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

(57) Abstract: The present invention relates to methods and compositions for monitoring, diagnosis, prognosis, and determination of treatment regimens in subjects suffering from or suspected of having a renal injury. In particular, the invention relates to using a one or more assays configured to detect a kidney injury marker selected from the group consisting of Coagulation factor X, Coagulation factor V, soluble Receptor tyrosine- protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde- 3 -phosphate dehydrogenase, Interferon omega- 1, Coagulation factor VIII, Thrombin- Antithrombin- III complex, and soluble Tumor necrosis factor ligand superfamily member 13B as diagnostic and prognostic biomarkers in renal injuries.

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

[0001] The present application claims priority to U.S. Provisional Patent Application No. 61/769,448, filed February 26, 2013, 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.

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

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

"Stage I": increase in serum creatinine of more than or equal to 0.3 mg/dL (≥ 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 one or more

biomarkers selected from the group consisting of Coagulation factor X, Coagulation factor V, Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and Tumor necrosis factor ligand superfamily member 13B (each referred to herein as a "kidney injury marker") can be used for diagnosis, prognosis, risk stratification, staging, monitoring, categorizing and determination of further diagnosis and treatment regimens in subjects suffering or at risk of suffering from an injury to renal function, reduced renal function, and/or acute renal failure (also called acute kidney injury).

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

[0015] In a first aspect, the present invention relates to methods for evaluating renal status in a subject. These methods comprise performing an assay method that is configured to detect one or more biomarkers selected from the group consisting of Coagulation factor X, Coagulation factor V, Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and Tumor necrosis factor ligand superfamily member 13B 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 measured concentration(s) of one or more biomarkers selected from the group consisting of Coagulation factor X, Coagulation

factor V, Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and Tumor necrosis factor ligand superfamily member 13B is/are correlated to the occurrence or nonoccurrence of a change in renal status. The following are preferred diagnostic embodiments.

[0025] In preferred diagnostic embodiments, these methods comprise diagnosing the occurrence or nonoccurrence of an injury to renal function, and the assay result(s) is/are correlated to the occurrence or nonoccurrence of such an injury. For example, each of the measured concentration(s) may be compared to a threshold value. For a positive going marker, an increased likelihood of the occurrence of an injury to renal function is assigned to the subject when the measured concentration is above the threshold (relative to the likelihood assigned when the measured concentration is below the threshold); alternatively, when the measured concentration is below the threshold, an increased likelihood of the nonoccurrence of an injury to renal function may be assigned to the subject (relative to the likelihood assigned when the measured concentration is above the threshold). For a negative going marker, an increased likelihood of the occurrence of an injury to renal function is assigned to the subject when the measured concentration is below the threshold (relative to the likelihood assigned when the measured concentration is above the threshold); alternatively, when the measured concentration is above the threshold, an increased likelihood of the nonoccurrence of an injury to renal function may be assigned to the subject (relative to the likelihood assigned when the measured concentration is below the threshold).

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

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

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

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

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

[0029] In still other preferred diagnostic embodiments, these methods comprise diagnosing a subject as being in need of renal transplantation, and the assay result(s0 is/are correlated to a need for renal transplantation. For example, each of the measured concentration(s) may be compared to a threshold value. For a positive going marker, an increased likelihood of the occurrence of an injury creating a need for renal transplantation is assigned to the subject when the measured concentration is above the threshold (relative to the likelihood assigned when the measured concentration is below the threshold); alternatively, when the measured concentration is below the threshold, an increased likelihood of the nonoccurrence of an injury creating a need for renal transplantation may be assigned to the subject (relative to the likelihood assigned when the measured concentration is above the threshold). For a negative going marker, an increased likelihood of the occurrence of an injury creating a need for renal transplantation is assigned to the subject when the measured concentration is below the threshold (relative to the likelihood assigned when the measured concentration is above the threshold); alternatively, when the measured concentration is above the threshold, an increased likelihood of the nonoccurrence of an injury creating a need for renal transplantation may be assigned to the subject (relative to the likelihood assigned when the measured concentration is below the threshold).

[0030] In still other embodiments, the methods for evaluating renal status described herein are methods for monitoring a renal injury in the subject; that is, assessing whether or not renal function is improving or worsening in a subject who has suffered from an injury to renal function, reduced renal function, or ARF. In these embodiments, the assay result(s), for example measured concentration(s) of one or more biomarkers selected from the group consisting of Coagulation factor X, Coagulation factor V, Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A,

Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and Tumor necrosis factor ligand superfamily member 13B is/are correlated to the occurrence or nonoccurrence of a change in renal status. The following are preferred monitoring embodiments.

[0031] In preferred monitoring embodiments, these methods comprise monitoring renal status in a subject suffering from an injury to renal function, and the assay result(s) is/are correlated to the occurrence or nonoccurrence of a change in renal status in the subject. For example, the measured concentration(s) may be compared to a threshold value. For a positive going marker, when the measured concentration is above the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is below the threshold, an improvement of renal function may be assigned to the subject. For a negative going marker, when the measured concentration is below the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is above the threshold, an improvement of renal function may be assigned to the subject.

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

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

function may be assigned to the subject. For a negative going marker, when the measured concentration is below the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is above the threshold, an improvement of renal function may be assigned to the subject.

[0034] In other additional preferred monitoring embodiments, these methods comprise monitoring renal status in a subject at risk of an injury to renal function due to the pre-existence of one or more known risk factors for prerenal, intrinsic renal, or postrenal ARF, and the assay result(s) is/are correlated to the occurrence or nonoccurrence of a change in renal status in the subject. For example, the measured concentration(s) may be compared to a threshold value. For a positive going marker, when the measured concentration is above the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is below the threshold, an improvement of renal function may be assigned to the subject. For a negative going marker, when the measured concentration is below the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is above the threshold, an improvement of renal function may be assigned to the subject.

[0035] In still other embodiments, the methods for evaluating renal status described herein are methods for classifying a renal injury in the subject; that is, determining whether a renal injury in a subject is prerenal, intrinsic renal, or postrenal; and/or further subdividing these classes into subclasses such as acute tubular injury, acute glomerulonephritis acute tubulointerstitial nephritis, acute vascular nephropathy, or infiltrative disease; and/or assigning a likelihood that a subject will progress to a particular RIFLE stage. In these embodiments, the assay result(s), for example measured concentration(s) of one or more biomarkers selected from the group consisting of Coagulation factor X, Coagulation factor V, Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and Tumor necrosis factor ligand superfamily member 13B is/are correlated to a particular class and/or subclass. The following are preferred classification embodiments.

[0036] In preferred classification embodiments, these methods comprise determining whether a renal injury in a subject is prerenal, intrinsic renal, or postrenal; and/or further subdividing these classes into subclasses such as acute tubular injury, acute

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

[0037] A variety of methods may be used by the skilled artisan to arrive at a desired threshold value for use in these methods. For example, the threshold value may be determined from a population of normal subjects by selecting a concentration representing the 75th, 85th, 90th, 95th, or 99th percentile of a kidney injury marker measured in such normal subjects. Alternatively, the threshold value may be determined from a "diseased" population of subjects, e.g., those suffering from an injury or having a predisposition for an injury (e.g., progression to ARF or some other clinical outcome such as death, dialysis, renal transplantation, etc.), by selecting a concentration representing the 75th, 85th, 90th, 95th, or 99th percentile of a kidney injury marker measured in such subjects. In another alternative, the threshold value may be determined from a prior measurement of a kidney injury marker in the same subject; that is, a temporal change in the level of a kidney injury marker in the subject may be used to assign risk to the subject.

[0038] The foregoing discussion is not meant to imply, however, that the kidney injury markers of the present invention must be compared to corresponding individual thresholds. Methods for combining assay results can comprise the use of multivariate logistical regression, loglinear modeling, neural network analysis, n-of-m analysis, decision tree analysis, calculating ratios of markers, etc. This list is not meant to be limiting. In these methods, a composite result which is determined by combining individual markers may be treated as if it is itself a marker; that is, a threshold may be determined for the composite result as described herein for individual markers, and the composite result for an individual patient compared to this threshold.

[0039] The ability of a particular test to distinguish two populations can be established using ROC analysis. For example, ROC curves established from a "first" subpopulation which is predisposed to one or more future changes in renal status, and a "second" subpopulation which is not so predisposed can be used to calculate a ROC curve, and the area under the curve provides a measure of the quality of the test.

Preferably, the tests described herein provide a ROC curve area greater than 0.5, preferably at least 0.6, more preferably 0.7, still more preferably at least 0.8, even more preferably at least 0.9, and most preferably at least 0.95.

[0040] In certain aspects, the measured concentration of one or more kidney injury markers, or a composite of such markers, may be treated as continuous variables. For example, any particular concentration can be converted into a corresponding probability of a future reduction in renal function for the subject, the occurrence of an injury, a classification, etc. In yet another alternative, a threshold that can provide an acceptable level of specificity and sensitivity in separating a population of subjects into "bins" such as a "first" subpopulation (e.g., which is predisposed to one or more future changes in renal status, the occurrence of an injury, a classification, etc.) and a "second" subpopulation which is not so predisposed. A threshold value is selected to separate this first and second population by one or more of the following measures of test accuracy:

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

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

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

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

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

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

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

[0041] Multiple thresholds may also be used to assess renal status in a subject. For example, a "first" subpopulation which is predisposed to one or more future changes in renal status, the occurrence of an injury, a classification, etc., and a "second" subpopulation which is not so predisposed can be combined into a single group. This group is then subdivided into three or more equal parts (known as tertiles, quartiles, quintiles, etc., depending on the number of subdivisions). An odds ratio is assigned to subjects based on which subdivision they fall into. If one considers a tertile, the lowest or highest tertile can be used as a reference for comparison of the other subdivisions. This reference subdivision is assigned an odds ratio of 1. The second tertile is assigned an odds ratio that is relative to that first tertile. That is, someone in the second tertile might be 3 times more likely to suffer one or more future changes in renal status in comparison to someone in the first tertile. The third tertile is also assigned an odds ratio that is relative to that first tertile.

[0042] In certain embodiments, the assay method is an immunoassay. Antibodies for use in such assays will specifically bind a full length kidney injury marker of interest, and may also bind one or more polypeptides that are "related" thereto, as that term is defined hereinafter. Numerous immunoassay formats are known to those of skill in the art. Preferred body fluid samples are selected from the group consisting of urine, blood, serum, saliva, tears, and plasma. In the case of those kidney injury markers which are membrane proteins as described hereinafter, preferred assays detect soluble forms thereof.

[0043] The foregoing method steps should not be interpreted to mean that the kidney injury marker assay result(s) is/are used in isolation in the methods described herein. Rather, additional variables or other clinical indicia may be included in the methods described herein. For example, a risk stratification, diagnostic, classification, monitoring,

etc. method may combine the assay result(s) with one or more variables measured for the subject selected from the group consisting of demographic information (e.g., weight, sex, age, race), medical history (e.g., family history, type of surgery, pre-existing disease such as aneurism, congestive heart failure, preeclampsia, eclampsia, diabetes mellitus, hypertension, coronary artery disease, proteinuria, renal insufficiency, or sepsis, type of toxin exposure such as NSAIDs, cyclosporines, tacrolimus, aminoglycosides, foscarnet, ethylene glycol, hemoglobin, myoglobin, ifosfamide, heavy metals, methotrexate, radiopaque contrast agents, or streptozotocin), clinical variables (e.g., blood pressure, temperature, respiration rate), risk scores (APACHE score, PREDICT score, TIMI Risk Score for UA/NSTEMI, Framingham Risk Score, risk scores of Thakar et al. (J. Am. Soc. Nephrol. 16: 162-68, 2005), Mehran et al. (J. Am. Coll. Cardiol. 44: 1393-99, 2004), Wijeysundera et al. (JAMA 297: 1801-9, 2007), Goldstein and Chawla (Clin. J. Am. Soc. Nephrol. 5: 943-49, 2010), or Chawla et al. (Kidney Intl. 68: 2274-80, 2005)), a glomerular filtration rate, an estimated glomerular filtration rate, a urine production rate, a serum or plasma creatinine concentration, a urine creatinine concentration, a fractional excretion of sodium, a urine sodium concentration, a urine creatinine to serum or plasma creatinine ratio, a urine specific gravity, a urine osmolality, a urine urea nitrogen to plasma urea nitrogen ratio, a plasma BUN to creatnine ratio, a renal failure index calculated as urine sodium / (urine creatinine / plasma creatinine), a serum or plasma neutrophil gelatinase (NGAL) concentration, a urine NGAL concentration, a serum or plasma cystatin C concentration, a serum or plasma cardiac troponin concentration, a serum or plasma BNP concentration, a serum or plasma NTproBNP concentration, and a serum or plasma proBNP concentration. Other measures of renal function which may be combined with one or more kidney injury marker assay result(s) are described hereinafter and in Harrison's Principles of Internal Medicine, 17$^{th}$ Ed., McGraw Hill, New York, pages 1741-1830, and Current Medical Diagnosis & Treatment 2008, 47$^{th}$ Ed, McGraw Hill, New York, pages 785-815, each of which are hereby incorporated by reference in their entirety.

[0044] When more than one marker is measured, the individual markers may be measured in samples obtained at the same time, or may be determined from samples obtained at different (e.g., an earlier or later) times. The individual markers may also be measured on the same or different body fluid samples. For example, one kidney injury marker may be measured in a serum or plasma sample and another kidney injury marker

may be measured in a urine sample. In addition, assignment of a likelihood may combine an individual kidney injury marker assay result with temporal changes in one or more additional variables.

[0045] In various related aspects, the present invention also relates to devices and kits for performing the methods described herein. Suitable kits comprise reagents sufficient for performing an assay for at least one of the described kidney injury markers, together with instructions for performing the described threshold comparisons.

[0046] In certain embodiments, reagents for performing such assays are provided in an assay device, and such assay devices may be included in such a kit. Preferred reagents can comprise one or more solid phase antibodies, the solid phase antibody comprising antibody that detects the intended biomarker target(s) bound to a solid support. In the case of sandwich immunoassays, such reagents can also include one or more detectably labeled antibodies, the detectably labeled antibody comprising antibody that detects the intended biomarker target(s) bound to a detectable label. Additional optional elements that may be provided as part of an assay device are described hereinafter.

[0047] Detectable labels may include molecules that are themselves detectable (e.g., fluorescent moieties, electrochemical labels, ecl (electrochemical luminescence) labels, metal chelates, colloidal metal particles, etc.) as well as molecules that may be indirectly detected by production of a detectable reaction product (e.g., enzymes such as horseradish peroxidase, alkaline phosphatase, etc.) or through the use of a specific binding molecule which itself may be detectable (e.g., a labeled antibody that binds to the second antibody, biotin, digoxigenin, maltose, oligohistidine, 2,4-dintrobenzene, phenylarsenate, ssDNA, dsDNA, etc.).

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

also be employed to determine the presence or amount of analytes that optionally eliminate the need for a labeled molecule.

## DETAILED DESCRIPTION OF THE INVENTION

[0049] The present invention relates to methods and compositions for diagnosis, differential diagnosis, risk stratification, monitoring, classifying and determination of treatment regimens in subjects suffering or at risk of suffering from injury to renal function, reduced renal function and/or acute renal failure through measurement of one or more kidney injury markers. In various embodiments, a measured concentration of one or more biomarkers selected from the group consisting of Coagulation factor X, Coagulation factor V, Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and Tumor necrosis factor ligand superfamily member 13B or one or more markers related thereto, are correlated to the renal status of the subject.

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

[0051] As used herein, an "injury to renal function" is an abrupt (within 14 days, preferably within 7 days, more preferably within 72 hours, and still more preferably within 48 hours) measurable reduction in a measure of renal function. Such an injury may be identified, for example, by a decrease in glomerular filtration rate or estimated GFR, a reduction in urine output, an increase in serum creatinine, an increase in serum cystatin C, a requirement for renal replacement therapy, *etc*. "Improvement in Renal Function" is an abrupt (within 14 days, preferably within 7 days, more preferably within 72 hours, and still more preferably within 48 hours) measurable increase in a measure of renal function. Preferred methods for measuring and/or estimating GFR are described hereinafter.

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

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

[0054] The following biomarkers (listed with the Swiss-Prot entry number of the human precursor) find use in the present invention as kidney injury markers.

[0055] As used herein, the term "Coagulation factor X" refers to one or more polypeptides present in a biological sample that are derived from the Coagulation factor X precursor (human precursor: Swiss-Prot P00742 (SEQ ID NO: 1))

```
        10         20         30         40         50         60
MGRPLHLVLL SASLAGLLLL GESLFIRREQ ANNILARVTR ANSFLEEMKK GHLERECMEE

        70         80         90        100        110        120
TCSYEEAREV FEDSDKTNEF WNKYKDGDQC ETSPCQNQGK CKDGLGEYTC TCLEGFEGKN

       130        140        150        160        170        180
CELFTRKLCS LDNGDCDQFC HEEQNSVVCS CARGYTLADN GKACIPTGPY PCGKQTLERR

       190        200        210        220        230        240
KRSVAQATSS SGEAPDSITW KPYDAADLDP TENPFDLLDF NQTQPERGDN NLTRIVGGQE

       250        260        270        280        290        300
CKDGECPWQA LLINEENEGF CGGTILSEFY ILTAAHCLYQ AKRFKVRVGD RNTEQEEGGE

       310        320        330        340        350        360
AVHEVEVVIK HNRFTKETYD FDIAVLRLKT PITFRMNVAP ACLPERDWAE STLMTQKTGI

       370        380        390        400        410        420
VSGFGRTHEK GRQSTRLKML EVPYVDRNSC KLSSSFIITQ NMFCAGYDTK QEDACQGDSG

       430        440        450        460        470        480
GPHVTRFKDT YFVTGIVSWG EGCARKGKYG IYTKVTAFLK WIDRSMKTRG LPKAKSHAPE

VITSSPLK
```

[0056] The following domains have been identified in Coagulation factor X:

| Residues | Length | Domain ID |
|---|---|---|
| 1-31 | 31 | Signal peptide |
| 32-40 | 9 | Propeptide |

| | | |
|---|---|---|
| 41-488 | 448 | Coagulation factor X |
| 41-179 | 139 | Factor X light chain |
| 183-488 | 306 | Factor X heavy chain |
| 183-234 | 52 | Activation peptide |
| 235-488 | 254 | Activated factor Xa heavy chain |

[0057] As used herein, the term "Coagulation factor V" refers to one or more polypeptides present in a biological sample that are derived from the Coagulation factor V precursor (human precursor: Swiss-Prot P12259 (SEQ ID NO: 2))

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        10         20         30         40         50         60
MFPGCPRLWV LVVLGTSWVG WGSQGTEAAQ LRQFYVAAQG ISWSYRPEPT NSSLNLSVTS

        70         80         90        100        110        120
FKKIVYREYE PYFKKEKPQS TISGLLGPTL YAEVGDIIKV HFKNKADKPL SIHPQGIRYS

       130        140        150        160        170        180
KLSEGASYLD HTFPAEKMDD AVAPGREYTY EWSISEDSGP THDDPPCLTH IYYSHENLIE

       190        200        210        220        230        240
DFNSGLIGPL LICKKGTLTE GGTQKTFDKQ IVLLFAVFDE SKSWSQSSSL MYTVNGYVNG

       250        260        270        280        290        300
TMPDITVCAH DHISWHLLGM SSGPELFSIH FNGQVLEQNH HKVSAITLVS ATSTTANMTV

       310        320        330        340        350        360
GPEGKWIISS LTPKHLQAGM QAYIDIKNCP KKTRNLKKIT REQRRHMKRW EYFIAAEEVI

       370        380        390        400        410        420
WDYAPVIPAN MDKKYRSQHL DNFSNQIGKH YKKVMYTQYE DESFTKHTVN PNMKEDGILG

       430        440        450        460        470        480
PIIRAQVRDT LKIVFKNMAS RPYSIYPHGV TFSPYEDEVN SSFTSGRNNT MIRAVQPGET

       490        500        510        520        530        540
YTYKWNILEF DEPTENDAQC LTRPYYSDVD IMRDIASGLI GLLLICKSRS LDRRGIQRAA

       550        560        570        580        590        600
DIEQQAVFAV FDENKSWYLE DNINKFCENP DEVKRDDPKF YESNIMSTIN GYVPESITTL

       610        620        630        640        650        660
GFCFDDTVQW HFCSVGTQNE ILTIHFTGHS FIYGKRHEDT LTLFPMRGES VTVTMDNVGT

       670        680        690        700        710        720
WMLTSMNSSP RSKKLRLKFR DVKCIPDDDE DSYEIFEPPE STVMATRKMH DRLEPEDEES

       730        740        750        760        770        780
DADYDYQNRL AAALGIRSFR NSSLNQEEEE FNLTALALEN GTEFVSSNTD IIVGSNYSSP

       790        800        810        820        830        840
SNISKFTVNN LAEPQKAPSH QQATTAGSPL RHLIGKNSVL NSSTAEHSSP YSEDPIEDPL

       850        860        870        880        890        900
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QPDVTGIRLL SLGAGEFKSQ EHAKHKGPKV ERDQAAKHRF SWMKLLAHKV GRHLSQDTGS

       910        920        930        940        950        960
PSGMRPWEDL PSQDTGSPSR MRPWKDPPSD LLLLKQSNSS KILVGRWHLA SEKGSYEIIQ

       970        980        990       1000       1010       1020
DTDEDTAVNN WLISPQNASR AWGESTPLAN KPGKQSGHPK FPRVRHKSLQ VRQDGGKSRL

      1030       1040       1050       1060       1070       1080
KKSQFLIKTR KKKKEKHTHH APLSPRTFHP LRSEAYNTFS ERRLKHSLVL HKSNETSLPT

      1090       1100       1110       1120       1130       1140
DLNQTLPSMD FGWIASLPDH NQNSSNDTGQ ASCPPGLYQT VPPEEHYQTF PIQDPDQMHS

      1150       1160       1170       1180       1190       1200
TSDPSHRSSS PELSEMLEYD RSHKSFPTDI SQMSPSSEHE VWQTVISPDL SQVTLSPELS

      1210       1220       1230       1240       1250       1260
QTNLSPDLSH TTLSPELIQR NLSPALGQMP ISPDLSHTTL SPDLSHTTLS LDLSQTNLSP

      1270       1280       1290       1300       1310       1320
ELSQTNLSPA LGQMPLSPDL SHTTLSLDFS QTNLSPELSH MTLSPELSQT NLSPALGQMP

      1330       1340       1350       1360       1370       1380
ISPDLSHTTL SLDFSQTNLS PELSQTNLSP ALGQMPLSPD PSHTTLSLDL SQTNLSPELS

      1390       1400       1410       1420       1430       1440
QTNLSPDLSE MPLFADLSQI PLTPDLDQMT LSPDLGETDL SPNFGQMSLS PDLSQVTLSP

      1450       1460       1470       1480       1490       1500
DISDTTLLPD LSQISPPPDL DQIFYPSESS QSLLLQEFNE SFPYPDLGQM PSPSSPTLND

      1510       1520       1530       1540       1550       1560
TFLSKEFNPL VIVGLSKDGT DYIEIIPKEE VQSSEDDYAE IDYVPYDDPY KTDVRTNINS

      1570       1580       1590       1600       1610       1620
SRDPDNIAAW YLRSNNGNRR NYYIAAEEIS WDYSEFVQRE TDIEDSDDIP EDTTYKKVVF

      1630       1640       1650       1660       1670       1680
RKYLDSTFTK RDPRGEYEEH LGILGPIIRA EVDDVIQVRF KNLASRPYSL HAHGLSYEKS

      1690       1700       1710       1720       1730       1740
SEGKTYEDDS PEWFKEDNAV QPNSSYTYVW HATERSGPES PGSACRAWAY YSAVNPEKDI

      1750       1760       1770       1780       1790       1800
HSGLIGPLLI CQKGILHKDS NMPMDMREFV LLFMTFDEKK SWYYEKKSRS SWRLTSSEMK

      1810       1820       1830       1840       1850       1860
KSHEFHAING MIYSLPGLKM YEQEWVRLHL LNIGGSQDIH VVHFHGQTLL ENGNKQHQLG

      1870       1880       1890       1900       1910       1920
VWPLLPGSFK TLEMKASKPG WWLLNTEVGE NQRAGMQTPF LIMDRDCRMP MGLSTGIISD

      1930       1940       1950       1960       1970       1980
SQIKASEFLG YWEPRLARLN NGGSYNAWSV EKLAAEFASK PWIQVDMQKE VIITGIQTQG

      1990       2000       2010       2020       2030       2040
AKHYLKSCYT TEFYVAYSSN QINWQIFKGN STRNVMYFNG NSDASTIKEN QFDPPIVARY

      2050       2060       2070       2080       2090       2100
IRISPTRAYN RPTLRLELQG CEVNGCSTPL GMENGKIENK QITASSFKKS WWGDYWEPFR
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      2110       2120       2130       2140       2150       2160
ARLNAQGRVN AWQAKANNNK QWLEIDLLKI KKITAIITQG CKSLSSEMYV KSYTIHYSEQ

      2170       2180       2190       2200       2210       2220
GVEWKPYRLK SSMVDKIFEG NTNTKGHVKN FFNPPIISRF IRVIPKTWNQ SIALRLELFG

CDIY
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[0058] The following domains have been identified in Coagulation factor V:

| Residues | Length | Domain ID |
|---|---|---|
| 1-28 | 28 | Signal peptide |
| 29-2224 | 2196 | Coagulation factor V |
| 41-179 | 139 | Factor V light chain |
| 29-737 | 709 | Factor V heavy chain |
| 738-1573 | 836 | Activation peptide |
| 1574-2224 | 651 | Activated factor Xa heavy chain |

[0059] As used herein, the term "Receptor tyrosine-protein kinase erbB-2" refers to one or more polypeptides present in a biological sample that are derived from the Receptor tyrosine-protein kinase erbB-2 precursor (human precursor: Swiss-Prot P04626 (SEQ ID NO: 3))

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        10         20         30         40         50         60
MELAALCRWG LLLALLPPGA ASTQVCTGTD MKLRLPASPE THLDMLRHLY QGCQVVQGNL

        70         80         90        100        110        120
ELTYLPTNAS LSFLQDIQEV QGYVLIAHNQ VRQVPLQRLR IVRGTQLFED NYALAVLDNG

       130        140        150        160        170        180
DPLNNTTPVT GASPGGLREL QLRSLTEILK GGVLIQRNPQ LCYQDTILWK DIFHKNNQLA

       190        200        210        220        230        240
LTLIDTNRSR ACHPCSPMCK GSRCWGESSE DCQSLTRTVC AGGCARCKGP LPTDCCHEQC

       250        260        270        280        290        300
AAGCTGPKHS DCLACLHFNH SGICELHCPA LVTYNTDTFE SMPNPEGRYT FGASCVTACP

       310        320        330        340        350        360
YNYLSTDVGS CTLVCPLHNQ EVTAEDGTQR CEKCSKPCAR VCYGLGMEHL REVRAVTSAN

       370        380        390        400        410        420
IQEFAGCKKI FGSLAFLPES FDGDPASNTA PLQPEQLQVF ETLEEITGYL YISAWPDSLP

       430        440        450        460        470        480
DLSVFQNLQV IRGRILHNGA YSLTLQGLGI SWLGLRSLRE LGSGLALIHH NTHLCFVHTV
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```
       490        500        510        520        530        540
PWDQLFRNPH QALLHTANRP EDECVGEGLA CHQLCARGHC WGPGPTQCVN CSQFLRGQEC

       550        560        570        580        590        600
VEECRVLQGL PREYVNARHC LPCHPECQPQ NGSVTCFGPE ADQCVACAHY KDPPFCVARC

       610        620        630        640        650        660
PSGVKPDLSY MPIWKFPDEE GACQPCPINC THSCVDLDDK GCPAEQRASP LTSIISAVVG

       670        680        690        700        710        720
ILLVVVLGVV FGILIKRRQQ KIRKYTMRRL LQETELVEPL TPSGAMPNQA QMRILKETEL

       730        740        750        760        770        780
RKVKVLGSGA FGTVYKGIWI PDGENVKIPV AIKVLRENTS PKANKEILDE AYVMAGVGSP

       790        800        810        820        830        840
YVSRLLGICL TSTVQLVTQL MPYGCLLDHV RENRGRLGSQ DLLNWCMQIA KGMSYLEDVR

       850        860        870        880        890        900
LVHRDLAARN VLVKSPNHVK ITDFGLARLL DIDETEYHAD GGKVPIKWMA LESILRRRFT

       910        920        930        940        950        960
HQSDVWSYGV TVWELMTFGA KPYDGIPARE IPDLLEKGER LPQPPICTID VYMIMVKCWM

       970        980        990       1000       1010       1020
IDSECRPRFR ELVSEFSRMA RDPQRFVVIQ NEDLGPASPL DSTFYRSLLE DDDMGDLVDA

      1030       1040       1050       1060       1070       1080
EEYLVPQQGF FCPDPAPGAG GMVHHRHRSS STRSGGGDLT LGLEPSEEEA PRSPLAPSEG

      1090       1100       1110       1120       1130       1140
AGSDVFDGDL GMGAAKGLQS LPTHDPSPLQ RYSEDPTVPL PSETDGYVAP LTCSPQPEYV

      1150       1160       1170       1180       1190       1200
NQPDVRPQPP SPREGPLPAA RPAGATLERP KTLSPGKNGV VKDVFAFGGA VENPEYLTPQ

      1210       1220       1230       1240       1250
GGAAPQPHPP PAFSPAFDNL YYWDQDPPER GAPPSTFKGT PTAENPEYLG LDVPV
```

[0060] Most preferably, the Receptor tyrosine-protein kinase erbB-2 assay detects one or more soluble forms of Receptor tyrosine-protein kinase erbB-2. Receptor tyrosine-protein kinase erbB-2 is a single-pass membrane protein having a large extracellular domain, most or all of which is present in soluble forms of Receptor tyrosine-protein kinase erbB-2 generated either through alternative splicing event which deletes all or a portion of the transmembrane domain, or by proteolysis of the membrane-bound form. In the case of an immunoassay, one or more antibodies that bind to epitopes within this extracellular domain may be used to detect these soluble form(s). The following domains have been identified in Receptor tyrosine-protein kinase erbB-2:

| Residues | Length | Domain ID |
|---|---|---|
| 1-22 | 22 | Signal peptide |

| | | |
|---|---|---|
| 23-1255 | 1233 | Receptor tyrosine-protein kinase erbB-2 |
| 23-652 | 630 | Extracellular |
| 653-675 | 23 | Transmembrane |
| 676-1255 | 580 | Cytoplasmic |
| 1-610 | | Missing in isoform 2 |
| 1-686 | | Missing in isoform 3 |
| 1-23 | | → MPRGSWKP (SEQ ID NO: 4) in isoform 4 |

[0061] As used herein, the term "Interferon beta" refers to one or more polypeptides present in a biological sample that are derived from the Interferon beta precursor (human precursor: Swiss-Prot P01574 (SEQ ID NO: 5))

```
        10         20         30         40         50         60
MTNKCLLQIA LLLCFSTTAL SMSYNLLGFL QRSSNFQCQK LLWQLNGRLE YCLKDRMNFD

        70         80         90        100        110        120
IPEEIKQLQQ FQKEDAALTI YEMLQNIFAI FRQDSSSTGW NETIVENLLA NVYHQINHLK

       130        140        150        160        170        180
TVLEEKLEKE DFTRGKLMSS LHLKRYYGRI LHYLKAKEYS HCAWTIVRVE ILRNFYFINR

LTGYLRN
```

[0062] The following domains have been identified in Interferon beta:

| Residues | Length | Domain ID |
|---|---|---|
| 1-21 | 21 | Signal peptide |
| 22-187 | 166 | Interferon beta |

[0063] As used herein, the term "C-type lectin domain family 11 member A" refers to one or more polypeptides present in a biological sample that are derived from the C-type lectin domain family 11 member A precursor (human precursor: Swiss-Prot Q9Y240 (SEQ ID NO: 6))

```
        10         20         30         40         50         60
MQAAWLLGAL VVPQLLGFGH GARGAEREWE GGWGGAQEEE REREALMLKH LQEALGLPAG

        70         80         90        100        110        120
RGDENPAGTV EGKEDWEMEE DQGEEEEEEA TPTPSSGPSP SPTPEDIVTY ILGRLAGLDA

       130        140        150        160        170        180
GLHQLHVRLH ALDTRVVELT QGLRQLRNAA GDTRDAVQAL QEAQGRAERE HGRLEGCLKG
```

```
       190        200        210        220        230        240
LRLGHKCFLL SRDFEAQAAA QARCTARGGS LAQPADRQQM EALTRYLRAA LAPYNWPVWL

       250        260        270        280        290        300
GVHDRRAEGL YLFENGQRVS FFAWHRSPRP ELGAQPSASP HPLSPDQPNG GTLENCVAQA

       310        320
SDDGSWWDHD CQRRLYYVCE FPF
```

[0064] The following domains have been identified in C-type lectin domain family 11 member A:

| Residues | Length | Domain ID |
|---|---|---|
| 1-21 | 21 | Signal peptide |
| 22-323 | 302 | C-type lectin domain family 11 member A |

[0065] As used herein, the term "Glyceraldehyde-3-phosphate dehydrogenase" refers to one or more polypeptides present in a biological sample that are derived from the Glyceraldehyde-3-phosphate dehydrogenase precursor (human precursor: Swiss-Prot P04406 (SEQ ID NO: 7))

```
        10         20         30         40         50         60
MGKVKVGVNG FGRIGRLVTR AAFNSGKVDI VAINDPFIDL NYMVYMFQYD STHGKFHGTV

        70         80         90        100        110        120
KAENGKLVIN GNPITIFQER DPSKIKWGDA GAEYVVESTG VFTTMEKAGA HLQGGAKRVI

       130        140        150        160        170        180
ISAPSADAPM FVMGVNHEKY DNSLKIISNA SCTTNCLAPL AKVIHDNFGI VEGLMTTVHA

       190        200        210        220        230        240
ITATQKTVDG PSGKLWRDGR GALQNIIPAS TGAAKAVGKV IPELNGKLTG MAFRVPTANV

       250        260        270        280        290        300
SVVDLTCRLE KPAKYDDIKK VVKQASEGPL KGILGYTEHQ VVSSDFNSDT HSSTFDAGAG

       310        320        330
IALNDHFVKL ISWYDNEFGY SNRVVDLMAH MASKE
```

[0066] The following domains have been identified in Glyceraldehyde-3-phosphate dehydrogenase:

| Residues | Length | Domain ID |
|---|---|---|
| 1 | 1 | Initiator methionine |
| 2-335 | 334 | Glyceraldehyde-3-phosphate dehydrogenase |

[0067] As used herein, the term "Interferon omega-1" refers to one or more polypeptides present in a biological sample that are derived from the Interferon omega-1 precursor (human precursor: Swiss-Prot P05000 (SEQ ID NO: 8))

```
        10         20         30         40         50         60
MALLFPLLAA LVMTSYSPVG SLGCDLPQNH GLLSRNTLVL LHQMRRISPF LCLKDRRDFR

        70         80         90        100        110        120
FPQEMVKGSQ LQKAHVMSVL HEMLQQIFSL FHTERSSAAW NMTLLDQLHT GLHQQLQHLE

       130        140        150        160        170        180
TCLLQVVGEG ESAGAISSPA LTLRRYFQGI RVYLKEKKYS DCAWEVVRME IMKSLFLSTN

       190
MQERLRSKDR DLGSS
```

[0068] The following domains have been identified in Interferon omega-1:

| Residues | Length | Domain ID |
|---|---|---|
| 1-21 | 21 | Signal peptide |
| 22-195 | 174 | Interferon omega-1 |

[0069] As used herein, the term "Coagulation factor VIII" refers to one or more polypeptides present in a biological sample that are derived from the Coagulation factor VIII precursor (human precursor: Swiss-Prot P00451 (SEQ ID NO: 9))

```
        10         20         30         40         50         60
MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP PRVPKSFPFN

        70         80         90        100        110        120
TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY DTVVITLKNM ASHPVSLHAV

       130        140        150        160        170        180
GVSYWKASEG AEYDDQTSQR EKEDDKVFPG GSHTYVWQVL KENGPMASDP LCLTYSYLSH

       190        200        210        220        230        240
VDLVKDLNSG LIGALLVCRE GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD

       250        260        270        280        290        300
AASARAWPKM HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH

       310        320        330        340        350        360
RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE EPQLRMKNNE

       370        380        390        400        410        420
EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT WVHYIAAEEE DWDYAPLVLA

       430        440        450        460        470        480
PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY TDETFKTREA IQHESGILGP LLYGEVGDTL

       490        500        510        520        530        540
LIIFKNQASR PYNIYPHGIT DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP
```

```
       550        560        570        580        590        600
TKSDPRCLTR YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE

       610        620        630        640        650        660
NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL HEVAYWYILS

       670        680        690        700        710        720
IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS MENPGLWILG CHNSDFRNRG

       730        740        750        760        770        780
MTALLKVSSC DKNTGDYYED SYEDISAYLL SKNNAIEPRS FSQNSRHPST RQKQFNATTI

       790        800        810        820        830        840
PENDIEKTDP WFAHRTPMPK IQNVSSSDLL MLLRQSPTPH GLSLSDLQEA KYETFSDDPS

       850        860        870        880        890        900
PGAIDSNNSL SEMTHFRPQL HHSGDMVFTP ESGLQLRLNE KLGTTAATEL KKLDFKVSST

       910        920        930        940        950        960
SNNLISTIPS DNLAAGTDNT SSLGPPSMPV HYDSQLDTTL FGKKSSPLTE SGGPLSLSEE

       970        980        990       1000       1010       1020
NNDSKLLESG LMNSQESSWG KNVSSTESGR LFKGKRAHGP ALLTKDNALF KVSISLLKTN

      1030       1040       1050       1060       1070       1080
KTSNNSATNR KTHIDGPSLL IENSPSVWQN ILESDTEFKK VTPLIHDRML MDKNATALRL

      1090       1100       1110       1120       1130       1140
NHMSNKTTSS KNMEMVQQKK EGPIPPDAQN PDMSFFKMLF LPESARWIQR THGKNSLNSG

      1150       1160       1170       1180       1190       1200
QGPSPKQLVS LGPEKSVEGQ NFLSEKNKVV VGKGEFTKDV GLKEMVFPSS RNLFLTNLDN

      1210       1220       1230       1240       1250       1260
LHENNTHNQE KKIQEEIEKK ETLIQENVVL PQIHTVTGTK NFMKNLFLLS TRQNVEGSYD

      1270       1280       1290       1300       1310       1320
GAYAPVLQDF RSLNDSTNRT KKHTAHFSKK GEEENLEGLG NQTKQIVEKY ACTTRISPNT

      1330       1340       1350       1360       1370       1380
SQQNFVTQRS KRALKQFRLP LEETELEKRI IVDDTSTQWS KNMKHLTPST LTQIDYNEKE

      1390       1400       1410       1420       1430       1440
KGAITQSPLS DCLTRSHSIP QANRSPLPIA KVSSFPSIRP IYLTRVLFQD NSSHLPAASY

      1450       1460       1470       1480       1490       1500
RKKDSGVQES SHFLQGAKKN NLSLAILTLE MTGDQREVGS LGTSATNSVT YKKVENTVLP

      1510       1520       1530       1540       1550       1560
KPDLPKTSGK VELLPKVHIY QKDLFPTETS NGSPGHLDLV EGSLLQGTEG AIKWNEANRP

      1570       1580       1590       1600       1610       1620
GKVPFLRVAT ESSAKTPSKL LDPLAWDNHY GTQIPKEEWK SQEKSPEKTA FKKKDTILSL

      1630       1640       1650       1660       1670       1680
NACESNHAIA AINEGQNKPE IEVTWAKQGR TERLCSQNPP VLKRHQREIT RTTLQSDQEE

      1690       1700       1710       1720       1730       1740
IDYDDTISVE MKKEDFDIYD EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR

      1750       1760       1770       1780       1790       1800
```

```
AQSGSVPQFK KVVFQEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR

      1810       1820       1830       1840       1850       1860
PYSFYSSLIS YEEDQRQGAE PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD CKAWAYFSDV

      1870       1880       1890       1900       1910       1920
DLEKDVHSGL IGPLLVCHTN TLNPAHGRQV TVQEFALFFT IFDETKSWYF TENMERNCRA

      1930       1940       1950       1960       1970       1980
PCNIQMEDPT FKENYRFHAI NGYIMDTLPG LVMAQDQRIR WYLLSMGSNE NIHSIHFSGH

      1990       2000       2010       2020       2030       2040
VFTVRKKEEY KMALYNLYPG VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC

      2050       2060       2070       2080       2090       2100
QTPLGMASGH IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII

      2110       2120       2130       2140       2150       2160
HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD SSGIKHNIFN

      2170       2180       2190       2200       2210       2220
PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME SKAISDAQIT ASSYFTNMFA

      2230       2240       2250       2260       2270       2280
TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ VDFQKTMKVT GVTTQGVKSL LTSMYVKEFL

      2290       2300       2310       2320       2330       2340
ISSSQDGHQW TLFFQNGKVK VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM

      2350
EVLGCEAQDL Y
```

[0070] The following domains have been identified in Coagulation factor VIII:

| Residues | Length | Domain ID |
|---|---|---|
| 1-19 | 19 | Signal peptide |
| 20-2351 | 2332 | Coagulation factor VIII |
| 20-1332 | 1313 | Factor VIIIa heavy chain, 200 kDa isoform |
| 20-759 | 740 | Factor VIIIa heavy chain, 92 kDa isoform |
| 760-1332 | 573 | Factor VIII B chain |
| 1668-2351 | 684 | Factor VIIIa light chain |

[0071] Free thrombin in plasma is inhibited by the formation of an irreversible, 1:1 complex with the anticoagulant protein, Antithrombin III. As used herein, the term "Thrombin-Antithrombin-III complex" refers to complexes formed following this complex formation. High plasma level of TAT complexes has been suggested to alter hemostatic activation in argentine hemorrhagic fever, chronic dialysis patients, and toxemia of pregnancy. Whereas low plasma level of TAT complexes is found in type 1

(insulin-dependent) diabetes, neonatal respiratory distress syndrome, and primary untreated cancer. TAT complexes are a useful marker to predict morphological changes in chronic aortic dissection.

[0072] As used herein, the term "Tumor necrosis factor ligand superfamily member 13B" refers to one or more polypeptides present in a biological sample that are derived from the Tumor necrosis factor ligand superfamily member 13B precursor (human precursor: Swiss-Prot Q9Y275 (SEQ ID NO: 10))

```
        10         20         30         40         50         60
MDDSTEREQS RLTSCLKKRE EMKLKECVSI LPRKESPSVR SSKDGKLLAA TLLLALLSCC

        70         80         90        100        110        120
LTVVSFYQVA ALQGDLASLR AELQGHHAEK LPAGAGAPKA GLEEAPAVTA GLKIFEPPAP

       130        140        150        160        170        180
GEGNSSQNSR NKRAVQGPEE TVTQDCLQLI ADSETPTIQK GSYTFVPWLL SFKRGSALEE

       190        200        210        220        230        240
KENKILVKET GYFFIYGQVL YTDKTYAMGH LIQRKKVHVF GDELSLVTLF RCIQNMPETL

       250        260        270        280
PNNSCYSAGI AKLEEGDELQ LAIPRENAQI SLDGDVTFFG ALKLL
```

[0073] Most preferably, the Tumor necrosis factor ligand superfamily member 13b assay detects one or more soluble forms of Tumor necrosis factor ligand superfamily member 13b. Tumor necrosis factor ligand superfamily member 13b is a single-pass membrane protein having a large extracellular domain, most or all of which is present in soluble forms of Tumor necrosis factor ligand superfamily member 13b generated either through alternative splicing event which deletes all or a portion of the transmembrane domain, or by proteolysis of the membrane-bound form. In the case of an immunoassay, one or more antibodies that bind to epitopes within this extracellular domain may be used to detect these soluble form(s). The following domains have been identified in Tumor necrosis factor ligand superfamily member 13b:

| Residues | Length | Domain ID |
|---|---|---|
| 1-285 | 285 | Tumor necrosis factor ligand superfamily member 13b |
| 134-285 | 251 | Tumor necrosis factor ligand superfamily member 13b, soluble form |
| 1-46 | 46 | Cytoplasmic |
| 47-67 | 21 | Transmembrane |

68-285 218 Extracellular

[0074] As used herein, the term "relating a signal to the presence or amount" of an analyte reflects the following understanding. Assay signals are typically related to the presence or amount of an analyte through the use of a standard curve calculated using known concentrations of the analyte of interest. As the term is used herein, an assay is "configured to detect" an analyte if an assay can generate a detectable signal indicative of the presence or amount of a physiologically relevant concentration of the analyte. Because an antibody epitope is on the order of 8 amino acids, an immunoassay configured to detect a marker of interest will also detect polypeptides related to the marker sequence, so long as those polypeptides contain the epitope(s) necessary to bind to the antibody or antibodies used in the assay. The term "related marker" as used herein with regard to a biomarker such as one of the kidney injury markers described herein refers to one or more fragments, variants, etc., of a particular marker or its biosynthetic parent that may be detected as a surrogate for the marker itself or as independent biomarkers. The term also refers to one or more polypeptides present in a biological sample that are derived from the biomarker precursor complexed to additional species, such as binding proteins, receptors, heparin, lipids, sugars, etc.

[0075] 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.

[0076] 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.

[0077] 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.

[0078] 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.

[0079] 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.

[0080] 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.

[0081] 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.

[0082] Marker Assays

[0083] 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.

[0084] 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.

[0085] 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.

[0086] 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.).

[0087] 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.

[0088] 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.

[0089] Antibodies

[0090] 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."

[0091] 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 $10^{12}$ $M^{-1}$ .

[0092] 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.

[0093] 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.

[0094] 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.

[0095] 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.

[0096] 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.

[0097] While the present application describes antibody-based binding assays in detail, alternatives to antibodies as binding species in assays are well known in the art. These include receptors for a particular target, aptamers, etc. Aptamers are oligonucleic acid or peptide molecules that bind to a specific target molecule. Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist. High-affinity aptamers containing modified nucleotides conferring improved characteristics on the ligand, such as improved in vivo stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions, and may include amino acid side chain functionalities.

[0098] Assay Correlations

[0099] 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.

[00100] 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.

[00101] Suitable thresholds may be determined in a variety of ways. For example, one recommended diagnostic threshold for the diagnosis of acute myocardial infarction using cardiac troponin is the 97.5th percentile of the concentration seen in a normal population.

Another method may be to look at serial samples from the same patient, where a prior "baseline" result is used to monitor for temporal changes in a biomarker level.

[00102] 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.

[00103] 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.

[00104] 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.

[0100] Measures of test accuracy may be obtained as described in Fischer *et al., Intensive Care Med.* 29: 1043-51, 2003, and used to determine the effectiveness of a given biomarker. These measures include sensitivity and specificity, predictive values, likelihood ratios, diagnostic odds ratios, and ROC curve areas. The area under the curve

("AUC") of a ROC plot is equal to the probability that a classifier will rank a randomly chosen positive instance higher than a randomly chosen negative one. The area under the ROC curve may be thought of as equivalent to the Mann-Whitney U test, which tests for the median difference between scores obtained in the two groups considered if the groups are of continuous data, or to the Wilcoxon test of ranks.

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

[0102] Additional clinical indicia may be combined with the kidney injury marker assay result(s) of the present invention. These include other biomarkers related to renal status. Examples include the following, which recite the common biomarker name,

followed by the Swiss-Prot entry number for that biomarker or its parent: Actin (P68133); Adenosine deaminase binding protein (DPP4, P27487); Alpha-1-acid glycoprotein 1 (P02763); Alpha-1-microglobulin (P02760); Albumin (P02768); Angiotensinogenase (Renin, P00797); Annexin A2 (P07355); Beta-glucuronidase (P08236); B-2-microglobulin (P61679); Beta-galactosidase (P16278); BMP-7 (P18075); Brain natriuretic peptide (proBNP, BNP-32, NTproBNP; P16860); Calcium-binding protein Beta (S100-beta, P04271); Carbonic anhydrase (Q16790); Casein Kinase 2 (P68400); Ceruloplasmin (P00450); Clusterin (P10909); Complement C3 (P01024); Cysteine-rich protein (CYR61, O00622); Cytochrome C (P99999); Epidermal growth factor (EGF, P01133); Endothelin-1 (P05305); Exosomal Fetuin-A (P02765); Fatty acid-binding protein, heart (FABP3, P05413); Fatty acid-binding protein, liver (P07148); Ferritin (light chain, P02793; heavy chain P02794); Fructose-1,6-biphosphatase (P09467); GRO-alpha (CXCL1, (P09341); Growth Hormone (P01241); Hepatocyte growth factor (P14210); Insulin-like growth factor I (P01343); Immunoglobulin G; Immunoglobulin Light Chains (Kappa and Lambda); Interferon gamma (P01308); Lysozyme (P61626); Interleukin-1alpha (P01583); Interleukin-2 (P60568); Interleukin-4 (P60568); Interleukin-9 (P15248); Interleukin-12p40 (P29460); Interleukin-13 (P35225); Interleukin-16 (Q14005); L1 cell adhesion molecule (P32004); Lactate dehydrogenase (P00338); Leucine Aminopeptidase (P28838); Meprin A-alpha subunit (Q16819); Meprin A-beta subunit (Q16820); Midkine (P21741); MIP2-alpha (CXCL2, P19875); MMP-2 (P08253); MMP-9 (P14780); Netrin-1 (O95631); Neutral endopeptidase (P08473); Osteopontin (P10451); Renal papillary antigen 1 (RPA1); Renal papillary antigen 2 (RPA2); Retinol binding protein (P09455); Ribonuclease; S100 calcium-binding protein A6 (P06703); Serum Amyloid P Component (P02743); Sodium/Hydrogen exchanger isoform (NHE3, P48764); Spermidine/spermine N1-acetyltransferase (P21673); TGF-Beta1 (P01137); Transferrin (P02787); Trefoil factor 3 (TFF3, Q07654); Toll-Like protein 4 (O00206); Total protein; Tubulointerstitial nephritis antigen (Q9UJW2); Uromodulin (Tamm-Horsfall protein, P07911).

[0103] For purposes of risk stratification, Adiponectin (Q15848); Alkaline phosphatase (P05186); Aminopeptidase N (P15144); CalbindinD28k (P05937); Cystatin C (P01034); 8 subunit of F1FO ATPase (P03928); Gamma-glutamyltransferase (P19440); GSTa (alpha-glutathione-S-transferase, P08263); GSTpi (Glutathione-S-transferase P; GST class-pi; P09211); IGFBP-1 (P08833); IGFBP-2 (P18065); IGFBP-6 (P24592); Integral membrane protein 1 (Itm1, P46977); Interleukin-6 (P05231); Interleukin-8

(P10145); Interleukin-18 (Q14116); IP-10 (10 kDa interferon-gamma-induced protein, P02778); IRPR (IFRD1, O00458); Isovaleryl-CoA dehydrogenase (IVD, P26440); I-TAC/CXCL11 (O14625); Keratin 19 (P08727); Kim-1 (Hepatitis A virus cellular receptor 1, O43656); L-arginine:glycine amidinotransferase (P50440); Leptin (P41159); Lipocalin2 (NGAL, P80188); MCP-1 (P13500); MIG (Gamma-interferon-induced monokine Q07325); MIP-1a (P10147); MIP-3a (P78556); MIP-1beta (P13236); MIP-1d (Q16663); NAG (N-acetyl-beta-D-glucosaminidase, P54802); Organic ion transporter (OCT2, O15244); Osteoprotegerin (O14788); P8 protein (O60356); Plasminogen activator inhibitor 1 (PAI-1, P05121); ProANP(1-98) (P01160); Protein phosphatase 1-beta (PPI-beta, P62140); Rab GDI-beta (P50395); Renal kallikrein (Q86U61 ); RT1.B-1 (alpha) chain of the integral membrane protein (Q5Y7A8); Soluble tumor necrosis factor receptor superfamily member 1A (sTNFR-I, P19438); Soluble tumor necrosis factor receptor superfamily member 1B (sTNFR-II, P20333); Tissue inhibitor of metalloproteinases 3 (TIMP-3, P35625); uPAR (Q03405) may be combined with the kidney injury marker assay result(s) of the present invention.

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

injury marker assay result(s) are described hereinafter and in Harrison's Principles of Internal Medicine, 17th Ed., McGraw Hill, New York, pages 1741-1830, and Current Medical Diagnosis & Treatment 2008, 47th Ed, McGraw Hill, New York, pages 785-815, each of which are hereby incorporated by reference in their entirety.

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

[0106] Diagnosis of Acute Renal Failure

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

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

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

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

[0110] Creatinine clearance (CCr) can be calculated if values for creatinine's urine concentration ($U_{Cr}$), urine flow rate (V), and creatinine's plasma concentration ($P_{Cr}$) are known. Since the product of urine concentration and urine flow rate yields creatinine's excretion rate, creatinine clearance is also said to be its excretion rate ($U_{Cr} \times V$) divided by its plasma concentration. This is commonly represented mathematically as:

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

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:

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

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:

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

[0111] 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.

[0112] 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).

[0113] Selecting a Treatment Regimen

[0114] 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.

[0115] 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.

[0116] Example 1: Contrast-induced nephropathy sample collection

[0117] 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.

[0118] 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.

[0119] 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).

[0120] 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%.

[0121] Example 2: Cardiac surgery sample collection

[0122] 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.

[0123] 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.

[0124] Example 3: Acutely ill subject sample collection

[0125] The objective of this study is to collect samples from acutely ill patients. Approximately 1900 adults expected to be in the ICU for at least 48 hours will be enrolled. To be enrolled in the study, each patient must meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria

males and females 18 years of age or older;

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

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

sepsis;

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

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

contrast media exposure within 24 hours of enrollment;

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

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

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

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

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

Exclusion Criteria

known pregnancy;

institutionalized individuals;

previous renal transplantation;

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

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

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

meets any of the following:

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

(ii) hemoglobin < 7 g/dL;

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

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

[0126] After obtaining informed consent, an EDTA anti-coagulated blood sample (10 mL) and a urine sample (25-50 mL) are collected from each patient. Blood and urine samples are then collected at 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), 36 (± 2), 48 (± 2), 60 (± 2), 72 (± 2), and 84 (± 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Blood is collected via direct venipuncture or via other available venous access, such as an existing femoral sheath, central venous line, peripheral intravenous line or hep-lock. These study blood samples are processed to plasma at the clinical site, frozen and shipped to Astute Medical, Inc., San Diego, CA. The study urine samples are frozen and shipped to Astute Medical, Inc.

[0127] Example 4. Immunoassay format

[0128] 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.

[0129] In the case of those kidney injury markers which are membrane proteins as described herein, the assays used in these examples detect soluble forms thereof.

[0130] Example 5. Apparently Healthy Donor and Chronic Disease Patient Samples

[0131] 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.

[0132] 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.

[0133] Example 6. Use of Kidney Injury Markers for evaluating renal status in patients

[0134] Patients from the intensive care unit (ICU) were enrolled in the following study. Each patient was classified by kidney status as non-injury (0), risk of injury (R), injury (I), and failure (F) according to the maximum stage reached within 7 days of enrollment as determined by the RIFLE criteria. EDTA anti-coagulated blood samples (10 mL) and a urine samples (25-30 mL) were collected from each patient at enrollment, 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), and 48 (± 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Markers were each measured by standard immunoassay methods using commercially available assay reagents in the urine samples and the plasma component of the blood samples collected.

[0135] Two cohorts were defined to represent a "diseased" and a "normal" population. While these terms are used for convenience, "diseased" and "normal" simply represent two cohorts for comparison (say RIFLE 0 vs RIFLE R, I and F; RIFLE 0 vs

RIFLE R; RIFLE 0 and R vs RIFLE I and F; etc.). The time "prior max stage" represents the time at which a sample is collected, relative to the time a particular patient reaches the lowest disease stage as defined for that cohort, binned into three groups which are +/- 12 hours. For example, "24 hr prior" which uses 0 vs R, I, F as the two cohorts would mean 24 hr (+/- 12 hours) prior to reaching stage R (or I if no sample at R, or F if no sample at R or I).

[0136] A receiver operating characteristic (ROC) curve was generated for each biomarker measured and the area under each ROC curve (AUC) is determined. Patients in Cohort 2 were also separated according to the reason for adjudication to cohort 2 as being based on serum creatinine measurements (sCr), being based on urine output (UO), or being based on either serum creatinine measurements or urine output. Using the same example discussed above (0 vs R, I, F), for those patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements alone, the stage 0 cohort may include patients adjudicated to stage R, I, or F on the basis of urine output; for those patients adjudicated to stage R, I, or F on the basis of urine output alone, the stage 0 cohort may include patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements; and for those patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements or urine output, the stage 0 cohort contains only patients in stage 0 for both serum creatinine measurements and urine output. Also, in the data for patients adjudicated on the basis of serum creatinine measurements or urine output, the adjudication method which yielded the most severe RIFLE stage is used.

[0137] The ability to distinguish cohort 1 from Cohort 2 was determined using ROC analysis. SE is the standard error of the AUC, n is the number of sample or individual patients ("pts," as indicated). Standard errors are calculated as described in Hanley, J. A., and McNeil, B.J., The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology (1982) 143: 29-36; p values are calculated with a two-tailed Z-test. An AUC < 0.5 is indicative of a negative going marker for the comparison, and an AUC > 0.5 is indicative of a positive going marker for the comparison.

[0138] Various threshold (or "cutoff") concentrations were selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 are determined. OR is the odds ratio calculated for the particular cutoff concentration, and 95% CI is the confidence interval for the odds ratio.

[0139] Example 7. Use of Kidney Injury Markers for evaluating recovery from AKI and ARF

[0140] Patients from the intensive care unit (ICU) who had kidney status classified as injury (I) and/or failure (F) as determined by the RIFLE criteria within 7 days of enrollment were considered in the recovery analysis. EDTA anti-coagulated blood samples (10 mL) and urine samples (25-30 mL) were collected from each patient at enrollment, 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), and 48 (± 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized.

[0141] Those sample collections that occurred while the patient was in RIFLE I or F were measured by standard immunoassay methods using commercially available assay reagents. Two cohorts were defined to represent a "recovered" and a "non-recovered" population. "Recovered" indicates those sample collections where the RIFLE stage, as determined by serum creatinine (sCr), reaches non-injury (RIFLE 0) during a period of time defined as follows: a "24 hour" group that reached RIFLE 0 within 24 hours and remained at RIFLE 0 for 24 hours following sample collection; a "48 hour" group that reached RIFLE 0 within 48 hours and remained at RIFLE 0 for 48 hours following sample collection; and a "72 hour" group that reached RIFLE 0 within 72 hours and remained at RIFLE 0 for 72 hours following sample collection. "Non-recovered" indicates those sample collections where the RIFLE stage, as determined by serum creatinine (sCr), during the applicable period of 24, 48 or 72 hours remains at "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", regardless of reaching RIFLE 0 during the applicable 24, 48 or 72 hour period. The ability to distinguish the "recovered" and "non-recovered" cohorts is determined using receiver operating characteristic (ROC) analysis.

[0142] Table 1: Comparison of marker concentrations in pg/mL and the area under the ROC curve (AUC) in urine samples for the "recovered" and "non-recovered" cohorts.

**Soluble Receptor tyrosine-protein kinase erbB-2**

| Recovery Period Duration (hr) | 24 | | 48 | | 72 | |
|---|---|---|---|---|---|---|
| Start of Recovery Period (hr) | 24 | | 48 | | 72 | |
| | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort |
| Median | 3.27 | 3.46 | 3.22 | 3.59 | 3.34 | 3.72 |
| Average | 3.11 | 3.98 | 3.13 | 4.17 | 3.19 | 4.41 |
| Stdev | 1.36 | 1.96 | 1.17 | 2.21 | 1.06 | 2.64 |
| Min | 1.58 | 2.18 | 1.58 | 2.18 | 1.58 | 2.18 |
| Max | 4.31 | 7.32 | 4.31 | 7.32 | 4.31 | 7.32 |
| n (Samples) | 4 | 5 | 5 | 4 | 6 | 3 |
| n (Patients) | 4 | 5 | 5 | 4 | 6 | 3 |

| Recovery Period Duration (hr) | 24 | 48 | 72 |
|---|---|---|---|
| Start of Recovery Period (hr) | 24 | 48 | 72 |
| AUC | 0.55 | 0.60 | 0.61 |
| SE | 0.202 | 0.201 | 0.214 |
| p | 8.0E-01 | 6.2E-01 | 6.0E-01 |
| nCohort Recovered | 4 | 5 | 6 |
| nCohort Non-recovered | 5 | 4 | 3 |

**Interferon Beta**

| Recovery Period Duration (hr) | 24 | | 48 | | 72 | |
|---|---|---|---|---|---|---|
| Start of Recovery Period (hr) | 24 | | 48 | | 72 | |
| | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort |
| Median | 22.8 | 76.7 | 24.3 | 66.9 | 29.6 | 76.7 |
| Average | 24.6 | 87.8 | 49.9 | 72.1 | 51.1 | 77.1 |
| Stdev | 7.28 | 39.3 | 56.8 | 20.3 | 50.9 | 21.6 |
| Min | 18.1 | 55.7 | 18.1 | 55.7 | 18.1 | 55.7 |
| Max | 34.9 | 150.8 | 150.8 | 98.9 | 150.8 | 98.9 |
| n (Samples) | 4 | 5 | 5 | 4 | 6 | 3 |
| n (Patients) | 4 | 5 | 5 | 4 | 6 | 3 |

| Recovery Period Duration (hr) | 24 | 48 | 72 |
|---|---|---|---|
| Start of Recovery Period (hr) | 24 | 48 | 72 |
| AUC | 1.00 | 0.80 | 0.78 |
| SE | 0.000 | 0.162 | 0.185 |
| p | 0.0E+00 | 6.4E-02 | 1.3E-01 |
| n (Recovered Cohort) | 4 | 5 | 6 |
| n (Non-recovered Cohort) | 5 | 4 | 3 |

**C-type lectin domain family 11 member A**

| Recovery Period Duration (hr) | 24 | | 48 | | 72 | |
|---|---|---|---|---|---|---|
| Start of Recovery Period (hr) | 24 | | 48 | | 72 | |
| | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort |
| Median | 854 | 1982 | 886 | 1854 | 1434 | 1727 |
| Average | 2574 | 1963 | 2520 | 1877 | 2430 | 1842 |
| Stdev | 3766 | 274 | 3264 | 226 | 2928 | 263 |
| Min | 374 | 1656 | 374 | 1656 | 374 | 1656 |
| Max | 8213 | 2306 | 8213 | 2143 | 8213 | 2143 |
| n (Samples) | 4 | 5 | 5 | 4 | 6 | 3 |
| n (Patients) | 4 | 5 | 5 | 4 | 6 | 3 |

| Recovery Period Duration (hr) | 24 | 48 | 72 |
|---|---|---|---|
| Start of Recovery Period (hr) | 24 | 48 | 72 |
| AUC | 0.75 | 0.60 | 0.56 |
| SE | 0.170 | 0.201 | 0.216 |
| p | 1.4E-01 | 6.2E-01 | 8.0E-01 |
| n (Recovered Cohort) | 4 | 5 | 6 |
| n (Non-recovered Cohort) | 5 | 4 | 3 |

**Glyceraldehyde-3-phosphate dehydrogenase**

| Recovery Period Duration (hr) | 24 | | 48 | | 72 | |
|---|---|---|---|---|---|---|
| Start of Recovery Period (hr) | 24 | | 48 | | 72 | |
| | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort |
| Median | 4530 | 1494 | 3038 | 3111 | 2339 | 4727 |
| Average | 22777 | 2402 | 18231 | 2991 | 15442 | 3490 |
| Stdev | 38464 | 2589 | 34827 | 2574 | 31891 | 2906 |
| Min | 1640 | 47 | 47 | 170 | 47 | 170 |
| Max | 80407 | 5573 | 80407 | 5573 | 80407 | 5573 |
| n (Samples) | 4 | 5 | 5 | 4 | 6 | 3 |
| n (Patients) | 4 | 5 | 5 | 4 | 6 | 3 |

| Recovery Period Duration (hr) | 24 | 48 | 72 |
|---|---|---|---|
| Start of Recovery Period (hr) | 24 | 48 | 72 |
| AUC | 0.20 | 0.40 | 0.50 |
| SE | 0.155 | 0.201 | 0.215 |
| p | 5.3E-02 | 6.2E-01 | 1.0E+00 |
| n (Recovered Cohort) | 4 | 5 | 6 |
| n (Non-recovered Cohort) | 5 | 4 | 3 |

[0143] Table 2: Comparison of marker levels and the area under the ROC curve (AUC) in EDTA samples for the "recovered" and "non-recovered" cohorts.

**Soluble Receptor tyrosine-protein kinase erbB-2**

| Recovery Period Duration (hr) | 24 | | 48 | | 72 | |
|---|---|---|---|---|---|---|
| Start of Recovery Period (hr) | 24 | | 48 | | 72 | |
| | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort |
| Median | 11.8 | 16.7 | 10.2 | 16.9 | 11.2 | 17.1 |
| Average | 12.4 | 15.0 | 12.0 | 16.3 | 12.0 | 17.6 |
| Stdev | 4.57 | 3.68 | 4.09 | 2.84 | 3.66 | 1.20 |
| Min | 7.83 | 10.1 | 7.83 | 12.3 | 7.83 | 16.7 |
| Max | 18.4 | 19.0 | 18.4 | 19.0 | 18.4 | 19.0 |
| n (Samples) | 4 | 5 | 5 | 4 | 6 | 3 |
| n (Patients) | 4 | 5 | 5 | 4 | 6 | 3 |

| Recovery Period Duration (hr) | 24 | 48 | 72 |
|---|---|---|---|
| Start of Recovery Period (hr) | 24 | 48 | 72 |
| AUC | 0.65 | 0.80 | 0.89 |
| SE | 0.191 | 0.162 | 0.140 |
| p | 4.3E-01 | 6.4E-02 | 5.3E-03 |
| n (Recovered Cohort) | 4 | 5 | 6 |
| n (Non-recovered Cohort) | 5 | 4 | 3 |

**Interferon Beta**

| Recovery Period Duration (hr) | 24 | | 48 | | 72 | |
|---|---|---|---|---|---|---|
| Start of Recovery Period (hr) | 24 | | 48 | | 72 | |
| | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort |
| Median | 0.82 | 5.49 | 1.64 | 5.56 | 2.52 | 5.62 |
| Average | 3.63 | 5.83 | 3.59 | 6.44 | 3.90 | 6.75 |
| Stdev | 6.22 | 4.26 | 5.39 | 4.66 | 4.88 | 5.65 |
| Min | 0.00 | 1.76 | 0.00 | 1.76 | 0.00 | 1.76 |
| Max | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 |
| n (Samples) | 4 | 5 | 5 | 4 | 6 | 3 |
| n (Patients) | 4 | 5 | 5 | 4 | 6 | 3 |

| Recovery Period Duration (hr) | 24 | 48 | 72 |
|---|---|---|---|
| Start of Recovery Period (hr) | 24 | 48 | 72 |
| AUC | 0.78 | 0.78 | 0.75 |
| SE | 0.163 | 0.170 | 0.192 |
| p | 9.1E-02 | 1.0E-01 | 1.9E-01 |
| n (Recovered Cohort) | 4 | 5 | 6 |
| n (Non-recovered Cohort) | 5 | 4 | 3 |

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| Recovery Period Duration (hr) | 24 | | 48 | | 72 | |
|---|---|---|---|---|---|---|
| Start of Recovery Period (hr) | 24 | | 48 | | 72 | |
| | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort |
| Median | 86603 | 79867 | 64193 | 86782 | 86603 | 79867 |
| Average | 133116 | 94443 | 118451 | 103106 | 131477 | 71940 |
| Stdev | 122850 | 60361 | 111330 | 66013 | 104563 | 26621 |
| Min | 46178 | 42255 | 46178 | 42255 | 46178 | 42255 |
| Max | 313080 | 196604 | 313080 | 196604 | 313080 | 93696 |
| n (Samples) | 4 | 5 | 5 | 4 | 6 | 3 |
| n (Patients) | 4 | 5 | 5 | 4 | 6 | 3 |

| Recovery Period Duration (hr) | 24 | 48 | 72 |
|---|---|---|---|
| Start of Recovery Period (hr) | 24 | 48 | 72 |
| AUC | 0.40 | 0.50 | 0.33 |
| SE | 0.198 | 0.204 | 0.208 |
| p | 6.1E-01 | 1.0E+00 | 4.2E-01 |
| n (Recovered Cohort) | 4 | 5 | 6 |
| n (Non-recovered Cohort) | 5 | 4 | 3 |

**Glyceraldehyde-3-phosphate dehydrogenase**

| Recovery Period Duration (hr) | 24 | | 48 | | 72 | |
|---|---|---|---|---|---|---|
| Start of Recovery Period (hr) | 24 | | 48 | | 72 | |
| | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort | Recovered Cohort | Non-recovered Cohort |
| Median | 19217 | 16359 | 17820 | 17463 | 18193 | 16359 |
| Average | 26256 | 17194 | 22644 | 19443 | 21965 | 19736 |
| Stdev | 20985 | 6924 | 19886 | 5497 | 17865 | 6694 |
| Min | 9641 | 8200 | 8200 | 15402 | 8200 | 15402 |
| Max | 56948 | 27445 | 56948 | 27445 | 56948 | 27445 |
| n (Samples) | 4 | 5 | 5 | 4 | 6 | 3 |
| n (Patients) | 4 | 5 | 5 | 4 | 6 | 3 |

| Recovery Period Duration (hr) | 24 | 48 | 72 |
|---|---|---|---|
| Start of Recovery Period (hr) | 24 | 48 | 72 |
| AUC | 0.35 | 0.55 | 0.50 |
| SE | 0.191 | 0.204 | 0.215 |
| p | 4.3E-01 | 8.1E-01 | 1.0E+00 |
| n (Recovered Cohort) | 4 | 5 | 6 |
| n (Non-recovered Cohort) | 5 | 4 | 3 |

[0144] 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.

[0145] 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.

[0146] 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.

[0147] 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.

[0148] Other embodiments are set forth within the following claims.

We claim:

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

performing one or more assays configured to detect one or more biomarkers selected from the group consisting of Coagulation factor X, Coagulation factor V, soluble Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and soluble Tumor necrosis factor ligand superfamily member 13B on a body fluid sample obtained from the subject to provide an assay result; and

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

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

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

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

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

a measured concentration of Coagulation factor X, a measured concentration of Coagulation factor V, a measured concentration of soluble Receptor tyrosine-protein kinase erbB-2, a measured concentration of Interferon beta, a measured concentration of C-type lectin domain family 11 member A, a measured concentration of Glyceraldehyde-3-phosphate dehydrogenase, a measured concentration of Interferon omega-1, a measured concentration of Coagulation factor VIII, a measured concentration of Thrombin-Antithrombin-III complex, and a measured concentration of soluble Tumor necrosis factor ligand superfamily member 13B.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

95. A method according to one of claims 1-94, wherein said method comprises performing assays that detect one, two or three, or more of Coagulation factor X, Coagulation factor V, soluble Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and soluble Tumor necrosis factor ligand superfamily member 13B .

96. Measurement of one or more biomarkers selected from the group consisting of Coagulation factor X, Coagulation factor V, soluble Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and soluble Tumor necrosis factor ligand superfamily member 13B for the risk stratification, prognosis, classifying and/or monitoring of renal injury.

97. Measurement of one or more biomarkers selected from the group consisting of Coagulation factor X, Coagulation factor V, soluble Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and soluble Tumor necrosis factor ligand superfamily member 13B for the risk stratification, prognosis, classifying and/or monitoring of acute renal injury.

98. A kit, comprising:

reagents for performing one or more assays configured to detect one or more kidney injury markers selected from the group consisting of Coagulation factor X, Coagulation factor V, soluble Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and soluble Tumor necrosis factor ligand superfamily member 13B.

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

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

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

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

103. A kit according to one of claims 98-102, wherein said one or more assays comprise assays that detect one, two or three, or more of Coagulation factor X, Coagulation factor V, soluble Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and soluble Tumor necrosis factor ligand superfamily member 13B.

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

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

performing a plurality of analyte binding assays configured to detect a plurality of biomarkers, one or more of which is selected from the group consisting of Coagulation factor X, Coagulation factor V, soluble Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and soluble Tumor necrosis factor ligand superfamily member 13B by introducing the urine sample obtained from the subject into an assay instrument which (i) contacts a plurality of reagents which specifically bind for detection the plurality of biomarkers with the urine sample, and (ii) generates one or more assay results indicative of binding of each biomarker which is assayed to a respective specific binding reagent in the plurality of reagents, wherein the subject is selected for evaluation based on a determination that the subject is in need of diagnosis, risk stratification, staging, prognosis, classifying or monitoring of the renal status of the subject.

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

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

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

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

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

110. A method according to claim 104, wherein the subject is selected for evaluation based on an existing diagnosis of one or more of congestive heart failure, preeclampsia, eclampsia, diabetes mellitus, hypertension, coronary artery disease, proteinuria, renal insufficiency, glomerular filtration below the normal range, cirrhosis, serum creatinine above the normal range, sepsis, injury to renal function, reduced renal function, or ARF, or based on undergoing or having undergone major vascular surgery, coronary artery bypass, or other cardiac surgery, or based on exposure to NSAIDs, cyclosporines, tacrolimus, aminoglycosides, foscarnet, ethylene glycol, hemoglobin, myoglobin, ifosfamide, heavy metals, methotrexate, radiopaque contrast agents, or streptozotocin.

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

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

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

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

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

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

117. A method according to claim 104, wherein at least one assay result is a measured concentration of Coagulation factor X, a measured concentration of Coagulation factor V, a measured concentration of soluble Receptor tyrosine-protein kinase erbB-2, a measured concentration of Interferon beta, a measured concentration of C-type lectin domain family 11 member A, a measured concentration of Glyceraldehyde-3-phosphate dehydrogenase, a measured concentration of Interferon omega-1, a measured concentration of Coagulation factor VIII, a measured concentration of Thrombin-Antithrombin-III complex, and a measured concentration of soluble Tumor necrosis factor ligand superfamily member 13B.

118. A system for evaluating biomarker levels, comprising:

a plurality of reagents which specifically bind for detection the plurality of biomarkers, one or more of which is selected from the group consisting of Coagulation factor X, Coagulation factor V, soluble Receptor tyrosine-protein kinase erbB-2, Interferon beta, C-type lectin domain family 11 member A, Glyceraldehyde-3-phosphate dehydrogenase, Interferon omega-1, Coagulation factor VIII, Thrombin-Antithrombin-III complex, and soluble Tumor necrosis factor ligand superfamily member 13B;

an assay instrument configured to receive a urine sample and contact the plurality of reagents with the urine sample and to generate one or more assay results indicative of binding of each biomarker which is assayed to a respective specific binding reagent in the plurality of reagents.

119. A system according to claim 118, wherein the reagents comprise a plurality of antibodies, at least one of which binds to each of the biomarkers which are assayed.

120. A system according to claim 119, wherein assay instrument comprises an assay device and an assay device reader, wherein the plurality of antibodies are immobilized at a plurality of predetermined locations within the assay device, wherein the assay device is configured to receive the urine sample such that the urine sample contacts the plurality of predetermined locations, and wherein the assay device reader interrogates the plurality of predetermined locations to generate the assay results.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/018804

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/566 (2014.01)
USPC - 506/9
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) - C40B 30/04, 60/12; C12M 1/34; G01N 33/566, 33/53, 33/573 (2014.01)
USPC - 422/69; 435/7.1, 7.4, 7.92, 287.2; 436/501; 506/9, 39

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

CPC - A61K 38/00; B01J 2219/00585, 2219/00659, 2219/00722; G01N 1/405, 33/564, 33/573 (2014.02)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Orbit, Google Patents, Google Scholar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 2004/0053309 A1 (HOLT et al) 18 March 2004 (18.03.2004) entire document | 96-106, 109-111, 117-120 |
| Y | | 1-9, 107, 108, 112-116 |
| Y | US 2012/0283128 A1 (ANDERBERG et al) 08 November 2012 (08.11.2012) entire document | 1-9, 107, 108, 112-116 |
| A | US 2011/0281280 A1 (VALKIRS et al) 17 November 2011 (17.11.2011) entire document | 1-5, 7-9, 96-120 |
| A | PERCO et al. "Biomarker candidates for cardiovascular disease and bone metabolism disorders in chronic kidney disease: a systems biology perspective," J Cell Mol Med. 08 February 2008 (08.02.2008), Vol. 12, No. 4, Pgs. 1177-1187. entire document | 1-5, 7-9, 96-120 |
| A | US 2012/0129265 A1 (LUNDIN et al) 24 May 2012 (24.05.2012) entire document | 1-5, 7-9, 96-120 |

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

* 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 |
|---|---|
| 08 May 2014 | 20 MAY 2014 |

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

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

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/018804

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

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

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

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

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

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

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

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

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

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

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

**Remark on Protest**

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

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

☐ No protest accompanied the payment of additional search fees.