(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 May 2016 (19.05.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/077484 A1

(51) International Patent Classification:
G01N 33/68 (2006.01)

(21) International Application Number:
PCT/US2015/060204

(22) International Filing Date:
11 November 2015 (11.11.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/078,347 11 November 2014 (11.11.2014) US

(71) Applicant: **ASTUTE MEDICAL, INC.** [US/US]; Blg 2, R. 645, 3550 General Atomics Court, San Diego, CA 92121 (US).

(72) Inventors: **ANDERBERG, Joseph**; 470 Delage Court, Encinitas, CA 92024 (US). **MCPHERSON, Paul**; 1449 Elva Court, Encinitas, CA 92024 (US). **GRAY, Jeff**; 417 Bay Meadows Way, Solana Beach, CA 92075 (US). **NAKAMURA, Kevin**; 2409 Newcastle Ave, Cardiff by the Sea, CA 92007 (US). **KAMPF, James, Patrick**; 5882 Gablewood Way, San Diego, CA 92130 (US).

(74) Agents: **WHITTAKER, Michael, A.** et al.; Acuity Law Group, P.C., 12707 High Bluff Drive, Suite 200, San Diego, CA 92130 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2016/077484 A1

(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 assays that detect Interleukin-18 -binding protein as diagnostic and prognostic biomarker assays in renal injuries.

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

[0001] The present application claims the benefit of United States Provisional Application 62/078,347 filed November 11, 2014, which is hereby incorporated by reference in its entirety.

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.

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.

[0009] More recently, Mehta *et al., Crit. Care* 11:R31 (doi:10.1186.cc5713), 2007, hereby incorporated by reference in its entirety, proposes the following similar classifications for stratifying AKI patients, which have been modified from RIFLE:

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

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

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

[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 Interleukin-

18-binding protein (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] Interleukin-18-binding protein 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 Interleukin-18-binding protein in a body fluid sample obtained from the subject. The assay result(s), for example a measured concentration of Interleukin-18-binding protein, 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

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 a measured concentration of Interleukin-18-binding protein, 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 a measured concentration of Interleukin-18-binding protein, 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 yet other preferred monitoring embodiments, these methods comprise monitoring renal status in a subject having, or at risk of, an injury to renal function for future persistence of acute kidney injury. "Future persistence" as used herein refers to an existing acute renal injury that will continue for a period selected from the group consisting of 21 days, 14 days, 7 days, 5 days, 96 hours, 72 hours, 48 hours, 36 hours, 24 hours, and 12 hours. In certain embodiments the subject has an acute kidney injury at the time the sample is obtained. This is not meant to imply that the subject must have an acute kidney injury at the time the sample is obtained, but rather that the subject, upon onset of an acute kidney injury, suffers from an acute kidney injury that will persist. In various embodiments, the assay result(s), for example a measured concentration of Interleukin-18-binding protein, is/are correlated to the future persistence of the acute kidney injury 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 future persistence of acute kidney injury may be assigned to the subject; alternatively, when the measured concentration is below the threshold, a future improvement of renal function may be assigned to the subject. For a negative going marker, when the measured concentration is below the threshold, a future persistence of acute kidney injury may be assigned to the subject; alternatively, when the measured concentration is above the threshold, a future improvement of renal function may be assigned to the subject.

[0036] In still other embodiments, the methods for evaluating renal status described herein are methods for classifying a renal injury in the subject; that is, determining whether a renal injury in a subject is prerenal, intrinsic renal, or postrenal; and/or further subdividing these classes into subclasses such as acute tubular injury, acute glomerulonephritis acute tubulointerstitial nephritis, acute vascular nephropathy, or infiltrative disease; and/or assigning a likelihood that a subject will progress to a particular RIFLE stage. In these embodiments, the assay result(s), for example a measured concentration of Interleukin-18-binding protein, is/are correlated to a particular class and/or subclass. The following are preferred classification embodiments.

[0037] In preferred classification embodiments, these methods comprise determining whether a renal injury in a subject is prerenal, intrinsic renal, or postrenal; and/or further subdividing these classes into subclasses such as acute tubular injury, acute glomerulonephritis acute tubulointerstitial nephritis, acute vascular nephropathy, or

infiltrative disease; and/or assigning a likelihood that a subject will progress to a particular RIFLE stage, and the assay result(s) is/are correlated to the injury classification for the subject. For example, the measured concentration may be compared to a threshold value, and when the measured concentration is above the threshold, a particular classification is assigned; alternatively, when the measured concentration is below the threshold, a different classification may be assigned to the subject.

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

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

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

preferably at least 0.6, more preferably 0.7, still more preferably at least 0.8, even more preferably at least 0.9, and most preferably at least 0.95.

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

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

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

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

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

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

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

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

[0042] Multiple thresholds may also be used to assess renal status in a subject. For example, a "first" subpopulation which is predisposed to one or more future changes in renal status, the occurrence of an injury, a classification, etc., and a "second" subpopulation which is not so predisposed can be combined into a single group. This group is then subdivided into three or more equal parts (known as tertiles, quartiles, quintiles, etc., depending on the number of subdivisions). An odds ratio is assigned to subjects based on which subdivision they fall into. If one considers a tertile, the lowest or highest tertile can be used as a reference for comparison of the other subdivisions. This reference subdivision is assigned an odds ratio of 1. The second tertile is assigned an odds ratio that is relative to that first tertile. That is, someone in the second tertile might be 3 times more likely to suffer one or more future changes in renal status in comparison to someone in the first tertile. The third tertile is also assigned an odds ratio that is relative to that first tertile.

[0043] 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.

[0044] The foregoing method steps should not be interpreted to mean that the kidney injury marker assay result(s) is/are used in isolation in the methods described herein. Rather, additional variables or other clinical indicia may be included in the methods described herein. For example, a risk stratification, diagnostic, classification, monitoring, etc. method may combine the assay result(s) with one or more variables measured for the

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

[0045] 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.

[0046] 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.

[0047] 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.

[0048] 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.*).

[0049] 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

[0050] 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 Interleukin-18-binding protein or one or more markers related thereto are correlated to the renal status of the subject.

[0051] For purposes of this document, the following definitions apply:

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.

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).

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."

[0052] 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.

[0053] As used herein, the term "Interleukin-18-binding protein" refers to one or more polypeptides present in a biological sample that are derived from the Interleukin-18-binding protein precursor (human sequence: Swiss-Prot O95998 (SEQ ID NO: 1)):

```
        10          20          30          40          50
MTMRHNWTPD LSPLWVLLLC AHVVTLLVRA TPVSQTTTAA TASVRSTKDP
        60          70          80          90         100
CPSQPPVFPA AKQCPALEVT WPEVEVPLNG TLSLSCVACS RFPNFSILYW
       110         120         130         140         150
LGNGSFIEHL PGRLWEGSTS RERGSTGTQL CKALVLEQLT PALHSTNFSC
       160         170         180         190
VLVDPEQVVQ RHVVLAQLWA GLRATLPPTQ EALPSSHSSP QQQG
```

[0054] The following domains have been identified in Interleukin-18-binding protein:

Residues	Length	Domain ID
1-30	30	Signal peptide
31-194	164	Interleukin-18-binding protein
116-194		Missing in IL-18BPB
79-115		→SWAEGNLAPHPRSPALQPQQSTAAGLR LSTGPAAAQP (SEQ IS NO: 2) in IL-18BPB
164-194		Missing in IL-18BPD
128-163		→ WAEGNLAPHPRSPALQPQQSTAAGLRLSTGP AAAQP (SEQ IS NO: 3) in IL-18BPD

[0055] As used herein, the term "relating a signal to the presence or amount" of an analyte reflects this understanding. Assay signals are typically related to the presence or amount of an analyte through the use of a standard curve calculated using known concentrations of the analyte of interest. 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.

[0056] 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.*

[0057] 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.

[0058] 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.

[0059] 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.

[0060] 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.

[0061] 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.

[0062] 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.

[0063] Marker Assays

[0064] 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.

[0065] 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.

[0066] 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.

[0067] 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.).

[0068] 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.

[0069] 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.

[0070] Antibodies

[0071] The term "antibody" as used herein refers to a peptide or polypeptide derived from, modeled after or substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, capable of specifically binding an antigen or epitope. *See, e.g.* Fundamental Immunology, 3rd Edition, W.E. Paul, ed., Raven Press, N.Y. (1993); Wilson (1994; J. Immunol. Methods 175:267-273; Yarmush (1992) J. Biochem. Biophys. Methods 25:85-97. The term antibody includes antigen-binding portions, i.e., "antigen binding sites," (e.g., fragments, subsequences, complementarity determining regions (CDRs)) that retain capacity to bind antigen, including (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CHl domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CHl domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Single chain antibodies are also included by reference in the term "antibody."

[0072] 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} .

[0073] 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.

[0074] 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.

[0075] 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.

[0076] 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.

[0077] 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.

[0078] Assay Correlations

[0079] 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.

[0080] 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.

[0081] 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 97.5^{th} 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.

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] 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

[0087] 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 (P61769); Beta-galactosidase (P16278); BMP-7 (P18075); Brain natriuretic peptide (proBNP, BNP-32, NTproBNP; P16860); Calcium-binding protein Beta (S100-beta, P04271); Carbonic anhydrase 9 (Q16790); Casein Kinase 2 (P68400); Cathepsin B (P07858); Ceruloplasmin (P00450); Clusterin (P10909); Complement C3 (P01024); Cysteine-rich protein (CYR61, O00622); Cytochrome C (P99999); Epidermal growth factor (EGF, P01133); Endothelin-1 (P05305); Exosomal Fetuin-A (P02765); Fatty acid-binding protein, heart (FABP3, P05413); Fatty acid-binding protein, liver (P07148); Ferritin (light chain, P02792; heavy chain P02794); Fructose-1,6-biphosphatase (P09467); GRO-alpha (CXCL1, (P09341); Growth Hormone (P01241); Hepatocyte growth factor (P14210); Insulin-like growth factor I (P05019); Immunoglobulin G; Immunoglobulin Light Chains (Kappa and Lambda); Interferon gamma (P01579); Lysozyme (P61626); Interleukin-1alpha (P01583); Interleukin-2 (P60568); Interleukin-4 (P05112); 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).

[0088] 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,Q96D42); 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 (O00300); 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 (P06870); RT1.B-1 (alpha) chain of the integral membrane protein (Q5Y7A8); Tumor necrosis factor receptor superfamily member 1A (sTNFR-I, P19438); 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.

[0089] 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.

[0090] 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.

[0091] Diagnosis of Acute Renal Failure

[0092] 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:

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

[0093] 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.

[0094] 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.

[0095] 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:

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

[0096] Commonly a 24 hour urine collection is undertaken, from empty-bladder one morning to the contents of the bladder the following morning, with a comparative blood test then taken:

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

[0097] 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:

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

[0098] 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.

[0099] 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).

[0100] Selecting a Treatment Regimen

[0101] 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.

[0102] 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.

[0103] Example 1: Contrast-induced nephropathy sample collection

[0104] 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.

[0105] 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.

[0106] 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).

[0107] 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%.

[0108] Example 2: Cardiac surgery sample collection

[0109] 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.

[0110] 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.

[0111] Example 3: Acutely ill subject sample collection

[0112] The objective of this study is to collect samples from acutely ill patients. Approximately 900 adults expected to be in the ICU for at least 48 hours will be enrolled. To be enrolled in the study, each patient must meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria

males and females 18 years of age or older;

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

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

sepsis;

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

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

contrast media exposure within 24 hours of enrollment;

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

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

Study population 3: approximately 300 patients

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

Exclusion Criteria

known pregnancy;

institutionalized individuals;

previous renal transplantation;

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

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

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

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

[0113] After providing informed consent, an EDTA anti-coagulated blood sample (10 mL) and a urine sample (25-30 mL) are collected from each patient. Blood and urine samples are then collected at 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), and 48 (± 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Blood is collected via direct venipuncture or via other available venous access, such as an existing femoral sheath, central venous line, peripheral intravenous line or hep-lock. These study blood samples are processed to plasma at the clinical site, frozen and shipped to Astute Medical, Inc., San Diego, CA. The study urine samples are frozen and shipped to Astute Medical, Inc.

[0114] Example 4. Immunoassay format

[0115] 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. Units for Interleukin-18-binding protein reported herein are pg/mL.

[0116] Example 5. Apparently Healthy Donor and Chronic Disease Patient Samples

[0117] 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.

[0118] 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.

[0119] Example 6. Interleukin-18-binding protein measurement in ICU patients

[0120] Patients from the intensive care unit (ICU) are enrolled in the following study. EDTA anti-coagulated blood samples (10 mL) and a urine samples (25-30 mL) are collected from each patient at enrollment, 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), and 48 (± 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Interleukin-18-binding protein is measured in the earliest samples collected while the patients were in RIFLE I or F by standard immunoassay methods using commercially available assay reagents.

[0121] Kidney status is assessed by RIFLE criteria based on serum creatinine, urine output, or both serum creatinine and urine output during a period starting at 12, 24, 48, or 72 hours after sample collection or at any time within 7 days after sample collection. Two cohorts are defined to represent a "recovered" and a "non-recovered" population. "Recovered" indicates those patients whose maximum RIFLE stage during a period of 24, 48 or 72 hours is non-injury (RIFLE 0). "Non-recovered" indicates those patients whose maximum RIFLE stage during a period of 24, 48 or 72 hours is risk of injury (R), injury

(I) or failure (F). If a patient dies or is placed on renal replacement therapy (RRT) within 9 days of enrollment, the patient is considered "non-recovered".

[0121] The ability to distinguish the "recovered" and "non-recovered" cohorts is determined using receiver operating characteristic (ROC) analysis.

[0122] Table 1: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "recovered" and "non-recovered" cohorts where recovery starts at 12 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	2760	406	2300	411	3170	458
Average	2700	1100	2470	1160	3170	1160
Stdev	1170	1520	1260	1550	875	1530
p (t-test)		0.0081		0.069		0.022
Min	498	0.744	498	0.744	2160	0.744
Max	4160	5540	4160	5540	4160	5540
n (Patient)	8	73	6	75	4	77

sCr only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	2040	121	1850	191	1850	200
Average	2200	706	2120	807	2130	849
Stdev	1650	1210	1670	1300	1730	1290
p (t-test)		9.4E-5		9.1E-4		0.0021
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	5390	5540	5390	5540	5390
n (Patient)	30	51	28	53	26	55

UO only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1280	392	1470	358	1670	399
Average	1700	1120	1850	1150	2000	1150
Stdev	1370	1580	1340	1610	1330	1590
p (t-test)		0.24		0.18		0.13
Min	191	1.06	191	1.06	191	1.06
Max	4160	5540	4160	5540	4160	5540
n (Patient)	11	61	10	62	9	64

Recovery Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.18	0.22	0.30	0.22	0.25	0.28	0.14	0.27	0.27
SE	0.063	0.051	0.077	0.079	0.054	0.077	0.068	0.056	0.078

p Value	4.2E-7	4.4E-8	0.011	3.5E-4	4.9E-6	0.0052	9.5E-8	5.1E-5	0.0030
nCohort Recovered	8	30	11	6	28	10	4	26	9
nCohort Non-recovered	73	51	61	75	53	62	77	55	64
Cutoff Quartile 2	25.7	25.7	30.1	25.7	25.7	30.1	25.7	25.7	31.6
Sensitivity	71%	65%	70%	72%	66%	71%	73%	67%	70%
Specificity	0%	10%	0%	0%	11%	0%	0%	12%	0%
Cutoff Quartile 3	603	603	478	603	603	478	603	603	498
Sensitivity	45%	35%	44%	47%	38%	44%	47%	40%	44%
Specificity	12%	27%	18%	17%	29%	10%	0%	31%	11%
Cutoff Quartile 4	2090	2090	1690	2090	2090	1810	2090	2090	1930
Sensitivity	19%	10%	23%	21%	13%	23%	21%	15%	22%
Specificity	25%	50%	64%	33%	54%	60%	0%	54%	56%
OR Quartile 2	0.144	0.204	0.102	0.195	0.233	0.115	0.292	0.268	0.123
p Value	0.19	0.018	0.12	0.27	0.031	0.14	0.42	0.052	0.16
Lower limit of 95% CI	0.00793	0.0542	0.00572	0.0105	0.0620	0.00638	0.0151	0.0710	0.00681
Upper limit of 95% CI	2.60	0.766	1.83	3.61	0.878	2.06	5.66	1.01	2.22
OR Quartile 3	0.118	0.198	0.176	0.175	0.242	0.0857	0.0977	0.296	0.0972
p Value	0.051	0.0014	0.035	0.12	0.0050	0.024	0.12	0.016	0.033
Lower limit of 95% CI	0.0138	0.0735	0.0352	0.0195	0.0900	0.0102	0.00509	0.110	0.0115
Upper limit of 95% CI	1.01	0.535	0.886	1.57	0.653	0.718	1.88	0.799	0.824
OR Quartile 4	0.0791	0.109	0.521	0.136	0.176	0.438	0.0298	0.199	0.350
p Value	0.0035	2.0E-4	0.35	0.028	0.0017	0.25	0.021	0.0032	0.15
Lower limit of 95% CI	0.0144	0.0338	0.133	0.0228	0.0591	0.108	0.00153	0.0677	0.0827
Upper limit of 95% CI	0.434	0.349	2.04	0.808	0.521	1.77	0.582	0.582	1.48

[0123] Table 2: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "recovered" and "non-recovered" cohorts where recovery starts at 24 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	2450	406	2610	409	2780	411
Average	2290	1080	2540	1110	2830	1100
Stdev	1450	1530	1460	1520	1330	1510
p (t-test)		0.028		0.038		0.018
Min	200	0.744	299	0.744	299	0.744
Max	4290	5540	4290	5540	4290	5540
n (Patient)	11	69	8	72	7	73

sCr only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1770	121	1710	258	1710	258
Average	2040	749	2010	841	2010	841
Stdev	1680	1270	1760	1290	1760	1290
p (t-test)		6.9E-4		0.0039		0.0039
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	5390	5540	5390	5540	5390
n (Patient)	31	49	28	52	28	52

UO only

Recovery Period Duration (hr)	24	48	72

	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	776	399	949	392	1110	406
Average	1410	1120	1500	1150	1590	1160
Stdev	1430	1600	1440	1630	1470	1600
p (t-test)		0.53		0.47		0.40
Min	19.4	1.06	19.4	1.06	19.4	1.06
Max	4290	5540	4290	5540	4290	5540
n (Patient)	14	54	13	55	12	57

Recovery Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.24	0.25	0.37	0.22	0.29	0.36	0.18	0.29	0.36
SE	0.066	0.054	0.079	0.071	0.058	0.080	0.066	0.058	0.082
p Value	7.1E-5	5.3E-6	0.087	6.8E-5	2.6E-4	0.076	1.3E-6	2.6E-4	0.080
nCohort Recovered	11	31	14	8	28	13	7	28	12
nCohort Non-recovered	69	49	54	72	52	55	73	52	57
Cutoff Quartile 2	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.7
Sensitivity	71%	65%	70%	72%	67%	71%	73%	67%	70%
Specificity	0%	10%	7%	0%	11%	8%	0%	11%	8%
Cutoff Quartile 3	551	551	434	551	551	434	551	551	458
Sensitivity	46%	39%	46%	47%	42%	45%	47%	42%	46%
Specificity	27%	32%	36%	25%	36%	31%	14%	36%	33%
Cutoff Quartile 4	1970	1970	1680	1970	1970	1810	1970	1970	1930
Sensitivity	20%	12%	22%	21%	15%	22%	21%	15%	23%
Specificity	45%	55%	64%	38%	57%	62%	29%	57%	67%
OR Quartile 2	0.105	0.202	0.183	0.151	0.247	0.203	0.174	0.247	0.214
p Value	0.12	0.018	0.12	0.20	0.039	0.14	0.24	0.039	0.15
Lower limit of 95% CI	0.00591	0.0534	0.0220	0.00831	0.0653	0.0243	0.00950	0.0653	0.0256
Upper limit of 95% CI	1.87	0.761	1.52	2.73	0.935	1.69	3.19	0.935	1.79
OR Quartile 3	0.324	0.302	0.479	0.298	0.407	0.370	0.145	0.407	0.419
p Value	0.12	0.013	0.24	0.15	0.064	0.13	0.081	0.064	0.19
Lower limit of 95% CI	0.0793	0.117	0.142	0.0564	0.158	0.102	0.0166	0.158	0.113
Upper limit of 95% CI	1.33	0.778	1.62	1.58	1.05	1.35	1.27	1.05	1.55
OR Quartile 4	0.212	0.169	0.514	0.158	0.242	0.447	0.103	0.242	0.591
p Value	0.022	0.0017	0.30	0.019	0.0089	0.22	0.010	0.0089	0.45
Lower limit of 95% CI	0.0564	0.0559	0.145	0.0338	0.0838	0.123	0.0182	0.0838	0.153
Upper limit of 95% CI	0.797	0.514	1.83	0.737	0.701	1.62	0.587	0.701	2.28

[0124] Table 3: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "recovered" and "non-recovered" cohorts where recovery starts at 48 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1280	392	1280	411	1280	411
Average	1610	1100	1640	1110	1640	1110
Stdev	1500	1580	1550	1560	1550	1560
p (t-test)		0.19		0.19		0.19
Min	5.23	0.744	5.23	0.744	5.23	0.744
Max	4790	5540	4790	5540	4790	5540
n (Patient)	23	57	21	59	21	59

sCr only

Recovery Period Duration (hr)	24	48	72

	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1400	392	1520	358	1520	358
Average	1750	901	1800	881	1800	881
Stdev	1720	1350	1720	1340	1720	1340
p (t-test)		0.023		0.014		0.014
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	5390	5540	5390	5540	5390
n (Patient)	34	45	33	46	33	46

UO only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	811	121	811	296	811	296
Average	1320	1090	1320	1130	1320	1130
Stdev	1340	1660	1380	1610	1380	1610
p (t-test)		0.57		0.65		0.65
Min	5.23	1.06	5.23	1.06	5.23	1.06
Max	4790	5540	4790	5540	4790	5540
n (Patient)	22	37	20	40	20	40

Recovery Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.35	0.35	0.35	0.36	0.32	0.37	0.36	0.32	0.37
SE	0.065	0.061	0.072	0.067	0.060	0.074	0.067	0.060	0.074
p Value	0.020	0.012	0.031	0.032	0.0032	0.086	0.032	0.0032	0.086
nCohort Recovered	23	34	22	21	33	20	21	33	20
nCohort Non-recovered	57	45	37	59	46	40	59	46	40
Cutoff Quartile 2	25.4	28.6	21.9	25.4	28.6	23.2	25.4	28.6	23.2
Sensitivity	68%	69%	65%	69%	67%	68%	69%	67%	68%
Specificity	9%	18%	9%	10%	15%	10%	10%	15%	10%
Cutoff Quartile 3	551	603	411	551	603	434	551	603	434
Sensitivity	46%	42%	41%	46%	41%	45%	46%	41%	45%
Specificity	39%	41%	36%	38%	39%	40%	38%	39%	40%
Cutoff Quartile 4	1970	2010	1850	1970	2010	1970	1970	2010	1970
Sensitivity	21%	16%	22%	22%	15%	25%	22%	15%	25%
Specificity	65%	62%	68%	67%	61%	75%	67%	61%	75%
OR Quartile 2	0.206	0.474	0.185	0.240	0.369	0.231	0.240	0.369	0.231
p Value	0.047	0.18	0.039	0.073	0.085	0.073	0.073	0.085	0.073
Lower limit of 95% CI	0.0436	0.160	0.0372	0.0504	0.119	0.0464	0.0504	0.119	0.0464
Upper limit of 95% CI	0.976	1.40	0.917	1.14	1.15	1.15	1.14	1.15	1.15
OR Quartile 3	0.539	0.512	0.390	0.519	0.457	0.545	0.519	0.457	0.545
p Value	0.22	0.15	0.090	0.21	0.093	0.28	0.21	0.093	0.28
Lower limit of 95% CI	0.201	0.207	0.131	0.187	0.184	0.183	0.187	0.184	0.183
Upper limit of 95% CI	1.45	1.26	1.16	1.44	1.14	1.62	1.44	1.14	1.62
OR Quartile 4	0.500	0.298	0.591	0.565	0.276	1.00	0.565	0.276	1.00
p Value	0.20	0.025	0.39	0.31	0.018	1.0	0.31	0.018	1.0
Lower limit of 95% CI	0.172	0.103	0.180	0.189	0.0952	0.289	0.189	0.0952	0.289
Upper limit of 95% CI	1.46	0.861	1.94	1.69	0.801	3.45	1.69	0.801	3.45

[0125] Table 4: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "recovered" and "non-recovered" cohorts where recovery starts at 72 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Recovery Period Duration (hr)	24	48	72

	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1400	392	1520	296	1520	296
Average	1860	970	1930	956	1930	956
Stdev	1750	1400	1750	1390	1750	1390
p (t-test)		0.031		0.021		0.021
Min	5.23	0.744	5.23	0.744	5.23	0.744
Max	5540	5390	5540	5390	5540	5390
n (Patient)	26	53	25	54	25	54

sCr only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1280	401	1400	392	1250	411
Average	1710	943	1760	921	1700	1000
Stdev	1720	1380	1720	1380	1750	1400
p (t-test)		0.041		0.026		0.070
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	5390	5540	5390	5540	5390
n (Patient)	35	42	34	43	32	45

UO only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	674	77.6	811	99.2	811	99.2
Average	1360	875	1430	856	1430	856
Stdev	1450	1350	1470	1340	1470	1340
p (t-test)		0.27		0.20		0.20
Min	5.23	1.06	5.23	1.06	5.23	1.06
Max	4790	5080	4790	5080	4790	5080
n (Patient)	17	35	16	36	16	36

Recovery Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.30	0.36	0.31	0.29	0.34	0.30	0.29	0.37	0.30
SE	0.060	0.063	0.075	0.060	0.062	0.075	0.060	0.064	0.075
p Value	0.0011	0.030	0.014	5.6E-4	0.011	0.0089	5.6E-4	0.046	0.0089
nCohort Recovered	26	35	17	25	34	16	25	32	16
nCohort Non-recovered	53	42	35	54	43	36	54	45	36
Cutoff Quartile 2	25.1	25.7	11.2	25.1	25.7	11.2	25.1	25.7	11.2
Sensitivity	66%	69%	66%	67%	67%	67%	67%	69%	67%
Specificity	8%	20%	6%	8%	18%	6%	8%	19%	6%
Cutoff Quartile 3	603	611	399	603	611	399	603	611	399
Sensitivity	43%	43%	43%	43%	42%	42%	43%	44%	42%
Specificity	38%	43%	35%	36%	41%	31%	36%	44%	31%
Cutoff Quartile 4	2010	2090	1560	2010	2090	1560	2010	2090	1560
Sensitivity	19%	17%	23%	19%	16%	22%	19%	18%	22%
Specificity	62%	66%	71%	60%	65%	69%	60%	66%	69%
OR Quartile 2	0.162	0.558	0.120	0.174	0.444	0.133	0.174	0.511	0.133
p Value	0.021	0.28	0.052	0.027	0.14	0.065	0.027	0.23	0.065
Lower limit of 95% CI	0.0344	0.194	0.0141	0.0368	0.149	0.0157	0.0368	0.172	0.0157
Upper limit of 95% CI	0.764	1.60	1.02	0.821	1.32	1.13	0.821	1.52	1.13
OR Quartile 3	0.479	0.562	0.409	0.417	0.504	0.325	0.417	0.622	0.325
p Value	0.13	0.21	0.14	0.080	0.14	0.077	0.080	0.31	0.077
Lower limit of 95% CI	0.184	0.227	0.123	0.157	0.202	0.0932	0.157	0.250	0.0932
Upper limit of 95% CI	1.25	1.39	1.36	1.11	1.26	1.13	1.11	1.55	1.13
OR Quartile 4	0.372	0.383	0.711	0.341	0.356	0.629	0.341	0.413	0.629
p Value	0.064	0.079	0.61	0.045	0.059	0.49	0.045	0.10	0.49
Lower limit of 95% CI	0.131	0.131	0.192	0.119	0.122	0.168	0.119	0.144	0.168
Upper limit of 95% CI	1.06	1.12	2.63	0.978	1.04	2.35	0.978	1.19	2.35

[0126] Table 5: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "recovered" and "non-recovered" cohorts where recovery starts within 7 days after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1490	90.5	1490	112	1490	156
Average	1770	778	1770	868	1760	906
Stdev	1590	1340	1610	1380	1630	1390
p (t-test)		0.0033		0.0099		0.015
Min	4.06	0.744	5.23	0.744	5.23	0.744
Max	5540	5390	5540	5390	5540	5390
n (Patient)	41	42	37	46	35	48

sCr only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1250	121	1080	191	1080	191
Average	1590	788	1550	886	1570	932
Stdev	1600	1390	1610	1430	1620	1430
p (t-test)		0.021		0.058		0.069
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	5390	5540	5390	5540	5390
n (Patient)	48	33	46	35	42	39

UO only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1490	231	1280	210	1380	299
Average	1720	992	1710	989	1760	979
Stdev	1590	1460	1590	1460	1600	1450
p (t-test)		0.047		0.048		0.037
Min	5.23	1.06	5.23	1.06	5.23	1.06
Max	5540	5390	5540	5390	5540	5390
n (Patient)	31	48	31	48	30	49

Recovery Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.25	0.33	0.31	0.27	0.36	0.30	0.29	0.36	0.30
SE	0.054	0.063	0.059	0.055	0.063	0.059	0.056	0.062	0.058
p Value	3.7E-6	0.0083	9.7E-4	3.4E-5	0.031	6.8E-4	1.2E-4	0.026	5.1E-4
nCohort Recovered	41	48	31	37	46	31	35	42	30
nCohort Non-recovered	42	33	48	46	35	48	48	39	49
Cutoff Quartile 2	28.6	25.7	31.7	28.6	25.7	31.7	28.6	25.7	31.7
Sensitivity	60%	67%	62%	61%	69%	62%	62%	67%	63%
Specificity	10%	21%	6%	8%	22%	6%	9%	19%	7%
Cutoff Quartile 3	611	603	611	611	603	611	611	603	611
Sensitivity	33%	36%	40%	37%	40%	40%	38%	41%	39%

Specificity	34%	42%	35%	35%	43%	35%	34%	43%	33%
Cutoff Quartile 4	2120	2090	2120	2120	2090	2120	2120	2090	2120
Sensitivity	14%	12%	19%	17%	14%	19%	19%	18%	18%
Specificity	63%	67%	65%	65%	67%	65%	66%	69%	63%
OR Quartile 2	0.159	0.526	0.115	0.137	0.606	0.115	0.156	0.471	0.123
p Value	0.0027	0.21	0.0061	0.0032	0.33	0.0061	0.0058	0.15	0.0080
Lower limit of 95% CI	0.0478	0.193	0.0245	0.0366	0.223	0.0245	0.0417	0.170	0.0262
Upper limit of 95% CI	0.529	1.44	0.540	0.514	1.65	0.540	0.585	1.30	0.578
OR Quartile 3	0.259	0.408	0.360	0.318	0.513	0.360	0.313	0.522	0.317
p Value	0.0036	0.054	0.033	0.013	0.14	0.033	0.012	0.15	0.018
Lower limit of 95% CI	0.104	0.164	0.141	0.129	0.210	0.141	0.126	0.216	0.122
Upper limit of 95% CI	0.644	1.02	0.919	0.783	1.25	0.919	0.778	1.26	0.821
OR Quartile 4	0.289	0.276	0.420	0.389	0.344	0.420	0.442	0.488	0.389
p Value	0.023	0.036	0.099	0.069	0.064	0.099	0.11	0.18	0.074
Lower limit of 95% CI	0.0988	0.0826	0.149	0.140	0.111	0.149	0.162	0.171	0.138
Upper limit of 95% CI	0.845	0.921	1.18	1.08	1.07	1.18	1.21	1.39	1.10

[0127] Example 7. Use of Interleukin-18-binding protein for evaluating renal status in patients admitted to the ICU: Recovery to RIFLE 0 and R from RIFLE I and F

[0128] Patients from the intensive care unit (ICU) are enrolled in the following study. EDTA anti-coagulated blood samples (10 mL) and a urine samples (25-30 mL) are collected from each patient at enrollment, 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), and 48 (± 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Interleukin-18-binding protein is measured in the earliest samples collected while the patients were in RIFLE I or F by standard immunoassay methods using commercially available assay reagents.

[0129] Kidney status is assessed by RIFLE criteria based on serum creatinine, urine output, or both serum creatinine and urine output during a period starting at 12, 24, 48, or 72 hours after sample collection or at any time within 7 days after sample collection. Two cohorts are defined to represent a "recovered" and a "non-recovered" population. "Recovered" indicates those patients whose maximum RIFLE stage during a period of 24, 48 or 72 hours is non-injury (RIFLE 0) or risk of injury (R). "Non-recovered" indicates those patients whose maximum RIFLE stage during a period of 24, 48 or 72 hours is injury (I) or failure (F). If a patient dies or is placed on renal replacement therapy (RRT) within 9 days of enrollment, the patient is considered "non-recovered".

[0130] The ability to distinguish the "recovered" and "non-recovered" cohorts is determined using receiver operating characteristic (ROC) analysis.

[0131] Table 6: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "recovered" and "non-recovered" cohorts where recovery starts at 12 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	2270	312	2220	324	2160	358
Average	2330	933	2250	977	2250	998
Stdev	1590	1410	1590	1440	1640	1440
p (t-test)		0.0023		0.0063		0.010
Min	19.4	0.744	19.4	0.744	19.4	0.744
Max	5390	5540	5390	5540	5390	5540
n (Patient)	19	62	18	63	17	64

sCr only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1280	156	1280	291	1280	291
Average	1690	674	1670	753	1670	753
Stdev	1710	1110	1710	1190	1710	1190
p (t-test)		0.0021		0.0064		0.0064
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	5080	5540	5080	5540	5080
n (Patient)	47	34	45	36	45	36

UO only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1110	312	1280	299	1110	324
Average	1810	970	1900	972	1810	1050
Stdev	1670	1460	1680	1470	1670	1500
p (t-test)		0.061		0.037		0.092
Min	19.4	1.06	19.4	1.06	19.4	1.06
Max	5390	5540	5390	5540	5390	5540
n (Patient)	20	52	21	51	20	53

Recovery Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.22	0.33	0.29	0.23	0.35	0.28	0.24	0.35	0.30
SE	0.054	0.062	0.064	0.056	0.062	0.062	0.059	0.062	0.065
p Value	1.7E-7	0.0055	9.7E-4	2.6E-6	0.014	3.2E-4	1.3E-5	0.014	0.0025
nCohort Recovered	19	47	20	18	45	21	17	45	20
nCohort Non-recovered	62	34	52	63	36	51	64	36	53
Cutoff Quartile 2	25.7	25.7	30.1	25.7	25.7	30.1	25.7	25.7	31.6
Sensitivity	68%	68%	67%	68%	69%	67%	69%	69%	66%
Specificity	5%	21%	5%	6%	22%	5%	6%	22%	5%
Cutoff Quartile 3	603	603	478	603	603	478	603	603	498
Sensitivity	40%	38%	40%	41%	39%	39%	42%	39%	42%
Specificity	21%	43%	25%	22%	42%	24%	24%	42%	30%
Cutoff Quartile 4	2090	2090	1690	2090	2090	1810	2090	2090	1930
Sensitivity	15%	9%	19%	16%	11%	18%	17%	11%	19%
Specificity	42%	64%	60%	44%	64%	57%	47%	64%	60%
OR Quartile 2	0.117	0.565	0.108	0.126	0.649	0.100	0.138	0.649	0.102
p Value	0.043	0.26	0.037	0.052	0.40	0.031	0.063	0.40	0.033
Lower limit of 95% CI	0.0145	0.207	0.0134	0.0157	0.239	0.0124	0.0170	0.239	0.0127
Upper limit of 95% CI	0.937	1.54	0.878	1.02	1.76	0.809	1.11	1.76	0.827
OR Quartile 3	0.180	0.459	0.226	0.201	0.465	0.202	0.225	0.465	0.304
p Value	0.0057	0.090	0.011	0.0098	0.093	0.0064	0.017	0.093	0.034
Lower limit of 95% CI	0.0535	0.186	0.0712	0.0593	0.190	0.0638	0.0659	0.190	0.101
Upper limit of 95% CI	0.607	1.13	0.716	0.679	1.14	0.637	0.765	1.14	0.915

OR Quartile 4	0.123	0.171	0.357	0.151	0.227	0.286	0.184	0.227	0.349
p Value	3.8E-4	0.0090	0.074	0.0013	0.016	0.029	0.0041	0.016	0.067
Lower limit of 95% CI	0.0390	0.0453	0.115	0.0478	0.0679	0.0928	0.0583	0.0679	0.113
Upper limit of 95% CI	0.391	0.643	1.11	0.476	0.756	0.880	0.584	0.756	1.08

[0132] Table 7: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "recovered" and "non-recovered" cohorts where recovery starts at 24 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	2010	156	1930	191	1800	291
Average	2090	888	2020	938	2010	961
Stdev	1640	1390	1640	1430	1680	1430
p (t-test)		0.0039		0.010		0.016
Min	19.4	0.744	19.4	0.744	19.4	0.744
Max	5390	5540	5390	5540	5390	5540
n (Patient)	24	56	23	57	22	58

sCr only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1280	191	1250	291	1280	191
Average	1700	668	1660	753	1690	732
Stdev	1730	1090	1730	1190	1730	1180
p (t-test)		0.0019		0.0079		0.0048
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	5080	5540	5080	5540	5080
n (Patient)	45	35	44	36	43	37

UO only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1080	108	1220	95.5	1080	121
Average	1720	920	1810	921	1710	1010
Stdev	1670	1450	1690	1460	1670	1500
p (t-test)		0.067		0.042		0.11
Min	19.4	1.06	19.4	1.06	19.4	1.06
Max	5390	5540	5390	5540	5390	5540
n (Patient)	22	46	23	45	22	47

Recovery Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.24	0.33	0.29	0.26	0.35	0.28	0.27	0.33	0.31
SE	0.054	0.062	0.064	0.057	0.062	0.062	0.058	0.061	0.065
p Value	2.2E-6	0.0073	9.8E-4	2.0E-5	0.019	3.5E-4	7.6E-5	0.0067	0.0030
nCohort Recovered	24	45	22	23	44	23	22	43	22
nCohort Non-recovered	56	35	46	57	36	45	58	37	47
Cutoff Quartile 2	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.4	25.7
Sensitivity	66%	69%	65%	67%	69%	64%	67%	68%	64%

Specificity	4%	20%	5%	4%	20%	4%	5%	19%	5%
Cutoff Quartile 3	551	551	434	551	551	434	551	551	458
Sensitivity	41%	40%	41%	42%	42%	40%	43%	41%	40%
Specificity	29%	42%	32%	30%	43%	30%	32%	42%	32%
Cutoff Quartile 4	1970	1970	1680	1970	1970	1810	1970	1970	1930
Sensitivity	14%	9%	17%	16%	11%	16%	17%	11%	19%
Specificity	50%	62%	59%	52%	64%	57%	55%	63%	64%
OR Quartile 2	0.0847	0.545	0.0893	0.0909	0.584	0.0824	0.0977	0.476	0.0840
p Value	0.020	0.24	0.024	0.024	0.30	0.020	0.028	0.16	0.020
Lower limit of 95% CI	0.0106	0.196	0.0110	0.0114	0.211	0.0101	0.0122	0.170	0.0104
Upper limit of 95% CI	0.676	1.51	0.726	0.726	1.62	0.669	0.782	1.34	0.681
OR Quartile 3	0.287	0.487	0.328	0.318	0.543	0.292	0.354	0.491	0.317
p Value	0.017	0.12	0.042	0.030	0.18	0.024	0.049	0.12	0.035
Lower limit of 95% CI	0.103	0.198	0.112	0.113	0.223	0.100	0.125	0.201	0.109
Upper limit of 95% CI	0.803	1.20	0.959	0.893	1.32	0.850	0.997	1.20	0.923
OR Quartile 4	0.167	0.154	0.304	0.205	0.219	0.239	0.250	0.205	0.414
p Value	0.0013	0.0058	0.041	0.0041	0.014	0.015	0.012	0.010	0.13
Lower limit of 95% CI	0.0557	0.0409	0.0971	0.0691	0.0654	0.0756	0.0848	0.0611	0.134
Upper limit of 95% CI	0.499	0.583	0.953	0.605	0.732	0.759	0.737	0.685	1.29

[0133] Table 8: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "recovered" and "non-recovered" cohorts where recovery starts at 48 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1280	112	1250	156	1220	191
Average	1630	909	1580	1010	1530	1060
Stdev	1550	1520	1550	1550	1540	1570
p (t-test)		0.042		0.11		0.19
Min	1.06	0.744	1.64	0.744	1.64	0.744
Max	5390	5540	5390	5540	5390	5540
n (Patient)	38	42	34	46	33	47

sCr only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1220	291	1250	191	1250	191
Average	1590	788	1630	764	1630	764
Stdev	1690	1240	1700	1230	1700	1230
p (t-test)		0.019		0.012		0.012
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	5080	5540	5080	5540	5080
n (Patient)	47	32	46	33	46	33

UO only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1280	33.7	949	77.6	811	99.2
Average	1510	859	1360	1080	1280	1140
Stdev	1430	1590	1330	1670	1300	1680
p (t-test)		0.11		0.48		0.73

Min	1.06	1.64	5.23	1.06	5.23	1.06
Max	4790	5540	4790	5540	4790	5540
n (Patient)	29	30	25	35	24	36

Recovery Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.32	0.37	0.28	0.34	0.35	0.33	0.36	0.35	0.35
SE	0.059	0.065	0.067	0.060	0.064	0.069	0.061	0.064	0.071
p Value	0.0024	0.048	0.0012	0.0074	0.020	0.015	0.019	0.020	0.041
nCohort Recovered	38	47	29	34	46	25	33	46	24
nCohort Non-recovered	42	32	30	46	33	35	47	33	36
Cutoff Quartile 2	25.4	28.6	21.9	25.4	28.6	23.2	25.4	28.6	23.2
Sensitivity	64%	72%	60%	65%	70%	63%	66%	70%	64%
Specificity	13%	23%	10%	12%	22%	8%	12%	22%	8%
Cutoff Quartile 3	551	603	411	551	603	434	551	603	434
Sensitivity	38%	41%	33%	41%	39%	40%	43%	39%	42%
Specificity	37%	45%	34%	38%	43%	36%	39%	43%	38%
Cutoff Quartile 4	1970	2010	1850	1970	2010	1970	1970	2010	1970
Sensitivity	17%	12%	17%	20%	12%	23%	21%	12%	25%
Specificity	66%	66%	66%	68%	65%	72%	70%	65%	75%
OR Quartile 2	0.273	0.781	0.173	0.250	0.639	0.147	0.267	0.639	0.161
p Value	0.025	0.64	0.014	0.024	0.39	0.019	0.032	0.39	0.025
Lower limit of 95% CI	0.0879	0.280	0.0427	0.0748	0.230	0.0297	0.0799	0.230	0.0325
Upper limit of 95% CI	0.847	2.18	0.702	0.836	1.77	0.728	0.894	1.77	0.796
OR Quartile 3	0.359	0.553	0.263	0.436	0.500	0.375	0.481	0.500	0.429
p Value	0.027	0.20	0.015	0.073	0.14	0.070	0.11	0.14	0.12
Lower limit of 95% CI	0.145	0.222	0.0895	0.176	0.201	0.130	0.194	0.201	0.149
Upper limit of 95% CI	0.889	1.37	0.774	1.08	1.24	1.08	1.19	1.24	1.24
OR Quartile 4	0.385	0.277	0.380	0.509	0.259	0.762	0.622	0.259	1.00
p Value	0.075	0.037	0.12	0.20	0.028	0.65	0.36	0.028	1.0
Lower limit of 95% CI	0.134	0.0826	0.111	0.183	0.0772	0.235	0.224	0.0772	0.303
Upper limit of 95% CI	1.10	0.927	1.30	1.41	0.866	2.47	1.72	0.866	3.30

[0134] Table 9: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "recovered" and "non-recovered" cohorts where recovery starts at 72 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1280	103	1280	112	1280	112
Average	1770	792	1730	851	1730	851
Stdev	1700	1290	1700	1330	1700	1330
p (t-test)		0.0059		0.014		0.014
Min	1.64	0.744	1.64	0.744	1.64	0.744
Max	5540	5080	5540	5080	5540	5080
n (Patient)	38	41	37	42	37	42

sCr only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1250	191	1250	191	1250	191
Average	1630	782	1630	782	1630	782
Stdev	1690	1270	1690	1270	1690	1270
p (t-test)		0.015		0.015		0.015

Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	5080	5540	5080	5540	5080
n (Patient)	46	31	46	31	46	31

UO only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	811	33.7	674	35.5	674	35.5
Average	1450	773	1350	850	1350	850
Stdev	1430	1330	1400	1380	1400	1380
p (t-test)		0.10		0.23		0.23
Min	5.23	1.06	5.23	1.06	5.23	1.06
Max	4790	5080	4790	5080	4790	5080
n (Patient)	20	32	19	33	19	33

Recovery Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.28	0.35	0.27	0.29	0.35	0.30	0.29	0.35	0.30
SE	0.057	0.065	0.069	0.058	0.065	0.072	0.058	0.065	0.072
p Value	8.6E-5	0.018	7.4E-4	3.9E-4	0.018	0.0046	3.9E-4	0.018	0.0046
nCohort Recovered	38	46	20	37	46	19	37	46	19
nCohort Non-recovered	41	31	32	42	31	33	42	31	33
Cutoff Quartile 2	25.1	25.7	11.2	25.1	25.7	11.2	25.1	25.7	11.2
Sensitivity	61%	68%	62%	62%	68%	64%	62%	68%	64%
Specificity	11%	22%	5%	11%	22%	5%	11%	22%	5%
Cutoff Quartile 3	603	611	399	603	611	399	603	611	399
Sensitivity	37%	35%	38%	38%	35%	39%	38%	35%	39%
Specificity	37%	41%	30%	38%	41%	32%	38%	41%	32%
Cutoff Quartile 4	2010	2090	1560	2010	2090	1560	2010	2090	1560
Sensitivity	15%	13%	19%	17%	13%	21%	17%	13%	21%
Specificity	63%	67%	65%	65%	67%	68%	65%	67%	68%
OR Quartile 2	0.184	0.583	0.0877	0.197	0.583	0.0972	0.197	0.583	0.0972
p Value	0.0061	0.30	0.025	0.0085	0.30	0.032	0.0085	0.30	0.032
Lower limit of 95% CI	0.0547	0.209	0.0104	0.0587	0.209	0.0115	0.0587	0.209	0.0115
Upper limit of 95% CI	0.617	1.63	0.741	0.661	1.63	0.822	0.661	1.63	0.822
OR Quartile 3	0.337	0.387	0.257	0.375	0.387	0.300	0.375	0.387	0.300
p Value	0.020	0.048	0.026	0.035	0.048	0.048	0.035	0.048	0.048
Lower limit of 95% CI	0.135	0.151	0.0779	0.151	0.151	0.0910	0.151	0.151	0.0910
Upper limit of 95% CI	0.841	0.992	0.849	0.931	0.992	0.989	0.931	0.992	0.989
OR Quartile 4	0.294	0.306	0.429	0.369	0.306	0.583	0.369	0.306	0.583
p Value	0.027	0.057	0.19	0.064	0.057	0.41	0.064	0.057	0.41
Lower limit of 95% CI	0.0990	0.0906	0.119	0.128	0.0906	0.163	0.128	0.0906	0.163
Upper limit of 95% CI	0.873	1.03	1.54	1.06	1.03	2.09	1.06	1.03	2.09

[0135] Table 10: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "recovered" and "non-recovered" cohorts where recovery starts within 7 days after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1280	90.5	1380	77.6	1380	77.6
Average	1650	659	1740	617	1740	617

Stdev	1640	1170	1640	1130	1640	1130
p (t-test)		0.0022		4.6E-4		4.6E-4
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	5080	5540	5080	5540	5080
n (Patient)	51	32	48	35	48	35

sCr only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	696	191	794	191	794	191
Average	1440	815	1480	777	1480	777
Stdev	1630	1290	1640	1250	1640	1250
p (t-test)		0.082		0.043		0.043
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	5080	5540	5080	5540	5080
n (Patient)	58	23	56	25	56	25

UO only

Recovery Period Duration (hr)	24		48		72	
	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort	Recovered Cohort	Non-recovered Cohort
Median	1250	77.6	1280	56.1	1280	76.7
Average	1670	876	1720	790	1710	823
Stdev	1610	1370	1620	1320	1590	1360
p (t-test)		0.023		0.0068		0.010
Min	1.06	1.64	1.06	1.64	1.06	1.64
Max	5540	5130	5540	5130	5540	5130
n (Patient)	40	39	41	38	40	39

Recovery Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.28	0.40	0.29	0.26	0.38	0.26	0.26	0.38	0.27
SE	0.060	0.072	0.058	0.056	0.069	0.056	0.056	0.069	0.057
p Value	3.1E-4	0.16	3.6E-4	1.4E-5	0.084	2.6E-5	1.4E-5	0.084	4.2E-5
nCohort Recovered	51	58	40	48	56	41	48	56	40
nCohort Non-recovered	32	23	39	35	25	38	35	25	39
Cutoff Quartile 2	28.6	25.7	31.7	28.6	25.7	31.7	28.6	25.7	31.7
Sensitivity	59%	74%	56%	60%	72%	55%	60%	72%	56%
Specificity	16%	26%	8%	15%	25%	7%	15%	25%	8%
Cutoff Quartile 3	611	603	611	611	603	611	611	603	611
Sensitivity	31%	39%	33%	29%	40%	32%	29%	40%	31%
Specificity	39%	47%	35%	35%	46%	34%	35%	46%	32%
Cutoff Quartile 4	2120	2090	2120	2120	2090	2120	2120	2090	2120
Sensitivity	12%	13%	18%	11%	12%	16%	11%	12%	18%
Specificity	67%	71%	68%	65%	70%	66%	65%	70%	68%
OR Quartile 2	0.272	0.988	0.105	0.256	0.857	0.0975	0.256	0.857	0.105
p Value	0.013	0.98	9.4E-4	0.011	0.78	6.5E-4	0.011	0.78	9.4E-4
Lower limit of 95% CI	0.0968	0.329	0.0276	0.0897	0.296	0.0256	0.0897	0.296	0.0276
Upper limit of 95% CI	0.764	2.97	0.399	0.731	2.48	0.372	0.731	2.48	0.399
OR Quartile 3	0.293	0.560	0.269	0.219	0.578	0.239	0.219	0.578	0.214
p Value	0.010	0.25	0.0057	0.0016	0.26	0.0029	0.0016	0.26	0.0014
Lower limit of 95% CI	0.115	0.209	0.106	0.0855	0.222	0.0934	0.0855	0.222	0.0829
Upper limit of 95% CI	0.747	1.50	0.683	0.563	1.50	0.613	0.563	1.50	0.553
OR Quartile 4	0.286	0.362	0.454	0.235	0.313	0.362	0.235	0.313	0.454
p Value	0.040	0.14	0.14	0.018	0.088	0.066	0.018	0.088	0.14
Lower limit of 95% CI	0.0862	0.0948	0.159	0.0710	0.0824	0.122	0.0710	0.0824	0.159
Upper limit of 95% CI	0.947	1.38	1.30	0.779	1.19	1.07	0.779	1.19	1.30

[0136] Example 8. Use of Interleukin-18-binding protein for evaluating renal status in patients admitted to the ICU: Persistent at RIFLE F

[0137] Patients from the intensive care unit (ICU) are enrolled in the following study. EDTA anti-coagulated blood samples (10 mL) and a urine samples (25-30 mL) are collected from each patient at enrollment, 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), and 48 (± 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Interleukin-18-binding protein is measured in the earliest samples collected while the patients were in RIFLE I or F by standard immunoassay methods using commercially available assay reagents.

[0138] Kidney status is assessed by RIFLE criteria based on serum creatinine, urine output, or both serum creatinine and urine output. Two cohorts are defined to represent a "persistent" and a "non-persistent" population. "Persistent" indicates those patients whose minimum RIFLE stage during a period of 24, 48 or 72 hours is failure (F) where the persistence period can start from the time of sample collection to 24, 48, 72, 96 or 168 hours after sample collection. "Non-persistent" indicates those patients who are not persistent at failure (F) and whose minimum RIFLE stage during a period of 24, 48 or 72 hours is non-injury (RIFLE 0), risk of injury (R), or injury (I) where the persistence period can start from the time of sample collection to 24, 48, 72, 96 or 168 hours after sample collection. If a patient dies after failure (F) or is placed on renal replacement therapy (RRT) at any time from sample collection to 24, 48, 72, 96 or 168 hours after sample collection, the patient is considered "persistent".

[0139] The ability to distinguish the "persistent" and "non-persistent" cohorts is determined using receiver operating characteristic (ROC) analysis.

[0140] Table 11: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "persistent" and "non-persistent" cohorts where persistence starts within 24 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	914	10.6	797	7.92	833	3.94
Average	1600	356	1450	360	1450	273
Stdev	1660	663	1620	755	1610	709
p (t-test)		0.0011		0.016		0.011
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	2560	5540	2560	5540	2560

n (Patient)	61	22	69	14	70	13

sCr only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	730	108	718	121	718	121
Average	1440	486	1400	493	1380	433
Stdev	1660	770	1640	819	1620	826
p (t-test)		0.039		0.077		0.090
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	2560	5540	2560	5540	2560
n (Patient)	66	14	69	11	71	9

UO only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	949	5.23	742	3.94	742	3.94
Average	1580	248	1420	300	1420	300
Stdev	1620	647	1590	849	1590	849
p (t-test)		0.0015		0.043		0.043
Min	1.06	1.64	1.06	1.64	1.06	1.64
Max	5540	2560	5540	2560	5540	2560
n (Patient)	61	17	69	9	69	9

Persistence Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.22	0.34	0.16	0.24	0.34	0.20	0.21	0.32	0.20
SE	0.063	0.085	0.063	0.079	0.095	0.091	0.078	0.10	0.091
p Value	1.2E-5	0.056	1.0E-7	8.1E-4	0.089	9.9E-4	1.5E-4	0.074	9.9E-4
nCohort Non-persistent	61	66	61	69	69	69	70	71	69
nCohort Persistent	22	14	17	14	11	9	13	9	9
Cutoff Quartile 2	28.6	25.4	31.6	28.6	25.4	31.6	28.6	25.4	31.6
Sensitivity	45%	64%	29%	43%	64%	22%	38%	56%	22%
Specificity	15%	23%	13%	19%	23%	19%	19%	23%	19%
Cutoff Quartile 3	611	607	623	611	607	623	611	607	623
Sensitivity	23%	29%	12%	21%	27%	11%	15%	22%	11%
Specificity	39%	45%	39%	43%	46%	45%	43%	46%	45%
Cutoff Quartile 4	2120	2110	2140	2120	2110	2140	2120	2110	2140
Sensitivity	5%	7%	6%	7%	9%	11%	8%	11%	11%
Specificity	67%	71%	69%	71%	72%	72%	71%	73%	72%
OR Quartile 2	0.144	0.529	0.0629	0.174	0.528	0.0663	0.143	0.364	0.0663
p Value	5.5E-4	0.31	2.3E-5	0.0049	0.35	0.0016	0.0026	0.16	0.0016
Lower limit of 95% CI	0.0481	0.154	0.0175	0.0515	0.137	0.0123	0.0401	0.0872	0.0123
Upper limit of 95% CI	0.432	1.82	0.226	0.589	2.04	0.357	0.507	1.52	0.357
OR Quartile 3	0.191	0.333	0.0865	0.210	0.324	0.102	0.136	0.248	0.102
p Value	0.0038	0.087	0.0021	0.025	0.12	0.036	0.013	0.096	0.036
Lower limit of 95% CI	0.0621	0.0949	0.0181	0.0537	0.0793	0.0121	0.0281	0.0482	0.0121
Upper limit of 95% CI	0.586	1.17	0.413	0.819	1.33	0.860	0.662	1.28	0.860
OR Quartile 4	0.0976	0.190	0.138	0.188	0.263	0.329	0.208	0.342	0.329
p Value	0.028	0.12	0.064	0.12	0.22	0.31	0.14	0.33	0.31
Lower limit of 95% CI	0.0122	0.0232	0.0171	0.0231	0.0315	0.0385	0.0254	0.0401	0.0385
Upper limit of 95% CI	0.778	1.56	1.12	1.54	2.20	2.81	1.71	2.92	2.81

[0141] Table 12: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "persistent" and "non-persistent" cohorts where persistence starts within 48 hours after sample collection and renal status is assessed by

serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1080	11.9	892	11.9	914	7.92
Average	1670	331	1510	322	1510	249
Stdev	1670	627	1630	690	1620	641
p (t-test)		2.1E-4		0.0044		0.0031
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	2560	5540	2560	5540	2560
n (Patient)	58	25	66	17	67	16

sCr only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	730	108	718	121	718	121
Average	1440	486	1400	493	1380	433
Stdev	1660	770	1640	819	1620	826
p (t-test)		0.039		0.077		0.090
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	2560	5540	2560	5540	2560
n (Patient)	66	14	69	11	71	9

UO only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1240	5.23	914	3.94	914	3.94
Average	1680	222	1500	241	1500	241
Stdev	1630	586	1610	706	1610	706
p (t-test)		1.5E-4		0.0074		0.0074
Min	1.06	1.64	1.06	1.64	1.06	1.64
Max	5540	2560	5540	2560	5540	2560
n (Patient)	57	21	65	13	65	13

Persistence Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.20	0.34	0.13	0.22	0.34	0.17	0.19	0.32	0.17
SE	0.058	0.085	0.053	0.070	0.095	0.074	0.069	0.10	0.074
p Value	3.4E-7	0.056	7.0E-12	7.5E-5	0.089	9.6E-6	1.1E-5	0.074	9.6E-6
nCohort Non-persistent	58	66	57	66	69	65	67	71	65
nCohort Persistent	25	14	21	17	11	13	16	9	13
Cutoff Quartile 2	28.6	25.4	31.6	28.6	25.4	31.6	28.6	25.4	31.6
Sensitivity	48%	64%	33%	47%	64%	31%	44%	56%	31%
Specificity	14%	23%	11%	18%	23%	17%	18%	23%	17%
Cutoff Quartile 3	611	607	623	611	607	623	611	607	623
Sensitivity	20%	29%	10%	18%	27%	8%	12%	22%	8%
Specificity	36%	45%	35%	41%	46%	42%	40%	46%	42%
Cutoff Quartile 4	2120	2110	2140	2120	2110	2140	2120	2110	2140
Sensitivity	4%	7%	5%	6%	9%	8%	6%	11%	8%
Specificity	66%	71%	67%	70%	72%	71%	70%	73%	71%
OR Quartile 2	0.148	0.529	0.0588	0.198	0.528	0.0905	0.170	0.364	0.0905
p Value	5.4E-4	0.31	7.6E-6	0.0053	0.35	4.6E-4	0.0029	0.16	4.6E-4

Lower limit of 95% CI	0.0500	0.154	0.0170	0.0632	0.137	0.0236	0.0527	0.0872	0.0236
Upper limit of 95% CI	0.436	1.82	0.203	0.617	2.04	0.347	0.546	1.52	0.347
OR Quartile 3	0.142	0.333	0.0569	0.148	0.324	0.0592	0.0964	0.248	0.0592
p Value	6.1E-4	0.087	3.0E-4	0.0053	0.12	0.0083	0.0033	0.096	0.0083
Lower limit of 95% CI	0.0464	0.0949	0.0120	0.0388	0.0793	0.00726	0.0203	0.0482	0.00726
Upper limit of 95% CI	0.433	1.17	0.269	0.567	1.33	0.483	0.459	1.28	0.483
OR Quartile 4	0.0792	0.190	0.100	0.144	0.263	0.202	0.157	0.342	0.202
p Value	0.016	0.12	0.030	0.069	0.22	0.14	0.082	0.33	0.14
Lower limit of 95% CI	0.00997	0.0232	0.0125	0.0178	0.0315	0.0245	0.0194	0.0401	0.0245
Upper limit of 95% CI	0.629	1.56	0.802	1.16	2.20	1.66	1.27	2.92	1.66

[0142] Table 13: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "persistent" and "non-persistent" cohorts where persistence starts within 72 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1220	21.7	914	7.92	931	3.94
Average	1690	335	1540	304	1530	234
Stdev	1680	615	1630	673	1620	624
p (t-test)		1.4E-4		0.0026		0.0018
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	2560	5540	2560	5540	2560
n (Patient)	57	26	65	18	66	17

sCr only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	742	121	718	121	718	121
Average	1460	484	1400	493	1380	433
Stdev	1670	742	1640	819	1620	826
p (t-test)		0.030		0.077		0.090
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	2560	5540	2560	5540	2560
n (Patient)	65	15	69	11	71	9

UO only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1260	7.24	949	3.94	931	3.78
Average	1700	232	1540	240	1520	224
Stdev	1640	574	1620	659	1610	681
p (t-test)		1.0E-4		0.0033		0.0043
Min	1.06	1.64	1.06	1.64	1.06	1.64
Max	5540	2560	5540	2560	5540	2560
n (Patient)	56	22	63	15	64	14

Persistence Period Duration (hr)	24	48	72

	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.21	0.34	0.14	0.20	0.34	0.17	0.18	0.32	0.15
SE	0.058	0.083	0.054	0.067	0.095	0.068	0.065	0.10	0.068
p Value	4.1E-7	0.058	2.1E-11	7.6E-6	0.089	9.8E-7	6.8E-7	0.074	3.1E-7
nCohort Non-persistent	57	65	56	65	69	63	66	71	64
nCohort Persistent	26	15	22	18	11	15	17	9	14
Cutoff Quartile 2	28.6	25.4	31.6	28.6	25.4	31.6	28.6	25.4	31.6
Sensitivity	50%	67%	36%	44%	64%	33%	41%	56%	29%
Specificity	14%	23%	11%	17%	23%	16%	17%	23%	16%
Cutoff Quartile 3	611	607	623	611	607	623	611	607	623
Sensitivity	19%	27%	9%	17%	27%	7%	12%	22%	7%
Specificity	35%	45%	34%	40%	46%	40%	39%	46%	41%
Cutoff Quartile 4	2120	2110	2140	2120	2110	2140	2120	2110	2140
Sensitivity	4%	7%	5%	6%	9%	7%	6%	11%	7%
Specificity	65%	71%	66%	69%	72%	70%	70%	73%	70%
OR Quartile 2	0.163	0.600	0.0686	0.163	0.528	0.0943	0.140	0.364	0.0741
p Value	9.2E-4	0.41	1.5E-5	0.0017	0.35	2.6E-4	9.2E-4	0.16	1.4E-4
Lower limit of 95% CI	0.0559	0.177	0.0204	0.0525	0.137	0.0265	0.0438	0.0872	0.0194
Upper limit of 95% CI	0.477	2.03	0.231	0.506	2.04	0.335	0.448	1.52	0.283
OR Quartile 3	0.129	0.293	0.0514	0.133	0.324	0.0470	0.0867	0.248	0.0526
p Value	3.2E-4	0.053	1.8E-4	0.0031	0.12	0.0041	0.0021	0.096	0.0059
Lower limit of 95% CI	0.0421	0.0844	0.0108	0.0351	0.0793	0.00581	0.0183	0.0482	0.00648
Upper limit of 95% CI	0.393	1.02	0.243	0.507	1.33	0.380	0.411	1.28	0.427
OR Quartile 4	0.0740	0.173	0.0927	0.132	0.263	0.165	0.144	0.342	0.182
p Value	0.014	0.10	0.025	0.057	0.22	0.093	0.069	0.33	0.11
Lower limit of 95% CI	0.00932	0.0212	0.0116	0.0165	0.0315	0.0203	0.0178	0.0401	0.0222
Upper limit of 95% CI	0.587	1.41	0.743	1.06	2.20	1.35	1.16	2.92	1.49

[0143] Table 14: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "persistent" and "non-persistent" cohorts where persistence starts within 96 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1220	21.7	931	11.9	949	7.92
Average	1690	335	1560	293	1560	226
Stdev	1680	615	1640	656	1620	606
p (t-test)		1.4E-4		0.0015		0.0011
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	2560	5540	2560	5540	2560
n (Patient)	57	26	64	19	65	18

sCr only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	742	121	730	108	730	108
Average	1460	484	1420	460	1400	399
Stdev	1670	742	1640	789	1630	786
p (t-test)		0.030		0.052		0.060
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	2560	5540	2560	5540	2560
n (Patient)	65	15	68	12	70	10

UO only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1260	7.24	972	7.92	949	3.94
Average	1700	232	1560	231	1540	215
Stdev	1640	574	1620	638	1610	657
p (t-test)		1.0E-4		0.0019		0.0026
Min	1.06	1.64	1.06	1.64	1.06	1.64
Max	5540	2560	5540	2560	5540	2560
n (Patient)	56	22	62	16	63	15

Persistence Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.21	0.34	0.14	0.20	0.33	0.17	0.18	0.31	0.16
SE	0.058	0.083	0.054	0.065	0.091	0.066	0.064	0.098	0.066
p Value	4.1E-7	0.058	2.1E-11	4.5E-6	0.068	6.2E-7	4.2E-7	0.056	2.2E-7
nCohort Non-persistent	57	65	56	64	68	62	65	70	63
nCohort Persistent	26	15	22	19	12	16	18	10	15
Cutoff Quartile 2	28.6	25.4	31.6	28.6	25.4	31.6	28.6	25.4	31.6
Sensitivity	50%	67%	36%	47%	67%	38%	44%	60%	33%
Specificity	14%	23%	11%	17%	24%	16%	17%	23%	16%
Cutoff Quartile 3	611	607	623	611	607	623	611	607	623
Sensitivity	19%	27%	9%	16%	25%	6%	11%	20%	7%
Specificity	35%	45%	34%	39%	46%	39%	38%	46%	40%
Cutoff Quartile 4	2120	2110	2140	2120	2110	2140	2120	2110	2140
Sensitivity	4%	7%	5%	5%	8%	6%	6%	10%	7%
Specificity	65%	71%	66%	69%	72%	69%	69%	73%	70%
OR Quartile 2	0.163	0.600	0.0686	0.187	0.615	0.115	0.163	0.444	0.0943
p Value	9.2E-4	0.41	1.5E-5	0.0031	0.47	5.1E-4	0.0017	0.25	2.6E-4
Lower limit of 95% CI	0.0559	0.177	0.0204	0.0615	0.164	0.0341	0.0525	0.112	0.0265
Upper limit of 95% CI	0.477	2.03	0.231	0.567	2.31	0.390	0.506	1.77	0.335
OR Quartile 3	0.129	0.293	0.0514	0.120	0.279	0.0421	0.0781	0.211	0.0470
p Value	3.2E-4	0.053	1.8E-4	0.0018	0.072	0.0029	0.0013	0.059	0.0041
Lower limit of 95% CI	0.0421	0.0844	0.0108	0.0317	0.0695	0.00522	0.0165	0.0417	0.00581
Upper limit of 95% CI	0.393	1.02	0.243	0.455	1.12	0.340	0.369	1.06	0.380
OR Quartile 4	0.0740	0.173	0.0927	0.122	0.234	0.151	0.132	0.298	0.165
p Value	0.014	0.10	0.025	0.048	0.18	0.077	0.057	0.27	0.093
Lower limit of 95% CI	0.00932	0.0212	0.0116	0.0152	0.0283	0.0186	0.0165	0.0354	0.0203
Upper limit of 95% CI	0.587	1.41	0.743	0.980	1.94	1.23	1.06	2.52	1.35

[0144] Table 15: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "persistent" and "non-persistent" cohorts where persistence starts within 168 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1260	31.6	1080	31.6	1080	31.6
Average	1780	318	1640	307	1610	265
Stdev	1680	587	1660	617	1640	586
p (t-test)		2.1E-5		3.4E-4		4.6E-4
Min	1.06	0.744	1.06	0.744	1.06	0.744

Max	5540	2560	5540	2560	5540	2560
n (Patient)	54	29	60	23	62	21

sCr only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	769	156	742	121	769	108
Average	1480	479	1440	456	1440	370
Stdev	1680	718	1650	755	1640	719
p (t-test)		0.023		0.040		0.030
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	2560	5540	2560	5540	2560
n (Patient)	64	16	67	13	68	12

UO only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1280	10.6	1230	21.7	1220	11.9
Average	1750	256	1630	285	1610	276
Stdev	1650	555	1650	601	1640	616
p (t-test)		4.9E-5		6.3E-4		8.7E-4
Min	1.06	1.64	1.06	1.64	1.06	1.64
Max	5540	2560	5540	2560	5540	2560
n (Patient)	54	24	58	20	59	19

Persistence Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.19	0.35	0.16	0.21	0.34	0.19	0.20	0.31	0.18
SE	0.054	0.081	0.054	0.060	0.088	0.063	0.062	0.090	0.063
p Value	6.7E-9	0.059	1.3E-10	1.1E-6	0.070	8.8E-7	2.0E-6	0.038	5.6E-7
nCohort Non-persistent	54	64	54	60	67	58	62	68	59
nCohort Persistent	29	16	24	23	13	20	21	12	19
Cutoff Quartile 2	28.6	25.4	31.6	28.6	25.4	31.6	28.6	25.4	31.6
Sensitivity	52%	69%	42%	52%	69%	45%	52%	67%	42%
Specificity	13%	23%	11%	17%	24%	16%	18%	24%	15%
Cutoff Quartile 3	611	607	623	611	607	623	611	607	623
Sensitivity	17%	25%	8%	17%	23%	10%	14%	17%	11%
Specificity	31%	44%	31%	37%	45%	36%	37%	44%	37%
Cutoff Quartile 4	2120	2110	2140	2120	2110	2140	2120	2110	2140
Sensitivity	3%	6%	4%	4%	8%	5%	5%	8%	5%
Specificity	63%	70%	65%	67%	72%	67%	68%	72%	68%
OR Quartile 2	0.160	0.673	0.0893	0.218	0.706	0.150	0.237	0.615	0.131
p Value	8.4E-4	0.52	5.5E-5	0.0050	0.60	0.0010	0.0088	0.47	5.6E-4
Lower limit of 95% CI	0.0543	0.202	0.0276	0.0754	0.191	0.0485	0.0809	0.164	0.0413
Upper limit of 95% CI	0.469	2.25	0.289	0.632	2.60	0.466	0.696	2.31	0.415
OR Quartile 3	0.0957	0.259	0.0418	0.122	0.243	0.0631	0.0983	0.158	0.0700
p Value	4.1E-5	0.032	6.4E-5	5.8E-4	0.044	5.0E-4	6.1E-4	0.023	8.1E-4
Lower limit of 95% CI	0.0312	0.0754	0.00880	0.0367	0.0614	0.0133	0.0261	0.0321	0.0147
Upper limit of 95% CI	0.294	0.891	0.198	0.404	0.964	0.299	0.370	0.776	0.332
OR Quartile 4	0.0607	0.158	0.0801	0.0909	0.211	0.108	0.105	0.234	0.117
p Value	0.0080	0.084	0.017	0.023	0.15	0.036	0.034	0.18	0.044
Lower limit of 95% CI	0.00766	0.0194	0.0100	0.0114	0.0256	0.0134	0.0131	0.0283	0.0145
Upper limit of 95% CI	0.481	1.28	0.640	0.724	1.73	0.869	0.839	1.94	0.942

[0145] Example 9. Use of Interleukin-18-binding protein for evaluating renal status in patients admitted to the ICU: Persistent at RIFLE I or F

[0146] Patients from the intensive care unit (ICU) are enrolled in the following study. EDTA anti-coagulated blood samples (10 mL) and a urine samples (25-30 mL) are collected from each patient at enrollment, 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), and 48 (± 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Interleukin-18-binding protein is measured in the earliest samples collected while the patients were in RIFLE I or F by standard immunoassay methods using commercially available assay reagents.

[0147] Kidney status is assessed by RIFLE criteria based on serum creatinine, urine output, or both serum creatinine and urine output. Two cohorts are defined to represent a "persistent" and a "non-persistent" population. "Persistent" indicates those patients whose minimum RIFLE stage during a period of 24, 48 or 72 hours is injury (I) or failure (F) where the persistence period can start from the time of sample collection to 24, 48, 72, 96 or 168 hours after sample collection. "Non-persistent" indicates those patients who are not persistent at injury (I) or failure (F) and whose minimum RIFLE stage during a period of 24, 48 or 72 hours is non-injury (RIFLE 0) or risk of injury (R) where the persistence period can start from the time of sample collection to 24, 48, 72, 96 or 168 hours after sample collection. If a patient dies after injury (I) or failure (F) or is placed on renal replacement therapy (RRT) at any time from sample collection to 24, 48, 72, 96 or 168 hours after sample collection, the patient is considered "persistent".

[0148] The ability to distinguish the "persistent" and "non-persistent" cohorts is determined using receiver operating characteristic (ROC) analysis.

[0149] Table 16: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "persistent" and "non-persistent" cohorts where persistence starts within 24 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1380	103	972	95.5	910	77.6
Average	1840	784	1530	733	1530	595
Stdev	1740	1210	1620	1290	1660	1010
p (t-test)		0.0017		0.029		0.014
Min	1.64	0.744	1.06	0.744	1.06	0.744
Max	5540	5130	5540	5130	5540	3830

n (Patient)	38	45	56	27	60	23

sCr only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	949	103	742	103	730	112
Average	1560	569	1490	589	1440	637
Stdev	1700	954	1670	1020	1650	1100
p (t-test)		0.010		0.029		0.070
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	3830	5540	3830	5540	3830
n (Patient)	57	23	61	19	64	16

UO only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	949	299	870	76.7	870	76.7
Average	1710	944	1510	920	1520	851
Stdev	1770	1290	1650	1360	1660	1280
p (t-test)		0.030		0.11		0.072
Min	1.98	1.06	1.06	1.64	1.06	1.64
Max	5540	5130	5540	5130	5540	5130
n (Patient)	35	43	49	29	51	27

Persistence Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.28	0.32	0.33	0.32	0.34	0.35	0.31	0.34	0.35
SE	0.056	0.069	0.061	0.065	0.075	0.066	0.068	0.081	0.067
p Value	9.3E-5	0.0080	0.0048	0.0065	0.031	0.021	0.0047	0.049	0.021
nCohort Non-persistent	38	57	35	56	61	49	60	64	51
nCohort Persistent	45	23	43	27	19	29	23	16	27
Cutoff Quartile 2	28.6	25.4	31.6	28.6	25.4	31.6	28.6	25.4	31.6
Sensitivity	62%	65%	60%	63%	68%	55%	61%	62%	56%
Specificity	11%	21%	9%	20%	23%	14%	20%	22%	16%
Cutoff Quartile 3	611	607	623	611	607	623	611	607	623
Sensitivity	40%	30%	42%	37%	32%	38%	35%	31%	37%
Specificity	37%	42%	40%	43%	44%	43%	43%	45%	43%
Cutoff Quartile 4	2120	2110	2140	2120	2110	2140	2120	2110	2140
Sensitivity	13%	9%	19%	15%	11%	21%	13%	12%	19%
Specificity	61%	68%	66%	70%	70%	71%	70%	72%	71%
OR Quartile 2	0.194	0.500	0.143	0.416	0.645	0.205	0.389	0.467	0.233
p Value	0.0073	0.20	0.0043	0.092	0.45	0.0042	0.078	0.20	0.0076
Lower limit of 95% CI	0.0584	0.172	0.0378	0.150	0.207	0.0694	0.136	0.144	0.0797
Upper limit of 95% CI	0.642	1.46	0.543	1.15	2.01	0.607	1.11	1.51	0.678
OR Quartile 3	0.389	0.318	0.480	0.441	0.367	0.458	0.408	0.377	0.446
p Value	0.037	0.030	0.11	0.089	0.071	0.10	0.078	0.10	0.099
Lower limit of 95% CI	0.160	0.113	0.194	0.172	0.123	0.179	0.150	0.117	0.171
Upper limit of 95% CI	0.946	0.893	1.19	1.13	1.09	1.17	1.11	1.21	1.16
OR Quartile 4	0.236	0.206	0.438	0.399	0.281	0.652	0.350	0.365	0.545
p Value	0.0086	0.047	0.12	0.14	0.11	0.44	0.12	0.21	0.30
Lower limit of 95% CI	0.0803	0.0436	0.155	0.120	0.0588	0.219	0.0923	0.0753	0.174
Upper limit of 95% CI	0.693	0.976	1.24	1.33	1.34	1.94	1.33	1.77	1.71

[0150] Table 17: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the “persistent” and “non-persistent” cohorts where persistence starts within 48 hours after sample collection and renal status is assessed by

serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	2010	121	1250	95.5	1110	77.6
Average	2170	760	1670	664	1660	545
Stdev	1790	1150	1660	1190	1700	926
p (t-test)		4.0E-5		0.0036		0.0016
Min	19.4	0.744	1.06	0.744	1.06	0.744
Max	5540	5130	5540	5130	5540	3830
n (Patient)	30	53	50	33	54	29

sCr only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1220	121	972	112	806	112
Average	1650	550	1550	560	1470	604
Stdev	1730	885	1690	956	1660	1040
p (t-test)		0.0028		0.012		0.040
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	3830	5540	3830	5540	3830
n (Patient)	53	27	58	22	62	18

UO only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1280	299	1110	56.1	1110	56.1
Average	1960	892	1660	811	1670	746
Stdev	1820	1250	1680	1280	1680	1200
p (t-test)		0.0030		0.017		0.0097
Min	19.4	1.06	1.06	1.64	1.06	1.64
Max	5540	5130	5540	5130	5540	5130
n (Patient)	29	49	44	34	46	32

Persistence Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.23	0.31	0.27	0.28	0.32	0.29	0.27	0.32	0.29
SE	0.050	0.065	0.056	0.059	0.071	0.060	0.061	0.076	0.061
p Value	5.2E-8	0.0031	4.5E-5	2.5E-4	0.013	6.5E-4	2.0E-4	0.021	7.3E-4
nCohort Non-persistent	30	53	29	50	58	44	54	62	46
nCohort Persistent	53	27	49	33	22	34	29	18	32
Cutoff Quartile 2	28.6	25.4	31.6	28.6	25.4	31.6	28.6	25.4	31.6
Sensitivity	64%	67%	61%	64%	68%	56%	62%	61%	56%
Specificity	7%	21%	3%	18%	22%	11%	19%	21%	13%
Cutoff Quartile 3	611	607	623	611	607	623	611	607	623
Sensitivity	40%	33%	39%	33%	32%	32%	31%	33%	31%
Specificity	30%	42%	31%	38%	43%	36%	39%	45%	37%
Cutoff Quartile 4	2120	2110	2140	2120	2110	2140	2120	2110	2140
Sensitivity	13%	7%	18%	12%	9%	18%	10%	11%	16%
Specificity	53%	66%	62%	66%	69%	68%	67%	71%	67%
OR Quartile 2	0.128	0.524	0.0564	0.384	0.619	0.162	0.372	0.417	0.193
p Value	0.0089	0.22	0.0066	0.064	0.39	0.0020	0.057	0.13	0.0036

Lower limit of 95% CI	0.0274	0.185	0.00707	0.140	0.208	0.0514	0.135	0.135	0.0638
Upper limit of 95% CI	0.597	1.48	0.449	1.06	1.84	0.513	1.03	1.29	0.583
OR Quartile 3	0.281	0.355	0.285	0.306	0.354	0.273	0.286	0.412	0.266
p Value	0.0093	0.036	0.012	0.012	0.049	0.0072	0.011	0.11	0.0068
Lower limit of 95% CI	0.108	0.135	0.108	0.122	0.125	0.106	0.110	0.137	0.102
Upper limit of 95% CI	0.731	0.935	0.755	0.770	0.997	0.703	0.747	1.24	0.694
OR Quartile 4	0.174	0.156	0.368	0.268	0.222	0.459	0.231	0.306	0.383
p Value	0.0014	0.019	0.060	0.031	0.058	0.16	0.030	0.14	0.098
Lower limit of 95% CI	0.0596	0.0331	0.130	0.0808	0.0469	0.155	0.0615	0.0636	0.123
Upper limit of 95% CI	0.507	0.732	1.04	0.887	1.05	1.36	0.866	1.47	1.19

[0151] Table 18: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "persistent" and "non-persistent" cohorts where persistence starts within 72 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1930	156	1250	95.5	1110	77.6
Average	2110	815	1660	732	1650	630
Stdev	1800	1210	1650	1270	1690	1070
p (t-test)		1.9E-4		0.0068		0.0034
Min	19.4	0.744	1.06	0.744	1.06	0.744
Max	5540	5130	5540	5130	5540	3830
n (Patient)	29	54	48	35	52	31

sCr only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1110	156	949	121	742	121
Average	1610	663	1510	697	1430	767
Stdev	1720	1050	1680	1140	1650	1240
p (t-test)		0.0100		0.037		0.11
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	3830	5540	3830	5540	3830
n (Patient)	52	28	57	23	61	19

UO only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1280	299	1220	35.5	1220	35.5
Average	1960	892	1700	788	1700	723
Stdev	1820	1250	1680	1270	1690	1190
p (t-test)		0.0030		0.0099		0.0055
Min	19.4	1.06	1.06	1.64	1.06	1.64
Max	5540	5130	5540	5130	5540	5130
n (Patient)	29	49	43	35	45	33

Persistence Period Duration (hr)	24	48	72

	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.24	0.33	0.27	0.28	0.35	0.27	0.28	0.36	0.27
SE	0.052	0.065	0.056	0.058	0.071	0.058	0.060	0.076	0.059
p Value	6.9E-7	0.0098	4.5E-5	2.1E-4	0.035	9.4E-5	1.9E-4	0.057	1.1E-4
nCohort Non-persistent	29	52	29	48	57	43	52	61	45
nCohort Persistent	54	28	49	35	23	35	31	19	33
Cutoff Quartile 2	28.6	25.4	31.6	28.6	25.4	31.6	28.6	25.4	31.6
Sensitivity	65%	68%	61%	63%	70%	54%	61%	63%	55%
Specificity	7%	21%	3%	17%	23%	9%	17%	21%	11%
Cutoff Quartile 3	611	607	623	611	607	623	611	607	623
Sensitivity	41%	36%	39%	34%	35%	31%	32%	37%	30%
Specificity	31%	42%	31%	38%	44%	35%	38%	46%	36%
Cutoff Quartile 4	2120	2110	2140	2120	2110	2140	2120	2110	2140
Sensitivity	15%	11%	18%	14%	13%	17%	13%	16%	15%
Specificity	55%	67%	62%	67%	70%	67%	67%	72%	67%
OR Quartile 2	0.136	0.566	0.0564	0.338	0.675	0.122	0.331	0.464	0.150
p Value	0.011	0.28	0.0066	0.038	0.48	7.6E-4	0.034	0.18	0.0013
Lower limit of 95% CI	0.0292	0.201	0.00707	0.122	0.229	0.0358	0.120	0.152	0.0473
Upper limit of 95% CI	0.637	1.60	0.449	0.941	1.99	0.415	0.918	1.42	0.476
OR Quartile 3	0.309	0.407	0.285	0.313	0.417	0.246	0.298	0.495	0.240
p Value	0.016	0.064	0.012	0.012	0.088	0.0038	0.011	0.19	0.0036
Lower limit of 95% CI	0.119	0.158	0.108	0.126	0.153	0.0950	0.117	0.172	0.0917
Upper limit of 95% CI	0.805	1.05	0.755	0.778	1.14	0.635	0.760	1.43	0.627
OR Quartile 4	0.214	0.247	0.368	0.333	0.353	0.429	0.305	0.485	0.357
p Value	0.0040	0.039	0.060	0.055	0.13	0.13	0.052	0.30	0.076
Lower limit of 95% CI	0.0750	0.0653	0.130	0.109	0.0924	0.145	0.0919	0.125	0.115
Upper limit of 95% CI	0.611	0.935	1.04	1.02	1.35	1.27	1.01	1.88	1.11

[0152] Table 19: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "persistent" and "non-persistent" cohorts where persistence starts within 96 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1930	291	1280	99.5	1250	95.5
Average	2070	884	1650	819	1670	718
Stdev	1740	1320	1590	1410	1650	1260
p (t-test)		9.4E-4		0.015		0.0054
Min	19.4	0.744	1.64	0.744	1.06	0.744
Max	5540	5130	5540	5130	5540	5080
n (Patient)	27	56	45	38	48	35

sCr only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1110	156	949	121	806	112
Average	1570	788	1470	845	1420	898
Stdev	1670	1310	1630	1410	1600	1500
p (t-test)		0.032		0.10		0.19
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	5080	5540	5080	5540	5080
n (Patient)	50	30	55	25	58	22

UO only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1280	392	1280	76.7	1250	77.1
Average	1900	965	1730	851	1680	873
Stdev	1760	1360	1620	1400	1620	1410
p (t-test)		0.011		0.012		0.021
Min	19.4	1.06	5.23	1.06	1.06	1.64
Max	5540	5130	5540	5130	5540	5130
n (Patient)	27	51	39	39	40	38

Persistence Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.25	0.34	0.28	0.28	0.36	0.26	0.28	0.36	0.29
SE	0.054	0.064	0.058	0.057	0.069	0.056	0.058	0.072	0.058
p Value	5.2E-6	0.013	2.2E-4	1.6E-4	0.044	2.1E-5	1.2E-4	0.055	2.4E-4
nCohort Non-persistent	27	50	27	45	55	39	48	58	40
nCohort Persistent	56	30	51	38	25	39	35	22	38
Cutoff Quartile 2	28.6	25.4	31.6	28.6	25.4	31.6	28.6	25.4	31.6
Sensitivity	66%	67%	63%	63%	68%	56%	63%	64%	58%
Specificity	7%	20%	4%	16%	22%	8%	17%	21%	10%
Cutoff Quartile 3	611	607	623	611	607	623	611	607	623
Sensitivity	41%	37%	39%	34%	36%	31%	31%	36%	32%
Specificity	30%	42%	30%	36%	44%	31%	35%	45%	32%
Cutoff Quartile 4	2120	2110	2140	2120	2110	2140	2120	2110	2140
Sensitivity	16%	13%	20%	16%	16%	18%	14%	18%	18%
Specificity	56%	68%	63%	67%	71%	67%	67%	72%	68%
OR Quartile 2	0.156	0.500	0.0648	0.316	0.593	0.108	0.338	0.457	0.153
p Value	0.018	0.19	0.0098	0.030	0.33	0.0011	0.038	0.15	0.0025
Lower limit of 95% CI	0.0333	0.179	0.00812	0.111	0.206	0.0283	0.122	0.156	0.0452
Upper limit of 95% CI	0.729	1.40	0.517	0.895	1.71	0.411	0.941	1.34	0.516
OR Quartile 3	0.293	0.419	0.272	0.287	0.435	0.198	0.251	0.464	0.222
p Value	0.014	0.067	0.011	0.0069	0.095	9.5E-4	0.0035	0.14	0.0020
Lower limit of 95% CI	0.110	0.165	0.100	0.116	0.164	0.0755	0.0995	0.169	0.0858
Upper limit of 95% CI	0.784	1.06	0.738	0.710	1.15	0.517	0.635	1.28	0.576
OR Quartile 4	0.239	0.327	0.415	0.375	0.464	0.437	0.333	0.583	0.469
p Value	0.0071	0.070	0.098	0.072	0.22	0.12	0.055	0.39	0.16
Lower limit of 95% CI	0.0845	0.0976	0.146	0.129	0.137	0.152	0.109	0.171	0.163
Upper limit of 95% CI	0.678	1.10	1.18	1.09	1.57	1.26	1.02	1.99	1.35

[0153] Table 20: Comparison of marker levels and the area under the ROC curve (AUC) in urine samples for the "persistent" and "non-persistent" cohorts where persistence starts within 168 hours after sample collection and renal status is assessed by serum creatinine (sCr) only, urine output (UO) only, or serum creatinine or urine output RIFLE criteria.

sCr or UO

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1930	291	1280	99.5	1280	95.5
Average	2100	910	1700	803	1720	706
Stdev	1740	1340	1610	1380	1660	1240
p (t-test)		0.0011		0.0081		0.0028
Min	19.4	0.744	1.64	0.744	1.06	0.744
Max	5540	5130	5540	5130	5540	5080

n (Patient)	25	58	43	40	46	37

sCr only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1220	191	972	156	910	112
Average	1590	776	1490	828	1460	842
Stdev	1680	1290	1630	1390	1610	1440
p (t-test)		0.023		0.078		0.11
Min	1.06	0.744	1.06	0.744	1.06	0.744
Max	5540	5080	5540	5080	5540	5080
n (Patient)	49	31	54	26	56	24

UO only

Persistence Period Duration (hr)	24		48		72	
	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort	Non-persistent Cohort	Persistent Cohort
Median	1250	399	1280	77.6	1280	77.6
Average	1850	1010	1700	914	1700	876
Stdev	1780	1380	1640	1410	1640	1390
p (t-test)		0.025		0.025		0.019
Min	19.4	1.06	5.23	1.06	1.06	1.64
Max	5540	5130	5540	5130	5540	5130
n (Patient)	26	52	37	41	39	39

Persistence Period Duration (hr)	24			48			72		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.25	0.34	0.30	0.28	0.36	0.28	0.27	0.35	0.29
SE	0.054	0.064	0.060	0.056	0.068	0.058	0.057	0.070	0.059
p Value	3.4E-6	0.012	9.5E-4	7.8E-5	0.042	1.7E-4	6.2E-5	0.034	3.5E-4
nCohort Non-persistent	25	49	26	43	54	37	46	56	39
nCohort Persistent	58	31	52	40	26	41	37	24	39
Cutoff Quartile 2	28.6	25.4	31.6	28.6	25.4	31.6	28.6	25.4	31.6
Sensitivity	66%	68%	63%	62%	69%	59%	62%	67%	59%
Specificity	4%	20%	4%	14%	22%	8%	15%	21%	10%
Cutoff Quartile 3	611	607	623	611	607	623	611	607	623
Sensitivity	41%	35%	40%	35%	35%	34%	32%	33%	33%
Specificity	28%	41%	31%	35%	43%	32%	35%	43%	33%
Cutoff Quartile 4	2120	2110	2140	2120	2110	2140	2120	2110	2140
Sensitivity	17%	13%	21%	15%	15%	20%	14%	17%	18%
Specificity	56%	67%	65%	65%	70%	68%	65%	71%	67%
OR Quartile 2	0.0792	0.538	0.0695	0.270	0.643	0.125	0.295	0.545	0.164
p Value	0.016	0.24	0.012	0.017	0.41	0.0022	0.022	0.26	0.0036
Lower limit of 95% CI	0.00997	0.193	0.00871	0.0923	0.225	0.0328	0.104	0.189	0.0487
Upper limit of 95% CI	0.629	1.50	0.554	0.791	1.84	0.473	0.837	1.58	0.554
OR Quartile 3	0.275	0.379	0.301	0.288	0.393	0.249	0.256	0.375	0.250
p Value	0.013	0.041	0.019	0.0070	0.059	0.0039	0.0036	0.055	0.0039
Lower limit of 95% CI	0.0992	0.150	0.111	0.117	0.149	0.0969	0.102	0.138	0.0975
Upper limit of 95% CI	0.759	0.962	0.819	0.712	1.04	0.639	0.641	1.02	0.641
OR Quartile 4	0.265	0.306	0.507	0.329	0.432	0.505	0.293	0.500	0.437
p Value	0.013	0.054	0.20	0.042	0.18	0.20	0.032	0.27	0.12
Lower limit of 95% CI	0.0934	0.0913	0.178	0.113	0.128	0.179	0.0955	0.148	0.152
Upper limit of 95% CI	0.752	1.02	1.44	0.961	1.46	1.42	0.899	1.69	1.26

[0154] Example 10. Interleukin-18-binding protein in ICU patients

[0155] Patients from the intensive care unit (ICU) are enrolled in the following study. Each patient is 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 urine samples (50 mL) are collected from each patient at enrollment, 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 while the subject is hospitalized. Interleukin-18-binding protein is measured by standard immunoassay methods using commercially available assay reagents in the urine samples and the plasma component of the blood samples collected.

[0156] Two cohorts are defined to represent a "diseased" and a "normal" population. While these terms are used for convenience, "diseased" and "normal" simply represent two cohorts for comparison (say RIFLE 0 vs RIFLE R, I and F; RIFLE 0 vs RIFLE R; RIFLE 0 and R vs RIFLE I and F; etc.). The time "prior max stage" represents the time at which a sample is collected, relative to the time a particular patient reaches the lowest disease stage as defined for that cohort, binned into three groups which are +/- 12 hours. For example, "24 hr prior" which uses 0 vs R, I, F as the two cohorts would mean 24 hr (+/- 12 hours) prior to reaching stage R (or I if no sample at R, or F if no sample at R or I).

[0157] A receiver operating characteristic (ROC) curve is generated for each biomarker and the area under the ROC curve (AUC) is determined. Patients in Cohort 2 are also separated according to the reason for adjudication to cohort 2 as being based on serum creatinine measurements (sCr), being based on urine output (UO), or being based on either serum creatinine measurements or urine output. Using the same example discussed above (0 vs R, I, F), for those patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements alone, the stage 0 cohort may include patients adjudicated to stage R, I, or F on the basis of urine output; for those patients adjudicated to stage R, I, or F on the basis of urine output alone, the stage 0 cohort may include patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements; and for those patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements or urine output, the stage 0 cohort contains only patients in stage 0 for both serum creatinine measurements and urine output. Also, in the data for patients adjudicated on the basis of serum creatinine measurements or urine output, the adjudication method which yielded the most severe RIFLE stage is used.

[0158] The ability to distinguish cohort 1 from Cohort 2 is determined using ROC analysis. SE is the standard error of the AUC, n is the number of sample or individual patients ("pts," as indicated). Standard errors are calculated as described in Hanley, J. A.,

and McNeil, B.J., The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology (1982) 143: 29-36; p values are calculated with a two-tailed Z-test. An AUC < 0.5 is indicative of a negative going marker for the comparison, and an AUC > 0.5 is indicative of a positive going marker for the comparison.

[0159] Various biomarker threshold (or "cutoff") concentrations are selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 are determined. OR is the odds ratio calculated for the particular cutoff concentration, and 95% CI is the confidence interval for the odds ratio.

[0160] Table 21: 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.

Interleukin-18-binding protein

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	1770	834	1770	1210	1770	2780
Average	1910	1580	1910	1400	1910	2410
Stdev	1400	1580	1400	1410	1400	1480
p(t-test)		0.35		0.18		0.42
Min	14.8	4.67	14.8	3.16	14.8	183
Max	4700	5110	4700	5360	4700	3950
n (Samp)	46	29	46	20	46	6
n (Patient)	22	29	22	20	22	6

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	1910	303	1910	1010	1910	1750
Average	2020	620	2020	988	2020	1480
Stdev	1440	949	1440	853	1440	1390
p(t-test)		0.0052		0.028		0.41
Min	4.67	5.92	4.67	13.6	4.67	3.16
Max	5360	3020	5360	2570	5360	3290
n (Samp)	95	9	95	10	95	5
n (Patient)	47	9	47	10	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	1790	1460	1790	1210	1790	1200
Average	1960	1710	1960	1480	1960	1820
Stdev	1430	1580	1430	1390	1430	1490
p(t-test)		0.49		0.20		0.78
Min	13.6	1.65	13.6	3.16	13.6	20.8
Max	4700	5110	4700	5360	4700	3950
n (Samp)	46	26	46	21	46	9
n (Patient)	22	26	22	21	22	9

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.41	0.22	0.43	0.36	0.29	0.38	0.59	0.39	0.46
SE	0.069	0.094	0.071	0.077	0.095	0.076	0.13	0.14	0.11
p	0.21	0.0029	0.30	0.078	0.025	0.10	0.48	0.40	0.69
nCohort 1	46	95	46	46	95	46	46	95	46
nCohort 2	29	9	26	20	10	21	6	5	9

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Cutoff 1	115	66.0	105	344	486	611	1200	115	611
Sens 1	72%	78%	73%	70%	70%	71%	83%	80%	78%
Spec 1	15%	12%	15%	22%	21%	28%	30%	16%	28%
Cutoff 2	52.2	19.6	49.5	8.47	76.4	14.8	1200	115	267
Sens 2	83%	89%	81%	85%	80%	81%	83%	80%	89%
Spec 2	7%	6%	7%	0%	14%	4%	30%	16%	20%
Cutoff 3	5.43	5.43	4.67	4.67	40.8	4.67	115	0	14.8
Sens 3	93%	100%	92%	90%	90%	90%	100%	100%	100%
Spec 3	0%	2%	0%	0%	7%	0%	15%	0%	4%
Cutoff 4	2800	2960	3010	2800	2960	3010	2800	2960	3010
Sens 4	28%	11%	23%	10%	0%	14%	50%	20%	33%
Spec 4	72%	71%	72%	72%	71%	72%	72%	71%	72%
Cutoff 5	3400	3400	3410	3400	3400	3410	3400	3400	3410
Sens 5	14%	0%	15%	10%	0%	10%	33%	0%	22%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	4000	4000	4000	4000	4000	4000	4000	4000	4000
Sens 6	10%	0%	12%	5%	0%	5%	0%	0%	0%
Spec 6	91%	91%	91%	91%	91%	91%	91%	91%	91%
OR Quart 2	0.79	>1.0	1.0	2.1	>2.2	2.5	1.0	1.0	0.28
p Value	0.73	<0.98	1.0	0.37	<0.52	0.25	1.0	1.0	0.30
95% CI of	0.21	>0.062	0.25	0.41	>0.19	0.52	0.056	0.059	0.026
OR Quart2	3.0	na	4.0	11	na	13	18	17	3.1
OR Quart 3	1.0	>2.2	1.0	2.5	>4.9	2.5	1.0	1.0	1.0
p Value	1.0	<0.54	1.0	0.25	<0.17	0.25	1.0	1.0	1.0
95% CI of	0.27	>0.18	0.25	0.52	>0.51	0.52	0.056	0.059	0.16
OR Quart3	3.7	na	4.0	13	na	13	18	17	6.1
OR Quart 4	1.7	>7.8	1.6	2.8	>4.9	2.8	3.6	2.1	0.67
p Value	0.42	<0.067	0.50	0.21	<0.17	0.21	0.30	0.56	0.69
95% CI of	0.46	>0.87	0.41	0.56	>0.51	0.56	0.32	0.18	0.093
OR Quart4	6.4	na	6.2	14	na	14	40	25	4.8

[0161] Table 22: 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.

Interleukin-18-binding protein

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	1870	655	1870	1310	nd	nd
Average	1970	1240	1970	1410	nd	nd
Stdev	1450	1410	1450	1170	nd	nd
p(t-test)		0.054		0.11	nd	nd
Min	8.47	1.65	8.47	3.16	nd	nd
Max	5360	3630	5360	3220	nd	nd
n (Samp)	91	18	91	20	nd	nd
n (Patient)	44	18	44	20	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1870	821	1870	1470	nd	nd
Average	1990	1310	1990	1480	nd	nd
Stdev	1480	1420	1480	1160	nd	nd
p(t-test)		0.087		0.16	nd	nd
Min	8.47	1.65	8.47	3.16	nd	nd
Max	5360	3630	5360	3220	nd	nd
n (Samp)	83	17	83	19	nd	nd
n (Patient)	40	17	40	19	nd	nd

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

	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	0.33	nd	0.35	0.38	nd	0.39	nd	nd	nd
SE	0.075	nd	0.078	0.073	nd	0.075	nd	nd	nd
p	0.021	nd	0.046	0.093	nd	0.13	nd	nd	nd
nCohort 1	91	nd	83	91	nd	83	nd	nd	nd
nCohort 2	18	nd	17	20	nd	19	nd	nd	nd
Cutoff 1	19.6	nd	40.8	562	nd	562	nd	nd	nd
Sens 1	72%	nd	71%	70%	nd	74%	nd	nd	nd
Spec 1	3%	nd	6%	24%	nd	24%	nd	nd	nd
Cutoff 2	5.43	nd	5.43	65.6	nd	14.8	nd	nd	nd
Sens 2	83%	nd	82%	80%	nd	84%	nd	nd	nd
Spec 2	0%	nd	0%	8%	nd	4%	nd	nd	nd
Cutoff 3	1.65	nd	1.65	8.47	nd	3.16	nd	nd	nd
Sens 3	94%	nd	94%	90%	nd	95%	nd	nd	nd
Spec 3	0%	nd	0%	1%	nd	0%	nd	nd	nd
Cutoff 4	2790	nd	3010	2790	nd	3010	nd	nd	nd
Sens 4	28%	nd	24%	15%	nd	11%	nd	nd	nd
Spec 4	70%	nd	71%	70%	nd	71%	nd	nd	nd
Cutoff 5	3410	nd	3570	3410	nd	3570	nd	nd	nd
Sens 5	11%	nd	12%	0%	nd	0%	nd	nd	nd
Spec 5	80%	nd	81%	80%	nd	81%	nd	nd	nd
Cutoff 6	4000	nd	4030	4000	nd	4030	nd	nd	nd
Sens 6	0%	nd	0%	0%	nd	0%	nd	nd	nd
Spec 6	90%	nd	90%	90%	nd	90%	nd	nd	nd
OR Quart 2	0.48	nd	1.0	3.5	nd	3.8	nd	nd	nd
p Value	0.42	nd	1.0	0.14	nd	0.13	nd	nd	nd
95% CI of	0.080	nd	0.18	0.65	nd	0.69	nd	nd	nd
OR Quart2	2.9	nd	5.5	19	nd	21	nd	nd	nd
OR Quart 3	1.0	nd	1.4	3.5	nd	3.6	nd	nd	nd
p Value	0.96	nd	0.68	0.14	nd	0.14	nd	nd	nd
95% CI of	0.23	nd	0.28	0.65	nd	0.65	nd	nd	nd
OR Quart3	4.7	nd	7.0	19	nd	20	nd	nd	nd
OR Quart 4	2.5	nd	2.9	3.7	nd	3.0	nd	nd	nd
p Value	0.18	nd	0.17	0.13	nd	0.22	nd	nd	nd
95% CI of	0.66	nd	0.64	0.68	nd	0.52	nd	nd	nd
OR Quart4	9.7	nd	13	20	nd	17	nd	nd	nd

[0162] Table 23: Comparison of marker levels in urine samples collected within 12 hours of reaching stage R from Cohort 1 (patients that reached, but did not progress beyond, RIFLE stage R) and from Cohort 2 (patients that reached RIFLE stage I or F).

Interleukin-18-binding protein

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1930	726	nd	nd	2570	1000
Average	2040	1240	nd	nd	2460	1400
Stdev	1610	1300	nd	nd	1660	1310
p(t-test)		0.15	nd	nd		0.11
Min	65.6	5.43	nd	nd	65.6	5.43
Max	5110	3610	nd	nd	5110	3610
n (Samp)	13	16	nd	nd	9	14
n (Patient)	13	16	nd	nd	9	14

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.34	nd	0.31
SE	0.10	nd	0.11
p	0.12	nd	0.088
nCohort 1	13	nd	9
nCohort 2	16	nd	14
Cutoff 1	65.6	nd	154
Sens 1	75%	nd	71%
Spec 1	8%	nd	22%
Cutoff 2	20.8	nd	5.92
Sens 2	81%	nd	86%

	At Enrollment		
	sCr or UO	sCr only	UO only
Spec 2	0%	nd	0%
Cutoff 3	5.43	nd	5.43
Sens 3	94%	nd	93%
Spec 3	0%	nd	0%
Cutoff 4	3020	nd	3240
Sens 4	12%	nd	7%
Spec 4	77%	nd	78%
Cutoff 5	3240	nd	4190
Sens 5	6%	nd	0%
Spec 5	85%	nd	89%
Cutoff 6	4190	nd	5110
Sens 6	0%	nd	0%
Spec 6	92%	nd	100%
OR Quart 2	0.75	nd	0.50
p Value	0.78	nd	0.56
95% CI of	0.098	nd	0.049
OR Quart2	5.8	nd	5.2
OR Quart 3	1.3	nd	5.0
p Value	0.78	nd	0.24
95% CI of	0.17	nd	0.34
OR Quart3	10	nd	73
OR Quart 4	2.5	nd	4.0
p Value	0.40	nd	0.32
95% CI of	0.29	nd	0.27
OR Quart4	21	nd	60

[0163] Table 24: Comparison of the maximum marker levels in urine samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0) and the maximum values in urine samples collected from subjects between enrollment and 0, 24 hours, and 48 hours prior to reaching stage F in Cohort 2.

Interleukin-18-binding protein

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	2370	435	2370	421	nd	nd
Average	2310	973	2310	967	nd	nd
Stdev	1470	1220	1470	1220	nd	nd
p(t-test)		0.029		0.028	nd	nd
Min	73.3	4.67	73.3	3.16	nd	nd
Max	4700	3220	4700	3220	nd	nd
n (Samp)	22	8	22	8	nd	nd
n (Patient)	22	8	22	8	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	2400	435	2400	421	nd	nd
Average	2380	973	2380	967	nd	nd
Stdev	1500	1220	1500	1220	nd	nd
p(t-test)		0.024		0.024	nd	nd
Min	73.3	4.67	73.3	3.16	nd	nd
Max	4700	3220	4700	3220	nd	nd
n (Samp)	22	8	22	8	nd	nd
n (Patient)	22	8	22	8	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.21	nd	0.20	0.21	nd	0.20	nd	nd	nd
SE	0.10	nd	0.10	0.10	nd	0.10	nd	nd	nd
p	0.0054	nd	0.0032	0.0054	nd	0.0032	nd	nd	nd
nCohort 1	22	nd	22	22	nd	22	nd	nd	nd
nCohort 2	8	nd	8	8	nd	8	nd	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Cutoff 1	13.6	nd	13.6	4.67	nd	4.67	nd	nd	nd
Sens 1	75%	nd	75%	75%	nd	75%	nd	nd	nd
Spec 1	0%	nd	0%	0%	nd	0%	nd	nd	nd
Cutoff 2	4.67	nd	4.67	3.16	nd	3.16	nd	nd	nd
Sens 2	88%	nd	88%	88%	nd	88%	nd	nd	nd
Spec 2	0%	nd	0%	0%	nd	0%	nd	nd	nd
Cutoff 3	0	nd	0	0	nd	0	nd	nd	nd
Sens 3	100%	nd	100%	100%	nd	100%	nd	nd	nd
Spec 3	0%	nd	0%	0%	nd	0%	nd	nd	nd
Cutoff 4	3600	nd	3680	3600	nd	3680	nd	nd	nd
Sens 4	0%	nd	0%	0%	nd	0%	nd	nd	nd
Spec 4	73%	nd	73%	73%	nd	73%	nd	nd	nd
Cutoff 5	4000	nd	4000	4000	nd	4000	nd	nd	nd
Sens 5	0%	nd	0%	0%	nd	0%	nd	nd	nd
Spec 5	82%	nd	82%	82%	nd	82%	nd	nd	nd
Cutoff 6	4050	nd	4050	4050	nd	4050	nd	nd	nd
Sens 6	0%	nd	0%	0%	nd	0%	nd	nd	nd
Spec 6	91%	nd	91%	91%	nd	91%	nd	nd	nd
OR Quart 2	1.2	nd	>3.2	1.2	nd	>3.2	nd	nd	nd
p Value	0.92	nd	<0.39	0.92	nd	<0.39	nd	nd	nd
95% CI of	0.059	nd	>0.23	0.059	nd	>0.23	nd	nd	nd
OR Quart2	23	nd	na	23	nd	na	nd	nd	nd
OR Quart 3	2.3	nd	>2.7	2.3	nd	>2.7	nd	nd	nd
p Value	0.53	nd	<0.46	0.53	nd	<0.46	nd	nd	nd
95% CI of	0.17	nd	>0.19	0.17	nd	>0.19	nd	nd	nd
OR Quart3	33	nd	na	33	nd	na	nd	nd	nd
OR Quart 4	9.3	nd	>11	9.3	nd	>11	nd	nd	nd
p Value	0.089	nd	<0.070	0.089	nd	<0.070	nd	nd	nd
95% CI of	0.71	nd	>0.82	0.71	nd	>0.82	nd	nd	nd
OR Quart4	120	nd	na	120	nd	na	nd	nd	nd

[0164] Table 25: 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.

Interleukin-18-binding protein

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	1240	840	1240	982	1240	894
Average	1120	1100	1120	1280	1120	924
Stdev	589	734	589	845	589	392
p(t-test)		0.87		0.40		0.47
Min	11.0	268	11.0	392	11.0	340
Max	2200	3420	2200	3600	2200	1330
n (Samp)	50	27	50	17	50	5
n (Patient)	25	27	25	17	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	962	1120	962	982	962	910
Average	1030	1130	1030	1420	1030	1390
Stdev	540	760	540	1070	540	1270
p(t-test)		0.62		0.087		0.18
Min	11.0	284	11.0	399	11.0	381
Max	2470	2200	2470	3420	2470	3600
n (Samp)	97	8	97	7	97	5
n (Patient)	49	8	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	1110	857	1110	1260	1110	823
Average	1090	1140	1090	1310	1090	792

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
Stdev	544	725	544	832	544	458
p(t-test)		0.74		0.21		0.17
Min	11.0	268	11.0	284	11.0	284
Max	2190	3420	2190	3600	2190	1410
n (Samp)	51	25	51	18	51	7
n (Patient)	26	25	26	18	26	7

[1]

	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	0.46	0.52	0.48	0.51	0.58	0.54	0.36	0.51	0.32
SE	0.070	0.11	0.071	0.082	0.12	0.080	0.14	0.13	0.12
p	0.54	0.82	0.83	0.94	0.49	0.58	0.32	0.93	0.14
nCohort 1	50	97	51	50	97	51	50	97	51
nCohort 2	27	8	25	17	7	18	5	5	7
Cutoff 1	601	449	665	777	777	777	777	822	449
Sens 1	70%	75%	72%	71%	71%	72%	80%	80%	71%
Spec 1	24%	14%	29%	32%	38%	31%	32%	39%	16%
Cutoff 2	449	284	566	539	539	476	777	822	308
Sens 2	81%	88%	80%	82%	86%	83%	80%	80%	86%
Spec 2	16%	6%	24%	20%	21%	18%	32%	39%	8%
Cutoff 3	284	268	362	392	391	390	88.6	340	66.3
Sens 3	93%	100%	92%	94%	100%	94%	100%	100%	100%
Spec 3	10%	6%	8%	12%	11%	10%	10%	9%	6%
Cutoff 4	1460	1360	1400	1460	1360	1400	1460	1360	1400
Sens 4	26%	50%	32%	29%	43%	44%	0%	20%	14%
Spec 4	70%	70%	71%	70%	70%	71%	70%	70%	71%
Cutoff 5	1600	1490	1590	1600	1490	1590	1600	1490	1590
Sens 5	22%	38%	24%	29%	43%	28%	0%	20%	0%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	1830	1760	1810	1830	1760	1810	1830	1760	1810
Sens 6	15%	25%	16%	18%	29%	17%	0%	20%	0%
Spec 6	90%	91%	90%	90%	91%	90%	90%	91%	90%
OR Quart 2	0.50	0.31	0.77	1.2	0.48	1.0	>2.3	2.0	>2.5
p Value	0.34	0.32	0.72	0.78	0.56	1.0	<0.51	0.58	<0.48
95% CI of	0.12	0.030	0.19	0.27	0.041	0.20	>0.19	0.17	>0.20
OR Quart2	2.1	3.2	3.2	5.8	5.6	4.9	na	24	na
OR Quart 3	1.7	0	1.6	0.64	0.48	1.0	>2.3	1.0	>2.3
p Value	0.43	na	0.50	0.61	0.56	1.0	<0.51	1.0	<0.51
95% CI of	0.46	na	0.42	0.12	0.041	0.20	>0.19	0.059	>0.19
OR Quart3	6.1	na	5.9	3.5	5.6	4.9	na	17	na
OR Quart 4	1.1	1.3	1.0	1.2	1.6	1.6	>1.2	0.96	>4.1
p Value	0.90	0.73	1.0	0.78	0.64	0.52	<0.92	0.98	<0.25
95% CI of	0.29	0.27	0.25	0.27	0.24	0.37	>0.066	0.057	>0.37
OR Quart4	4.0	6.6	3.9	5.8	10	7.2	na	16	na

[0165] Table 26: 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.

Interleukin-18-binding protein

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	981	1030	981	910	nd	nd
Average	1030	1180	1030	1110	nd	nd
Stdev	539	786	539	792	nd	nd
p(t-test)		0.31		0.55	nd	nd
Min	11.0	320	11.0	268	nd	nd
Max	2470	3420	2470	3600	nd	nd
n (Samp)	90	18	90	21	nd	nd
n (Patient)	45	18	45	21	nd	nd

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

	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	980	948	980	962	nd	nd
Average	1040	1170	1040	1150	nd	nd
Stdev	520	810	520	824	nd	nd
p(t-test)		0.38		0.43	nd	nd
Min	11.0	320	11.0	268	nd	nd
Max	2470	3420	2470	3600	nd	nd
n (Samp)	83	17	83	19	nd	nd
n (Patient)	42	17	42	19	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.53	nd	0.51	0.50	nd	0.51	nd	nd	nd
SE	0.076	nd	0.078	0.070	nd	0.074	nd	nd	nd
p	0.74	nd	0.89	0.95	nd	0.92	nd	nd	nd
nCohort 1	90	nd	83	90	nd	83	nd	nd	nd
nCohort 2	18	nd	17	21	nd	19	nd	nd	nd
Cutoff 1	665	nd	665	566	nd	488	nd	nd	nd
Sens 1	72%	nd	71%	71%	nd	74%	nd	nd	nd
Spec 1	30%	nd	29%	26%	nd	18%	nd	nd	nd
Cutoff 2	566	nd	566	476	nd	464	nd	nd	nd
Sens 2	83%	nd	82%	81%	nd	84%	nd	nd	nd
Spec 2	26%	nd	24%	19%	nd	17%	nd	nd	nd
Cutoff 3	320	nd	320	399	nd	268	nd	nd	nd
Sens 3	94%	nd	94%	90%	nd	95%	nd	nd	nd
Spec 3	7%	nd	5%	13%	nd	4%	nd	nd	nd
Cutoff 4	1340	nd	1360	1340	nd	1360	nd	nd	nd
Sens 4	33%	nd	35%	33%	nd	37%	nd	nd	nd
Spec 4	70%	nd	71%	70%	nd	71%	nd	nd	nd
Cutoff 5	1460	nd	1490	1460	nd	1490	nd	nd	nd
Sens 5	28%	nd	29%	24%	nd	26%	nd	nd	nd
Spec 5	80%	nd	81%	80%	nd	81%	nd	nd	nd
Cutoff 6	1760	nd	1760	1760	nd	1760	nd	nd	nd
Sens 6	17%	nd	18%	14%	nd	16%	nd	nd	nd
Spec 6	90%	nd	90%	90%	nd	90%	nd	nd	nd
OR Quart 2	1.3	nd	1.3	0.23	nd	0.58	nd	nd	nd
p Value	0.72	nd	0.71	0.086	nd	0.44	nd	nd	nd
95% CI of	0.31	nd	0.31	0.043	nd	0.14	nd	nd	nd
OR Quart2	5.5	nd	5.6	1.2	nd	2.3	nd	nd	nd
OR Quart 3	1.0	nd	0.72	0.82	nd	0.28	nd	nd	nd
p Value	1.0	nd	0.68	0.75	nd	0.14	nd	nd	nd
95% CI of	0.22	nd	0.14	0.24	nd	0.050	nd	nd	nd
OR Quart3	4.5	nd	3.6	2.8	nd	1.5	nd	nd	nd
OR Quart 4	1.3	nd	1.3	0.86	nd	1.2	nd	nd	nd
p Value	0.72	nd	0.71	0.81	nd	0.81	nd	nd	nd
95% CI of	0.31	nd	0.31	0.25	nd	0.33	nd	nd	nd
OR Quart4	5.5	nd	5.6	3.0	nd	4.1	nd	nd	nd

[0166] Table 27: Comparison of marker levels in EDTA samples collected within 12 hours of reaching stage R from Cohort 1 (patients that reached, but did not progress beyond, RIFLE stage R) and from Cohort 2 (patients that reached RIFLE stage I or F).

Interleukin-18-binding protein

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	751	857	nd	nd	831	1020
Average	874	1140	nd	nd	921	1220
Stdev	510	833	nd	nd	448	852
p(t-test)		0.33	nd	nd		0.34
Min	308	268	nd	nd	381	268
Max	1720	3420	nd	nd	1720	3420
n (Samp)	12	17	nd	nd	9	15
n (Patient)	12	17	nd	nd	9	15

	At Enrollment		
	sCr or UO	sCr only	UO only

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.58	nd	0.57
SE	0.11	nd	0.12
p	0.47	nd	0.56
nCohort 1	12	nd	9
nCohort 2	17	nd	15
Cutoff 1	601	nd	601
Sens 1	71%	nd	73%
Spec 1	42%	nd	22%
Cutoff 2	459	nd	532
Sens 2	82%	nd	80%
Spec 2	33%	nd	22%
Cutoff 3	268	nd	268
Sens 3	94%	nd	93%
Spec 3	0%	nd	0%
Cutoff 4	1370	nd	1260
Sens 4	29%	nd	33%
Spec 4	75%	nd	78%
Cutoff 5	1410	nd	1370
Sens 5	29%	nd	33%
Spec 5	83%	nd	89%
Cutoff 6	1570	nd	1720
Sens 6	24%	nd	27%
Spec 6	92%	nd	100%
OR Quart 2	1.8	nd	0.25
p Value	0.59	nd	0.26
95% CI of	0.21	nd	0.023
OR Quart2	15	nd	2.8
OR Quart 3	3.3	nd	1.0
p Value	0.29	nd	1.0
95% CI of	0.36	nd	0.091
OR Quart3	31	nd	11
OR Quart 4	2.2	nd	2.5
p Value	0.45	nd	0.51
95% CI of	0.28	nd	0.16
OR Quart4	18	nd	39

[0167] Table 28: Comparison of the maximum marker levels in EDTA samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0) and the maximum values in EDTA samples collected from subjects between enrollment and 0, 24 hours, and 48 hours prior to reaching stage F in Cohort 2.

Interleukin-18-binding protein

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	1310	1180	1310	1180	nd	nd
Average	1210	1450	1210	1440	nd	nd
Stdev	641	1050	641	1060	nd	nd
p(t-test)		0.45		0.47	nd	nd
Min	11.0	362	11.0	284	nd	nd
Max	2200	3600	2200	3600	nd	nd
n (Samp)	25	8	25	8	nd	nd
n (Patient)	25	8	25	8	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1240	1180	1240	1180	nd	nd
Average	1150	1450	1150	1440	nd	nd
Stdev	585	1050	585	1060	nd	nd
p(t-test)		0.31		0.33	nd	nd
Min	11.0	362	11.0	284	nd	nd
Max	2190	3600	2190	3600	nd	nd
n (Samp)	26	8	26	8	nd	nd
n (Patient)	26	8	26	8	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.52	nd	0.54	0.52	nd	0.54	nd	nd	nd
SE	0.12	nd	0.12	0.12	nd	0.12	nd	nd	nd
p	0.90	nd	0.75	0.90	nd	0.75	nd	nd	nd
nCohort 1	25	nd	26	25	nd	26	nd	nd	nd
nCohort 2	8	nd	8	8	nd	8	nd	nd	nd
Cutoff 1	679	nd	679	679	nd	679	nd	nd	nd
Sens 1	75%	nd	75%	75%	nd	75%	nd	nd	nd
Spec 1	24%	nd	27%	24%	nd	27%	nd	nd	nd
Cutoff 2	566	nd	636	566	nd	636	nd	nd	nd
Sens 2	88%	nd	88%	88%	nd	88%	nd	nd	nd
Spec 2	24%	nd	27%	24%	nd	27%	nd	nd	nd
Cutoff 3	88.6	nd	66.3	88.6	nd	66.3	nd	nd	nd
Sens 3	100%	nd	100%	100%	nd	100%	nd	nd	nd
Spec 3	12%	nd	8%	12%	nd	8%	nd	nd	nd
Cutoff 4	1590	nd	1520	1590	nd	1520	nd	nd	nd
Sens 4	38%	nd	38%	38%	nd	38%	nd	nd	nd
Spec 4	72%	nd	73%	72%	nd	73%	nd	nd	nd
Cutoff 5	1810	nd	1760	1810	nd	1760	nd	nd	nd
Sens 5	25%	nd	25%	25%	nd	25%	nd	nd	nd
Spec 5	80%	nd	81%	80%	nd	81%	nd	nd	nd
Cutoff 6	1890	nd	1890	1890	nd	1890	nd	nd	nd
Sens 6	25%	nd	25%	25%	nd	25%	nd	nd	nd
Spec 6	92%	nd	92%	92%	nd	92%	nd	nd	nd
OR Quart 2	1.0	nd	3.5	1.0	nd	3.5	nd	nd	nd
p Value	1.0	nd	0.33	1.0	nd	0.33	nd	nd	nd
95% CI of	0.10	nd	0.28	0.10	nd	0.28	nd	nd	nd
OR Quart2	9.6	nd	43	9.6	nd	43	nd	nd	nd
OR Quart 3	1.0	nd	1.0	1.0	nd	1.0	nd	nd	nd
p Value	1.0	nd	1.0	1.0	nd	1.0	nd	nd	nd
95% CI of	0.10	nd	0.052	0.10	nd	0.052	nd	nd	nd
OR Quart3	9.6	nd	19	9.6	nd	19	nd	nd	nd
OR Quart 4	0.86	nd	3.5	0.86	nd	3.5	nd	nd	nd
p Value	0.89	nd	0.33	0.89	nd	0.33	nd	nd	nd
95% CI of	0.091	nd	0.28	0.091	nd	0.28	nd	nd	nd
OR Quart4	8.1	nd	43	8.1	nd	43	nd	nd	nd

[0168] 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.

[0169] 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.

[0170] 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.

[0171] 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.

[0172] Other embodiments are set forth within the following claims.

We claim:

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

performing an assay method configured to detect Interleukin-18-binding protein on a body fluid sample obtained from the subject to provide an assay result; and

correlating the assay result to the renal status of the subject.

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

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

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 Interleukin-18-binding protein.

6. A method according to one of claims 1-5, wherein said correlating step comprises combining a plurality of assay results using a function that converts the plurality of assay results into a single composite result.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

100. A method according to one of claims 1-99, wherein the assay method comprises introducing the body fluid sample obtained from the subject into an assay instrument which (i) contacts the urine sample with a reagent which specifically binds for detection Interleukin-18-binding protein and generates an assay result indicative of Interleukin-18-binding protein in the urine sample, (ii) uses the assay result to assign the subject to a predetermined subpopulation of individuals having a known predisposition of a current or future acute renal injury or current or future acute renal failure.

101. A method according to claim 100, further comprising treating the subject based on the predetermined subpopulation of individuals to which the patient is assigned, wherein the treatment comprises one or more of initiating renal replacement therapy, withdrawing delivery of compounds that are known to be damaging to the kidney, delaying or avoiding procedures that are known to be damaging to the kidney, and modifying diuretic administration.

102. A method according to claim 100 or 101, wherein the subject does not have a current acute renal injury or acute renal failure, and the assay result is used to assign the subject to a predetermined subpopulation of individuals having a known predisposition of a future acute renal injury or future acute renal failure.

103. A method according to claim 102, wherein the future acute renal injury or future acute renal failure is within a period selected from the group consisting of 21 days, 14 days, 7 days, 5 days, 96 hours, 72 hours, 48 hours, 36 hours, 24 hours, and 12 hours

104. A method according to claim 100 or 101, wherein the subject has a current acute renal injury or acute renal failure, and the assay result is used to assign the subject to a predetermined subpopulation of individuals having a known predisposition of future recovery from acute renal injury or acute renal failure.

105. A method according to claim 104, wherein the future recovery from acute renal injury or acute renal failure is within a period selected from the group consisting of 21 days, 14 days, 7 days, 5 days, 96 hours, 72 hours, 48 hours, 36 hours, 24 hours, and 12 hours

106. Measurement of Interleukin-18-binding protein for the evaluation of renal injury.

107. Measurement of Interleukin-18-binding protein for the evaluation of acute renal injury.

108. A kit, comprising:

reagents for performing an assay configured to detect Interleukin-18-binding protein, and

a device which contains an encoded calibration curve for correlating results from performing said assay to a concentration of Interleukin-18-binding protein, wherein the concentration range of said calibration curve comprises a normal concentration of Interleukin-18-binding protein and a threshold concentration of Interleukin-18-binding protein used to indicate renal injury in a human.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/060204

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/68 (2016.01)
CPC - G01N 33/68 (2016.02)
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 33/48, 33/53, 33/58, 33/68 (2016.01)
CPC - G01N 33/48, 33/487, 33/493, 33/53, 33/566, 33/58, 33/581, 33/68, 33/6672, 33/6893 (2016.02)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/7.1, 7.2, 9; 506/9, 10, 18 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, Google Patents, Google Scholar
Search terms used: renal failure, ARI, AKI, prognosis, classification, diagnosis, staging, assay, interleukin, il-18, il-18bp, il18bp

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2014/0038203 A1 (MUSC FOUNDATION FOR RESEARCH DEVELOPMENT) 06 February 2014 (06.02.2014) entire document	1-3, 5, 106, 107 --- 4, 7-9, 108
Y	US 2012/0156701 A1 (ANDERBERG et al) 21 June 2012 (21.06.2012) entire document	4, 7-9, 108
A	WO 2012/102963 A1 (UNIVERSITY OF PITTSBURGH - OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION) 02 August 2012 (02.08.2012) entire document	1-5, 7-9, 106-108

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"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
"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)
"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
"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
"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
"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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 February 2016	03 MAR 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/060204

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, 10-105
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.