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

(57) **Abstract**: The present invention relates to methods and compositions for monitoring, diagnosis, prognosis, and determination of treatment regimens in subjects suffering from or suspected of having a renal injury. In particular, the invention relates to using a one or more assays configured to detect a kidney injury marker selected from the group consisting of Ceruloplasmin and Annexin A2 as diagnostic and prognostic biomarkers in renal injuries.

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

[0001]   The present application claims priority to provisional U.S. patent applications 61/679,545 filed August 3, 2012; and 61/679,514 filed August 3, 2012, each of which is hereby incorporated in its entirety including all tables, figures, and claims.

BACKGROUND OF THE INVENTION

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

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

[0004]   Acute renal failure (ARF, also known as acute kidney injury, or AKI) is an abrupt (typically detected within about 48 hours to 1 week)reduction in glomerular filtration. This loss of filtration capacity results in retention of nitrogenous (urea and creatinine) and non-nitrogenous waste products that are normally excreted by the kidney, a reduction in urine output, or both. It is reported that ARF complicates about 5% of hospital admissions, 4-15% of cardiopulmonary bypass surgeries, and up to 30% of intensive care admissions. ARF may be categorized as prerenal, intrinsic renal, or postrenal in causation. Intrinsic renal disease can be further divided into glomerular,

tubular, interstitial, and vascular abnormalities. Major causes of ARF are described in the following table, which is adapted from the Merck Manual, 17th ed., Chapter 222, and which is hereby incorporated by reference in their entirety:

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

Type	Risk Factors
	ingestion, myeloma protein, myoglobin
Ureteral obstruction	Intrinsic: Calculi, clots, sloughed renal tissue, fungus ball, edema, malignancy, congenital defects; Extrinsic: Malignancy, retroperitoneal fibrosis, ureteral trauma during surgery or high impact injury
Bladder obstruction	Mechanical: Benign prostatic hyperplasia, prostate cancer, bladder cancer, urethral strictures, phimosis, paraphimosis, urethral valves, obstructed indwelling urinary catheter; Neurogenic: Anticholinergic drugs, upper or lower motor neuron lesion

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

[0006]   Acute kidney injury caused by radiocontrast agents (also called contrast media) and other nephrotoxins such as cyclosporine, antibiotics including aminoglycosides and anticancer drugs such as cisplatin manifests over a period of days to about a week. Contrast induced nephropathy (CIN, which is AKI caused by radiocontrast agents) is thought to be caused by intrarenal vasoconstriction (leading to ischemic injury) and from the generation of reactive oxygen species that are directly toxic to renal tubular epithelial cells. CIN classically presents as an acute (onset within 24-48h) but reversible (peak 3-5 days, resolution within 1 week) rise in blood urea nitrogen and serum creatinine.

[0007]   A commonly reported criteria for defining and detecting AKI is an abrupt (typically within about 2-7 days or within a period of hospitalization) elevation of serum creatinine. Although the use of serum creatinine elevation to define and detect AKI is well established, the magnitude of the serum creatinine elevation and the time over which

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

[0008]   One study (Lassnigg et all, J Am Soc Nephrol 15:1597-1605, 2004, hereby incorporated by reference in its entirety) investigated both increases and decreases in serum creatinine. Patients with a mild fall in serum creatinine of -0.1 to -0.3 mg/dL following heart surgery had the lowest mortality rate. Patients with a larger fall in serum creatinine (more than or equal to -0.4 mg/dL) or any increase in serum creatinine had a larger mortality rate. These findings caused the authors to conclude that even very subtle changes in renal function (as detected by small creatinine changes within 48 hours of surgery) seriously effect patient's outcomes. In an effort to reach consensus on a unified classification system for using serum creatinine to define AKI in clinical trials and in clinical practice, Bellomo *et al., Crit Care*. 8(4):R204-12, 2004, which is hereby

incorporated by reference in its entirety, proposes the following classifications for stratifying AKI patients:

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

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

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

And included two clinical outcomes:

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

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

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

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

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

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

"Stage III": increase in serum creatinine to more than 300% (> 3-fold) from baseline OR serum creatinine $\geq$ 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.

[0010]   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.

[0011]   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.

[0012]   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

[0013]   It is an object of the invention to provide methods and compositions for evaluating renal function in a subject. As described herein, measurement of one or more

biomarkers selected from the group consisting of Ceruloplasmin and Annexin A2 (each referred to herein as a "kidney injury marker") can be used for diagnosis, prognosis, risk stratification, staging, monitoring, categorizing and determination of further diagnosis and treatment regimens in subjects suffering or at risk of suffering from an injury to renal function, reduced renal function, and/or acute renal failure (also called acute kidney injury).

[0014]   The kidney injury markers of the present invention may be used, individually or in panels comprising a plurality of kidney injury markers, for risk stratification (that is, to identify subjects at risk for a future injury to renal function, for future progression to reduced renal function, for future progression to ARF, for future improvement in renal function, *etc.*); for diagnosis of existing disease (that is, to identify subjects who have suffered an injury to renal function, who have progressed to reduced renal function, who have progressed to ARF, *etc.*); for monitoring for deterioration or improvement of renal function; and for predicting a future medical outcome, such as improved or worsening renal function, a decreased or increased mortality risk, a decreased or increased risk that a subject will require renal replacement therapy (*i.e.*, hemodialysis, peritoneal dialysis, hemofiltration, and/or renal transplantation, a decreased or increased risk that a subject will recover from an injury to renal function, a decreased or increased risk that a subject will recover from ARF, a decreased or increased risk that a subject will progress to end stage renal disease, a decreased or increased risk that a subject will progress to chronic renal failure, a decreased or increased risk that a subject will suffer rejection of a transplanted kidney, *etc.*

[0015]   In a first aspect, the present invention relates to methods for evaluating renal status in a subject. These methods comprise performing an assay method that is configured to detect one or more biomarkers selected from the group consisting of Ceruloplasmin and Annexin A2 is/are then correlated to the renal status of the subject. This correlation to renal status may include correlating the assay result(s) to one or more of risk stratification, diagnosis, prognosis, staging, classifying and monitoring of the subject as described herein. Thus, the present invention utilizes one or more kidney injury markers of the present invention for the evaluation of renal injury.

[0016]   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.

[0017]   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.

[0018]   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.

[0019]   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

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when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold.

[0020]   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.

[0021]   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.

[0022]   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.

[0023]   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.

[0024]   In other embodiments, the methods for evaluating renal status described herein are methods for diagnosing a renal injury in the subject; that is, assessing whether or not a subject has suffered from an injury to renal function, reduced renal function, or ARF. In these embodiments, the assay result(s), for example measured concentration(s) of one or more biomarkers selected from the group consisting of Ceruloplasmin and Annexin A2 is/are correlated to the occurrence or nonoccurrence of a change in renal status. The following are preferred diagnostic embodiments.

[0025]   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).

[0026]   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).

[0027]   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).

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

[0029]   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).

[0030]   In still other embodiments, the methods for evaluating renal status described herein are methods for monitoring a renal injury in the subject; that is, assessing whether or not renal function is improving or worsening in a subject who has suffered from an injury to renal function, reduced renal function, or ARF. In these embodiments, the assay result(s), for example measured concentration(s) of one or more biomarkers selected from the group consisting of Ceruloplasmin and Annexin A2 is/are correlated to the occurrence or nonoccurrence of a change in renal status. The following are preferred monitoring embodiments.

[0031]   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.

[0032]   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.

[0033]   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.

[0034]   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.

[0035]　In still other embodiments, the methods for evaluating renal status described herein are methods for classifying a renal injury in the subject; that is, determining whether a renal injury in a subject is prerenal, intrinsic renal, or postrenal; and/or further subdividing these classes into subclasses such as acute tubular injury, acute glomerulonephritis acute tubulointerstitial nephritis, acute vascular nephropathy, or infiltrative disease; and/or assigning a likelihood that a subject will progress to a particular RIFLE stage. In these embodiments, the assay result(s), for example measured concentration(s) of one or more biomarkers selected from the group consisting of Ceruloplasmin and Annexin A2 is/are correlated to a particular class and/or subclass. The following are preferred classification embodiments.

[0036]　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.

[0037]　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.

[0038]   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.

[0039]   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.

[0040]   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.

[0041] 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

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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.

[0042]   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. In the case of those kidney injury markers which are membrane proteins as described hereinafter, preferred assays detect soluble forms thereof.

[0043]   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, risk scores of Thakar et al. (J. Am. Soc. Nephrol. 16: 162-68, 2005), Mehran et al. (J. Am. Coll. Cardiol. 44: 1393-99, 2004), Wijeysundera et al. (JAMA 297: 1801-9, 2007), Goldstein and Chawla (Clin. J. Am. Soc. Nephrol. 5: 943-49, 2010), or Chawla et al. (Kidney Intl. 68: 2274-80, 2005)), a glomerular filtration rate, an estimated glomerular filtration rate, a urine production rate, a serum or plasma creatinine concentration, a urine creatinine concentration, a fractional excretion of sodium, a urine sodium concentration, a urine creatinine to serum or plasma

creatinine ratio, a urine specific gravity, a urine osmolality, a urine urea nitrogen to plasma urea nitrogen ratio, a plasma BUN to creatnine ratio, a renal failure index calculated as urine sodium / (urine creatinine / plasma creatinine), a serum or plasma neutrophil gelatinase (NGAL) concentration, a urine NGAL concentration, a serum or plasma cystatin C concentration, a serum or plasma cardiac troponin concentration, a serum or plasma BNP concentration, a serum or plasma NTproBNP concentration, and a serum or plasma proBNP concentration. Other measures of renal function which may be combined with one or more kidney injury marker assay result(s) are described hereinafter and in Harrison's Principles of Internal Medicine, 17th Ed., McGraw Hill, New York, pages 1741-1830, and Current Medical Diagnosis & Treatment 2008, 47th Ed, McGraw Hill, New York, pages 785-815, each of which are hereby incorporated by reference in their entirety.

[0044]   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.

[0045]   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.

[0046]   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.

[0047]   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.).

[0048]   Generation of a signal from the signal development element can be performed using various optical, acoustical, and electrochemical methods well known in the art. Examples of detection modes include fluorescence, radiochemical detection, reflectance, absorbance, amperometry, conductance, impedance, interferometry, ellipsometry, etc. In certain of these methods, the solid phase antibody is coupled to a transducer (e.g., a diffraction grating, electrochemical sensor, etc) for generation of a signal, while in others, a signal is generated by a transducer that is spatially separate from the solid phase antibody (e.g., a fluorometer that employs an excitation light source and an optical detector). This list is not meant to be limiting. Antibody-based biosensors may also be employed to determine the presence or amount of analytes that optionally eliminate the need for a labeled molecule.

DETAILED DESCRIPTION OF THE INVENTION

[0049]   The present invention relates to methods and compositions for diagnosis, differential diagnosis, risk stratification, monitoring, classifying and determination of treatment regimens in subjects suffering or at risk of suffering from injury to renal function, reduced renal function and/or acute renal failure through measurement of one or more kidney injury markers. In various embodiments, a measured concentration of one or more biomarkers selected from the group consisting of Ceruloplasmin and Annexin A2 or one or more markers related thereto, are correlated to the renal status of the subject.

[0050]   For purposes of this document, the following definitions apply:

[0051]   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.

[0052]   As used herein, "reduced renal function" is an abrupt (within 14 days, preferably within 7 days, more preferably within 72 hours, and still more preferably within 48 hours) reduction in kidney function identified by an absolute increase in serum creatinine of greater than or equal to 0.1 mg/dL ($\geq$ 8.8 µmol/L), a percentage increase in serum creatinine of greater than or equal to 20% (1.2-fold from baseline), or a reduction in urine output (documented oliguria of less than 0. 5 ml/kg per hour).

[0053]   As used herein, "acute renal failure" or "ARF" is an abrupt (within 14 days, preferably within 7 days, more preferably within 72 hours, and still more preferably within 48 hours) reduction in kidney function identified by an absolute increase in serum creatinine of greater than or equal to 0.3 mg/dl ($\geq$ 26.4 µmol/l), a percentage increase in serum creatinine of greater than or equal to 50% (1. 5-fold from baseline), or a reduction in urine output (documented oliguria of less than 0.5 ml/kg per hour for at least 6 hours). This term is synonymous with "acute kidney injury" or "AKI."

[0054]   As used herein, the term "Ceruloplasmin" refers to one or more polypeptides present in a biological sample that are derived from the Ceruloplasmin precursor (human sequence: Swiss-Prot P00450 (SEQ ID NO: 1)):

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         10         20         30         40         50         60
MKILILGIFL FLCSTPAWAK EKHYYIGIIE TTWDYASDHG EKKLISVDTE HSNIYLQNGP

         70         80         90        100        110        120
DRIGRLYKKA LYLQYTDETF RTTIEKPVWL GFLGPIIKAE TGDKVYVHLK NLASRPYTFH

        130        140        150        160        170        180
SHGITYYKEH EGAIYPDNTT DFQRADDKVY PGEQYTYMLL ATEEQSPGEG DGNCVTRIYH

        190        200        210        220        230        240
SHIDAPKDIA SGLIGPLIIC KKDSLDKEKE KHIDREFVVM FSVVDENFSW YLEDNIKTYC

        250        260        270        280        290        300
SEPEKVDKDN EDFQESNRMY SVNGYTFGSL PGLSMCAEDR VKWYLFGMGN EVDVHAAFFH

        310        320        330        340        350        360
GQALTNKNYR IDTINLFPAT LFDAYMVAQN PGEWMLSCQN LNHLKAGLQA FFQVQECNKS

        370        380        390        400        410        420
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SSKDNIRGKH VRHYYIAAEE IIWNYAPSGI DIFTKENLTA PGSDSAVFFE QGTTRIGGSY

       430        440        450        460        470        480
KKLVYREYTD ASFTNRKERG PEEEHLGILG PVIWAEVGDT IRVTFHNKGA YPLSIEPIGV

       490        500        510        520        530        540
RFNKNNEGTY YSPNYNPQSR SVPPSASHVA PTETFTYEWT VPKEVGPTNA DPVCLAKMYY

       550        560        570        580        590        600
SAVDPTKDIF TGLIGPMKIC KKGSLHANGR QKDVDKEFYL FPTVFDENES LLLEDNIRMF

       610        620        630        640        650        660
TTAPDQVDKE DEDFQESNKM HSMNGFMYGN QPGLTMCKGD SVVWYLFSAG NEADVHGIYF

       670        680        690        700        710        720
SGNTYLWRGE RRDTANLFPQ TSLTLHMWPD TEGTFNVECL TTDHYTGGMK QKYTVNQCRR

       730        740        750        760        770        780
QSEDSTFYLG ERTYYIAAVE VEWDYSPQRE WEKELHHLQE QNVSNAFLDK GEFYIGSKYK

       790        800        810        820        830        840
KVVYRQYTDS TFRVPVERKA EEEHLGILGP QLHADVGDKV KIIFKNMATR PYSIHAHGVQ

       850        860        870        880        890        900
TESSTVTPTL PGETLTYVWK IPERSGAGTE DSACIPWAYY STVDQVKDLY SGLIGPLIVC

       910        920        930        940        950        960
RRPYLKVFNP RRKLEFALLF LVFDENESWY LDDNIKTYSD HPEKVNKDDE EFIESNKMHA

       970        980        990       1000       1010       1020
INGRMFGNLQ GLTMHVGDEV NWYLMGMGNE IDLHTVHFHG HSFQYKHRGV YSSDVFDIFP

      1030       1040       1050       1060
GTYQTLEMFP RTPGIWLLHC HVTDHIHAGM ETTYTVLQNE DTKSG
```

[0055]   The following domains have been identified in Ceruloplasmin:

Residues   Length   Domain ID

1-19   19   Signal peptiden

20-1065   1046   Ceruloplasmin

[0056]   As used herein, the term "Annexin A2" refers to one or more polypeptides present in a biological sample that are derived from the Annexin A2 precursor (human sequence: Swiss-Prot P07355 (SEQ ID NO: 2)):

```
        10         20         30         40         50         60
MSTVHEILCK LSLEGDHSTP PSAYGSVKAY TNFDAERDAL NIETAIKTKG VDEVTIVNIL

        70         80         90        100        110        120
TNRSNAQRQD IAFAYQRRTK KELASALKSA LSGHLETVIL GLLKTPAQYD ASELKASMKG
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          130        140        150        160        170        180
LGTDEDSLIE IICSRTNQEL QEINRVYKEM YKTDLEKDII SDTSGDFRKL MVALAKGRRA

          190        200        210        220        230        240
EDGSVIDYEL IDQDARDLYD AGVKRKGTDV PKWISIMTER SVPHLQKVFD RYKSYSPYDM

          250        260        270        280        290        300
LESIRKEVKG DLENAFLNLV QCIQNKPLYF ADRLYDSMKG KGTRDKVLIR IMVSRSEVDM

          310        320        330
LKIRSEFKRK YGKSLYYYIQ QDTKGDYQKA LLYLCGGDD
```

[0057]   The following domains have been identified in Annexin A2:

Residues	Length	Domain ID
1	1	Initiator methionine
2-339	338	Annexin A2
1		→ MGRQLAGCGDAGKKASFKM (SEQ ID NO: 3) in isoform 2

[0058]   As used herein, the term "relating a signal to the presence or amount" of an analyte reflects the following understanding. Assay signals are typically related to the presence or amount of an analyte through the use of a standard curve calculated using known concentrations of the analyte of interest. As the term is used herein, an assay is "configured to detect" an analyte if an assay can generate a detectable signal indicative of the presence or amount of a physiologically relevant concentration of the analyte. Because an antibody epitope is on the order of 8 amino acids, an immunoassay configured to detect a marker of interest will also detect polypeptides related to the marker sequence, so long as those polypeptides contain the epitope(s) necessary to bind to the antibody or antibodies used in the assay. The term "related marker" as used herein with regard to a biomarker such as one of the kidney injury markers described herein refers to one or more fragments, variants, etc., of a particular marker or its biosynthetic parent that may be detected as a surrogate for the marker itself or as independent biomarkers. The term also refers to one or more polypeptides present in a biological sample that are derived from the biomarker precursor complexed to additional species, such as binding proteins, receptors, heparin, lipids, sugars, etc.

[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 quatitation). This list is not meant to be limiting.

[0060]   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.

[0061]   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.

[0062]   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.

[0063]   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.

[0064]   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.

[0065]   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.

[0066]   Marker Assays

[0067]   In general, immunoassays involve contacting a sample containing or suspected of containing a biomarker of interest with at least one antibody that specifically binds to the biomarker. A signal is then generated indicative of the presence or amount of complexes formed by the binding of polypeptides in the sample to the antibody. The signal is then related to the presence or amount of the biomarker in the sample. Numerous methods and devices are well known to the skilled artisan for the detection and analysis of biomarkers. *See, e.g.,* U.S. Patents 6,143,576; 6,113,855; 6,019,944; 5,985,579;

5,947,124; 5,939,272; 5,922,615; 5,885,527; 5,851,776; 5,824,799; 5,679,526; 5,525,524; and 5,480,792, and *The Immunoassay Handbook*, David Wild, ed. Stockton Press, New York, 1994, each of which is hereby incorporated by reference in its entirety, including all tables, figures and claims.

[0068]   The assay devices and methods known in the art can utilize labeled molecules in various sandwich, competitive, or non-competitive assay formats, to generate a signal that is related to the presence or amount of the biomarker of interest. Suitable assay formats also include chromatographic, mass spectrographic, and protein "blotting" methods. Additionally, certain methods and devices, such as biosensors and optical immunoassays, may be employed to determine the presence or amount of analytes without the need for a labeled molecule. *See, e.g.*, U.S. Patents 5,631,171; and 5,955,377, each of which is hereby incorporated by reference in its entirety, including all tables, figures and claims. One skilled in the art also recognizes that robotic instrumentation including but not limited to Beckman ACCESS®, Abbott AXSYM®, Roche ELECSYS®, Dade Behring STRATUS® systems are among the immunoassay analyzers that are capable of performing immunoassays. But any suitable immunoassay may be utilized, for example, enzyme-linked immunoassays (ELISA), radioimmunoassays (RIAs), competitive binding assays, and the like.

[0069]   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.

[0070]   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.).

[0071]   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.

[0072]   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.

[0073]   Antibodies

[0074]   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."

[0075]   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} .

[0076]   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.

[0077]   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.

[0078]   Numerous publications discuss the use of phage display technology to produce and screen libraries of polypeptides for binding to a selected analyte. *See, e.g*, Cwirla *et al., Proc. Natl. Acad. Sci. USA* 87, 6378-82, 1990; Devlin *et al., Science* 249, 404-6, 1990, Scott and Smith, *Science* 249, 386-88, 1990; and Ladner *et al.*, U.S. Pat. No. 5,571,698. A basic concept of phage display methods is the establishment of a physical association between DNA encoding a polypeptide to be screened and the polypeptide. This physical association is provided by the phage particle, which displays a polypeptide as part of a capsid enclosing the phage genome which encodes the polypeptide. The establishment of a physical association between polypeptides and their genetic material allows simultaneous mass screening of very large numbers of phage bearing different polypeptides. Phage displaying a polypeptide with affinity to a target bind to the target and these phage are enriched by affinity screening to the target. The identity of polypeptides displayed from these phage can be determined from their respective genomes. Using these methods a polypeptide identified as having a binding affinity for a desired target can then be synthesized in bulk by conventional means. *See, e.g.*, U.S. Patent No. 6,057,098, which is hereby incorporated in its entirety, including all tables, figures, and claims.

[0079]   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.

[0080]   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.

[0081]   While the present application describes antibody-based binding assays in detail, alternatives to antibodies as binding species in assays are well known in the art. These include receptors for a particular target, aptamers, etc. Aptamers are oligonucleic acid or peptide molecules that bind to a specific target molecule. Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist. High-affinity aptamers containing modified nucleotides conferring improved characteristics on the ligand, such as improved in vivo stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions, and may include amino acid side chain functionalities.

[0082]   Assay Correlations

[0083]   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.

[0084]   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.

[0085]   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.

[0086]   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.

[0087]   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.

[0088]   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.

[0089]   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.

[0100]   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

[0101]   Additional clinical indicia may be combined with the kidney injury marker assay result(s) of the present invention. These include other biomarkers related to renal status. Examples include the following, which recite the common biomarker name, followed by the Swiss-Prot entry number for that biomarker or its parent: Actin (P68133); Adenosine deaminase binding protein (DPP4, P27487); Alpha-1-acid glycoprotein 1 (P02763); Alpha-1-microglobulin (P02760); Albumin (P02768); Angiotensinogenase (Renin, P00797); Annexin A2 (P07355); Beta-glucuronidase (P08236); B-2-microglobulin (P61679); Beta-galactosidase (P16278); BMP-7 (P18075); Brain natriuretic peptide (proBNP, BNP-32, NTproBNP; P16860); Calcium-binding protein Beta (S100-beta, P04271); Carbonic anhydrase (Q16790); Casein Kinase 2 (P68400); Ceruloplasmin (P00450); Clusterin (P10909); Complement C3 (P01024); Cysteine-rich protein (CYR61, O00622); Cytochrome C (P99999); Epidermal growth factor (EGF, P01133); Endothelin-1 (P05305); Exosomal Fetuin-A (P02765); Fatty acid-binding protein, heart (FABP3, P05413); Fatty acid-binding protein, liver (P07148); Ferritin (light chain, P02793; heavy chain P02794); Fructose-1,6-biphosphatase (P09467); GRO-alpha (CXCL1, (P09341); Growth Hormone (P01241); Hepatocyte growth factor (P14210);

Insulin-like growth factor I (P01343); Immunoglobulin G; Immunoglobulin Light Chains (Kappa and Lambda); Interferon gamma (P01308); Lysozyme (P61626); Interleukin-1alpha (P01583); Interleukin-2 (P60568); Interleukin-4 (P60568); Interleukin-9 (P15248); Interleukin-12p40 (P29460); Interleukin-13 (P35225); Interleukin-16 (Q14005); L1 cell adhesion molecule (P32004); Lactate dehydrogenase (P00338); Leucine Aminopeptidase (P28838); Meprin A-alpha subunit (Q16819); Meprin A-beta subunit (Q16820); Midkine (P21741); MIP2-alpha (CXCL2, P19875); MMP-2 (P08253); MMP-9 (P14780); Netrin-1 (O95631); Neutral endopeptidase (P08473); Osteopontin (P10451); Renal papillary antigen 1 (RPA1); Renal papillary antigen 2 (RPA2); Retinol binding protein (P09455); Ribonuclease; S100 calcium-binding protein A6 (P06703); Serum Amyloid P Component (P02743); Sodium/Hydrogen exchanger isoform (NHE3, P48764); Spermidine/spermine N1-acetyltransferase (P21673); TGF-Beta1 (P01137); Transferrin (P02787); Trefoil factor 3 (TFF3, Q07654); Toll-Like protein 4 (O00206); Total protein; Tubulointerstitial nephritis antigen (Q9UJW2); Uromodulin (Tamm-Horsfall protein, P07911).

[0102]   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.

[0103]   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.

[0104]   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.

[0090]   Diagnosis of Acute Renal Failure

[0105]   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:

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

[0106]   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.

[0107]   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.

[0108]   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:

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

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:

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

[0109]   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.

[0110]   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).

[0111]   Selecting a Treatment Regimen

[0112]   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.

[0113]   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.

[0114]   Example 1:   Contrast-induced nephropathy sample collection

[0115]   The objective of this sample collection study is to collect samples of plasma and urine and clinical data from patients before and after receiving intravascular contrast media. Approximately 250 adults undergoing radiographic/angiographic procedures involving intravascular administration of iodinated contrast media are enrolled. To be enrolled in the study, each patient must meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria

males and females 18 years of age or older;

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

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

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

Exclusion Criteria

renal transplant recipients;

acutely worsening renal function prior to the contrast procedure;

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

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

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

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

[0116]   Immediately prior to the first contrast administration (and after any pre-procedure hydration), an EDTA anti-coagulated blood sample (10 mL) and a urine sample (10 mL) are collected from each patient. Blood and urine samples are then collected at 4 (±0.5), 8 (±1), 24 (±2) 48 (±2), and 72 (±2) hrs following the last administration of contrast media during the index contrast procedure. Blood is collected via direct venipuncture or via other available venous access, such as an existing femoral sheath, central venous line, peripheral intravenous line or hep-lock. These study blood samples are processed to plasma at the clinical site, frozen and shipped to Astute Medical, Inc., San Diego, CA. The study urine samples are frozen and shipped to Astute Medical, Inc.

[0117]   Serum creatinine is assessed at the site immediately prior to the first contrast administration (after any pre-procedure hydration) and at 4 (±0.5), 8 (±1), 24 (±2) and 48 (±2)), and 72 (±2) hours following the last administration of contrast (ideally at the same time as the study samples are obtained). In addition, each patient's status is evaluated through day 30 with regard to additional serum and urine creatinine measurements, a need for dialysis, hospitalization status, and adverse clinical outcomes (including mortality).

[0118]   Prior to contrast administration, each patient is assigned a risk based on the following assessment: systolic blood pressure <80 mm Hg = 5 points; intra-arterial balloon pump = 5 points; congestive heart failure (Class III-IV or history of pulmonary edema) = 5 points; age >75 yrs = 4 points; hematocrit level <39% for men, <35% for women = 3 points; diabetes = 3 points; contrast media volume = 1 point for each 100 mL; serum creatinine level >1.5 g/dL = 4 points OR estimated GFR 40–60 mL/min/1.73 m^2 = 2 points, 20–40 mL/min/1.73 m^2 = 4 points, < 20 mL/min/1.73 m^2 = 6 points. The risks assigned are as follows: risk for CIN and dialysis: 5 or less total points = risk of CIN -

7.5%, risk of dialysis - 0.04%; 6–10 total points = risk of CIN - 14%, risk of dialysis - 0.12%; 11–16 total points = risk of CIN - 26.1%, risk of dialysis - 1.09%; >16 total points = risk of CIN - 57.3%, risk of dialysis - 12.8%.

[0119]   Example 2:   Cardiac surgery sample collection

[0120]   The objective of this sample collection study is to collect samples of plasma and urine and clinical data from patients before and after undergoing cardiovascular surgery, a procedure known to be potentially damaging to kidney function. Approximately 900 adults undergoing such surgery are enrolled. To be enrolled in the study, each patient must meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria

males and females 18 years of age or older;

undergoing cardiovascular surgery;

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

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

Exclusion Criteria

known pregnancy;

previous renal transplantation;

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

RIFLE criteria);

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

currently enrolled in another clinical study or expected to be enrolled in another clinical study within 7 days of cardiac surgery that involves drug infusion or a therapeutic intervention for AKI;

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

[0121]   Within 3 hours prior to the first incision (and after any pre-procedure hydration), an EDTA anti-coagulated blood sample (10 mL), whole blood (3 mL), and a urine sample (35 mL) are collected from each patient. Blood and urine samples are then collected at 3 (±0.5), 6 (±0.5), 12 (±1), 24 (±2) and 48 (±2) hrs following the procedure and then daily on days 3 through 7 if the subject remains in the hospital. Blood is collected via direct venipuncture or via other available venous access, such as an existing femoral sheath, central venous line, peripheral intravenous line or hep-lock. These study blood samples are frozen and shipped to Astute Medical, Inc., San Diego, CA. The study urine samples are frozen and shipped to Astute Medical, Inc.

[0122]   Example 3:   Acutely ill subject sample collection

[0123]   The objective of this study is to collect samples from acutely ill patients. Approximately 1900 adults expected to be in the ICU for at least 48 hours will be enrolled. To be enrolled in the study, each patient must meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria

males and females 18 years of age or older;

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

shock (SBP < 90 mmHg and/or need for vasopressor support to maintain MAP > 60 mmHg and/or documented drop in SBP of at least 40 mmHg); and

sepsis;

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

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

contrast media exposure within 24 hours of enrollment;

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

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

Study population 3: approximately 300 patients expected to be hospitalized through acute care setting (ICU or ED) with a known risk factor for acute renal injury (e.g. sepsis, hypotension/shock (Shock = systolic BP < 90 mmHg and/or the need for vasopressor

support to maintain a MAP > 60 mmHg and/or a documented drop in SBP > 40 mmHg), major trauma, hemorrhage, or major surgery); and/or expected to be hospitalized to the ICU for at least 24 hours after enrollment;

Study population 4: approximately 1000 patients that are 21 years of age or older, within 24 hours of being admitted into the ICU, expected to have an indwelling urinary catheter for at least 48 hours after enrollment, and have at least one of the following acute conditions within 24 hours prior to enrollment:

(i) respiratory SOFA score of ≥ 2 (PaO2/FiO2 <300), (ii) cardiovascular SOFA score of ≥ 1 (MAP < 70 mm Hg and/or any vasopressor required).

Exclusion Criteria

known pregnancy;

institutionalized individuals;

previous renal transplantation;

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

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

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

meets any of the following:

(i)   active bleeding with an anticipated need for > 4 units PRBC in a day;

(ii)   hemoglobin < 7 g/dL;

(iii)   any other condition that in the physician's opinion would contraindicate drawing serial blood samples for clinical study purposes;

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

[0124]   After obtaining informed consent, an EDTA anti-coagulated blood sample (10 mL) and a urine sample (25-50 mL) are collected from each patient. Blood and urine samples are then collected at 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), 36 (± 2), 48 (± 2), 60 (± 2), 72 (± 2), and 84 (± 2) hours

after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Blood is collected via direct venipuncture or via other available venous access, such as an existing femoral sheath, central venous line, peripheral intravenous line or hep-lock. These study blood samples are processed to plasma at the clinical site, frozen and shipped to Astute Medical, Inc., San Diego, CA. The study urine samples are frozen and shipped to Astute Medical, Inc.

[0125]   Example 4.   Immunoassay format

[0126]   Analytes are measured using standard sandwich enzyme immunoassay techniques. A first antibody which binds the analyte is immobilized in wells of a 96 well polystyrene microplate. Analyte standards and test samples are pipetted into the appropriate wells and any analyte present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase-conjugated second antibody which binds the analyte is added to the wells, thereby forming sandwich complexes with the analyte (if present) and the first antibody. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution comprising tetramethylbenzidine and hydrogen peroxide is added to the wells. Color develops in proportion to the amount of analyte present in the sample. The color development is stopped and the intensity of the color is measured at 540 nm or 570 nm. An analyte concentration is assigned to the test sample by comparison to a standard curve determined from the analyte standards.

[0127]   In the case of those kidney injury markers which are membrane proteins as described herein, the assays used in these examples detect soluble forms thereof. Concentrations reported below are in ng/mL for all markers measured.

[0128]   Example 5.   Apparently Healthy Donor and Chronic Disease Patient Samples

[0129]   Human urine samples from donors with no known chronic or acute disease ("Apparently Healthy Donors") were purchased from two vendors (Golden West Biologicals, Inc., 27625 Commerce Center Dr., Temecula, CA 92590 and Virginia Medical Research, Inc., 915 First Colonial Rd., Virginia Beach, VA 23454). The urine samples were shipped and stored frozen at less than -20° C. The vendors supplied demographic information for the individual donors including gender, race (Black /White), smoking status and age.

[0130]   Human urine samples from donors with various chronic diseases ("Chronic Disease Patients") including congestive heart failure, coronary artery disease, chronic kidney disease, chronic obstructive pulmonary disease, diabetes mellitus and hypertension were purchased from Virginia Medical Research, Inc., 915 First Colonial Rd., Virginia Beach, VA 23454. The urine samples were shipped and stored frozen at less than -20 degrees centigrade. The vendor provided a case report form for each individual donor with age, gender, race (Black/White), smoking status and alcohol use, height, weight, chronic disease(s) diagnosis, current medications and previous surgeries.

[0131]   Example 6.   Use of Kidney Injury Markers for evaluating renal status in patients

[0132]   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 ($\pm$ 0.5) and 8 ($\pm$ 1) hours after contrast administration (if applicable); at 12 ($\pm$ 1), 24 ($\pm$ 2), and 48 ($\pm$ 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject was hospitalized. Markers were each measured by standard immunoassay methods using commercially available assay reagents in the urine samples and the plasma component of the blood samples collected.

[0133]   Two cohorts were defined to represent a "diseased" and a "normal" population. While these terms were used for convenience, "diseased" and "normal" simply represent two cohorts for comparison (say RIFLE 0 vs RIFLE R, I and F; RIFLE 0 vs RIFLE R; RIFLE 0 and R vs RIFLE I and F; etc.). The time "prior max stage" represents the time at which a sample was collected, relative to the time a particular patient reaches the lowest disease stage as defined for that cohort, binned into three groups which were +/- 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).

[0134]   A receiver operating characteristic (ROC) curve was generated for each biomarker measured 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 include patients adjudicated to stage R, I, or F on the basis of urine output; for those patients adjudicated to stage R, I, or F on the basis of urine output alone, the stage 0 cohort may include patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements; and for those patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements or urine output, the stage 0 cohort contains only patients in stage 0 for both serum creatinine measurements and urine output. Also, in the data for patients adjudicated on the basis of serum creatinine measurements or urine output, the adjudication method which yielded the most severe RIFLE stage was used.

[0135]   The ability to distinguish cohort 1 from Cohort 2 was determined using ROC analysis. SE was the standard error of the AUC, n was 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 was indicative of a negative going marker for the comparison, and an AUC > 0.5 was indicative of a positive going marker for the comparison.

[0136]   Various threshold (or "cutoff") concentrations were selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 were determined. OR was the odds ratio calculated for the particular cutoff concentration, and 95% CI was the confidence interval for the odds ratio.

[0137]   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.

Ceruloplasmin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	450	1260	450	753	450	511
Average	1610	6560	1610	3490	1610	631
Stdev	4670	11100	4670	7230	4670	208
p(t-test)		0.011		0.21		0.65

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Min	34.3	28.5	34.3	84.9	34.3	467
Max	30000	30000	30000	30000	30000	946
n (Samp)	47	22	47	20	47	5
n (Patient)	22	22	22	20	22	5

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	611	1010	611	2020	611	946
Average	2550	6660	2550	6390	2550	964
Stdev	6270	11700	6270	10300	6270	539
p(t-test)		0.18		0.12		0.57
Min	6.77	568	6.77	485	6.77	404
Max	30000	27500	30000	30000	30000	1670
n (Samp)	98	5	98	8	98	5
n (Patient)	47	5	47	8	47	5

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	450	1260	450	631	450	499
Average	1770	5790	1770	2960	1770	4830
Stdev	4930	10500	4930	6930	4930	9560
p(t-test)		0.037		0.43		0.17
Min	34.3	28.5	34.3	84.9	34.3	145
Max	30000	30000	30000	30000	30000	27500
n (Samp)	47	20	47	20	47	8
n (Patient)	22	20	22	20	22	8

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.74	0.71	0.73	0.63	0.74	0.61	0.61	0.56	0.61
SE	0.068	0.13	0.072	0.077	0.10	0.077	0.14	0.14	0.11
p	4.7E-4	0.12	0.0017	0.11	0.020	0.14	0.42	0.65	0.33
nCohort 1	47	98	47	47	98	47	47	98	47
nCohort 2	22	5	20	20	8	20	5	5	8
Cutoff 1	836	836	836	404	836	404	467	467	459
Sens 1	73%	80%	70%	70%	75%	70%	80%	80%	75%
Spec 1	72%	58%	72%	45%	58%	45%	53%	41%	53%
Cutoff 2	459	836	459	322	624	322	467	467	426
Sens 2	82%	80%	80%	80%	88%	80%	80%	80%	88%
Spec 2	53%	58%	53%	40%	53%	40%	53%	41%	49%
Cutoff 3	322	564	322	146	467	146	459	361	137
Sens 3	91%	100%	90%	90%	100%	90%	100%	100%	100%
Spec 3	40%	48%	40%	17%	41%	17%	53%	34%	17%

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Cutoff 4	728	1220	728	728	1220	728	728	1220	728
Sens 4	73%	40%	70%	50%	62%	40%	40%	40%	38%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	1170	1950	1080	1170	1950	1080	1170	1950	1080
Sens 5	55%	40%	60%	45%	50%	40%	0%	0%	25%
Spec 5	81%	81%	81%	81%	81%	81%	81%	81%	81%
Cutoff 6	2510	3590	2420	2510	3590	2420	2510	3590	2420
Sens 6	27%	20%	30%	15%	25%	15%	0%	0%	25%
Spec 6	91%	91%	91%	91%	91%	91%	91%	91%	91%
OR Quart 2	1.6	>1.0	2.2	1.8	>1.0	1.8	>1.1	>2.1	0.92
	0.63	<1.0	0.42	0.48	<1.0	0.48	<0.96	<0.56	0.96
p Value	0.23	>0.059	0.34	0.35	>0.059	0.35	>0.061	>0.18	0.052
95% CI of OR Quart2	11	na	14	9.2	na	9.2	na	na	16
OR Quart 3	5.2	>2.1	2.9	1.3	>2.2	1.3	>3.9	>2.1	4.8
	0.065	<0.56	0.25	0.74	<0.54	0.74	<0.27	<0.56	0.19
p Value	0.90	>0.18	0.48	0.25	>0.18	0.25	>0.35	>0.18	0.46
95% CI of OR Quart3	31	na	18	7.2	na	7.2	na	na	50
OR Quart 4	9.4	>2.1	7.9	3.9	>5.9	3.9	>1.1	>1.0	2.0
	0.012	<0.56	0.022	0.093	<0.12	0.093	<0.96	<1.0	0.59
p Value	1.6	>0.18	1.4	0.80	>0.64	0.80	>0.061	>0.059	0.16
95% CI of OR Quart4	54	na	46	19	na	19	na	na	25

[0138]   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.

Ceruloplasmin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	562	1160	562	1180
Average	2740	3130	2740	2030
Stdev	6850	6910	6850	3920
p(t-test)		0.83		0.66
Min	28.5	6.77	28.5	31.7
Max	30000	30000	30000	18200

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
n (Samp)	92	18	92	20
n (Patient)	44	18	44	20

sCr only	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	621	446	621	638
Average	2810	446	2810	1360
Stdev	6620	573	6620	1800
p(t-test)		0.62		0.71
Min	6.77	40.5	6.77	31.7
Max	30000	851	30000	3410
n (Samp)	124	2	124	3
n (Patient)	60	2	60	3

UO only	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	550	1290	550	1180
Average	2860	3310	2860	2140
Stdev	7150	7080	7150	4000
p(t-test)		0.81		0.67
Min	28.5	6.77	28.5	84.9
Max	30000	30000	30000	18200
n (Samp)	84	17	84	19
n (Patient)	40	17	40	19

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.59	0.30	0.63	0.61	0.47	0.65
SE	0.076	0.21	0.078	0.073	0.17	0.074
p	0.23	0.35	0.087	0.14	0.88	0.042
nCohort 1	92	124	84	92	124	84
nCohort 2	18	2	17	20	3	19
Cutoff 1	436	35.4	488	585	28.5	585
Sens 1	72%	100%	71%	70%	100%	74%
Spec 1	39%	3%	46%	53%	2%	55%
Cutoff 2	322	35.4	410	400	28.5	400

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Sens 2	83%	100%	82%	80%	100%	84%
Spec 2	30%	3%	38%	35%	2%	36%
Cutoff 3	34.3	35.4	34.3	146	28.5	146
Sens 3	94%	100%	94%	90%	100%	95%
Spec 3	2%	3%	4%	14%	2%	17%
Cutoff 4	961	1290	946	961	1290	946
Sens 4	56%	0%	59%	60%	33%	63%
Spec 4	71%	70%	70%	71%	70%	70%
Cutoff 5	1810	2090	1810	1810	2090	1810
Sens 5	39%	0%	41%	25%	33%	26%
Spec 5	80%	81%	81%	80%	81%	81%
Cutoff 6	3590	4670	4670	3590	4670	4670
Sens 6	11%	0%	12%	10%	0%	5%
Spec 6	90%	90%	90%	90%	90%	90%
OR	1.3	>1.1	2.2	1.4	1.0	1.5
Quart 2	0.72	<0.96	0.39	0.69	1.0	0.67
p Value	0.27	>0.064	0.36	0.28	0.060	0.23
95% CI of OR Quart2	6.6	na	13	6.9	17	9.8
OR	1.0	>0	1.6	2.3	0	3.4
Quart 3	1.0	<na	0.64	0.28	na	0.16
p Value	0.18	>na	0.24	0.51	na	0.62
95% CI of OR Quart3	5.5	na	10	10	na	19
OR	3.2	>1.1	5.1	2.8	1.0	5.1
Quart 4	0.12	<0.96	0.055	0.17	0.98	0.055
p Value	0.75	>0.064	0.96	0.64	0.062	0.96
95% CI of OR Quart4	14	na	27	12	17	27

[0139]   Table 3: 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.

Ceruloplasmin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	404000	414000	404000	457000	404000	425000
Average	487000	463000	487000	507000	487000	427000
Stdev	478000	171000	478000	223000	478000	99500
p(t-test)		0.83		0.86		0.78
Min	15500	229000	15500	189000	15500	305000
Max	3450000	906000	3450000	1010000	3450000	537000
n (Samp)	50	21	50	18	50	5
n (Patient)	25	21	25	18	25	5

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	411000	413000	411000	531000	411000	537000
Average	460000	510000	460000	563000	460000	551000
Stdev	360000	221000	360000	274000	360000	119000
p(t-test)		0.79		0.46		0.58
Min	15500	374000	15500	224000	15500	402000
Max	3450000	840000	3450000	1010000	3450000	731000
n (Samp)	99	4	99	7	99	5
n (Patient)	49	4	49	7	49	5

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	414000	422000	414000	399000	414000	354000
Average	498000	446000	498000	450000	498000	397000
Stdev	479000	151000	479000	217000	479000	140000
p(t-test)		0.64		0.68		0.58
Min	15500	229000	15500	189000	15500	199000
Max	3450000	906000	3450000	1010000	3450000	609000
n (Samp)	51	20	51	19	51	7
n (Patient)	26	20	26	19	26	7

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.56	0.59	0.53	0.59	0.65	0.49	0.54	0.76	0.45
SE	0.076	0.15	0.077	0.081	0.12	0.078	0.14	0.13	0.12
p	0.41	0.55	0.75	0.25	0.20	0.85	0.80	0.043	0.68
nCohort 1	50	99	51	50	99	51	50	99	51
nCohort 2	21	4	20	18	7	19	5	5	7

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Cutoff 1	383000	411000	383000	393000	462000	316000	340000	505000	340000
Sens 1	71%	75%	70%	72%	71%	74%	80%	80%	71%
Spec 1	46%	51%	45%	48%	67%	29%	34%	76%	33%
Cutoff 2	369000	369000	369000	316000	316000	283000	340000	505000	299000
Sens 2	81%	100%	80%	83%	86%	84%	80%	80%	86%
Spec 2	42%	40%	41%	30%	29%	22%	34%	76%	25%
Cutoff 3	262000	369000	262000	196000	196000	189000	299000	402000	196000
Sens 3	90%	100%	90%	94%	100%	95%	100%	100%	100%
Spec 3	20%	40%	20%	12%	8%	12%	26%	48%	14%
Cutoff 4	473000	477000	505000	473000	477000	505000	473000	477000	505000
Sens 4	33%	25%	25%	39%	57%	26%	40%	80%	29%
Spec 4	70%	71%	71%	70%	71%	71%	70%	71%	71%
Cutoff 5	561000	559000	591000	561000	559000	591000	561000	559000	591000
Sens 5	14%	25%	10%	33%	43%	16%	0%	40%	14%
Spec 5	80%	81%	82%	80%	81%	82%	80%	81%	82%
Cutoff 6	610000	649000	649000	610000	649000	649000	610000	649000	649000
Sens 6	14%	25%	10%	22%	29%	16%	0%	20%	0%
Spec 6	90%	91%	90%	90%	91%	90%	90%	91%	90%
OR Quart 2	1.8	>1.0	2.3	1.0	0.96	1.5	>2.2	>1.0	2.3
	0.48	<1.0	0.30	1.0	0.98	0.63	<0.55	<0.98	0.51
p Value	0.36	>0.059	0.48	0.17	0.057	0.32	>0.17	>0.062	0.19
95% CI of OR Quart2	9.1	na	11	5.8	16	6.7	na	na	29
OR Quart 3	3.0	>2.1	3.0	2.5	1.0	1.8	>1.0	>1.0	3.5
	0.17	<0.56	0.17	0.25	1.0	0.46	<1.0	<0.98	0.30
p Value	0.62	>0.18	0.62	0.52	0.059	0.40	>0.056	>0.062	0.32
95% CI of OR Quart3	14	na	14	13	17	7.7	na	na	38
OR Quart 4	2.3	>1.0	1.3	2.5	4.3	1.1	>2.2	>3.4	1.1
	0.30	<1.0	0.74	0.25	0.20	0.93	<0.55	<0.30	0.96
p Value	0.48	>0.059	0.25	0.52	0.45	0.22	>0.17	>0.33	0.061
95% CI of OR Quart4	11	na	7.1	13	42	5.2	na	na	19

[0140]   Table 4: 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.

Ceruloplasmin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	414000	412000	414000	380000
Average	469000	420000	469000	440000
Stdev	375000	156000	375000	215000
p(t-test)		0.58		0.74
Min	15500	188000	15500	190000
Max	3450000	779000	3450000	923000
n (Samp)	91	19	91	20
n (Patient)	45	19	45	20

sCr only	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	414000	412000	414000	327000
Average	462000	437000	462000	297000
Stdev	336000	143000	336000	95500
p(t-test)		0.90		0.40
Min	15500	309000	15500	190000
Max	3450000	591000	3450000	374000
n (Samp)	123	3	123	3
n (Patient)	61	3	61	3

UO only	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	410000	412000	410000	382000
Average	470000	417000	470000	453000
Stdev	389000	158000	389000	212000
p(t-test)		0.58		0.85
Min	15500	188000	15500	224000
Max	3450000	779000	3450000	923000
n (Samp)	84	17	84	19
n (Patient)	42	17	42	19

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.48	0.53	0.47	0.47	0.25	0.50
SE	0.074	0.17	0.078	0.072	0.16	0.074
p	0.76	0.84	0.74	0.73	0.12	0.97

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
nCohort 1	91	123	84	91	123	84
nCohort 2	19	3	17	20	3	19
Cutoff 1	297000	305000	297000	316000	189000	316000
Sens 1						
Spec 1	74%	100%	71%	70%	100%	74%
	22%	26%	21%	27%	7%	29%
Cutoff 2	271000	305000	271000	232000	189000	232000
Sens 2						
Spec 2	84%	100%	82%	85%	100%	89%
	15%	26%	17%	12%	7%	14%
Cutoff 3	244000	305000	244000	224000	189000	224000
Sens 3						
Spec 3	95%	100%	94%	90%	100%	95%
	13%	26%	15%	11%	7%	13%
Cutoff 4	477000	479000	477000	477000	479000	477000
Sens 4						
Spec 4	32%	33%	29%	30%	0%	32%
	70%	71%	70%	70%	71%	70%
Cutoff 5	561000	561000	567000	561000	561000	567000
Sens 5						
Spec 5	16%	33%	12%	25%	0%	21%
	80%	80%	81%	80%	80%	81%
Cutoff 6	610000	674000	610000	610000	674000	610000
Sens 6						
Spec 6	11%	0%	12%	15%	0%	16%
	90%	90%	90%	90%	90%	90%
OR Quart 2	0.80	>2.1	1.4	0.44	>0	0.95
p Value	0.76	<0.56	0.67	0.28	<na	0.94
95% CI of	0.19	>0.18	0.32	0.098	>na	0.24
OR Quart2	3.4	na	5.8	2.0	na	3.8
OR Quart 3	0.77	>0	0.48	0.80	>2.1	0.52
p Value	0.72	<na	0.42	0.74	<0.54	0.41
95% CI of	0.18	>na	0.079	0.21	>0.18	0.11
OR Quart3	3.2	na	2.9	3.0	na	2.5
OR Quart 4	1.3	>1.0	1.7	1.0	>1.1	1.2
p Value	0.69	<1.0	0.44	0.94	<0.96	0.79
95% CI of	0.35	>0.060	0.43	0.29	>0.064	0.31
OR Quart4	5.0	na	7.1	3.8	na	4.6

[0141] Table 5: 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.

Annexin A2

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.000221	0.000221	0.000221	0.000183
Average	0.0282	0.104	0.0282	0.109
Stdev	0.116	0.230	0.116	0.219
p(t-test)		0.14		0.079
Min	0.000144	0.000144	0.000144	0.000144
Max	0.888	0.571	0.888	0.599
n (Samp)	116	6	116	8
n (Patient)	56	6	56	8

UO only	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.000221	0.000221	0.000221	0.000183
Average	0.0289	0.104	0.0289	0.109
Stdev	0.120	0.230	0.120	0.219
p(t-test)		0.16		0.093
Min	0.000144	0.000144	0.000144	0.000144
Max	0.888	0.571	0.888	0.599
n (Samp)	106	6	106	8
n (Patient)	51	6	51	8

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.61	nd	0.61	0.52	nd	0.52
SE	0.13	nd	0.13	0.11	nd	0.11
p	0.36	nd	0.37	0.84	nd	0.86
nCohort 1	116	nd	106	116	nd	106
nCohort 2	6	nd	6	8	nd	8
Cutoff 1	0	nd	0	0	nd	0
Sens 1	100%	nd	100%	100%	nd	100%
Spec 1	0%	nd	0%	0%	nd	0%

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Cutoff 2	0	nd	0	0	nd	0
Sens 2	100%	nd	100%	100%	nd	100%
Spec 2	0%	nd	0%	0%	nd	0%
Cutoff 3	0	nd	0	0	nd	0
Sens 3	100%	nd	100%	100%	nd	100%
Spec 3	0%	nd	0%	0%	nd	0%
Cutoff 4	0.000221	nd	0.000221	0.000221	nd	0.000221
Sens 4	33%	nd	33%	25%	nd	25%
Spec 4	92%	nd	92%	92%	nd	92%
Cutoff 5	0.000221	nd	0.000221	0.000221	nd	0.000221
Sens 5	33%	nd	33%	25%	nd	25%
Spec 5	92%	nd	92%	92%	nd	92%
Cutoff 6	0.000221	nd	0.000221	0.000221	nd	0.000221
Sens 6	33%	nd	33%	25%	nd	25%
Spec 6	92%	nd	92%	92%	nd	92%
OR Quart 2	0	nd	0	0	nd	0.21
	na	nd	na	na	nd	0.18
p Value	na	nd	na	na	nd	0.022
95% CI of OR Quart2	na	nd	na	na	nd	2.0
OR Quart 3	1.0	nd	1.0	0.47	nd	0.22
	1.0	nd	1.0	0.40	nd	0.19
p Value	0.13	nd	0.13	0.079	nd	0.023
95% CI of OR Quart3	7.6	nd	7.6	2.8	nd	2.1
OR Quart 4	0.97	nd	1.0	0.47	nd	0.44
	0.97	nd	1.0	0.40	nd	0.37
p Value	0.13	nd	0.13	0.079	nd	0.075
95% CI of OR Quart4	7.3	nd	7.6	2.8	nd	2.6

Ceruloplasmin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	611	940	611	905
Average	2590	6570	2590	1220
Stdev	6310	11700	6310	1190
p(t-test)		0.15		0.54
Min	6.77	324	6.77	84.9
Max	30000	30000	30000	3860
n (Samp)	116	6	116	8
n (Patient)	56	6	56	8

UO only	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	611	940	611	905
Average	2720	6570	2720	1220
Stdev	6570	11700	6570	1190
p(t-test)		0.19		0.52
Min	6.77	324	6.77	84.9
Max	30000	30000	30000	3860
n (Samp)	106	6	106	8
n (Patient)	51	6	51	8

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.63	nd	0.63	0.55	nd	0.55
SE	0.13	nd	0.13	0.11	nd	0.11
p	0.31	nd	0.32	0.61	nd	0.62
nCohort 1	116	nd	106	116	nd	106
nCohort 2	6	nd	6	8	nd	8
Cutoff 1	410	nd	410	522	nd	511
Sens 1	83%	nd	83%	75%	nd	75%
Spec 1	34%	nd	34%	45%	nd	44%
Cutoff 2	410	nd	410	400	nd	400
Sens 2	83%	nd	83%	88%	nd	88%
Spec 2	34%	nd	34%	33%	nd	32%
Cutoff 3	322	nd	322	84.1	nd	84.1
Sens 3	100%	nd	100%	100%	nd	100%

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Spec 3	28%	nd	28%	8%	nd	8%
Cutoff 4	1290	nd	1290	1290	nd	1290
Sens 4	33%	nd	33%	38%	nd	38%
Spec 4	71%	nd	71%	71%	nd	71%
Cutoff 5	1960	nd	1960	1960	nd	1960
Sens 5	33%	nd	33%	12%	nd	12%
Spec 5	80%	nd	80%	80%	nd	80%
Cutoff 6	3410	nd	3410	3410	nd	3410
Sens 6	33%	nd	33%	12%	nd	12%
Spec 6	91%	nd	91%	91%	nd	91%
OR Quart 2 p Value 95% CI of OR Quart2	>2.1 <0.56 >0.18 na	nd nd nd nd	>2.2 <0.54 >0.18 na	2.1 0.56 0.18 24	nd nd nd nd	2.0 0.58 0.17 23
OR Quart 3 p Value 95% CI of OR Quart3	>2.1 <0.54 >0.18 na	nd nd nd nd	>2.2 <0.54 >0.18 na	4.4 0.19 0.47 42	nd nd nd nd	4.5 0.19 0.47 43
OR Quart 4 p Value 95% CI of OR Quart4	>2.1 <0.56 >0.18 na	nd nd nd nd	>2.2 <0.54 >0.18 na	1.0 1.0 0.060 17	nd nd nd nd	0.96 0.98 0.057 16

[0142]   Table 6: 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.

Annexin A2

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

	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.330	0.0951	0.330	0.176
Average	2.83	0.182	2.83	0.396
Stdev	8.99	0.277	8.99	0.668
p(t-test)		0.47		0.45
Min	6.45E-5	8.63E-5	6.45E-5	8.63E-5
Max	52.3	0.724	52.3	2.03
n (Samp)	116	6	116	8
n (Patient)	58	6	58	8

UO only	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.349	0.0951	0.349	0.176
Average	3.09	0.182	3.09	0.396
Stdev	9.36	0.277	9.36	0.668
p(t-test)		0.45		0.42
Min	6.45E-5	8.63E-5	6.45E-5	8.63E-5
Max	52.3	0.724	52.3	2.03
n (Samp)	106	6	106	8
n (Patient)	53	6	53	8

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.31	nd	0.28	0.40	nd	0.37
SE	0.12	nd	0.12	0.11	nd	0.11
p	0.13	nd	0.072	0.37	nd	0.25
nCohort 1	116	nd	106	116	nd	106
nCohort 2	6	nd	6	8	nd	8
Cutoff 1	6.45E-5	nd	6.45E-5	0.0982	nd	0.0894
Sens 1	100%	nd	100%	75%	nd	75%
Spec 1	9%	nd	8%	30%	nd	26%
Cutoff 2	6.45E-5	nd	6.45E-5	0.0289	nd	0.0289
Sens 2	100%	nd	100%	88%	nd	88%
Spec 2	9%	nd	8%	24%	nd	21%
Cutoff 3	6.45E-5	nd	6.45E-5	6.45E-5	nd	6.45E-5
Sens 3	100%	nd	100%	100%	nd	100%
Spec 3	9%	nd	8%	9%	nd	8%
Cutoff 4	0.789	nd	0.879	0.789	nd	0.879

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Sens 4	0%	nd	0%	12%	nd	12%
Spec 4	71%	nd	71%	71%	nd	71%
Cutoff 5	1.43	nd	1.61	1.43	nd	1.61
Sens 5	0%	nd	0%	12%	nd	12%
Spec 5	80%	nd	80%	80%	nd	80%
Cutoff 6	3.93	nd	4.23	3.93	nd	4.23
Sens 6	0%	nd	0%	0%	nd	0%
Spec 6	91%	nd	91%	91%	nd	91%
OR	>1.1	nd	>1.0	1.0	nd	0
Quart 2	<0.96	nd	<0.98	1.0	nd	na
p Value	>0.064	nd	>0.062	0.060	nd	na
95% CI of OR Quart2	na	nd	na	17	nd	na
OR	>2.1	nd	>2.2	4.4	nd	5.8
Quart 3	<0.54	nd	<0.54	0.19	nd	0.12
p Value	>0.18	nd	>0.18	0.47	nd	0.64
95% CI of OR Quart3	na	nd	na	42	nd	53
OR	>3.4	nd	>3.4	2.1	nd	2.2
Quart 4	<0.30	nd	<0.31	0.56	nd	0.54
p Value	>0.34	nd	>0.33	0.18	nd	0.18
95% CI of OR Quart4	na	nd	na	24	nd	25

Ceruloplasmin

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	400000	495000	400000	511000
Average	450000	480000	450000	550000
Stdev	343000	135000	343000	197000
p(t-test)		0.83		0.42
Min	15500	271000	15500	327000
Max	3450000	674000	3450000	923000

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
n (Samp)	116	6	116	8
n (Patient)	58	6	58	8

UO only	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	397000	495000	397000	511000
Average	452000	480000	452000	550000
Stdev	357000	135000	357000	197000
p(t-test)		0.85		0.45
Min	15500	271000	15500	327000
Max	3450000	674000	3450000	923000
n (Samp)	106	6	106	8
n (Patient)	53	6	53	8

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.64	nd	0.64	0.70	nd	0.69
SE	0.13	nd	0.13	0.11	nd	0.11
p	0.27	nd	0.26	0.067	nd	0.069
nCohort 1	116	nd	106	116	nd	106
nCohort 2	6	nd	6	8	nd	8
Cutoff 1	411000	nd	406000	444000	nd	444000
Sens 1	83%	nd	83%	75%	nd	75%
Spec 1	53%	nd	54%	60%	nd	60%
Cutoff 2	411000	nd	406000	377000	nd	377000
Sens 2	83%	nd	83%	88%	nd	88%
Spec 2	53%	nd	54%	45%	nd	46%
Cutoff 3	262000	nd	262000	316000	nd	316000
Sens 3	100%	nd	100%	100%	nd	100%
Spec 3	18%	nd	19%	32%	nd	32%
Cutoff 4	473000	nd	473000	473000	nd	473000
Sens 4	50%	nd	50%	50%	nd	50%
Spec 4	71%	nd	71%	71%	nd	71%
Cutoff 5	549000	nd	549000	549000	nd	549000
Sens 5	17%	nd	17%	50%	nd	50%
Spec 5	80%	nd	80%	80%	nd	80%

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Cutoff 6	610000	nd	610000	610000	nd	610000
Sens 6	17%	nd	17%	25%	nd	25%
Spec 6	91%	nd	91%	91%	nd	91%
OR	0	nd	0	>2.1	nd	>2.1
Quart 2	na	nd	na	<0.54	nd	<0.56
p Value	na	nd	na	>0.18	nd	>0.18
95% CI of OR Quart2	na	nd	na	na	nd	na
OR	2.1	nd	2.1	>2.1	nd	>2.2
Quart 3	0.56	nd	0.56	<0.54	nd	<0.54
p Value	0.18	nd	0.18	>0.18	nd	>0.18
95% CI of OR Quart3	24	nd	24	na	nd	na
OR	3.1	nd	3.2	>4.6	nd	>4.5
Quart 4	0.34	nd	0.32	<0.18	nd	<0.19
p Value	0.30	nd	0.32	>0.48	nd	>0.47
95% CI of OR Quart4	32	nd	33	na	nd	na

[0143]   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.

[0144]   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.

[0145] 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.

[0146] 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.

[0147] Other embodiments are set forth within the following claims.

We claim:

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

performing one or more assays configured to detect one or more biomarkers selected from the group consisting of Ceruloplasmin and Annexin A2 on a body fluid sample obtained from the subject to provide an assay result; and

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

2.   A method according to claim 1, wherein said correlation step comprises correlating the assay result(s) to prognosis of the renal status of the subject.

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

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

5.   A method according to one of claims 1-4, wherein said assay results comprise a measured concentration of Ceruloplasmin and/or a measured concentration of Annexin A2.

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

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

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

9. A method according to claim 8, wherein the likelihood of one or more future changes in renal status is that an event of interest is more or less likely to occur within a period selected from the group consisting of 21 days, 14 days, 7 days, 5 days, 96 hours, 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, and 12 hours.

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

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

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

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

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

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

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

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

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

19.   A method according to one of claims 1-5, wherein said one or more future changes in renal status comprise one or more of a future injury to renal function, future reduced renal function, future improvement in renal function, and future acute renal failure (ARF) within 21 days, 14 days, 7 days, 5 days, 96 hours, 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, or 12 hours of the time at which the body fluid sample is obtained.

20.   A method according to one of claims 1-5, wherein said one or more future changes in renal status comprise one or more of a future injury to renal function, future reduced renal function, future improvement in renal function, and future acute renal failure (ARF) within 48 hours of the time at which the body fluid sample is obtained.

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

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

23.   A method according to claim 22, wherein the subject is in RIFLE stage 0, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage R, I or F within 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, or 12 hours.

24.   A method according to claim 23, wherein the subject is in RIFLE stage 0, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, or 12 hours.

25.   A method according to claim 23, wherein the subject is in RIFLE stage 0, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, or 12 hours.

26.   A method according to claim 22, wherein the subject is in RIFLE stage 0 or R, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, or 12 hours.

27.   A method according to claim 26, wherein the subject is in RIFLE stage 0 or R, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, or 12 hours.

28.   A method according to claim 22, wherein the subject is in RIFLE stage R, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, or 12 hours.

29.   A method according to claim 28, wherein the subject is in RIFLE stage R, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, or 12 hours.

30.   A method according to one of claims 1-5, wherein the subject is in RIFLE stage 0, R, or I, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, or 12 hours.

31.   A method according to claim 30, wherein the subject is in RIFLE stage I, and said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, or 12 hours.

32.   A method according to claim 23, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage R, I or F within 48 hours.

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

34.   A method according to claim 25, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 48 hours.

35.   A method according to claim 26, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 48 hours.

36.　　A method according to claim 27, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 48 hours.

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

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

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

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

41.　　A method according to claim 23, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage R, I or F within 24 hours.

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

43.　　A method according to claim 25, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 24 hours.

44.　　A method according to claim 26, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage I or F within 24 hours.

45.　　A method according to claim 27, wherein said correlating step comprises assigning a likelihood that the subject will reach RIFLE stage F within 24 hours.

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

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

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

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

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

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

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

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

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

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

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

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

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

59.   A method according to claim 58, wherein said correlating step comprises assigning one or more of: a likelihood that within 48 hours the subject will (i) experience a 1.5-fold or greater increase in serum creatinine (ii) have a urine output of less than 0.5 ml/kg/hr over a 6 hour period, or (iii) experience an increase of 0.3 mg/dL or greater in serum creatinine.

60.   A method according to claim 58, wherein said correlating step comprises assigning one or more of: a likelihood that within 24 hours the subject will (i) experience a 1.5-fold or greater increase in serum creatinine (ii) have a urine output of less than 0.5 ml/kg/hr over a 6 hour period, or (iii) experience an increase of 0.3 mg/dL or greater in serum creatinine.

61.   A method according to claim 58, wherein said correlating step comprises assigning a likelihood that within 72 hours the subject will experience a 1.5-fold or greater increase in serum creatinine.

62.   A method according to claim 58, wherein said correlating step comprises assigning a likelihood that within 72 hours the subject will have a urine output of less than 0.5 ml/kg/hr over a 6 hour period.

63.   A method according to claim 58, wherein said correlating step comprises assigning a likelihood that within 72 hours the subject will experience an increase of 0.3 mg/dL or greater in serum creatinine.

64.   A method according to claim 58, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will experience a 1.5-fold or greater increase in serum creatinine.

65.   A method according to claim 58, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will have a urine output of less than 0.5 ml/kg/hr over a 6 hour period.

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66.   A method according to claim 58, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will experience an increase of 0.3 mg/dL or greater in serum creatinine.

67.   A method according to claim 58, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will experience a 1.5-fold or greater increase in serum creatinine.

68.   A method according to claim 58, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will have a urine output of less than 0.5 ml/kg/hr over a 6 hour period.

69.   A method according to claim 58, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will experience an increase of 0.3 mg/dL or greater in serum creatinine.

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

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

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

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

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

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

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

77.   A method according to claim 76, wherein said correlating step comprises
assigning one or more of: a likelihood that within 48 hours the subject will (i) experience
a 2-fold or greater increase in serum creatinine (ii) have a urine output of less than 0.5
ml/kg/hr over a 6 hour period, or (iii) experience an increase of 0.3 mg/dL or greater in
serum creatinine.

78.   A method according to claim 76, wherein said correlating step comprises
assigning one or more of: a likelihood that within 24 hours the subject will (i) experience
a 2-fold or greater increase in serum creatinine, or (ii) have a urine output of less than 0.5
ml/kg/hr over a 6 hour period.

79.   A method according to claim 76, wherein said correlating step comprises
assigning a likelihood that within 72 hours the subject will experience a 2-fold or greater
increase in serum creatinine.

80.   A method according to claim 76, wherein said correlating step comprises
assigning a likelihood that within 72 hours the subject will have a urine output of less
than 0.5 ml/kg/hr over a 6 hour period.

81.   A method according to claim 76, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will experience a 2-fold or greater increase in serum creatinine.

82.   A method according to claim 76, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will have a urine output of less than 0.5 ml/kg/hr over a 6 hour period.

83.   A method according to claim 76, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will experience a 2-fold or greater increase in serum creatinine.

84.   A method according to claim 76, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will have a urine output of less than 0.5 ml/kg/hr over a 6 hour period.

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

86.   A method according to claim 85, wherein said correlating step comprises assigning one or more of: a likelihood that within 48 hours the subject will (i) experience a 3-fold or greater increase in serum creatinine, or (ii) have a urine output of less than 0.3 ml/kg/hr over a 24 hour period or anuria over a 12 hour period.

87.   A method according to claim 85, wherein said correlating step comprises assigning one or more of: a likelihood that within 24 hours the subject will (i) experience a 3-fold or greater increase in serum creatinine, or (ii) have a urine output of less than 0.3 ml/kg/hr over a 24 hour period or anuria over a 12 hour period.

88.   A method according to claim 85, wherein said correlating step comprises assigning a likelihood that within 72 hours the subject will experience a 3-fold or greater increase in serum creatinine.

89.   A method according to claim 85, wherein said correlating step comprises assigning a likelihood that within 72 hours the subject will have a urine output of less than 0.3 ml/kg/hr over a 24 hour period or anuria over a 12 hour period.

90. A method according to claim 85, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will experience a 3-fold or greater increase in serum creatinine.

91. A method according to claim 85, wherein said correlating step comprises assigning a likelihood that within 48 hours the subject will have a urine output of less than 0.3 ml/kg/hr over a 24 hour period or anuria over a 12 hour period.

92. A method according to claim 85, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will experience a 3-fold or greater increase in serum creatinine.

93. A method according to claim 85, wherein said correlating step comprises assigning a likelihood that within 24 hours the subject will have a urine output of less than 0.3 ml/kg/hr over a 24 hour period or anuria over a 12 hour period.

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

95. A method according to one of claims 1-94, wherein said method comprises performing assays that detect one, two or three, or more of Ceruloplasmin and Annexin A2.

96. Measurement of one or more biomarkers selected from the group consisting of Ceruloplasmin and Annexin A2 for the risk stratification, prognosis, classifying and/or monitoring of renal injury.

97. Measurement of one or more biomarkers selected from the group consisting of Ceruloplasmin and Annexin A2 for the risk stratification, prognosis, classifying and/or monitoring of acute renal injury.

98. A kit, comprising:

reagents for performing one or more assays configured to detect one or more kidney injury markers selected from the group consisting of Ceruloplasmin and Annexin A2.

99. A kit according to claim 98, wherein said reagents comprise one or more binding reagents, each of which specifically binds one of said of kidney injury markers.

100. A kit according to claim 99, wherein a plurality of binding reagents are contained in a single assay device.

101.   A kit according to claim 99, wherein at least one of said assays is configured as a sandwich binding assay.

102.   A kit according to claim 99, wherein at least one of said assays is configured as a competitive binding assay.

103.   A kit according to one of claims 98-102, wherein said one or more assays comprise an assay that detects Ceruloplasmin and an assay that detects Annexin A2.

104.   A method for evaluating biomarker levels in a body fluid sample, comprising:

obtaining a urine sample from a subject selected for evaluation based on a determination that the subject is at risk of a future or current acute renal injury; and

performing one or more analyte binding assays configured to detect one or more biomarkers, one or more of which is selected from the group consisting of Ceruloplasmin and Annexin A2 by introducing the urine sample obtained from the subject into an assay instrument which (i) contacts a plurality of reagents which specifically bind for detection the plurality of biomarkers with the urine sample, and generates one or more assay results indicative of binding of each biomarker which is assayed to a respective specific binding reagent in the plurality of reagents, (iii) generates from the one or more assay results an indication of a risk of a future or current acute renal injury, and (iv) displays the indication of a risk of a future or current acute renal injury.

105.   A method according to claim 104, wherein the subject is selected for evaluation based on a determination that the subject is at risk of a future acute renal injury.

106.   A method according to claim 105, wherein the subject is selected for evaluation based on a determination that the subject is at risk of a future injury to renal function, future reduced renal function, future improvement in renal function, and future acute renal failure (ARF).

107.   A method according to claim 105, wherein the subject is selected for evaluation based on a determination that the subject is at risk of a future acute renal injury within 30 days of the time at which the urine sample is obtained from the subject.

108.   A method according to claim 107, wherein the subject is selected for evaluation based on a determination that the subject is at risk of a future acute renal injury within a period selected from the group consisting of 21 days, 14 days, 7 days, 5 days, 96 hours, 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, and 12 hours.

109.   A method according to claim 104, wherein the subject is selected for based on the pre-existence in the subject of one or more known risk factors for prerenal, intrinsic renal, or postrenal ARF.

110.   A method according to claim 104, wherein the subject is selected for evaluation 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.

111.   A method according to claim 104, wherein the plurality of assays are immunoassays performed by (i) introducing the urine sample into an assay device comprising a plurality of antibodies, at least one of which binds to each biomarker which is assayed, and (ii) generating an assay result indicative of binding of each biomarker to its respective antibody.

112.   A method according to claim 104, wherein the subject is selected for evaluation based on a determination that the subject is at risk of one or more future changes in renal status selected from the group consisting of a future injury to renal function, future reduced renal function, future improvement in renal function, and future acute renal failure (ARF) within 72 hours of the time at which the urine sample is obtained.

113.   A method according to claim 104, wherein the subject is selected for evaluation based on a determination that the subject is at risk of one or more future changes in renal status selected from the group consisting of a future injury to renal function, future reduced renal function, future improvement in renal function, and future acute renal failure (ARF) within 48 hours of the time at which the urine sample is obtained.

114.   A method according to claim 104, wherein the subject is selected for evaluation based on a determination that the subject is at risk of one or more future changes in renal status selected from the group consisting of a future injury to renal function, future reduced renal function, future improvement in renal function, and future acute renal failure (ARF) within 24 hours of the time at which the urine sample is obtained.

115.   A method according to claim 104, wherein the subject is in RIFLE stage 0 or R.

116   A method according to claim 104, wherein the subject is in RIFLE stage 0, R, or I.

117.   A method according to claim 104, wherein at least one assay result is a measured concentration of Cerulopasmin or a measured concentration of Annexin A2.

118.   A method according to claim 104, further comprising treating the subject to mitigate the risk of a future acute renal injury.

119.   A system for evaluating biomarker levels, comprising:

a plurality of reagents which specifically bind for detection the plurality of biomarkers, one or more of which is selected from the group consisting of Ceruloplasmin and Annexin A2;

an assay instrument configured to receive a urine sample and contact the plurality of reagents with the urine sample, to generate one or more assay results indicative of binding of each biomarker which is assayed to a respective specific binding reagent in the plurality of reagents, to generate from the one or more assay results an indication of a risk of a future or current acute renal injury, and to display the indication of a risk of a future or current acute renal injury.

120.   A system according to claim 119, wherein the reagents comprise a plurality of antibodies, at least one of which binds to each of the biomarkers which are assayed.

121.   A system according to claim 120, wherein assay instrument comprises an assay device and an assay device reader, wherein the plurality of antibodies are immobilized at a plurality of predetermined locations within the assay device, wherein the assay device is configured to receive the urine sample such that the urine sample contacts the plurality of predetermined locations, and wherein the assay device reader interrogates the plurality of predetermined locations to generate the assay results.

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/50 (2013.01)
USPC - 435/7.94
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - G01N 33/50, 33/53, 33/74, 33/92 (2013.01)
USPC - 435/7.92, 7.93, 7.94, 287.2; 436/501; 506/9

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - G01N 33/68, 2800/52, 2800/347 (2013.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, Patbase, Google Patents, Google Scholar, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012102963 A1 (SINGBARTL) 02 August 2012 (02.08.2012) entire document	1-5, 7-9, 96-103
X	WO 2011075744 A1 (ANERBERG) 23 June 2011 (23.06.2011) entire document	104-121
A	De Geus et al. ' Biomarkers for the prediction of acute kidney injury: a narrative review on current status and future challenges". Clinical Kidney Journal. Vol. 5, Issue 2. Pages 102-108. April 2012. entire document	1-5, 7-9, 96-121

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
12 December 2013	**0 3 JAN 2014**

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450	Blaine R. Copenheaver
Facsimile No. 571-273-3201	PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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

INTERNATIONAL SEARCH REPORT

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

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

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

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

3. ☒ Claims Nos.: 6, 10-95
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

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