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(54) Title: METHODS AND COMPOSITIONS FOR DIAGNOSIS AND PROGNOSIS OF RENAL INJURY AND RENAL FAILURE

(57) Abstract: The present invention relates to methods and compositions for monitoring, diagnosis, prognosis, and determination of treatment regimens in subjects suffering from or suspected of having a renal injury. In particular, the invention relates to using a one or more assays configured to detect a kidney injury marker selected from the group consisting of Cathepsin B, Renin, Dipeptidyl Peptidase IV, Neprilysin, Beta-2- microglobulin, Carbonic anhydrase IX, and C-X-C motif chemokine 2 as diagnostic and prognostic biomarkers in renal injuries.

METHODS AND COMPOSITIONS FOR DIAGNOSIS AND PROGNOSIS OF RENAL INJURY AND RENAL FAILURE

[0001]   The present application claims priority to U.S. Provisional Patent Application No. 61/259,163 filed November 7, 2009; U.S. Provisional Patent Application No. 61/259,540 filed November 9, 2009; U.S. Provisional Patent Application No. 61/259,140 filed November 7, 2009; U.S. Provisional Patent Application No. 61/259,142 filed November 7, 2009; U.S. Provisional Patent Application No. 61/259,143 filed November 7, 2009; U.S. Provisional Patent Application No. 61/259,141 filed November 7, 2009; and U.S. Provisional Patent Application No. 61/259,511 filed November 9, 2009, each of which is hereby incorporated in its entirety including all tables, figures, and claims

BACKGROUND OF THE INVENTION

[0002]   The following discussion of the background of the invention is merely provided to aid the reader in understanding the invention and is not admitted to describe or constitute prior art to the present invention.

[0003]   The kidney is responsible for water and solute excretion from the body. Its functions include maintenance of acid-base balance, regulation of electrolyte concentrations, control of blood volume, and regulation of blood pressure. As such, loss of kidney function through injury and/or disease results in substantial morbidity and mortality. A detailed discussion of renal injuries is provided in Harrison's Principles of Internal Medicine, 17th Ed., McGraw Hill, New York, pages 1741-1830, which are hereby incorporated by reference in their entirety. Renal disease and/or injury may be acute or chronic. Acute and chronic kidney disease are described as follows (from Current Medical Diagnosis & Treatment 2008, 47th Ed, McGraw Hill, New York, pages 785-815, which are hereby incorporated by reference in their entirety): "Acute renal failure is worsening of renal function over hours to days, resulting in the retention of nitrogenous wastes (such as urea nitrogen) and creatinine in the blood. Retention of these substances is called azotemia. Chronic renal failure (chronic kidney disease) results from an abnormal loss of renal function over months to years".

[0004]   Acute renal failure (ARF, also known as acute kidney injury, or AKI) is an abrupt (typically detected within about 48 hours to 1 week)reduction in glomerular filtration. This loss of filtration capacity results in retention of nitrogenous (urea and

creatinine) and non-nitrogenous waste products that are normally excreted by the kidney, a reduction in urine output, or both. It is reported that ARF complicates about 5% of hospital admissions, 4-15% of cardiopulmonary bypass surgeries, and up to 30% of intensive care admissions. ARF may be categorized as prerenal, intrinsic renal, or postrenal in causation. Intrinsic renal disease can be further divided into glomerular, tubular, interstitial, and vascular abnormalities. Major causes of ARF are described in the following table, which is adapted from the Merck Manual, 17th ed., Chapter 222, and which is hereby incorporated by reference in their entirety:

Type	Risk Factors
Prerenal	
ECF volume depletion	Excessive diuresis, hemorrhage, GI losses, loss of intravascular fluid into the extravascular space (due to ascites, peritonitis, pancreatitis, or burns), loss of skin and mucus membranes, renal salt- and water-wasting states
Low cardiac output	Cardiomyopathy, MI, cardiac tamponade, pulmonary embolism, pulmonary hypertension, positive-pressure mechanical ventilation
Low systemic vascular resistance	Septic shock, liver failure, antihypertensive drugs
Increased renal vascular resistance	NSAIDs, cyclosporines, tacrolimus, hypercalcemia, anaphylaxis, anesthetics, renal artery obstruction, renal vein thrombosis, sepsis, hepatorenal syndrome
Decreased efferent arteriolar tone (leading to decreased GFR from reduced glomerular transcapillary pressure, especially in patients with bilateral renal artery stenosis)	ACE inhibitors or angiotensin II receptor blockers
Intrinsic Renal	
Acute tubular injury	Ischemia (prolonged or severe prerenal state): surgery, hemorrhage, arterial or venous obstruction; Toxins: NSAIDs, cyclosporines, tacrolimus, aminoglycosides, foscarnet, ethylene glycol, hemoglobin, myoglobin, ifosfamide, heavy metals, methotrexate, radiopaque contrast agents, streptozotocin
Acute glomerulonephritis	ANCA-associated: Crescentic glomerulonephritis, polyarteritis nodosa, Wegener's granulomatosis; Anti-GBM glomerulonephritis: Goodpasture's syndrome; Immune-complex: Lupus glomerulonephritis, postinfectious glomerulonephritis, cryoglobulinemic glomerulonephritis
Acute tubulointerstitial	Drug reaction (eg, β-lactams, NSAIDs, sulfonamides,

Type	Risk Factors
nephritis	ciprofloxacin, thiazide diuretics, furosemide, phenytoin, allopurinol, pyelonephritis, papillary necrosis
Acute vascular nephropathy	Vasculitis, malignant hypertension, thrombotic microangiopathies, scleroderma, atheroembolism
Infiltrative diseases	Lymphoma, sarcoidosis, leukemia
Postrenal	
Tubular precipitation	Uric acid (tumor lysis), sulfonamides, triamterene, acyclovir, indinavir, methotrexate, ethylene glycol ingestion, myeloma protein, myoglobin
Ureteral obstruction	Intrinsic: Calculi, clots, sloughed renal tissue, fungus ball, edema, malignancy, congenital defects; Extrinsic: Malignancy, retroperitoneal fibrosis, ureteral trauma during surgery or high impact injury
Bladder obstruction	Mechanical: Benign prostatic hyperplasia, prostate cancer, bladder cancer, urethral strictures, phimosis, paraphimosis, urethral valves, obstructed indwelling urinary catheter; Neurogenic: Anticholinergic drugs, upper or lower motor neuron lesion

[0005]   In the case of ischemic ARF, the course of the disease may be divided into four phases. During an initiation phase, which lasts hours to days, reduced perfusion of the kidney is evolving into injury. Glomerular ultrafiltration reduces, the flow of filtrate is reduced due to debris within the tubules, and back leakage of filtrate through injured epithelium occurs. Renal injury can be mediated during this phase by reperfusion of the kidney. Initiation is followed by an extension phase which is characterized by continued ischemic injury and inflammation and may involve endothelial damage and vascular congestion. During the maintenance phase, lasting from 1 to 2 weeks, renal cell injury occurs, and glomerular filtration and urine output reaches a minimum. A recovery phase can follow in which the renal epithelium is repaired and GFR gradually recovers. Despite this, the survival rate of subjects with ARF may be as low as about 60%.

[0006]   Acute kidney injury caused by radiocontrast agents (also called contrast media) and other nephrotoxins such as cyclosporine, antibiotics including aminoglycosides and anticancer drugs such as cisplatin manifests over a period of days to about a week. Contrast induced nephropathy (CIN, which is AKI caused by radiocontrast agents) is thought to be caused by intrarenal vasoconstriction (leading to ischemic injury) and from the generation of reactive oxygen species that are directly toxic to renal tubular epithelial cells. CIN classically presents as an acute (onset within 24-48h) but reversible

(peak 3-5 days, resolution within 1 week) rise in blood urea nitrogen and serum creatinine.

[0007]   A commonly reported criteria for defining and detecting AKI is an abrupt (typically within about 2-7 days or within a period of hospitalization) elevation of serum creatinine. Although the use of serum creatinine elevation to define and detect AKI is well established, the magnitude of the serum creatinine elevation and the time over which it is measured to define AKI varies considerably among publications. Traditionally, relatively large increases in serum creatinine such as 100%, 200%, an increase of at least 100% to a value over 2 mg/dL and other definitions were used to define AKI. However, the recent trend has been towards using smaller serum creatinine rises to define AKI. The relationship between serum creatinine rise, AKI and the associated health risks are reviewed in Praught and Shlipak, *Curr Opin Nephrol Hypertens* 14:265-270, 2005 and Chertow et al, *J Am Soc Nephrol* 16: 3365-3370, 2005, which, with the references listed therein, are hereby incorporated by reference in their entirety. As described in these publications, acute worsening renal function (AKI) and increased risk of death and other detrimental outcomes are now known to be associated with very small increases in serum creatinine. These increases may be determined as a relative (percent) value or a nominal value. Relative increases in serum creatinine as small as 20% from the pre-injury value have been reported to indicate acutely worsening renal function (AKI) and increased health risk, but the more commonly reported value to define AKI and increased health risk is a relative increase of at least 25%. Nominal increases as small as 0.3 mg/dL, 0.2 mg/dL or even 0.1 mg/dL have been reported to indicate worsening renal function and increased risk of death. Various time periods for the serum creatinine to rise to these threshold values have been used to define AKI, for example, ranging from 2 days, 3 days, 7 days, or a variable period defined as the time the patient is in the hospital or intensive care unit. These studies indicate there is not a particular threshold serum creatinine rise (or time period for the rise) for worsening renal function or AKI, but rather a continuous increase in risk with increasing magnitude of serum creatinine rise.

[0008]   One study (Lassnigg et all, J Am Soc Nephrol 15:1597-1605, 2004, hereby incorporated by reference in its entirety) investigated both increases and decreases in serum creatinine. Patients with a mild fall in serum creatinine of -0.1 to -0.3 mg/dL following heart surgery had the lowest mortality rate. Patients with a larger fall in serum creatinine (more than or equal to -0.4 mg/dL) or any increase in serum creatinine had a

larger mortality rate. These findings caused the authors to conclude that even very subtle changes in renal function (as detected by small creatinine changes within 48 hours of surgery) seriously effect patient's outcomes. In an effort to reach consensus on a unified classification system for using serum creatinine to define AKI in clinical trials and in clinical practice, Bellomo *et al., Crit Care*. 8(4):R204-12, 2004, which is hereby incorporated by reference in its entirety, proposes the following classifications for stratifying AKI patients:

"Risk": serum creatinine increased 1.5 fold from baseline OR urine production of <0.5 ml/kg body weight/hr for 6 hours;

"Injury": serum creatinine increased 2.0 fold from baseline OR urine production <0.5 ml/kg/hr for 12 h;

"Failure": serum creatinine increased 3.0 fold from baseline OR creatinine >355 µmol/l (with a rise of >44) or urine output below 0.3 ml/kg/hr for 24 h or anuria for at least 12 hours;

And included two clinical outcomes:

"Loss": persistent need for renal replacement therapy for more than four weeks.

"ESRD": end stage renal disease—the need for dialysis for more than 3 months.

[0009]   These criteria are called the RIFLE criteria, which provide a useful clinical tool to classify renal status. As discussed in Kellum, *Crit. Care Med*. 36: S141-45, 2008 and Ricci *et al., Kidney Int*. 73, 538-546, 2008, each hereby incorporated by reference in its entirety, the RIFLE criteria provide a uniform definition of AKI which has been validated in numerous studies.

[0010]   More recently, Mehta *et al., Crit. Care* 11:R31 (doi:10.1186.cc5713), 2007, hereby incorporated by reference in its entirety, proposes the following similar classifications for stratifying AKI patients, which have been modified from RIFLE:

"Stage I": increase in serum creatinine of more than or equal to 0.3 mg/dL ($\geq$ 26.4 µmol/L) or increase to more than or equal to 150% (1.5-fold) from baseline OR urine output less than 0.5 mL/kg per hour for more than 6 hours;

"Stage II": increase in serum creatinine to more than 200% (> 2-fold) from baseline OR urine output less than 0.5 mL/kg per hour for more than 12 hours;

"Stage III": increase in serum creatinine to more than 300% (> 3-fold) from baseline OR serum creatinine ≥ 354 μmol/L accompanied by an acute increase of at least 44 μmol/L OR urine output less than 0.3 mL/kg per hour for 24 hours or anuria for 12 hours.

[0011] The CIN Consensus Working Panel (McCollough et al, Rev Cardiovasc Med. *2006;7(4):177-197,* hereby incorporated by reference in its entirety*)* uses a serum creatinine rise of 25% to define Contrast induced nephropathy (which is a type of AKI).Although various groups propose slightly different criteria for using serum creatinine to detect AKI, the consensus is that small changes in serum creatinine, such as 0.3 mg/dL or 25%, are sufficient to detect AKI (worsening renal function) and that the magnitude of the serum creatinine change is an indicator of the severity of the AKI and mortality risk.

[0012] Although serial measurement of serum creatinine over a period of days is an accepted method of detecting and diagnosing AKI and is considered one of the most important tools to evaluate AKI patients, serum creatinine is generally regarded to have several limitations in the diagnosis, assessment and monitoring of AKI patients. The time period for serum creatinine to rise to values (e.g., a 0.3 mg/dL or 25% rise) considered diagnostic for AKI can be 48 hours or longer depending on the definition used. Since cellular injury in AKI can occur over a period of hours, serum creatinine elevations detected at 48 hours or longer can be a late indicator of injury, and relying on serum creatinine can thus delay diagnosis of AKI. Furthermore, serum creatinine is not a good indicator of the exact kidney status and treatment needs during the most acute phases of AKI when kidney function is changing rapidly. Some patients with AKI will recover fully, some will need dialysis (either short term or long term) and some will have other detrimental outcomes including death, major adverse cardiac events and chronic kidney disease. Because serum creatinine is a marker of filtration rate, it does not differentiate between the causes of AKI (pre-renal, intrinsic renal, post-renal obstruction, atheroembolic, etc) or the category or location of injury in intrinsic renal disease (for example, tubular, glomerular or interstitial in origin). Urine output is similarly limited, Knowing these things can be of vital importance in managing and treating patients with AKI.

[0013] These limitations underscore the need for better methods to detect and assess AKI, particularly in the early and subclinical stages, but also in later stages when

recovery and repair of the kidney can occur. Furthermore, there is a need to better identify patients who are at risk of having an AKI.

BRIEF SUMMARY OF THE INVENTION

[0014]   It is an object of the invention to provide methods and compositions for evaluating renal function in a subject. As described herein, measurement of one or more biomarkers selected from the group consisting of Cathepsin B, Renin, Dipeptidyl Peptidase IV, Neprilysin, Beta-2-microglobulin, Carbonic anhydrase IX, and C-X-C motif chemokine 2 (each referred to herein as a "kidney injury marker") can be used for diagnosis, prognosis, risk stratification, staging, monitoring, categorizing and determination of further diagnosis and treatment regimens in subjects suffering or at risk of suffering from an injury to renal function, reduced renal function, and/or acute renal failure (also called acute kidney injury).

[0015]   The kidney injury markers of the present invention may be used, individually or in panels comprising a plurality of kidney injury markers, for risk stratification (that is, to identify subjects at risk for a future injury to renal function, for future progression to reduced renal function, for future progression to ARF, for future improvement in renal function, *etc.*); for diagnosis of existing disease (that is, to identify subjects who have suffered an injury to renal function, who have progressed to reduced renal function, who have progressed to ARF, *etc.*); for monitoring for deterioration or improvement of renal function; and for predicting a future medical outcome, such as improved or worsening renal function, a decreased or increased mortality risk, a decreased or increased risk that a subject will require renal replacement therapy (*i.e.*, hemodialysis, peritoneal dialysis, hemofiltration, and/or renal transplantation, a decreased or increased risk that a subject will recover from an injury to renal function, a decreased or increased risk that a subject will recover from ARF, a decreased or increased risk that a subject will progress to end stage renal disease, a decreased or increased risk that a subject will progress to chronic renal failure, a decreased or increased risk that a subject will suffer rejection of a transplanted kidney, *etc.*

[0016]   In a first aspect, the present invention relates to methods for evaluating renal status in a subject. These methods comprise performing an assay method that is configured to detect one or more biomarkers selected from the group consisting of Cathepsin B, Renin, Dipeptidyl Peptidase IV, Neprilysin, Beta-2-microglobulin, Carbonic

anhydrase IX, and C-X-C motif chemokine 2 is/are then correlated to the renal status of the subject. This correlation to renal status may include correlating the assay result(s) to one or more of risk stratification, diagnosis, prognosis, staging, classifying and monitoring of the subject as described herein. Thus, the present invention utilizes one or more kidney injury markers of the present invention for the evaluation of renal injury.

[0017]   In certain embodiments, the methods for evaluating renal status described herein are methods for risk stratification of the subject; that is, assigning a likelihood of one or more future changes in renal status to the subject. In these embodiments, the assay result(s) is/are correlated to one or more such future changes. The following are preferred risk stratification embodiments.

[0018]   In preferred risk stratification embodiments, these methods comprise determining a subject's risk for a future injury to renal function, and the assay result(s) is/are correlated to a likelihood of such a future injury to renal function. For example, the measured concentration(s) may each be compared to a threshold value. For a "positive going" kidney injury marker, an increased likelihood of suffering a future injury to renal function is assigned to the subject when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold. For a "negative going" kidney injury marker, an increased likelihood of suffering a future injury to renal function is assigned to the subject when the measured concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold.

[0019]   In other preferred risk stratification embodiments, these methods comprise determining a subject's risk for future reduced renal function, and the assay result(s) is/are correlated to a likelihood of such reduced renal function. For example, the measured concentrations may each be compared to a threshold value. For a "positive going" kidney injury marker, an increased likelihood of suffering a future reduced renal function is assigned to the subject when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold. For a "negative going" kidney injury marker, an increased likelihood of future reduced renal function is assigned to the subject when the measured concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold.

[0020]   In still other preferred risk stratification embodiments, these methods comprise determining a subject's likelihood for a future improvement in renal function, and the assay result(s) is/are correlated to a likelihood of such a future improvement in renal function. For example, the measured concentration(s) may each be compared to a threshold value. For a "positive going" kidney injury marker, an increased likelihood of a future improvement in renal function is assigned to the subject when the measured concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold. For a "negative going" kidney injury marker, an increased likelihood of a future improvement in renal function is assigned to the subject when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold.

[0021]   In yet other preferred risk stratification embodiments, these methods comprise determining a subject's risk for progression to ARF, and the result(s) is/are correlated to a likelihood of such progression to ARF. For example, the measured concentration(s) may each be compared to a threshold value. For a "positive going" kidney injury marker, an increased likelihood of progression to ARF is assigned to the subject when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold. For a "negative going" kidney injury marker, an increased likelihood of progression to ARF is assigned to the subject when the measured concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold.

[0022]   And in other preferred risk stratification embodiments, these methods comprise determining a subject's outcome risk, and the assay result(s) is/are correlated to a likelihood of the occurrence of a clinical outcome related to a renal injury suffered by the subject. For example, the measured concentration(s) may each be compared to a threshold value. For a "positive going" kidney injury marker, an increased likelihood of one or more of: acute kidney injury, progression to a worsening stage of AKI, mortality, a requirement for renal replacement therapy, a requirement for withdrawal of renal toxins, end stage renal disease, heart failure, stroke, myocardial infarction, progression to chronic kidney disease, *etc.*, is assigned to the subject when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold. For a "negative going" kidney injury marker, an increased likelihood of one or more of: acute kidney injury, progression to a worsening stage of AKI, mortality, a

requirement for renal replacement therapy, a requirement for withdrawal of renal toxins, end stage renal disease, heart failure, stroke, myocardial infarction, progression to chronic kidney disease, *etc.*, is assigned to the subject when the measured concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold.

[0023]   In such risk stratification embodiments, preferably the likelihood or risk assigned is that an event of interest is more or less likely to occur within 180 days of the time at which the body fluid sample is obtained from the subject. In particularly preferred embodiments, the likelihood or risk assigned relates to an event of interest occurring within a shorter time period such as 18 months, 120 days, 90 days, 60 days, 45 days, 30 days, 21 days, 14 days, 7 days, 5 days, 96 hours, 72 hours, 48 hours, 36 hours, 24 hours, 12 hours, or less. A risk at 0 hours of the time at which the body fluid sample is obtained from the subject is equivalent to diagnosis of a current condition.

[0024]   In preferred risk stratification embodiments, the subject is selected for risk stratification based on the pre-existence in the subject of one or more known risk factors for prerenal, intrinsic renal, or postrenal ARF. For example, a subject undergoing or having undergone major vascular surgery, coronary artery bypass, or other cardiac surgery; a subject having pre-existing congestive heart failure, preeclampsia, eclampsia, diabetes mellitus, hypertension, coronary artery disease, proteinuria, renal insufficiency, glomerular filtration below the normal range, cirrhosis, serum creatinine above the normal range, or sepsis; or a subject exposed to NSAIDs, cyclosporines, tacrolimus, aminoglycosides, foscarnet, ethylene glycol, hemoglobin, myoglobin, ifosfamide, heavy metals, methotrexate, radiopaque contrast agents, or streptozotocin are all preferred subjects for monitoring risks according to the methods described herein. This list is not meant to be limiting. By "pre-existence" in this context is meant that the risk factor exists at the time the body fluid sample is obtained from the subject. In particularly preferred embodiments, a subject is chosen for risk stratification based on an existing diagnosis of injury to renal function, reduced renal function, or ARF.

[0025]   In other embodiments, the methods for evaluating renal status described herein are methods for diagnosing a renal injury in the subject; that is, assessing whether or not a subject has suffered from an injury to renal function, reduced renal function, or ARF. In these embodiments, the assay result(s), for example measured concentration(s) of one or more biomarkers selected from the group consisting of Cathepsin B, Renin, Dipeptidyl

Peptidase IV, Neprilysin, Beta-2-microglobulin, Carbonic anhydrase IX, and C-X-C motif chemokine 2 is/are correlated to the occurrence or nonoccurrence of a change in renal status. The following are preferred diagnostic embodiments.

[0026]   In preferred diagnostic embodiments, these methods comprise diagnosing the occurrence or nonoccurrence of an injury to renal function, and the assay result(s) is/are correlated to the occurrence or nonoccurrence of such an injury. For example, each of the measured concentration(s) may be compared to a threshold value. For a positive going marker, an increased likelihood of the occurrence of an injury to renal function is assigned to the subject when the measured concentration is above the threshold (relative to the likelihood assigned when the measured concentration is below the threshold); alternatively, when the measured concentration is below the threshold, an increased likelihood of the nonoccurrence of an injury to renal function may be assigned to the subject (relative to the likelihood assigned when the measured concentration is above the threshold). For a negative going marker, an increased likelihood of the occurrence of an injury to renal function is assigned to the subject when the measured concentration is below the threshold (relative to the likelihood assigned when the measured concentration is above the threshold); alternatively, when the measured concentration is above the threshold, an increased likelihood of the nonoccurrence of an injury to renal function may be assigned to the subject (relative to the likelihood assigned when the measured concentration is below the threshold).

[0027]   In other preferred diagnostic embodiments, these methods comprise diagnosing the occurrence or nonoccurrence of reduced renal function, and the assay result(s) is/are correlated to the occurrence or nonoccurrence of an injury causing reduced renal function. For example, each of the measured concentration(s) may be compared to a threshold value. For a positive going marker, an increased likelihood of the occurrence of an injury causing reduced renal function is assigned to the subject when the measured concentration is above the threshold (relative to the likelihood assigned when the measured concentration is below the threshold); alternatively, when the measured concentration is below the threshold, an increased likelihood of the nonoccurrence of an injury causing reduced renal function may be assigned to the subject (relative to the likelihood assigned when the measured concentration is above the threshold). For a negative going marker, an increased likelihood of the occurrence of an injury causing reduced renal function is assigned to the subject when the measured concentration is

below the threshold (relative to the likelihood assigned when the measured concentration is above the threshold); alternatively, when the measured concentration is above the threshold, an increased likelihood of the nonoccurrence of an injury causing reduced renal function may be assigned to the subject (relative to the likelihood assigned when the measured concentration is below the threshold).

[0028]   In yet other preferred diagnostic embodiments, these methods comprise diagnosing the occurrence or nonoccurrence of ARF, and the assay result(s) is/are correlated to the occurrence or nonoccurrence of an injury causing ARF. For example, each of the measured concentration(s) may be compared to a threshold value. For a positive going marker, an increased likelihood of the occurrence of ARF is assigned to the subject when the measured concentration is above the threshold (relative to the likelihood assigned when the measured concentration is below the threshold); alternatively, when the measured concentration is below the threshold, an increased likelihood of the nonoccurrence of ARF may be assigned to the subject (relative to the likelihood assigned when the measured concentration is above the threshold). For a negative going marker, an increased likelihood of the occurrence of ARF is assigned to the subject when the measured concentration is below the threshold (relative to the likelihood assigned when the measured concentration is above the threshold); alternatively, when the measured concentration is above the threshold, an increased likelihood of the nonoccurrence of ARF may be assigned to the subject (relative to the likelihood assigned when the measured concentration is below the threshold).

[0029]   In still other preferred diagnostic embodiments, these methods comprise diagnosing a subject as being in need of renal replacement therapy, and the assay result(s) is/are correlated to a need for renal replacement therapy. For example, each of the measured concentration(s) may be compared to a threshold value. For a positive going marker, an increased likelihood of the occurrence of an injury creating a need for renal replacement therapy is assigned to the subject when the measured concentration is above the threshold (relative to the likelihood assigned when the measured concentration is below the threshold); alternatively, when the measured concentration is below the threshold, an increased likelihood of the nonoccurrence of an injury creating a need for renal replacement therapy may be assigned to the subject (relative to the likelihood assigned when the measured concentration is above the threshold). For a negative going marker, an increased likelihood of the occurrence of an injury creating a need for renal

replacement therapy is assigned to the subject when the measured concentration is below the threshold (relative to the likelihood assigned when the measured concentration is above the threshold); alternatively, when the measured concentration is above the threshold, an increased likelihood of the nonoccurrence of an injury creating a need for renal replacement therapy may be assigned to the subject (relative to the likelihood assigned when the measured concentration is below the threshold).

[0030]   In still other preferred diagnostic embodiments, these methods comprise diagnosing a subject as being in need of renal transplantation, and the assay result(s0 is/are correlated to a need for renal transplantation. For example, each of the measured concentration(s) may be compared to a threshold value. For a positive going marker, an increased likelihood of the occurrence of an injury creating a need for renal transplantation is assigned to the subject when the measured concentration is above the threshold (relative to the likelihood assigned when the measured concentration is below the threshold); alternatively, when the measured concentration is below the threshold, an increased likelihood of the nonoccurrence of an injury creating a need for renal transplantation may be assigned to the subject (relative to the likelihood assigned when the measured concentration is above the threshold). For a negative going marker, an increased likelihood of the occurrence of an injury creating a need for renal transplantation is assigned to the subject when the measured concentration is below the threshold (relative to the likelihood assigned when the measured concentration is above the threshold); alternatively, when the measured concentration is above the threshold, an increased likelihood of the nonoccurrence of an injury creating a need for renal transplantation may be assigned to the subject (relative to the likelihood assigned when the measured concentration is below the threshold).

[0031]   In still other embodiments, the methods for evaluating renal status described herein are methods for monitoring a renal injury in the subject; that is, assessing whether or not renal function is improving or worsening in a subject who has suffered from an injury to renal function, reduced renal function, or ARF. In these embodiments, the assay result(s), for example measured concentration(s) of one or more biomarkers selected from the group consisting of Cathepsin B, Renin, Dipeptidyl Peptidase IV, Neprilysin, Beta-2-microglobulin, Carbonic anhydrase IX, and C-X-C motif chemokine 2 is/are correlated to the occurrence or nonoccurrence of a change in renal status. The following are preferred monitoring embodiments.

[0032]   In preferred monitoring embodiments, these methods comprise monitoring renal status in a subject suffering from an injury to renal function, and the assay result(s) is/are correlated to the occurrence or nonoccurrence of a change in renal status in the subject. For example, the measured concentration(s) may be compared to a threshold value. For a positive going marker, when the measured concentration is above the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is below the threshold, an improvement of renal function may be assigned to the subject. For a negative going marker, when the measured concentration is below the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is above the threshold, an improvement of renal function may be assigned to the subject.

[0033]   In other preferred monitoring embodiments, these methods comprise monitoring renal status in a subject suffering from reduced renal function, and the assay result(s) is/are correlated to the occurrence or nonoccurrence of a change in renal status in the subject. For example, the measured concentration(s) may be compared to a threshold value. For a positive going marker, when the measured concentration is above the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is below the threshold, an improvement of renal function may be assigned to the subject. For a negative going marker, when the measured concentration is below the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is above the threshold, an improvement of renal function may be assigned to the subject.

[0034]   In yet other preferred monitoring embodiments, these methods comprise monitoring renal status in a subject suffering from acute renal failure, and the assay result(s) is/are correlated to the occurrence or nonoccurrence of a change in renal status in the subject. For example, the measured concentration(s) may be compared to a threshold value. For a positive going marker, when the measured concentration is above the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is below the threshold, an improvement of renal function may be assigned to the subject. For a negative going marker, when the measured concentration is below the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is above the threshold, an improvement of renal function may be assigned to the subject.

[0035]   In other additional preferred monitoring embodiments, these methods comprise monitoring renal status in a subject at risk of an injury to renal function due to the pre-existence of one or more known risk factors for prerenal, intrinsic renal, or postrenal ARF, and the assay result(s) is/are correlated to the occurrence or nonoccurrence of a change in renal status in the subject. For example, the measured concentration(s) may be compared to a threshold value. For a positive going marker, when the measured concentration is above the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is below the threshold, an improvement of renal function may be assigned to the subject. For a negative going marker, when the measured concentration is below the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is above the threshold, an improvement of renal function may be assigned to the subject.

[0036]   In still other embodiments, the methods for evaluating renal status described herein are methods for classifying a renal injury in the subject; that is, determining whether a renal injury in a subject is prerenal, intrinsic renal, or postrenal; and/or further subdividing these classes into subclasses such as acute tubular injury, acute glomerulonephritis acute tubulointerstitial nephritis, acute vascular nephropathy, or infiltrative disease; and/or assigning a likelihood that a subject will progress to a particular RIFLE stage. In these embodiments, the assay result(s), for example measured concentration(s) of one or more biomarkers selected from the group consisting of Cathepsin B, Renin, Dipeptidyl Peptidase IV, Neprilysin, Beta-2-microglobulin, Carbonic anhydrase IX, and C-X-C motif chemokine 2 is/are correlated to a particular class and/or subclass. The following are preferred classification embodiments.

[0037]   In preferred classification embodiments, these methods comprise determining whether a renal injury in a subject is prerenal, intrinsic renal, or postrenal; and/or further subdividing these classes into subclasses such as acute tubular injury, acute glomerulonephritis acute tubulointerstitial nephritis, acute vascular nephropathy, or infiltrative disease; and/or assigning a likelihood that a subject will progress to a particular RIFLE stage, and the assay result(s) is/are correlated to the injury classification for the subject. For example, the measured concentration may be compared to a threshold value, and when the measured concentration is above the threshold, a particular

classification is assigned; alternatively, when the measured concentration is below the threshold, a different classification may be assigned to the subject.

[0038]   A variety of methods may be used by the skilled artisan to arrive at a desired threshold value for use in these methods. For example, the threshold value may be determined from a population of normal subjects by selecting a concentration representing the 75th, 85th, 90th, 95th, or 99th percentile of a kidney injury marker measured in such normal subjects. Alternatively, the threshold value may be determined from a "diseased" population of subjects, e.g., those suffering from an injury or having a predisposition for an injury (e.g., progression to ARF or some other clinical outcome such as death, dialysis, renal transplantation, *etc.*), by selecting a concentration representing the 75th, 85th, 90th, 95th, or 99th percentile of a kidney injury marker measured in such subjects. In another alternative, the threshold value may be determined from a prior measurement of a kidney injury marker in the same subject; that is, a temporal change in the level of a kidney injury marker in the subject may be used to assign risk to the subject.

[0039]   The foregoing discussion is not meant to imply, however, that the kidney injury markers of the present invention must be compared to corresponding individual thresholds. Methods for combining assay results can comprise the use of multivariate logistical regression, loglinear modeling, neural network analysis, n-of-m analysis, decision tree analysis, calculating ratios of markers, *etc*. This list is not meant to be limiting. In these methods, a composite result which is determined by combining individual markers may be treated as if it is itself a marker; that is, a threshold may be determined for the composite result as described herein for individual markers, and the composite result for an individual patient compared to this threshold.

[0040]   The ability of a particular test to distinguish two populations can be established using ROC analysis. For example, ROC curves established from a "first" subpopulation which is predisposed to one or more future changes in renal status, and a "second" subpopulation which is not so predisposed can be used to calculate a ROC curve, and the area under the curve provides a measure of the quality of the test. Preferably, the tests described herein provide a ROC curve area greater than 0.5, preferably at least 0.6, more preferably 0.7, still more preferably at least 0.8, even more preferably at least 0.9, and most preferably at least 0.95.

[0041]   In certain aspects, the measured concentration of one or more kidney injury markers, or a composite of such markers, may be treated as continuous variables. For example, any particular concentration can be converted into a corresponding probability of a future reduction in renal function for the subject, the occurrence of an injury, a classification, etc. In yet another alternative, a threshold that can provide an acceptable level of specificity and sensitivity in separating a population of subjects into "bins" such as a "first" subpopulation (e.g., which is predisposed to one or more future changes in renal status, the occurrence of an injury, a classification, etc.) and a "second" subpopulation which is not so predisposed. A threshold value is selected to separate this first and second population by one or more of the following measures of test accuracy:

an odds ratio greater than 1, preferably at least about 2 or more or about 0.5 or less, more preferably at least about 3 or more or about 0.33 or less, still more preferably at least about 4 or more or about 0.25 or less, even more preferably at least about 5 or more or about 0.2 or less, and most preferably at least about 10 or more or about 0.1 or less;

a specificity of greater than 0.5, preferably at least about 0.6, more preferably at least about 0.7, still more preferably at least about 0.8, even more preferably at least about 0.9 and most preferably at least about 0.95, with a corresponding sensitivity greater than 0.2, preferably greater than about 0.3, more preferably greater than about 0.4, still more preferably at least about 0.5, even more preferably about 0.6, yet more preferably greater than about 0.7, still more preferably greater than about 0.8, more preferably greater than about 0.9, and most preferably greater than about 0.95;

a sensitivity of greater than 0.5, preferably at least about 0.6, more preferably at least about 0.7, still more preferably at least about 0.8, even more preferably at least about 0.9 and most preferably at least about 0.95, with a corresponding specificity greater than 0.2, preferably greater than about 0.3, more preferably greater than about 0.4, still more preferably at least about 0.5, even more preferably about 0.6, yet more preferably greater than about 0.7, still more preferably greater than about 0.8, more preferably greater than about 0.9, and most preferably greater than about 0.95;

at least about 75% sensitivity, combined with at least about 75% specificity;

a positive likelihood ratio (calculated as sensitivity/(1-specificity)) of greater than 1, at least about 2, more preferably at least about 3, still more preferably at least about 5, and most preferably at least about 10; or

a negative likelihood ratio (calculated as (1-sensitivity)/specificity) of less than 1, less than or equal to about 0.5, more preferably less than or equal to about 0.3, and most preferably less than or equal to about 0.1.

[0042] The term "about" in the context of any of the above measurements refers to +/- 5% of a given measurement.

[0043] Multiple thresholds may also be used to assess renal status in a subject. For example, a "first" subpopulation which is predisposed to one or more future changes in renal status, the occurrence of an injury, a classification, etc., and a "second" subpopulation which is not so predisposed can be combined into a single group. This group is then subdivided into three or more equal parts (known as tertiles, quartiles, quintiles, etc., depending on the number of subdivisions). An odds ratio is assigned to subjects based on which subdivision they fall into. If one considers a tertile, the lowest or highest tertile can be used as a reference for comparison of the other subdivisions. This reference subdivision is assigned an odds ratio of 1. The second tertile is assigned an odds ratio that is relative to that first tertile. That is, someone in the second tertile might be 3 times more likely to suffer one or more future changes in renal status in comparison to someone in the first tertile. The third tertile is also assigned an odds ratio that is relative to that first tertile.

[0044] In certain embodiments, the assay method is an immunoassay. Antibodies for use in such assays will specifically bind a full length kidney injury marker of interest, and may also bind one or more polypeptides that are "related" thereto, as that term is defined hereinafter. Numerous immunoassay formats are known to those of skill in the art. Preferred body fluid samples are selected from the group consisting of urine, blood, serum, saliva, tears, and plasma.

[0045] The foregoing method steps should not be interpreted to mean that the kidney injury marker assay result(s) is/are used in isolation in the methods described herein. Rather, additional variables or other clinical indicia may be included in the methods described herein. For example, a risk stratification, diagnostic, classification, monitoring, etc. method may combine the assay result(s) with one or more variables measured for the subject selected from the group consisting of demographic information (e.g., weight, sex, age, race), medical history (e.g., family history, type of surgery, pre-existing disease such as aneurism, congestive heart failure, preeclampsia, eclampsia, diabetes mellitus,

hypertension, coronary artery disease, proteinuria, renal insufficiency, or sepsis, type of toxin exposure such as NSAIDs, cyclosporines, tacrolimus, aminoglycosides, foscarnet, ethylene glycol, hemoglobin, myoglobin, ifosfamide, heavy metals, methotrexate, radiopaque contrast agents, or streptozotocin), clinical variables (e.g., blood pressure, temperature, respiration rate), risk scores (APACHE score, PREDICT score, TIMI Risk Score for UA/NSTEMI, Framingham Risk Score), a glomerular filtration rate, an estimated glomerular filtration rate, a urine production rate, a serum or plasma creatinine concentration, a urine creatinine concentration, a fractional excretion of sodium, a urine sodium concentration, a urine creatinine to serum or plasma creatinine ratio, a urine specific gravity, a urine osmolality, a urine urea nitrogen to plasma urea nitrogen ratio, a plasma BUN to creatnine ratio, a renal failure index calculated as urine sodium / (urine creatinine / plasma creatinine), a serum or plasma neutrophil gelatinase (NGAL) concentration, a urine NGAL concentration, a serum or plasma cystatin C concentration, a serum or plasma cardiac troponin concentration, a serum or plasma BNP concentration, a serum or plasma NTproBNP concentration, and a serum or plasma proBNP concentration. Other measures of renal function which may be combined with one or more kidney injury marker assay result(s) are described hereinafter and in Harrison's Principles of Internal Medicine, 17th Ed., McGraw Hill, New York, pages 1741-1830, and Current Medical Diagnosis & Treatment 2008, 47th Ed, McGraw Hill, New York, pages 785-815, each of which are hereby incorporated by reference in their entirety.

[0046]   When more than one marker is measured, the individual markers may be measured in samples obtained at the same time, or may be determined from samples obtained at different (e.g., an earlier or later) times. The individual markers may also be measured on the same or different body fluid samples. For example, one kidney injury marker may be measured in a serum or plasma sample and another kidney injury marker may be measured in a urine sample. In addition, assignment of a likelihood may combine an individual kidney injury marker assay result with temporal changes in one or more additional variables.

[0047]   In various related aspects, the present invention also relates to devices and kits for performing the methods described herein. Suitable kits comprise reagents sufficient for performing an assay for at least one of the described kidney injury markers, together with instructions for performing the described threshold comparisons.

[0048]   In certain embodiments, reagents for performing such assays are provided in an assay device, and such assay devices may be included in such a kit. Preferred reagents can comprise one or more solid phase antibodies, the solid phase antibody comprising antibody that detects the intended biomarker target(s) bound to a solid support. In the case of sandwich immunoassays, such reagents can also include one or more detectably labeled antibodies, the detectably labeled antibody comprising antibody that detects the intended biomarker target(s) bound to a detectable label. Additional optional elements that may be provided as part of an assay device are described hereinafter.

[0049]   Detectable labels may include molecules that are themselves detectable (e.g., fluorescent moieties, electrochemical labels, ecl (electrochemical luminescence) labels, metal chelates, colloidal metal particles, *etc.*) as well as molecules that may be indirectly detected by production of a detectable reaction product (*e.g.*, enzymes such as horseradish peroxidase, alkaline phosphatase, *etc.*) or through the use of a specific binding molecule which itself may be detectable (*e.g.*, a labeled antibody that binds to the second antibody, biotin, digoxigenin, maltose, oligohistidine, 2,4-dintrobenzene, phenylarsenate, ssDNA, dsDNA, etc.).

[0050]   Generation of a signal from the signal development element can be performed using various optical, acoustical, and electrochemical methods well known in the art. Examples of detection modes include fluorescence, radiochemical detection, reflectance, absorbance, amperometry, conductance, impedance, interferometry, ellipsometry, *etc.* In certain of these methods, the solid phase antibody is coupled to a transducer (*e.g.*, a diffraction grating, electrochemical sensor, etc) for generation of a signal, while in others, a signal is generated by a transducer that is spatially separate from the solid phase antibody (*e.g.*, a fluorometer that employs an excitation light source and an optical detector). This list is not meant to be limiting. Antibody-based biosensors may also be employed to determine the presence or amount of analytes that optionally eliminate the need for a labeled molecule.

DETAILED DESCRIPTION OF THE INVENTION

[0051]   The present invention relates to methods and compositions for diagnosis, differential diagnosis, risk stratification, monitoring, classifying and determination of treatment regimens in subjects suffering or at risk of suffering from injury to renal function, reduced renal function and/or acute renal failure through measurement of one or

more kidney injury markers. In various embodiments, a measured concentration of one or more biomarkers selected from the group consisting of Cathepsin B, Renin, Dipeptidyl Peptidase IV, Neprilysin, Beta-2-microglobulin, Carbonic anhydrase IX, and C-X-C motif chemokine 2 or one or more markers related thereto, are correlated to the renal status of the subject.

[0052]   For purposes of this document, the following definitions apply:

[0053]   As used herein, an "injury to renal function" is an abrupt (within 14 days, preferably within 7 days, more preferably within 72 hours, and still more preferably within 48 hours) measurable reduction in a measure of renal function. Such an injury may be identified, for example, by a decrease in glomerular filtration rate or estimated GFR, a reduction in urine output, an increase in serum creatinine, an increase in serum cystatin C, a requirement for renal replacement therapy, *etc*. "Improvement in Renal Function" is an abrupt (within 14 days, preferably within 7 days, more preferably within 72 hours, and still more preferably within 48 hours) measurable increase in a measure of renal function. Preferred methods for measuring and/or estimating GFR are described hereinafter.

[0054]   As used herein, "reduced renal function" is an abrupt (within 14 days, preferably within 7 days, more preferably within 72 hours, and still more preferably within 48 hours) reduction in kidney function identified by an absolute increase in serum creatinine of greater than or equal to 0.1 mg/dL ($\geq$ 8.8 μmol/L), a percentage increase in serum creatinine of greater than or equal to 20% (1.2-fold from baseline), or a reduction in urine output (documented oliguria of less than 0. 5 ml/kg per hour).

[0055]   As used herein, "acute renal failure" or "ARF" is an abrupt (within 14 days, preferably within 7 days, more preferably within 72 hours, and still more preferably within 48 hours) reduction in kidney function identified by an absolute increase in serum creatinine of greater than or equal to 0.3 mg/dl ($\geq$ 26.4 μmol/l), a percentage increase in serum creatinine of greater than or equal to 50% (1. 5-fold from baseline), or a reduction in urine output (documented oliguria of less than 0.5 ml/kg per hour for at least 6 hours). This term is synonymous with "acute kidney injury" or "AKI."

[0056]   As used herein, the term "Cathepsin B" refers to one or more polypeptides present in a biological sample that are derived from the Cathepsin B precursor (Swiss-Prot P07858 (SEQ ID NO: 1))

```
           10         20         30         40         50         60
MWQLWASLCC LLVLANARSR PSFHPLSDEL VNYVNKRNTT WQAGHNFYNV DMSYLKRLCG

           70         80         90        100        110        120
TFLGGPKPPQ RVMFTEDLKL PASFDAREQW PQCPTIKEIR DQGSCGSCWA FGAVEAISDR

          130        140        150        160        170        180
ICIHTNAHVS VEVSAEDLLT CCGSMCGDGC NGGYPAEAWN FWTRKGLVSG GLYESHVGCR

          190        200        210        220        230        240
PYSIPPCEHH VNGSRPPCTG EGDTPKCSKI CEPGYSPTYK QDKHYGYNSY SVSNSEKDIM

          250        260        270        280        290        300
AEIYKNGPVE GAFSVYSDFL LYKSGVYQHV TGEMMGGHAI RILGWGVENG TPYWLVANSW

          310        320        330
NTDWGDNGFF KILRGQDHCG IESEVVAGIP RTDQYWEKI
```

[0057]   The following domains have been identified in Cathepsin B:

Residues	Length	Domain ID
1-17	17	signal peptide
18-79	62	activation peptide
334-339	6	propeptide
80-333	254	Cathepsin B
80-126	47	Cathepsin B light chain
129-333	205	Cathepsin B heavy chain

[0058]   As used herein, the term "Renin" refers to one or more polypeptides present in a biological sample that are derived from the Renin precursor (Swiss-Prot P00797 (SEQ ID NO: 2)).

```
           10         20         30         40         50         60
MDGWRRMPRW GLLLLLWGSC TFGLPTDTTT FKRIFLKRMP SIRESLKERG VDMARLGPEW

           70         80         90        100        110        120
SQPMKRLTLG NTTSSVILTN YMDTQYYGEI GIGTPPQTFK VVFDTGSSNV WVPSSKCSRL

          130        140        150        160        170        180
YTACVYHKLF DASDSSSYKH NGTELTLRYS TGTVSGFLSQ DIITVGGITV TQMFGEVTEM

          190        200        210        220        230        240
PALPFMLAEF DGVVGMGFIE QAIGRVTPIF DNIISQGVLK EDVFSFYYNR DSENSQSLGG
```

```
            250        260        270        280        290        300
       QIVLGGSDPQ HYEGNFHYIN LIKTGVWQIQ MKGVSVGSST LLCEDGCLAL VDTGASYISG

            310        320        330        340        350        360
       STSSIEKLME ALGAKKRLFD YVVKCNEGPT LPDISFHLGG KEYTLTSADY VFQESYSSKK

            370        380        390        400
       LCTLAIHAMD IPPPTGPTWA LGATFIRKFY TEFDRRNNRI GFALAR
```

[0059]   The following domains have been identified in Renin:

Residues	Length	Domain ID
1-23	23	Propeptide
24-66	43	Activation peptide
67-406	340	Renin
231-233	3	Missing in isoform 2

[0060]   As used herein, the term "Dipeptidyl peptidase 4" refers to one or more polypeptides present in a biological sample that are derived from the Dipeptidyl peptidase 4 precursor (Swiss-Prot P27487 (SEQ ID NO: 3))

```
            10         20         30         40         50         60
       MKTPWKVLLG LLGAAALVTI ITVPVVLLNK GTDDATADSR KTYTLTDYLK NTYRLKLYSL

            70         80         90        100        110        120
       RWISDHEYLY KQENNILVFN AEYGNSSVFL ENSTFDEFGH SINDYSISPD GQFILLEYNY

           130        140        150        160        170        180
       VKQWRHSYTA SYDIYDLNKR QLITEERIPN NTQWVTWSPV GHKLAYVWNN DIYVKIEPNL

           190        200        210        220        230        240
       PSYRITWTGK EDIIYNGITD WVYEEEVFSA YSALWWSPNG TFLAYAQFND TEVPLIEYSF

           250        260        270        280        290        300
       YSDESLQYPK TVRVPYPKAG AVNPTVKFFV VNTDSLSSVT NATSIQITAP ASMLIGDHYL

           310        320        330        340        350        360
       CDVTWATQER ISLQWLRRIQ NYSVMDICDY DESSGRWNCL VARQHIEMST TGWVGRFRPS

           370        380        390        400        410        420
       EPHFTLDGNS FYKIISNEEG YRHICYFQID KKDCTFITKG TWEVIGIEAL TSDYLYYISN

           430        440        450        460        470        480
       EYKGMPGGRN LYKIQLSDYT KVTCLSCELN PERCQYYSVS FSKEAKYYQL RCSGPGLPLY
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```
            490        500        510        520        530        540
TLHSSVNDKG LRVLEDNSAL DKMLQNVQMP SKKLDFIILN ETKFWYQMIL PPHFDKSKKY

            550        560        570        580        590        600
PLLLDVYAGP CSQKADTVFR LNWATYLAST ENIIVASFDG RGSGYQGDKI MHAINRRLGT

            610        620        630        640        650        660
FEVEDQIEAA RQFSKMGFVD NKRIAIWGWS YGGYVTSMVL GSGSGVFKCG IAVAPVSRWE

            670        680        690        700        710        720
YYDSVYTERY MGLPTPEDNL DHYRNSTVMS RAENFKQVEY LLIHGTADDN VHFQQSAQIS

            730        740        750        760
KALVDVGVDF QAMWYTDEDH GIASSTAHQH IYTHMSHFIK QCFSLP
```

[0061] Most preferably, the Dipeptidyl peptidase 4 assay detects one or more soluble forms of Dipeptidyl peptidase 4. Dipeptidyl peptidase 4 is a type II membrane protein having a large extracellular domain, most or all of which is present in soluble forms of Dipeptidyl peptidase 4 generated either through alternative splicing event which deletes all or a portion of the transmembrane domain, or by proteolysis of the membrane-bound form. In the case of an immunoassay, one or more antibodies that bind to epitopes within this extracellular domain may be used to detect these soluble form(s). The following domains have been identified in Dipeptidyl peptidase 4:

Residues	Length	Domain ID
Residues	Length	Domain ID
1-766	766	Dipeptidyl peptidase 4
39-766	728	Dipeptidyl peptidase 4, soluble form
1-6	6	Cytoplasmic
7-28	22	Anchor signal
29-766	738	Extracellular

[0062] As used herein, the term "Neprilysin" refers to one or more polypeptides present in a biological sample that are derived from the Neprilysin precursor (Swiss-Prot P08473 (SEQ ID NO: 4))

```
            10         20         30         40         50         60
MGKSESQMDI TDINTPKPKK KQRWTPLEIS LSVLVLLLTI IAVTMIALYA TYDDGICKSS
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        70          80          90         100         110         120
DCIKSAARLI  QNMDATTEPC  TDFFKYACGG  WLKRNVIPET  SSRYGNFDIL  RDELEVVLKD

       130         140         150         160         170         180
VLQEPKTEDI  VAVQKAKALY  RSCINESAID  SRGGEPLLKL  LPDIYGWPVA  TENWEQKYGA

       190         200         210         220         230         240
SWTAEKAIAQ  LNSKYGKKVL  INLFVGTDDK  NSVNHVIHID  QPRLGLPSRD  YYECTGIYKE

       250         260         270         280         290         300
ACTAYVDFMI  SVARLIRQEE  RLPIDENQLA  LEMNKVMELE  KEIANATAKP  EDRNDPMLLY

       310         320         330         340         350         360
NKMTLAQIQN  NFSLEINGKP  FSWLNFTNEI  MSTVNISITN  EEDVVVYAPE  YLTKLKPILT

       370         380         390         400         410         420
KYSARDLQNL  MSWRFIMDLV  SSLSRTYKES  RNAFRKALYG  TTSETATWRR  CANYVNGNME

       430         440         450         460         470         480
NAVGRLYVEA  AFAGESKHVV  EDLIAQIREV  FIQTLDDLTW  MDAETKKRAE  EKALAIKERI

       490         500         510         520         530         540
GYPDDIVSND  NKLNNEYLEL  NYKEDEYFEN  IIQNLKFSQS  KQLKKLREKV  DKDEWISGAA

       550         560         570         580         590         600
VVNAFYSSGR  NQIVFPAGIL  QPPFFSAQQS  NSLNYGGIGM  VIGHEITHGF  DDNGRNFNKD

       610         620         630         640         650         660
GDLVDWWTQQ  SASNFKEQSQ  CMVYQYGNFS  WDLAGGQHLN  GINTLGENIA  DNGGLGQAYR

       670         680         690         700         710         720
AYQNYIKKNG  EEKLLPGLDL  NHKQLFFLNF  AQVWCGTYRP  EYAVNSIKTD  VHSPGNFRII

       730         740         750
GTLQNSAEFS  EAFHCRKNSY  MNPEKKCRVW
```

[0063] Most preferably, the Neprilysin assay detects one or more soluble forms of Neprilysin. Neprilysin is a type II membrane protein having a large extracellular domain, most or all of which is present in soluble forms of Neprilysin generated either through alternative splicing event which deletes all or a portion of the transmembrane domain, or by proteolysis of the membrane-bound form. In the case of an immunoassay, one or more antibodies that bind to epitopes within this extracellular domain may be used to detect these soluble form(s). The following domains have been identified in Neprilysin:

Residues	Length	Domain ID
1	1	initiator methionine
2-750	749	Neprilysin

2-28	27	Cytoplasmic
29-51	23	Anchor signal
52-750	699	Extracellular

[0064]   As used herein, the term "Beta-2-microglobulin" refers to one or more polypeptides present in a biological sample that are derived from the Beta-2-microglobulin precursor (Swiss-Prot P61769 (SEQ ID NO: 5))

```
          10         20         30         40         50         60
MSRSVALAVL ALLSLSGLEA IQRTPKIQVY SRHPAENGKS NFLNCYVSGF HPSDIEVDLL

          70         80         90        100        110
KNGERIEKVE HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSQP KIVKWDRDM
```

[0065]   The following domains have been identified in Beta-2-microglobulin:

Residues	Length	Domain ID
1-20	20	signal peptide
21-119	99	Beta-2-microglobulin
22-119	21	Beta-2-microglobulin form pI 5.3

[0066]   As used herein, the term "Carbonic anhydrase IX" refers to one or more polypeptides present in a biological sample that are derived from the Carbonic anhydrase IX precursor (Swiss-Prot Q16790 (SEQ ID NO: 6))

```
          10         20         30         40         50         60
MAPLCPSPWL PLLIPAPAPG LTVQLLLSLL LLVPVHPQRL PRMQEDSPLG GGSSGEDDPL

          70         80         90        100        110        120
GEEDLPSEED SPREEDPPGE EDLPGEEDLP GEEDLPEVKP KSEEEGSLKL EDLPTVEAPG

         130        140        150        160        170        180
DPQEPQNNAH RDKEGDDQSH WRYGGDPPWP RVSPACAGRF QSPVDIRPQL AAFCPALRPL

         190        200        210        220        230        240
ELLGFQLPPL PELRLRNNGH SVQLTLPPGL EMALGPGREY RALQLHLHWG AAGRPGSEHT

         250        260        270        280        290        300
VEGHRFPAEI HVVHLSTAFA RVDEALGRPG GLAVLAAFLE EGPEENSAYE QLLSRLEEIA

         310        320        330        340        350        360
EEGSETQVPG LDISALLPSD FSRYFQYEGS LTTPPCAQGV IWTVFNQTVM LSAKQLHTLS

         370        380        390        400        410        420
DTLWGPGDSR LQLNFRATQP LNGRVIEASF PAGVDSSPRA AEPVQLNSCL AAGDILALVF
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        430        440        450
GLLFAVTSVA FLVQMRRQHR RGTKGGVSYR PAEVAETGA
```

[0067]   Most preferably, the Carbonic anhydrase IX assay detects one or more soluble forms of Carbonic anhydrase IX. Carbonic anhydrase IX is a type I membrane protein having a large extracellular domain, most or all of which is present in soluble forms of Carbonic anhydrase IX generated either through alternative splicing event which deletes all or a portion of the transmembrane domain, or by proteolysis of the membrane-bound form. In the case of an immunoassay, one or more antibodies that bind to epitopes within this extracellular domain may be used to detect these soluble form(s). The following domains have been identified in Carbonic anhydrase IX:

Residues	Length	Domain ID
1-37	37	Signal peptide
38-459	422	Carbonic anhydrase IX
38-414	377	Extracellular
415-435	21	Transmembrane domain
436-459	699	Cytoplasmic

[0068]   As used herein, the term "C-X-C motif chemokine 2" refers to one or more polypeptides present in a biological sample that are derived from the C-X-C motif chemokine 2 precursor (Swiss-Prot P19875 (SEQ ID NO: 7)).

```
        10         20         30         40         50         60
MARATLSAAP SNPRLLRVAL LLLLLVAASR RAAGAPLATE LRCQCLQTLQ GIHLKNIQSV

        70         80         90        100
KVKSPGPHCA QTEVIATLKN GQKACLNPAS PMVKKIIEKM LKNGKSN
```

[0069]   The following domains have been identified in C-X-C motif chemokine 2:

Residues	Length	Domain ID
1-34	34	Signal peptide
35-107	73	C-X-C motif chemokine 2
39-107	69	GRO-beta

[0070]   As used herein, the term "relating a signal to the presence or amount" of an analyte reflects the following understanding. Assay signals are typically related to the

presence or amount of an analyte through the use of a standard curve calculated using known concentrations of the analyte of interest. As the term is used herein, an assay is "configured to detect" an analyte if an assay can generate a detectable signal indicative of the presence or amount of a physiologically relevant concentration of the analyte. Because an antibody epitope is on the order of 8 amino acids, an immunoassay configured to detect a marker of interest will also detect polypeptides related to the marker sequence, so long as those polypeptides contain the epitope(s) necessary to bind to the antibody or antibodies used in the assay. The term "related marker" as used herein with regard to a biomarker such as one of the kidney injury markers described herein refers to one or more fragments, variants, etc., of a particular marker or its biosynthetic parent that may be detected as a surrogate for the marker itself or as independent biomarkers. The term also refers to one or more polypeptides present in a biological sample that are derived from the biomarker precursor complexed to additional species, such as binding proteins, receptors, heparin, lipids, sugars, etc.

[0071]   In this regard, the skilled artisan will understand that the signals obtained from an immunoassay are a direct result of complexes formed between one or more antibodies and the target biomolecule (*i.e.*, the analyte) and polypeptides containing the necessary epitope(s) to which the antibodies bind. While such assays may detect the full length biomarker and the assay result be expressed as a concentration of a biomarker of interest, the signal from the assay is actually a result of all such "immunoreactive" polypeptides present in the sample. Expression of biomarkers may also be determined by means other than immunoassays, including protein measurements (such as dot blots, western blots, chromatographic methods, mass spectrometry, *etc.*) and nucleic acid measurements (mRNA quatitation). This list is not meant to be limiting.

[0072]   The term "positive going" marker as that term is used herein refer to a marker that is determined to be elevated in subjects suffering from a disease or condition, relative to subjects not suffering from that disease or condition. The term "negative going" marker as that term is used herein refer to a marker that is determined to be reduced in subjects suffering from a disease or condition, relative to subjects not suffering from that disease or condition.

[0073]   The term "subject" as used herein refers to a human or non-human organism. Thus, the methods and compositions described herein are applicable to both human and veterinary disease. Further, while a subject is preferably a living organism, the invention

described herein may be used in post-mortem analysis as well. Preferred subjects are humans, and most preferably "patients," which as used herein refers to living humans that are receiving medical care for a disease or condition. This includes persons with no defined illness who are being investigated for signs of pathology.

[0074]   Preferably, an analyte is measured in a sample. Such a sample may be obtained from a subject, or may be obtained from biological materials intended to be provided to the subject. For example, a sample may be obtained from a kidney being evaluated for possible transplantation into a subject, and an analyte measurement used to evaluate the kidney for preexisting damage. Preferred samples are body fluid samples.

[0075]   The term "body fluid sample" as used herein refers to a sample of bodily fluid obtained for the purpose of diagnosis, prognosis, classification or evaluation of a subject of interest, such as a patient or transplant donor. In certain embodiments, such a sample may be obtained for the purpose of determining the outcome of an ongoing condition or the effect of a treatment regimen on a condition. Preferred body fluid samples include blood, serum, plasma, cerebrospinal fluid, urine, saliva, sputum, and pleural effusions. In addition, one of skill in the art would realize that certain body fluid samples would be more readily analyzed following a fractionation or purification procedure, for example, separation of whole blood into serum or plasma components.

[0076]   The term "diagnosis" as used herein refers to methods by which the skilled artisan can estimate and/or determine the probability ("a likelihood") of whether or not a patient is suffering from a given disease or condition. In the case of the present invention, "diagnosis" includes using the results of an assay, most preferably an immunoassay, for a kidney injury marker of the present invention, optionally together with other clinical characteristics, to arrive at a diagnosis (that is, the occurrence or nonoccurrence) of an acute renal injury or ARF for the subject from which a sample was obtained and assayed. That such a diagnosis is "determined" is not meant to imply that the diagnosis is 100% accurate. Many biomarkers are indicative of multiple conditions. The skilled clinician does not use biomarker results in an informational vacuum, but rather test results are used together with other clinical indicia to arrive at a diagnosis. Thus, a measured biomarker level on one side of a predetermined diagnostic threshold indicates a greater likelihood of the occurrence of disease in the subject relative to a measured level on the other side of the predetermined diagnostic threshold.

[0077]   Similarly, a prognostic risk signals a probability ("a likelihood") that a given course or outcome will occur. A level or a change in level of a prognostic indicator, which in turn is associated with an increased probability of morbidity (e.g., worsening renal function, future ARF, or death) is referred to as being "indicative of an increased likelihood" of an adverse outcome in a patient.

[0078]   Marker Assays

[0079]   In general, immunoassays involve contacting a sample containing or suspected of containing a biomarker of interest with at least one antibody that specifically binds to the biomarker. A signal is then generated indicative of the presence or amount of complexes formed by the binding of polypeptides in the sample to the antibody. The signal is then related to the presence or amount of the biomarker in the sample. Numerous methods and devices are well known to the skilled artisan for the detection and analysis of biomarkers. *See, e.g.*, U.S. Patents 6,143,576; 6,113,855; 6,019,944; 5,985,579; 5,947,124; 5,939,272; 5,922,615; 5,885,527; 5,851,776; 5,824,799; 5,679,526; 5,525,524; and 5,480,792, and *The Immunoassay Handbook*, David Wild, ed. Stockton Press, New York, 1994, each of which is hereby incorporated by reference in its entirety, including all tables, figures and claims.

[0080]   The assay devices and methods known in the art can utilize labeled molecules in various sandwich, competitive, or non-competitive assay formats, to generate a signal that is related to the presence or amount of the biomarker of interest. Suitable assay formats also include chromatographic, mass spectrographic, and protein "blotting" methods. Additionally, certain methods and devices, such as biosensors and optical immunoassays, may be employed to determine the presence or amount of analytes without the need for a labeled molecule. *See, e.g.*, U.S. Patents 5,631,171; and 5,955,377, each of which is hereby incorporated by reference in its entirety, including all tables, figures and claims. One skilled in the art also recognizes that robotic instrumentation including but not limited to Beckman ACCESS®, Abbott AXSYM®, Roche ELECSYS®, Dade Behring STRATUS® systems are among the immunoassay analyzers that are capable of performing immunoassays. But any suitable immunoassay may be utilized, for example, enzyme-linked immunoassays (ELISA), radioimmunoassays (RIAs), competitive binding assays, and the like.

[0081]   Antibodies or other polypeptides may be immobilized onto a variety of solid supports for use in assays. Solid phases that may be used to immobilize specific binding members include include those developed and/or used as solid phases in solid phase binding assays. Examples of suitable solid phases include membrane filters, cellulose-based papers, beads (including polymeric, latex and paramagnetic particles), glass, silicon wafers, microparticles, nanoparticles, TentaGels, AgroGels, PEGA gels, SPOCC gels, and multiple-well plates. An assay strip could be prepared by coating the antibody or a plurality of antibodies in an array on solid support. This strip could then be dipped into the test sample and then processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot. Antibodies or other polypeptides may be bound to specific zones of assay devices either by conjugating directly to an assay device surface, or by indirect binding. In an example of the later case, antibodies or other polypeptides may be immobilized on particles or other solid supports, and that solid support immobilized to the device surface.

[0082]   Biological assays require methods for detection, and one of the most common methods for quantitation of results is to conjugate a detectable label to a protein or nucleic acid that has affinity for one of the components in the biological system being studied. Detectable labels may include molecules that are themselves detectable (*e.g.*, fluorescent moieties, electrochemical labels, metal chelates, *etc.*) as well as molecules that may be indirectly detected by production of a detectable reaction product (*e.g.*, enzymes such as horseradish peroxidase, alkaline phosphatase, *etc.*) or by a specific binding molecule which itself may be detectable (e.g., biotin, digoxigenin, maltose, oligohistidine, 2,4-dintrobenzene, phenylarsenate, ssDNA, dsDNA, etc.).

[0083]   Preparation of solid phases and detectable label conjugates often comprise the use of chemical cross-linkers. Cross-linking reagents contain at least two reactive groups, and are divided generally into homofunctional cross-linkers (containing identical reactive groups) and heterofunctional cross-linkers (containing non-identical reactive groups). Homobifunctional cross-linkers that couple through amines, sulfhydryls or react non-specifically are available from many commercial sources. Maleimides, alkyl and aryl halides, alpha-haloacyls and pyridyl disulfides are thiol reactive groups. Maleimides, alkyl and aryl halides, and alpha-haloacyls react with sulfhydryls to form thiol ether bonds, while pyridyl disulfides react with sulfhydryls to produce mixed disulfides. The pyridyl disulfide product is cleavable. Imidoesters are also very useful for protein-protein

cross-links. A variety of heterobifunctional cross-linkers, each combining different attributes for successful conjugation, are commercially available.

[0084]   In certain aspects, the present invention provides kits for the analysis of the described kidney injury markers. The kit comprises reagents for the analysis of at least one test sample which comprise at least one antibody that a kidney injury marker. The kit can also include devices and instructions for performing one or more of the diagnostic and/or prognostic correlations described herein. Preferred kits will comprise an antibody pair for performing a sandwich assay, or a labeled species for performing a competitive assay, for the analyte. Preferably, an antibody pair comprises a first antibody conjugated to a solid phase and a second antibody conjugated to a detectable label, wherein each of the first and second antibodies that bind a kidney injury marker. Most preferably each of the antibodies are monoclonal antibodies. The instructions for use of the kit and performing the correlations can be in the form of labeling, which refers to any written or recorded material that is attached to, or otherwise accompanies a kit at any time during its manufacture, transport, sale or use. For example, the term labeling encompasses advertising leaflets and brochures, packaging materials, instructions, audio or video cassettes, computer discs, as well as writing imprinted directly on kits.

[0085]   Antibodies

[0086]   The term "antibody" as used herein refers to a peptide or polypeptide derived from, modeled after or substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, capable of specifically binding an antigen or epitope. See, e.g. Fundamental Immunology, 3rd Edition, W.E. Paul, ed., Raven Press, N.Y. (1993); Wilson (1994; J. Immunol. Methods 175:267-273; Yarmush (1992) J. Biochem. Biophys. Methods 25:85-97. The term antibody includes antigen-binding portions, i.e., "antigen binding sites," (e.g., fragments, subsequences, complementarity determining regions (CDRs)) that retain capacity to bind antigen, including (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CHl domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CHl domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Single chain antibodies are also included by reference in the term "antibody."

[0087]   Antibodies used in the immunoassays described herein preferably specifically bind to a kidney injury marker of the present invention. The term "specifically binds" is not intended to indicate that an antibody binds exclusively to its intended target since, as noted above, an antibody binds to any polypeptide displaying the epitope(s) to which the antibody binds. Rather, an antibody "specifically binds" if its affinity for its intended target is about 5-fold greater when compared to its affinity for a non-target molecule which does not display the appropriate epitope(s). Preferably the affinity of the antibody will be at least about 5 fold, preferably 10 fold, more preferably 25-fold, even more preferably 50-fold, and most preferably 100-fold or more, greater for a target molecule than its affinity for a non-target molecule. In preferred embodiments, Preferred antibodies bind with affinities of at least about 10^7 M^{-1}, and preferably between about 10^8 M^{-1} to about 10^9 M^{-1}, about 10^9 M^{-1} to about 10^{10} M^{-1}, or about 10^{10} M^{-1} to about 10^{12} M^{-1} .

[0088]   Affinity is calculated as $K_d = k_{off}/k_{on}$ (k_{off} is the dissociation rate constant, K_{on} is the association rate constant and K_d is the equilibrium constant). Affinity can be determined at equilibrium by measuring the fraction bound (r) of labeled ligand at various concentrations (c). The data are graphed using the Scatchard equation: r/c = K(n-r): where r = moles of bound ligand/mole of receptor at equilibrium; c = free ligand concentration at equilibrium; K = equilibrium association constant; and n = number of ligand binding sites per receptor molecule. By graphical analysis, r/c is plotted on the Y-axis versus r on the X-axis, thus producing a Scatchard plot. Antibody affinity measurement by Scatchard analysis is well known in the art. *See, e.g.*, van Erp *et al., J. Immunoassay* 12: 425-43, 1991; Nelson and Griswold, *Comput. Methods Programs Biomed.* 27: 65-8, 1988.

[0089]   The term "epitope" refers to an antigenic determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[0090]   Numerous publications discuss the use of phage display technology to produce and screen libraries of polypeptides for binding to a selected analyte. *See, e.g,* Cwirla *et al., Proc. Natl. Acad. Sci. USA* 87, 6378-82, 1990; Devlin *et al., Science* 249, 404-6, 1990, Scott and Smith, *Science* 249, 386-88, 1990; and Ladner *et al.,* U.S. Pat. No. 5,571,698. A basic concept of phage display methods is the establishment of a physical

association between DNA encoding a polypeptide to be screened and the polypeptide. This physical association is provided by the phage particle, which displays a polypeptide as part of a capsid enclosing the phage genome which encodes the polypeptide. The establishment of a physical association between polypeptides and their genetic material allows simultaneous mass screening of very large numbers of phage bearing different polypeptides. Phage displaying a polypeptide with affinity to a target bind to the target and these phage are enriched by affinity screening to the target. The identity of polypeptides displayed from these phage can be determined from their respective genomes. Using these methods a polypeptide identified as having a binding affinity for a desired target can then be synthesized in bulk by conventional means. *See, e.g.,* U.S. Patent No. 6,057,098, which is hereby incorporated in its entirety, including all tables, figures, and claims.

[0091]   The antibodies that are generated by these methods may then be selected by first screening for affinity and specificity with the purified polypeptide of interest and, if required, comparing the results to the affinity and specificity of the antibodies with polypeptides that are desired to be excluded from binding. The screening procedure can involve immobilization of the purified polypeptides in separate wells of microtiter plates. The solution containing a potential antibody or groups of antibodies is then placed into the respective microtiter wells and incubated for about 30 min to 2 h. The microtiter wells are then washed and a labeled secondary antibody (for example, an anti-mouse antibody conjugated to alkaline phosphatase if the raised antibodies are mouse antibodies) is added to the wells and incubated for about 30 min and then washed. Substrate is added to the wells and a color reaction will appear where antibody to the immobilized polypeptide(s) are present.

[0092]   The antibodies so identified may then be further analyzed for affinity and specificity in the assay design selected. In the development of immunoassays for a target protein, the purified target protein acts as a standard with which to judge the sensitivity and specificity of the immunoassay using the antibodies that have been selected. Because the binding affinity of various antibodies may differ; certain antibody pairs (*e.g.,* in sandwich assays) may interfere with one another sterically, *etc.,* assay performance of an antibody may be a more important measure than absolute affinity and specificity of an antibody.

[0093]   While the present application describes antibody-based binding assays in detail, alternatives to antibodies as binding species in assays are well known in the art. These include receptors for a particular target, aptamers, etc. Aptamers are oligonucleic acid or peptide molecules that bind to a specific target molecule. Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist. High-affinity aptamers containing modified nucleotides conferring improved characteristics on the ligand, such as improved in vivo stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions, and may include amino acid side chain functionalities.

[0094]   Assay Correlations

[0095]   The term "correlating" as used herein in reference to the use of biomarkers refers to comparing the presence or amount of the biomarker(s) in a patient to its presence or amount in persons known to suffer from, or known to be at risk of, a given condition; or in persons known to be free of a given condition. Often, this takes the form of comparing an assay result in the form of a biomarker concentration to a predetermined threshold selected to be indicative of the occurrence or nonoccurrence of a disease or the likelihood of some future outcome.

[0096]   Selecting a diagnostic threshold involves, among other things, consideration of the probability of disease, distribution of true and false diagnoses at different test thresholds, and estimates of the consequences of treatment (or a failure to treat) based on the diagnosis. For example, when considering administering a specific therapy which is highly efficacious and has a low level of risk, few tests are needed because clinicians can accept substantial diagnostic uncertainty. On the other hand, in situations where treatment options are less effective and more risky, clinicians often need a higher degree of diagnostic certainty. Thus, cost/benefit analysis is involved in selecting a diagnostic threshold.

[0097]   Suitable thresholds may be determined in a variety of ways. For example, one recommended diagnostic threshold for the diagnosis of acute myocardial infarction using cardiac troponin is the 97.5th percentile of the concentration seen in a normal population. Another method may be to look at serial samples from the same patient, where a prior "baseline" result is used to monitor for temporal changes in a biomarker level.

[0098] Population studies may also be used to select a decision threshold. Reciever Operating Characteristic ("ROC") arose from the field of signal dectection therory developed during World War II for the analysis of radar images, and ROC analysis is often used to select a threshold able to best distinguish a "diseased" subpopulation from a "nondiseased" subpopulation. A false positive in this case occurs when the person tests positive, but actually does not have the disease. A false negative, on the other hand, occurs when the person tests negative, suggesting they are healthy, when they actually do have the disease. To draw a ROC curve, the true positive rate (TPR) and false positive rate (FPR) are determined as the decision threshold is varied continuously. Since TPR is equivalent with sensitivity and FPR is equal to 1 - specificity, the ROC graph is sometimes called the sensitivity vs (1 - specificity) plot. A perfect test will have an area under the ROC curve of 1.0; a random test will have an area of 0.5. A threshold is selected to provide an acceptable level of specificity and sensitivity.

[0099] In this context, "diseased" is meant to refer to a population having one characteristic (the presence of a disease or condition or the occurrence of some outcome) and "nondiseased" is meant to refer to a population lacking the characteristic. While a single decision threshold is the simplest application of such a method, multiple decision thresholds may be used. For example, below a first threshold, the absence of disease may be assigned with relatively high confidence, and above a second threshold the presence of disease may also be assigned with relatively high confidence. Between the two thresholds may be considered indeterminate. This is meant to be exemplary in nature only.

[0100] In addition to threshold comparisons, other methods for correlating assay results to a patient classification (occurrence or nonoccurrence of disease, likelihood of an outcome, etc.) include decision trees, rule sets, Bayesian methods, and neural network methods. These methods can produce probability values representing the degree to which a subject belongs to one classification out of a plurality of classifications.

Measures of test accuracy may be obtained as described in Fischer *et al., Intensive Care Med.* 29: 1043-51, 2003, and used to determine the effectiveness of a given biomarker. These measures include sensitivity and specificity, predictive values, likelihood ratios, diagnostic odds ratios, and ROC curve areas. The area under the curve ("AUC") of a ROC plot is equal to the probability that a classifier will rank a randomly chosen positive instance higher than a randomly chosen negative one. The area under the ROC curve may be thought of as equivalent to the Mann-Whitney U test, which tests for the median

difference between scores obtained in the two groups considered if the groups are of continuous data, or to the Wilcoxon test of ranks.

[0101]   As discussed above, suitable tests may exhibit one or more of the following results on these various measures: a specificity of greater than 0.5, preferably at least 0.6, more preferably at least 0.7, still more preferably at least 0.8, even more preferably at least 0.9 and most preferably at least 0.95, with a corresponding sensitivity greater than 0.2, preferably greater than 0.3, more preferably greater than 0.4, still more preferably at least 0.5, even more preferably 0.6, yet more preferably greater than 0.7, still more preferably greater than 0.8, more preferably greater than 0.9, and most preferably greater than 0.95; a sensitivity of greater than 0.5, preferably at least 0.6, more preferably at least 0.7, still more preferably at least 0.8, even more preferably at least 0.9 and most preferably at least 0.95, with a corresponding specificity greater than 0.2, preferably greater than 0.3, more preferably greater than 0.4, still more preferably at least 0.5, even more preferably 0.6, yet more preferably greater than 0.7, still more preferably greater than 0.8, more preferably greater than 0.9, and most preferably greater than 0.95; at least 75% sensitivity, combined with at least 75% specificity; a ROC curve area of greater than 0.5, preferably at least 0.6, more preferably 0.7, still more preferably at least 0.8, even more preferably at least 0.9, and most preferably at least 0.95; an odds ratio different from 1, preferably at least about 2 or more or about 0.5 or less, more preferably at least about 3 or more or about 0.33 or less, still more preferably at least about 4 or more or about 0.25 or less, even more preferably at least about 5 or more or about 0.2 or less, and most preferably at least about 10 or more or about 0.1 or less; a positive likelihood ratio (calculated as sensitivity/(1-specificity)) of greater than 1, at least 2, more preferably at least 3, still more preferably at least 5, and most preferably at least 10; and or a negative likelihood ratio (calculated as (1-sensitivity)/specificity) of less than 1, less than or equal to 0.5, more preferably less than or equal to 0.3, and most preferably less than or equal to 0.1

[0102]   Additional clinical indicia may be combined with the kidney injury marker assay result(s) of the present invention. These include other biomarkers related to renal status. Examples include the following, which recite the common biomarker name, followed by the Swiss-Prot entry number for that biomarker or its parent: Actin (P68133); Adenosine deaminase binding protein (DPP4, P27487); Alpha-1-acid glycoprotein 1 (P02763); Alpha-1-microglobulin (P02760); Albumin (P02768); Angiotensinogenase

(Renin, P00797); Annexin A2 (P07355); Beta-glucuronidase (P08236); B-2-microglobulin (P61679); Beta-galactosidase (P16278); BMP-7 (P18075); Brain natriuretic peptide (proBNP, BNP-32, NTproBNP; P16860); Calcium-binding protein Beta (S100-beta, P04271); Carbonic anhydrase (Q16790); Casein Kinase 2 (P68400); Ceruloplasmin (P00450); Clusterin (P10909); Complement C3 (P01024); Cysteine-rich protein (CYR61, O00622); Cytochrome C (P99999); Epidermal growth factor (EGF, P01133); Endothelin-1 (P05305); Exosomal Fetuin-A (P02765); Fatty acid-binding protein, heart (FABP3, P05413); Fatty acid-binding protein, liver (P07148); Ferritin (light chain, P02793; heavy chain P02794); Fructose-1,6-biphosphatase (P09467); GRO-alpha (CXCL1, (P09341); Growth Hormone (P01241); Hepatocyte growth factor (P14210); Insulin-like growth factor I (P01343); Immunoglobulin G; Immunoglobulin Light Chains (Kappa and Lambda); Interferon gamma (P01308); Lysozyme (P61626); Interleukin-1alpha (P01583); Interleukin-2 (P60568); Interleukin-4 (P60568); Interleukin-9 (P15248); Interleukin-12p40 (P29460); Interleukin-13 (P35225); Interleukin-16 (Q14005); L1 cell adhesion molecule (P32004); Lactate dehydrogenase (P00338); Leucine Aminopeptidase (P28838); Meprin A-alpha subunit (Q16819); Meprin A-beta subunit (Q16820); Midkine (P21741); MIP2-alpha (CXCL2, P19875); MMP-2 (P08253); MMP-9 (P14780); Netrin-1 (O95631); Neutral endopeptidase (P08473); Osteopontin (P10451); Renal papillary antigen 1 (RPA1); Renal papillary antigen 2 (RPA2); Retinol binding protein (P09455); Ribonuclease; S100 calcium-binding protein A6 (P06703); Serum Amyloid P Component (P02743); Sodium/Hydrogen exchanger isoform (NHE3, P48764); Spermidine/spermine N1-acetyltransferase (P21673); TGF-Beta1 (P01137); Transferrin (P02787); Trefoil factor 3 (TFF3, Q07654); Toll-Like protein 4 (O00206); Total protein; Tubulointerstitial nephritis antigen (Q9UJW2); Uromodulin (Tamm-Horsfall protein, P07911).

[0103]   For purposes of risk stratification, Adiponectin (Q15848); Alkaline phosphatase (P05186); Aminopeptidase N (P15144); CalbindinD28k (P05937); Cystatin C (P01034); 8 subunit of F1FO ATPase (P03928); Gamma-glutamyltransferase (P19440); GSTa (alpha-glutathione-S-transferase, P08263); GSTpi (Glutathione-S-transferase P; GST class-pi; P09211); IGFBP-1 (P08833); IGFBP-2 (P18065); IGFBP-6 (P24592); Integral membrane protein 1 (Itm1, P46977); Interleukin-6 (P05231); Interleukin-8 (P10145); Interleukin-18 (Q14116); IP-10 (10 kDa interferon-gamma-induced protein, P02778); IRPR (IFRD1, O00458); Isovaleryl-CoA dehydrogenase (IVD, P26440); I-TAC/CXCL11 (O14625); Keratin 19 (P08727); Kim-1 (Hepatitis A virus cellular

receptor 1, O43656); L-arginine:glycine amidinotransferase (P50440); Leptin (P41159); Lipocalin2 (NGAL, P80188); MCP-1 (P13500); MIG (Gamma-interferon-induced monokine Q07325); MIP-1a (P10147); MIP-3a (P78556); MIP-1beta (P13236); MIP-1d (Q16663); NAG (N-acetyl-beta-D-glucosaminidase, P54802); Organic ion transporter (OCT2, O15244); Osteoprotegerin (O14788); P8 protein (O60356); Plasminogen activator inhibitor 1 (PAI-1, P05121); ProANP(1-98) (P01160); Protein phosphatase 1-beta (PPI-beta, P62140); Rab GDI-beta (P50395); Renal kallikrein (Q86U61); RT1.B-1 (alpha) chain of the integral membrane protein (Q5Y7A8); Soluble tumor necrosis factor receptor superfamily member 1A (sTNFR-I, P19438); Soluble tumor necrosis factor receptor superfamily member 1B (sTNFR-II, P20333); Tissue inhibitor of metalloproteinases 3 (TIMP-3, P35625); uPAR (Q03405) may be combined with the kidney injury marker assay result(s) of the present invention.

[0104]   Other clinical indicia which may be combined with the kidney injury marker assay result(s) of the present invention includes demographic information (e.g., weight, sex, age, race), medical history (e.g., family history, type of surgery, pre-existing disease such as aneurism, congestive heart failure, preeclampsia, eclampsia, diabetes mellitus, hypertension, coronary artery disease, proteinuria, renal insufficiency, or sepsis, type of toxin exposure such as NSAIDs, cyclosporines, tacrolimus, aminoglycosides, foscarnet, ethylene glycol, hemoglobin, myoglobin, ifosfamide, heavy metals, methotrexate, radiopaque contrast agents, or streptozotocin), clinical variables (*e.g.*, blood pressure, temperature, respiration rate), risk scores (APACHE score, PREDICT score, TIMI Risk Score for UA/NSTEMI, Framingham Risk Score), a urine total protein measurement, a glomerular filtration rate, an estimated glomerular filtration rate, a urine production rate, a serum or plasma creatinine concentration, a renal papillary antigen 1 (RPA1) measurement; a renal papillary antigen 2 (RPA2) measurement; a urine creatinine concentration, a fractional excretion of sodium, a urine sodium concentration, a urine creatinine to serum or plasma creatinine ratio, a urine specific gravity, a urine osmolality, a urine urea nitrogen to plasma urea nitrogen ratio, a plasma BUN to creatnine ratio, and/or a renal failure index calculated as urine sodium / (urine creatinine / plasma creatinine). Other measures of renal function which may be combined with the kidney injury marker assay result(s) are described hereinafter and in Harrison's Principles of Internal Medicine, 17th Ed., McGraw Hill, New York, pages 1741-1830, and Current

Medical Diagnosis & Treatment 2008, 47th Ed, McGraw Hill, New York, pages 785-815, each of which are hereby incorporated by reference in their entirety.

[0105]   Combining assay results/clinical indicia in this manner can comprise the use of multivariate logistical regression, loglinear modeling, neural network analysis, n-of-m analysis, decision tree analysis, etc. This list is not meant to be limiting.

[0106]   Diagnosis of Acute Renal Failure

[0107]   As noted above, the terms "acute renal (or kidney) injury" and "acute renal (or kidney) failure" as used herein are defined in part in terms of changes in serum creatinine from a baseline value. Most definitions of ARF have common elements, including the use of serum creatinine and, often, urine output. Patients may present with renal dysfunction without an available baseline measure of renal function for use in this comparison. In such an event, one may estimate a baseline serum creatinine value by assuming the patient initially had a normal GFR. Glomerular filtration rate (GFR) is the volume of fluid filtered from the renal (kidney) glomerular capillaries into the Bowman's capsule per unit time. Glomerular filtration rate (GFR) can be calculated by measuring any chemical that has a steady level in the blood, and is freely filtered but neither reabsorbed nor secreted by the kidneys. GFR is typically expressed in units of ml/min:

$$GFR = \frac{\text{Urine Concentration} \times \text{Urine Flow}}{\text{Plasma Concentration}}$$

[0108]   By normalizing the GFR to the body surface area, a GFR of approximately 75–100 ml/min per 1.73 m^2 can be assumed. The rate therefore measured is the quantity of the substance in the urine that originated from a calculable volume of blood.

[0109]   There are several different techniques used to calculate or estimate the glomerular filtration rate (GFR or eGFR). In clinical practice, however, creatinine clearance is used to measure GFR. Creatinine is produced naturally by the body (creatinine is a metabolite of creatine, which is found in muscle). It is freely filtered by the glomerulus, but also actively secreted by the renal tubules in very small amounts such that creatinine clearance overestimates actual GFR by 10-20%. This margin of error is acceptable considering the ease with which creatinine clearance is measured.

[0110]   Creatinine clearance (CCr) can be calculated if values for creatinine's urine concentration (U_{Cr}), urine flow rate (V), and creatinine's plasma concentration (P_{Cr}) are

known. Since the product of urine concentration and urine flow rate yields creatinine's excretion rate, creatinine clearance is also said to be its excretion rate ($U_{Cr} \times V$) divided by its plasma concentration. This is commonly represented mathematically as:

$$C_{Cr} = \frac{U_{Cr} \times V}{P_{Cr}}$$

[0111]   Commonly a 24 hour urine collection is undertaken, from empty-bladder one morning to the contents of the bladder the following morning, with a comparative blood test then taken:

$$C_{Cr} = \frac{U_{Cr} \times 24\text{-hour volume}}{P_{Cr} \times 24 \times 60 mins}$$

[0112]   To allow comparison of results between people of different sizes, the CCr is often corrected for the body surface area (BSA) and expressed compared to the average sized man as ml/min/1.73 m2. While most adults have a BSA that approaches 1.7 (1.6-1.9), extremely obese or slim patients should have their CCr corrected for their actual BSA:

$$C_{Cr-corrected} = \frac{C_{Cr} \times 1.73}{BSA}$$

[0113]   The accuracy of a creatinine clearance measurement (even when collection is complete) is limited because as glomerular filtration rate (GFR) falls creatinine secretion is increased, and thus the rise in serum creatinine is less. Thus, creatinine excretion is much greater than the filtered load, resulting in a potentially large overestimation of the GFR (as much as a twofold difference). However, for clinical purposes it is important to determine whether renal function is stable or getting worse or better. This is often determined by monitoring serum creatinine alone. Like creatinine clearance, the serum creatinine will not be an accurate reflection of GFR in the non-steady-state condition of ARF. Nonetheless, the degree to which serum creatinine changes from baseline will reflect the change in GFR. Serum creatinine is readily and easily measured and it is specific for renal function.

[0114]   For purposes of determining urine output on a Urine output on a mL/kg/hr basis, hourly urine collection and measurement is adequate. In the case where, for example, only a cumulative 24-h output was available and no patient weights are

provided, minor modifications of the RIFLE urine output criteria have been described. For example, Bagshaw *et al., Nephrol. Dial. Transplant.* 23: 1203–1210, 2008, assumes an average patient weight of 70 kg, and patients are assigned a RIFLE classification based on the following: <35 mL/h (Risk), <21 mL/h (Injury) or <4 mL/h (Failure).

[0115]   Selecting a Treatment Regimen

[0116]   Once a diagnosis is obtained, the clinician can readily select a treatment regimen that is compatible with the diagnosis, such as initiating renal replacement therapy, withdrawing delivery of compounds that are known to be damaging to the kidney, kidney transplantation, delaying or avoiding procedures that are known to be damaging to the kidney, modifying diuretic administration, initiating goal directed therapy, etc. The skilled artisan is aware of appropriate treatments for numerous diseases discussed in relation to the methods of diagnosis described herein. See, e.g., Merck Manual of Diagnosis and Therapy, 17th Ed. Merck Research Laboratories, Whitehouse Station, NJ, 1999. In addition, since the methods and compositions described herein provide prognostic information, the markers of the present invention may be used to monitor a course of treatment. For example, improved or worsened prognostic state may indicate that a particular treatment is or is not efficacious.

[0117]   One skilled in the art readily appreciates that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The examples provided herein are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention.

[0118]   <u>Example 1:   Contrast-induced nephropathy sample collection</u>

[0119]   The objective of this sample collection study is to collect samples of plasma and urine and clinical data from patients before and after receiving intravascular contrast media. Approximately 250 adults undergoing radiographic/angiographic procedures involving intravascular administration of iodinated contrast media are enrolled. To be enrolled in the study, each patient must meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria

males and females 18 years of age or older;

undergoing a radiographic / angiographic procedure (such as a CT scan or coronary intervention) involving the intravascular administration of contrast media;

expected to be hospitalized for at least 48 hours after contrast administration.

able and willing to provide written informed consent for study participation and to comply with all study procedures.

Exclusion Criteria

renal transplant recipients;

acutely worsening renal function prior to the contrast procedure;

already receiving dialysis (either acute or chronic) or in imminent need of dialysis at enrollment;

expected to undergo a major surgical procedure (such as involving cardiopulmonary bypass) or an additional imaging procedure with contrast media with significant risk for further renal insult within the 48 hrs following contrast administration;

participation in an interventional clinical study with an experimental therapy within the previous 30 days;

known infection with human immunodeficiency virus (HIV) or a hepatitis virus.

[0120]   Immediately prior to the first contrast administration (and after any pre-procedure hydration), an EDTA anti-coagulated blood sample (10 mL) and a urine sample (10 mL) are collected from each patient. Blood and urine samples are then collected at 4 (±0.5), 8 (±1), 24 (±2) 48 (±2), and 72 (±2) hrs following the last administration of contrast media during the index contrast procedure. Blood is collected via direct venipuncture or via other available venous access, such as an existing femoral sheath, central venous line, peripheral intravenous line or hep-lock. These study blood samples are processed to plasma at the clinical site, frozen and shipped to Astute Medical, Inc., San Diego, CA. The study urine samples are frozen and shipped to Astute Medical, Inc.

[0121]   Serum creatinine is assessed at the site immediately prior to the first contrast administration (after any pre-procedure hydration) and at 4 (±0.5), 8 (±1), 24 (±2) and 48 (±2)), and 72 (±2) hours following the last administration of contrast (ideally at the same time as the study samples are obtained). In addition, each patient's status is evaluated

through day 30 with regard to additional serum and urine creatinine measurements, a need for dialysis, hospitalization status, and adverse clinical outcomes (including mortality).

[0122]   Prior to contrast administration, each patient is assigned a risk based on the following assessment: systolic blood pressure <80 mm Hg = 5 points; intra-arterial balloon pump = 5 points; congestive heart failure (Class III-IV or history of pulmonary edema) = 5 points; age >75 yrs = 4 points; hematocrit level <39% for men, <35% for women = 3 points; diabetes = 3 points; contrast media volume = 1 point for each 100 mL; serum creatinine level >1.5 g/dL = 4 points OR estimated GFR 40–60 mL/min/1.73 m^2 = 2 points, 20–40 mL/min/1.73 m^2 = 4 points, < 20 mL/min/1.73 m^2 = 6 points. The risks assigned are as follows: risk for CIN and dialysis: 5 or less total points = risk of CIN - 7.5%, risk of dialysis - 0.04%; 6–10 total points = risk of CIN - 14%, risk of dialysis - 0.12%; 11–16 total points = risk of CIN - 26.1%, risk of dialysis - 1.09%; >16 total points = risk of CIN - 57.3%, risk of dialysis - 12.8%.

[0123]   Example 2:   Cardiac surgery sample collection

[0124]   The objective of this sample collection study is to collect samples of plasma and urine and clinical data from patients before and after undergoing cardiovascular surgery, a procedure known to be potentially damaging to kidney function. Approximately 900 adults undergoing such surgery are enrolled. To be enrolled in the study, each patient must meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria

males and females 18 years of age or older;

undergoing cardiovascular surgery;

Toronto/Ottawa Predictive Risk Index for Renal Replacement risk score of at least 2 (Wijeysundera *et al., JAMA* 297: 1801-9, 2007); and

able and willing to provide written informed consent for study participation and to comply with all study procedures.

Exclusion Criteria

known pregnancy;

previous renal transplantation;

acutely worsening renal function prior to enrollment (e.g., any category of

RIFLE criteria);

already receiving dialysis (either acute or chronic) or in imminent need of dialysis at

enrollment;

currently enrolled in another clinical study or expected to be enrolled in another clinical

study within 7 days of cardiac surgery that involves drug infusion or a therapeutic

intervention for AKI;

known infection with human immunodeficiency virus (HIV) or a hepatitis virus.

[0125]   Within 3 hours prior to the first incision (and after any pre-procedure

hydration), an EDTA anti-coagulated blood sample (10 mL), whole blood (3 mL), and a

urine sample (35 mL) are collected from each patient. Blood and urine samples are then

collected at 3 (±0.5), 6 (±0.5), 12 (±1), 24 (±2) and 48 (±2) hrs following the procedure

and then daily on days 3 through 7 if the subject remains in the hospital. Blood is

collected via direct venipuncture or via other available venous access, such as an existing

femoral sheath, central venous line, peripheral intravenous line or hep-lock. These study

blood samples are frozen and shipped to Astute Medical, Inc., San Diego, CA. The study

urine samples are frozen and shipped to Astute Medical, Inc.

[0126]   Example 3:   Acutely ill subject sample collection

[0127]   The objective of this study is to collect samples from acutely ill patients.

Approximately 900 adults expected to be in the ICU for at least 48 hours will be enrolled.

To be enrolled in the study, each patient must meet all of the following inclusion criteria

and none of the following exclusion criteria:

Inclusion Criteria

males and females 18 years of age or older;

Study population 1: approximately 300 patients that have at least one of:

shock (SBP < 90 mmHg and/or need for vasopressor support to maintain MAP > 60

mmHg and/or documented drop in SBP of at least 40 mmHg); and

sepsis;

Study population 2: approximately 300 patients that have at least one of:

IV antibiotics ordered in computerized physician order entry (CPOE) within 24 hours of enrollment;

contrast media exposure within 24 hours of enrollment;

increased Intra-Abdominal Pressure with acute decompensated heart failure; and

severe trauma as the primary reason for ICU admission and likely to be hospitalized in the ICU for 48 hours after enrollment;

Study population 3: approximately 300 patients expected to be hospitalized through acute care setting (ICU or ED) with a known risk factor for acute renal injury (*e.g.* sepsis, hypotension/shock (Shock = systolic BP < 90 mmHg and/or the need for vasopressor support to maintain a MAP > 60 mmHg and/or a documented drop in SBP > 40 mmHg), major trauma, hemorrhage, or major surgery); and/or expected to be hospitalized to the ICU for at least 24 hours after enrollment.

Exclusion Criteria

known pregnancy;

institutionalized individuals;

previous renal transplantation;

known acutely worsening renal function prior to enrollment (e.g., any category of RIFLE criteria);

received dialysis (either acute or chronic) within 5 days prior to enrollment or in imminent need of dialysis at the time of enrollment;

known infection with human immunodeficiency virus (HIV) or a hepatitis virus;

meets only the SBP < 90 mmHg inclusion criterion set forth above, and does not have shock in the attending physician's or principal investigator's opinion.

[0128]   After providing informed consent, an EDTA anti-coagulated blood sample (10 mL) and a urine sample (25-30 mL) are collected from each patient. Blood and urine samples are then collected at 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), and 48 (± 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Blood is collected via direct venipuncture or via other available venous access, such as an existing femoral sheath, central venous line, peripheral intravenous line or hep-lock. These study blood samples

are processed to plasma at the clinical site, frozen and shipped to Astute Medical, Inc., San Diego, CA. The study urine samples are frozen and shipped to Astute Medical, Inc.

[0129]   Example 4.   Immunoassay format

[0130]   Analytes are measured using standard sandwich enzyme immunoassay techniques. A first antibody which binds the analyte is immobilized in wells of a 96 well polystyrene microplate. Analyte standards and test samples are pipetted into the appropriate wells and any analyte present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase-conjugated second antibody which binds the analyte is added to the wells, thereby forming sandwich complexes with the analyte (if present) and the first antibody. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution comprising tetramethylbenzidine and hydrogen peroxide is added to the wells. Color develops in proportion to the amount of analyte present in the sample. The color development is stopped and the intensity of the color is measured at 540 nm or 570 nm. An analyte concentration is assigned to the test sample by comparison to a standard curve determined from the analyte standards. Concentrations reported below are as follows: Cathepsin B ng/mL; Renin pg/mL; Dipeptidyl Peptidase IV (soluble form) ng/mL; Neprilysin (soluble form) ng/mL; Beta-2-microglobulin μg/mL; Carbonic anhydrase IX (soluble form) ng/mL; and C-X-C motif chemokine 2 pg/mL.

[0131]   Example 5.   Apparently Healthy Donor and Chronic Disease Patient Samples

[0132]   Human urine samples from donors with no known chronic or acute disease ("Apparently Healthy Donors") were purchased from two vendors (Golden West Biologicals, Inc., 27625 Commerce Center Dr., Temecula, CA 92590 and Virginia Medical Research, Inc., 915 First Colonial Rd., Virginia Beach, VA 23454). The urine samples were shipped and stored frozen at less than -20° C. The vendors supplied demographic information for the individual donors including gender, race (Black /White), smoking status and age.

[0133]   Human urine samples from donors with various chronic diseases ("Chronic Disease Patients") including congestive heart failure, coronary artery disease, chronic kidney disease, chronic obstructive pulmonary disease, diabetes mellitus and hypertension were purchased from Virginia Medical Research, Inc., 915 First Colonial

Rd., Virginia Beach, VA 23454. The urine samples were shipped and stored frozen at less than -20 degrees centigrade. The vendor provided a case report form for each individual donor with age, gender, race (Black/White), smoking status and alcohol use, height, weight, chronic disease(s) diagnosis, current medications and previous surgeries.

[0134]   Example 6.   Use of Kidney Injury Markers for evaluating renal status in patients

[0135]   Patients from the intensive care unit (ICU) were enrolled in the following study. Each patient was classified by kidney status as non-injury (0), risk of injury (R), injury (I), and failure (F) according to the maximum stage reached within 7 days of enrollment as determined by the RIFLE criteria. EDTA anti-coagulated blood samples (10 mL) and a urine samples (25-30 mL) were collected from each patient at enrollment, 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), and 48 (± 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Markers were each measured by standard immunoassay methods using commercially available assay reagents in the urine samples and the plasma component of the blood samples collected.

[0136]   Two cohorts were defined to represent a "diseased" and a "normal" population. While these terms are used for convenience, "diseased" and "normal" simply represent two cohorts for comparison (say RIFLE 0 vs RIFLE R, I and F; RIFLE 0 vs RIFLE R; RIFLE 0 and R vs RIFLE I and F; etc.). The time "prior max stage" represents the time at which a sample is collected, relative to the time a particular patient reaches the lowest disease stage as defined for that cohort, binned into three groups which are +/- 12 hours. For example, "24 hr prior" which uses 0 vs R, I, F as the two cohorts would mean 24 hr (+/- 12 hours) prior to reaching stage R (or I if no sample at R, or F if no sample at R or I).

[0137]   A receiver operating characteristic (ROC) curve was generated for each biomarker measured and the area under each ROC curve (AUC) is determined. Patients in Cohort 2 were also separated according to the reason for adjudication to cohort 2 as being based on serum creatinine measurements (sCr), being based on urine output (UO), or being based on either serum creatinine measurements or urine output. Using the same example discussed above (0 vs R, I, F), for those patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements alone, the stage 0 cohort may include

patients adjudicated to stage R, I, or F on the basis of urine output; for those patients adjudicated to stage R, I, or F on the basis of urine output alone, the stage 0 cohort may include patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements; and for those patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements or urine output, the stage 0 cohort contains only patients in stage 0 for both serum creatinine measurements and urine output. Also, in the data for patients adjudicated on the basis of serum creatinine measurements or urine output, the adjudication method which yielded the most severe RIFLE stage is used.

[0138]   The ability to distinguish cohort 1 from Cohort 2 was determined using ROC analysis. SE is the standard error of the AUC, n is the number of sample or individual patients ("pts," as indicated). Standard errors are calculated as described in Hanley, J. A., and McNeil, B.J., The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology (1982) 143: 29-36; p values are calculated with a two-tailed Z-test. An AUC < 0.5 is indicative of a negative going marker for the comparison, and an AUC > 0.5 is indicative of a positive going marker for the comparison.

[0139]   Various threshold (or "cutoff") concentrations were selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 are determined. OR is the odds ratio calculated for the particular cutoff concentration, and 95% CI is the confidence interval for the odds ratio.

[0140]   Table 1: Comparison of marker levels in urine samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0) and in urine samples collected from subjects at 0, 24 hours, and 48 hours prior to reaching stage R, I or F in Cohort 2.

Beta-2-microglobulin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1.44	4.76	1.44	2.61	1.44	4.70
Average	2.57	3.52	2.57	2.83	2.57	3.36
Stdev	2.43	2.58	2.43	2.53	2.43	2.39
p(t-test)		0.014		0.48		0.11
Min	0.00152	0.00403	0.00152	0.00212	0.00152	0.0378
Max	6.10	6.10	6.10	6.07	6.10	5.80
n (Samp)	255	48	255	57	255	27
n (Patient)	103	48	103	57	103	27

sCr only	0hr prior to AKI stage	24hr prior to AKI stage	48hr prior to AKI stage

	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1.43	0.462	1.43	0.763	1.43	1.24
Average	2.59	2.48	2.59	2.51	2.59	2.07
Stdev	2.45	2.78	2.45	2.68	2.45	2.33
p(t-test)		0.86		0.89		0.45
Min	0.00152	0.00925	0.00152	0.0114	0.00152	0.0324
Max	6.10	6.10	6.10	5.80	6.10	5.80
n (Samp)	447	16	447	21	447	13
n (Patient)	170	16	170	21	170	13

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1.39	4.57	1.39	2.61	1.39	4.82
Average	2.56	3.58	2.56	2.90	2.56	3.51
Stdev	2.44	2.45	2.44	2.50	2.44	2.41
p(t-test)		0.010		0.37		0.064
Min	0.00152	0.00403	0.00152	0.00212	0.00152	0.0378
Max	6.10	6.10	6.10	6.07	6.10	5.80
n (Samp)	218	46	218	51	218	25
n (Patient)	87	46	87	51	87	25

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.59	0.45	0.60	0.51	0.46	0.51	0.57	0.45	0.58
SE	0.046	0.075	0.048	0.043	0.066	0.045	0.060	0.083	0.063
p	0.044	0.53	0.031	0.83	0.59	0.79	0.25	0.55	0.19
nCohort 1	255	447	218	255	447	218	255	447	218
nCohort 2	48	16	46	57	21	51	27	13	25
Cutoff 1	0.734	0.0999	1.48	0.278	0.153	0.278	1.22	0.171	1.02
Sens 1	71%	75%	72%	70%	71%	71%	70%	77%	72%
Spec 1	40%	17%	51%	27%	21%	27%	48%	23%	46%
Cutoff 2	0.0999	0.0513	0.278	0.0999	0.0851	0.152	0.213	0.0743	0.354
Sens 2	81%	81%	80%	81%	81%	80%	81%	85%	80%
Spec 2	14%	11%	27%	14%	15%	19%	24%	14%	31%
Cutoff 3	0.0273	0.0197	0.0298	0.0246	0.0372	0.0168	0.0683	0.0683	0.141
Sens 3	92%	94%	91%	91%	90%	90%	93%	92%	92%
Spec 3	4%	4%	5%	3%	8%	2%	10%	13%	18%
Cutoff 4	5.08	5.13	5.16	5.08	5.13	5.16	5.08	5.13	5.16
Sens 4	48%	38%	43%	37%	38%	37%	41%	23%	36%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	5.80	5.80	5.80	5.80	5.80	5.80	5.80	5.80	5.80
Sens 5	17%	6%	17%	2%	0%	2%	0%	0%	0%
Spec 5	95%	95%	94%	95%	95%	94%	95%	95%	94%
Cutoff 6	5.80	5.80	5.80	5.80	5.80	5.80	5.80	5.80	5.80
Sens 6	17%	6%	17%	2%	0%	2%	0%	0%	0%
Spec 6	95%	95%	94%	95%	95%	94%	95%	95%	94%
OR Quart 2	0.41	0.39	0.52	0.57	0.49	0.51	0.47	1.0	0.57
p Value	0.12	0.27	0.26	0.20	0.32	0.17	0.30	1.0	0.45
95% CI of	0.14	0.074	0.16	0.24	0.12	0.20	0.11	0.20	0.13

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
OR Quart2	1.2	2.0	1.6	1.3	2.0	1.3	2.0	5.1	2.5
OR Quart 3	1.1	0.59	2.2	0.85	0.83	1.4	1.8	1.0	1.9
p Value	0.85	0.48	0.084	0.68	0.76	0.42	0.29	1.0	0.28
95% CI of	0.45	0.14	0.90	0.38	0.24	0.63	0.61	0.20	0.60
OR Quart3	2.7	2.5	5.4	1.9	2.8	3.1	5.2	5.1	6.1
OR Quart 4	2.1	1.2	1.9	1.1	1.2	0.73	1.4	1.3	1.7
p Value	0.080	0.75	0.18	0.84	0.78	0.48	0.59	0.70	0.40
95% CI of	0.92	0.36	0.75	0.50	0.38	0.30	0.44	0.29	0.51
OR Quart4	4.7	4.1	4.6	2.3	3.6	1.8	4.1	6.2	5.4

C-X-C motif chemokine 2

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.135	2.51	0.135	4.01	0.135	0.0260
Average	8.01	11.4	8.01	12.4	8.01	7.85
Stdev	26.5	27.8	26.5	30.9	26.5	19.6
p(t-test)		0.32		0.17		0.97
Min	0.00804	0.0260	0.00804	0.0260	0.00804	0.0260
Max	266	217	266	217	266	111
n (Samp)	360	75	360	91	360	43
n (Patient)	190	75	190	91	190	43

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.536	2.64	0.536	4.37	0.536	3.34
Average	8.92	17.6	8.92	17.4	8.92	6.53
Stdev	25.5	42.4	25.5	40.9	25.5	8.89
p(t-test)		0.083		0.058		0.65
Min	0.00804	0.0260	0.00804	0.0260	0.00804	0.0260
Max	266	217	266	217	266	36.2
n (Samp)	755	29	755	37	755	23
n (Patient)	295	29	295	37	295	23

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.262	2.64	0.262	5.28	0.262	0.0260
Average	7.67	12.4	7.67	14.9	7.67	9.52
Stdev	25.1	29.6	25.1	33.2	25.1	21.4
p(t-test)		0.18		0.035		0.67
Min	0.00804	0.0260	0.00804	0.0260	0.00804	0.0260
Max	266	217	266	217	266	111
n (Samp)	315	65	315	78	315	36
n (Patient)	134	65	134	78	134	36

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.61	0.61	0.62	0.62	0.61	0.64	0.49	0.57	0.49
SE	0.037	0.057	0.040	0.034	0.050	0.037	0.047	0.063	0.051
p	0.0039	0.049	0.0025	5.8E-4	0.025	9.7E-5	0.82	0.27	0.83
nCohort 1	360	755	315	360	755	315	360	755	315
nCohort 2	75	29	65	91	37	78	43	23	36
Cutoff 1	0.00804	0.244	0.299	0.00804	0.00804	0.299	0.00804	0.00804	0.00804
Sens 1	100%	72%	71%	100%	100%	71%	100%	100%	100%
Spec 1	0%	46%	51%	0%	0%	51%	0%	0%	0%
Cutoff 2	0.00804	0.00804	0.00804	0.00804	0.00804	0.00804	0.00804	0.00804	0.00804
Sens 2	100%	100%	100%	100%	100%	100%	100%	100%	100%
Spec 2	0%	0%	0%	0%	0%	0%	0%	0%	0%
Cutoff 3	0.00804	0.00804	0.00804	0.00804	0.00804	0.00804	0.00804	0.00804	0.00804
Sens 3	100%	100%	100%	100%	100%	100%	100%	100%	100%
Spec 3	0%	0%	0%	0%	0%	0%	0%	0%	0%
Cutoff 4	3.08	3.96	3.82	3.08	3.96	3.82	3.08	3.96	3.82
Sens 4	45%	38%	45%	52%	54%	55%	37%	43%	28%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	6.49	7.98	7.00	6.49	7.98	7.00	6.49	7.98	7.00
Sens 5	36%	31%	38%	37%	35%	40%	23%	30%	25%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	15.4	20.2	14.9	15.4	20.2	14.9	15.4	20.2	14.9
Sens 6	23%	21%	25%	19%	22%	24%	19%	4%	25%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	0.14	>9.4	0.25	>44	>13	3.1	0.89	>9.4	0.76
p Value	4.8E-4	<0.034	0.010	<2.2E-4	<0.015	0.015	0.81	<0.034	0.60
95% CI of OR Quart2	0.047 0.42	>1.2 na	0.090 0.72	>5.9 na	>1.6 na	1.2 7.8	0.35 2.3	>1.2 na	0.27 2.1
OR Quart 3	0.78	>12	1.1	>30	>11	3.3	0.78	>5.1	0.10
p Value	0.47	<0.019	0.85	<9.4E-4	<0.025	0.0097	0.62	<0.14	0.031
95% CI of OR Quart3	0.40 1.5	>1.5 na	0.51 2.2	>4.0 na	>1.3 na	1.3 8.3	0.30 2.1	>0.59 na	0.012 0.81
OR Quart 4	1.3	>9.4	1.6	>50	>16	6.2	1.7	>9.4	2.5
p Value	0.36	<0.034	0.16	<1.3E-4	<0.0073	4.4E-5	0.20	<0.034	0.040
95% CI of OR Quart4	0.72 2.5	>1.2 na	0.82 3.3	>6.7 na	>2.1 na	2.6 15	0.75 4.0	>1.2 na	1.0 5.8

Renin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	319	494	319	483	319	323
Average	750	886	750	1350	750	1120
Stdev	1270	1110	1270	3110	1270	1660
p(t-test)		0.53		0.073		0.20
Min	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9
Max	9810	5150	9810	21100	9810	5550
n (Samp)	121	45	121	50	121	26
n (Patient)	98	45	98	50	98	26

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	394	254	394	565	394	304
Average	978	1150	978	1340	978	985
Stdev	1840	1760	1840	1670	1840	1460
p(t-test)		0.75		0.40		0.99
Min	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9
Max	21100	4570	21100	5380	21100	4250
n (Samp)	259	12	259	19	259	13
n (Patient)	159	12	159	19	159	13

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	297	638	297	606	297	385
Average	758	996	758	1540	758	1290
Stdev	1340	1100	1340	3230	1340	1710
p(t-test)		0.31		0.035		0.10
Min	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9
Max	9810	5150	9810	21100	9810	5550
n (Samp)	106	42	106	46	106	23
n (Patient)	82	42	82	46	82	23

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.58	0.46	0.64	0.57	0.56	0.63	0.53	0.47	0.59
SE	0.051	0.087	0.052	0.049	0.070	0.051	0.063	0.083	0.068
p	0.12	0.65	0.0072	0.13	0.42	0.012	0.59	0.76	0.21
nCohort 1	121	259	106	121	259	106	121	259	106
nCohort 2	45	12	42	50	19	46	26	13	23
Cutoff 1	234	161	279	203	84.2	211	123	137	123
Sens 1	71%	75%	71%	70%	74%	72%	73%	77%	74%
Spec 1	40%	29%	46%	38%	20%	41%	31%	28%	32%
Cutoff 2	177	34.5	224	111	34.5	134	10.6	0	10.6
Sens 2	80%	83%	81%	80%	84%	80%	81%	100%	83%
Spec 2	36%	15%	41%	30%	15%	33%	21%	0%	20%
Cutoff 3	10.1	0	131	65.6	0	88.6	0	0	7.32
Sens 3	91%	100%	90%	90%	100%	91%	100%	100%	91%
Spec 3	21%	0%	33%	27%	0%	28%	0%	0%	18%
Cutoff 4	606	881	600	606	881	600	606	881	600
Sens 4	44%	25%	52%	42%	42%	50%	31%	31%	39%
Spec 4	70%	70%	71%	70%	70%	71%	70%	70%	71%
Cutoff 5	1130	1590	1130	1130	1590	1130	1130	1590	1130
Sens 5	22%	25%	29%	30%	37%	35%	31%	23%	39%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	1990	2390	2060	1990	2390	2060	1990	2390	2060
Sens 6	13%	25%	14%	16%	16%	22%	23%	15%	22%
Spec 6	90%	90%	91%	90%	90%	91%	90%	90%	91%
OR Quart 2	2.9	0.66	6.1	2.1	0.15	2.5	0.97	0.66	0.56
p Value	0.052	0.65	0.0089	0.16	0.085	0.11	0.96	0.65	0.45

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
95% CI of	0.99	0.11	1.6	0.75	0.018	0.81	0.28	0.11	0.12
OR Quart2	8.6	4.1	24	5.6	1.3	7.5	3.3	4.1	2.6
OR Quart 3	2.1	1.4	5.4	1.6	0.65	2.5	0.97	1.7	1.2
p Value	0.18	0.70	0.015	0.34	0.51	0.11	0.96	0.47	0.74
95% CI of	0.71	0.29	1.4	0.59	0.17	0.81	0.28	0.39	0.34
OR Quart3	6.5	6.3	21	4.6	2.4	7.5	3.3	7.5	4.6
OR Quart 4	2.9	1.0	6.9	2.5	1.4	3.9	1.4	1.0	2.0
p Value	0.052	0.99	0.0052	0.067	0.59	0.014	0.59	1.0	0.26
95% CI of	0.99	0.20	1.8	0.94	0.44	1.3	0.43	0.19	0.60
OR Quart4	8.6	5.2	27	6.8	4.1	11	4.5	5.1	6.9

Cathepsin B

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.504	0.628	0.504	1.15	0.504	0.649
Average	2.26	1.84	2.26	5.00	2.26	4.06
Stdev	8.48	2.68	8.48	11.8	8.48	15.2
p(t-test)		0.74		0.084		0.40
Min	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9
Max	72.4	11.0	72.4	64.9	72.4	78.1
n (Samp)	131	47	131	50	131	26
n (Patient)	102	47	102	50	102	26

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.657	0.485	0.657	1.92	0.657	0.338
Average	2.89	1.92	2.89	4.95	2.89	1.27
Stdev	9.57	2.68	9.57	7.48	9.57	1.85
p(t-test)		0.71		0.36		0.55
Min	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9	0.0682
Max	78.1	7.84	78.1	29.7	78.1	6.19
n (Samp)	269	14	269	19	269	13
n (Patient)	162	14	162	19	162	13

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.539	0.951	0.539	1.32	0.539	0.846
Average	2.37	2.21	2.37	5.79	2.37	5.24
Stdev	8.90	2.91	8.90	12.6	8.90	16.1
p(t-test)		0.91		0.052		0.23
Min	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9
Max	72.4	11.6	72.4	64.9	72.4	78.1
n (Samp)	118	44	118	46	118	23
n (Patient)	87	44	87	46	87	23

	0hr prior to AKI stage	24hr prior to AKI stage	48hr prior to AKI stage

	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.56	0.48	0.62	0.63	0.67	0.65	0.50	0.46	0.56
SE	0.050	0.080	0.051	0.048	0.070	0.050	0.062	0.084	0.067
p	0.25	0.83	0.021	0.0072	0.015	0.0032	0.98	0.64	0.41
nCohort 1	131	269	118	131	269	118	131	269	118
nCohort 2	47	14	44	50	19	46	26	13	23
Cutoff 1	0.339	0.142	0.546	0.665	0.797	0.665	0.185	0.265	0.185
Sens 1	70%	71%	70%	70%	74%	72%	73%	77%	74%
Spec 1	40%	18%	51%	58%	55%	56%	21%	28%	21%
Cutoff 2	0.140	0.0513	0.309	0.245	0.265	0.268	0.105	0.128	0.0764
Sens 2	81%	86%	82%	80%	84%	80%	81%	85%	83%
Spec 2	18%	14%	37%	30%	28%	31%	17%	17%	12%
Cutoff 3	0	0	0.0764	0	0	0	0	0.0901	0
Sens 3	100%	100%	91%	100%	100%	100%	100%	92%	100%
Spec 3	0%	0%	12%	0%	0%	0%	0%	15%	0%
Cutoff 4	1.16	1.35	1.17	1.16	1.35	1.17	1.16	1.35	1.17
Sens 4	36%	36%	45%	50%	63%	54%	27%	23%	39%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	1.81	2.07	2.02	1.81	2.07	2.02	1.81	2.07	2.02
Sens 5	28%	36%	30%	38%	42%	41%	19%	23%	30%
Spec 5	80%	80%	81%	80%	80%	81%	80%	80%	81%
Cutoff 6	3.53	4.65	3.53	3.53	4.65	3.53	3.53	4.65	3.53
Sens 6	19%	14%	20%	26%	26%	28%	15%	8%	30%
Spec 6	90%	90%	91%	90%	90%	91%	90%	90%	91%
OR Quart 2	0.85	0.19	2.9	0.44	1.5	0.49	1.7	1.0	0.24
p Value	0.75	0.13	0.071	0.16	0.65	0.25	0.36	0.99	0.092
95% CI of OR Quart2	0.31 2.3	0.021 1.7	0.91 9.2	0.14 1.4	0.25 9.4	0.15 1.6	0.54 5.3	0.20 5.2	0.047 1.3
OR Quart 3	1.4	0.38	3.0	1.9	1.5	1.8	0.47	1.4	1.0
p Value	0.47	0.26	0.062	0.17	0.65	0.22	0.31	0.70	1.0
95% CI of OR Quart3	0.55 3.7	0.072 2.0	0.94 9.5	0.76 4.9	0.25 9.4	0.69 4.9	0.11 2.0	0.29 6.3	0.31 3.2
OR Quart 4	1.7	1.2	4.0	2.5	6.3	2.8	1.5	1.0	0.97
p Value	0.27	0.74	0.016	0.054	0.019	0.037	0.52	0.99	0.95
95% CI of OR Quart4	0.66 4.3	0.36 4.3	1.3 13	0.99 6.2	1.3 30	1.1 7.3	0.46 4.7	0.20 5.2	0.30 3.1

Neprilysin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1.26	0.949	1.26	1.03	nd	nd
Average	4.09	2.29	4.09	5.48	nd	nd
Stdev	8.95	3.70	8.95	13.1	nd	nd
p(t-test)		0.40		0.60	nd	nd
Min	0.0532	0.0313	0.0532	0.0902	nd	nd
Max	51.0	16.0	51.0	46.3	nd	nd
n (Samp)	62	19	62	19	nd	nd
n (Patient)	50	19	50	19	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.963	0.949	0.963	0.896	nd	nd
Average	2.98	2.47	2.98	5.23	nd	nd
Stdev	6.67	4.11	6.67	12.8	nd	nd
p(t-test)		0.78		0.33	nd	nd
Min	0.0532	0.0313	0.0532	0.0203	nd	nd
Max	35.3	16.0	35.3	46.3	nd	nd
n (Samp)	52	15	52	20	nd	nd
n (Patient)	41	15	41	20	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.43	nd	0.47	0.47	nd	0.50	nd	nd	nd
SE	0.077	nd	0.086	0.077	nd	0.077	nd	nd	nd
p	0.38	nd	0.69	0.74	nd	0.99	nd	nd	nd
nCohort 1	62	nd	52	62	nd	52	nd	nd	nd
nCohort 2	19	nd	15	19	nd	20	nd	nd	nd
Cutoff 1	0.279	nd	0.376	0.586	nd	0.690	nd	nd	nd
Sens 1	74%	nd	73%	74%	nd	70%	nd	nd	nd
Spec 1	8%	nd	15%	23%	nd	31%	nd	nd	nd
Cutoff 2	0.238	nd	0.251	0.351	nd	0.461	nd	nd	nd
Sens 2	84%	nd	80%	84%	nd	80%	nd	nd	nd
Spec 2	8%	nd	8%	15%	nd	21%	nd	nd	nd
Cutoff 3	0.117	nd	0.0869	0.178	nd	0.306	nd	nd	nd
Sens 3	95%	nd	93%	95%	nd	90%	nd	nd	nd
Spec 3	5%	nd	4%	6%	nd	10%	nd	nd	nd
Cutoff 4	1.99	nd	1.45	1.99	nd	1.45	nd	nd	nd
Sens 4	37%	nd	33%	21%	nd	35%	nd	nd	nd
Spec 4	71%	nd	71%	71%	nd	71%	nd	nd	nd
Cutoff 5	3.50	nd	2.26	3.50	nd	2.26	nd	nd	nd
Sens 5	21%	nd	33%	16%	nd	15%	nd	nd	nd
Spec 5	81%	nd	81%	81%	nd	81%	nd	nd	nd
Cutoff 6	10.1	nd	4.43	10.1	nd	4.43	nd	nd	nd
Sens 6	5%	nd	13%	11%	nd	10%	nd	nd	nd
Spec 6	90%	nd	90%	90%	nd	90%	nd	nd	nd
OR Quart 2	0.44	nd	0.51	1.4	nd	1.8	nd	nd	nd
p Value	0.30	nd	0.42	0.65	nd	0.46	nd	nd	nd
95% CI of OR Quart2	0.094 2.1	nd	0.10 2.6	0.32 6.3	nd	0.40 7.7	nd	nd	nd
OR Quart 3	0.44	nd	0.32	1.1	nd	1.3	nd	nd	nd
p Value	0.30	nd	0.22	0.94	nd	0.70	nd	nd	nd
95% CI of OR Quart3	0.094 2.1	nd	0.053 1.9	0.23 5.0	nd	0.30 6.1	nd	nd	nd
OR Quart 4	1.3	nd	1.1	1.8	nd	1.3	nd	nd	nd
p Value	0.66	nd	0.91	0.42	nd	0.70	nd	nd	nd
95% CI of OR Quart4	0.36 5.0	nd	0.25 4.8	0.43 7.8	nd	0.30 6.1	nd	nd	nd

Carbonic anhydrase IX

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.00525	0.00780	0.00525	0.0170	nd	nd
Average	0.0125	0.0158	0.0125	0.0706	nd	nd
Stdev	0.0182	0.0196	0.0182	0.193	nd	nd
p(t-test)		0.51		0.020	nd	nd
Min	1.00E-9	0.000522	1.00E-9	0.00216	nd	nd
Max	0.119	0.0760	0.119	0.859	nd	nd
n (Samp)	62	19	62	19	nd	nd
n (Patient)	50	19	50	19	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.00432	0.0111	0.00432	0.0204	nd	nd
Average	0.0112	0.0178	0.0112	0.0693	nd	nd
Stdev	0.0189	0.0211	0.0189	0.188	nd	nd
p(t-test)		0.25		0.029	nd	nd
Min	1.00E-9	0.000522	1.00E-9	0.00216	nd	nd
Max	0.119	0.0760	0.119	0.859	nd	nd
n (Samp)	52	15	52	20	nd	nd
n (Patient)	41	15	41	20	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.54	nd	0.63	0.73	nd	0.81	nd	nd	nd
SE	0.077	nd	0.085	0.072	nd	0.063	nd	nd	nd
p	0.60	nd	0.12	0.0017	nd	8.1E-7	nd	nd	nd
nCohort 1	62	nd	52	62	nd	52	nd	nd	nd
nCohort 2	19	nd	15	19	nd	20	nd	nd	nd
Cutoff 1	0.00269	nd	0.00444	0.0102	nd	0.0158	nd	nd	nd
Sens 1	74%	nd	73%	74%	nd	70%	nd	nd	nd
Spec 1	26%	nd	52%	61%	nd	83%	nd	nd	nd
Cutoff 2	0.00187	nd	0.00269	0.00548	nd	0.0114	nd	nd	nd
Sens 2	84%	nd	80%	84%	nd	80%	nd	nd	nd
Spec 2	15%	nd	35%	52%	nd	71%	nd	nd	nd
Cutoff 3	0.000712	nd	0.00105	0.00337	nd	0.00548	nd	nd	nd
Sens 3	95%	nd	93%	95%	nd	90%	nd	nd	nd
Spec 3	6%	nd	13%	32%	nd	56%	nd	nd	nd
Cutoff 4	0.0121	nd	0.0114	0.0121	nd	0.0114	nd	nd	nd
Sens 4	42%	nd	47%	58%	nd	80%	nd	nd	nd
Spec 4	71%	nd	71%	71%	nd	71%	nd	nd	nd
Cutoff 5	0.0183	nd	0.0143	0.0183	nd	0.0143	nd	nd	nd
Sens 5	32%	nd	47%	42%	nd	75%	nd	nd	nd
Spec 5	81%	nd	81%	81%	nd	81%	nd	nd	nd
Cutoff 6	0.0293	nd	0.0212	0.0293	nd	0.0212	nd	nd	nd
Sens 6	16%	nd	20%	26%	nd	50%	nd	nd	nd
Spec 6	90%	nd	90%	90%	nd	90%	nd	nd	nd
OR Quart 2	1.0	nd	1.5	3.4	nd	2.1	nd	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
p Value	1.0	nd	0.68	0.31	nd	0.55	nd	nd	nd
95% CI of	0.21	nd	0.22	0.32	nd	0.18	nd	nd	nd
OR Quart2	4.7	nd	10	35	nd	26	nd	nd	nd
OR Quart 3	1.3	nd	1.5	10	nd	8.5	nd	nd	nd
p Value	0.71	nd	0.68	0.039	nd	0.061	nd	nd	nd
95% CI of	0.30	nd	0.22	1.1	nd	0.90	nd	nd	nd
OR Quart3	5.9	nd	10	93	nd	80	nd	nd	nd
OR Quart 4	1.6	nd	4.9	12	nd	27	nd	nd	nd
p Value	0.52	nd	0.078	0.028	nd	0.0039	nd	nd	nd
95% CI of	0.38	nd	0.84	1.3	nd	2.9	nd	nd	nd
OR Quart4	6.8	nd	29	110	nd	250	nd	nd	nd

Dipeptidyl peptidase IV

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	147	435	147	237	147	239
Average	7480	1090	7480	423	7480	852
Stdev	67000	2170	67000	468	67000	1110
p(t-test)		0.60		0.53		0.64
Min	0.200	0.899	0.200	3.65	0.200	2.62
Max	677000	11000	677000	1480	677000	4320
n (Samp)	102	31	102	36	102	22
n (Patient)	82	31	82	36	82	22

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	266	147	266	130	266	96.7
Average	4260	927	4260	672	4260	1030
Stdev	47800	2100	47800	1270	47800	1670
p(t-test)		0.82		0.80		0.84
Min	0.200	6.52	0.200	18.2	0.200	10.1
Max	677000	7150	677000	4460	677000	4950
n (Samp)	201	11	201	12	201	9
n (Patient)	131	11	131	12	131	9

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	121	577	121	312	121	328
Average	536	1260	536	463	536	888
Stdev	1240	2270	1240	451	1240	1120
p(t-test)		0.030		0.74		0.24
Min	0.200	0.899	0.200	3.65	0.200	2.62
Max	9980	11000	9980	1410	9980	4320
n (Samp)	93	28	93	35	93	20
n (Patient)	71	28	71	35	71	20

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.57	0.48	0.63	0.53	0.49	0.60	0.61	0.53	0.65
SE	0.060	0.091	0.063	0.057	0.086	0.058	0.069	0.100	0.072
p	0.23	0.79	0.038	0.60	0.91	0.086	0.12	0.79	0.038
nCohort 1	102	201	93	102	201	93	102	201	93
nCohort 2	31	11	28	36	12	35	22	9	20
Cutoff 1	58.9	58.9	62.9	58.9	52.2	104	93.8	78.9	95.2
Sens 1	71%	73%	71%	72%	75%	71%	73%	78%	70%
Spec 1	37%	32%	39%	37%	31%	49%	44%	35%	47%
Cutoff 2	20.7	52.2	19.1	36.5	42.6	52.2	83.4	52.2	83.4
Sens 2	81%	82%	82%	81%	83%	80%	82%	89%	80%
Spec 2	24%	31%	23%	29%	29%	35%	41%	31%	44%
Cutoff 3	6.52	12.4	6.36	9.55	36.5	9.55	42.6	9.55	42.6
Sens 3	90%	91%	93%	92%	92%	91%	91%	100%	90%
Spec 3	12%	15%	12%	15%	26%	15%	34%	13%	33%
Cutoff 4	538	677	407	538	677	407	538	677	407
Sens 4	48%	27%	61%	33%	25%	40%	36%	33%	45%
Spec 4	71%	70%	71%	71%	70%	71%	71%	70%	71%
Cutoff 5	1110	1110	698	1110	1110	698	1110	1110	698
Sens 5	23%	18%	43%	11%	17%	26%	32%	33%	40%
Spec 5	80%	80%	81%	80%	80%	81%	80%	80%	81%
Cutoff 6	1960	1990	1560	1960	1990	1560	1960	1990	1560
Sens 6	13%	9%	18%	0%	8%	0%	14%	11%	25%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	0.83	1.0	0.23	1.9	1.0	0.66	4.2	5.3	2.8
p Value	0.76	1.0	0.088	0.29	0.98	0.52	0.089	0.13	0.24
95% CI of OR Quart2	0.24 2.8	0.14 7.4	0.044 1.2	0.59 5.9	0.14 7.5	0.19 2.4	0.80 22	0.60 47	0.50 16
OR Quart 3	1.2	2.7	1.0	1.9	4.0	2.1	2.8	0	2.8
p Value	0.77	0.26	1.0	0.26	0.096	0.18	0.24	na	0.24
95% CI of OR Quart3	0.38 3.8	0.49 14	0.30 3.3	0.62 6.1	0.78 20	0.71 6.5	0.50 16	na na	0.50 16
OR Quart 4	1.5	1.0	2.1	1.9	0.50	1.9	5.0	3.1	5.0
p Value	0.44	1.0	0.20	0.29	0.58	0.27	0.054	0.34	0.058
95% CI of OR Quart4	0.51 4.7	0.14 7.4	0.68 6.3	0.59 5.9	0.044 5.7	0.62 5.7	0.98 26	0.31 30	0.95 26

[0141]   Table 2: Comparison of marker levels in urine samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0 or R) and in urine samples collected from subjects at 0, 24 hours, and 48 hours prior to reaching stage I or F in Cohort 2.

Beta-2-microglobulin

sCr or UO	0hr prior to AKI stage	24hr prior to AKI stage	48hr prior to AKI stage

	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1.75	3.29	1.75	0.538	1.75	0.362
Average	2.67	2.85	2.67	2.11	2.67	1.46
Stdev	2.47	2.38	2.47	2.56	2.47	1.95
p(t-test)		0.73		0.21		0.046
Min	0.00152	0.00181	0.00152	0.00212	0.00152	0.00546
Max	6.10	6.10	6.10	6.10	6.10	5.80
n (Samp)	421	25	421	33	421	17
n (Patient)	165	25	165	33	165	17

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	nd	nd	1.47	3.95	1.47	0.763
Average	nd	nd	2.57	3.27	2.57	1.33
Stdev	nd	nd	2.45	2.75	2.45	1.42
p(t-test)	nd	nd		0.43		0.18
Min	nd	nd	0.00152	0.0843	0.00152	0.0324
Max	nd	nd	6.10	5.80	6.10	3.49
n (Samp)	nd	nd	511	8	511	7
n (Patient)	nd	nd	198	8	198	7

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1.81	3.29	1.81	0.721	1.81	0.261
Average	2.73	2.89	2.73	1.92	2.73	1.84
Stdev	2.48	2.35	2.48	2.38	2.48	2.40
p(t-test)		0.74		0.093		0.15
Min	0.00152	0.00181	0.00152	0.00212	0.00152	0.00546
Max	6.10	6.10	6.10	6.10	6.10	5.80
n (Samp)	357	25	357	29	357	17
n (Patient)	135	25	135	29	135	17

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.52	nd	0.51	0.43	0.57	0.41	0.33	0.39	0.35
SE	0.060	nd	0.060	0.054	0.11	0.057	0.073	0.11	0.074
p	0.75	nd	0.84	0.21	0.48	0.11	0.024	0.35	0.040
nCohort 1	421	nd	357	421	511	357	421	511	357
nCohort 2	25	nd	25	33	8	29	17	7	17
Cutoff 1	0.363	nd	0.363	0.153	0.518	0.152	0.107	0.476	0.107
Sens 1	72%	nd	72%	73%	75%	72%	71%	71%	71%
Spec 1	33%	nd	31%	22%	37%	19%	17%	36%	15%
Cutoff 2	0.278	nd	0.278	0.0572	0.200	0.0497	0.0376	0.153	0.0351

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Sens 2	80%	nd	80%	82%	88%	83%	82%	86%	82%
Spec 2	29%	nd	27%	10%	26%	9%	7%	23%	7%
Cutoff 3	0.0351	nd	0.0351	0.0319	0.0830	0.00312	0.0124	0.0319	0.0124
Sens 3	92%	nd	92%	91%	100%	93%	94%	100%	94%
Spec 3	7%	nd	7%	6%	16%	1%	3%	7%	2%
Cutoff 4	5.48	nd	5.57	5.48	5.13	5.57	5.48	5.13	5.57
Sens 4	24%	nd	20%	30%	50%	24%	6%	0%	18%
Spec 4	70%	nd	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	5.80	nd	5.80	5.80	5.80	5.80	5.80	5.80	5.80
Sens 5	12%	nd	12%	6%	0%	7%	0%	0%	0%
Spec 5	94%	nd	94%	94%	94%	94%	94%	94%	94%
Cutoff 6	5.80	nd	5.80	5.80	5.80	5.80	5.80	5.80	5.80
Sens 6	12%	nd	12%	6%	0%	7%	0%	0%	0%
Spec 6	94%	nd	94%	94%	94%	94%	94%	94%	94%
OR Quart 2	1.8	nd	0.99	0.19	2.0	0.66	4.2	>2.0	2.1
p Value	0.37	nd	0.99	0.033	0.57	0.53	0.21	<0.56	0.41
95% CI of OR Quart2	0.51	nd	0.28	0.040	0.18	0.18	0.46	>0.18	0.37
	6.3	nd	3.5	0.88	22	2.4	38	na	12
OR Quart 3	2.6	nd	2.6	0.89	2.0	1.4	4.1	>3.1	1.5
p Value	0.11	nd	0.084	0.81	0.57	0.58	0.21	<0.33	0.65
95% CI of OR Quart3	0.80	nd	0.88	0.35	0.18	0.45	0.45	>0.32	0.25
	8.7	nd	7.7	2.3	22	4.1	37	na	9.3
OR Quart 4	0.99	nd	0.58	1.2	3.0	2.0	8.6	>2.0	4.3
p Value	0.99	nd	0.47	0.64	0.34	0.20	0.044	<0.56	0.069
95% CI of OR Quart4	0.24	nd	0.13	0.51	0.31	0.70	1.1	>0.18	0.89
	4.1	nd	2.5	3.0	29	5.5	70	na	21

C-X-C motif chemokine 2

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.485	1.87	0.485	6.90	0.485	0.889
Average	8.64	16.6	8.64	23.6	8.64	5.37
Stdev	24.6	39.6	24.6	44.3	24.6	7.31
p(t-test)		0.066		1.4E-4		0.48
Min	0.00804	0.0260	0.00804	0.0260	0.00804	0.0260
Max	266	217	266	217	266	20.9
n (Samp)	690	37	690	48	690	28
n (Patient)	279	37	279	48	279	28

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.687	11.6	0.687	15.2	0.687	3.34
Average	8.64	42.0	8.64	33.1	8.64	10.1
Stdev	24.0	69.5	24.0	58.6	24.0	12.5
p(t-test)		6.6E-5		4.4E-4		0.83
Min	0.00804	0.0260	0.00804	0.0260	0.00804	0.0260

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Max	266	217	266	217	266	31.3
n (Samp)	899	9	899	13	899	13
n (Patient)	335	9	335	13	335	13

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.671	1.65	0.671	6.97	0.671	2.64
Average	9.13	16.4	9.13	25.2	9.13	5.99
Stdev	24.9	40.7	24.9	46.4	24.9	7.43
p(t-test)		0.11		1.7E-4		0.53
Min	0.00804	0.0260	0.00804	0.0260	0.00804	0.0260
Max	266	217	266	217	266	20.9
n (Samp)	578	35	578	43	578	25
n (Patient)	205	35	205	43	205	25

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.58	0.72	0.55	0.68	0.73	0.68	0.52	0.58	0.55
SE	0.050	0.097	0.051	0.044	0.080	0.046	0.056	0.083	0.060
p	0.12	0.025	0.35	3.3E-5	0.0045	1.1E-4	0.73	0.34	0.38
nCohort 1	690	899	578	690	899	578	690	899	578
nCohort 2	37	9	35	48	13	43	28	13	25
Cutoff 1	0.00804	1.86	0.00804	1.45	5.86	1.45	0.00804	0.00804	0.00804
Sens 1	100%	78%	100%	71%	77%	72%	100%	100%	100%
Spec 1	0%	57%	0%	57%	73%	54%	0%	0%	0%
Cutoff 2	0.00804	0.00804	0.00804	0.121	0.00804	0.299	0.00804	0.00804	0.00804
Sens 2	100%	100%	100%	81%	100%	81%	100%	100%	100%
Spec 2	0%	0%	0%	45%	0%	46%	0%	0%	0%
Cutoff 3	0.00804	0.00804	0.00804	0.00804	0.00804	0.00804	0.00804	0.00804	0.00804
Sens 3	100%	100%	100%	100%	100%	100%	100%	100%	100%
Spec 3	0%	0%	0%	0%	0%	0%	0%	0%	0%
Cutoff 4	4.12	4.66	4.76	4.12	4.66	4.76	4.12	4.66	4.76
Sens 4	38%	67%	34%	56%	77%	56%	36%	46%	40%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	8.05	8.68	8.50	8.05	8.68	8.50	8.05	8.68	8.50
Sens 5	32%	56%	29%	48%	62%	49%	29%	46%	28%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	19.6	19.7	21.7	19.6	19.7	21.7	19.6	19.7	21.7
Sens 6	16%	44%	14%	31%	46%	30%	4%	23%	0%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	0.16	0	>15	>14	0	>12	0.16	0	0.24
p Value	0.016	na	<0.0087	<0.012	na	<0.019	0.016	na	0.073
95% CI of OR Quart2	0.035	na	>2.0	>1.8	na	>1.5	0.034	na	0.050
	0.71	na	na	na	na	na	0.71	na	1.1
OR Quart 3	0.82	0.50	>11	>11	0.66	>12	0.48	0.39	0.99
p Value	0.65	0.57	<0.025	<0.025	0.66	<0.019	0.15	0.27	0.99
95% CI of	0.34	0.045	>1.4	>1.3	0.11	>1.5	0.18	0.076	0.36

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
OR Quart3	1.9	5.5	na	na	4.0	na	1.3	2.1	2.7
OR Quart 4	1.1	3.1	>12	>29	2.7	>24	0.65	1.2	0.86
p Value	0.85	0.17	<0.019	<0.0011	0.14	<0.0020	0.35	0.76	0.78
95% CI of	0.48	0.61	>1.5	>3.9	0.71	>3.2	0.26	0.36	0.30
OR Quart4	2.4	15	na	na	10	na	1.6	4.0	2.4

Renin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	343	669	343	1180	343	380
Average	808	1130	808	2210	808	1220
Stdev	1290	1140	1290	3740	1290	1780
p(t-test)		0.25		3.1E-5		0.23
Min	1.00E-9	18.7	1.00E-9	1.00E-9	1.00E-9	1.00E-9
Max	9810	4050	9810	21100	9810	5550
n (Samp)	239	22	239	32	239	16
n (Patient)	157	22	157	32	157	16

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	nd	nd	394	1010	394	2150
Average	nd	nd	954	1350	954	2600
Stdev	nd	nd	1740	1410	1740	2150
p(t-test)	nd	nd		0.58		0.014
Min	nd	nd	1.00E-9	1.00E-9	1.00E-9	1.00E-9
Max	nd	nd	21100	3390	21100	5380
n (Samp)	nd	nd	305	6	305	7
n (Patient)	nd	nd	184	6	184	7

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	346	699	346	1180	346	435
Average	847	1160	847	2270	847	1220
Stdev	1350	1120	1350	3900	1350	1790
p(t-test)		0.30		1.3E-4		0.31
Min	1.00E-9	18.7	1.00E-9	1.00E-9	1.00E-9	1.00E-9
Max	9810	4050	9810	21100	9810	5550
n (Samp)	207	22	207	29	207	15
n (Patient)	130	22	130	29	130	15

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.64	nd	0.64	0.70	0.58	0.71	0.52	0.68	0.54
SE	0.066	nd	0.066	0.054	0.12	0.057	0.075	0.11	0.079
p	0.033	nd	0.030	1.6E-4	0.50	2.3E-4	0.82	0.10	0.63
nCohort 1	239	nd	207	239	305	207	239	305	207

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
nCohort 2	22	nd	22	32	6	29	16	7	15
Cutoff 1	337	nd	397	453	38.3	397	88.6	2070	134
Sens 1	73%	nd	73%	72%	83%	72%	75%	71%	73%
Spec 1	50%	nd	55%	57%	16%	55%	24%	88%	28%
Cutoff 2	266	nd	266	380	38.3	310	69.5	0	118
Sens 2	82%	nd	82%	81%	83%	83%	81%	100%	80%
Spec 2	44%	nd	43%	52%	16%	48%	23%	0%	25%
Cutoff 3	69.5	nd	69.5	96.1	0	125	0	0	69.5
Sens 3	91%	nd	91%	91%	100%	93%	100%	100%	93%
Spec 3	23%	nd	21%	25%	0%	26%	0%	0%	21%
Cutoff 4	730	nd	743	730	879	743	730	879	743
Sens 4	45%	nd	45%	66%	50%	66%	38%	71%	33%
Spec 4	70%	nd	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	1280	nd	1410	1280	1590	1410	1280	1590	1410
Sens 5	32%	nd	32%	47%	33%	45%	38%	71%	27%
Spec 5	80%	nd	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	2070	nd	2140	2070	2390	2140	2070	2390	2140
Sens 6	18%	nd	18%	38%	33%	38%	19%	43%	13%
Spec 6	90%	nd	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	1.4	nd	1.0	0.73	0	1.4	0.57	0	1.3
p Value	0.70	nd	1.0	0.68	na	0.70	0.46	na	0.72
95% CI of	0.29	nd	0.19	0.16	na	0.29	0.13	na	0.28
OR Quart2	6.3	nd	5.2	3.4	na	6.3	2.5	na	6.3
OR Quart 3	2.5	nd	2.9	2.1	0.49	3.4	0.37	0	1.0
p Value	0.20	nd	0.13	0.25	0.56	0.081	0.25	na	1.0
95% CI of	0.62	nd	0.74	0.60	0.043	0.86	0.070	na	0.19
OR Quart3	10	nd	12	7.3	5.5	13	2.0	na	5.2
OR Quart 4	2.9	nd	2.9	5.2	1.5	5.3	1.2	2.6	1.7
p Value	0.14	nd	0.13	0.0047	0.66	0.013	0.77	0.26	0.48
95% CI of	0.72	nd	0.72	1.7	0.24	1.4	0.35	0.49	0.39
OR Quart4	11	nd	11	17	9.2	20	4.2	14	7.5

Cathepsin B

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.661	0.911	0.661	1.10	0.661	0.312
Average	2.94	1.16	2.94	5.27	2.94	1.34
Stdev	9.10	1.30	9.10	12.7	9.10	1.86
p(t-test)		0.36		0.19		0.48
Min	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9
Max	78.1	5.47	78.1	64.9	78.1	4.98
n (Samp)	256	22	256	32	256	16
n (Patient)	163	22	163	32	163	16

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	nd	nd	0.648	1.62	0.648	2.27

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Average	nd	nd	2.81	6.92	2.81	5.45
Stdev	nd	nd	8.88	9.65	8.88	10.8
p(t-test)	nd	nd		0.26		0.44
Min	nd	nd	1.00E-9	0.593	1.00E-9	0.0682
Max	nd	nd	78.1	24.2	78.1	29.7
n (Samp)	nd	nd	322	6	322	7
n (Patient)	nd	nd	190	6	190	7

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.768	0.911	0.768	1.20	0.768	0.605
Average	3.26	1.14	3.26	5.03	3.26	2.90
Stdev	9.69	1.27	9.69	12.8	9.69	6.17
p(t-test)		0.31		0.38		0.89
Min	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9	1.00E-9
Max	78.1	5.29	78.1	64.9	78.1	24.2
n (Samp)	222	22	222	29	222	15
n (Patient)	135	22	135	29	135	15

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.50	nd	0.47	0.62	0.73	0.59	0.42	0.60	0.44
SE	0.064	nd	0.066	0.056	0.12	0.059	0.077	0.11	0.079
p	0.96	nd	0.62	0.032	0.056	0.14	0.30	0.37	0.46
nCohort 1	256	nd	222	256	322	222	256	322	222
nCohort 2	22	nd	22	32	6	29	16	7	15
Cutoff 1	0.296	nd	0.296	0.572	0.797	0.522	0.0513	0.319	0.158
Sens 1	73%	nd	73%	72%	83%	72%	75%	71%	73%
Spec 1	32%	nd	29%	48%	55%	39%	13%	34%	18%
Cutoff 2	0.140	nd	0.140	0.463	0.797	0.460	0	0.309	0
Sens 2	82%	nd	82%	81%	83%	83%	100%	86%	100%
Spec 2	19%	nd	17%	40%	55%	35%	0%	33%	0%
Cutoff 3	1.00E-9	nd	1.00E-9	0.290	0.572	0.236	0	0.0513	0
Sens 3	91%	nd	91%	91%	100%	93%	100%	100%	100%
Spec 3	12%	nd	10%	31%	48%	23%	0%	14%	0%
Cutoff 4	1.46	nd	1.64	1.46	1.41	1.64	1.46	1.41	1.64
Sens 4	27%	nd	23%	38%	50%	38%	31%	57%	33%
Spec 4	70%	nd	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	2.37	nd	2.74	2.37	2.37	2.74	2.37	2.37	2.74
Sens 5	14%	nd	9%	25%	33%	28%	25%	43%	27%
Spec 5	80%	nd	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	5.61	nd	6.20	5.61	4.86	6.20	5.61	4.86	6.20
Sens 6	0%	nd	0%	16%	33%	14%	0%	14%	7%
Spec 6	90%	nd	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	1.7	nd	3.3	2.9	>1.0	2.9	0.19	2.0	0
p Value	0.37	nd	0.082	0.13	<0.99	0.13	0.13	0.57	na
95% CI of	0.53	nd	0.86	0.73	>0.062	0.72	0.021	0.18	na

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
OR Quart2	5.5	nd	13	11	na	11	1.7	23	na
OR Quart 3	0.79	nd	1.7	3.7	>2.0	2.9	0.79	0	0.80
p Value	0.73	nd	0.47	0.054	<0.56	0.13	0.73	na	0.75
95% CI of	0.20	nd	0.39	0.98	>0.18	0.72	0.20	na	0.20
OR Quart3	3.1	nd	7.6	14	na	11	3.1	na	3.1
OR Quart 4	1.0	nd	1.7	4.1	>3.1	3.7	1.2	4.1	1.2
p Value	0.98	nd	0.47	0.035	<0.33	0.056	0.75	0.21	0.73
95% CI of	0.28	nd	0.39	1.1	>0.32	0.97	0.35	0.45	0.36
OR Quart4	3.7	nd	7.6	16	na	14	4.2	38	4.3

Dipeptidyl peptidase IV

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	157	473	157	662	157	455
Average	4080	1160	4080	1750	4080	758
Stdev	47500	1640	47500	3170	47500	826
p(t-test)		0.81		0.83		0.82
Min	0.200	7.95	0.200	10.1	0.200	17.0
Max	677000	5420	677000	12500	677000	2280
n (Samp)	203	16	203	19	203	11
n (Patient)	135	16	135	19	135	11

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	151	603	151	639	151	556
Average	619	1230	619	1480	619	836
Stdev	1350	1630	1350	2970	1350	777
p(t-test)		0.091		0.028		0.60
Min	0.200	7.95	0.200	10.1	0.200	17.0
Max	11000	5420	11000	12500	11000	2280
n (Samp)	174	16	174	18	174	11
n (Patient)	114	16	114	18	114	11

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.61	nd	0.63	0.66	nd	0.65	0.61	nd	0.68
SE	0.077	nd	0.078	0.071	nd	0.073	0.093	nd	0.092
p	0.14	nd	0.086	0.028	nd	0.039	0.24	nd	0.055
nCohort 1	203	nd	174	203	nd	174	203	nd	174
nCohort 2	16	nd	16	19	nd	18	11	nd	11
Cutoff 1	63.4	nd	52.3	294	nd	294	161	nd	407
Sens 1	75%	nd	75%	74%	nd	72%	73%	nd	73%
Spec 1	33%	nd	32%	60%	nd	61%	51%	nd	65%
Cutoff 2	52.3	nd	31.7	48.0	nd	48.0	52.3	nd	161
Sens 2	81%	nd	81%	84%	nd	83%	82%	nd	82%
Spec 2	31%	nd	25%	29%	nd	29%	31%	nd	52%

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Cutoff 3	18.2	nd	18.2	18.2	nd	18.2	39.0	nd	39.0
Sens 3	94%	nd	94%	95%	nd	94%	91%	nd	91%
Spec 3	19%	nd	20%	19%	nd	20%	27%	nd	27%
Cutoff 4	560	nd	529	560	nd	529	560	nd	529
Sens 4	44%	nd	50%	58%	nd	61%	36%	nd	55%
Spec 4	70%	nd	70%	70%	nd	70%	70%	nd	70%
Cutoff 5	1010	nd	889	1010	nd	889	1010	nd	889
Sens 5	25%	nd	31%	26%	nd	33%	36%	nd	36%
Spec 5	80%	nd	80%	80%	nd	80%	80%	nd	80%
Cutoff 6	1560	nd	1530	1560	nd	1530	1560	nd	1530
Sens 6	25%	nd	31%	16%	nd	11%	18%	nd	18%
Spec 6	90%	nd	90%	90%	nd	90%	90%	nd	90%
OR Quart 2	0.64	nd	0.64	0.64	nd	0.65	3.1	nd	1.0
p Value	0.63	nd	0.63	0.63	nd	0.65	0.34	nd	1.0
95% CI of	0.10	nd	0.10	0.10	nd	0.10	0.31	nd	0.061
OR Quart2	4.0	nd	4.0	4.0	nd	4.1	30	nd	16
OR Quart 3	2.1	nd	1.4	2.5	nd	1.7	3.1	nd	4.3
p Value	0.32	nd	0.70	0.20	nd	0.46	0.33	nd	0.20
95% CI of	0.49	nd	0.29	0.62	nd	0.39	0.31	nd	0.46
OR Quart3	8.8	nd	6.5	10	nd	7.7	31	nd	40
OR Quart 4	1.7	nd	2.5	2.5	nd	3.0	4.2	nd	5.4
p Value	0.48	nd	0.20	0.21	nd	0.12	0.21	nd	0.13
95% CI of	0.39	nd	0.61	0.61	nd	0.74	0.45	nd	0.60
OR Quart4	7.5	nd	10	10	nd	12	39	nd	48

[0142]   Table 3: Comparison of the maximum marker levels in urine samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0) and the maximum values in urine samples collected from subjects between enrollment and 0, 24 hours, and 48 hours prior to reaching stage F in Cohort 2.

Beta-2-microglobulin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	4.78	2.99	4.78	3.49	4.78	2.99
Average	3.59	2.92	3.59	3.09	3.59	2.83
Stdev	2.43	2.66	2.43	2.64	2.43	2.50
p(t-test)		0.31		0.46		0.40
Min	0.00270	0.00133	0.00270	0.00133	0.00270	0.0324
Max	6.10	5.80	6.10	5.80	6.10	5.80
n (Samp)	103	16	103	15	103	8
n (Patient)	103	16	103	15	103	8

sCr only	0hr prior to AKI stage	24hr prior to AKI stage	48hr prior to AKI stage

	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	4.50	5.24	4.50	5.16	nd	nd
Average	3.52	3.82	3.52	3.80	nd	nd
Stdev	2.47	2.57	2.47	2.55	nd	nd
p(t-test)		0.74		0.75	nd	nd
Min	0.00270	0.00133	0.00270	0.00133	nd	nd
Max	6.10	5.80	6.10	5.80	nd	nd
n (Samp)	170	8	170	8	nd	nd
n (Patient)	170	8	170	8	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	4.20	0.348	4.20	0.480	4.20	1.83
Average	3.52	2.21	3.52	2.43	3.52	2.57
Stdev	2.42	2.68	2.42	2.74	2.42	2.82
p(t-test)		0.11		0.21		0.36
Min	0.00270	0.0324	0.00270	0.0324	0.00270	0.0324
Max	6.10	5.80	6.10	5.80	6.10	5.80
n (Samp)	87	10	87	9	87	6
n (Patient)	87	10	87	9	87	6

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.41	0.49	0.32	0.41	0.47	0.35	0.38	nd	0.36
SE	0.080	0.11	0.098	0.082	0.11	0.10	0.11	nd	0.13
p	0.24	0.95	0.073	0.29	0.81	0.14	0.28	nd	0.25
nCohort 1	103	170	87	103	170	87	103	nd	87
nCohort 2	16	8	10	15	8	9	8	nd	6
Cutoff 1	0.170	2.39	0.170	0.170	2.39	0.152	0.170	nd	0.0851
Sens 1	75%	75%	70%	73%	75%	78%	75%	nd	83%
Spec 1	13%	38%	10%	13%	38%	9%	13%	nd	9%
Cutoff 2	0.152	0.152	0.152	0.152	0.152	0.0851	0.0851	nd	0.0851
Sens 2	81%	88%	80%	80%	88%	89%	88%	nd	83%
Spec 2	12%	13%	9%	12%	13%	9%	12%	nd	9%
Cutoff 3	0.0319	0	0.0851	0.0319	0	0.0273	0.0319	nd	0.0273
Sens 3	94%	100%	90%	93%	100%	100%	100%	nd	100%
Spec 3	6%	0%	9%	6%	0%	5%	6%	nd	5%
Cutoff 4	5.80	5.80	5.80	5.80	5.80	5.80	5.80	nd	5.80
Sens 4	0%	0%	0%	0%	0%	0%	0%	nd	0%
Spec 4	89%	88%	86%	89%	88%	86%	89%	nd	86%
Cutoff 5	5.80	5.80	5.80	5.80	5.80	5.80	5.80	nd	5.80
Sens 5	0%	0%	0%	0%	0%	0%	0%	nd	0%
Spec 5	89%	88%	86%	89%	88%	86%	89%	nd	86%
Cutoff 6	5.84	6.10	6.10	5.84	6.10	6.10	5.84	nd	6.10
Sens 6	0%	0%	0%	0%	0%	0%	0%	nd	0%
Spec 6	90%	100%	100%	90%	100%	100%	90%	nd	100%
OR Quart 2	>9.1	>5.8	>3.6	>9.5	>5.8	>3.4	>3.4	nd	1.0
p Value	<0.045	<0.12	<0.29	<0.041	<0.12	<0.30	<0.31	nd	0.98
95% CI of	>1.0	>0.65	>0.35	>1.1	>0.65	>0.33	>0.33	nd	0.062

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
OR Quart2	na	na	na	na	na	na	na	nd	18
OR Quart 3	>2.1	>1.0	>1.1	>2.1	>1.0	>1.0	>2.2	nd	1.0
p Value	<0.54	<0.99	<0.95	<0.54	<0.99	<0.98	<0.54	nd	0.98
95% CI of	>0.18	>0.062	>0.064	>0.18	>0.062	>0.062	>0.18	nd	0.062
OR Quart3	na	na	na	na	na	na	na	nd	18
OR Quart 4	>9.5	>2.1	>8.3	>7.8	>2.1	>6.3	>3.5	nd	3.4
p Value	<0.041	<0.54	<0.059	<0.065	<0.54	<0.11	<0.29	nd	0.30
95% CI of	>1.1	>0.19	>0.92	>0.88	>0.19	>0.68	>0.34	nd	0.33
OR Quart4	na	na	na	na	na	na	na	nd	36

C-X-C motif chemokine 2

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1.04	28.7	1.04	27.8	1.04	3.34
Average	9.16	42.4	9.16	37.7	9.16	12.4
Stdev	30.2	57.0	30.2	56.5	30.2	14.7
p(t-test)		2.7E-5		2.9E-4		0.73
Min	0.00804	0.0260	0.00804	0.0260	0.00804	0.0260
Max	266	217	266	217	266	36.2
n (Samp)	190	21	190	21	190	11
n (Patient)	190	21	190	21	190	11

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1.90	27.8	1.90	27.8	1.90	6.02
Average	13.1	38.9	13.1	38.9	13.1	11.5
Stdev	33.8	63.2	33.8	63.2	33.8	13.5
p(t-test)		0.017		0.017		0.91
Min	0.00804	0.0260	0.00804	0.0260	0.00804	0.135
Max	266	217	266	217	266	31.3
n (Samp)	295	11	295	11	295	6
n (Patient)	295	11	295	11	295	6

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1.60	35.3	1.60	29.0	1.60	3.34
Average	9.22	53.2	9.22	46.6	9.22	14.0
Stdev	29.8	64.3	29.8	64.7	29.8	15.7
p(t-test)		6.7E-6		1.2E-4		0.64
Min	0.00804	0.534	0.00804	0.534	0.00804	0.0260
Max	266	217	266	217	266	36.2
n (Samp)	134	15	134	15	134	9
n (Patient)	134	15	134	15	134	9

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.79	0.70	0.81	0.79	0.70	0.80	0.68	0.65	0.67
SE	0.061	0.089	0.070	0.061	0.089	0.070	0.091	0.12	0.10
p	1.9E-6	0.022	1.0E-5	2.7E-6	0.023	1.5E-5	0.042	0.23	0.089
nCohort 1	190	295	134	190	295	134	190	295	134
nCohort 2	21	11	15	21	11	15	11	6	9
Cutoff 1	3.33	1.95	3.33	3.33	1.95	3.33	1.52	0.570	1.52
Sens 1	71%	73%	73%	71%	73%	73%	73%	83%	78%
Spec 1	65%	51%	60%	65%	51%	60%	54%	41%	49%
Cutoff 2	1.52	0.570	1.95	1.52	0.570	1.95	0.541	0.570	0.544
Sens 2	81%	82%	80%	81%	82%	80%	82%	83%	89%
Spec 2	54%	41%	56%	54%	41%	56%	48%	41%	45%
Cutoff 3	0.371	0.134	0.544	0.371	0.134	0.544	0.134	0.134	0.00804
Sens 3	90%	91%	93%	90%	91%	93%	91%	100%	100%
Spec 3	48%	37%	45%	48%	37%	45%	45%	37%	1%
Cutoff 4	4.12	6.43	4.69	4.12	6.43	4.69	4.12	6.43	4.69
Sens 4	67%	64%	67%	67%	55%	67%	45%	50%	44%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	6.66	11.6	8.08	6.66	11.6	8.08	6.66	11.6	8.08
Sens 5	62%	55%	67%	62%	55%	67%	45%	33%	44%
Spec 5	80%	80%	81%	80%	80%	81%	80%	80%	81%
Cutoff 6	14.8	28.5	14.8	14.8	28.5	14.8	14.8	28.5	14.8
Sens 6	57%	45%	60%	57%	45%	60%	36%	17%	44%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	>4.2	2.0	>3.3	>4.2	2.0	>3.3	>3.2	>2.1	>3.2
p Value	<0.20	0.57	<0.32	<0.20	0.57	<0.32	<0.32	<0.56	<0.33
95% CI of OR Quart2	>0.46 na	0.18 23	>0.32 na	>0.46 na	0.18 23	>0.32 na	>0.32 na	>0.18 na	>0.31 na
OR Quart 3	>4.2	2.0	>2.1	>4.2	2.0	>2.1	>3.2	>1.0	>2.1
p Value	<0.20	0.57	<0.55	<0.20	0.57	<0.55	<0.32	<0.99	<0.56
95% CI of OR Quart3	>0.46 na	0.18 23	>0.18 na	>0.46 na	0.18 23	>0.18 na	>0.32 na	>0.062 na	>0.18 na
OR Quart 4	>17	6.3	>13	>17	6.3	>13	>5.4	>3.1	>4.4
p Value	<0.0076	0.091	<0.017	<0.0076	0.091	<0.017	<0.13	<0.33	<0.20
95% CI of OR Quart4	>2.1 na	0.74 54	>1.6 na	>2.1 na	0.74 54	>1.6 na	>0.61 na	>0.31 na	>0.46 na

Renin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	382	3580	382	3580	382	2150
Average	828	5520	828	4430	828	2820
Stdev	1360	6880	1360	5560	1360	1670
p(t-test)		2.4E-8		4.5E-7		3.6E-4
Min	1.00E-9	99.0	1.00E-9	99.0	1.00E-9	326
Max	9810	21100	9810	21100	9810	5370
n (Samp)	98	12	98	12	98	7
n (Patient)	98	12	98	12	98	7

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	494	3030	494	3030	nd	nd
Average	1230	5100	1230	5100	nd	nd
Stdev	2230	6800	2230	6800	nd	nd
p(t-test)		2.6E-4		2.6E-4	nd	nd
Min	1.00E-9	99.0	1.00E-9	99.0	nd	nd
Max	21100	18400	21100	18400	nd	nd
n (Samp)	159	6	159	6	nd	nd
n (Patient)	159	6	159	6	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	371	4150	371	4150	371	2930
Average	873	7420	873	5790	873	2930
Stdev	1480	7810	1480	6420	1480	1810
p(t-test)		1.5E-9		1.1E-7		0.0016
Min	1.00E-9	326	1.00E-9	326	1.00E-9	326
Max	9810	21100	9810	21100	9810	5370
n (Samp)	82	8	82	8	82	6
n (Patient)	82	8	82	8	82	6

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.84	0.75	0.90	0.84	0.75	0.90	0.87	nd	0.85
SE	0.073	0.12	0.074	0.074	0.12	0.075	0.088	nd	0.10
p	3.6E-6	0.030	7.6E-8	4.4E-6	0.030	1.4E-7	2.9E-5	nd	5.0E-4
nCohort 1	98	159	82	98	159	82	98	nd	82
nCohort 2	12	6	8	12	6	8	7	nd	6
Cutoff 1	2070	606	3280	2070	606	3280	2070	nd	1840
Sens 1	75%	83%	75%	75%	83%	75%	71%	nd	83%
Spec 1	91%	58%	95%	91%	58%	95%	91%	nd	87%
Cutoff 2	606	606	2070	606	606	2070	1990	nd	1840
Sens 2	83%	83%	88%	83%	83%	88%	86%	nd	83%
Spec 2	68%	58%	89%	68%	58%	89%	89%	nd	87%
Cutoff 3	310	88.6	310	310	88.6	310	310	nd	310
Sens 3	92%	100%	100%	92%	100%	100%	100%	nd	100%
Spec 3	45%	18%	46%	45%	18%	46%	45%	nd	46%
Cutoff 4	730	1150	695	730	1150	695	730	nd	695
Sens 4	75%	67%	88%	75%	67%	88%	86%	nd	83%
Spec 4	70%	70%	71%	70%	70%	71%	70%	nd	71%
Cutoff 5	1280	1840	1280	1280	1840	1280	1280	nd	1280
Sens 5	75%	67%	88%	75%	67%	88%	86%	nd	83%
Spec 5	81%	81%	80%	81%	81%	80%	81%	nd	80%
Cutoff 6	2070	3280	2140	2070	3280	2140	2070	nd	2140
Sens 6	75%	50%	75%	75%	50%	75%	71%	nd	50%
Spec 6	91%	91%	90%	91%	91%	90%	91%	nd	90%
OR Quart 2	0.96	0	>1.0	0.96	0	>1.0	>1.0	nd	>1.0

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
p Value	0.98	na	<1.0	0.98	na	<1.0	<0.98	nd	<0.97
95% CI of OR Quart2	0.057	na	>0.059	0.057	na	>0.059	>0.062	nd	>0.061
OR Quart2	16	na	na	16	na	na	na	nd	na
OR Quart 3	1.0	1.0	>0	1.0	1.0	>0	>0	nd	>0
p Value	1.0	1.0	<na	1.0	1.0	<na	<na	nd	<na
95% CI of OR Quart3	0.059	0.060	>na	0.059	0.060	>na	>na	nd	>na
OR Quart3	17	17	na	17	17	na	na	nd	na
OR Quart 4	12	4.2	>9.6	12	4.2	>9.6	>7.4	nd	>6.5
p Value	0.022	0.21	<0.043	0.022	0.21	<0.043	<0.073	nd	<0.10
95% CI of OR Quart4	1.4	0.45	>1.1	1.4	0.45	>1.1	>0.83	nd	>0.69
OR Quart4	110	39	na	110	39	na	na	nd	na

Cathepsin B

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.556	4.74	0.556	4.74	0.556	2.27
Average	2.13	12.7	2.13	12.7	2.13	2.62
Stdev	7.41	19.1	7.41	19.1	7.41	2.21
p(t-test)		2.7E-4		2.7E-4		0.86
Min	1.00E-9	0.310	1.00E-9	0.310	1.00E-9	0.310
Max	72.4	64.9	72.4	64.9	72.4	6.19
n (Samp)	102	12	102	12	102	7
n (Patient)	102	12	102	12	102	7

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.897	9.47	0.897	9.47	nd	nd
Average	3.16	12.6	3.16	12.6	nd	nd
Stdev	9.92	12.0	9.92	12.0	nd	nd
p(t-test)		0.025		0.025	nd	nd
Min	1.00E-9	0.405	1.00E-9	0.405	nd	nd
Max	78.1	29.7	78.1	29.7	nd	nd
n (Samp)	162	6	162	6	nd	nd
n (Patient)	162	6	162	6	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.677	4.74	0.677	4.74	0.677	2.13
Average	2.26	14.2	2.26	14.2	2.26	2.68
Stdev	7.97	22.7	7.97	22.7	7.97	2.42
p(t-test)		0.0015		0.0015		0.90
Min	1.00E-9	0.310	1.00E-9	0.310	1.00E-9	0.310
Max	72.4	64.9	72.4	64.9	72.4	6.19
n (Samp)	87	8	87	8	87	6
n (Patient)	87	8	87	8	87	6

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.79	0.82	0.77	0.79	0.82	0.77	0.71	nd	0.69
SE	0.081	0.11	0.10	0.081	0.11	0.10	0.11	nd	0.12
p	4.2E-4	0.0030	0.0064	4.2E-4	0.0030	0.0064	0.059	nd	0.13
nCohort 1	102	162	87	102	162	87	102	nd	87
nCohort 2	12	6	8	12	6	8	7	nd	6
Cutoff 1	0.949	2.16	0.949	0.949	2.16	0.949	0.888	nd	0.604
Sens 1	75%	83%	75%	75%	83%	75%	71%	nd	83%
Spec 1	58%	77%	56%	58%	77%	56%	58%	nd	49%
Cutoff 2	0.888	2.16	0.888	0.888	2.16	0.888	0.604	nd	0.604
Sens 2	83%	83%	88%	83%	83%	88%	86%	nd	83%
Spec 2	58%	77%	56%	58%	77%	56%	53%	nd	49%
Cutoff 3	0.397	0.397	0.309	0.397	0.397	0.309	0.309	nd	0.309
Sens 3	92%	100%	100%	92%	100%	100%	100%	nd	100%
Spec 3	40%	31%	31%	40%	31%	31%	32%	nd	31%
Cutoff 4	1.42	1.67	1.49	1.42	1.67	1.49	1.42	nd	1.49
Sens 4	67%	83%	62%	67%	83%	62%	57%	nd	50%
Spec 4	71%	70%	70%	71%	70%	70%	71%	nd	70%
Cutoff 5	2.04	2.74	2.04	2.04	2.74	2.04	2.04	nd	2.04
Sens 5	67%	67%	62%	67%	67%	62%	57%	nd	50%
Spec 5	80%	80%	80%	80%	80%	80%	80%	nd	80%
Cutoff 6	3.88	5.61	3.76	3.88	5.61	3.76	3.88	nd	3.76
Sens 6	50%	67%	50%	50%	67%	50%	29%	nd	33%
Spec 6	90%	90%	91%	90%	90%	91%	90%	nd	91%
OR Quart 2	>2.1	>1.0	>1.0	>2.1	>1.0	>1.0	>1.0	nd	>2.2
p Value	<0.56	<0.99	<1.0	<0.56	<0.99	<1.0	<0.98	nd	<0.53
95% CI of OR Quart2	>0.18 na	>0.062 na	>0.059 na	>0.18 na	>0.062 na	>0.059 na	>0.062 na	nd	>0.18 na
OR Quart 3	>2.2	>1.0	>2.1	>2.2	>1.0	>2.1	>2.2	nd	>1.0
p Value	<0.54	<0.99	<0.56	<0.54	<0.99	<0.56	<0.54	nd	<0.98
95% CI of OR Quart3	>0.18 na	>0.062 na	>0.18 na	>0.18 na	>0.062 na	>0.18 na	>0.18 na	nd	>0.062 na
OR Quart 4	>11	>4.4	>6.1	>11	>4.4	>6.1	>4.5	nd	>3.3
p Value	<0.031	<0.19	<0.11	<0.031	<0.19	<0.11	<0.19	nd	<0.32
95% CI of OR Quart4	>1.2 na	>0.47 na	>0.65 na	>1.2 na	>0.47 na	>0.65 na	>0.47 na	nd	>0.32 na

Dipeptidyl peptidase IV

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	143	2270	143	2270	143	2270
Average	9210	2860	9210	2860	9210	2490
Stdev	74700	2490	74700	2490	74700	2330
p(t-test)		0.79		0.79		0.83
Min	0.200	0.651	0.200	0.651	0.200	10.1
Max	677000	7150	677000	7150	677000	5420
n (Samp)	82	10	82	10	82	6
n (Patient)	82	10	82	10	82	6

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	103	2270	103	2270	nd	nd
Average	604	2720	604	2720	nd	nd
Stdev	1400	2100	1400	2100	nd	nd
p(t-test)		1.0E-3		1.0E-3	nd	nd
Min	0.200	10.1	0.200	10.1	nd	nd
Max	9980	5420	9980	5420	nd	nd
n (Samp)	71	6	71	6	nd	nd
n (Patient)	71	6	71	6	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.74	nd	0.81	0.74	nd	0.81	0.68	nd	nd
SE	0.094	nd	0.11	0.094	nd	0.11	0.12	nd	nd
p	0.012	nd	0.0037	0.012	nd	0.0037	0.15	nd	nd
nCohort 1	82	nd	71	82	nd	71	82	nd	nd
nCohort 2	10	nd	6	10	nd	6	6	nd	nd
Cutoff 1	1210	nd	1190	1210	nd	1190	12.4	nd	nd
Sens 1	70%	nd	83%	70%	nd	83%	83%	nd	nd
Spec 1	82%	nd	86%	82%	nd	86%	18%	nd	nd
Cutoff 2	659	nd	1190	659	nd	1190	12.4	nd	nd
Sens 2	80%	nd	83%	80%	nd	83%	83%	nd	nd
Spec 2	73%	nd	86%	73%	nd	86%	18%	nd	nd
Cutoff 3	9.55	nd	9.55	9.55	nd	9.55	9.55	nd	nd
Sens 3	90%	nd	100%	90%	nd	100%	100%	nd	nd
Spec 3	15%	nd	17%	15%	nd	17%	15%	nd	nd
Cutoff 4	545	nd	407	545	nd	407	545	nd	nd
Sens 4	80%	nd	83%	80%	nd	83%	67%	nd	nd
Spec 4	71%	nd	70%	71%	nd	70%	71%	nd	nd
Cutoff 5	1190	nd	942	1190	nd	942	1190	nd	nd
Sens 5	70%	nd	83%	70%	nd	83%	67%	nd	nd
Spec 5	80%	nd	80%	80%	nd	80%	80%	nd	nd
Cutoff 6	2020	nd	1810	2020	nd	1810	2020	nd	nd
Sens 6	50%	nd	67%	50%	nd	67%	50%	nd	nd
Spec 6	90%	nd	90%	90%	nd	90%	90%	nd	nd
OR Quart 2	0	nd	0	0	nd	0	0	nd	nd
p Value	na	nd	na	na	nd	na	na	nd	nd
95% CI of OR Quart2	na	nd	na	na	nd	na	na	nd	nd
OR Quart 3	0.48	nd	0	0.48	nd	0	0	nd	nd
p Value	0.56	nd	na	0.56	nd	na	na	nd	nd
95% CI of OR Quart3	0.040 5.7	nd	na	0.040 5.7	nd	na	na	nd	nd
OR Quart 4	4.6	nd	6.0	4.6	nd	6.0	2.2	nd	nd
p Value	0.079	nd	0.12	0.079	nd	0.12	0.39	nd	nd
95% CI of OR Quart4	0.84 25	nd	0.63 57	0.84 25	nd	0.63 57	0.36 14	nd	nd

[0143]   Table 4: Comparison of marker levels in EDTA samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0) and in EDTA samples collected from subjects at 0, 24 hours, and 48 hours prior to reaching stage R, I or F in Cohort 2.

Beta-2-microglobulin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	2.38	2.60	2.38	2.37	2.38	2.46
Average	2.90	3.66	2.90	3.86	2.90	3.17
Stdev	1.90	3.11	1.90	3.82	1.90	2.08
p(t-test)		0.020		0.0058		0.49
Min	0.850	0.759	0.850	1.14	0.850	1.04
Max	12.1	16.2	12.1	22.7	12.1	9.51
n (Samp)	263	51	263	56	263	26
n (Patient)	111	51	111	56	111	26

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	2.38	4.85	2.38	4.23	2.38	4.37
Average	2.98	5.96	2.98	5.76	2.98	4.24
Stdev	2.03	4.33	2.03	5.04	2.03	2.01
p(t-test)		1.4E-8		3.7E-8		0.027
Min	0.729	0.759	0.729	1.51	0.729	1.54
Max	16.7	16.2	16.7	22.7	16.7	7.60
n (Samp)	466	18	466	21	466	13
n (Patient)	180	18	180	21	180	13

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	2.46	3.00	2.46	2.54	2.46	2.45
Average	2.96	3.80	2.96	3.78	2.96	3.41
Stdev	1.87	2.87	1.87	3.19	1.87	3.29
p(t-test)		0.011		0.016		0.32
Min	0.899	1.00	0.899	1.14	0.899	1.04
Max	12.1	14.6	12.1	16.5	12.1	16.2
n (Samp)	221	50	221	52	221	23
n (Patient)	91	50	91	52	91	23

	0hr prior to AKI stage	24hr prior to AKI stage	48hr prior to AKI stage

	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.57	0.74	0.59	0.55	0.72	0.55	0.55	0.71	0.52
SE	0.045	0.068	0.046	0.043	0.064	0.045	0.061	0.082	0.064
p	0.14	4.4E-4	0.051	0.24	7.0E-4	0.22	0.45	0.0093	0.81
nCohort 1	263	466	221	263	466	221	263	466	221
nCohort 2	51	18	50	56	21	52	26	13	23
Cutoff 1	1.93	2.86	2.08	1.80	2.73	1.88	2.10	2.45	1.66
Sens 1	71%	72%	70%	71%	71%	71%	73%	77%	74%
Spec 1	37%	62%	38%	32%	60%	33%	41%	52%	27%
Cutoff 2	1.70	2.13	1.74	1.62	1.88	1.62	1.59	2.19	1.54
Sens 2	82%	83%	80%	80%	81%	81%	81%	85%	83%
Spec 2	29%	42%	29%	26%	33%	26%	25%	44%	23%
Cutoff 3	1.40	1.70	1.59	1.46	1.76	1.43	1.37	1.88	1.37
Sens 3	90%	94%	90%	91%	90%	90%	92%	92%	91%
Spec 3	19%	27%	25%	20%	30%	19%	18%	33%	19%
Cutoff 4	3.23	3.27	3.43	3.23	3.27	3.43	3.23	3.27	3.43
Sens 4	37%	67%	40%	32%	67%	35%	35%	62%	35%
Spec 4	70%	70%	71%	70%	70%	71%	70%	70%	71%
Cutoff 5	3.85	4.11	4.15	3.85	4.11	4.15	3.85	4.11	4.15
Sens 5	29%	61%	28%	29%	57%	27%	23%	62%	13%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	5.37	5.50	5.50	5.37	5.50	5.50	5.37	5.50	5.50
Sens 6	18%	44%	16%	18%	29%	19%	12%	23%	9%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	2.4	3.1	1.8	1.7	1.5	1.3	1.2	2.0	1.5
p Value	0.077	0.34	0.23	0.23	0.66	0.51	0.75	0.57	0.54
95% CI of OR Quart2	0.91 6.2	0.31 30	0.68 5.0	0.73 3.8	0.25 9.1	0.56 3.2	0.35 4.2	0.18 22	0.43 4.9
OR Quart 3	2.0	2.0	2.4	0.99	0.99	0.89	1.2	2.0	1.0
p Value	0.16	0.57	0.073	0.97	0.99	0.81	0.75	0.57	1.0
95% CI of OR Quart3	0.76 5.4	0.18 23	0.92 6.4	0.40 2.4	0.14 7.2	0.35 2.3	0.35 4.2	0.18 22	0.27 3.6
OR Quart 4	2.6	13	2.6	1.7	7.7	1.7	1.9	8.4	1.2
p Value	0.051	0.014	0.048	0.23	0.0078	0.22	0.28	0.046	0.75
95% CI of OR Quart4	1.00 6.7	1.7 100	1.0 6.9	0.73 3.8	1.7 35	0.73 3.9	0.60 5.9	1.0 68	0.35 4.2

Renin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	3630	3850	3630	3360	3630	4440
Average	3940	4030	3940	4520	3940	4580
Stdev	1330	1360	1330	4260	1330	2250
p(t-test)		0.81		0.37		0.26
Min	1700	1120	1700	2100	1700	1870
Max	7950	6070	7950	23300	7950	8860
n (Samp)	55	16	55	24	55	8
n (Patient)	54	16	54	24	54	8

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	4180	3800	4180	3360	4180	4410
Average	4290	4040	4290	4510	4290	3960
Stdev	1610	1490	1610	4260	1610	1550
p(t-test)		0.61		0.75		0.62
Min	1700	1120	1700	2100	1700	1870
Max	9230	6070	9230	23300	9230	6200
n (Samp)	49	14	49	24	49	7
n (Patient)	45	14	45	24	45	7

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.55	nd	0.48	0.46	nd	0.41	0.60	nd	0.49
SE	0.084	nd	0.089	0.072	nd	0.073	0.11	nd	0.12
p	0.57	nd	0.79	0.54	nd	0.24	0.36	nd	0.91
nCohort 1	55	nd	49	55	nd	49	55	nd	49
nCohort 2	16	nd	14	24	nd	24	8	nd	7
Cutoff 1	3490	nd	3310	2630	nd	2630	3630	nd	3630
Sens 1	75%	nd	71%	71%	nd	71%	75%	nd	71%
Spec 1	45%	nd	33%	11%	nd	12%	51%	nd	37%
Cutoff 2	2770	nd	2510	2110	nd	2110	1900	nd	1900
Sens 2	81%	nd	86%	83%	nd	83%	88%	nd	86%
Spec 2	13%	nd	12%	5%	nd	6%	5%	nd	6%
Cutoff 3	2240	nd	2240	2100	nd	2100	1840	nd	1840
Sens 3	94%	nd	93%	92%	nd	92%	100%	nd	100%
Spec 3	9%	nd	10%	5%	nd	6%	4%	nd	4%
Cutoff 4	4320	nd	4690	4320	nd	4690	4320	nd	4690
Sens 4	44%	nd	36%	38%	nd	29%	62%	nd	29%
Spec 4	71%	nd	71%	71%	nd	71%	71%	nd	71%
Cutoff 5	4690	nd	5460	4690	nd	5460	4690	nd	5460
Sens 5	38%	nd	29%	29%	nd	21%	38%	nd	14%
Spec 5	80%	nd	82%	80%	nd	82%	80%	nd	82%
Cutoff 6	5580	nd	6490	5580	nd	6490	5580	nd	6490
Sens 6	12%	nd	0%	17%	nd	4%	25%	nd	0%
Spec 6	91%	nd	92%	91%	nd	92%	91%	nd	92%
OR Quart 2	0.65	nd	0.43	0.17	nd	0.43	0.43	nd	1.0
p Value	0.61	nd	0.37	0.040	nd	0.30	0.51	nd	1.0
95% CI of OR Quart2	0.12	nd	0.066	0.030	nd	0.090	0.035	nd	0.12
	3.5	nd	2.8	0.92	nd	2.1	5.3	nd	8.3
OR Quart 3	0.65	nd	1.0	0.64	nd	0.83	0.93	nd	0.46
p Value	0.61	nd	1.0	0.51	nd	0.80	0.94	nd	0.55
95% CI of OR Quart3	0.12	nd	0.20	0.17	nd	0.20	0.11	nd	0.037
	3.5	nd	5.0	2.4	nd	3.4	7.6	nd	5.8
OR Quart 4	1.6	nd	1.1	1.1	nd	2.7	1.5	nd	1.0
p Value	0.52	nd	0.92	0.89	nd	0.15	0.68	nd	1.0
95% CI of OR Quart4	0.37	nd	0.22	0.30	nd	0.71	0.21	nd	0.12
	7.2	nd	5.5	3.9	nd	10	11	nd	8.3

Neprilysin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.689	0.927	0.689	0.827	0.689	0.605
Average	1.18	1.27	1.18	1.20	1.18	0.816
Stdev	1.12	0.993	1.12	1.10	1.12	0.785
p(t-test)		0.76		0.93		0.38
Min	0.0870	0.183	0.0870	0.191	0.0870	0.244
Max	4.92	3.50	4.92	4.43	4.92	2.73
n (Samp)	53	16	53	24	53	8
n (Patient)	52	16	52	24	52	8

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.830	0.839	0.830	0.771	0.830	0.604
Average	1.35	1.12	1.35	1.11	1.35	0.542
Stdev	1.17	0.997	1.17	1.06	1.17	0.148
p(t-test)		0.51		0.40		0.076
Min	0.0870	0.183	0.0870	0.191	0.0870	0.244
Max	4.92	3.50	4.92	4.43	4.92	0.684
n (Samp)	48	14	48	24	48	7
n (Patient)	44	14	44	24	44	7

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.55	nd	0.44	0.52	nd	0.45	0.40	nd	0.29
SE	0.084	nd	0.089	0.072	nd	0.073	0.11	nd	0.12
p	0.57	nd	0.53	0.80	nd	0.45	0.37	nd	0.063
nCohort 1	53	nd	48	53	nd	48	53	nd	48
nCohort 2	16	nd	14	24	nd	24	8	nd	7
Cutoff 1	0.658	nd	0.510	0.540	nd	0.540	0.540	nd	0.540
Sens 1	75%	nd	71%	71%	nd	71%	75%	nd	71%
Spec 1	45%	nd	27%	36%	nd	31%	36%	nd	31%
Cutoff 2	0.510	nd	0.308	0.340	nd	0.340	0.454	nd	0.454
Sens 2	81%	nd	86%	83%	nd	83%	88%	nd	86%
Spec 2	32%	nd	6%	19%	nd	12%	28%	nd	25%
Cutoff 3	0.183	nd	0.183	0.319	nd	0.308	0.236	nd	0.0870
Sens 3	94%	nd	93%	92%	nd	92%	100%	nd	100%
Spec 3	2%	nd	2%	13%	nd	6%	6%	nd	2%
Cutoff 4	1.22	nd	1.67	1.22	nd	1.67	1.22	nd	1.67
Sens 4	31%	nd	21%	29%	nd	21%	12%	nd	0%
Spec 4	72%	nd	71%	72%	nd	71%	72%	nd	71%
Cutoff 5	1.82	nd	2.14	1.82	nd	2.14	1.82	nd	2.14
Sens 5	25%	nd	21%	25%	nd	12%	12%	nd	0%
Spec 5	81%	nd	81%	81%	nd	81%	81%	nd	81%
Cutoff 6	3.14	nd	3.57	3.14	nd	3.57	3.14	nd	3.57
Sens 6	6%	nd	0%	8%	nd	4%	0%	nd	0%
Spec 6	91%	nd	92%	91%	nd	92%	91%	nd	92%
OR Quart 2	1.4	nd	1.6	1.3	nd	1.3	1.1	nd	>0

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
p Value	0.67	nd	0.60	0.72	nd	0.72	0.96	nd	<na
95% CI of	0.27	nd	0.29	0.32	nd	0.31	0.061	nd	>na
OR Quart2	7.7	nd	8.6	5.3	nd	5.4	19	nd	na
OR Quart 3	1.4	nd	1.4	1.6	nd	2.1	7.5	nd	>10
p Value	0.67	nd	0.67	0.49	nd	0.30	0.085	nd	<0.044
95% CI of	0.27	nd	0.27	0.41	nd	0.52	0.76	nd	>1.1
OR Quart3	7.7	nd	7.8	6.5	nd	8.3	74	nd	na
OR Quart 4	1.8	nd	1.1	1.2	nd	1.0	1.1	nd	>1.2
p Value	0.48	nd	0.93	0.80	nd	1.0	0.96	nd	<0.92
95% CI of	0.36	nd	0.18	0.30	nd	0.23	0.061	nd	>0.066
OR Quart4	9.1	nd	6.4	4.9	nd	4.3	19	nd	na

Carbonic anhydrase IX

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.115	0.121	0.115	0.107	0.115	0.141
Average	0.155	0.126	0.155	0.209	0.155	0.146
Stdev	0.139	0.0833	0.139	0.374	0.139	0.0668
p(t-test)		0.44		0.35		0.86
Min	0.0145	0.00992	0.0145	0.0142	0.0145	0.0579
Max	0.796	0.310	0.796	1.90	0.796	0.246
n (Samp)	55	16	55	24	55	8
n (Patient)	54	16	54	24	54	8

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.124	0.156	0.124	0.117	0.124	0.135
Average	0.170	0.148	0.170	0.214	0.170	0.134
Stdev	0.158	0.0908	0.158	0.373	0.158	0.0629
p(t-test)		0.61		0.48		0.56
Min	0.0145	0.00992	0.0145	0.0142	0.0145	0.0579
Max	0.796	0.310	0.796	1.90	0.796	0.246
n (Samp)	49	14	49	24	49	7
n (Patient)	45	14	45	24	45	7

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.47	nd	0.51	0.48	nd	0.48	0.57	nd	0.51
SE	0.083	nd	0.089	0.071	nd	0.073	0.11	nd	0.12
p	0.72	nd	0.88	0.78	nd	0.76	0.56	nd	0.93
nCohort 1	55	nd	49	55	nd	49	55	nd	49
nCohort 2	16	nd	14	24	nd	24	8	nd	7
Cutoff 1	0.0795	nd	0.0877	0.0780	nd	0.0780	0.0919	nd	0.0901
Sens 1	75%	nd	71%	71%	nd	71%	75%	nd	71%
Spec 1	27%	nd	33%	25%	nd	24%	36%	nd	35%
Cutoff 2	0.0577	nd	0.0577	0.0665	nd	0.0635	0.0877	nd	0.0877

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Sens 2	81%	nd	86%	83%	nd	83%	88%	nd	86%
Spec 2	18%	nd	18%	24%	nd	20%	33%	nd	33%
Cutoff 3	0.0156	nd	0.0156	0.0467	nd	0.0467	0.0577	nd	0.0577
Sens 3	94%	nd	93%	92%	nd	92%	100%	nd	100%
Spec 3	4%	nd	4%	15%	nd	12%	18%	nd	18%
Cutoff 4	0.166	nd	0.212	0.166	nd	0.212	0.166	nd	0.212
Sens 4	31%	nd	21%	29%	nd	29%	38%	nd	14%
Spec 4	71%	nd	71%	71%	nd	71%	71%	nd	71%
Cutoff 5	0.231	nd	0.238	0.231	nd	0.238	0.231	nd	0.238
Sens 5	12%	nd	21%	29%	nd	25%	12%	nd	14%
Spec 5	80%	nd	82%	80%	nd	82%	80%	nd	82%
Cutoff 6	0.325	nd	0.392	0.325	nd	0.392	0.325	nd	0.392
Sens 6	0%	nd	0%	8%	nd	8%	0%	nd	0%
Spec 6	91%	nd	92%	91%	nd	92%	91%	nd	92%
OR Quart 2	1.9	nd	0.92	0.62	nd	0.49	2.0	nd	2.2
p Value	0.43	nd	0.93	0.49	nd	0.33	0.59	nd	0.55
95% CI of OR Quart2	0.38 9.6	nd	0.16 5.5	0.16 2.4	nd	0.11 2.1	0.16 25	nd	0.17 27
OR Quart 3	1.4	nd	1.8	1.0	nd	1.1	3.2	nd	3.5
p Value	0.67	nd	0.48	1.0	nd	0.90	0.34	nd	0.30
95% CI of OR Quart3	0.27 7.5	nd	0.35 9.5	0.27 3.7	nd	0.29 4.1	0.30 35	nd	0.32 39
OR Quart 4	1.5	nd	0.92	0.66	nd	0.86	2.0	nd	1.0
p Value	0.61	nd	0.93	0.56	nd	0.82	0.59	nd	1.0
95% CI of OR Quart4	0.29 8.2	nd	0.16 5.5	0.17 2.6	nd	0.22 3.3	0.16 25	nd	0.056 18

[0144]   Table 5: Comparison of marker levels in EDTA samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0 or R) and in EDTA samples collected from subjects at 0, 24 hours, and 48 hours prior to reaching stage I or F in Cohort 2.

Beta-2-microglobulin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	2.35	3.84	2.35	3.30	2.35	3.65
Average	2.95	4.81	2.95	4.65	2.95	4.18
Stdev	2.08	3.91	2.08	4.22	2.08	2.85
p(t-test)		4.1E-5		4.8E-5		0.019
Min	0.759	0.780	0.759	1.12	0.759	0.945
Max	16.5	16.7	16.5	22.7	16.5	11.3
n (Samp)	437	26	437	33	437	17
n (Patient)	174	26	174	33	174	17

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	2.46	6.68	2.46	4.26	2.46	4.60
Average	3.12	5.93	3.12	7.26	3.12	5.82
Stdev	2.23	2.51	2.23	7.08	2.23	3.55
p(t-test)		0.0023		2.9E-7		0.0017
Min	0.729	2.87	0.729	1.84	0.729	1.90
Max	16.7	8.27	16.7	22.7	16.7	10.6
n (Samp)	535	6	535	9	535	7
n (Patient)	207	6	207	9	207	7

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	2.41	3.84	2.41	3.30	2.41	3.65
Average	3.03	4.81	3.03	4.24	3.03	4.32
Stdev	2.17	3.87	2.17	2.81	2.17	2.90
p(t-test)		1.7E-4		0.0039		0.019
Min	0.899	0.780	0.899	1.12	0.899	0.945
Max	16.5	16.7	16.5	11.9	16.5	11.3
n (Samp)	362	26	362	31	362	17
n (Patient)	140	26	140	31	140	17

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.69	0.83	0.69	0.65	0.72	0.65	0.65	0.75	0.66
SE	0.059	0.10	0.059	0.053	0.097	0.055	0.074	0.11	0.074
p	0.0014	0.0011	0.0017	0.0052	0.024	0.0056	0.048	0.018	0.033
nCohort 1	437	535	362	437	535	362	437	535	362
nCohort 2	26	6	26	33	9	31	17	7	17
Cutoff 1	2.60	2.93	2.60	2.10	2.27	2.52	2.33	4.35	2.45
Sens 1	73%	83%	73%	73%	78%	71%	71%	71%	71%
Spec 1	56%	60%	55%	41%	44%	53%	50%	81%	52%
Cutoff 2	2.38	2.93	2.38	1.88	1.88	1.96	1.88	2.19	2.27
Sens 2	81%	83%	81%	82%	89%	81%	82%	86%	82%
Spec 2	50%	60%	49%	33%	31%	36%	33%	42%	45%
Cutoff 3	1.47	2.86	1.47	1.56	1.83	1.56	1.43	1.88	1.43
Sens 3	92%	100%	92%	91%	100%	90%	94%	100%	94%
Spec 3	18%	59%	18%	22%	30%	21%	16%	31%	17%
Cutoff 4	3.17	3.47	3.31	3.17	3.47	3.31	3.17	3.47	3.31
Sens 4	58%	67%	62%	55%	67%	48%	59%	71%	59%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	3.83	4.30	3.95	3.83	4.30	3.95	3.83	4.30	3.95
Sens 5	50%	67%	42%	45%	44%	45%	41%	71%	35%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	5.37	5.58	5.50	5.37	5.58	5.50	5.37	5.58	5.50
Sens 6	23%	67%	19%	27%	33%	26%	24%	43%	24%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	0.99	>0	1.0	2.1	>3.1	1.3	0.99	>2.0	0.65
p Value	0.99	<na	1.0	0.25	<0.33	0.73	0.99	<0.57	0.64

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
95% CI of OR Quart2	0.20 5.0	>na na	0.20 5.1	0.60 7.0	>0.32 na	0.33 4.9	0.20 5.0	>0.18 na	0.11 4.0
OR Quart 3 p Value	2.0 0.32	>2.0 <0.57	1.7 0.47	1.3 0.73	>0 <na	2.1 0.24	0.33 0.34	>0 <na	0.99 0.99
95% CI of OR Quart3	0.50 8.3	>0.18 na	0.40 7.3	0.33 4.8	>na na	0.61 7.2	0.034 3.2	>na na	0.19 5.0
OR Quart 4 p Value	5.1 0.012	>4.1 <0.21	5.7 0.0073	4.4 0.0097	>6.3 <0.091	3.9 0.021	3.5 0.061	>5.2 <0.14	3.2 0.091
95% CI of OR Quart4	1.4 18	>0.45 na	1.6 21	1.4 14	>0.75 na	1.2 12	0.94 13	>0.59 na	0.83 12

Renin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	nd	nd	3740	4690	nd	nd
Average	nd	nd	4070	6680	nd	nd
Stdev	nd	nd	1650	6470	nd	nd
p(t-test)	nd	nd		0.0015	nd	nd
Min	nd	nd	1120	2100	nd	nd
Max	nd	nd	10400	23300	nd	nd
n (Samp)	nd	nd	113	9	nd	nd
n (Patient)	nd	nd	92	9	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	nd	nd	3980	4690	nd	nd
Average	nd	nd	4160	6680	nd	nd
Stdev	nd	nd	1700	6470	nd	nd
p(t-test)	nd	nd		0.0035	nd	nd
Min	nd	nd	1120	2100	nd	nd
Max	nd	nd	10400	23300	nd	nd
n (Samp)	nd	nd	99	9	nd	nd
n (Patient)	nd	nd	77	9	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	nd	nd	nd	0.64	nd	0.62	nd	nd	nd
SE	nd	nd	nd	0.10	nd	0.10	nd	nd	nd
p	nd	nd	nd	0.17	nd	0.25	nd	nd	nd
nCohort 1	nd	nd	nd	113	nd	99	nd	nd	nd
nCohort 2	nd	nd	nd	9	nd	9	nd	nd	nd
Cutoff 1	nd	nd	nd	3620	nd	3580	nd	nd	nd
Sens 1	nd	nd	nd	78%	nd	78%	nd	nd	nd
Spec 1	nd	nd	nd	47%	nd	41%	nd	nd	nd
Cutoff 2	nd	nd	nd	3360	nd	3340	nd	nd	nd
Sens 2	nd	nd	nd	89%	nd	89%	nd	nd	nd
Spec 2	nd	nd	nd	41%	nd	38%	nd	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Cutoff 3	nd	nd	nd	2100	nd	2100	nd	nd	nd
Sens 3	nd	nd	nd	100%	nd	100%	nd	nd	nd
Spec 3	nd	nd	nd	5%	nd	6%	nd	nd	nd
Cutoff 4	nd	nd	nd	4690	nd	4900	nd	nd	nd
Sens 4	nd	nd	nd	56%	nd	33%	nd	nd	nd
Spec 4	nd	nd	nd	71%	nd	71%	nd	nd	nd
Cutoff 5	nd	nd	nd	5340	nd	5480	nd	nd	nd
Sens 5	nd	nd	nd	33%	nd	33%	nd	nd	nd
Spec 5	nd	nd	nd	81%	nd	81%	nd	nd	nd
Cutoff 6	nd	nd	nd	6200	nd	6380	nd	nd	nd
Sens 6	nd	nd	nd	33%	nd	22%	nd	nd	nd
Spec 6	nd	nd	nd	90%	nd	91%	nd	nd	nd
OR Quart 2	nd	nd	nd	2.0	nd	3.2	nd	nd	nd
p Value	nd	nd	nd	0.58	nd	0.32	nd	nd	nd
95% CI of	nd	nd	nd	0.17	nd	0.32	nd	nd	nd
OR Quart2	nd	nd	nd	23	nd	33	nd	nd	nd
OR Quart 3	nd	nd	nd	3.2	nd	2.1	nd	nd	nd
p Value	nd	nd	nd	0.32	nd	0.56	nd	nd	nd
95% CI of	nd	nd	nd	0.32	nd	0.18	nd	nd	nd
OR Quart3	nd	nd	nd	33	nd	24	nd	nd	nd
OR Quart 4	nd	nd	nd	3.1	nd	3.2	nd	nd	nd
p Value	nd	nd	nd	0.34	nd	0.32	nd	nd	nd
95% CI of	nd	nd	nd	0.30	nd	0.32	nd	nd	nd
OR Quart4	nd	nd	nd	32	nd	33	nd	nd	nd

[0145]   Table 6: Comparison of the maximum marker levels in EDTA samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0) and the maximum values in EDTA samples collected from subjects between enrollment and 0, 24 hours, and 48 hours prior to reaching stage F in Cohort 2.

Beta-2-microglobulin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	2.63	7.26	2.63	6.24	2.63	5.57
Average	3.24	8.21	3.24	7.37	3.24	5.98
Stdev	2.18	5.22	2.18	5.28	2.18	3.16
p(t-test)		3.7E-10		1.1E-7		0.0012
Min	0.951	2.40	0.951	2.19	0.951	2.34
Max	12.1	22.7	12.1	22.7	12.1	11.3
n (Samp)	111	16	111	16	111	8
n (Patient)	111	16	111	16	111	8

sCr only	0hr prior to AKI stage	24hr prior to AKI stage	48hr prior to AKI stage

	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	2.62	8.11	2.62	6.44	nd	nd
Average	3.34	9.20	3.34	8.54	nd	nd
Stdev	2.33	6.94	2.33	7.37	nd	nd
p(t-test)		5.7E-9		2.7E-7	nd	nd
Min	0.951	2.40	0.951	2.19	nd	nd
Max	16.7	22.7	16.7	22.7	nd	nd
n (Samp)	180	8	180	8	nd	nd
n (Patient)	180	8	180	8	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	2.66	7.62	2.66	6.90	2.66	5.57
Average	3.09	8.36	3.09	7.55	3.09	6.46
Stdev	1.97	3.54	1.97	3.23	1.97	3.33
p(t-test)		6.8E-11		7.4E-9		2.0E-4
Min	0.951	3.32	0.951	2.34	0.951	2.34
Max	12.1	14.6	12.1	11.9	12.1	11.3
n (Samp)	91	10	91	10	91	6
n (Patient)	91	10	91	10	91	6

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.86	0.82	0.93	0.80	0.74	0.89	0.79	nd	0.83
SE	0.060	0.092	0.058	0.069	0.10	0.069	0.098	nd	0.11
p	1.6E-9	4.2E-4	1.5E-13	1.5E-5	0.018	2.2E-8	0.0035	nd	0.0018
nCohort 1	111	180	91	111	180	91	111	nd	91
nCohort 2	16	8	10	16	8	10	8	nd	6
Cutoff 1	4.35	3.32	6.82	4.16	2.38	5.62	4.35	nd	4.35
Sens 1	75%	75%	70%	75%	75%	70%	75%	nd	83%
Spec 1	77%	66%	97%	76%	44%	88%	77%	nd	81%
Cutoff 2	3.32	2.93	5.62	2.38	2.27	5.39	2.38	nd	4.35
Sens 2	81%	88%	80%	81%	88%	80%	88%	nd	83%
Spec 2	67%	57%	88%	46%	41%	87%	46%	nd	81%
Cutoff 3	2.93	2.38	4.35	2.26	2.17	4.35	2.30	nd	2.30
Sens 3	94%	100%	90%	94%	100%	90%	100%	nd	100%
Spec 3	59%	44%	81%	42%	37%	81%	44%	nd	43%
Cutoff 4	3.61	3.62	3.43	3.61	3.62	3.43	3.61	nd	3.43
Sens 4	75%	62%	90%	75%	62%	90%	75%	nd	83%
Spec 4	70%	70%	70%	70%	70%	70%	70%	nd	70%
Cutoff 5	4.76	4.76	4.31	4.76	4.76	4.31	4.76	nd	4.31
Sens 5	69%	62%	90%	62%	50%	90%	62%	nd	83%
Spec 5	81%	80%	80%	81%	80%	80%	81%	nd	80%
Cutoff 6	6.14	6.17	6.04	6.14	6.17	6.04	6.14	nd	6.04
Sens 6	62%	62%	70%	50%	50%	60%	38%	nd	33%
Spec 6	90%	90%	90%	90%	90%	90%	90%	nd	90%
OR Quart 2	>1.0	>1.0	>0	>4.4	>3.2	>1.0	>2.1	nd	>1.0
p Value	<1.0	<0.99	<na	<0.19	<0.32	<0.98	<0.56	nd	<0.98
95% CI of	>0.060	>0.062	>na	>0.47	>0.32	>0.062	>0.18	nd	>0.062

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
OR Quart2	na	na	na	na	na	na	na	nd	na
OR Quart 3	>4.4	>2.1	>1.0	>2.1	>1.0	>0	>1.0	nd	>0
p Value	<0.19	<0.55	<0.98	<0.56	<0.99	<na	<1.0	nd	<na
95% CI of OR Quart3	>0.47	>0.18	>0.062	>0.18	>0.062	>na	>0.060	nd	>na
	na	na	na	na	na	na	na	nd	na
OR Quart 4	>16	>5.6	>13	>14	>4.4	>13	>5.8	nd	>6.0
p Value	<0.0100	<0.12	<0.019	<0.015	<0.19	<0.019	<0.12	nd	<0.11
95% CI of OR Quart4	>1.9	>0.63	>1.5	>1.7	>0.47	>1.5	>0.63	nd	>0.65
	na	na	na	na	na	na	na	nd	na

[0146]   Table 7: Comparison of marker levels in urine samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0, R, or I) and in urine samples collected from Cohort 2 (subjects who progress to RIFLE stage F) at 0, 24 hours, and 48 hours prior to the subject reaching RIFLE stage I.

Beta-2-microglobulin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1.51	0.990	1.51	0.202	nd	nd
Average	2.57	2.02	2.57	1.80	nd	nd
Stdev	2.44	2.22	2.44	2.59	nd	nd
p(t-test)		0.48		0.30	nd	nd
Min	0.00152	0.00133	0.00152	0.00212	nd	nd
Max	6.10	5.64	6.10	5.80	nd	nd
n (Samp)	534	10	534	11	nd	nd
n (Patient)	204	10	204	11	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1.60	0.480	1.60	0.163	nd	nd
Average	2.61	1.86	2.61	0.998	nd	nd
Stdev	2.44	2.11	2.44	1.98	nd	nd
p(t-test)		0.42		0.064	nd	nd
Min	0.00152	0.00403	0.00152	0.00212	nd	nd
Max	6.10	4.76	6.10	5.80	nd	nd
n (Samp)	454	7	454	8	nd	nd
n (Patient)	168	7	168	8	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.40	nd	0.38	0.40	nd	0.29	nd	nd	nd
SE	0.096	nd	0.11	0.091	nd	0.10	nd	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
p	0.29	nd	0.31	0.25	nd	0.044	nd	nd	nd
nCohort 1	534	nd	454	534	nd	454	nd	nd	nd
nCohort 2	10	nd	7	11	nd	8	nd	nd	nd
Cutoff 1	0.260	nd	0.214	0.153	nd	0.0999	nd	nd	nd
Sens 1	70%	nd	71%	73%	nd	75%	nd	nd	nd
Spec 1	28%	nd	25%	22%	nd	17%	nd	nd	nd
Cutoff 2	0.0899	nd	0.0899	0.100	nd	0.0301	nd	nd	nd
Sens 2	80%	nd	86%	82%	nd	88%	nd	nd	nd
Spec 2	17%	nd	16%	18%	nd	7%	nd	nd	nd
Cutoff 3	0.00347	nd	0.00347	0.0319	nd	0.00181	nd	nd	nd
Sens 3	90%	nd	100%	91%	nd	100%	nd	nd	nd
Spec 3	1%	nd	1%	7%	nd	0%	nd	nd	nd
Cutoff 4	5.13	nd	5.20	5.13	nd	5.20	nd	nd	nd
Sens 4	10%	nd	0%	27%	nd	12%	nd	nd	nd
Spec 4	70%	nd	70%	70%	nd	70%	nd	nd	nd
Cutoff 5	5.80	nd	5.80	5.80	nd	5.80	nd	nd	nd
Sens 5	0%	nd	0%	0%	nd	0%	nd	nd	nd
Spec 5	95%	nd	94%	95%	nd	94%	nd	nd	nd
Cutoff 6	5.80	nd	5.80	5.80	nd	5.80	nd	nd	nd
Sens 6	0%	nd	0%	0%	nd	0%	nd	nd	nd
Spec 6	95%	nd	94%	95%	nd	94%	nd	nd	nd
OR Quart 2	>5.2	nd	>3.1	0	nd	0	nd	nd	nd
p Value	<0.14	nd	<0.33	na	nd	na	nd	nd	nd
95% CI of OR Quart2	>0.60 na	nd	>0.32 na	na na	nd	na na	nd	nd	nd
OR Quart 3	>2.0	nd	>1.0	1.0	nd	2.0	nd	nd	nd
p Value	<0.57	nd	<0.99	0.99	nd	0.57	nd	nd	nd
95% CI of OR Quart3	>0.18 na	nd	>0.063 na	0.20 5.1	nd	0.18 23	nd	nd	nd
OR Quart 4	>3.1	nd	>3.1	1.7	nd	5.2	nd	nd	nd
p Value	<0.33	nd	<0.33	0.47	nd	0.13	nd	nd	nd
95% CI of OR Quart4	>0.32 na	nd	>0.32 na	0.40 7.3	nd	0.60 45	nd	nd	nd

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sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.689	11.6	0.689	27.9	0.689	3.59
Average	8.05	33.9	8.05	45.6	8.05	7.16
Stdev	22.2	60.3	22.2	62.5	22.2	8.31
p(t-test)		6.7E-5		3.2E-10		0.92
Min	0.00804	0.0260	0.00804	0.534	0.00804	0.0260
Max	266	217	266	217	266	18.5
n (Samp)	932	13	932	16	932	7
n (Patient)	343	13	343	16	343	7

sCr only	0hr prior to AKI stage	24hr prior to AKI stage	48hr prior to AKI stage

	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.895	27.8	nd	nd	nd	nd
Average	8.75	52.6	nd	nd	nd	nd
Stdev	23.4	76.5	nd	nd	nd	nd
p(t-test)		2.0E-6	nd	nd	nd	nd
Min	0.00804	0.0260	nd	nd	nd	nd
Max	266	217	nd	nd	nd	nd
n (Samp)	969	7	nd	nd	nd	nd
n (Patient)	353	7	nd	nd	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.805	9.01	0.805	28.6	nd	nd
Average	8.30	46.4	8.30	49.6	nd	nd
Stdev	22.4	75.1	22.4	66.0	nd	nd
p(t-test)		4.9E-6		1.8E-10	nd	nd
Min	0.00804	0.0260	0.00804	0.534	nd	nd
Max	266	217	266	217	nd	nd
n (Samp)	797	8	797	14	nd	nd
n (Patient)	260	8	260	14	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.63	0.73	0.62	0.83	nd	0.82	0.59	nd	nd
SE	0.084	0.11	0.11	0.064	nd	0.069	0.11	nd	nd
p	0.13	0.039	0.25	2.1E-7	nd	2.6E-6	0.44	nd	nd
nCohort 1	932	969	797	932	nd	797	932	nd	nd
nCohort 2	13	7	8	16	nd	14	7	nd	nd
Cutoff 1	0.00804	11.6	0.00804	6.36	nd	11.6	0.135	nd	nd
Sens 1	100%	71%	100%	75%	nd	71%	71%	nd	nd
Spec 1	0%	82%	0%	75%	nd	83%	43%	nd	nd
Cutoff 2	0.00804	0.00804	0.00804	1.64	nd	1.54	0.00804	nd	nd
Sens 2	100%	100%	100%	81%	nd	86%	100%	nd	nd
Spec 2	0%	0%	0%	56%	nd	54%	0%	nd	nd
Cutoff 3	0.00804	0.00804	0.00804	1.45	nd	1.45	0.00804	nd	nd
Sens 3	100%	100%	100%	94%	nd	93%	100%	nd	nd
Spec 3	0%	0%	0%	54%	nd	53%	0%	nd	nd
Cutoff 4	4.46	5.17	4.82	4.46	nd	4.82	4.46	nd	nd
Sens 4	54%	71%	50%	75%	nd	71%	43%	nd	nd
Spec 4	70%	70%	70%	70%	nd	70%	70%	nd	nd
Cutoff 5	8.41	9.64	8.68	8.41	nd	8.68	8.41	nd	nd
Sens 5	54%	71%	50%	69%	nd	71%	43%	nd	nd
Spec 5	80%	80%	80%	80%	nd	80%	80%	nd	nd
Cutoff 6	19.3	20.9	20.1	19.3	nd	20.1	19.3	nd	nd
Sens 6	38%	57%	38%	62%	nd	64%	0%	nd	nd
Spec 6	90%	90%	90%	90%	nd	90%	90%	nd	nd
OR Quart 2	0	>2.0	>3.0	>1.0	nd	>1.0	0.50	nd	nd
p Value	na	<0.57	<0.34	<1.00	nd	<1.0	0.57	nd	nd
95% CI of	na	>0.18	>0.31	>0.062	nd	>0.062	0.045	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
OR Quart2	na	na	na	na	nd	na	5.5	nd	nd
OR Quart 3	0.20	>0	>1.0	>4.1	nd	>3.0	0.50	nd	nd
p Value	0.14	<na	<1.00	<0.21	nd	<0.34	0.57	nd	nd
95% CI of	0.023	>na	>0.062	>0.45	nd	>0.31	0.045	nd	nd
OR Quart3	1.7	na	na	na	nd	na	5.5	nd	nd
OR Quart 4	1.4	>5.1	>4.1	>12	nd	>10	1.5	nd	nd
p Value	0.57	<0.14	<0.21	<0.020	nd	<0.026	0.66	nd	nd
95% CI of	0.44	>0.59	>0.45	>1.5	nd	>1.3	0.25	nd	nd
OR Quart4	4.5	na	na	na	nd	na	9.1	nd	nd

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sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	384	670	384	3140	nd	nd
Average	850	1270	850	4360	nd	nd
Stdev	1270	1520	1270	6140	nd	nd
p(t-test)		0.39		6.2E-11	nd	nd
Min	1.00E-9	78.9	1.00E-9	226	nd	nd
Max	9810	4050	9810	21100	nd	nd
n (Samp)	322	7	322	10	nd	nd
n (Patient)	188	7	188	10	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	nd	nd	396	3330	nd	nd
Average	nd	nd	905	4940	nd	nd
Stdev	nd	nd	1320	6790	nd	nd
p(t-test)	nd	nd		1.3E-10	nd	nd
Min	nd	nd	1.00E-9	226	nd	nd
Max	nd	nd	9810	21100	nd	nd
n (Samp)	nd	nd	280	8	nd	nd
n (Patient)	nd	nd	157	8	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.60	nd	nd	0.82	nd	0.81	nd	nd	nd
SE	0.11	nd	nd	0.083	nd	0.093	nd	nd	nd
p	0.38	nd	nd	1.3E-4	nd	8.4E-4	nd	nd	nd
nCohort 1	322	nd	nd	322	nd	280	nd	nd	nd
nCohort 2	7	nd	nd	10	nd	8	nd	nd	nd
Cutoff 1	310	nd	nd	1480	nd	1480	nd	nd	nd
Sens 1	71%	nd	nd	70%	nd	75%	nd	nd	nd
Spec 1	46%	nd	nd	81%	nd	80%	nd	nd	nd
Cutoff 2	97.3	nd	nd	667	nd	407	nd	nd	nd
Sens 2	86%	nd	nd	80%	nd	88%	nd	nd	nd
Spec 2	24%	nd	nd	66%	nd	51%	nd	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Cutoff 3	69.5	nd	nd	407	nd	225	nd	nd	nd
Sens 3	100%	nd	nd	90%	nd	100%	nd	nd	nd
Spec 3	22%	nd	nd	53%	nd	34%	nd	nd	nd
Cutoff 4	804	nd	nd	804	nd	865	nd	nd	nd
Sens 4	43%	nd	nd	70%	nd	75%	nd	nd	nd
Spec 4	70%	nd	nd	70%	nd	70%	nd	nd	nd
Cutoff 5	1440	nd	nd	1440	nd	1520	nd	nd	nd
Sens 5	29%	nd	nd	70%	nd	62%	nd	nd	nd
Spec 5	80%	nd	nd	80%	nd	80%	nd	nd	nd
Cutoff 6	2280	nd	nd	2280	nd	2350	nd	nd	nd
Sens 6	29%	nd	nd	60%	nd	62%	nd	nd	nd
Spec 6	90%	nd	nd	90%	nd	90%	nd	nd	nd
OR Quart 2	0.49	nd	nd	>1.0	nd	>2.1	nd	nd	nd
p Value	0.57	nd	nd	<0.99	nd	<0.56	nd	nd	nd
95% CI of	0.044	nd	nd	>0.062	nd	>0.18	nd	nd	nd
OR Quart2	5.6	nd	nd	na	nd	na	nd	nd	nd
OR Quart 3	1.0	nd	nd	>2.0	nd	>0	nd	nd	nd
p Value	1.0	nd	nd	<0.56	nd	<na	nd	nd	nd
95% CI of	0.14	nd	nd	>0.18	nd	>na	nd	nd	nd
OR Quart3	7.3	nd	nd	na	nd	na	nd	nd	nd
OR Quart 4	0.99	nd	nd	>7.6	nd	>6.5	nd	nd	nd
p Value	0.99	nd	nd	<0.060	nd	<0.086	nd	nd	nd
95% CI of	0.14	nd	nd	>0.92	nd	>0.77	nd	nd	nd
OR Quart4	7.2	nd	nd	na	nd	na	nd	nd	nd

Cathepsin B

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.632	1.86	0.632	2.76	nd	nd
Average	2.59	3.31	2.59	13.5	nd	nd
Stdev	8.00	4.58	8.00	20.9	nd	nd
p(t-test)		0.81		9.4E-5	nd	nd
Min	1.00E-9	1.00E-9	1.00E-9	1.00E-9	nd	nd
Max	78.1	12.7	78.1	64.9	nd	nd
n (Samp)	339	7	339	10	nd	nd
n (Patient)	194	7	194	10	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	nd	nd	0.726	2.35	nd	nd
Average	nd	nd	2.90	13.5	nd	nd
Stdev	nd	nd	8.59	23.0	nd	nd
p(t-test)	nd	nd		0.0014	nd	nd
Min	nd	nd	1.00E-9	1.00E-9	nd	nd
Max	nd	nd	78.1	64.9	nd	nd
n (Samp)	nd	nd	295	8	nd	nd
n (Patient)	nd	nd	162	8	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.58	nd	nd	0.75	nd	0.70	nd	nd	nd
SE	0.11	nd	nd	0.091	nd	0.11	nd	nd	nd
p	0.51	nd	nd	0.0065	nd	0.059	nd	nd	nd
nCohort 1	339	nd	nd	339	nd	295	nd	nd	nd
nCohort 2	7	nd	nd	10	nd	8	nd	nd	nd
Cutoff 1	0.397	nd	nd	1.39	nd	0.973	nd	nd	nd
Sens 1	71%	nd	nd	70%	nd	75%	nd	nd	nd
Spec 1	38%	nd	nd	70%	nd	58%	nd	nd	nd
Cutoff 2	0.0840	nd	nd	0.973	nd	0.924	nd	nd	nd
Sens 2	86%	nd	nd	80%	nd	88%	nd	nd	nd
Spec 2	15%	nd	nd	60%	nd	57%	nd	nd	nd
Cutoff 3	0	nd	nd	0.924	nd	0	nd	nd	nd
Sens 3	100%	nd	nd	90%	nd	100%	nd	nd	nd
Spec 3	0%	nd	nd	59%	nd	0%	nd	nd	nd
Cutoff 4	1.41	nd	nd	1.41	nd	1.57	nd	nd	nd
Sens 4	57%	nd	nd	60%	nd	50%	nd	nd	nd
Spec 4	70%	nd	nd	70%	nd	70%	nd	nd	nd
Cutoff 5	2.48	nd	nd	2.48	nd	2.74	nd	nd	nd
Sens 5	43%	nd	nd	50%	nd	50%	nd	nd	nd
Spec 5	80%	nd	nd	80%	nd	80%	nd	nd	nd
Cutoff 6	5.07	nd	nd	5.07	nd	5.47	nd	nd	nd
Sens 6	29%	nd	nd	40%	nd	38%	nd	nd	nd
Spec 6	90%	nd	nd	90%	nd	90%	nd	nd	nd
OR Quart 2	0.49	nd	nd	0	nd	0	nd	nd	nd
p Value	0.56	nd	nd	na	nd	na	nd	nd	nd
95% CI of OR Quart2	0.043	nd	nd	na	nd	na	nd	nd	nd
	5.5	nd	nd	na	nd	na	nd	nd	nd
OR Quart 3	0.49	nd	nd	3.1	nd	3.0	nd	nd	nd
p Value	0.57	nd	nd	0.34	nd	0.34	nd	nd	nd
95% CI of OR Quart3	0.044	nd	nd	0.31	nd	0.31	nd	nd	nd
	5.6	nd	nd	30	nd	30	nd	nd	nd
OR Quart 4	1.5	nd	nd	6.3	nd	4.1	nd	nd	nd
p Value	0.66	nd	nd	0.092	nd	0.21	nd	nd	nd
95% CI of OR Quart4	0.24	nd	nd	0.74	nd	0.45	nd	nd	nd
	9.2	nd	nd	53	nd	38	nd	nd	nd

Dipeptidyl peptidase IV

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	197	1310	197	1010	nd	nd
Average	3440	2090	3440	2020	nd	nd
Stdev	42400	2430	42400	2600	nd	nd
p(t-test)		0.94		0.92	nd	nd
Min	0.200	0.651	0.200	10.1	nd	nd
Max	677000	5420	677000	7150	nd	nd
n (Samp)	255	6	255	8	nd	nd
n (Patient)	158	6	158	8	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	nd	nd	197	847	nd	nd
Average	nd	nd	706	1390	nd	nd
Stdev	nd	nd	1510	1830	nd	nd
p(t-test)	nd	nd		0.28	nd	nd
Min	nd	nd	0.200	10.1	nd	nd
Max	nd	nd	12500	4950	nd	nd
n (Samp)	nd	nd	221	6	nd	nd
n (Patient)	nd	nd	134	6	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.59	nd	nd	0.71	nd	0.67	nd	nd	nd
SE	0.12	nd	nd	0.10	nd	0.12	nd	nd	nd
p	0.49	nd	nd	0.049	nd	0.18	nd	nd	nd
nCohort 1	255	nd	nd	255	nd	221	nd	nd	nd
nCohort 2	6	nd	nd	8	nd	6	nd	nd	nd
Cutoff 1	52.3	nd	nd	337	nd	294	nd	nd	nd
Sens 1	83%	nd	nd	75%	nd	83%	nd	nd	nd
Spec 1	29%	nd	nd	58%	nd	57%	nd	nd	nd
Cutoff 2	52.3	nd	nd	294	nd	294	nd	nd	nd
Sens 2	83%	nd	nd	88%	nd	83%	nd	nd	nd
Spec 2	29%	nd	nd	56%	nd	57%	nd	nd	nd
Cutoff 3	0.202	nd	nd	9.55	nd	9.55	nd	nd	nd
Sens 3	100%	nd	nd	100%	nd	100%	nd	nd	nd
Spec 3	1%	nd	nd	11%	nd	12%	nd	nd	nd
Cutoff 4	623	nd	nd	623	nd	623	nd	nd	nd
Sens 4	50%	nd	nd	62%	nd	50%	nd	nd	nd
Spec 4	70%	nd	nd	70%	nd	70%	nd	nd	nd
Cutoff 5	1020	nd	nd	1020	nd	942	nd	nd	nd
Sens 5	50%	nd	nd	50%	nd	50%	nd	nd	nd
Spec 5	80%	nd	nd	80%	nd	80%	nd	nd	nd
Cutoff 6	1810	nd	nd	1810	nd	1580	nd	nd	nd
Sens 6	50%	nd	nd	25%	nd	17%	nd	nd	nd
Spec 6	90%	nd	nd	90%	nd	90%	nd	nd	nd
OR Quart 2	2.0	nd	nd	0	nd	0	nd	nd	nd
p Value	0.57	nd	nd	na	nd	na	nd	nd	nd
95% CI of	0.18	nd	nd	na	nd	na	nd	nd	nd
OR Quart2	23	nd	nd	na	nd	na	nd	nd	nd
OR Quart 3	0	nd	nd	3.0	nd	2.0	nd	nd	nd
p Value	na	nd	nd	0.34	nd	0.58	nd	nd	nd
95% CI of	na	nd	nd	0.31	nd	0.18	nd	nd	nd
OR Quart3	na	nd	nd	30	nd	23	nd	nd	nd
OR Quart 4	3.0	nd	nd	4.1	nd	3.1	nd	nd	nd
p Value	0.34	nd	nd	0.21	nd	0.34	nd	nd	nd
95% CI of	0.31	nd	nd	0.45	nd	0.31	nd	nd	nd
OR Quart4	30	nd	nd	38	nd	30	nd	nd	nd

[0147] Table 8: Comparison of marker levels in EDTA samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0, R, or I) and in EDTA samples collected from Cohort 2 (subjects who progress to RIFLE stage F) at 0, 24 hours, and 48 hours prior to the subject reaching RIFLE stage I.

Beta-2-microglobulin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	2.46	5.00	2.46	5.64	nd	nd
Average	3.12	6.46	3.12	7.49	nd	nd
Stdev	2.19	3.84	2.19	6.07	nd	nd
p(t-test)		3.9E-7		1.0E-9	nd	nd
Min	0.729	2.19	0.729	1.52	nd	nd
Max	16.7	14.6	16.7	22.7	nd	nd
n (Samp)	551	12	551	11	nd	nd
n (Patient)	213	12	213	11	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	2.54	6.19	2.54	5.64	nd	nd
Average	3.15	7.19	3.15	6.53	nd	nd
Stdev	2.22	3.93	2.22	3.65	nd	nd
p(t-test)		7.7E-7		1.1E-5	nd	nd
Min	0.729	3.32	0.729	1.52	nd	nd
Max	16.7	14.6	16.7	11.9	nd	nd
n (Samp)	464	8	464	9	nd	nd
n (Patient)	173	8	173	9	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.82	nd	0.88	0.78	nd	0.79	nd	nd	nd
SE	0.075	nd	0.079	0.083	nd	0.090	nd	nd	nd
p	1.7E-5	nd	1.8E-6	6.7E-4	nd	0.0011	nd	nd	nd
nCohort 1	551	nd	464	551	nd	464	nd	nd	nd
nCohort 2	12	nd	8	11	nd	9	nd	nd	nd
Cutoff 1	3.81	nd	4.59	4.43	nd	4.43	nd	nd	nd
Sens 1	75%	nd	75%	73%	nd	78%	nd	nd	nd
Spec 1	75%	nd	83%	82%	nd	82%	nd	nd	nd
Cutoff 2	3.31	nd	3.81	2.31	nd	2.31	nd	nd	nd
Sens 2	83%	nd	88%	82%	nd	89%	nd	nd	nd
Spec 2	68%	nd	74%	46%	nd	44%	nd	nd	nd
Cutoff 3	2.93	nd	3.31	2.27	nd	1.51	nd	nd	nd
Sens 3	92%	nd	100%	91%	nd	100%	nd	nd	nd
Spec 3	60%	nd	67%	44%	nd	18%	nd	nd	nd
Cutoff 4	3.48	nd	3.58	3.48	nd	3.58	nd	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Sens 4	75%	nd	88%	73%	nd	78%	nd	nd	nd
Spec 4	70%	nd	70%	70%	nd	70%	nd	nd	nd
Cutoff 5	4.31	nd	4.35	4.31	nd	4.35	nd	nd	nd
Sens 5	58%	nd	75%	73%	nd	78%	nd	nd	nd
Spec 5	80%	nd	80%	80%	nd	80%	nd	nd	nd
Cutoff 6	5.57	nd	5.57	5.57	nd	5.57	nd	nd	nd
Sens 6	42%	nd	50%	55%	nd	56%	nd	nd	nd
Spec 6	90%	nd	90%	90%	nd	90%	nd	nd	nd
OR Quart 2	>1.0	nd	>0	2.0	nd	1.0	nd	nd	nd
p Value	<1.0	nd	<na	0.57	nd	1.0	nd	nd	nd
95% CI of	>0.062	nd	>na	0.18	nd	0.062	nd	nd	nd
OR Quart2	na	nd	na	22	nd	16	nd	nd	nd
OR Quart 3	>3.0	nd	>2.0	0	nd	0	nd	nd	nd
p Value	<0.34	nd	<0.56	na	nd	na	nd	nd	nd
95% CI of	>0.31	nd	>0.18	na	nd	na	nd	nd	nd
OR Quart3	na	nd	na	na	nd	na	nd	nd	nd
OR Quart 4	>8.4	nd	>6.3	8.4	nd	7.3	nd	nd	nd
p Value	<0.046	nd	<0.090	0.047	nd	0.065	nd	nd	nd
95% CI of	>1.0	nd	>0.75	1.0	nd	0.89	nd	nd	nd
OR Quart4	na	nd	na	68	nd	60	nd	nd	nd

[0148]   Table 9: Comparison of marker levels in enroll urine samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0 or R within 48hrs) and in enroll urine samples collected from Cohort 2 (subjects reaching RIFLE stage I or F within 48hrs). Enroll samples from patients already at RIFLE stage I or F were included in Cohort 2.

Beta-2-microglobulin

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1.58	0.320	1.10	0.745	1.48	0.289
Average	2.51	1.59	2.33	2.38	2.49	1.43
Stdev	2.46	2.27	2.44	2.80	2.47	2.15
p(t-test)		0.046		0.95		0.034
Min	0.00152	0.00133	0.00152	0.00133	0.00152	0.00212
Max	6.10	6.07	6.10	5.80	6.10	6.07
n (Samp)	138	35	164	8	111	30
n (Patient)	138	35	164	8	111	30

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.37	0.49	0.35

| | At Enrollment | | |
	sCr or UO	sCr only	UO only
SE	0.055	0.11	0.060
p	0.017	0.93	0.014
nCohort 1	138	164	111
nCohort 2	35	8	30
Cutoff 1	0.0372	0.255	0.0372
Sens 1	71%	75%	70%
Spec 1	10%	35%	11%
Cutoff 2	0.0182	0.0627	0.0182
Sens 2	80%	88%	80%
Spec 2	5%	19%	5%
Cutoff 3	0.00270	0	0.00408
Sens 3	91%	100%	90%
Spec 3	1%	0%	3%
Cutoff 4	5.08	4.82	5.08
Sens 4	20%	38%	17%
Spec 4	70%	70%	70%
Cutoff 5	5.80	5.80	5.80
Sens 5	3%	0%	3%
Spec 5	95%	95%	95%
Cutoff 6	5.80	5.80	5.80
Sens 6	3%	0%	3%
Spec 6	95%	95%	95%
OR Quart 2	0.70	0	1.3
p Value	0.56	na	0.71
95% CI of	0.20	na	0.35
OR Quart2	2.4	na	4.7
OR Quart 3	1.6	1.0	1.8
p Value	0.39	1.0	0.33
95% CI of	0.55	0.19	0.54
OR Quart3	4.7	5.3	6.3
OR Quart 4	2.3	0.65	2.8
p Value	0.12	0.65	0.084
95% CI of	0.81	0.10	0.87
OR Quart4	6.5	4.1	9.3

C-X-C motif chemokine 2

| | sCr or UO | | sCr only | | UO only | |
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.129	5.17	0.279	17.3	0.137	4.46
Average	8.07	22.1	9.66	35.0	8.19	23.9
Stdev	26.5	45.2	29.3	59.8	26.5	47.2
p(t-test)		0.0012		0.0052		0.0013
Min	0.00804	0.0260	0.00804	0.0260	0.00804	0.0260
Max	266	217	266	217	266	217
n (Samp)	292	61	338	12	211	55
n (Patient)	292	61	338	12	211	55

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.66	0.77	0.66
SE	0.041	0.081	0.044
p	7.9E-5	9.7E-4	1.8E-4
nCohort 1	292	338	211
nCohort 2	61	12	55
Cutoff 1	0.297	6.27	0.297
Sens 1	70%	75%	71%
Spec 1	54%	75%	54%
Cutoff 2	0.00804	5.62	0.00804
Sens 2	100%	83%	100%
Spec 2	0%	72%	0%
Cutoff 3	0.00804	0.00804	0.00804
Sens 3	100%	100%	100%
Spec 3	0%	0%	0%
Cutoff 4	3.89	4.33	3.95
Sens 4	52%	83%	51%
Spec 4	70%	70%	70%
Cutoff 5	6.98	8.37	7.79
Sens 5	43%	67%	44%
Spec 5	80%	80%	80%
Cutoff 6	16.4	20.5	14.8
Sens 6	30%	50%	36%
Spec 6	90%	90%	90%
OR Quart 2	0.16	0	0.17
p Value	0.0046	na	0.0084
95% CI of	0.044	na	0.047
OR Quart2	0.57	na	0.64
OR Quart 3	0.85	1.0	1.0
p Value	0.69	1.0	1.0
95% CI of	0.39	0.14	0.43
OR Quart3	1.9	7.3	2.3
OR Quart 4	2.1	4.2	2.1
p Value	0.043	0.073	0.065
95% CI of	1.0	0.88	0.96
OR Quart4	4.2	21	4.5

Renin

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	346	1010	nd	nd	344	1250
Average	771	2410	nd	nd	800	2590
Stdev	1050	4300	nd	nd	1090	4450
p(t-test)		5.2E-4	nd	nd		7.8E-4
Min	1.00E-9	92.1	nd	nd	1.00E-9	92.1
Max	4830	21100	nd	nd	4830	21100
n (Samp)	109	24	nd	nd	90	22
n (Patient)	109	24	nd	nd	90	22

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.70	nd	0.72
SE	0.064	nd	0.066
p	0.0018	nd	0.0012
nCohort 1	109	nd	90
nCohort 2	24	nd	22
Cutoff 1	394	nd	394
Sens 1	71%	nd	73%
Spec 1	52%	nd	52%
Cutoff 2	310	nd	346
Sens 2	83%	nd	82%
Spec 2	48%	nd	51%
Cutoff 3	97.3	nd	114
Sens 3	92%	nd	91%
Spec 3	27%	nd	28%
Cutoff 4	730	nd	730
Sens 4	58%	nd	64%
Spec 4	71%	nd	70%
Cutoff 5	1440	nd	1440
Sens 5	42%	nd	45%
Spec 5	81%	nd	80%
Cutoff 6	2150	nd	2150
Sens 6	38%	nd	41%
Spec 6	91%	nd	90%
OR Quart 2	1.8	nd	1.8
p Value	0.46	nd	0.45
95% CI of	0.39	nd	0.39
OR Quart2	8.2	nd	8.4
OR Quart 3	2.2	nd	1.4
p Value	0.29	nd	0.69
95% CI of	0.51	nd	0.28
OR Quart3	9.8	nd	6.9
OR Quart 4	4.2	nd	4.6
p Value	0.045	nd	0.035
95% CI of	1.0	nd	1.1
OR Quart4	17	nd	19

Cathepsin B

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.640	1.39	nd	nd	0.760	1.39
Average	2.41	7.35	nd	nd	2.72	6.90
Stdev	5.89	14.4	nd	nd	6.66	14.6
p(t-test)		0.0073	nd	nd		0.046
Min	1.00E-9	1.00E-9	nd	nd	1.00E-9	1.00E-9
Max	51.8	64.9	nd	nd	51.8	64.9
n (Samp)	110	24	nd	nd	91	22

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
n (Patient)	110	24	nd	nd	91	22

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.64	nd	0.63
SE	0.066	nd	0.069
p	0.029	nd	0.061
nCohort 1	110	nd	91
nCohort 2	24	nd	22
Cutoff 1	0.604	nd	0.604
Sens 1	71%	nd	73%
Spec 1	50%	nd	47%
Cutoff 2	0.388	nd	0.460
Sens 2	83%	nd	82%
Spec 2	37%	nd	37%
Cutoff 3	0.148	nd	0.148
Sens 3	92%	nd	91%
Spec 3	18%	nd	18%
Cutoff 4	1.29	nd	1.29
Sens 4	54%	nd	55%
Spec 4	70%	nd	70%
Cutoff 5	2.34	nd	2.34
Sens 5	42%	nd	41%
Spec 5	80%	nd	80%
Cutoff 6	5.45	nd	7.18
Sens 6	25%	nd	23%
Spec 6	90%	nd	90%
OR Quart 2	1.2	nd	1.0
p Value	0.76	nd	1.0
95% CI of	0.30	nd	0.22
OR Quart2	5.1	nd	4.5
OR Quart 3	1.3	nd	1.3
p Value	0.72	nd	0.72
95% CI of	0.31	nd	0.31
OR Quart3	5.3	nd	5.5
OR Quart 4	3.0	nd	2.7
p Value	0.090	nd	0.14
95% CI of	0.84	nd	0.72
OR Quart4	11	nd	10

Neprilysin

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1.28	1.52	nd	nd	1.28	1.32
Average	6.20	17.1	nd	nd	5.94	19.1
Stdev	16.9	25.3	nd	nd	17.0	26.3
p(t-test)		0.11	nd	nd		0.074

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Min	0.0869	0.0203	nd	nd	0.0869	0.0203
Max	98.9	64.3	nd	nd	98.9	64.3
n (Samp)	50	9	nd	nd	42	8
n (Patient)	50	9	nd	nd	42	8

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.57	nd	0.57
SE	0.11	nd	0.11
p	0.51	nd	0.53
nCohort 1	50	nd	42
nCohort 2	9	nd	8
Cutoff 1	0.820	nd	0.820
Sens 1	78%	nd	75%
Spec 1	40%	nd	43%
Cutoff 2	0.238	nd	0.238
Sens 2	89%	nd	88%
Spec 2	10%	nd	10%
Cutoff 3	0	nd	0
Sens 3	100%	nd	100%
Spec 3	0%	nd	0%
Cutoff 4	1.68	nd	1.68
Sens 4	33%	nd	38%
Spec 4	70%	nd	71%
Cutoff 5	3.30	nd	3.50
Sens 5	33%	nd	38%
Spec 5	80%	nd	81%
Cutoff 6	10.8	nd	10.8
Sens 6	33%	nd	38%
Spec 6	90%	nd	90%
OR Quart 2	0.92	nd	0.91
p Value	0.94	nd	0.93
95% CI of	0.11	nd	0.11
OR Quart2	7.6	nd	7.7
OR Quart 3	0.92	nd	0.45
p Value	0.94	nd	0.54
95% CI of	0.11	nd	0.036
OR Quart3	7.6	nd	5.8
OR Quart 4	1.5	nd	1.5
p Value	0.69	nd	0.69
95% CI of	0.21	nd	0.20
OR Quart4	11	nd	11

Carbonic anhydrase IX

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.0111	0.0219	nd	nd	0.0113	0.0232

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Average	0.0193	0.114	nd	nd	0.0192	0.126
Stdev	0.0252	0.280	nd	nd	0.0259	0.297
p(t-test)		0.018	nd	nd		0.021
Min	0.000386	0.00345	nd	nd	0.00102	0.00345
Max	0.119	0.859	nd	nd	0.119	0.859
n (Samp)	50	9	nd	nd	42	8
n (Patient)	50	9	nd	nd	42	8

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.70	nd	0.71
SE	0.10	nd	0.11
p	0.054	nd	0.058
nCohort 1	50	nd	42
nCohort 2	9	nd	8
Cutoff 1	0.0179	nd	0.0179
Sens 1	78%	nd	75%
Spec 1	68%	nd	69%
Cutoff 2	0.0121	nd	0.0121
Sens 2	89%	nd	88%
Spec 2	58%	nd	57%
Cutoff 3	0.00296	nd	0.00296
Sens 3	100%	nd	100%
Spec 3	26%	nd	24%
Cutoff 4	0.0205	nd	0.0205
Sens 4	56%	nd	50%
Spec 4	70%	nd	71%
Cutoff 5	0.0284	nd	0.0274
Sens 5	22%	nd	38%
Spec 5	80%	nd	81%
Cutoff 6	0.0421	nd	0.0358
Sens 6	11%	nd	12%
Spec 6	90%	nd	90%
OR Quart 2	0	nd	0
p Value	na	nd	na
95% CI of	na	nd	na
OR Quart2	na	nd	na
OR Quart 3	6.5	nd	3.7
p Value	0.11	nd	0.29
95% CI of	0.65	nd	0.32
OR Quart3	65	nd	42
OR Quart 4	3.2	nd	4.9
p Value	0.33	nd	0.19
95% CI of	0.30	nd	0.46
OR Quart4	36	nd	52

Dipeptidyl peptidase IV

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	130	793	nd	nd	129	1050
Average	513	1820	nd	nd	486	2010
Stdev	904	3030	nd	nd	840	3170
p(t-test)		8.5E-4	nd	nd		4.7E-4
Min	0.924	0.651	nd	nd	0.924	10.1
Max	5010	12500	nd	nd	5010	12500
n (Samp)	89	18	nd	nd	74	16
n (Patient)	89	18	nd	nd	74	16

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.67	nd	0.71
SE	0.075	nd	0.078
p	0.027	nd	0.0079
nCohort 1	89	nd	74
nCohort 2	18	nd	16
Cutoff 1	173	nd	161
Sens 1	72%	nd	75%
Spec 1	56%	nd	57%
Cutoff 2	18.2	nd	48.0
Sens 2	83%	nd	81%
Spec 2	22%	nd	35%
Cutoff 3	8.88	nd	15.1
Sens 3	94%	nd	94%
Spec 3	12%	nd	22%
Cutoff 4	457	nd	457
Sens 4	61%	nd	62%
Spec 4	71%	nd	70%
Cutoff 5	837	nd	698
Sens 5	50%	nd	56%
Spec 5	81%	nd	81%
Cutoff 6	1810	nd	1810
Sens 6	28%	nd	31%
Spec 6	91%	nd	91%
OR Quart 2	0.21	nd	0.29
p Value	0.18	nd	0.30
95% CI of	0.022	nd	0.028
OR Quart2	2.0	nd	3.0
OR Quart 3	0.96	nd	1.0
p Value	0.95	nd	1.0
95% CI of	0.21	nd	0.18
OR Quart3	4.3	nd	5.6
OR Quart 4	2.8	nd	4.1
p Value	0.14	nd	0.063
95% CI of	0.73	nd	0.93
OR Quart4	10	nd	18

[0149]   Table 10: Comparison of marker levels in enroll EDTA samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0 or R within 48hrs) and in enroll EDTA samples collected from Cohort 2 (subjects reaching RIFLE stage I or

F within 48hrs). Enroll samples from patients already at stage I or F were included in Cohort 2.

Beta-2-microglobulin

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	2.23	3.61	2.34	4.39	2.30	3.64
Average	3.01	4.59	3.14	6.99	2.96	4.13
Stdev	2.15	4.09	2.26	6.39	2.14	2.79
p(t-test)		0.0018		2.7E-5		0.014
Min	0.824	1.38	0.824	2.19	0.824	1.38
Max	13.8	22.7	13.8	22.7	13.8	11.3
n (Samp)	139	36	165	9	110	31
n (Patient)	139	36	165	9	110	31

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.64	0.79	0.64
SE	0.054	0.092	0.059
p	0.0077	0.0017	0.020
nCohort 1	139	165	110
nCohort 2	36	9	31
Cutoff 1	2.19	3.52	2.19
Sens 1	72%	78%	71%
Spec 1	49%	71%	47%
Cutoff 2	1.81	2.23	1.76
Sens 2	81%	89%	81%
Spec 2	31%	48%	28%
Cutoff 3	1.51	2.18	1.50
Sens 3	94%	100%	94%
Spec 3	21%	46%	22%
Cutoff 4	3.15	3.48	3.00
Sens 4	53%	78%	55%
Spec 4	71%	70%	70%
Cutoff 5	4.30	4.41	4.15
Sens 5	36%	44%	39%
Spec 5	81%	80%	80%
Cutoff 6	5.95	6.04	5.49
Sens 6	22%	44%	26%
Spec 6	91%	91%	90%
OR Quart 2	1.2	>2.0	0.81
p Value	0.80	<0.56	0.74
95% CI of	0.36	>0.18	0.22
OR Quart2	3.8	na	2.9
OR Quart 3	1.6	>1.0	1.2
p Value	0.42	<0.99	0.76
95% CI of	0.51	>0.062	0.36
OR Quart3	4.9	na	4.0

	At Enrollment		
	sCr or UO	sCr only	UO only
OR Quart 4	2.9	>6.8	2.7
p Value	0.053	<0.082	0.076
95% CI of	0.99	>0.78	0.90
OR Quart4	8.4	na	8.3

Renin

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	3930	3630	nd	nd	4300	3630
Average	4190	5880	nd	nd	4310	5880
Stdev	1810	6690	nd	nd	1930	6690
p(t-test)		0.15	nd	nd		0.22
Min	1840	2100	nd	nd	1840	2100
Max	10400	23300	nd	nd	10400	23300
n (Samp)	43	9	nd	nd	36	9
n (Patient)	43	9	nd	nd	36	9

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.50	nd	0.48
SE	0.11	nd	0.11
p	0.99	nd	0.89
nCohort 1	43	nd	36
nCohort 2	9	nd	9
Cutoff 1	2560	nd	2560
Sens 1	78%	nd	78%
Spec 1	23%	nd	25%
Cutoff 2	2110	nd	2110
Sens 2	89%	nd	89%
Spec 2	9%	nd	11%
Cutoff 3	2100	nd	2100
Sens 3	100%	nd	100%
Spec 3	7%	nd	8%
Cutoff 4	4690	nd	4900
Sens 4	22%	nd	22%
Spec 4	72%	nd	72%
Cutoff 5	5340	nd	5550
Sens 5	22%	nd	22%
Spec 5	81%	nd	81%
Cutoff 6	6480	nd	6490
Sens 6	11%	nd	11%
Spec 6	91%	nd	92%
OR Quart 2	1.0	nd	1.1
p Value	1.0	nd	0.92
95% CI of	0.12	nd	0.13
OR Quart2	8.4	nd	9.6
OR Quart 3	1.0	nd	1.9

	At Enrollment		
	sCr or UO	sCr only	UO only
p Value	1.0	nd	0.54
95% CI of	0.12	nd	0.25
OR Quart3	8.4	nd	14
OR Quart 4	1.6	nd	1.1
p Value	0.62	nd	0.92
95% CI of	0.23	nd	0.13
OR Quart4	12	nd	9.6

Neprilysin

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.607	0.869	nd	nd	0.605	0.869
Average	0.878	1.43	nd	nd	0.862	1.43
Stdev	0.838	1.32	nd	nd	0.864	1.32
p(t-test)		0.12	nd	nd		0.12
Min	0.0682	0.312	nd	nd	0.0682	0.312
Max	3.76	4.43	nd	nd	3.76	4.43
n (Samp)	42	9	nd	nd	36	9
n (Patient)	42	9	nd	nd	36	9

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.66	nd	0.68
SE	0.11	nd	0.11
p	0.14	nd	0.095
nCohort 1	42	nd	36
nCohort 2	9	nd	9
Cutoff 1	0.685	nd	0.684
Sens 1	78%	nd	78%
Spec 1	69%	nd	72%
Cutoff 2	0.337	nd	0.337
Sens 2	89%	nd	89%
Spec 2	10%	nd	8%
Cutoff 3	0.308	nd	0.308
Sens 3	100%	nd	100%
Spec 3	5%	nd	6%
Cutoff 4	0.806	nd	0.684
Sens 4	67%	nd	78%
Spec 4	71%	nd	72%
Cutoff 5	0.992	nd	0.821
Sens 5	33%	nd	67%
Spec 5	81%	nd	81%
Cutoff 6	1.84	nd	1.84
Sens 6	33%	nd	33%
Spec 6	90%	nd	92%
OR Quart 2	0	nd	0
p Value	na	nd	na

	At Enrollment		
	sCr or UO	sCr only	UO only
95% CI of	na	nd	na
OR Quart2	na	nd	na
OR Quart 3	2.2	nd	1.0
p Value	0.42	nd	1.0
95% CI of	0.33	nd	0.11
OR Quart3	15	nd	8.7
OR Quart 4	1.5	nd	3.2
p Value	0.69	nd	0.23
95% CI of	0.20	nd	0.47
OR Quart4	11	nd	22

Carbonic anhydrase IX

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.116	0.113	nd	nd	0.122	0.113
Average	0.145	0.340	nd	nd	0.150	0.340
Stdev	0.132	0.598	nd	nd	0.138	0.598
p(t-test)		0.053	nd	nd		0.083
Min	0.0202	0.0472	nd	nd	0.0202	0.0472
Max	0.657	1.90	nd	nd	0.657	1.90
n (Samp)	43	9	nd	nd	36	9
n (Patient)	43	9	nd	nd	36	9

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.51	nd	0.49
SE	0.11	nd	0.11
p	0.95	nd	0.95
nCohort 1	43	nd	36
nCohort 2	9	nd	9
Cutoff 1	0.0653	nd	0.0579
Sens 1	78%	nd	78%
Spec 1	21%	nd	19%
Cutoff 2	0.0472	nd	0.0472
Sens 2	89%	nd	89%
Spec 2	14%	nd	14%
Cutoff 3	0.0467	nd	0.0467
Sens 3	100%	nd	100%
Spec 3	14%	nd	14%
Cutoff 4	0.147	nd	0.155
Sens 4	33%	nd	33%
Spec 4	72%	nd	72%
Cutoff 5	0.183	nd	0.183
Sens 5	33%	nd	33%
Spec 5	81%	nd	81%
Cutoff 6	0.241	nd	0.241
Sens 6	33%	nd	33%

	At Enrollment		
	sCr or UO	sCr only	UO only
Spec 6	91%	nd	92%
OR Quart 2	0.61	nd	0.30
p Value	0.62	nd	0.33
95% CI of	0.083	nd	0.026
OR Quart2	4.4	nd	3.4
OR Quart 3	0.28	nd	0.67
p Value	0.30	nd	0.69
95% CI of	0.025	nd	0.089
OR Quart3	3.1	nd	5.0
OR Quart 4	1.0	nd	1.1
p Value	1.0	nd	0.90
95% CI of	0.16	nd	0.17
OR Quart4	6.2	nd	7.2

[0150]   While the invention has been described and exemplified in sufficient detail for those skilled in this art to make and use it, various alternatives, modifications, and improvements should be apparent without departing from the spirit and scope of the invention. The examples provided herein are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Modifications therein and other uses will occur to those skilled in the art. These modifications are encompassed within the spirit of the invention and are defined by the scope of the claims.

[0151]   It will be readily apparent to a person skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

[0152]   All patents and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

[0153]   The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as

terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[0154]   Other embodiments are set forth within the following claims.

We claim:

1.   A method for evaluating renal status in a subject, comprising:

performing one or more assays configured to detect one or more biomarkers selected from the group consisting of Cathepsin B, Renin, soluble Dipeptidyl Peptidase IV, soluble Neprilysin, Beta-2-microglobulin, soluble Carbonic anhydrase IX, and C-X-C motif chemokine 2 on a body fluid sample obtained from the subject to provide an assay result; and

correlating the assay result(s) to the renal status of the subject.

2.   A method according to claim 1, wherein said correlation step comprises correlating the assay result(s) to one or more of risk stratification, diagnosis, staging, prognosis, classifying and monitoring of the renal status of the subject.

3.   A method according to claim 1, wherein said correlating step comprises assigning a likelihood of one or more future changes in renal status to the subject based on the assay result(s).

4.   A method according to claim 3, wherein said one or more future changes in renal status comprise one or more of a future injury to renal function, future reduced renal function, future improvement in renal function, and future acute renal failure (ARF).

5.   A method according to one of claims 1-4, wherein said assay results comprise at least 2, 3, 4, or 5 of:

a measured concentration of Cathepsin B,

a measured concentration of Renin,

a measured concentration of soluble Dipeptidyl Peptidase IV,

a measured concentration of soluble Neprilysin,

a measured concentration of Beta-2-microglobulin,

a measured concentration of soluble Carbonic anhydrase IX, and

a measured concentration of C-X-C motif chemokine 2.

6.   A method according to one of claims 1-5, wherein a plurality of assay results are combined using a function that converts the plurality of assay results into a single composite result.

7.   A method according to claim 3, wherein said one or more future changes in renal status comprise a clinical outcome related to a renal injury suffered by the subject.

8.   A method according to claim 3, wherein the likelihood of one or more future changes in renal status is that an event of interest is more or less likely to occur within 30 days of the time at which the body fluid sample is obtained from the subject.

9.   A method according to claim 8, wherein the likelihood of one or more future changes in renal status is that an event of interest is more or less likely to occur within a period selected from the group consisting of 21 days, 14 days, 7 days, 5 days, 96 hours, 72 hours, 48 hours, 36 hours, 24 hours, and 12 hours.

10.   A method according to one of claims 1-5, wherein the subject is selected for evaluation of renal status based on the pre-existence in the subject of one or more known risk factors for prerenal, intrinsic renal, or postrenal ARF.

11.   A method according to one of claims 1-5, wherein the subject is selected for evaluation of renal status based on an existing diagnosis of one or more of congestive heart failure, preeclampsia, eclampsia, diabetes mellitus, hypertension, coronary artery disease, proteinuria, renal insufficiency, glomerular filtration below the normal range, cirrhosis, serum creatinine above the normal range, sepsis, injury to renal function, reduced renal function, or ARF, or based on undergoing or having undergone major vascular surgery, coronary artery bypass, or other cardiac surgery, or based on exposure to NSAIDs, cyclosporines, tacrolimus, aminoglycosides, foscarnet, ethylene glycol, hemoglobin, myoglobin, ifosfamide, heavy metals, methotrexate, radiopaque contrast agents, or streptozotocin.

12.   A method according to one of claims 1-5, wherein said correlating step comprises assigning a diagnosis of the occurrence or nonoccurrence of one or more of an injury to renal function, reduced renal function, or ARF to the subject based on the assay result(s).

13.   A method according to one of claims 1-5, wherein said correlating step comprises assessing whether or not renal function is improving or worsening in a subject who has suffered from an injury to renal function, reduced renal function, or ARF based on the assay result(s).

14.   A method according to one of claims 1-5, wherein said method is a method of diagnosing the occurrence or nonoccurrence of an injury to renal function in said subject.

15.   A method according to one of claims 1-5, wherein said method is a method of diagnosing the occurrence or nonoccurrence of reduced renal function in said subject.

16.   A method according to one of claims 1-5, wherein said method is a method of diagnosing the occurrence or nonoccurrence of acute renal failure in said subject.

17.   A method according to one of claims 1-5, wherein said method is a method of diagnosing the occurrence or nonoccurrence of a need for renal replacement therapy in said subject.

18.   A method according to one of claims 1-5, wherein said method is a method of diagnosing the occurrence or nonoccurrence of a need for renal transplantation in said subject.

19.   A method according to one of claims 1-5, wherein said method is a method of assigning a risk of the future occurrence or nonoccurrence of an injury to renal function in said subject.

20.   A method according to one of claims 1-5, wherein said method is a method of assigning a risk of the future occurrence or nonoccurrence of reduced renal function in said subject.

21.   A method according to one of claims 1-5, wherein said method is a method of assigning a risk of the future occurrence or nonoccurrence of acute renal failure in said subject.

22.   A method according to one of claims 1-5, wherein said method is a method of assigning a risk of the future occurrence or nonoccurrence of a need for renal replacement therapy in said subject.

23.   A method according to one of claims 1-5, wherein said method is a method of assigning a risk of the future occurrence or nonoccurrence of a need for renal transplantation in said subject.

24.   A method according to one of claims 1-5, wherein said one or more future changes in renal status comprise one or more of a future injury to renal function, future reduced renal function, future improvement in renal function, and future acute renal failure (ARF) within 72 hours of the time at which the body fluid sample is obtained.

25.   A method according to one of claims 1-5, wherein said one or more future changes in renal status comprise one or more of a future injury to renal function, future

reduced renal function, future improvement in renal function, and future acute renal failure (ARF) within 48 hours of the time at which the body fluid sample is obtained.

26.   A method according to one of claims 1-5, wherein said one or more future changes in renal status comprise one or more of a future injury to renal function, future reduced renal function, future improvement in renal function, and future acute renal failure (ARF) within 24 hours of the time at which the body fluid sample is obtained.

27.   A method according to one of claims 1-5, wherein the subject is in RIFLE stage 0 or R.

28.   A method according to claim 27, wherein the subject is in RIFLE stage 0, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage R, I or F within 72 hours.

29.   A method according to claim 28, wherein the subject is in RIFLE stage 0, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 72 hours.

30.   A method according to claim 28, wherein the subject is in RIFLE stage 0, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 72 hours.

31.   A method according to claim 27, wherein the subject is in RIFLE stage 0 or R, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 72 hours.

32.   A method according to claim 31, wherein the subject is in RIFLE stage 0 or R, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 72 hours.

33.   A method according to claim 27, wherein the subject is in RIFLE stage R, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 72 hours.

34.   A method according to claim 33, wherein the subject is in RIFLE stage R, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 72 hours.

35.   A method according to one of claims 1-5, wherein the subject is in RIFLE stage 0, R, or I, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 72 hours.

36.   A method according to claim 35, wherein the subject is in RIFLE stage I, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 72 hours.

37.   A method according to claim 28, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage R, I or F within 48 hours.

38.   A method according to claim 29, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 48 hours.

39.   A method according to claim 30, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 48 hours.

40.   A method according to claim 31, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 48 hours.

41.   A method according to claim 32, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 48 hours.

42.   A method according to claim 33, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 48 hours.

43.   A method according to claim 34, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 48 hours.

44.   A method according to claim 35, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 48 hours.

45.   A method according to claim 36, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 48 hours.

46.   A method according to claim 28, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage R, I or F within 24 hours.

47.   A method according to claim 29, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 24 hours.

48.   A method according to claim 30, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 24 hours.

49.   A method according to claim 31, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 24 hours.

50.   A method according to claim 32, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 24 hours.

51.   A method according to claim 33, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 24 hours.

52.   A method according to claim 34, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 24 hours.

53.   A method according to claim 35, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 24 hours.

54.   A method according to claim 36, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 24 hours.

55.   A method according to one of claims 1-5, wherein the subject is not in acute renal failure.

56.   A method according to one of claims 1-5, wherein the subject has not experienced a 1.5-fold or greater increase in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained.

57.   A method according to one of claims 1-5, wherein the subject has a urine output of at least 0.5 ml/kg/hr over the 6 hours preceding the time at which the body fluid sample is obtained.

58.   A method according to one of claims 1-5, wherein the subject has not experienced an increase of 0.3 mg/dL or greater in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained.

59.   A method according to one of claims 1-5, wherein the subject (i) has not experienced a 1.5-fold or greater increase in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained, (ii) has a urine output of at least 0.5 ml/kg/hr over the 6 hours preceding the time at which the body fluid sample is obtained, and (iii) has not experienced an increase of 0.3 mg/dL or greater in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained.

60.   A method according to one of claims 1-5, wherein the subject has not experienced a 1.5-fold or greater increase in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained.

61.   A method according to one of claims 1-5, wherein the subject has a urine output of at least 0.5 ml/kg/hr over the 6 hours preceding the time at which the body fluid sample is obtained.

62.   A method according to one of claims 1-5, wherein the subject (i) has not experienced a 1.5-fold or greater increase in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained, (ii) has a urine output of at least 0.5 ml/kg/hr over the 12 hours preceding the time at which the body fluid sample is obtained, and (iii) has not experienced an increase of 0.3 mg/dL or greater in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained.

63.   A method according to one of claims 1-5, wherein said correlating step comprises assigning one or more of: a likelihood that within 72 hours the subject will (i) experience a 1.5-fold or greater increase in serum creatinine (ii) have a urine output of less than 0.5 ml/kg/hr over a 6 hour period, or (iii) experience an increase of 0.3 mg/dL or greater in serum creatinine.

64.   A method according to claim 63, wherein said correlating step comprises assigning one or more of: a likelihood that within 48 hours the subject will (i) experience a 1.5-fold or greater increase in serum creatinine (ii) have a urine output of less than 0.5 ml/kg/hr over a 6 hour period, or (iii) experience an increase of 0.3 mg/dL or greater in serum creatinine.

65.   A method according to claim 63, wherein said correlating step comprises assigning one or more of: a likelihood that within 24 hours the subject will (i) experience a 1.5-fold or greater increase in serum creatinine (ii) have a urine output of less than 0.5 ml/kg/hr over a 6 hour period, or (iii) experience an increase of 0.3 mg/dL or greater in serum creatinine.

66.   A method according to claim 63, wherein said correlating step comprises assigning a likelihood that within 72 hours the subject will experience a 1.5-fold or greater increase in serum creatinine.

67.   A method according to claim 63, wherein said correlating step comprises assigning a likelihood that within 72 hours the subject will have a urine output of less than 0.5 ml/kg/hr over a 6 hour period.

68.   A method according to claim 63, wherein said correlating step comprises assigning a likelihood that within 72 hours the subject will experience an increase of 0.3 mg/dL or greater in serum creatinine.

69.   A method according to claim 63, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will experience a 1.5-fold or greater increase in serum creatinine.

70.   A method according to claim 63, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will have a urine output of less than 0.5 ml/kg/hr over a 6 hour period.

71.   A method according to claim 63, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will experience an increase of 0.3 mg/dL or greater in serum creatinine.

72.   A method according to claim 63, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will experience a 1.5-fold or greater increase in serum creatinine.

73.   A method according to claim 63, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will have a urine output of less than 0.5 ml/kg/hr over a 6 hour period.

74.   A method according to claim 63, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will experience an increase of 0.3 mg/dL or greater in serum creatinine.

75.   A method according to one of claims 1-5, wherein the subject has not experienced a 2-fold or greater increase in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained.

76.   A method according to one of claims 1-5, wherein the subject has a urine output of at least 0.5 ml/kg/hr over the 12 hours preceding the time at which the body fluid sample is obtained.

77.   A method according to one of claims 1-5, wherein the subject (i) has not experienced a 2-fold or greater increase in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained, (ii) has a urine output of at least 0.5 ml/kg/hr over the 2 hours preceding the time at which the body fluid sample is obtained, and (iii) has not experienced an increase of 0.3 mg/dL or greater in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained.

78.   A method according to one of claims 1-5, wherein the subject has not experienced a 3-fold or greater increase in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained.

79.   A method according to one of claims 1-5, wherein the subject has a urine output of at least 0.3 ml/kg/hr over the 24 hours preceding the time at which the body fluid sample is obtained, or anuria over the 12 hours preceding the time at which the body fluid sample is obtained.

80.   A method according to one of claims 1-5, wherein the subject (i) has not experienced a 3-fold or greater increase in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained, (ii) has a urine output of at least 0.3 ml/kg/hr over the 24 hours preceding the time at which the body fluid sample is obtained, or anuria over the 12 hours preceding the time at which the body fluid sample is obtained, and (iii) has not experienced an increase of 0.3 mg/dL or greater in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained.

81.   A method according to one of claims 1-5, wherein said correlating step comprises assigning one or more of: a likelihood that within 72 hours the subject will (i) experience a 2-fold or greater increase in serum creatinine (ii) have a urine output of less than 0.5 ml/kg/hr over a 12 hour period, or (iii) experience an increase of 0.3 mg/dL or greater in serum creatinine.

82.   A method according to claim 81, wherein said correlating step comprises assigning one or more of: a likelihood that within 48 hours the subject will (i) experience a 2-fold or greater increase in serum creatinine (ii) have a urine output of less than 0.5 ml/kg/hr over a 6 hour period, or (iii) experience an increase of 0.3 mg/dL or greater in serum creatinine.

83.   A method according to claim 81, wherein said correlating step comprises assigning one or more of: a likelihood that within 24 hours the subject will (i) experience a 2-fold or greater increase in serum creatinine, or (ii) have a urine output of less than 0.5 ml/kg/hr over a 6 hour period.

84.   A method according to claim 81, wherein said correlating step comprises assigning a likelihood that within 72 hours the subject will experience a 2-fold or greater increase in serum creatinine.

85.   A method according to claim 81, wherein said correlating step comprises assigning a likelihood that within 72 hours the subject will have a urine output of less than 0.5 ml/kg/hr over a 6 hour period.

86.   A method according to claim 81, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will experience a 2-fold or greater increase in serum creatinine.

87.   A method according to claim 81, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will have a urine output of less than 0.5 ml/kg/hr over a 6 hour period.

88.   A method according to claim 81, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will experience a 2-fold or greater increase in serum creatinine.

89.   A method according to claim 81, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will have a urine output of less than 0.5 ml/kg/hr over a 6 hour period.

90.   A method according to one of claims 1-5, wherein said correlating step comprises assigning one or more of: a likelihood that within 72 hours the subject will (i) experience a 3-fold or greater increase in serum creatinine, or (ii) have a urine output of less than 0.3 ml/kg/hr over a 24 hour period or anuria over a 12 hour period.

91.   A method according to claim 90, wherein said correlating step comprises assigning one or more of: a likelihood that within 48 hours the subject will (i) experience a 3-fold or greater increase in serum creatinine, or (ii) have a urine output of less than 0.3 ml/kg/hr over a 24 hour period or anuria over a 12 hour period.

92.   A method according to claim 90, wherein said correlating step comprises assigning one or more of: a likelihood that within 24 hours the subject will (i) experience a 3-fold or greater increase in serum creatinine, or (ii) have a urine output of less than 0.3 ml/kg/hr over a 24 hour period or anuria over a 12 hour period.

93.   A method according to claim 90, wherein said correlating step comprises assigning a likelihood that within 72 hours the subject will experience a 3-fold or greater increase in serum creatinine.

94.   A method according to claim 90, wherein said correlating step comprises assigning a likelihood that within 72 hours the subject will have a urine output of less than 0.3 ml/kg/hr over a 24 hour period or anuria over a 12 hour period.

95.   A method according to claim 90, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will experience a 3-fold or greater increase in serum creatinine.

96.   A method according to claim 90, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will have a urine output of less than 0.3 ml/kg/hr over a 24 hour period or anuria over a 12 hour period.

97.   A method according to claim 90, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will experience a 3-fold or greater increase in serum creatinine.

98.   A method according to claim 90, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will have a urine output of less than 0.3 ml/kg/hr over a 24 hour period or anuria over a 12 hour period.

99.   A method according to one of claims 1-98, wherein the body fluid sample is a urine sample.

100.   A method according to one of claims 1-99, wherein said method comprises performing assays that detect one, two or three, or more of Cathepsin B, Renin, soluble Dipeptidyl Peptidase IV, and C-X-C motif chemokine 2.

101.   Measurement of one or more biomarkers selected from the group consisting of Cathepsin B, Renin, soluble Dipeptidyl Peptidase IV, soluble Neprilysin, Beta-2-microglobulin, soluble Carbonic anhydrase IX, and C-X-C motif chemokine 2 for the evaluation of renal injury.

102.   Measurement of one or more biomarkers selected from the group consisting of Cathepsin B, Renin, soluble Dipeptidyl Peptidase IV, soluble Neprilysin, Beta-2-microglobulin, soluble Carbonic anhydrase IX, and C-X-C motif chemokine 2 for the evaluation of acute renal injury.

103.   A kit, comprising:

reagents for performing one or more assays configured to detect one or more kidney injury markers selected from the group consisting of Cathepsin B, Renin, soluble Dipeptidyl Peptidase IV, soluble Neprilysin, Beta-2-microglobulin, soluble Carbonic anhydrase IX, and C-X-C motif chemokine 2.

104.   A kit according to claim 103, wherein said reagents comprise one or more binding reagents, each of which specifically binds one of said of kidney injury markers.

105.   A kit according to claim 104, wherein a plurality of binding reagents are contained in a single assay device.

106.   A kit according to claim 103, wherein at least one of said assays is configured as a sandwich binding assay.

107.   A kit according to claim 103, wherein at least one of said assays is configured as a competitive binding assay.

108.   A kit according to one of claims 103-107, wherein said one or more assays comprise assays that detect one, two or three, or more of Cathepsin B, Renin, soluble Dipeptidyl Peptidase IV, soluble Neprilysin, Beta-2-microglobulin, soluble Carbonic anhydrase IX, and C-X-C motif chemokine 2.

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/53 (2011.01) USPC - 435/7.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols) IPC(8): G01N 33/53 (2011.01) USPC: 435/7.1
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC: 435/6; 435/7.94; 436/63
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) --- See continuation on extra sheet ----

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2006/083986 A2 (BARRETT et al.) 10 August 2006 (10.08.2006), pg 3, ln 24-26; pg 4, ln 11-16; pg 5, ln 1-3; pg 5, ln 4-7; pg 9, ln 13-16; pg 21, ln 4-5; pg 23, ln 6-12; pg 44, ln 31-33; pg 57, ln 9-11; pg 62, ln 22-27; pg 67, ln 17-22; pg 230; pg 249; Table 15. This document can be viewed by entering the doc number at the following url: http://ep.espacenet.com/numberSearch?locale=en_EP	1-5, 101-108 ------------------ 7-9
Y	US 2007/0248989 A1 (DEVARAJAN) 25 October 2007 (25.10.2007), abstract; para [0008], [0056], [0069].	7-9
X	KEHOE et al. Elevated Plasma Renin Activity Associated with Renal Dysfunction. Nephron 1986; 44:51-57 (abstract only)	1-5, 101-108

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 January 2011 (11.01.11)	08 FEB 2011
Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450	Lee W. Young
Facsimile No. 571-273-3201	PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

| Box No. II | Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet) |

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6 and 10-100
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

| Box No. III | Observations where unity of invention is lacking (Continuation of item 3 of first sheet) |

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

Continuation of (B) Fields Searched: Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) -

WEST (PGPB,USPT,EPAB,JPAB): biomarker, marker, renal, status, kidney, renin, future, event, change, clinical outcome, anderberg, HNFJ2, FLJ10761, REN, more or less likely, CTSB, APPS, APP secretase, cysteine protease, neprilysin, MME, DKFZp469N0914, B2M, GROb, Gro2, Mip2, Scyb, "MIP-2", Scyb2, "MIP-2a", "Mgsa-b", "CINC-2a", Cxcl2

Google Scholar: renal status biomarker renin cathepsin B neprilysin

esp@cenet: Astute, renal, Joseph Anderberg