentaacetic acid, and ethylenediaminetetraacetic acid (Stevens et al., 2006). However, measuring the clearance of these exogenous markers can be expensive, complex, and difficult to do routinely in the clinic (Mohanram & Toto, 2005). In addition, studies have shown that there are variations in clearance measurements, with errors as high as 20 percent, when using these exogenous markers to calculate GFR whether procedures are done on the same day or different days, and these variations increase at higher absolute scale GFR measurements (Stevens et al., 2006). And although inulin meets the requirements that the marker to measure GFR should be freely filtered in the glomerus while not being secreted, reabsorbed, or metabolized, it needs to be infused intravenously, leading to its use mainly as a research tool and not in clinical practice (Perrone et al., 1992).

Serum Creatinine

It has been widely interpreted over many years that serum creatinine concentration is a measure of GFR and that GFR is an overall indication of renal function in the clinical setting (Perrone et al., 1992). In fact, there have been many studies that support the reciprocal relationship GFR has with the serum creatinine level and the direct relationship GFR has to creatinine clearance (Stevens & Levey, 2005). However, creatinine is far from a perfect tool in measuring renal function through GFR. There are innate differences in the level of serum creatinine among different racial, ethnic, geographic, and age groups which is most probably attributed to the fact that muscle mass and dietary habits primarily determine how much creatinine is generated (Stevens et

al., 2006). Table 2 lists examples of the effect age, gender, race, body type, illness, and diet have on serum creatinine levels.

Table 2 Factors That Affect The Generation of Creatinine. Variation in muscle mass predominantly accounts for differences in creatinine production. Caucasian race was used as a reference when comparing ethnic groups. Table taken from (Stevens et al., 2006).

Factor	Effect on Serum Creatinine
Aging	Decreased
Female sex	Decreased
Race or ethnic group	
Black	Increased
Hispanic	Decreased
Asian	Decreased
Body habitus	
Muscular	Increased
Amputation	Decreased
Obesity	No change
Chronic illness	
Malnutrition, inflammation, deconditioning (e.g., cancer, severe cardiovascular disease, hospitalized patients)	Decreased
Neuromuscular diseases	Decreased
Diet	
Vegetarian diet	Decreased
Ingestion of cooked meat	Increased

Creatinine is derived from amino acids, has a molecular mass of 113 Daltons, and is freely filtered in the glomerulus (Stevens et al., 2006). However, if it is used as a marker for GFR, it is assumed that 2 criteria need to be met:

1. Creatinine must be a perfect filtration marker (Perrone et al., 1992).

2. The metabolism of creatinine must be constant over time among different individuals while the renal extraction rate of creatinine equals the production rate (Perrone et al., 1992).

From Table 2, it is already apparent creatinine production can vary greatly among different individuals. And while creatinine fulfills many of the requirements to be a perfect filtration marker, it does not meet them all (Perrone et al., 1992). Creatinine is not protein bound, is filtered freely, is not subject to renal metabolism, and is physiologically non-reactive (Perrone et al., 1992). However, since the proximal tubular cells in the nephron secrete creatinine while simultaneously being filtered freely by the glomerulus, the clearance of creatinine usually exceeds the actual GFR (Stevens et al., 2006). In fact, the proximal tubular secretion of creatinine can result in the creatinine clearance exceeding inulin clearance by up to 40 percent (Perrone et al., 1992). This overestimation of renal function based on a falsely high creatinine clearance can be potentially dangerous in the clinical setting. This is because a higher creatinine clearance based GFR calculation can inaccurately estimate the level of renal functionality and fail to identify the beginning of renal insufficiency and also result in incorrect medication dosages being prescribed to patients suffering from chronic kidney disease (CKD) (Levey et al., 1999). Nonetheless, creatinine clearance has been used as the standard for measuring GFR and overall renal function. In clinic, creatinine clearance can physically be computed by using timed 24-hour urine collections along with concurrent blood sampling (Stevens et al., 2006). However, these timed collections are easily subjected to human error and are

cumbersome to those involved, so this type of measurement is no longer a recommended way to routinely estimate the level of renal function (Stevens et al., 2006).

Equations To Estimate GFR

In order to account for the many variables affecting GFR measurements such as age, gender, ethnicity, and serum creatinine, estimating equations have been developed and used widely to overcome the limitations of using serum creatinine alone (Stevens et al., 2006). In fact, studies have shown that these equations based on the serum creatinine level in conjunction with other variables performed much better in correctly calculating GFR when compared to using serum creatinine levels alone (Coresh et al., 2002). Table 3 lists out the 7 different GFR estimating equations that were compared in a 1999 study investigating the accuracy of GFR prediction from serum creatinine levels. The two most commonly used equations are the Cockroft-Gault formula and the MDRD study equation which are represented by equations 2 and 7 respectively in Table 3. The Cockcroft-Gault formula was created in 1973 while the MDRD study equation was created in 1999 (Stevens et al., 2006).

Although the Cockcroft-Gault equation was used widely in the past, it overestimated GFR because it did not take into account the tubular secretion of creatinine (Stevens et al., 2006). Because it did not take into account the adjustments needed for body-surface area, there needs to be additional measurements of height, calculation of body-surface area, and a re-calculation to 1.73 m^2 in order to compare the Cockcroft-

Gault equation to normal creatinine clearance values ("Frequently Asked Questions About GFR Estimates," n.d.).

Table 3 Equations Using Serum Creatinine Concentration to Predict Glomerular Filtration Rate. (ml/min per 1.73 m^2) Alb = serum albumin concentration (g/dl); C_{Cr} = creatinine clearance (ml/min per 1.73 m^2); C_{urea} = urea clearance (ml/min per 1.73 m^2); P_{Cr} = serum creatinine concentration (mg/dl); SUN = serum urea nitrogen concentration (mg/dl); UUN = urine urea nitrogen concentration (g/d); S_{Cr} = serum creatinine concentration (mg/100ml). Cockcroft-Gault formula is $C_{Cr} = [(140\text{-age})(\text{wt kg})]/72 \times S_{Cr}$ (Cockcroft & Gault, 1976). Table taken from (Andrew S. Levey et al., 1999).

Equation 1: Serum creatinine $GFR = 0.69 \times [100/P_{cr}]$
Equation 2: Cockcroft–Gault formula $GFR = 0.84 \times$ [Cockcroft–Gault formula]
Equation 3: Creatinine clearance $GFR = 0.81 \times [C_{cr}]$
Equation 4: Average of creatinine and urea clearance $GFR = 1.11 \times [(C_{cr} + C_{urea})/2]$
Equation 5: Creatinine clearance, urea clearance, and demographic variables $GFR = 1.04 \times [C_{cr}]^{+0.751} \times [C_{urea}]^{+0.226} \times$ [1.109 if patient is black]
Equation 6: Demographic, serum, and urine variables $GFR = 198 \times [P_{cr}]^{-0.858} \times [Age]^{-0.167} \times$ [0.822 if patient is female] $\times$ [1.178 if patient is black] $\times [SUN]^{-0.293} \times [UUN]^{+0.249}$
Equation 7: Demographic and serum variables only $GFR = 170 \times [P_{cr}]^{-0.999} \times [Age]^{-0.176} \times$ [0.762 if patient is female] $\times$ [1.180 if patient is black] $\times [SUN]^{-0.170} \times [Alb]^{+0.318}$

Conversely, the MDRD study equation has already adjusted for body-surface area in calculating the GFR (Levey et al., 2000). Out of all 7 equations tested, the ones that used estimated or measured creatinine as a basis for GFR calculation tended to overestimate GFR (Levey et al., 1999). Even after correcting for these systematic errors in GFR overestimation, the MDRD study equation produced the least variation when comparing the predicted GFR with the measured GFR (Levey et al., 1999). In fact, the MDRD study

equation provides reasonably accurate GFR measurements for non-hospitalized patients who had been diagnosed with chronic kidney disease (Stevens et al., 2006). These equations provide another advantage in the clinical setting because it can be difficult to quantify and appreciate the degree and rate of change in GFR by only measuring changes in serum creatinine levels because of the reciprocal relationship between serum creatinine levels and GFR (Stevens et al., 2006). For example, if you take a 50-year-old Caucasian male:

1. An increase in serum creatinine from 1.0 to 2.0 mg/dl (88.4 to 176.8 μmol/l) correlates to a GFR decrease of 46 ml/min per 1.73 m^2 (Stevens et al., 2006).
2. However, a further increase in serum creatinine from 2.0 to 3.0 mg/dl (265.2 μmol/l) only correlates to a GFR decrease of 14 ml/min 1.73 m^2 (Stevens et al., 2006).

Therefore, these equations provide the advantage of directly calculating for GFR instead of creating the need to extrapolate and interpret the magnitude of GFR change from reciprocal changes in serum creatinine levels.

Limitations of Traditional Biomarkers

Although serum creatinine and blood urea nitrogen (BUN) have been used extensively in the past as biomarkers for kidney injury, their utility has limitations. As mentioned earlier, serum creatinine levels innately fluctuate among individuals based on age, body type, ethnicity, and other factors. Therefore, serum creatinine levels do not present great sensitivity or specificity in early detection of renal injury (Lattanzio &

Kopyt, 2009). Similarly, BUN levels are not sensitive or specific enough in diagnosing AKI because its levels are also affected by renal and non-renal factors alike, independent of both kidney injury or function (Urbschat et al., 2011). The production of these biomarkers does not necessarily differentiate between normal renal function and an active lesion that may indicate active kidney damage (Mori & Nakao, 2007). For example, urea nitrogen production is not constant because it can increase with a diet high in protein or with enhanced breakdown of tissue from a trauma, but it can also decrease with a diet low in protein or advanced liver disease, all without a change in GFR (Proulx et al., 2005).

Serum creatinine concentration has its own fluctuations with body characteristics, but it presents another challenge in being used to identify kidney injury because its levels may not change until kidney function has already decreased to an appreciable degree (Urbschat et al., 2011). This means that kidney injury has already occurred by the time serum creatinine levels are elevated. Another physiologic limitation of even greater importance is that because of the tubular secretion of creatinine, renal function is overestimated especially at lower GFR values (Urbschat et al., 2011). Lower GFR values already indicate more advanced renal injury, so an overestimation of kidney function at this stage could potentially amplify other health risks. For example, a study has shown that creatinine production as a whole was dramatically decreased in mice with sepsis, so this artificially low serum creatinine level overestimated renal function and made it an even poorer tool in evaluating renal damage (Doi et al., 2009).

There have been benefits in using equations to estimate GFR instead of only relying on serum creatinine measurements, but these equations also have their limitations because patients need to be stable or have a chronic kidney dysfunction in order for the equations to have any utility (Urbschat et al., 2011). It is difficult to use the GFR equations in acute cases such as AKI because GFR is already reduced significantly in a short time before serum creatinine has the opportunity to accumulate (Urbschat et al., 2011). Nevertheless, the historical use of serum creatinine levels and BUN in conjunction with GFR estimation equations to identify renal injury has set a foundation for AKI diagnosis and treatment, and over the years, methods have been modified to create a standard in diagnostics that will help perpetuate further advancements in the field.

Historical Classification of AKI

Before the 1800s, there were few references to AKI (Srisawat et al., 2010). However, even in the second century AD, the investigation of one man named Galen created the foundation for using anatomically accurate models to understand the functions of the human body (Eknoyan, 1989). By essentially laying down the groundwork for experimental physiology, Galen defined the differential diagnosis of ischuria, the suppression of urine output, using a physical examination to see if a patient presents with a distended bladder or not (Eknoyan, 1989). By the end of the 18th century, the famous pathologist and anatomist Batista Morgagni introduced, for the first time, terminology that defined ischuria based on kidney pathology (Eknoyan, 2002). These terms included ischuria renalis, ischuria ureterica, uschuria vesicalis, and ischuria

urethralis (Eknoyan, 2002). By the beginning of the 1900s, acute renal failure (ARF) was called acute Bright's disease, and during this time, extensive microscopic and macroscopic pathological study in conjunction with major contributions by military medicine in the area of traumatic shock added to the development of study in this field (Srisawat et al., 2010). During World War II, there were cases of impaired function of the kidney following crush injuries, and an examination of the pathology of the kidney showed that there was pervasive damage to the renal tubules along with pigmented casts inside the tubules (Srisawat et al., 2010). Once these studies created the groundwork for future investigation into acute renal failure, the actual term "acute renal failure" was finally introduced in 1951 by Homer W. Smith in his textbook *The Kidney. Structure and Function in Health and Disease* (Srisawat et al., 2010). During the 1950s, three physicians by the names of William J. Kolff, John P. Merril, and George E. Schreiner greatly added to the knowledge on ARF by respectively making contributions like inventing the artificial kidney, creating the management and clinical course of ARF, and describing and encouraging the treatment of ARF (Srisawat et al., 2010). Figure 2 provides a short timeline illustrating all these milestones.

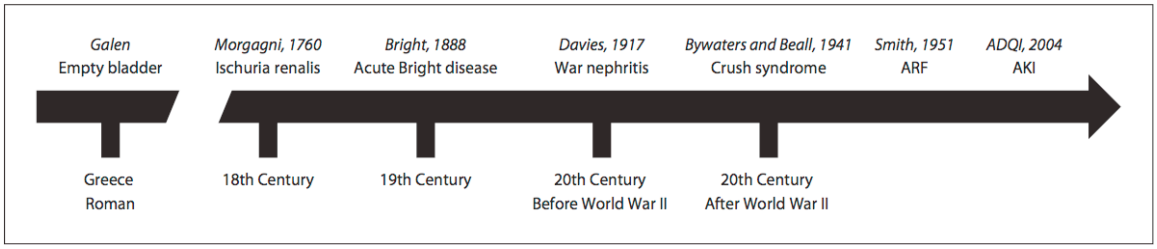


Figure 2 Timeline Illustrating Milestones in the History of AKI. Figure taken from (Srisawat et al., 2010).

Even with the advancements made throughout history in the study of kidney injury, one of the recent points of emphasis was developing a clear definition of AKI because realistically, a patient can neither be diagnosed nor cleared of AKI until there is a universally accepted set of criteria to follow (Ricci et al., 2007). The creation of this criteria was extremely important because even a relatively small impairment in kidney function needs to be considered as an independent mortality risk factor (Joannidis et al., 2009). In fact, studies have shown that even a rise in serum creatinine of 0.5 mg/dl can increase the odds of death due to kidney injury by a factor of six (Chertow et al., 2005). Not only that, but even in instances of survival, a serum creatinine increase of 2.0 mg/dl was correlated with an increase of $34,000 in total hospital costs (Chertow et al., 2005).

Nevertheless, before 2004, there were over 35 different definitions ARF in medical literature resulting in a lack of diagnostic criteria to follow (Srisawat et al., 2010). This lack of uniformity has limited and impaired studies looking into the epidemiology and outcomes for critically ill patients (Chang et al., 2010). For example, studies measuring the rate of AKI in patients admitted for acute decompensated heart failure (ADHF) have ranged from 10 to 40% because of the varying definitions for AKI in the ADHF population, and other outcomes such as in-hospital death and readmission due to heart failure can vary wildly between studies (Roy et al., 2013). Conventionally, the term ARF was reserved for patients who required acute dialysis support and were usually admitted to the ICU, but because of the clinical importance of even small increases in serum creatinine, there was a need to classify the entire spectrum of kidney injury (Srisawat et al., 2010). Therefore, in 2004, the Acute Dialysis Quality Initiative

(ADQI) created a working definition and classification system for AKI that included the following features:

1. Simplicity and clinical applicability across different healthcare centers (Bellomo et al., 2004).
2. Specificity and sensitivity in various populations and relevance to questions in research (Bellomo et al., 2004).
3. Evaluation of changes in creatinine levels from a measured baseline value (Bellomo et al., 2004).
4. Creation of a classification system for acute on chronic renal disease (Bellomo et al., 2004).

This new classification system should distinguish both between early or late cases and mild or severe cases, thereby effectively categorizing and detecting patients with mildly affected renal function and patients with severely affected renal function (Ricci et al., 2007).

RIFLE Criteria

In 2004, the ADQI group in conjunction with representatives from the American Society of Nephrology (ASN), the International Society of Nephrology (ISN), the National Kidney Foundation (NKF), and the European Society of Intensive Care Medicine (ESICM) proposed the terminology “acute kidney injury” to describe how acute renal dysfunction is actually composed of an entire spectrum of severity that usually follows an injury to the kidney affecting structural and functional changes in the

kidney (Ricci et al., 2007). They looked to standardize the definition of AKI in a way that was already done in two other syndromes common to the ICU: sepsis and Acute Respiratory Distress Syndrome (ARDS) (Srisawat et al., 2010). The study that this group conducted was focused on first creating a clear definition of ARF, but it also looked to form a foundation for future research by focusing on other things such as an evaluation of different animal models in ARF research and an assessment on the appropriate use of physiological and clinical end-points in testing new ARF treatments (Bellomo et al., 2004). This group was able to successfully reach a consensus on a total of 47 questions integrating the intended points of emphasis by reviewing the evidence available concerning optimal practice in these areas (Bellomo et al., 2004).

First, a definition and classification system was developed for ARF because without a set of universally accepted criteria, ARF occurrence ranged from 1% to 25% in critically ill patients, and death ranged from 28% to 90% depending on which population was studied and what criteria was used to diagnose ARF (Bellomo et al., 2004). It was first determined to use measures of serum creatinine and urine output in determining the severity of ARF because these functions are unique to the kidney with values that are easily measured (Bellomo et al., 2004). Measures of BUN were excluded because it is not as specific a marker of renal function when compared to creatinine (Bellomo et al., 2004). Figure 1 illustrates the numerical values associated with the severity scale for both the GFR criteria and the urine output criteria. The criteria that leads to the worst stage outcome is the one used in grading ARF (Bellomo et al., 2004). As Figure 1 shows, the acronym RIFLE refers to the Risk of renal dysfunction, Injury to the kidney, Failure of

kidney function, Loss of kidney function, and End-stage kidney disease (ESKD) (Bellomo et al., 2004). The first 3 categories are used to assess the severity of renal dysfunction while the latter 2 categories indicate clinical outcomes (Bellomo et al., 2004). The 2 different clinical outcomes acknowledge that there is a difference in adaptations that must occur in ESKD that are not observed is persistent ARF (Bellomo et al., 2004). For example, while ESKD is defined by the required use of dialysis for greater than 3 months, persistent ARF is defined as the necessary use of renal replacement therapy (RRT) for greater than 4 weeks (Bellomo et al., 2004). The RIFLE criteria was created to have high sensitivity starting from the Risk assessment and move down to have high specificity at the ESKD level (Bellomo et al., 2004).

One hurdle this classification system faces is the need for a baseline measurement of renal function. This is because many patients are diagnosed with acute dysfunction in their kidneys without ever having a baseline measurement to use as a reference and make assessments (Bellomo et al., 2004). Therefore, it was determined to use the MDRD formula to create theoretical baseline serum creatinine values, assuming normal GFR, for patients differing in age, race, and gender (Bellomo et al., 2004). Table 4 gives examples for estimated baseline serum creatinine levels for a few different populations.

The original intention for creating the RIFLE classification was to create a standard definition and severity scale for AKI, but other studies have shown that the RIFLE criteria also holds some predictive value in the clinic (Ricci et al., 2007). For example, a systematic review was done to evaluate the ability the RIFLE criteria had in predicting mortality in patients from the ICU, inside the hospital but not in the ICU, after

cardiac surgery, and in the pediatric ward (Ricci et al., 2007). While these patients had not presented to the hospital suffering from AKI, there was an increasing related risk for death when the RIFLE criteria was used to classify these patients from Risk to Failure (Ricci et al., 2007). The one population where this trend did not hold was in patients who already required RRT, but it was suggested that this was probably because for those patients that are already severely ill, the RIFLE criteria is not able to discriminate between the Risk, Injury, and Failure classes (Ricci et al., 2007).

Table 4 Estimated Baseline Serum Creatinine Levels Using the MDRD Formula. Estimated GFR = 75 ml/min per 1.73 m^2 = 186 × (serum creatinine [S_{Cr}]) – 1.154 × (age) – 0.203 × (0.742 if female) × (1.210 if black) = exp(5.228 – 1.154 × ln[S_{Cr}]) – 0.203 × ln(age) – (0.299 if female) + (0.192 if black). Table taken from (Bellomo et al., 2004).

Age (years)	Black males (mg/dl [µmol/l])	Other males (mg/dl [µmol/l])	Black females (mg/dl [µmol/l])	Other females (mg/dl [µmol/l])
20–24	1.5 (133)	1.3 (115)	1.2 (106)	1.0 (88)
25–29	1.5 (133)	1.2 (106)	1.1 (97)	1.0 (88)
30–39	1.4 (124)	1.2 (106)	1.1 (97)	0.9 (80)
40–54	1.3 (115)	1.1 (97)	1.0 (88)	0.9 (80)
55–65	1.3 (115)	1.1 (97)	1.0 (88)	0.8 (71)
>65	1.2 (106)	1.0 (88)	0.9 (80)	0.8 (71)

The RIFLE criteria made significant and necessary advancements in creating a standardized set of diagnostic measurements for AKI, but it still had limitations and room for improvement. One limitation was that while urine output is very specific and sensitive to the kidney, its variability due to external factors has led to its omission in many studies (Ricci et al., 2007). Some problems include:

1. The use of diuretics can compromise the sensitivity and specificity of urine output for grading AKI (Ricci et al., 2007).

2. Urine output needs to be accurately assessed, but this is usually only possible with a urinary catheter making proper urine output measurement feasible in the ICU but less so in the wards (Ricci et al., 2007).
3. The urine output criteria, being too sensitive and not specific enough, has not been proven to be well balanced with the serum creatinine criteria when defining the Risk, Injury, and Failure categories. This has led to patients diagnosed using the serum creatinine criteria being more severely ill in each category than patients diagnosed using the urine output criteria (Ricci et al., 2007).

Another limitation of the RIFLE criteria is that a baseline serum creatinine level measurement is needed as a reference point (Ricci et al., 2007). Although the MDRD formula has provided theoretical baseline values for different populations, the values are inherently non-universal for all individuals, and the validity of these values has been questioned (Hoste & Kellum, 2006). Finally, the RIFLE criteria fails to account for the influence etiology of AKI has on the course of diagnosis and treatment, and it also does not consider the need for RRT as a factor in ranking the Risk, Injury, and Failure categories (Ricci et al., 2007). Therefore, subsequent modifications were necessary to account for some of these shortcomings.

AKIN Modification to RIFLE Criteria

When the ADQI created the RIFLE criteria, they also established the Acute Kidney Injury Network (AKIN), which was comprised of experts from the leading kidney research societies, to work as a collaborative network and use their expertise to

facilitate international, interdisciplinary, and intersocietal collaboration in order to advance the field of AKI research (Mehta et al., 2007). When this group met for their first conference in 2005, they worked to create additional criteria in defining and classifying AKI. The focused on 6 concepts in creating this diagnostic criteria:

1. The definition has to be broad enough to encompass and recognize deviations in clinical presentation across different age groups, geographic locations, and clinical situations (Mehta et al., 2007).
2. Since serum creatinine levels and urine output can be influenced by other factors besides GFR, other biomarkers that are more sensitive and specific to kidney injury need to be the future of diagnosis and prognosis (Mehta et al., 2007).
3. Small variations in serum creatinine levels can have adverse outcomes too, but current clinical practices usually attributes this to lab variations and does not give much attention to it (Mehta et al., 2007).
4. It was observed that adverse outcomes occurred when small changes in serum creatinine presented within 24 to 48 hours. Therefore, a time constraint for diagnosis was placed at 48 hours in order to eliminate "non-acute" increases in serum creatinine that may change by 0.3 mg/dl over a longer period of time (Mehta et al., 2007).
5. Further evaluation needs to be done for patients with CKD to see if the serum creatinine concentration elevation of 0.3 mg/dl is still applicable to them since AKI can often present superimposed with pre-existing CKD (Mehta et al., 2007).

6. Urine output criteria needs to be included because it can be used as a diagnostic measure for critically ill patients when renal dysfunction is present before there is enough time for serum creatinine levels to increase (Mehta et al., 2007).

There was some dissent among a small subset of AKIN members who argued that a urine output reduction of 0.5 ml/kg per hour over 6 hours was not specific enough to diagnose AKI, and that the difficulty to accurately measure urine in non-ICU conditions in conjunction with the effect of diuretics on urine output made it a problematic measure for AKI (Mehta et al., 2007). Nevertheless, it was determined that even with the possible increase in false-positive results, it was still an improvement on the current state of diagnosis where AKI was under-recognized and many patients were identified in late stages of their illness (Mehta et al., 2007). Since the purpose of this diagnostic criteria was to increase the clinical awareness and diagnosis of AKI, even though some patients labeled with AKI may not actually have the condition, it allows the opportunity for prevention and procedures to prevent further kidney damage (Mehta et al., 2007). Table 5 shows the changes proposed by the AKIN criteria in both diagnostic measurements and naming of stages.

The AKIN criteria made some changes to the RIFLE criteria but also retained many of its recommendations. Since the importance of small changes in serum creatinine levels had been emphasized, the AKIN criteria included that modification in Stage 1. Even though AKI is diagnosed over a period of 48 hours, progression through the stages occurs over a longer time period, and the AKIN criteria maintained the time frame of one week that was proposed by the ADQI group (Mehta et al., 2007). While the RIFLE

criteria included the therapy required in treating AKI to define the Loss and ESKD categories, the AKIN group consciously did not include them because they were viewed as outcomes of AKI as opposed to stages of AKI (Mehta et al., 2007). While Stage 1 in the AKIN criteria was made analogous to the Risk category in the RIFLE criteria, Stage 2 and Stage 3 were equated to the Injury and Failure categories respectively (Mehta et al., 2007). Finally, all patients requiring the administration of RRT were automatically placed in Stage 3 of the AKIN criteria because the variability in commencing RRT in different populations and countries made it difficult to use it as a staging criteria between levels (Mehta et al., 2007).

Table 5 Stages of Acute Kidney Injury As Defined by the RIFLE and AKIN Criteria. Table taken from (Palevsky, 2012).

RIFLE Stages	Increase in Serum Creatinine* in RIFLE Criteria	Urine Output* RIFLE and AKIN Criteria	Increase in Serum Creatinine* in AKIN Criteria	AKIN Stages
Risk	≥150% of baseline	<0.5 mL/kg/hour for >6 hours	≥0.3 mg/dL over baseline; or ≥150% of baseline	Stage 1
Injury	≥200% of baseline	<0.5 mL/kg/hour for >12 hours	≥200% of baseline	Stage 2
Failure	≥300% of baseline; or ≥0.5 mg/dL to a level >4.0 mg/dL	<0.3 mL/kg/hour for >24 hours; or Anuria for >12 hours	≥300% of baseline; or ≥0.5 mg/dL to a level >4.0 mg/dL	Stage 3
Loss	Need for renal replacement therapy as a result of acute kidney injury for >4 weeks			
End stage	Need for renal replacement therapy as a result of acute kidney injury for >3 months			

*Stage determined by highest severity of serum creatinine and urine output criteria.

Comparing RIFLE and AKIN Criteria

One of the main goals of the AKIN criteria was to increase the sensitivity of AKI detection by including the diagnostic measurement of an increase in serum creatinine of 0.3 mg/dl or more within a 48-hour window. This new addition succeeded in its intended purpose while still maintaining some of the original benefits of the RIFLE criteria. There have been conflicting results where some studies showed that the AKIN criteria may not actually improve upon the RIFLE criteria's sensitivity or predictive ability while other studies showed that the AKIN criteria showed superior sensitivity in detecting AKI but was inferior in its ability to predict outcomes in critically ill patients (Chang et al., 2010). Nevertheless, a study by Chang et al. showed that the AKIN criteria identified 7.9% more AKI patients than the RIFLE criteria (Chang et al., 2010). In addition, the AKIN criteria maintained the patient predictive ability as the mortality rates all increased when moving from Stage 1 to Stage 2 to Stage 3 as had been observed when moving from Risk to Injury to Failure in the RIFLE criteria (Chang et al., 2010). Even in a study specific to patients undergoing cardiac surgery, the AKIN criteria diagnosed significantly more patients with AKI when compared to the RIFLE criteria (Englberger et al., 2011).

However, this is not always the case. Table 6 shows the distribution of critically ill patients classified in the different categories of both the RIFLE and AKIN criteria. As shown in the table, 14,356 patients were classified into different stages of AKI severity using these 2 classification systems. The horizontal rows define the AKIN stages while the vertical columns define the RIFLE stages. The boxes highlighted in yellow that make

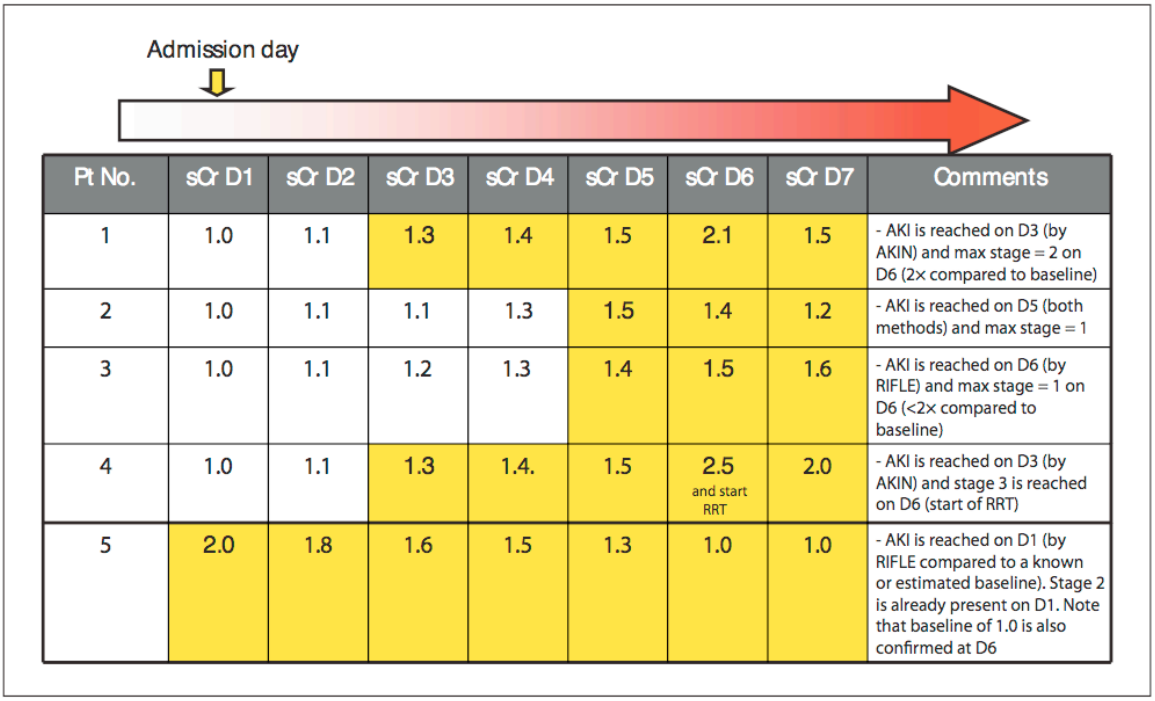
the horizontal line across the table indicate the subpopulation of patients that were categorized to the analogous stages in both the RIFLE and AKIN staging levels. By adding those numbers up, we can see that 82.7% of patients were staged in consensus between these two classification systems.

Table 6 Distribution of Critically Ill Patients Classified by the RIFLE and AKIN Criteria. A total of 14,356 patients were cross-classified using the RIFLE and AKIN criteria. The boxes highlighted in yellow represent the subpopulation of patients that were categorized to the same stage using the two classification systems. The bracketed percentages represent the mortality rate of each group. Table taken from (Joannidis et al., 2009).

AKIN		RIFLE					Total (AKIN)
		non-AKI	Risk	Injury	Failure		
non-AKI	n *	8759 (12.9%)	781 (27.7%)	452 (37.4%)	271 (41.3%)		10263 (15.9%)
Stage 1	n *	457 (25.2%)	282 (33.0%)	243 (44.0%)	95 (60.0%)		1077 (34.5%)
Stage 2	n *	36 (30.6%)	21 (47.6%)	885 (25.9%)	91 (54.9%)		1033 (29.0%)
Stage 3	n *	11 (18.2%)	8 (12.5%)	16 (62.5%)	1948 (41.3%)		1983 (41.2%)
Total (RIFLE)	n *	9263 (13.6%)	1092 (29.2%)	1596 (32.3%)	2405 (42.6%)		14356 (21.7%)

Looking deeper into the table shows that the AKIN criteria does not necessarily show an increase in sensitivity when compared to the RIFLE criteria. By adding up the Risk, Injury, and Failure totals in the non-AKI AKIN row, it is obvious that 1,504 patients that were classified as non-AKI using the AKIN criteria were subsequently classified as AKI under the RIFLE criteria, with roughly half of them falling in the Risk category (Joannidis et al., 2009). Conversely when adding up the Stage 1, Stage 2, and Stage 3 totals in the non-AKI RIFLE column, 504 patients that were classified as non-AKI using

the RIFLE criteria were subsequently classified as AKI under the AKIN criteria, with nearly all of them falling in Stage 1 (Joannidis et al., 2009). This shows that using either criteria alone leaves room for missing diagnoses in patients, and depending on the patient population, either the RIFLE or the AKIN criteria may be more sensitive. Figure 3 illustrates how this may be the case.



Pt No.	sCr D1	sCr D2	sCr D3	sCr D4	sCr D5	sCr D6	sCr D7	Comments
1	1.0	1.1	1.3	1.4	1.5	2.1	1.5	- AKI is reached on D3 (by AKIN) and max stage = 2 on D6 (2× compared to baseline)
2	1.0	1.1	1.1	1.3	1.5	1.4	1.2	- AKI is reached on D5 (both methods) and max stage = 1
3	1.0	1.1	1.2	1.3	1.4	1.5	1.6	- AKI is reached on D6 (by RIFLE) and max stage = 1 on D6 (<2× compared to baseline)
4	1.0	1.1	1.3	1.4.	1.5	2.5 and start RRT	2.0	- AKI is reached on D3 (by AKIN) and stage 3 is reached on D6 (start of RRT)
5	2.0	1.8	1.6	1.5	1.3	1.0	1.0	- AKI is reached on D1 (by RIFLE compared to a known or estimated baseline). Stage 2 is already present on D1. Note that baseline of 1.0 is also confirmed at D6

Figure 3 Illustrative Cases on the Diagnosis of AKI Using the RIFLE and AKIN Staging Criteria. All patients have a baseline serum creatinine level of 1.0 mg/dl. Figure taken from (Srisawat et al., 2010).

Figure 3 follows the serum creatinine concentration of 5 hypothetical patients for seven days following admission to the hospital, all with a baseline serum creatinine level of 1.0 mg/dl.

1. Case 1 shows how the AKIN modifications can allow for earlier detection of AKI (Srisawat et al., 2010). Using the AKIN criteria, patient 1 could be diagnosed with AKI as early as day 3 since a serum creatinine level of 1.3 mg/dl meets the Stage

1 requirement of a 0.3 mg/dl increase over baseline within a 48 hour period (Srisawat et al., 2010). Conversely, the RIFLE criteria would not have diagnosed AKI in the Risk category until day 5 because a 150% baseline measurement of serum creatinine would not be reached until it hit the value 1.5 mg/dl (Srisawat et al., 2010). Moreover, if the patient's serum creatinine level had peaked at 1.4 mg/dl, the RIFLE criteria would have never even classified this as a case of AKI, thus potentially missing the progression of a deadly disease (Srisawat et al., 2010). The severity of AKI reaches a maximum on day 6 at Stage 2 using the AKIN criteria and Injury using the RIFLE criteria.

2. Case 2 shows an instance where AKI is diagnosed on the same day regardless of which classification system is used (Srisawat et al., 2010). The RIFLE criteria cannot diagnose AKI until day 5 because the serum creatinine does not reach 1.5 mg/dl until then. Interestingly, this is a case where the AKIN criteria of an increase in serum creatinine of 0.3 mg/dl within a 48 hour period is not helpful in early diagnosis. Because of this requirement, AKI cannot be diagnosed using the AKIN criteria until day 5 as well because the serum creatinine increase is not acute enough from days 1-3 to classify as AKI. It is only when the serum creatinine concentration increases from 1.1 mg/dl on day 3 to 1.5 mg/dl on day 5 that the AKIN criteria for Stage 1 is met.
3. Case 3 shows how using the AKIN criteria alone could miss a case of AKI altogether. By applying the 48-hour time window rule alone, it is not possible to diagnose AKI because the serum creatinine concentration never rises 0.3 mg/dl

within that short a period of time (Srisawat et al., 2010). However, by also including the 150% baseline measurement of serum creatinine criteria, Stage 1 and the Risk category are both diagnosed on day 6.

4. Case 4 shows how the AKIN modification in using RRT treatment to automatically categorize to Stage 3 severity of AKI is utilized regardless of serum creatinine concentrations (Srisawat et al., 2010). Once again, the AKIN criteria diagnoses AKI on day 3 with an increase in serum creatinine of 0.3 mg/dl within 48 hours, and the RIFLE criteria diagnoses AKI on day 5 once the serum creatinine concentration reaches 150% of the baseline measurement. However, the AKIN criteria will automatically place this patient in Stage 3 because RRT is started on day 6.
5. Case 5 shows an instance of CA-AKI where the peak serum creatinine concentration is reached at or before admission into the hospital (Srisawat et al., 2010). In this case, the patient can be diagnosed with AKI if the baseline of 1.0 mg/dl is known or can be estimated using the MDRD equation (Srisawat et al., 2010). However, even without knowing the baseline value or using the estimating equation, the stable serum creatinine measurement of 1.0 mg/dl on day 6 and 7 confirm that this was, in fact, a case of AKI (Srisawat et al., 2010).

As discussed, there have been significant advancements made in the recognition and classification of AKI in recent history. Current methods of measuring renal function using serum creatinine measurements and urine output have been good tools, but they have inherent limitations in detecting early kidney injury and creating a course of

treatment based on the etiology of kidney injury. The RIFLE criteria has set the foundation for future kidney research by creating a universal definition and staging system that was further modified to increase sensitivity by the AKIN criteria. Now, the next step is to find even more specific and sensitive biomarkers that can help detect AKI sooner, thus improving the prognosis, and apply these new tools to create beneficial modifications to the existing RIFLE and AKIN criteria.

OBJECTIVES

The objective of this study is to investigate ways in which to build upon the RIFLE criteria and AKIN criteria and propose modifications that can be made and tested in future studies. These modifications will focus on increasing the sensitivity of AKI detection, increasing the ability to discover renal injury earlier, and finally helping to create a better prognosis for AKI patients by developing more specific and personalized treatments. Current methods of kidney injury detection focus on the physiological effects that come after kidney injury has already led to a decrease in organ function, but a better diagnostic approach would be to find a way to detect structural and function damage to the kidney before the secondary effects take place. This opens up a discussion on the new novel biomarkers that are presently being researched as signs of renal cell injury to conclude whether the RIFLE criteria should be modified further using these new biomarkers to allow earlier detection of AKI and hopefully lead to a better overall prognosis.

Change in serum creatinine concentrations is a common measure of AKI development, but the concentration of these new biomarkers seem to change earlier than that of creatinine. Cystatin C concentration has been shown to measure changes in glomerular filtration rate, whereas kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin concentration have been shown to be related to tubular stress or injury. The hope is that these new biomarkers can be utilized to provide earlier indications of AKI and integrated into the RIFLE criteria, thereby resulting in better prognoses for at risk patients.

PRESENTATION OF PUBLISHED RESULTS

As with many systemic diseases, a key factor in the treatment of AKI is early detection. However, the current standard of using serum creatinine concentration and urine output to measure renal function does not allow for this early detection because in some cases the damage to renal tubules may not be sufficient enough to create an appreciable change in serum creatinine (Han et al., 2007). In cases of more severe damage to the renal tubules, there is a time delay between the moment of injury and a measurable increase in serum creatinine (Han et al., 2007). Early detection is important for purposes of quick treatment, but it is also important because the lowest level of severity in the RIFLE and AKIN criteria presents the potential of reversibility in the physical and functional damages to the kidney (Srisawat et al., 2010). Therefore, in recent years, novel biomarkers that present with changes closer to the time of renal injury have been researched with the hope that they will help with earlier detection of kidney

injury as well as help elucidate the location of the intrarenal injury, differentiating among the proximal tubule, distal tubule, intersitium, and vasculature (Urbschat et al., 2011). A combination of early diagnosis and specific knowledge of the location of the injury can result in more personalized treatment and the potential for a better outcome for the patient.

The preclinical assessment of nephrotoxicity has been greatly advanced by the use of urinary biomarkers in detecting AKI in its early stages (Haase & Mertens, 2010). This is because this knowledge will help to assess and predict kidney injury through preclinical testing before overwhelming nephrotoxicity and loss of renal function become apparent (Haase & Mertens, 2010). The field of AKI is not the first to look for unique biomarkers as signs of injury to an organ. In fact, in the field of cardiovascular disease, serum troponin levels have been used to accurately diagnose and assess myocardial infarctions (Adiyanti & Loho, 2012). When a patient suffers from angina pectoris, the damaged myosin in the cardiac tissue releases troponin, so monitoring serum troponin levels can help to directly identify acute myocardial injury (Adiyanti & Loho, 2012). There is a hopeful outlook that the same can be done in the field of AKI.

The body's natural inflammatory response plays a major role in the initiation and progression of AKI irrespective of whether the injury results as a side effect of surgery or the patient is admitted to the ICU for a different condition (Mårtensson et al., 2012). In fact, AKI has in part been defined by the local and system inflammatory response following renal insult where many of the cytokines affect the kidney and other distant organs mediated by the systemic release of leukocytes from the kidneys and renal tubular

cells (Bihorac et al., 2013). Figure 4 illustrates the inflammatory mechanisms that lead to AKI and subsequent repair of the renal cells.

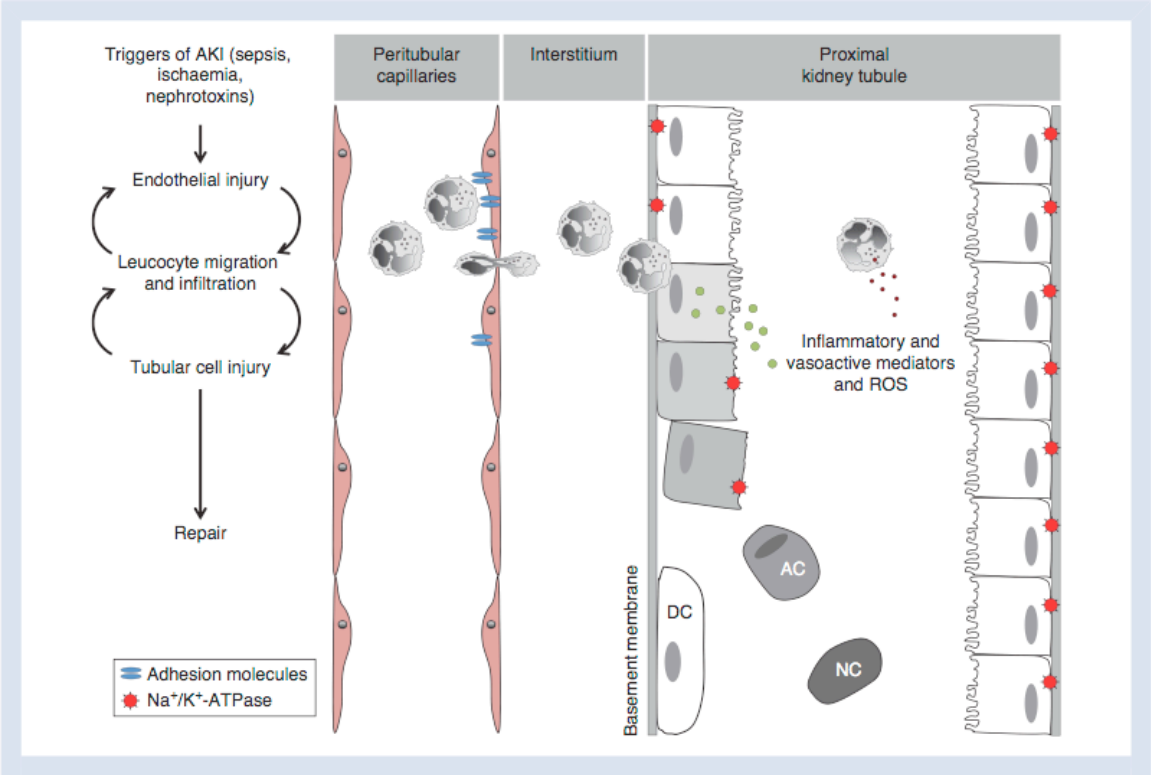


Figure 4 Pathophysiological Mechanisms of AKI and Repair. The number of adhesion molecules on the surface of peritubular capillary endothelial cells increases which helps neutrophils to migrate into the interstitium and tubular lumen. Then inflammatory mediators, inflammatory mediators, and reactive oxygen species damage the tubular cells leading to the sloughing off of the brush border, improper location of Na+/K+ ATPase, and the beginning of apoptosis and necrosis. In the case of sever injury, parts of the basement membrane is left denuded as cells are desquamated. The pathophysiological changes worsen as even more inflammatory and vasoactive substances are released from the damaged tubular cells. If the renal cells are able to recover, viable cells differentiate to cover up the sections of bare basement membrane and begin to restore functionality in the nephron. AC = apoptotic cell, DC = differentiating cell, NC = necrotic cell. Figure taken from (Mårtensson et al., 2012).

Ischemia, bacterial endotoxins, and nephrotoxins are some examples of triggers of AKI that lead to the release of inflammatory mediators from the endothelial cells of the capillaries and the tubular cells that make up the kidney (Mårtensson et al., 2012). These

cytokines and chemokines trigger the migration of neutrophils and other leukocytes to the inflammation site and facilitate the transfer across the interstitium to the injured tubular cells (Mårtensson et al., 2012). Even more leukocytes pass through the interstitium as the endothelial inflammatory response increases permeability across these barriers (Mårtensson et al., 2012). Once the leukocytes reach the tubular lumen, they release other pro-inflammatory cytokines that propagate tubular injury and eventually lead to the desquamation of cells, necrosis, and apoptosis (Mårtensson et al., 2012). Therefore, being able to track the levels of these kidney inflammation biomarkers can lead to early detection of tubular injury.

Novel Biomarkers

The following have been suggested as properties an ideal biomarker would have in early detection of AKI:

1. The biomarker must come from the damaged cells and be specific to the organ experiencing the injury (Mårtensson et al., 2012).
2. As the extent of damage increases, the concentration of the biomarker must increase proportionally as well (Mårtensson et al., 2012).
3. The temporal release of the biomarker must be close to the time of organ injury as this timing would help detect when the damage is still potentially reversible (Mårtensson et al., 2012).

4. Once the acute injury episode has subsided, the concentration of the biomarker should also decrease quickly in order for it to be used as a therapeutic monitoring tool (Mårtensson et al., 2012).
5. The biomarker should be able to be measured rapidly and reliably (Mårtensson et al., 2012).

Table 7 lists many different biomarkers that are currently being investigated, but this paper will focus on the properties and potential of three novel biomarkers that have recently shown promise in fulfilling these qualities: cystatin C, kidney injury molecule-1 (KIM-1), and neutrophil gelatinase associated lipocalin (NGAL).

Table 7 Novel Biomarkers for AKI Detection and Their Methods of Measurement. Table taken from (Adiyanti & Loho, 2012).

Biomarker	Description	Renal function	Method of measurement
NAG	Proximal tubular lisosomal enzyme, more stable than other urine enzymes.	Tubular	Colorimetry
β2-microglobulin	MHC-I light chain in all nucleated cell. Unstable in urine with pH < 6	Tubular	ELISA, nephelometer
RBP	Synthesized by liver, plays a role in vitamin A transport, stable in acid pH urine.	Tubular	ELISA, nephelometer
Cystatin C	Cystein protease inhibitor	Glomerulus filtration	ELISA
KIM-1	Type I membrane glycoprotein, highly specific and sensitive	Tubular	ELISA, Luminex based assay
Clusterin	Expressed in tubular epithelial cell, highly sensitive, no clinical studies yet.	Tubular	ELISA
NGAL	Initially detected in neutrophil-gelatinase, and also induced in epithelial cells which experience inflammation	Tubular	ELISA, Luminex based assay
NHE3	The most abundant sodium transporter in tubular, sample examination process has not been optimal.	Tubular	Immunoblotting
Exosomal Fetuin A	Acute-phase protein synthesized in liver, sample examination process has not been optimal.	Tubular	Immunoblotting

Cystatin C

Plasma cystatin C, a protease inhibitor, is known to be a good measure of kidney function because it is a more robust endogenous marker of GFR when compared to creatinine (Mårtensson et al., 2012). In addition to being constantly produced by all nucleated cells, this is the case for many reasons (Adiyanti & Loho, 2012):

1. The production of cystatin C is relatively constant, and it is released into the plasma (Zhang et al., 2011).
2. >99% of cystatin C is freely filtered by the glomeruli (Zhang et al., 2011).
3. Cystatin C is not significantly bound to any proteins (Zhang et al., 2011).
4. Cystatin C is not placed back into systemic circulation after being filtered because it is almost completely reabsorbed and catabolized in the renal tubules (Song et al., 2009).

AKI was shown to be diagnosed one or two stages earlier based on the RIFLE criteria when measuring serum cystatin C levels as opposed to serum creatinine levels because cystatin C increases at a greater rate when exposed to contrast media (Adiyanti & Loho, 2012). A study that compared the increase of cystatin C, serum creatinine, and BUN levels as a percentage of their baseline values in mouse models showed that cystatin C levels exhibited a significantly sharper and earlier increase in mice that had undergone bilateral nephrectomy (Song et al., 2009). 2 hours after the bilateral nephrectomy procedure, both the serum creatinine and BUN levels had increased similarly to around 50% above their baseline values (Song et al., 2009). However, cystatin C levels had already increased to almost 300% over its baseline value (Song et al., 2009). Likewise,

after 12 hours, cystatin C levels had increased over 700% above its baseline value while serum creatinine and BUN had only increased 200%-300% above their baseline values (Song et al., 2009). After 24 hrs, cystatin C levels had reached nearly 1000% its baseline value while serum creatinine and BUN roughly maintained the 200%-300% increase it had already present with at the 12 hour mark (Song et al., 2009). Therefore, cystatin C has shown promise in its ability to detect renal damage significantly earlier than serum creatinine or BUN (Song et al., 2009). In fact, a study that investigated cystatin C's ability to predict AKI concluded that cystatin C presented with its best diagnostic accuracy as early as 10 hours after admission into a hospital (Zhang et al., 2011).

However, just as serum creatinine had limitations due to external factors affecting creatinine production in different individuals, cystatin C is also influenced by factors not related to renal function (Knight et al., 2004). Cystatin C levels were observed to be affected by age, gender, race, presence of diabetes, white blood cell count, serum albumin, and C-reactive protein (Stevens et al., 2008). Therefore, it was proposed that the best way to estimate GFR may be to use a combination of serum creatinine and serum cystatin levels with the hope that this would minimize the effect extrarenal physiological processes have on these biomarker levels (Stevens et al., 2008).

KIM-1

While cystatin C is seen as a marker of kidney function, KIM-1 is seen as a marker of kidney injury (Mårtensson et al., 2012). Studies have shown that when urinary KIM-1 levels were used to predict histopathological changes in the renal tubules, it

significantly outperformed the predictive abilities of serum creatinine and BUN (Mårtensson et al., 2012). KIM-1 is a type I cell membrane glycoprotein that is composed of a immunoglobulin-like domain uniquely made up of six cysteine residues (Vaidya et al., 2010). While the KIM-1 gene and associated protein are not expressed in the normal kidney, mice models have shown that gene expression and mRNA for KIM-1 increase more than any other known gene 24-48 hours after ischemia (Adiyanti & Loho, 2012). Epithelial cells express phosphatidylserine on their surface after undergoing apoptosis, and KIM-1 is used as a phosphatidylserine receptor on phagocytes, signaling them to dispose of the dead cells (Adiyanti & Loho, 2012). KIM-1 proteins are highly localized on the proximal tubular apical membrane in the region that was most affected by the injury (Vaidya et al., 2010). The ectoderm region of the KIM-1 protein is shed from the epithelial cells of the proximal tubule after the injury, and they are eventually excreted in the urine (Vaidya et al., 2010). Therefore, KIM-1 has shown great promise in mice models in serving as an early diagnostic indicator for kidney injury (Vaidya et al., 2010).

A study by Vaidya et al. has concluded that the ability of KIM-1 to indicate kidney injury makes it useful as a marker of nephrotoxicity, which means it can help in preventing organ damage during clinical drug development while simultaneously helping to monitor post-market nephrotoxicity as a side affect of the drug (Vaidya et al., 2010). N-acetyl-β-glucosaminidase (NAG) is a proximal tubular lysosomal enzyme that has also been shown to increase in urine concentration after exposure to nephrotoxic drugs (Adiyanti & Loho, 2012). Therefore, an increase in NAG can also indicates some form of tubular injury (Mårtensson et al., 2012). However, after being exposed to nephrotoxicants

and hepatotoxicants, urinary KIM-1 measurements still performed better than serum creatinine, BUN, and NAG in early detection of kidney injury with high specificity and sensitivity (Vaidya et al., 2010). However, because KIM-1 is an indication of cell death, it is important to evaluate how specific its presence is to renal cell death as opposed to cell death in other parts of the body. Interestingly enough, the KIM-1 levels in control groups and those exposed to hepatotoxic and cardiotoxic chemicals were similar (Vaidya et al., 2010). The fact that urinary KIM-1 level changes were unremarkable in cases of hepatotoxicity and cardiotoxicity in rat models shows that KIM-1 shows high specificity for kidney damage (Vaidya et al., 2010).

Ischemia and reperfusion injuries in rat models also showed that KIM-1 has an advantage over serum creatinine, BUN, and NAG. When looking at the histological changes following ischemia and reperfusion injury, there was low grade damage at 3-6 hours, and after 9 hours, there was evidence of single cell necrosis, dilation of the tubules, and the sloughing off of dead cells (Vaidya et al., 2010). At 12-24 hours, histological slices showed that there was substantial necrosis in the proximal tubule with associated inflammation (Vaidya et al., 2010). There were only small transient increases in serum creatinine, BUN, and NAG between 3-9 hours, and statistically significant increases occurred much later: serum creatinine increased ~2.4 fold at 18 hours, BUN increased ~2.1 fold at 18 hours, and NAG increased ~5.5 fold at 12 hours (Vaidya et al., 2010). Conversely, KIM-1 levels already showed up to ~6 fold increases at 3-6 hours, peaked at 24 hours with a ~700 fold increase, and leveled off at ~70 fold increase up to 120 hours after reperfusion (Vaidya et al., 2010).

NGAL

As was the case with KIM-1, NGAL is also loosely categorized as a marker of kidney injury (Mårtensson et al., 2012). NGAL is a protein that is located in the secondary granules of neutrophils, and the secondary granules are released into the bloodstream in response to both bacterial and viral infections (Mårtensson et al., 2012). NGAL is normally expressed only in low amounts in different human tissues, but once there is some type of injury to epithelial cells like those in the kidney, the NGAL level rises significantly (Adiyanti & Loho, 2012). NGAL is filtered in the glomerulus and reabsorbed in the proximal tubules, so NGAL is detected in the urine either when there is damage to the proximal tubules to disrupt reabsorption or when there is an increase in NGAL synthesis (Adiyanti & Loho, 2012). It has been documented that NGAL mRNA expression can be increased up to 1000 fold in the ascending loop of the loop of Henle and the collecting ducts (Adiyanti & Loho, 2012).

One of the important roles NGAL has is helping to transport iron from cell to cell (Mårtensson et al., 2012). NGAL binds to siderophores, small iron-binding molecules, and its delivery of iron to the renal tubule cells induces the differentiation of progenitor cells into the epithelial cells of the renal tubules, thus helping in the injury-repair process (Mårtensson et al., 2012). Bacteria also produce siderophores in order to get the iron necessary for growth in surrounding tissues, so NGAL blocks the supply of iron to bacteria by binding to siderophores and may have a secondary effect as a bacteriostatic agent (Mårtensson et al., 2012).

A characteristic that would be extremely beneficial in any new biomarker for AKI would be the ability to differentiate between the type of kidney injury, and NGAL has shown some promise in discriminating between pre-renal and intrinsic AKI (Mehta, 2011). Although this area of study is still new, clinicians found that after reviewing clinical data, patients who presented with a concentration of NGAL less than 47 μg/l were identified as having pre-renal AKI, and patients who presented with a concentration of NGAL greater than 104 μg/l were identified as having intrinsic AKI (Mehta, 2011). This is important because the type of kidney injury can have an effect on the efficacy and course of treatment. This use of NGAL is analogous to the use of N-terminal brain natriuretic peptide (BNP) to discriminate between heart failure and other cause of pulmonary congestion (Mehta, 2011). However, further research needs to be conducted to use NGAL effectively in this way.

NGAL has been shown to detect AKI 36-48 hours earlier than serum creatinine, but that does not mean that AKI diagnosis cannot be improved by using beneficial aspects of both physiological measurements (McCullough et al., 2011). Studies showed that if only serum creatinine were used to diagnose AKI, up to 41% of patients with AKI would have been missed (McCullough et al., 2011). However, if patients present with elevated levels of both serum creatinine and urinary NGAL, they stayed in the ICU and hospital twice as long, required more dialysis, and experienced mortality three times higher than patients negative for both biomarkers (McCullough et al., 2011). A combination of these 2 measurements can also help to identify subgroups within the AKI patient population. For example, patients who were negative for NGAL but positive for

serum creatinine were likely to suffer from pre-renal azotemia or malfunctioning tubulo-glomerular feedback without actually experiencing any acute tubular necrosis (McCullough et al., 2011). Conversely, patients who were positive for NGAL but negative for serum creatinine could indicate the presence of iron-dependant oxidative stress before any decrease in organ function is measureable (McCullough et al., 2011).

DISCUSSION AND CONCLUSION

An investigation into the properties of cystatin C, KIM-1, and NGAL has shown that each of these new biomarkers provide a noticeable advantage in early detection of AKI over the traditional methods of tracking serum creatinine concentration and urine output. It is important to continue to make progress in the detection and treatment of AKI because it is becoming clearer that AKI should no longer just be considered in determining the severity of injury in critically ill patients (Bihorac et al., 2013). Injury to the kidney can have secondary effects in other organs like the lungs through the release of inflammatory mediators and leukocytes from the renal tubular cells (Bihorac et al., 2013). Even with the advantages each new biomarker provides, a single biomarker by itself probably will not be adequate in providing all the necessary information such as the site of injury and projected course of treatment (Adiyanti & Loho, 2012). Therefore, there needs to be further research to see if a combination or panel of biomarkers can be used to provide the entire spectrum of information to effectively diagnose and treat AKI (Han et al., 2007). Figure 5 gives an illustration as to how new biomarker measurements could be incorporated into the already established RIFLE criteria. The important thing to notice is

that changes to multiple different markers of injury need to be used to balance out the shortcomings and innate biases that may be present from any single biomarker on its own.

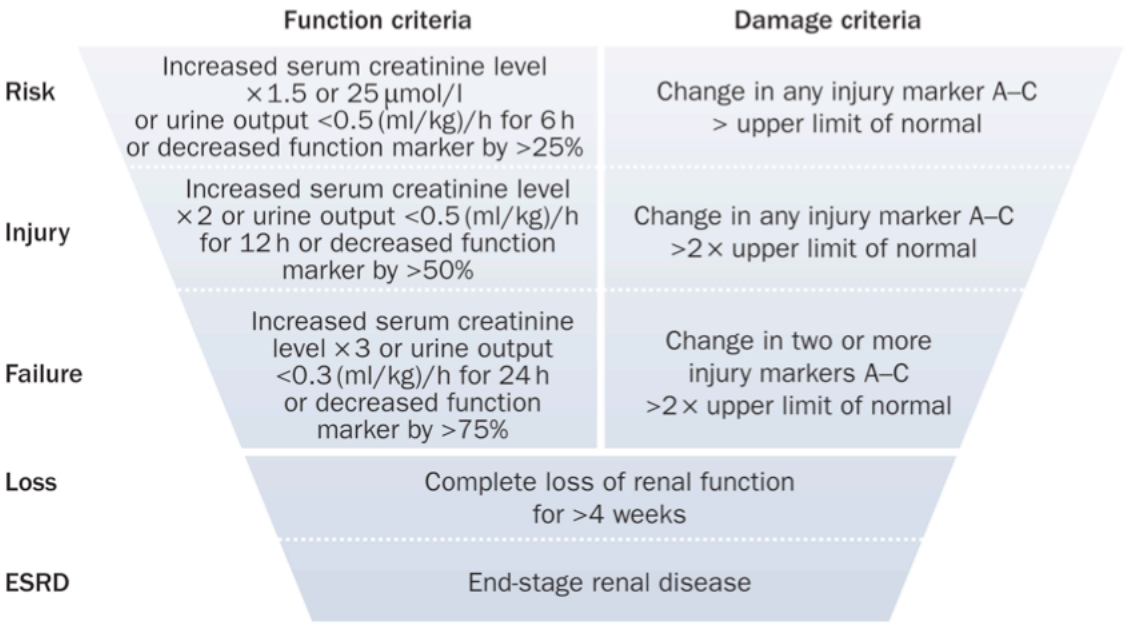


Figure 5 Theoretical Classification for the Next Generation in AKI Diagnosis. This new classification system could retain the function criteria provided by the current RIFLE criteria. A set of damage criteria could be added to measure the structural changes occurring in the renal tubules as markers for cell injury are monitored. Figure taken from (Murugan & Kellum, 2011).

As discussed, an ideal biomarker should be composed of certain characteristics. Cystatin C, KIM-1, and NGAL fulfill some of these requirements while leaving a void in others, and this opens up the possibility for future research in this field. First, it was suggested that the biomarker come from the cells that were damaged and be specific to the organ that was damaged (Mårtensson et al., 2012). Since cystatin C is produced by all nucleated cells, injury to the renal epithelial cells will be released. However, cystatin C has the limitation of being influenced by factors outside of renal functioning such as age, gender, and ethnicity. That is where a biomarker panel including measurements of KIM-1

and NGAL can be beneficial. Even though KIM-1 and NGAL are released by cells mounting an inflammatory response, they are closely associated enough with the actual site of injury to be applicable. Also, KIM-1 release has been shown to be very specific to kidney injury as opposed to injuries to other organs, so it can be used confirm damage to renal parenchyma cells as opposed to those of the liver or heart. Secondly, it was suggested that the concentration of the biomarker should increase proportionally as the extend of damage increases (Mårtensson et al., 2012). Studies have not investigated what level of increase in cystatin C, KIM-1, and NGAL is proportional to a certain level of renal injury, but each biomarker was shown to increase to a much higher degree than current measures of serum creatinine. In the cases of cystatin C and KIM-1, their levels increased 10-100 times the baseline measurement while serum creatinine and other traditional measurements maxed out at around 3-5 times the baseline measurement. Increases in serum creatinine also seems to plateau off earlier than these new biomarkers, so it would be interesting to investigate which biomarkers stop increasing while physical renal injury continues and which biomarkers increase throughout the entirety of the event. Thirdly, it was suggested that the biomarker be released as soon as possible after the moment of injury in order to diagnose AKI early when the damage is potentially reversible (Mårtensson et al., 2012). Studies showed that NGAL was able to detect AKI up to 48 hours earlier than serum creatinine, and both cystatin C and KIM-1 not only reached higher levels but also reached those levels at a much higher rate that serum creatinine. While there was no measurement as to how close to the time of injury these biomarkers were released, it is evident that they are detectable much earlier than

traditional methods. Finally, it was suggested that the biomarker levels decrease quickly after the acute injury has subsided as this can help in monitoring the patient therapeutically (Mårtensson et al., 2012). There has not been much research in this area, so the timing of biomarker level decrease can be a future area of study.

Knowing the properties of cystatin C, KIM-1, and NGAL provides a great platform for innovation in AKI diagnosis and treatment, but only have documentation that these biomarker levels increase quicker and to a much higher value than current measurements of serum creatinine is not sufficient. There needs to be further research into discovering what the cutoff values need to be for new biomarkers to be effective predictors of AKI (Zhang et al., 2011). There is some data that suggests that patients with a serum cystatin C level of less than 0.8 mg/l are less likely to develop AKI after some form of renal injury and that patients with a level greater than 2.04 mg/l have an increased risk of subsequently developing AKI (Zhang et al., 2011). However, these types of proposals need to be repeated across different clinical settings and a set of standardized values need to be agreed upon for all new biomarkers before they can provide universal benefit in the clinic. Moreover, even when testing these biomarkers, there needs to be caution in using creatinine as the "gold standard" to see their potential as injury markers (Mårtensson et al., 2012). Serum creatinine has been the standard for a long time, but because of its inherent limitations, it may be more effective to use less variable markers of kidney function like inulin in the controlled laboratory setting. Finally, many of the studies already conducted have evaluated the predictive properties of these biomarkers once the injury has already occurred. However, to truly study the ability

of these biomarkers to predict AKI, a more appropriate model would be a prospective case-controlled study (Mårtensson et al., 2012). Admittedly, this would be difficult to do in the ICU as most patients already present with AKI, but ideally, this study model would help estimate the predictive capabilities of these biomarkers even further.

The diagnosis and classification of AKI has come a long way in the last decade. Once a universal definition and set of criteria was set, the road for future research was laid out. Even with these advances, the incidence of mortality once AKI is sustained is undesirably high. The consensus has been that this is because physical injury cannot be detected early enough, and the window of reversible injury is usually missed. That is a direct result of using a secondary marker such as creatinine to measure the functional changes after physical damage has occurred. Therefore, these new biomarkers have great potential to improve detection and prognosis of AKI as the change in their levels is closer to the time of injury on the spectrum of measuring the physical and functional. Because the kidney plays a vital role in maintaining homeostasis in the entire body, advances in the field of AKI will have a positive impact on the outcome of patients in many fields other than its own.

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