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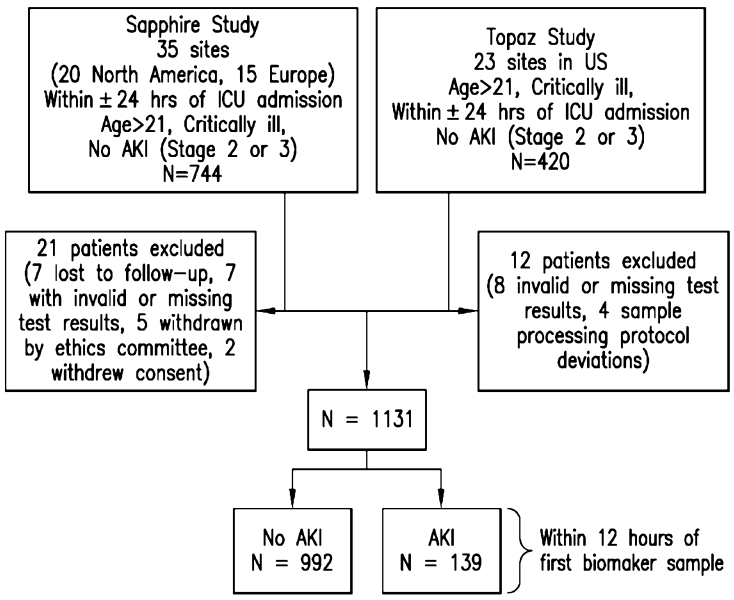

FIG. 1

(57) Abstract: The present invention provides methods and compositions for identifying patients at risk of kidney injury. A risk score, which is a composite of a urinary concentration of IGFBP7 (insulin-like growth factor-binding protein 7) and a urinary concentration of TIMP-2 (tissue inhibitor of metalloproteinase 2), is determined obtained from the patient, and is used to manage patient treatment.

WO 2017/214203 A1

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MANAGEMENT OF ACUTE KIDNEY INJURY USING INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN 7 AND TISSUE INHIBITOR OF METALLOPROTEINASE 2

[0001] The present application claims the benefit of U.S. Provisional Patent Application 62/346,381 filed June 6, 2016, which is hereby incorporated by reference in its entirety including all tables, figures and claims.

BACKGROUND OF THE INVENTION

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

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

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

postrenal in causation. Intrinsic renal disease can be further divided into glomerular, tubular, interstitial, and vascular abnormalities. Major causes of ARF are described in the following table, which is adapted from the Merck Manual, 17th ed., Chapter 222, and which is hereby incorporated by reference in their entirety:

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

Type	Risk Factors
	contrast agents, streptozotocin
Acute glomerulonephritis	ANCA-associated: Crescentic glomerulonephritis, polyarteritis nodosa, Wegener's granulomatosis; Anti-GBM glomerulonephritis: Goodpasture's syndrome; Immune-complex: Lupus glomerulonephritis, postinfectious glomerulonephritis, cryoglobulinemic glomerulonephritis
Acute tubulointerstitial nephritis	Drug reaction (eg, β-lactams, NSAIDs, sulfonamides, ciprofloxacin, thiazide diuretics, furosemide, phenytoin, allopurinol, pyelonephritis, papillary necrosis
Acute vascular nephropathy	Vasculitis, malignant hypertension, thrombotic microangiopathies, scleroderma, atheroembolism
Infiltrative diseases	Lymphoma, sarcoidosis, leukemia
Postrenal	
Tubular precipitation	Uric acid (tumor lysis), sulfonamides, triamterene, acyclovir, indinavir, methotrexate, ethylene glycol ingestion, myeloma protein, myoglobin
Ureteral obstruction	Intrinsic: Calculi, clots, sloughed renal tissue, fungus ball, edema, malignancy, congenital defects; Extrinsic: Malignancy, retroperitoneal fibrosis, ureteral trauma during surgery or high impact injury
Bladder obstruction	Mechanical: Benign prostatic hyperplasia, prostate cancer, bladder cancer, urethral strictures, phimosis, paraphimosis, urethral valves, obstructed indwelling urinary catheter; Neurogenic: Anticholinergic drugs, upper or lower motor neuron lesion

[0005] 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

[0006] 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.

[0007] 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.

[0008] 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.

[0009] 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.

[0010] In contrast, chronic kidney disease (CKD) is a different clinical entity characterized by irreversible nephron loss. A progressive decline in renal function is observed over a period of months or years with few, if any, symptoms until the chronic

injury is far advanced. CKD is characterized histologically by the concurrent development of glomerulosclerosis and tubulointerstitial fibrosis. Podocyte damage and loss has been identified as a key mechanism, at which a number of glomerular pathomechanisms converge to result in glomerulosclerosis. The mesangial cell is the major matrix forming cell in the glomerulus and is also pivotal to the glomerulosclerotic process, while the activated (alpha-smooth muscle actin-positive) interstitial fibroblast or myofibroblast is central to the development of tubulointerstitial fibrosis. In chronic renal failure, the tubules become scarred causing water loss. In contrast to the oliguria seen in AKI, CKD typically results in polyruria (increased urine volume).

[0011] The Merck Manual discusses the need to distinguish between acute renal failure and chronic renal disease, as these are different conditions with different therapies (see, inter alia, page 1846, right hand column, section "Diagnosis", first sentence "the first step is to determine whether the renal failure is acute, chronic or super-imposed on chronic, and Table 222-4 on page 1847 "Classification of Acute Versus Chronic Renal Failure). Recently, a prospective, multicenter investigation in which two novel biomarkers for AKI were identified in a discovery cohort of critically ill adult patients and subsequently validated using a clinical assay and compared to existing markers of AKI in an independent validation cohort of heterogeneous critically ill patients. Urinary insulin-like growth factor binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase 2 (TIMP-2) robust markers that have improved performance characteristics when directly compared with existing methods for detecting risk for AKI, but also provide significant additional information over clinical data. It is notable that IGFBP7 and TIMP-2 are each involved with the phenomenon of G_1 cell cycle arrest during the very early phases of cell injury, it has been shown that renal tubular cells enter a short period of G1 cell-cycle arrest following injury from experimental sepsis or ischemia. See, e.g., Yang et al., J. Infect. 58:459-464, 2009; Witzgall et al., J. Clin. Invest. 93:2175-2188, 1994.

BRIEF SUMMARY OF THE INVENTION

[0012] It is an object of the present invention to provide methods and compositions for identification of subjects at risk of having CKD, and the short term risk of AKI superimposed on CKD.

[0013] In a first aspect, the present invention relates to methods for managing a patient not suffering from acute kidney injury (AKI) prior to and following a medical surgical procedure which compromises kidney function. These methods comprise:

calculating a risk score which is a composite of a urinary concentration of IGFBP7 (insulin-like growth factor-binding protein 7) and a urinary concentration of TIMP-2 (tissue inhibitor of metalloproteinase 2) by measuring each of an IGFBP7 concentration and TIMP-2 concentration in a urine sample obtained from the patient immediately prior to performing the medical or surgical procedure and mathematically combining the IGFBP7 and TIMP-2 concentrations to provide the risk score;

comparing the risk score to a risk score threshold value, wherein when the risk score is below the risk score threshold value the patient is determined to be at a higher risk of having CKD as compared to when the risk score is above the risk score threshold value.

[0014] If an elevated risk of CKD is identified, the patient may be managed according to clinical practice for the CKD patient. See, e.g., Kidney Intl. 3(1), 1-136, January 2013, which is hereby incorporated by reference. This can include, but is not limited to, assessment of GFR and albuminuria, manage blood pressure to a target, monitoring for acute kidney injury, use of an angiotensin receptor blockers (ARB) or ACE inhibitor (ACE-I).

[0015] Alternatively, the risk score threshold value is selected in terms of relative risk. For example, when the risk score is below the risk score threshold value the patient is determined to be at an at least 1.5-fold higher risk of having CKD as compared to when the risk score is above the risk score threshold value. This is not meant to be limiting. Thus, in various embodiments, the increased relative risk may be at least a 2-fold increased risk, at least a 3-fold increased risk, etc.

[0016] Various methods may be used to determine risk score thresholds. By way of example, the risk score threshold is selected based on the results of a population study of individuals which includes a individuals having CKD and individuals not having CKD, wherein a urine sample is obtained from each of the individuals, and a risk score is calculated for each of the individuals from the urine sample, and wherein the risk score threshold value is selected to separate the population into a first subpopulation of the individuals with a risk score that is less than the risk score threshold value and a second subpopulation of the individuals with a risk score that is greater than the risk score

threshold value, wherein the first subpopulation has an at least 1.5-fold higher risk of having CKD as compared to the second subpopulation.

[0017] In certain embodiments, the methods comprise mathematically combining the concentrations comprises multiplying the IGFBP7 concentration or a value obtained therefrom and the TIMP-2 concentration or a value obtained therefrom. For example, the risk score may be expressed mathematically as ([TIMP-2]•[IGFBP7])/1000. In these embodiments, the first risk score threshold value can be about 0.06 and the second risk score threshold value can be about 0.3.

[0018] In a related aspect, the present invention relates to methods for identifying and treating a subject as having a frail kidney, wherein the subject is not identified as having an acute kidney injury or chronic kidney disease (CKD). These methods comprise:

calculating a risk score which is a composite of a urinary concentration of IGFBP7 (insulin-like growth factor-binding protein 7) and a urinary concentration of TIMP-2 (tissue inhibitor of metalloproteinase 2) by measuring each of an IGFBP7 concentration and TIMP-2 concentration in a urine sample obtained from the patient and mathematically combining the IGFBP7 and TIMP-2 concentrations to provide the risk score;

comparing the risk score to a risk score threshold value, wherein when the risk score is below the risk score threshold value the patient is determined to be at a higher risk of having a frail kidney as compared to when the risk score is above the risk score threshold value; and

if the risk score is below the threshold value, optionally managing the subject as a CKD patient.

[0019] In certain embodiments, the risk score threshold is selected based on the results of a population study of individuals which includes a individuals having CKD and individuals not having CKD, wherein a urine sample is obtained from each of the individuals, and a risk score is calculated for each of the individuals from the urine sample, and wherein the risk score threshold value is selected to separate the population into a first subpopulation of the individuals with a risk score that is less than the risk score threshold value and a second subpopulation of the individuals with a risk score that is

greater than the risk score threshold value, wherein the first subpopulation has an at least 1.5-fold higher risk of having CKD as compared to the second subpopulation.

[0020] In alternative embodiments, the risk score threshold is selected based on the results of a population study of healthy individuals, wherein a urine sample is obtained from each of the individuals, and a risk score is calculated for each of the individuals from the urine sample, and wherein the risk score threshold value is represented by low risk scores in the population. By way of example, a suitable risk score threshold value may be the upper limit of the lowest 20% of values in the population, lowest 15% of values, lowest 10% of values, lowest 5% of values, etc.

[0021] In certain embodiments, mathematically combining the concentrations comprises multiplying the IGFBP7 concentration or a value obtained therefrom and the TIMP-2 concentration or a value obtained therefrom. In preferred embodiments, the risk score is expressed mathematically as ([TIMP-2]•[IGFBP7])/1000, and the risk score threshold value between about 0.12 to about 0.04, preferably between about 0.09 to about 0.04, and is most preferably is about 0.07 or less.

[0022] The term "about" as used herein refers to +/- 10% of a given value.

[0023] In certain embodiments, the urinary concentration of IGFBP7 and the urinary concentration of TIMP-2 are measured using an instrument that receives the urine sample, performs a specific binding assay, and reports the assay result(s) in a form readable by the operator. In one example, the concentrations may be obtained by introducing the urine sample obtained from the patient into an immunoassay instrument; wherein the immunoassay instrument comprises a solid phase, an IGFBP7 antibody immobilized at a first location on the solid phase, and a TIMP-2 antibody immobilized at a second location on the solid phase; wherein the instrument causes the urine sample to contact the first location and the second location; wherein the instrument measures the amount of IGFBP7 which binds to the IGFBP7 antibody immobilized at the first location and determines therefrom the concentration of IGFBP7 in the urine sample; wherein the instrument measures the amount of TIMP-2 which binds to the TIMP-2 antibody immobilized at the second location and determines therefrom the concentration of TIMP-2 in the urine sample; wherein the instrument mathematically combines the concentration of IGFBP7 and the concentration of TIMP-2 in the urine sample into the risk score; and wherein the instrument reports the risk score in a human readable form.

[0024] Preferred are sandwich immunoassays. In these embodiments, the urine sample obtained from the patient may be further contacted with a second IGFBP7 antibody conjugated to detectable label and a second TIMP-2 antibody conjugated to detectable label; wherein first sandwich complexes are formed between the IGFBP7 antibody, IGFBP7 present in the urine sample, and the second IGFBP7 antibody; wherein second sandwich complexes are formed between the TIMP-2 antibody, TIMP-2 present in the urine sample, and the second TIMP-2 antibody; wherein the amount of IGFBP7 which binds to the IGFBP7 antibody is determined by the instrument detecting the detectable label bound at the first location; and wherein the amount of TIMP-2 which binds to the TIMP-2 antibody is determined by the instrument detecting the detectable label bound at the second location.

[0025] Managing the patient as high risk for future AKI may be performed as described in the KDIGO Clinical Practice Guideline for Acute Kidney Injury, Kidney Intl. 2 (Suppl. 1), March 2012, pp 1-138. In certain embodiments, management can comprise one or more of discontinuing or avoiding one or more nephrotoxic agents, maintaining kidney oxygen perfusion during the surgical procedure, maintaining a protein intake of at least 0.8 g/kg/day, maintaining plasma glucose within the range of 110–149mg/dL, and performing intravenous administration of isotonic solutions to maintain hemodynamic status. As discussed above, the methods described herein may be used prophylactically in advance of, or as a treatment following, various treatments or conditions that are known to be injurious to the kidney.

[0026] Managing the patient for CKD may be performed as described in Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney inter., Suppl.* 2013; 3: 1–150. In certain embodiments, management can comprise one or more of management of blood pressure, renin-angiotensin-aldosterone system ("RAAS") interruption, glycemic control and dietary/lifestyle manipulations which have been examined in the context of delaying progression of CKD; management of anemia in CKD; managing calcium abnormalities associated with CKD; managing metabolic acidosis; and managing AKI risks.

[0027] In certain embodiments, the subject is selected for risk stratification based on the 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, tacrolimus, aminoglycosides, foscarnet, ethylene glycol, hemoglobin, myoglobin, ifosfamide, heavy metals, methotrexate, radiopaque contrast agents, or streptozotocin.

[0028] Additional clinical indicia of health status, and particularly of renal sufficiency, may be combined with the IGFBP7 and/or TIMP-2 measurements in the methods described herein. Such clinical indicia may include one or more of: a baseline urine output value for the patient, a baseline change in serum creatinine for the patient, 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), other 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 IGFBP7 and/or TIMP-2 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.

[0029] As noted above, various methods may be used to evaluate the IGFBP7 and/or TIMP-2 biomarker results. By way of example, a cutoff for a biomarker or a combination of biomarkers may be selected which has been predetermined to divide a relevant population into two or more groups. A first group, often called the "nondiseased" population for convenience, represents those patients which have a high risk of CKD or CKD superimposed with AKI. A second group represents those patients with a risk of CKD or CKD superimposed with AKI is lower as measured by the biomarker result. A relative risk of CKD or CKD superimposed with AKI for the second group is determined relative to the risk in the first group. A relative risk of 1 means there is no difference in risk between the two groups; while a relative risk of >1 means the risk is higher in the second group.

[0030] 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.

[0031] In certain aspects, the measured IGFBP7 and/or TIMP-2 concentrations 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.

[0032] 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] Figure 1 shows the flow of patient recruitment from both the Sapphire and Topaz studies.

DETAILED DESCRIPTION OF THE INVENTION

[0034] For purposes of this document, the following definitions apply:

[0035] 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.

[0036] 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).

[0037] 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."

[0038] As used herein, chronic kidney disease or "CKD" is CKD is defined as abnormalities of kidney structure or function, present for >3 months, with implications for health. Approximately 11% of U.S. adults reportedly have CKD, many of whom are elderly. The condition is usually asymptomatic until its advanced stages.

[0039] 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.

[0040] 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.

[0041] 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. A body fluid sample is obtained "immediately prior to" a procedure if it is obtained within 72 hours of initiating

the procedure, and preferably within 48 hours, 24 hours, 18 hours, 12 hours, or 6 hours thereof.

[0042] 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.

[0043] 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.

[0044] IGFBP7 and TIMP-2 Assays

[0045] In general, immunoassays are specific binding assay that 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.

[0046] 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), lateral flow assays, competitive binding assays, and the like.

[0047] 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.

[0048] Such 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.).

[0049] 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.

[0050] In certain aspects, the present invention provides kits for the analysis of IGFBP7 and/or TIMP-2. The kit comprises reagents for the analysis of at least one test sample which comprise at least one antibody that bind each biomarker being assayed. 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.

[0051] Antibodies

[0052] 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."

[0053] 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} .

[0054] 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.

[0055] 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.

[0056] 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.

[0057] 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.

[0058] 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.

[0059] 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.

[0060] Assay Correlations

[0061] 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.

[0062] 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.

[0063] 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.

[0064] 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.

[0065] 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.

[0066] 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.

[0067] 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.

[0068] 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

[0069] 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 in the methods of the present invention 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.

[0070] 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.

[0071] Diagnosis of Acute Renal Failure

[0072] 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:

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

[0073] 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.

[0074] 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.

[0075] 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:

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

[0076] 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.

[0077] 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).

[0078] Selecting a Treatment Regimen

[0079] 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.

[0080] The distinction between prerenal AKI and instrinsic AKI is an important clinical assessment that directs the therapeutic intervention(s). Patients who are prerenal need therapies directed at hemodynamics to improve renal blood flow. These therapies are often involve inotropes, intravenous fluids and/or vasopressors. Each of these interventions have potential side effects (e.g. arrhythmias, volume overload, vasoconstriction) and would not be advisable to implement these therapies if they are not destined to improve renal function. Thus, the distinction between prerenal AKI and intrinsic AKI helps determine the therapy which should be prescribed. If prerenal AKI is not present, therapy is directed at mitigating AKI and providing supportive care.

[0081] Prerenal acute renal failure occurs when a sudden reduction in blood flow to the kidney camera (renal hypoperfusion) causes a loss of kidney function. Causes can include low blood volume, low blood pressure, shunting of blood from the kidney, heart failure, and local changes to the blood vessels supplying the kidney. In prerenal acute renal failure, there is nothing wrong with the kidney itself. Treatment focuses on correcting the cause of the prerenal acute renal failure.

[0082] In prerenal AKI without fluid overload, administration of intravenous fluids is typically the first step to improve renal function. This is particularly used in patients in

whom prerenal AKI develops as the result of intravascular volume depletion in order to restore normal circulating blood volume. Volume status may be monitored to avoid over- or under-replacement of fluid as described herein. Fluids with colloidal particles such as albumin may be preferred over simple saline infusion. In a prerenal condition wherein the forward flow is compromised, drugs directed at augmenting cardiac output are typically employed.

[0083] In patients with congestive heart failure in whom AKI has developed as a result of excessive diuresis, withholding of diuretics and cautious volume replacement may be sufficient to restore kidney function. Inotropes such as norepinephrine and dobutamine may be given to improve cardiac output and hence renal perfusion.

[0084] Hospitalized fluid overload patients are typically treated with fluid restriction, IV diuretics, inotropes (e.g., milrinone or dobutamine) and combination therapies. The loop diuretic furosemide is the most frequently prescribed diuretic for treatment of volume overload in HF. Initial oral doses of 20 to 40 mg once a day should be administered to patients with dyspnea on exertion and signs of volume overload who do not have indications for acute hospitalization. Severe overload and pulmonary edema are indications for hospitalization and intravenous furosemide. Some patients with mild HF can be treated effectively with thiazide diuretics. Those who have persistent volume overload on a thiazide diuretic should be switched to an oral loop diuretic. In patients with severe kidney injury, diuretics may not result in significant diuresis. Ultrafiltration, also called aquapheresis, may be used to treat fluid overload in such cases.

[0085] In contrast to prerenal AKI, the main goal of treatment of acute tubular necrosis (ATN) is to prevent further injury to the kidney. Ischemic ATN can be caused when the kidneys are not sufficiently perfused for a long period of time (e.g. due to renal artery stenosis) or by shock. Sepsis causes 30% to 70% of deaths in patients with ATN; therefore, avoidance of intravenous lines, bladder catheters, and respirators is recommended. Because septic patients are vasodilated, large volumes of administered fluid accumulate in the lung interstitium of these patients. Extracellular fluid volume should be assessed promptly, and repletion of any deficit should be initiated promptly. Hemodynamic status should be modified by appropriate fluid therapy, giving vasopressors and/or inotropes and treating any underlying sepsis. All possible nephrotoxic drugs should be stopped. In addition, doses of all medications that are eliminated by the kidney should be adjusted.

[0086] 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.

[0087] Example 1: Study design and participants

[0088] A secondary analysis of data collected from two multi-center clinical trials used to validate [TIMP2]•[IGFBP7] in AKI, the "Sapphire" and "Topaz" studies, was conducted. The Sapphire study enrolled 744 critically ill adult (>21 years) patients who were at risk for development of AKI. In the Topaz study, 420 critically ill adult patients were enrolled, with similar inclusion/exclusion criteria to the Sapphire study. Specifically, subjects in both studies were required to have evidence of significant pulmonary (respiratory SOFA score > 2) or cardiovascular (cardiac SOFA score > 1) dysfunction, and could not yet have met criteria for moderate-severe AKI (KDIGO stage 2 or 3). Patient recruitment occurred within 24 hours of intensive care unit (ICU) admission. More detailed descriptions of both studies have previously been published. Both the Sapphire and Topaz studies were approved by the Western Institutional Review Board (Olympia, Washington, USA) as well as individual site investigational review boards if required, and written informed consent was obtained from all subjects (or their legally-authorized representatives).

[0089] Examples 2: Procedures

[0090] Clinical data collection included patient demographics, reason for ICU admission, APACHE III score variables, hourly urine output and laboratory testing results. Comorbid conditions were determined based on review of available medical records and based on diagnostic codes. Estimated GFR was determined using the CKD-EPI equation; patients with missing or unknown race were considered not to be black. Each site extracted data from clinical sources and input the data into an online electronic case-report form in a de-identified password-protected dataset. Study data was stored at an independent server site (Medidata Solutions, New York, NY).

[0091] The primary outcome was moderate to severe AKI as defined by KDIGO criteria (KDIGO Clinical Practice Guideline for Acute Kidney Injury, 2012) for stage 2 or 3 AKI developing within 12 hours of enrollment. In the TOPAZ study, final

determination of AKI was adjudicated by an expert panel of 3 independent nephrologists who were blinded to biomarker results.

[0092] Example 3: Analysis

[0093] Urine and blood samples were collected and processed using standard methods. Sample supernatants were frozen within 2 hours of collection and stored at -70°C before batched transport to a central lab for analysis. Samples were thawed immediately before analysis. Testing for [TIMP2]•[IGFBP7] was performed using a clinical immunoassay (NEPHROCHECK Test and ASTUTE140 Meter; Astute Medical Inc., San Diego, CA) and lab technicians performing the testing were blinded to patient outcomes. For subjects recruited in the Sapphire study, [TIMP2]•[IGFBP7] testing was performed at Astute Medical Inc. laboratories; in the Topaz study, testing was performed in triplicate at 3 independent laboratories (University of California at San Diego, University of Louisville, and ARUP Laboratories in Salt Lake City). Test results for [TIMP2]•[IGFBP7] are uniformly reported in units of (ng/ml)2/1000 throughout the text.

[0094] Continuous variables were compared between AKI groups using Wilcoxon rank sum test or t-test. Categorical variables were compared between AKI groups using Chi-square test. To assess the effect of comorbidity on levels of [TIMP2]•[IGFBP7], we performed multiple linear regression analysis for each comorbidity where the response variable is rank transformed [TIMP2]•[IGFBP7] and the explanatory variables are AKI status, comorbidity status, and the interaction between them. AUC calculation and testing for difference in two AUCs were based on the Delong method, using R package "pROC".12 When necessary P values were adjusted for multiple testing by Benjamini-Hochberg method. A multivariate logistic regression model was constructed to predict the primary outcome of AKI accounting for comorbid conditions. Backward selection was used to eliminate variables with $p<0.10$ and arrive at the final model. Statistical analyses were performed using R version 3.1.0. (R Foundation, www.r-project.org/) and SAS 9.3 (SAS Institute, Cary, NC).

[0095] Example 4: Results

[0096] Figure 1 shows the flow of patient recruitment from both the Sapphire and Topaz studies. A total of 1164 patients were recruited; in the Sapphire study, 21 (2.8%) patients were excluded after enrollment, while 12 (2.9%) patients were excluded in the Topaz study. Patient characteristics were similar between the two studies. The final

cohort consisted of 1131 patients, of whom 139 (12.3%) developed moderate-severe AKI (KDIGO stage 2 or 3).5 For simplicity, throughout the example AKI will refer to KDIGO stage 2 or 3 AKI, while no AKI will indicate either no AKI or stage 1 AKI.

[0097] Patient characteristics stratified by AKI status are shown in Table 1. A greater percentage of patients who developed AKI had pre-existing diabetes and hypertension compared to patients without AKI. The percentage of patients with underlying CKD was similar between AKI groups, but median enrollment serum creatinine was higher in patients who developed AKI.

[0098] Patients characteristics, stratified by acute kidney injury status. Categorical variables are shown as N (%) and numerical as mean (standard deviation) or median (interquartile range).

	No AKI or Stage 1	**AKI Stage 2 or 3**	**P-value**
All patients	992	139	
Male	589 (59%)	74 (53%)	0.169
Age, Years	62 (16)	65 (15)	0.081
Body Mass Index, kg/m²	27 (24-32)	31 (26-38)	<0.001
Race			0.948
Black	127 (13%)	16 (12%)	
White	794 (80%)	113 (81%)	
Other/Unknown	70 (7%)	10 (7%)	
Medical History			
Chronic Kidney Disease	84 (8%)	13 (9%)	0.746
Diabetes Mellitus	274 (28%)	52 (37%)	0.021
Congestive Heart Failure	174 (18%)	32 (23%)	0.127
Coronary Artery Disease	296 (30%)	40 (29%)	0.843
Hypertension	599 (60%)	101 (73%)	0.005
Chronic Obstructive Pulmonary Disease	213 (21%)	23 (17%)	0.220
Admitted to ICU from			0.244
ED	380 (38%)	62 (45%)	
Floor	175 (18%)	18 (13%)	

	No AKI or Stage 1	AKI Stage 2 or 3	P-value
OR	275 (28%)	30 (22%)	
Other Hospital	140 (14%)	25 (18%)	
Other ICU	10 (1%)	1 (1%)	
Unknown	12 (1%)	3 (2%)	
Reason for ICU Admission			
Respiratory	447 (45%)	67 (48%)	0.525
Surgery	340 (34%)	35 (25%)	0.034
Cardiovascular	345 (35%)	58 (42%)	0.130
Sepsis	192 (19%)	40 (29%)	0.013
Neurological	112 (11%)	10 (7%)	0.188
Trauma	89 (9%)	10 (7%)	0.630
Other	207 (21%)	40 (29%)	0.038
Time from ICU admission to biomarker sample collection, Hours	16 (7-20)	16 (11-20)	0.427
Non-Renal Apache III	57 (43-78)	69 (50-87)	<0.001
Enrollment eGFR*, mL/min/1.73m^2	82 (55-101)	52 (31-84)	<0.001
Enrollment serum creatinine**, mg/dL	0.9 (0.7-1.2)	1.3 (0.9-1.8)	<0.001
6-hour cumulative urine output at enrollment†, mL	424 (280-705)	185 (115-303)	<0.001
Radiocontrast agents‡	354 (36%)	47 (34%)	0.706
Blood transfusions§			
PRBC	283 (29%)	32 (23%)	0.190
Platelets	98 (10%)	19 (14%)	0.180
Fresh Frozen Plasma	139 (14%)	25 (18%)	0.246
Albumin	143 (14%)	25 (18%)	0.255
Cryoprecipitate	24 (2%)	2 (1%)	0.761

AKI: acute kidney injury, defined by KDIGO criteria; ED: emergency department; OR: operating room; ICU: intensive care unit; eGFR: estimated glomerular filtration rate

*Calculated from enrollment serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

**Value from medical record closest to time of enrollment.

†15% of No AKI or Stage 1 and 12% of AKI Stage 2 or 3 did not have urine output data for 6 hours prior to enrollment.

‡Number of patients receiving IV or IA contrast administered within 5 days prior to and including the day of enrollment

§Number of patients receiving blood products within 5 days prior to and including the day of enrollment

[0099] In the overall cohort, median [TIMP2]•[IGFBP7] was significantly higher in AKI patients compared to non-AKI patients (1.5 [IQR 0.6-2.8] vs. 0.3 [IQR 0.1-0.7], $p<0.001$). These values were consistent across a variety of comorbid states, and remained statistically different between AKI and non-AKI patients.

[00100] In the cohort of 97 patients with CKD, the AUC for [TIMP2]•[IGFBP7] prediction of moderate-severe AKI was 0.91 (95% CI 0.85-0.97). The relative risk for AKI with a [TIMP2]•[IGFBP7] value above the previously validated cut-off of 0.3 was 32.4 (95% CI 3.7-284.8). In non-AKI patients, the presence of CKD was associated with lower [TIMP2]•[IGFBP7].The AUC (95% CI) of [TIMP2]•[IGFBP7] for discrimination of CKD from non-CKD in non-AKI patients was 0.60 (0.54-0.66), $p = 0.001$.

[TIMP2]•[IGFBP7] in patients without moderate-severe AKI by CKD status.

CKD Status	**Number of Patients**	**[TIMP2]•[IGFBP7]**			**p-value***
		Median	25th percentile	75th percentile	
Yes	84	0.22	0.08	0.44	0.002
No	908	0.31	0.13	0.74	

*Wilcoxon rank sum test

[0100] The percentage of patients with CKD decreased from 12.1% to 5.6% across [TIMP2]•[IGFBP7] quartiles, $p = 0.006$ for Cochran-Armitage trend test. The odds ratio (OR) for CKD was 2.3, $p = 0.013$, for the first quartile relative to the fourth quartile.

[0101] Percentage and odds ratio for CKD by [TIMP2]•[IGFBP7] quartile in patients without moderate-severe AKI.

Quartile	[TIMP-2]•[IGFBP7]	Number without CKD	Number with CKD	Percent with CKD	OR relative to Q4	95% Confidence Interval	p
1	<=0.13	218	30	12.1%	2.30	1.19-4.45	0.013
2	>0.13 to <=0.3	226	23	9.2%	1.70	0.85-3.39	0.131
3	>0.30 to <=0.73	230	17	6.9%	1.24	0.60-2.56	0.571
4	>0.73	234	14	5.6%	1	-	-

[0102] Example 5: [TIMP2]•[IGFBP7] in Healthy Donors

[0103] Urine sample were collected from 378 healthy donors and tested for [TIMP2]•[IGFBP7] as described in Chindarkar, NS, et al. "[IGFBP7]·[TIMP2] in apparently healthy subjects and chronic comorbid subjects without AKI", Clin Chim Acta. 2016 Jan 15;452:32-7. [TIMP2]•[IGFBP7] values corresponding to percentiles ranging from the 2.5th to 97.5th percentile in 2.5% increments are shown in the table below. The reportable range of the [TIMP2]•[IGFBP7] assay is from 0.04 to 10.00 $(ng/mL)^2/1000$.

Percentile	[TIMP-2]•[IGFBP7], $(ng/mL)^2/1000$	Percentile	[TIMP-2]•[IGFBP7], $(ng/mL)^2/1000$
2.5%	0.04	52.5%	0.36
5.0%	0.05	55.0%	0.40
7.5%	0.06	57.5%	0.43
10.0%	0.07	60.0%	0.45
12.5%	0.08	62.5%	0.47
15.0%	0.09	65.0%	0.51
17.5%	0.11	67.5%	0.55
20.0%	0.12	70.0%	0.59
22.5%	0.13	72.5%	0.66
25.0%	0.14	75.0%	0.74
27.5%	0.16	77.5%	0.80
30.0%	0.17	80.0%	0.89

Percentile	[TIMP-2]•[IGFBP7], (ng/mL)^2/1000	Percentile	[TIMP-2]•[IGFBP7], (ng/mL)^2/1000
32.5%	0.19	82.5%	0.95
35.0%	0.21	85.0%	1.06
37.5%	0.22	87.5%	1.18
40.0%	0.24	90.0%	1.29
42.5%	0.26	92.5%	1.47
45.0%	0.28	95.0%	1.85
47.5%	0.30	97.5%	2.25
50.0%	0.32		

[0104] Patients in the lowest percentiles of [TIMP2]•[IGFBP7] values indicate a potential for cryptic CKD existing in these patients, a state referred to herein as having a "frail kidney." While not wishing to be bound by any theory, patients with low [TIMP2]•[IGFBP7] values may be unable to respond effectively to kidney stress, thus resulting in an increased risk of damage from an insult. Treating such individuals (e.g., those in the lowest 10% of values) as if they suffer from CKD can avoid such damage to these individuals.

[0105] Example 6: Pre-operative evaluation of frail kidney

[0106] The following is an example of a pre-operative evaluation in a stable (not acutely ill) patient. For a patient who has not been diagnosed with CKD and is undergoing a procedure with a high (>5%) risk of acute kidney injury (e.g. coronary artery bypass).

[TIMP2]•[IGFBP7]	Interpretation	Action
< 0.07	Frail kidney	Treat as CKD
0.07 – 0.5	Normal risk	Treat as per normal risk
>0.5	Possible AKI	Review exposures monitor for 48h

[0107] For those with frail kidney, the following actions should be considered:

- Consider alternatives to surgery as consistent with patient goals and preferences
- Preoperative nephrology consultation

- Preform remote ischemic preconditioning
- Perform surgery using off-pump technique
- Start an ACE inhibitor and recheck test in 3-4 weeks

[0108] Example 7: Routine evaluation of frail kidney

[0109] The following is an example of a routine health screen (**e.g. annual physical).**

Test result	**Interpretation**	**Action**
< 0.07	Frail kidney	Treat as CKD
0.07 – 0.5	Normal risk	None
>0.5	Possible AKI	Review for kidney stress**

[0110] For those with frail kidney, the following actions should be considered:

- Test urine for albumin and measure serum creatinine every 3-6 months
- Screen for hypertension, diabetes –follow management guidelines for CKD
- Avoid nephrotoxic medications (e.g. NSAIDS); switch to less nephrotoxic meds
- Start an ACE inhibitor and recheck test in 3-4 months

**The following actions could be considered:

- Review medications for potential source of stress –discontinue offending agent
- Review lifestyle (e.g. extreme exercise) and modify accordingly

[0111] 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.

[0112] 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.

[0113] 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.

[0114] 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.

[0115] Other embodiments are set forth within the following claims.

We claim:

1. A method for identifying and treating a subject as having a frail kidney, wherein the subject is not identified as having an acute kidney injury or chronic kidney disease (CKD), comprising:

calculating a risk score which is a composite of a urinary concentration of IGFBP7 (insulin-like growth factor-binding protein 7) and a urinary concentration of TIMP-2 (tissue inhibitor of metalloproteinase 2) by measuring each of an IGFBP7 concentration and TIMP-2 concentration in a urine sample obtained from the patient and mathematically combining the IGFBP7 and TIMP-2 concentrations to provide the risk score;

comparing the risk score to a risk score threshold value, wherein when the risk score is below the risk score threshold value the patient is determined to be at a higher risk of having a frail kidney as compared to when the risk score is above the risk score threshold value; and

if the risk score is below the threshold value, optionally managing the subject as a CKD patient.

2. A method according to claim 1, wherein the risk score threshold is selected based on the results of a population study of individuals which includes individuals having CKD and individuals not having CKD, wherein a urine sample is obtained from each of the individuals, and a risk score is calculated for each of the individuals from the urine sample, and wherein the risk score threshold value is selected to separate the population into a first subpopulation of the individuals with a risk score that is less than the risk score threshold value and a second subpopulation of the individuals with a risk score that is greater than the risk score threshold value, wherein the first subpopulation has an at least 1.5-fold higher risk of having CKD as compared to the second subpopulation.

3. A method according to claim 1, wherein the risk score threshold is selected based on the results of a population study of healthy individuals, wherein a urine sample is obtained from each of the individuals, and a risk score is calculated for each of the individuals from the urine sample, and wherein the risk score threshold value is the risk score value for the lowest tenth percentile of risk scores in the population.

4. A method according to one of claims 1-3, wherein mathematically combining the concentrations comprises multiplying the IGFBP7 concentration or a value obtained therefrom and the TIMP-2 concentration or a value obtained therefrom.

5. A method according to claim 4, wherein the risk score is expressed mathematically as ([TIMP-2]•[IGFBP7])/1000, and the risk score threshold value is about 0.07.

6. A method according to one of claims 1-5, wherein the urinary concentration of IGFBP7 and the urinary concentration of TIMP-2 are measured by introducing the urine sample obtained from the patient into an immunoassay instrument; wherein the immunoassay instrument comprises a solid phase, an IGFBP7 antibody immobilized at a first location on the solid phase, and a TIMP-2 antibody immobilized at a second location on the solid phase; wherein the instrument causes the urine sample to contact the first location and the second location; wherein the instrument measures the amount of IGFBP7 which binds to the IGFBP7 antibody immobilized at the first location and determines therefrom the concentration of IGFBP7 in the urine sample; wherein the instrument measures the amount of TIMP-2 which binds to the TIMP-2 antibody immobilized at the second location and determines therefrom the concentration of TIMP-2 in the urine sample; wherein the instrument mathematically combines the concentration of IGFBP7 and the concentration of TIMP-2 in the urine sample into the risk score; and wherein the instrument reports the risk score in a human readable form.

7. A method according to claim 6, wherein the urine sample obtained from the patient is further contacted with a second IGFBP7 antibody conjugated to detectable label and a second TIMP-2 antibody conjugated to detectable label; wherein first sandwich complexes are formed between the IGFBP7 antibody, IGFBP7 present in the urine sample, and the second IGFBP7 antibody; wherein second sandwich complexes are formed between the TIMP-2 antibody, TIMP-2 present in the urine sample, and the second TIMP-2 antibody; wherein the amount of IGFBP7 which binds to the IGFBP7 antibody is determined by the instrument detecting the detectable label bound at the first location; and wherein the amount of TIMP-2 which binds to the TIMP-2 antibody is determined by the instrument detecting the detectable label bound at the second location.

8. A method according to one of claims 1-7, wherein managing the patient as a CKD patient comprises one or more of assessment GFR and albuminuria, management of blood pressure to a target, monitoring for acute kidney injury, use of an angiotensin receptor blockers (ARB), and use of an ACE inhibitor (ACE-I).

9. A method for identifying chronic kidney disease (CKD) risk in a subject, comprising:

calculating a risk score which is a composite of a urinary concentration of IGFBP7 (insulin-like growth factor-binding protein 7) and a urinary concentration of TIMP-2 (tissue inhibitor of metalloproteinase 2) by measuring each of an IGFBP7 concentration and TIMP-2 concentration in a urine sample obtained from the patient and mathematically combining the IGFBP7 and TIMP-2 concentrations to provide the risk score;

comparing the risk score to a risk score threshold value, wherein when the risk score is below the risk score threshold value the patient is determined to be at a higher risk of having CKD as compared to when the risk score is above the risk score threshold value.

10. A method according to claim 9, wherein when the risk score is below the risk score threshold value the patient is determined to be at an at least 1.5-fold higher risk of having CKD as compared to when the risk score is above the risk score threshold value.

11. A method according to claim 10, wherein the risk score threshold is selected based on the results of a population study of individuals which includes individuals having CKD and individuals not having CKD, wherein a urine sample is obtained from each of the individuals, and a risk score is calculated for each of the individuals from the urine sample, and wherein the risk score threshold value is selected to separate the population into a first subpopulation of the individuals with a risk score that is less than the risk score threshold value and a second subpopulation of the individuals with a risk score that is greater than the risk score threshold value, wherein the first subpopulation has an at least 1.5-fold higher risk of having CKD as compared to the second subpopulation.

12. A method according to one of claims 9-11, wherein mathematically combining the concentrations comprises multiplying the IGFBP7 concentration or a value obtained therefrom and the TIMP-2 concentration or a value obtained therefrom.

13. A method according to claim 12, wherein the risk score is expressed mathematically as ([TIMP-2]•[IGFBP7])/1000, and the risk score threshold value is about 0.07.

14. A method according to one of claims 9-13, wherein the urinary concentration of IGFBP7 and the urinary concentration of TIMP-2 are measured by introducing the urine sample obtained from the patient into an immunoassay instrument; wherein the immunoassay instrument comprises a solid phase, an IGFBP7 antibody immobilized at a first location on the solid phase, and a TIMP-2 antibody immobilized at a second location on the solid phase; wherein the instrument causes the urine sample to contact the first location and the second location; wherein the instrument measures the amount of IGFBP7 which binds to the IGFBP7 antibody immobilized at the first location and determines therefrom the concentration of IGFBP7 in the urine sample; wherein the instrument measures the amount of TIMP-2 which binds to the TIMP-2 antibody immobilized at the second location and determines therefrom the concentration of TIMP-2 in the urine sample; wherein the instrument mathematically combines the concentration of IGFBP7 and the concentration of TIMP-2 in the urine sample into the risk score; and wherein the instrument reports the risk score in a human readable form.

15. A method according to claim 14, wherein the urine sample obtained from the patient is further contacted with a second IGFBP7 antibody conjugated to detectable label and a second TIMP-2 antibody conjugated to detectable label; wherein first sandwich complexes are formed between the IGFBP7 antibody, IGFBP7 present in the urine sample, and the second IGFBP7 antibody; wherein second sandwich complexes are formed between the TIMP-2 antibody, TIMP-2 present in the urine sample, and the second TIMP-2 antibody; wherein the amount of IGFBP7 which binds to the IGFBP7 antibody is determined by the instrument detecting the detectable label bound at the first location; and wherein the amount of TIMP-2 which binds to the TIMP-2 antibody is determined by the instrument detecting the detectable label bound at the second location.

16. A method according to one of claims 9-15, wherein managing the patient at higher risk of CKD comprises one or more of assessment of GFR and albuminuria, management of blood pressure to a target, monitoring for acute kidney injury, use of an angiotensin receptor blockers (ARB), and use of an ACE inhibitor (ACE-I).

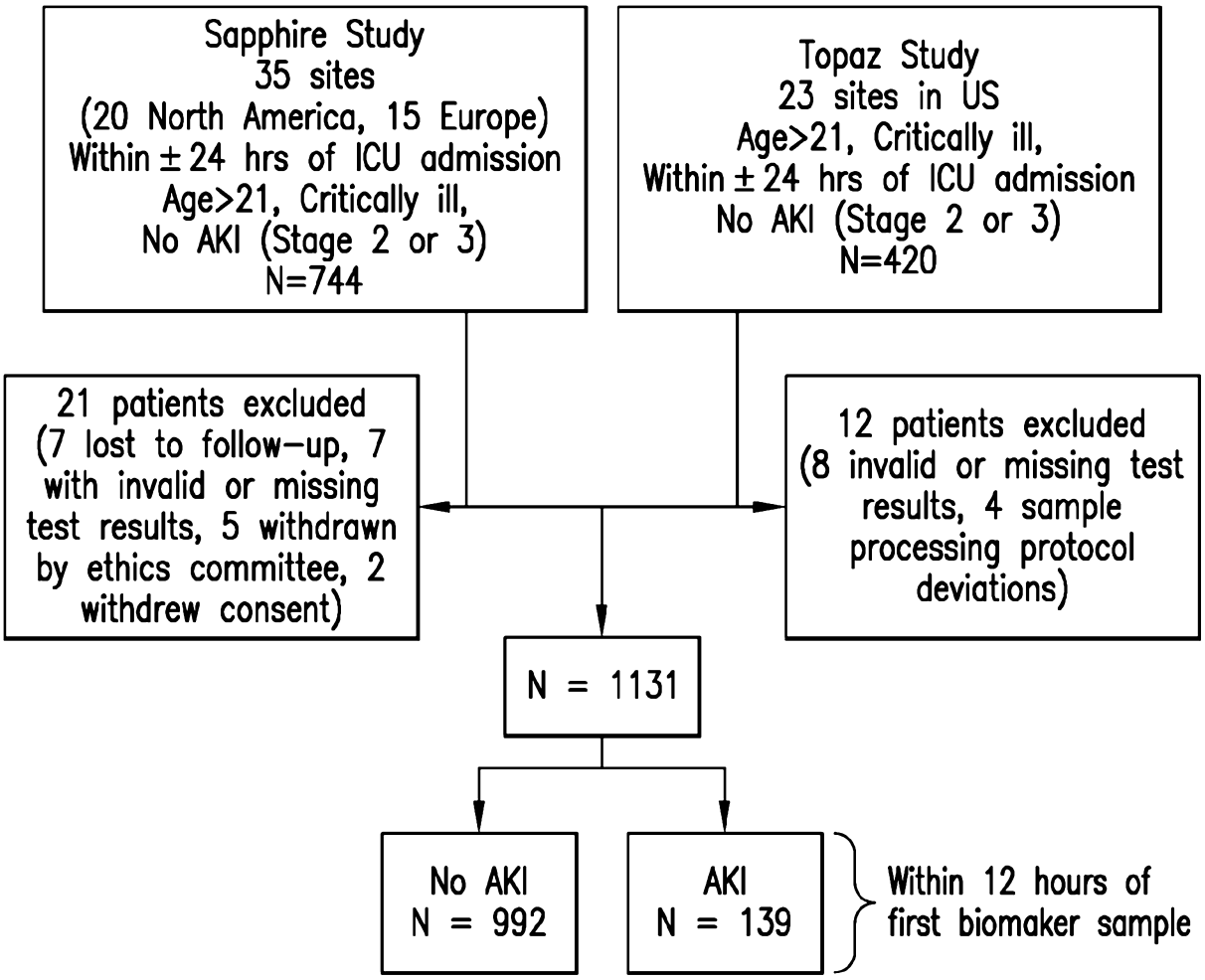
Sapphire Study
35 sites
(20 North America, 15 Europe)
Within ± 24 hrs of ICU admission
Age>21, Critically ill,
No AKI (Stage 2 or 3)
N=744
Topaz Study
23 sites in US
Age>21, Critically ill,
Within ± 24 hrs of ICU admission
No AKI (Stage 2 or 3)
N=420
21 patients excluded
(7 lost to follow-up, 7 with invalid or missing test results, 5 withdrawn by ethics committee, 2 withdrew consent)
12 patients excluded
(8 invalid or missing test results, 4 sample processing protocol deviations)
N = 1131
No AKI
N = 992
AKI
N = 139
Within 12 hours of first biomaker sample

FIG.1