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(54) **METHODS AND COMPOSITIONS FOR THE EVALUATION OF RENAL INJURY USING HYALURONIC ACID**

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(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 assays that detect one or more of hyaluronic acid (HA) as diagnostic and prognostic biomarker assays in renal injuries.

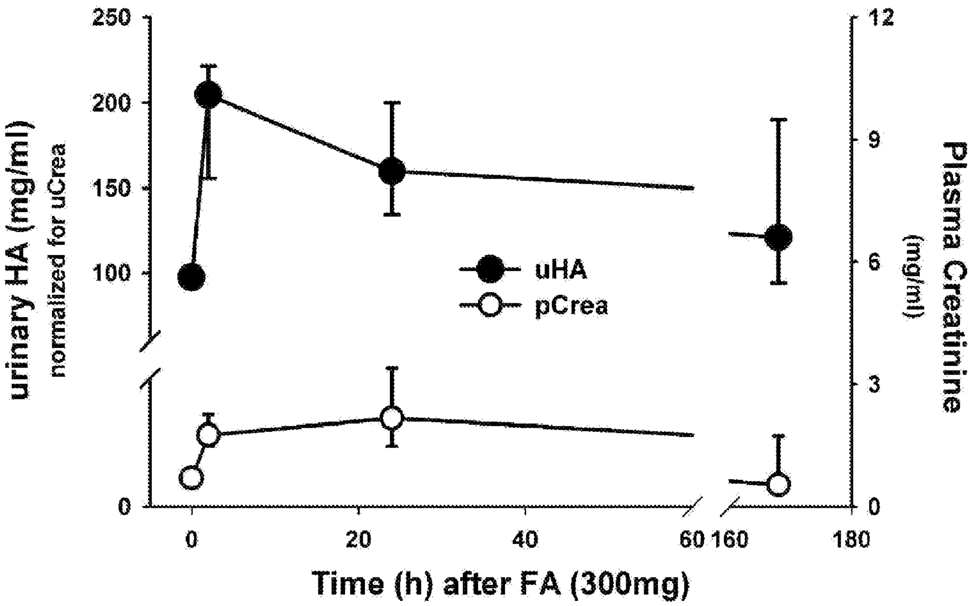
urinary HA (mg/ml)
normalized for uCrea
250
200
150
100
0
Plasma Creatinine
(mg/ml)
12
9
6
3
0
uHA
pCrea
0
20
40
60
160
180
Time (h) after FA (300mg)

FIGURE 1

METHODS AND COMPOSITIONS FOR THE EVALUATION OF RENAL INJURY USING HYALURONIC ACID

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a continuation-in-part of U.S. patent application Ser. No. 13/825,675 filed May 31, 2013, which is a national stage filing of PCT/US2011/053015 filed Sep. 23, 2011, and which claims priority to U.S. Provisional Patent Application 61/386,421 filed Sep. 24, 2010; and of U.S. patent application Ser. No. 13/517,244 filed Dec. 20, 2010, which is a national stage filing of PCT/US2010/061377 filed Dec. 20, 2010, and which claims priority to U.S. Provisional Patent Application 61/288,327 filed Dec. 20, 2009, U.S. Provisional Patent Application 61/308,861 filed Feb. 26, 2010, U.S. Provisional Patent Application 61/410,875 filed Nov. 6, 2010, U.S. Provisional Patent Application 61/410,878 filed Nov. 6, 2010, U.S. Provisional Patent Application 61/410,879 filed Nov. 6, 2010, and U.S. Provisional Patent Application 61/410,880 filed Nov. 6, 2010; each of which is hereby incorporated in its entirety including all tables, figures, and claims.

STATEMENT OF GOVERNMENTAL SUPPORT

[0002] This invention was made with government support under Grant/Contract No. 5R01DK070910-035R01DK070910-03 awarded by the National Institutes of Diabetes and Digestive and Kidney Diseases. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] 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.

[0004] 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".

[0005] 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,

-continued

Type	Risk Factors
	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 nephritis	Drug reaction (eg, β-lactams, NSAIDs, sulfonamides, 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

[0006] 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%.

[0007] 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 24-48 h) but reversible (peak 3-5 days, resolution within 1 week) rise in blood urea nitrogen and serum creatinine.

[0008] 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.

[0009] 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.

[0010] 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. For purposes of the present invention, "RIFLE stage 0" refers to a patient that does not fall within the RIFLE R, I or F criteria, and so is "pre-risk."

[0011] 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 (≥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.

[0012] 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.

[0013] 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.

[0014] 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

[0015] It is an object of the invention to provide methods and compositions for evaluating renal function in a subject. As described herein, measurement of the kidney injury markers described herein 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).

[0016] These kidney injury markers 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 initiation or continuation of 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.

[0017] 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 hyaluronic acid (HA) in a body fluid sample obtained from the subject. The assay result(s), for example a measured concentration of HA, is 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. Preferred subjects are those with relatively normal kidney function, including those not receiving renal replacement therapy. This includes subjects in RIFLE stage 0 or R at the time the sample being tested is obtained from the subject.

[0018] 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.

[0019] 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.

[0020] 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.

[0021] 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.

[0022] 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.

[0023] 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.

[0024] 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.

[0025] 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.

[0026] 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 a measured concentration of HA, is/are correlated to the occurrence or nonoccurrence of a change in renal status. The following are preferred diagnostic embodiments.

[0027] 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).

[0028] 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).

[0029] 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).

[0030] 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 non-occurrence 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).

[0031] 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).

[0032] 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 a measured concentration of HA, is/are correlated to the occurrence or nonoccurrence of a change in renal status. The following are preferred monitoring embodiments.

[0033] 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.

[0034] 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.

[0035] 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.

[0036] 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.

[0037] 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 a measured concentration of HA, is/are correlated to a particular class and/or subclass. The following are preferred classification embodiments.

[0038] 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.

[0039] 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.

[0040] 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.

[0041] 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.

[0042] 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.

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, 17*th* Ed., McGraw Hill, New York, pages 1741-1830, and Current Medical Diagnosis & Treatment 2008, 47*th* 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.

BRIEF DESCRIPTION OF THE FIGURES

[0051] FIG. **1** depicts the change in normalized urinary concentration of hyaluronic acid in response to a chemically induced acute kidney injury.

DETAILED DESCRIPTION OF THE INVENTION

[0052] 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 of the present invention.

[0053] The following is a brief description of the kidney injury marker of the present invention.

[0054] Hyaluronic acid (HA) is a ubiquitous connective tissue glycosaminoglycan that in vivo is present as a high molecular mass component of most extracellular matrices. Although HA is not a major constituent of the normal renal corticointerstitium,3 it is expressed around renal proximal tubular epithelial cells (PTC) after both acute and chronic renal injury that is caused by numerous diseases.4, 5 Furthermore, increased deposition of interstitial HA correlates with both proteinuria and renal function in progressive renal disease.6 Binding of HA to its principle receptor, CD44, promotes inflammation through interaction between HA and CD44, expressed on inflammatory cells.7 HA/CD44 binding activates the mitogen-activated protein kinase (MAPK) pathway and enhances PTC migration, a process that is implicated in epithelial cell-fibroblast transdifferentiation and progressive renal fibrosis.8 In ischemic kidneys from diabetic subjects, the renal HA-content started to increases already after 24 hours and significantly so 1-8 weeks after ischemia/reperfusion (I/R).9

[0055] For purposes of this document, the following definitions apply:

[0056] 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.

[0057] 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 (≥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).

[0058] 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 (≥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."

[0059] 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 quantitation). This list is not meant to be limiting.

[0060] As used herein, the term "relating a signal to the presence or amount" of an analyte reflects this 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. The skilled artisan will understand that the signals obtained from an assay are often 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 quantitation). This list is not meant to be limiting.

[0061] 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.

[0062] 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.

[0063] 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.

[0064] 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.

[0065] 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.

[0066] 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.

[0067] 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.

[0068] Marker Assays

[0069] 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. Pat. Nos. 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.

[0070] 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. Pat. Nos. 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.

[0071] 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.

[0072] 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.).

[0073] 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.

[0074] 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.

[0075] Antibodies

[0076] 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 CH1 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 CH1 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."

[0077] 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}.

[0078] 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.

[0079] 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.

[0080] 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. Pat. No. 6,057,098, which is hereby incorporated in its entirety, including all tables, figures, and claims.

[0081] 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.

[0082] 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.

[0083] Assay Correlations

[0084] 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.

[0085] 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.

[0086] 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.

[0087] 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.

[0088] 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.

[0089] 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.

[0090] 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.

[0091] 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

[0092] 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); Cadherin-3 (P07858); 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).

[0093] 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.

[0094] 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.

[0095] 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.

[0096] Diagnosis of Acute Renal Failure

[0097] 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:

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

[0098] 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.

[0099] 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.

[0100] 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:

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

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:

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

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:

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

[0101] 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.

[0102] 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).

[0103] Selecting a Treatment Regimen

[0104] 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.

[0105] 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.

Example 1

HA as a Diagnostic Marker of AKI

[0106] Urinary HA and plasma creatinine were measured in mice after administration of folic acid, a known nephrotoxin. Intraperitoneal injections of folic acid (FA, 300 mg/kg dissolved in $NaHCO_3$) was selected as a suitable dose to induce AKI (time=0 h) based on pilot studies which indicated that this dose was effective to cause increases in plasma creatinine levels indicative of AKI, but without FA leading to severe illness or death. Control animals received an equivalent volume of vehicle ($NaHCO_3$) i.p. Plasma creatinine and blood urea nitrogen (BUN) were measured to assess renal function using commercially available assays (creatinine kit from Diazyme (San Diego, Calif.), BUN kit from Sigma (St. Louis, Mo.)). Urinary HA levels were normalized by expressing the HA concentration per mg of urinary creatinine.

[0107] The results of this analysis are depicted in FIG. **1**. As can be seen, normalized HA levels are reflective of creatinine levels indicative of AKI in this induced AKI model system.

Example 2

Use of HA as a Prognostic and Diagnostic Marker

[0108] 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. HA was measured by standard immunoassay methods using commercially available assay reagents in the urine samples and the plasma component of the blood samples collected.

[0109] Two cohorts were defined as described in the introduction to each of the following tables. In the following tables, 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).

[0110] A receiver operating characteristic (ROC) curve was generated for HA and the area under each ROC curve (AUC) was 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 have included 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 have included 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 was used.

[0111] 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 were 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 were 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.

[0112] Various HA threshold (or "cutoff") concentrations were selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 were determined. OR is the odds ratio calculated for the particular cutoff concentration, and 95% CI is the confidence interval for the odds ratio.

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.

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
sCr or UO						
Median	979	1840	979	1280	979	1330
Average	1290	2010	1290	1870	1290	2030
Stdev	1090	1300	1090	1460	1090	1540
p (t-test)		2.3E−13		3.4E−8		3.0E−6
Min	41.6	151	41.6	77.8	41.6	126
Max	6400	5710	6400	6300	6400	5450
n (Samp)	570	189	570	170	570	58
n (Patient)	259	189	259	170	259	58
sCr only						
Median	1280	1600	1280	1550	1280	1150
Average	1700	1720	1700	1850	1700	1750
Stdev	1350	1120	1350	1290	1350	1440
p (t-test)		0.87		0.39		0.82

TABLE 1-continued

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.

Min	41.6	151	41.6	77.8	41.6	152
Max	6400	6400	6400	5710	6400	5910
n (Samp)	1322	59	1322	60	1322	36
n (Patient)	467	59	467	60	467	36
UO only						
Median	1040	2020	1040	1560	1040	1500
Average	1370	2230	1370	2090	1370	2130
Stdev	1130	1400	1130	1580	1130	1550
p (t-test)		4.7E−16		1.5E−10		6.0E−6
Min	41.6	168	41.6	91.1	41.6	126
Max	5540	6400	5540	6390	5540	6190
n (Samp)	587	173	587	161	587	54
n (Patient)	223	173	223	161	223	54

	0 hr prior to AKI stage			24 hr prior to AKI stage			48 hr 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	0.54	0.71	0.62	0.56	0.64	0.63	0.51	0.64
SE	0.024	0.039	0.024	0.025	0.039	0.026	0.041	0.049	0.042
p	4.0E−15	0.26	0	1.9E−6	0.16	8.9E−8	9.4E−4	0.88	5.9E−4
nCohort 1	570	1322	587	570	1322	587	570	1322	587
nCohort 2	189	59	173	170	60	161	58	36	54
Cutoff 1	1180	1040	1360	886	1100	964	854	849	976
Sens 1	70%	71%	71%	70%	70%	70%	71%	72%	70%
Spec 1	58%	40%	64%	45%	42%	46%	43%	32%	47%
Cutoff 2	893	640	1020	690	770	741	719	648	776
Sens 2	80%	81%	80%	80%	80%	80%	81%	81%	81%
Spec 2	46%	22%	49%	35%	29%	35%	37%	22%	38%
Cutoff 3	451	358	583	392	389	465	437	477	437
Sens 3	90%	92%	90%	90%	90%	90%	91%	92%	91%
Spec 3	19%	9%	25%	16%	10%	18%	19%	15%	17%
Cutoff 4	1480	2010	1600	1480	2010	1600	1480	2010	1600
Sens 4	61%	37%	65%	46%	37%	49%	47%	36%	48%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	1820	2610	2010	1820	2610	2010	1820	2610	2010
Sens 5	52%	19%	50%	42%	20%	42%	45%	19%	46%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	2660	3790	2890	2660	3790	2890	2660	3790	2890
Sens 6	25%	2%	24%	25%	8%	27%	34%	11%	33%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	1.1	0.66	1.3	1.6	1.6	2.6	1.8	1.1	1.3
p Value	0.79	0.37	0.43	0.10	0.24	0.0013	0.19	0.82	0.63
95% CI of	0.61	0.27	0.69	0.91	0.73	1.4	0.74	0.45	0.49
OR Quart 2	1.9	1.6	2.4	2.7	3.6	4.6	4.5	2.8	3.3
OR Quart 3	1.9	1.8	2.7	1.4	1.6	1.5	1.3	0.89	1.4
p Value	0.015	0.11	5.9E−4	0.21	0.23	0.22	0.63	0.81	0.48
95% CI of	1.1	0.87	1.5	0.82	0.73	0.80	0.49	0.34	0.55
OR Quart 3	3.2	3.7	4.8	2.5	3.6	2.7	3.3	2.3	3.6
OR Quart 4	5.1	1.5	6.5	3.8	1.8	4.9	3.7	1.00	3.5
p Value	1.6E−10	0.27	2.0E−11	2.7E−7	0.13	1.7E−8	0.0019	0.99	0.0031
95% CI of	3.1	0.72	3.7	2.3	0.84	2.8	1.6	0.39	1.5
OR Quart 4	8.3	3.2	11	6.3	4.0	8.5	8.4	2.5	8.0

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.

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
sCr or UO						
Median	1180	2190	1180	2050	1180	1880
Average	1500	2440	1500	2450	1500	2100
Stdev	1190	1460	1190	1650	1190	1620
p (t-test)		1.3E−13		7.7E−14		2.0E−4

TABLE 2-continued

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.

Min	41.6	89.4	41.6	110	41.6	81.2
Max	6400	6400	6400	6400	6400	6190
n (Samp)	1183	102	1183	106	1183	61
n (Patient)	444	102	444	106	444	61
sCr only						
Median	1330	1760	1330	2010	1330	1550
Average	1740	2060	1740	2430	1740	1970
Stdev	1380	1260	1380	1470	1380	1530
p (t-test)		0.28		0.0085		0.42
Min	41.6	404	41.6	340	41.6	324
Max	6400	6400	6400	6400	6400	6400
n (Samp)	1617	22	1617	29	1617	25
n (Patient)	556	22	556	29	556	25
UO only						
Median	1220	2330	1220	2180	1220	1950
Average	1550	2600	1550	2510	1550	2290
Stdev	1200	1530	1200	1700	1200	1700
p (t-test)		5.8E−15		5.4E−13		2.3E−5
Min	41.6	89.4	41.6	110	41.6	81.2
Max	6400	6400	6400	6400	6400	6190
n (Samp)	1118	93	1118	97	1118	52
n (Patient)	382	93	382	97	382	52

	0 hr prior to AKI stage			24 hr prior to AKI stage			48 hr 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	0.71	0.62	0.71	0.68	0.66	0.67	0.60	0.55	0.62
SE	0.030	0.064	0.031	0.030	0.056	0.031	0.039	0.060	0.042
p	2.0E−12	0.069	3.6E−12	1.5E−9	0.0033	2.0E−8	0.012	0.36	0.0051
nCohort 1	1183	1617	1118	1183	1617	1118	1183	1617	1118
nCohort 2	102	22	93	106	29	97	61	25	52
Cutoff 1	1590	1340	1660	1330	1710	1400	886	1100	957
Sens 1	71%	73%	71%	71%	72%	70%	70%	72%	71%
Spec 1	65%	50%	65%	57%	61%	57%	37%	41%	38%
Cutoff 2	1160	1070	1190	923	1150	819	582	770	648
Sens 2	80%	82%	81%	80%	83%	80%	80%	80%	81%
Spec 2	49%	40%	49%	39%	42%	32%	22%	28%	23%
Cutoff 3	671	1020	671	515	641	513	469	537	470
Sens 3	90%	91%	90%	91%	93%	91%	90%	92%	90%
Spec 3	26%	38%	25%	18%	21%	17%	16%	17%	15%
Cutoff 4	1770	2050	1850	1770	2050	1850	1770	2050	1850
Sens 4	62%	41%	66%	58%	48%	59%	51%	36%	54%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	2160	2700	2280	2160	2700	2280	2160	2700	2280
Sens 5	51%	18%	51%	48%	31%	47%	39%	16%	40%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	3280	3830	3390	3280	3830	3390	3280	3830	3390
Sens 6	24%	5%	27%	24%	14%	26%	26%	12%	29%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	1.3	5.0	1.2	1.1	1.00	1.2	0.59	1.2	0.49
p Value	0.53	0.14	0.67	0.85	1.00	0.71	0.22	0.76	0.16
95% CI of	0.57	0.59	0.51	0.51	0.20	0.54	0.25	0.36	0.18
OR Quart 2	3.0	43	2.8	2.3	5.0	2.5	1.4	4.0	1.3
OR Quart 3	2.5	8.1	1.8	1.8	3.7	1.5	0.59	1.6	0.91
p Value	0.017	0.049	0.13	0.098	0.044	0.28	0.22	0.41	0.83
95% CI of	1.2	1.0	0.84	0.90	1.0	0.72	0.25	0.52	0.40
OR Quart 3	5.3	65	4.1	3.5	14	3.1	1.4	5.0	2.1
OR Quart 4	6.4	8.1	6.2	4.3	4.1	4.4	2.0	1.2	2.0
p Value	1.5E−7	0.049	2.9E−7	2.7E−6	0.030	4.6E−6	0.043	0.76	0.061
95% CI of	3.2	1.0	3.1	2.3	1.1	2.3	1.0	0.36	0.97
OR Quart 4	13	65	12	8.0	15	8.3	3.7	4.0	4.1

TABLE 3

Comparison of marker levels in urine samples collected within 12 hours of reaching stage R from Cohort 1 (patients that reached, but did not progress beyond, RIFLE stage R) and from Cohort 2 (patients that reached RIFLE stage I or F).

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1680	2050	1600	2430	1850	1870
Average	1830	2300	1950	2470	1950	2220
Stdev	1160	1540	1410	1360	1130	1550
p (t-test)		0.0071		0.15		0.16
Min	151	183	151	183	168	190
Max	5180	6350	6400	5250	5180	6400
n (Samp)	169	84	65	20	142	64
n (Patient)	169	84	65	20	142	64

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.58	0.62	0.53
SE	0.039	0.075	0.044
p	0.033	0.097	0.55
nCohort 1	169	65	142
nCohort 2	84	20	64
Cutoff 1	1270	1940	1270
Sens 1	70%	70%	70%
Spec 1	36%	60%	31%
Cutoff 2	945	1400	1000
Sens 2	81%	80%	81%
Spec 2	23%	38%	22%
Cutoff 3	550	842	582
Sens 3	90%	90%	91%
Spec 3	13%	26%	10%
Cutoff 4	2150	2560	2280
Sens 4	48%	50%	34%
Spec 4	70%	71%	70%
Cutoff 5	2700	2940	2770
Sens 5	32%	35%	23%
Spec 5	80%	80%	80%
Cutoff 6	3530	3790	3470
Sens 6	18%	15%	17%
Spec 6	91%	91%	90%
OR Quart 2	0.93	0.63	1.4
p Value	0.84	0.64	0.43
95% CI of	0.43	0.094	0.60
OR Quart 2	2.0	4.2	3.2
OR Quart 3	1.0	3.7	1.1
p Value	1.0	0.089	0.83
95% CI of	0.47	0.82	0.47
OR Quart 3	2.1	17	2.6
OR Quart 4	1.8	2.8	1.3
p Value	0.11	0.18	0.56
95% CI of	0.87	0.61	0.55
OR Quart 4	3.7	13	3.0

TABLE 4

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.

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
sCr or UO						
Median	1250	3410	1250	3300	1250	3210
Average	1570	3520	1570	3470	1570	3050
Stdev	1190	1570	1190	1580	1190	1230
p (t-test)		3.9E−19		5.2E−18		1.6E−8

TABLE 4-continued

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.

Min	69.2	565	69.2	565	69.2	1020
Max	6400	6400	6400	6400	6400	6190
n (Samp)	259	44	259	43	259	24
n (Patient)	259	44	259	43	259	24
sCr only						
Median	1760	3240	1760	3170	1760	3110
Average	2100	3070	2100	2930	2100	2860
Stdev	1470	1530	1470	1380	1470	945
p (t-test)		0.0033		0.012		0.056
Min	69.2	565	69.2	565	69.2	1330
Max	6400	6400	6400	5080	6400	4360
n (Samp)	467	21	467	21	467	14
n (Patient)	467	21	467	21	467	14
UO only						
Median	1400	3720	1400	3600	1400	3130
Average	1790	3850	1790	3800	1790	3090
Stdev	1250	1590	1250	1610	1250	1400
p (t-test)		3.0E−15		2.8E−14		3.7E−5
Min	113	687	113	687	113	1020
Max	5540	6400	5540	6400	5540	6190
n (Samp)	223	32	223	31	223	18
n (Patient)	223	32	223	31	223	18

	0 hr prior to AKI stage			24 hr prior to AKI stage			48 hr 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	0.69	0.85	0.83	0.68	0.84	0.83	0.71	0.78
SE	0.039	0.065	0.044	0.040	0.066	0.045	0.053	0.079	0.066
p	0	0.0035	5.6E−15	0	0.0064	7.5E−14	3.5E−10	0.0095	2.3E−5
nCohort 1	259	467	223	259	467	223	259	467	223
nCohort 2	44	21	32	43	21	31	24	14	18
Cutoff 1	2720	2210	2910	2710	2210	2800	2200	2210	2170
Sens 1	70%	71%	72%	72%	71%	71%	71%	71%	72%
Spec 1	85%	65%	84%	85%	65%	83%	79%	65%	73%
Cutoff 2	2170	1810	2470	2170	1810	2470	1810	1810	1660
Sens 2	82%	81%	81%	81%	81%	81%	83%	86%	83%
Spec 2	79%	52%	77%	79%	52%	77%	70%	52%	58%
Cutoff 3	1060	1060	1810	1060	1060	1810	1590	1590	1320
Sens 3	91%	90%	91%	91%	90%	90%	92%	93%	94%
Spec 3	42%	28%	63%	42%	28%	63%	63%	45%	48%
Cutoff 4	1860	2450	2120	1860	2450	2120	1860	2450	2120
Sens 4	84%	67%	88%	84%	67%	87%	79%	64%	72%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	2270	3280	2630	2270	3280	2630	2270	3280	2630
Sens 5	77%	43%	78%	77%	38%	77%	67%	36%	67%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	3260	4350	3660	3260	4350	3660	3260	4350	3660
Sens 6	57%	19%	50%	53%	19%	48%	46%	7%	22%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	2.0	1.0	2.0	0.99	1.0	2.0	>1.0	>2.0	>2.1
p Value	0.42	1.0	0.58	0.99	1.0	0.58	<1.0	<0.56	<0.56
95% CI of	0.36	0.14	0.18	0.19	0.14	0.18	>0.061	>0.18	>0.18
OR Quart 2	11	7.2	23	5.0	7.2	23	na	na	na
OR Quart 3	3.1	2.6	5.3	2.1	2.6	4.2	>7.7	>4.1	>4.3
p Value	0.17	0.27	0.14	0.31	0.27	0.20	<0.060	<0.21	<0.20
95% CI of	0.61	0.49	0.60	0.50	0.49	0.46	>0.92	>0.46	>0.46
OR Quart 3	16	13	46	8.7	13	39	na	na	na
OR Quart 4	27	6.5	37	17	6.5	37	>20	>8.5	>15
p Value	1.4E−5	0.015	5.1E−4	9.6E−6	0.015	5.1E−4	<0.0040	<0.045	<0.011
95% CI of	6.1	1.4	4.8	4.8	1.4	4.8	>2.6	>1.0	>1.8
OR Quart 4	120	30	290	57	30	290	na	na	na

TABLE 5

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.

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
sCr or UO						
Median	284	335	284	331	284	428
Average	501	570	501	708	501	930
Stdev	627	641	627	839	627	999
p (t-test)		0.43		0.053		0.021
Min	86.8	74.7	86.8	63.6	86.8	132
Max	3370	3170	3370	3200	3370	3200
n (Samp)	162	77	162	56	162	14
n (Patient)	90	77	90	56	90	14
sCr only						
Median	290	350	290	573	290	309
Average	619	505	619	540	619	374
Stdev	764	488	764	251	764	269
p (t-test)		0.50		0.71		0.43
Min	48.0	105	48.0	183	48.0	112
Max	3370	2060	3370	1020	3370	832
n (Samp)	378	21	378	13	378	6
n (Patient)	178	21	178	13	178	6
UO only						
Median	323	384	323	330	323	499
Average	544	626	544	724	544	1070
Stdev	603	688	603	863	603	1080
p (t-test)		0.36		0.075		0.0012
Min	86.8	74.7	86.8	63.6	86.8	132
Max	3370	3170	3370	3200	3370	3200
n (Samp)	187	66	187	59	187	18
n (Patient)	94	66	94	59	94	18

	0 hr prior to AKI stage			24 hr prior to AKI stage			48 hr 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	0.52	0.54	0.57	0.65	0.53	0.65	0.45	0.65
SE	0.040	0.066	0.042	0.045	0.084	0.044	0.082	0.12	0.073
p	0.16	0.73	0.33	0.12	0.077	0.56	0.070	0.70	0.039
nCohort 1	162	378	187	162	378	187	162	378	187
nCohort 2	77	21	66	56	13	59	14	6	18
Cutoff 1	246	280	248	217	326	217	317	156	317
Sens 1	70%	71%	71%	71%	77%	71%	71%	83%	72%
Spec 1	40%	47%	36%	33%	55%	28%	55%	15%	49%
Cutoff 2	198	194	217	190	318	190	182	156	212
Sens 2	81%	81%	80%	80%	85%	81%	86%	83%	83%
Spec 2	28%	25%	28%	27%	53%	22%	26%	15%	27%
Cutoff 3	124	124	141	150	232	141	168	111	168
Sens 3	91%	90%	91%	91%	92%	92%	93%	100%	94%
Spec 3	10%	8%	12%	17%	35%	12%	23%	4%	19%
Cutoff 4	409	491	501	409	491	501	409	491	501
Sens 4	40%	33%	29%	48%	54%	36%	50%	33%	50%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	578	833	751	578	833	751	578	833	751
Sens 5	25%	14%	26%	32%	15%	24%	43%	0%	44%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	1030	1820	1320	1030	1820	1320	1030	1820	1320
Sens 6	12%	5%	12%	20%	0%	20%	29%	0%	28%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	1.4	0.73	1.4	1.5	0.99	0.89	1.0	2.0	1.0
p Value	0.45	0.69	0.40	0.40	0.99	0.79	1.0	0.57	1.0
95% CI of	0.61	0.16	0.62	0.60	0.061	0.39	0.13	0.18	0.19
OR Quart 2	3.0	3.4	3.2	3.6	16	2.1	7.4	23	5.2
OR Quart 3	1.6	2.3	1.5	1.0	5.2	0.83	2.1	1.0	1.4
p Value	0.26	0.17	0.30	1.0	0.14	0.67	0.41	1.0	0.70
95% CI of	0.72	0.70	0.68	0.39	0.59	0.36	0.36	0.062	0.29
OR Quart 3	3.5	7.9	3.5	2.6	45	1.9	12	16	6.4
OR Quart 4	1.7	1.2	1.5	2.1	6.3	1.2	3.3	2.0	2.9
p Value	0.19	0.75	0.33	0.10	0.092	0.72	0.16	0.57	0.13

TABLE 5-continued

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.

95% CI of OR Quart 4	0.77 3.7	0.33 4.8	0.66 3.4	0.87 4.9	0.74 53	0.52 2.6	0.63 17	0.18 23	0.73 12

TABLE 6

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.

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
sCr or UO						
Median	317	318	317	318	317	524
Average	581	651	581	739	581	729
Stdev	680	806	680	882	680	794
p (t-test)		0.61		0.19		0.36
Min	74.7	113	74.7	48.0	74.7	112
Max	3370	2880	3370	3200	3370	2810
n (Samp)	357	28	357	37	357	19
n (Patient)	179	28	179	37	179	19
sCr only						
Median	nd	nd	nd	nd	333	469
Average	nd	nd	nd	nd	647	452
Stdev	nd	nd	nd	nd	751	285
p (t-test)	nd	nd	nd	nd		0.53
Min	nd	nd	nd	nd	48.0	112
Max	nd	nd	nd	nd	3370	832
n (Samp)	nd	nd	nd	nd	477	6
n (Patient)	nd	nd	nd	nd	216	6
UO only						
Median	325	303	325	314	325	524
Average	586	638	586	745	586	760
Stdev	671	810	671	893	671	827
p (t-test)		0.70		0.19		0.30
Min	74.7	113	74.7	48.0	74.7	119
Max	3370	2880	3370	3200	3370	2810
n (Samp)	347	28	347	36	347	17
n (Patient)	167	28	167	36	167	17

	0 hr prior to AKI stage			24 hr prior to AKI stage			48 hr 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	0.49	0.54	nd	0.52	0.54	0.50	0.55
SE	0.057	nd	0.057	0.051	nd	0.051	0.069	0.12	0.073
p	0.76	nd	0.89	0.48	nd	0.67	0.54	0.97	0.53
nCohort 1	357	nd	347	357	nd	347	357	477	347
nCohort 2	28	nd	28	37	nd	36	19	6	17
Cutoff 1	246	nd	246	228	nd	227	184	194	194
Sens 1	71%	nd	71%	70%	nd	72%	74%	83%	71%
Spec 1	37%	nd	35%	33%	nd	31%	22%	23%	22%
Cutoff 2	168	nd	168	191	nd	191	141	194	183
Sens 2	82%	nd	82%	81%	nd	81%	84%	83%	82%
Spec 2	19%	nd	17%	24%	nd	22%	13%	23%	20%
Cutoff 3	141	nd	141	112	nd	111	118	111	128
Sens 3	93%	nd	93%	92%	nd	92%	95%	100%	94%
Spec 3	13%	nd	11%	4%	nd	3%	6%	4%	8%
Cutoff 4	502	nd	512	502	nd	512	502	535	512
Sens 4	29%	nd	25%	41%	nd	42%	53%	50%	53%
Spec 4	70%	nd	70%	70%	nd	70%	70%	70%	70%
Cutoff 5	833	nd	841	833	nd	841	833	940	841
Sens 5	18%	nd	18%	24%	nd	25%	21%	0%	24%
Spec 5	80%	nd	80%	80%	nd	80%	80%	80%	80%
Cutoff 6	1410	nd	1400	1410	nd	1400	1410	1860	1400

TABLE 6-continued

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.

Sens 6	14%	nd	14%	19%	nd	19%	16%	0%	18%
Spec 6	90%	nd	90%	90%	nd	90%	90%	90%	90%
OR Quart 2	1.0	nd	1.2	0.99	nd	1.2	0.32	2.0	0.32
p Value	1.0	nd	0.77	0.98	nd	0.65	0.17	0.57	0.17
95% CI of	0.34	nd	0.38	0.38	nd	0.49	0.063	0.18	0.063
OR Quart 2	3.0	nd	3.7	2.6	nd	3.1	1.6	23	1.6
OR Quart 3	1.0	nd	1.4	0.88	nd	0.64	0.65	1.0	0.65
p Value	1.0	nd	0.58	0.80	nd	0.41	0.52	1.0	0.52
95% CI of	0.34	nd	0.45	0.32	nd	0.22	0.18	0.062	0.18
OR Quart 3	3.0	nd	4.1	2.4	nd	1.9	2.4	16	2.4
OR Quart 4	0.99	nd	1.2	1.2	nd	1.1	1.2	2.0	0.82
p Value	0.98	nd	0.76	0.65	nd	0.83	0.77	0.56	0.76
95% CI of	0.33	nd	0.39	0.49	nd	0.43	0.38	0.18	0.24
OR Quart 4	2.9	nd	3.7	3.1	nd	2.9	3.7	23	2.8

TABLE 7

Comparison of marker levels in EDTA samples collected within 12 hours of reaching stage R from Cohort 1 (patients that reached, but did not progress beyond, RIFLE stage R) and from Cohort 2 (patients that reached RIFLE stage I or F).

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	316	336	nd	nd	335	348
Average	608	776	nd	nd	591	728
Stdev	666	936	nd	nd	664	883
p (t-test)		0.32	nd	nd		0.45
Min	74.7	110	nd	nd	74.7	110
Max	3200	3170	nd	nd	3200	3170
n (Samp)	67	30	nd	nd	51	26
n (Patient)	67	30	nd	nd	51	26

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.53	nd	0.52
SE	0.064	nd	0.070
p	0.65	nd	0.75
nCohort 1	67	nd	51
nCohort 2	30	nd	26
Cutoff 1	262	nd	219
Sens 1	70%	nd	73%
Spec 1	39%	nd	27%
Cutoff 2	194	nd	186

TABLE 7-continued

Comparison of marker levels in EDTA samples collected within 12 hours of reaching stage R from Cohort 1 (patients that reached, but did not progress beyond, RIFLE stage R) and from Cohort 2 (patients that reached RIFLE stage I or F).

Sens 2	80%	nd	81%
Spec 2	19%	nd	18%
Cutoff 3	173	nd	159
Sens 3	90%	nd	92%
Spec 3	19%	nd	18%
Cutoff 4	685	nd	538
Sens 4	27%	nd	27%
Spec 4	70%	nd	71%
Cutoff 5	900	nd	849
Sens 5	27%	nd	23%
Spec 5	81%	nd	80%
Cutoff 6	1410	nd	1200
Sens 6	20%	nd	19%
Spec 6	91%	nd	90%
OR Quart 2	1.0	nd	1.3
p Value	1.0	nd	0.73
95% CI of	0.29	nd	0.33
OR Quart 2	3.5	nd	4.8
OR Quart 3	1.2	nd	1.3
p Value	0.76	nd	0.73
95% CI of	0.36	nd	0.33
OR Quart 3	4.1	nd	4.8
OR Quart 4	1.1	nd	0.93
p Value	0.83	nd	0.91
95% CI of	0.34	nd	0.24
OR Quart 4	3.9	nd	3.6

TABLE 8

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.

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
sCr or UO						
Median	345	698	345	698	345	663
Average	612	1270	612	1090	612	578
Stdev	733	1050	733	877	733	270
p (t-test)		0.0093		0.047		0.91

TABLE 8-continued

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.

Min	86.8	231	86.8	231	86.8	231
Max	3370	3200	3370	3200	3370	932
n (Samp)	90	11	90	11	90	6
n (Patient)	90	11	90	11	90	6
sCr only						
Median	338	655	338	655	338	655
Average	707	576	707	576	707	576
Stdev	844	270	844	270	844	270
p (t-test)		0.70		0.70		0.70
Min	86.8	231	86.8	231	86.8	231
Max	3370	932	3370	932	3370	932
n (Samp)	178	6	178	6	178	6
n (Patient)	178	6	178	6	178	6
UO only						
Median	355	1390	355	1280	nd	nd
Average	632	1680	632	1410	nd	nd
Stdev	702	1110	702	948	nd	nd
p (t-test)		4.0E−4		0.0068	nd	nd
Min	86.8	618	86.8	618	nd	nd
Max	3370	3200	3370	3200	nd	nd
n (Samp)	94	7	94	7	nd	nd
n (Patient)	94	7	94	7	nd	nd

	0 hr prior to AKI stage			24 hr prior to AKI stage			48 hr 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	0.76	0.62	0.85	0.75	0.62	0.83	0.64	0.62	nd
SE	0.087	0.12	0.093	0.088	0.12	0.097	0.13	0.12	nd
p	0.0029	0.35	1.8E−4	0.0046	0.35	7.3E−4	0.25	0.35	nd
nCohort 1	90	178	94	90	178	94	90	178	nd
nCohort 2	11	6	7	11	6	7	6	6	nd
Cutoff 1	626	280	689	626	280	689	278	280	nd
Sens 1	73%	83%	71%	73%	83%	71%	83%	83%	nd
Spec 1	74%	43%	74%	74%	43%	74%	42%	43%	nd
Cutoff 2	591	280	626	591	280	626	278	280	nd
Sens 2	82%	83%	86%	82%	83%	86%	83%	83%	nd
Spec 2	73%	43%	71%	73%	43%	71%	42%	43%	nd
Cutoff 3	278	228	591	278	228	591	228	228	nd
Sens 3	91%	100%	100%	91%	100%	100%	100%	100%	nd
Spec 3	42%	30%	70%	42%	30%	70%	34%	30%	nd
Cutoff 4	475	578	591	475	578	591	475	578	nd
Sens 4	82%	67%	100%	82%	67%	100%	67%	67%	nd
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	nd
Cutoff 5	754	946	933	754	946	933	754	946	nd
Sens 5	45%	0%	57%	45%	0%	57%	17%	0%	nd
Spec 5	80%	80%	81%	80%	80%	81%	80%	80%	nd
Cutoff 6	1660	2040	1810	1660	2040	1810	1660	2040	nd
Sens 6	27%	0%	43%	18%	0%	29%	0%	0%	nd
Spec 6	90%	90%	91%	90%	90%	91%	90%	90%	nd
OR Quart 2	>2.2	>2.1	>0	>2.2	>2.1	>0	>2.2	>2.1	nd
p Value	<0.54	<0.55	<na	<0.54	<0.55	<na	<0.54	<0.55	nd
95% CI of	>0.18	>0.18	>na	>0.18	>0.18	>na	>0.18	>0.18	nd
OR Quart 2	na	na	na	na	na	na	na	na	nd
OR Quart 3	>4.8	>3.2	>3.4	>4.8	>3.2	>3.4	>1.0	>3.2	nd
p Value	<0.18	<0.32	<0.30	<0.18	<0.32	<0.30	<0.98	<0.32	nd
95% CI of	>0.49	>0.32	>0.33	>0.49	>0.32	>0.33	>0.062	>0.32	nd
OR Quart 3	na	na	na	na	na	na	na	na	nd
OR Quart 4	>6.0	>1.0	>4.5	>6.0	>1.0	>4.5	>3.4	>1.0	nd
p Value	<0.12	<0.99	<0.19	<0.12	<0.99	<0.19	<0.30	<0.99	nd
95% CI of	>0.64	>0.062	>0.47	>0.64	>0.062	>0.47	>0.33	>0.062	nd
OR Quart 4	na	na	na	na	na	na	na	na	nd

TABLE 9

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.

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
sCr or UO						
Median	1300	2590	1300	3200	1300	2010
Average	1670	2900	1670	3320	1670	2430
Stdev	1300	1820	1300	1750	1300	1860
p (t-test)		1.9E−7		4.1E−11		0.020
Min	41.6	390	41.6	687	41.6	81.2
Max	6400	6400	6400	6400	6400	6190
n (Samp)	1703	31	1703	28	1703	16
n (Patient)	580	31	580	28	580	16
sCr only						
Median	1360	2480	1360	2500	1360	1880
Average	1750	2480	1750	2860	1750	2240
Stdev	1390	1900	1390	1150	1390	1120
p (t-test)		0.083		0.012		0.30
Min	41.6	565	41.6	1430	41.6	1040
Max	6400	6400	6400	5000	6400	4360
n (Samp)	1782	11	1782	10	1782	9
n (Patient)	600	11	600	10	600	9
UO only						
Median	1380	3210	1380	3220	1380	3480
Average	1720	3380	1720	3530	1720	3040
Stdev	1300	1950	1300	1930	1300	2330
p (t-test)		2.4E−8		1.1E−11		0.0045
Min	41.6	390	41.6	687	41.6	379
Max	6400	6400	6400	6400	6400	6190
n (Samp)	1587	20	1587	25	1587	8
n (Patient)	499	20	499	25	499	8

	0 hr prior to AKI stage			24 hr prior to AKI stage			48 hr 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	0.71	0.62	0.75	0.78	0.78	0.78	0.61	0.67	0.63
SE	0.053	0.091	0.063	0.052	0.087	0.055	0.075	0.100	0.11
p	7.5E−5	0.20	7.1E−5	4.9E−8	0.0015	5.5E−7	0.14	0.097	0.22
nCohort 1	1703	1782	1587	1703	1782	1587	1703	1782	1587
nCohort 2	31	11	20	28	10	25	16	9	8
Cutoff 1	1660	1070	2470	2450	2270	2450	1040	1420	480
Sens 1	71%	73%	70%	71%	70%	72%	75%	78%	75%
Spec 1	62%	40%	78%	79%	74%	78%	39%	52%	14%
Cutoff 2	1030	934	1660	1440	2010	1540	480	1310	471
Sens 2	81%	82%	80%	82%	80%	80%	81%	89%	88%
Spec 2	39%	34%	60%	55%	68%	56%	15%	49%	13%
Cutoff 3	874	577	874	819	1960	808	378	1040	378
Sens 3	90%	91%	90%	93%	90%	92%	94%	100%	100%
Spec 3	32%	19%	30%	30%	67%	28%	10%	38%	9%
Cutoff 4	1990	2070	2050	1990	2070	2050	1990	2070	2050
Sens 4	65%	55%	70%	75%	70%	76%	50%	44%	62%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	2560	2710	2650	2560	2710	2650	2560	2710	2650
Sens 5	52%	36%	55%	61%	40%	64%	38%	22%	62%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	3620	3850	3690	3620	3850	3690	3620	3850	3690
Sens 6	29%	18%	45%	39%	20%	48%	25%	11%	38%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	1.7	1.0	3.0	2.0	>0	4.0	0.25	>2.0	0
p Value	0.48	1.0	0.34	0.57	<na	0.21	0.21	<0.57	na
95% CI of	0.40	0.14	0.31	0.18	>na	0.45	0.028	>0.18	na
OR Quart 2	7.0	7.1	29	22	na	36	2.2	na	na
OR Quart 3	1.3	0.50	2.0	5.0	>4.0	2.0	1.00	>4.0	0
p Value	0.71	0.57	0.57	0.14	<0.21	0.57	1.00	<0.21	na
95% CI of	0.30	0.045	0.18	0.59	>0.45	0.18	0.25	>0.45	na
OR Quart 3	6.0	5.5	22	43	na	22	4.0	na	na

TABLE 9-continued

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.

OR Quart 4	6.6	3.0	14	21	>6.1	19	1.8	>3.0	1.7
p Value	0.0026	0.18	0.010	0.0031	<0.095	0.0044	0.37	<0.34	0.48
95% CI of	1.9	0.61	1.9	2.8	>0.73	2.5	0.51	>0.31	0.40
OR Quart 4	22	15	110	160	na	140	6.1	na	7.0

TABLE 10

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.

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
sCr or UO						
Median	nd	nd	326	618	nd	nd
Average	nd	nd	606	1130	nd	nd
Stdev	nd	nd	706	1140	nd	nd
p (t-test)	nd	nd		0.054	nd	nd
Min	nd	nd	48.0	190	nd	nd
Max	nd	nd	3370	3200	nd	nd
n (Samp)	nd	nd	489	7	nd	nd
n (Patient)	nd	nd	222	7	nd	nd
UO only						
Median	nd	nd	326	1000	nd	nd
Average	nd	nd	604	1340	nd	nd
Stdev	nd	nd	698	1110	nd	nd
p (t-test)	nd	nd		0.011	nd	nd
Min	nd	nd	48.0	279	nd	nd
Max	nd	nd	3370	3200	nd	nd
n (Samp)	nd	nd	485	6	nd	nd
n (Patient)	nd	nd	208	6	nd	nd

	0 hr prior to AKI stage			24 hr prior to AKI stage			48 hr 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	0.78	nd	nd	nd
SE	nd	nd	nd	0.11	nd	0.11	nd	nd	nd
p	nd	nd	nd	0.21	nd	0.012	nd	nd	nd
nCohort 1	nd	nd	nd	489	nd	485	nd	nd	nd
nCohort 2	nd	nd	nd	7	nd	6	nd	nd	nd
Cutoff 1	nd	nd	nd	278	nd	560	nd	nd	nd
Sens 1	nd	nd	nd	71%	nd	83%	nd	nd	nd
Spec 1	nd	nd	nd	42%	nd	73%	nd	nd	nd
Cutoff 2	nd	nd	nd	228	nd	560	nd	nd	nd
Sens 2	nd	nd	nd	86%	nd	83%	nd	nd	nd
Spec 2	nd	nd	nd	31%	nd	73%	nd	nd	nd
Cutoff 3	nd	nd	nd	190	nd	278	nd	nd	nd
Sens 3	nd	nd	nd	100%	nd	100%	nd	nd	nd
Spec 3	nd	nd	nd	22%	nd	42%	nd	nd	nd
Cutoff 4	nd	nd	nd	515	nd	518	nd	nd	nd
Sens 4	nd	nd	nd	57%	nd	83%	nd	nd	nd
Spec 4	nd	nd	nd	70%	nd	70%	nd	nd	nd
Cutoff 5	nd	nd	nd	845	nd	833	nd	nd	nd
Sens 5	nd	nd	nd	43%	nd	50%	nd	nd	nd
Spec 5	nd	nd	nd	80%	nd	80%	nd	nd	nd
Cutoff 6	nd	nd	nd	1670	nd	1660	nd	nd	nd
Sens 6	nd	nd	nd	29%	nd	33%	nd	nd	nd
Spec 6	nd	nd	nd	90%	nd	90%	nd	nd	nd
OR Quart 2	nd	nd	nd	2.0	nd	>1.0	nd	nd	nd
p Value	nd	nd	nd	0.57	nd	<1.0	nd	nd	nd
95% CI of	nd	nd	nd	0.18	nd	>0.062	nd	nd	nd

TABLE 10-continued

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.

OR Quart 2	nd	nd	nd	23	nd	na	nd	nd	nd
OR Quart 3	nd	nd	nd	1.0	nd	>2.0	nd	nd	nd
p Value	nd	nd	nd	1.0	nd	<0.57	nd	nd	nd
95% CI of	nd	nd	nd	0.062	nd	>0.18	nd	nd	nd
OR Quart 3	nd	nd	nd	16	nd	na	nd	nd	nd
OR Quart 4	nd	nd	nd	3.0	nd	>3.0	nd	nd	nd
p Value	nd	nd	nd	0.34	nd	<0.34	nd	nd	nd
95% CI of	nd	nd	nd	0.31	nd	>0.31	nd	nd	nd
OR Quart 4	nd	nd	nd	30	nd	na	nd	nd	nd

TABLE 11

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

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1170	2300	1260	2800	1220	2220
Average	1480	2660	1680	2830	1560	2680
Stdev	1160	1770	1360	1780	1180	1810
p (t-test)		2.1E−18		2.0E−5		3.6E−14
Min	41.6	81.2	41.6	197	41.6	81.2
Max	6300	6400	6400	6390	5430	6400
n (Samp)	484	129	576	28	406	110
n (Patient)	484	129	576	28	406	110

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.70	0.69	0.69
SE	0.028	0.057	0.030
p	3.5E−13	7.6E−4	6.8E−10
nCohort 1	484	576	406
nCohort 2	129	28	110
Cutoff 1	1380	1450	1380
Sens 1	71%	71%	70%
Spec 1	58%	57%	56%
Cutoff 2	886	808	949
Sens 2	81%	82%	80%
Spec 2	38%	32%	38%
Cutoff 3	616	551	674
Sens 3	91%	93%	90%
Spec 3	24%	19%	25%
Cutoff 4	1760	1990	1880
Sens 4	59%	68%	57%
Spec 4	70%	70%	70%
Cutoff 5	2280	2660	2440
Sens 5	50%	61%	46%
Spec 5	80%	80%	80%
Cutoff 6	3190	3790	3310
Sens 6	36%	29%	34%
Spec 6	90%	90%	90%
OR Quart 2	1.1	0.39	1.1
p Value	0.86	0.27	0.85
95% CI of	0.53	0.075	0.52
OR Quart 2	2.2	2.1	2.2
OR Quart 3	2.0	0.79	1.9
p Value	0.030	0.74	0.069
95% CI of	1.1	0.21	0.95
OR Quart 3	3.9	3.0	3.7
OR Quart 4	5.5	3.7	4.5
p Value	2.0E−8	0.012	3.4E−6

TABLE 11-continued

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

95% CI of	3.0	1.3	2.4
OR Quart 4	10	10	8.4

TABLE 12

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

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	309	266	nd	nd	354	247
Average	651	674	nd	nd	647	679
Stdev	791	841	nd	nd	774	856
p (t-test)		0.89	nd	nd		0.85
Min	76.0	48.0	nd	nd	76.0	48.0
Max	3350	3200	nd	nd	3350	3200
n (Samp)	140	29	nd	nd	133	28
n (Patient)	140	29	nd	nd	133	28

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.48	nd	0.47
SE	0.059	nd	0.061
p	0.79	nd	0.61
nCohort 1	140	nd	133
nCohort 2	29	nd	28
Cutoff 1	184	nd	184
Sens 1	72%	nd	71%
Spec 1	23%	nd	21%
Cutoff 2	140	nd	140
Sens 2	83%	nd	82%
Spec 2	14%	nd	11%
Cutoff 3	93.7	nd	93.7
Sens 3	93%	nd	93%
Spec 3	3%	nd	3%
Cutoff 4	517	nd	538
Sens 4	41%	nd	36%
Spec 4	70%	nd	71%
Cutoff 5	882	nd	882
Sens 5	21%	nd	21%
Spec 5	80%	nd	80%
Cutoff 6	1860	nd	1860
Sens 6	10%	nd	11%
Spec 6	90%	nd	90%
OR Quart 2	1.5	nd	1.2
p Value	0.53	nd	0.73
95% CI of	0.46	nd	0.38
OR Quart 2	4.6	nd	4.1
OR Quart 3	1.0	nd	1.0
p Value	0.96	nd	0.96
95% CI of	0.30	nd	0.30
OR Quart 3	3.5	nd	3.5
OR Quart 4	1.7	nd	1.7
p Value	0.37	nd	0.37
95% CI of	0.54	nd	0.54
OR Quart 4	5.2	nd	5.3

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[0134] 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.

[0135] 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.

[0136] 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.

[0137] 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.

[0138] Other embodiments are set forth within the following claims.

We claim:

1. A method for evaluating renal status in a subject not receiving renal replacement therapy and that is characterized as being in RIFLE 0 or R, and treating the subject based on the evaluation, comprising:

performing an assay configured to detect hyaluronic acid (HA) on a urine sample obtained from the subject by introducing the urine sample into an assay instrument which (i) contacts the urine sample with an antibody that specifically binds for detection HA present in the urine sample, and (ii) generates an assay result indicative of binding of HA to the antibody;

correlating the assay result to the renal status of the subject by using the assay result to assign the patient to a predetermined subpopulation of individuals having a known predisposition of a future acute assignment made by comparing the assay result or a value derived therefrom to a threshold assay value obtained from a population study, wherein the threshold separates the population into a first subpopulation above the renal injury characterized as being in RIFLE I or F occurring within 48 hours of the time at which the body fluid sample is obtained from the subject, the threshold which is at an increased predisposition for having acute renal failure characterized as being in RIFLE I or F within 48 hours relative to a second subpopulation below the threshold; and

wherein when the assay result is above the threshold assay value the subject is treated by one or more of initiating renal replacement therapy, withdrawing delivery of compounds that are known to be damaging to the kidney, delaying or avoiding procedures that are known to be damaging to the kidney, and modifying diuretic administration.

2. A method according to claim **1**, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 48 hours.

3. A method according to claim **2**, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 24 hours.

4. A method according to claim **1**, wherein said assay result is a measured urine concentration of HA

5. A method according to claim **1**, wherein the subject is selected for evaluation of renal status based on the preexistence in the subject of one or more known risk factors for prerenal, intrinsic renal, or postrenal ARF.

6. A method according to claim **1**, 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.

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