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

(57) Abstract: The present invention relates to methods and compositions for monitoring, diagnosis, prognosis, and determination of treatment regimens in subjects suffering from or suspected of having a renal injury. In particular, the invention relates to using a one or more assays configured to detect a kidney injury marker selected from the group consisting of Heat shock protein beta- 1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein as diagnostic and prognostic biomarkers in renal injuries.

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

[0001] The present application claims priority to provisional U.S. patent application 61/506,038 filed July 9, 2011, which is hereby incorporated in its entirety including all tables, figures, and claims.

BACKGROUND OF THE INVENTION

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

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

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

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

Type	Risk Factors
Prerenal	
ECF volume depletion	Excessive diuresis, hemorrhage, GI losses, loss of intravascular fluid into the extravascular space (due to ascites, peritonitis, pancreatitis, or burns), loss of skin and mucus membranes, renal salt- and water-wasting states
Low cardiac output	Cardiomyopathy, MI, cardiac tamponade, pulmonary embolism, pulmonary hypertension, positive-pressure mechanical ventilation
Low systemic vascular resistance	Septic shock, liver failure, antihypertensive drugs
Increased renal vascular resistance	NSAIDs, cyclosporines, tacrolimus, hypercalcemia, anaphylaxis, anesthetics, renal artery obstruction, renal vein thrombosis, sepsis, hepatorenal syndrome
Decreased efferent arteriolar tone (leading to decreased GFR from reduced glomerular transcapillary pressure, especially in patients with bilateral renal artery stenosis)	ACE inhibitors or angiotensin II receptor blockers
Intrinsic Renal	
Acute tubular injury	Ischemia (prolonged or severe prerenal state): surgery, hemorrhage, arterial or venous obstruction; Toxins: NSAIDs, cyclosporines, tacrolimus, aminoglycosides, foscarnet, ethylene glycol, hemoglobin, myoglobin, ifosfamide, heavy metals, methotrexate, radiopaque contrast agents, streptozotocin
Acute glomerulonephritis	ANCA-associated: Crescentic glomerulonephritis, polyarteritis nodosa, Wegener's granulomatosis; Anti-GBM glomerulonephritis: Goodpasture's syndrome; Immune-complex: Lupus glomerulonephritis, postinfectious glomerulonephritis, cryoglobulinemic glomerulonephritis
Acute tubulointerstitial nephritis	Drug reaction (eg, β-lactams, NSAIDs, sulfonamides, ciprofloxacin, thiazide diuretics, furosemide, phenytoin, allopurinol, pyelonephritis, papillary necrosis
Acute vascular nephropathy	Vasculitis, malignant hypertension, thrombotic microangiopathies, scleroderma, atheroembolism
Infiltrative diseases	Lymphoma, sarcoidosis, leukemia
Postrenal	
Tubular precipitation	Uric acid (tumor lysis), sulfonamides, triamterene, acyclovir, indinavir, methotrexate, ethylene glycol

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

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

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

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

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

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

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

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

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

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

And included two clinical outcomes:

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

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

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

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

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

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

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

[0010] The CIN Consensus Working Panel (McCollough et al, Rev Cardiovasc Med. *2006;7(4):177-197,* hereby incorporated by reference in its entirety*)* uses a serum

creatinine rise of 25% to define Contrast induced nephropathy (which is a type of AKI).Although various groups propose slightly different criteria for using serum creatinine to detect AKI, the consensus is that small changes in serum creatinine, such as 0.3 mg/dL or 25%, are sufficient to detect AKI (worsening renal function) and that the magnitude of the serum creatinine change is an indicator of the severity of the AKI and mortality risk.

[0011] Although serial measurement of serum creatinine over a period of days is an accepted method of detecting and diagnosing AKI and is considered one of the most important tools to evaluate AKI patients, serum creatinine is generally regarded to have several limitations in the diagnosis, assessment and monitoring of AKI patients. The time period for serum creatinine to rise to values (e.g., a 0.3 mg/dL or 25% rise) considered diagnostic for AKI can be 48 hours or longer depending on the definition used. Since cellular injury in AKI can occur over a period of hours, serum creatinine elevations detected at 48 hours or longer can be a late indicator of injury, and relying on serum creatinine can thus delay diagnosis of AKI. Furthermore, serum creatinine is not a good indicator of the exact kidney status and treatment needs during the most acute phases of AKI when kidney function is changing rapidly. Some patients with AKI will recover fully, some will need dialysis (either short term or long term) and some will have other detrimental outcomes including death, major adverse cardiac events and chronic kidney disease. Because serum creatinine is a marker of filtration rate, it does not differentiate between the causes of AKI (pre-renal, intrinsic renal, post-renal obstruction, atheroembolic, etc) or the category or location of injury in intrinsic renal disease (for example, tubular, glomerular or interstitial in origin). Urine output is similarly limited, Knowing these things can be of vital importance in managing and treating patients with AKI.

[0012] These limitations underscore the need for better methods to detect and assess AKI, particularly in the early and subclinical stages, but also in later stages when recovery and repair of the kidney can occur. Furthermore, there is a need to better identify patients who are at risk of having an AKI.

BRIEF SUMMARY OF THE INVENTION

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

biomarkers selected from the group consisting of Heat shock protein beta-1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein (each referred to herein as a "kidney injury marker") can be used for diagnosis, prognosis, risk stratification, staging, monitoring, categorizing and determination of further diagnosis and treatment regimens in subjects suffering or at risk of suffering from an injury to renal function, reduced renal function, and/or acute renal failure (also called acute kidney injury).

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

[0015] In a first aspect, the present invention relates to methods for evaluating renal status in a subject. These methods comprise performing an assay method that is configured to detect one or more biomarkers selected from the group consisting of Heat shock protein beta-1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein is/are then correlated to the renal status of the subject. This correlation to renal status may include correlating the assay result(s) to one or more of risk stratification, diagnosis, prognosis, staging, classifying and monitoring of the subject as described herein. Thus, the present invention utilizes one or more kidney injury markers of the present invention for the evaluation of renal injury.

[0016] In certain embodiments, the methods for evaluating renal status described herein are methods for risk stratification of the subject; that is, assigning a likelihood of one or more future changes in renal status to the subject. In these embodiments, the assay result(s) is/are correlated to one or more such future changes. The following are preferred risk stratification embodiments.

[0017] In preferred risk stratification embodiments, these methods comprise determining a subject's risk for a future injury to renal function, and the assay result(s) is/are correlated to a likelihood of such a future injury to renal function. For example, the measured concentration(s) may each be compared to a threshold value. For a "positive going" kidney injury marker, an increased likelihood of suffering a future injury to renal function is assigned to the subject when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold. For a "negative going" kidney injury marker, an increased likelihood of suffering a future injury to renal function is assigned to the subject when the measured concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold.

[0018] In other preferred risk stratification embodiments, these methods comprise determining a subject's risk for future reduced renal function, and the assay result(s) is/are correlated to a likelihood of such reduced renal function. For example, the measured concentrations may each be compared to a threshold value. For a "positive going" kidney injury marker, an increased likelihood of suffering a future reduced renal function is assigned to the subject when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold. For a "negative going" kidney injury marker, an increased likelihood of future reduced renal function is assigned to the subject when the measured concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold.

[0019] In still other preferred risk stratification embodiments, these methods comprise determining a subject's likelihood for a future improvement in renal function, and the assay result(s) is/are correlated to a likelihood of such a future improvement in renal function. For example, the measured concentration(s) may each be compared to a threshold value. For a "positive going" kidney injury marker, an increased likelihood of a future improvement in renal function is assigned to the subject when the measured

concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold. For a "negative going" kidney injury marker, an increased likelihood of a future improvement in renal function is assigned to the subject when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold.

[0020] In yet other preferred risk stratification embodiments, these methods comprise determining a subject's risk for progression to ARF, and the result(s) is/are correlated to a likelihood of such progression to ARF. For example, the measured concentration(s) may each be compared to a threshold value. For a "positive going" kidney injury marker, an increased likelihood of progression to ARF is assigned to the subject when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold. For a "negative going" kidney injury marker, an increased likelihood of progression to ARF is assigned to the subject when the measured concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold.

[0021] And in other preferred risk stratification embodiments, these methods comprise determining a subject's outcome risk, and the assay result(s) is/are correlated to a likelihood of the occurrence of a clinical outcome related to a renal injury suffered by the subject. For example, the measured concentration(s) may each be compared to a threshold value. For a "positive going" kidney injury marker, an increased likelihood of one or more of: acute kidney injury, progression to a worsening stage of AKI, mortality, a requirement for renal replacement therapy, a requirement for withdrawal of renal toxins, end stage renal disease, heart failure, stroke, myocardial infarction, progression to chronic kidney disease, etc., is assigned to the subject when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold. For a "negative going" kidney injury marker, an increased likelihood of one or more of: acute kidney injury, progression to a worsening stage of AKI, mortality, a requirement for renal replacement therapy, a requirement for withdrawal of renal toxins, end stage renal disease, heart failure, stroke, myocardial infarction, progression to chronic kidney disease, etc., is assigned to the subject when the measured concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold.

[0022] In such risk stratification embodiments, preferably the likelihood or risk assigned is that an event of interest is more or less likely to occur within 180 days of the time at which the body fluid sample is obtained from the subject. In particularly preferred embodiments, the likelihood or risk assigned relates to an event of interest occurring within a shorter time period such as 18 months, 120 days, 90 days, 60 days, 45 days, 30 days, 21 days, 14 days, 7 days, 5 days, 96 hours, 72 hours, 48 hours, 36 hours, 24 hours, 12 hours, or less. A risk at 0 hours of the time at which the body fluid sample is obtained from the subject is equivalent to diagnosis of a current condition.

[0023] In preferred risk stratification embodiments, the subject is selected for risk stratification based on the pre-existence in the subject of one or more known risk factors for prerenal, intrinsic renal, or postrenal ARF. For example, a subject undergoing or having undergone major vascular surgery, coronary artery bypass, or other cardiac surgery; a subject having pre-existing congestive heart failure, preeclampsia, eclampsia, diabetes mellitus, hypertension, coronary artery disease, proteinuria, renal insufficiency, glomerular filtration below the normal range, cirrhosis, serum creatinine above the normal range, or sepsis; or a subject exposed to NSAIDs, cyclosporines, tacrolimus, aminoglycosides, foscarnet, ethylene glycol, hemoglobin, myoglobin, ifosfamide, heavy metals, methotrexate, radiopaque contrast agents, or streptozotocin are all preferred subjects for monitoring risks according to the methods described herein. This list is not meant to be limiting. By "pre-existence" in this context is meant that the risk factor exists at the time the body fluid sample is obtained from the subject. In particularly preferred embodiments, a subject is chosen for risk stratification based on an existing diagnosis of injury to renal function, reduced renal function, or ARF.

[0024] In other embodiments, the methods for evaluating renal status described herein are methods for diagnosing a renal injury in the subject; that is, assessing whether or not a subject has suffered from an injury to renal function, reduced renal function, or ARF. In these embodiments, the assay result(s), for example measured concentration(s) of one or more biomarkers selected from the group consisting of Heat shock protein beta-1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein is/are correlated to the occurrence or nonoccurrence of a change in renal status. The following are preferred diagnostic embodiments.

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

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

function may be assigned to the subject (relative to the likelihood assigned when the measured concentration is below the threshold).

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

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

threshold, an increased likelihood of the nonoccurrence of an injury creating a need for renal replacement therapy may be assigned to the subject (relative to the likelihood assigned when the measured concentration is below the threshold).

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

[0030] In still other embodiments, the methods for evaluating renal status described herein are methods for monitoring a renal injury in the subject; that is, assessing whether or not renal function is improving or worsening in a subject who has suffered from an injury to renal function, reduced renal function, or ARF. In these embodiments, the assay result(s), for example measured concentration(s) of one or more biomarkers selected from the group consisting of Heat shock protein beta-1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein is/are correlated to the occurrence or nonoccurrence of a change in renal status. The following are preferred monitoring embodiments.

[0031] In preferred monitoring embodiments, these methods comprise monitoring renal status in a subject suffering from an injury to renal function, and the assay result(s) is/are correlated to the occurrence or nonoccurrence of a change in renal status in the

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

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

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

[0034] In other additional preferred monitoring embodiments, these methods comprise monitoring renal status in a subject at risk of an injury to renal function due to the pre-existence of one or more known risk factors for prerenal, intrinsic renal, or

postrenal ARF, and the assay result(s) is/are correlated to the occurrence or nonoccurrence of a change in renal status in the subject. For example, the measured concentration(s) may be compared to a threshold value. For a positive going marker, when the measured concentration is above the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is below the threshold, an improvement of renal function may be assigned to the subject. For a negative going marker, when the measured concentration is below the threshold, a worsening of renal function may be assigned to the subject; alternatively, when the measured concentration is above the threshold, an improvement of renal function may be assigned to the subject.

[0035] In still other embodiments, the methods for evaluating renal status described herein are methods for classifying a renal injury in the subject; that is, determining whether a renal injury in a subject is prerenal, intrinsic renal, or postrenal; and/or further subdividing these classes into subclasses such as acute tubular injury, acute glomerulonephritis acute tubulointerstitial nephritis, acute vascular nephropathy, or infiltrative disease; and/or assigning a likelihood that a subject will progress to a particular RIFLE stage. In these embodiments, the assay result(s), for example measured concentration(s) of one or more biomarkers selected from the group consisting of Heat shock protein beta-1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein is/are correlated to a particular class and/or subclass. The following are preferred classification embodiments.

[0036] In preferred classification embodiments, these methods comprise determining whether a renal injury in a subject is prerenal, intrinsic renal, or postrenal; and/or further subdividing these classes into subclasses such as acute tubular injury, acute glomerulonephritis acute tubulointerstitial nephritis, acute vascular nephropathy, or infiltrative disease; and/or assigning a likelihood that a subject will progress to a particular RIFLE stage, and the assay result(s) is/are correlated to the injury classification for the subject. For example, the measured concentration may be compared to a threshold value, and when the measured concentration is above the threshold, a particular classification is assigned; alternatively, when the measured concentration is below the threshold, a different classification may be assigned to the subject.

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

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

[0039] The ability of a particular test to distinguish two populations can be established using ROC analysis. For example, ROC curves established from a "first" subpopulation which is predisposed to one or more future changes in renal status, and a "second" subpopulation which is not so predisposed can be used to calculate a ROC curve, and the area under the curve provides a measure of the quality of the test. Preferably, the tests described herein provide a ROC curve area greater than 0.5, preferably at least 0.6, more preferably 0.7, still more preferably at least 0.8, even more preferably at least 0.9, and most preferably at least 0.95.

[0040] In certain aspects, the measured concentration of one or more kidney injury markers, or a composite of such markers, may be treated as continuous variables. For example, any particular concentration can be converted into a corresponding probability of a future reduction in renal function for the subject, the occurrence of an injury, a

classification, etc. In yet another alternative, a threshold that can provide an acceptable level of specificity and sensitivity in separating a population of subjects into "bins" such as a "first" subpopulation (e.g., which is predisposed to one or more future changes in renal status, the occurrence of an injury, a classification, etc.) and a "second" subpopulation which is not so predisposed. A threshold value is selected to separate this first and second population by one or more of the following measures of test accuracy:

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

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

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

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

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

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

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

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

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

[0043] The foregoing method steps should not be interpreted to mean that the kidney injury marker assay result(s) is/are used in isolation in the methods described herein. Rather, additional variables or other clinical indicia may be included in the methods described herein. For example, a risk stratification, diagnostic, classification, monitoring, etc. method may combine the assay result(s) with one or more variables measured for the subject selected from the group consisting of demographic information (e.g., weight, sex, age, race), medical history (e.g., family history, type of surgery, pre-existing disease such as aneurism, congestive heart failure, preeclampsia, eclampsia, diabetes mellitus, hypertension, coronary artery disease, proteinuria, renal insufficiency, or sepsis, type of toxin exposure such as NSAIDs, cyclosporines, tacrolimus, aminoglycosides, foscarnet, ethylene glycol, hemoglobin, myoglobin, ifosfamide, heavy metals, methotrexate,

radiopaque contrast agents, or streptozotocin), clinical variables (e.g., blood pressure, temperature, respiration rate), risk scores (APACHE score, PREDICT score, TIMI Risk Score for UA/NSTEMI, Framingham Risk Score, risk scores of Thakar et al. (J. Am. Soc. Nephrol. 16: 162-68, 2005), Mehran et al. (J. Am. Coll. Cardiol. 44: 1393-99, 2004), Wijeysundera et al. (JAMA 297: 1801-9, 2007), Goldstein and Chawla (Clin. J. Am. Soc. Nephrol. 5: 943-49, 2010), or Chawla et al. (Kidney Intl. 68: 2274-80, 2005)), a glomerular filtration rate, an estimated glomerular filtration rate, a urine production rate, a serum or plasma creatinine concentration, a urine creatinine concentration, a fractional excretion of sodium, a urine sodium concentration, a urine creatinine to serum or plasma creatinine ratio, a urine specific gravity, a urine osmolality, a urine urea nitrogen to plasma urea nitrogen ratio, a plasma BUN to creatnine ratio, a renal failure index calculated as urine sodium / (urine creatinine / plasma creatinine), a serum or plasma neutrophil gelatinase (NGAL) concentration, a urine NGAL concentration, a serum or plasma cystatin C concentration, a serum or plasma cardiac troponin concentration, a serum or plasma BNP concentration, a serum or plasma NTproBNP concentration, and a serum or plasma proBNP concentration. Other measures of renal function which may be combined with one or more kidney injury marker assay result(s) are described hereinafter and in Harrison's Principles of Internal Medicine, 17th Ed., McGraw Hill, New York, pages 1741-1830, and Current Medical Diagnosis & Treatment 2008, 47th Ed, McGraw Hill, New York, pages 785-815, each of which are hereby incorporated by reference in their entirety.

[0044] When more than one marker is measured, the individual markers may be measured in samples obtained at the same time, or may be determined from samples obtained at different (e.g., an earlier or later) times. The individual markers may also be measured on the same or different body fluid samples. For example, one kidney injury marker may be measured in a serum or plasma sample and another kidney injury marker may be measured in a urine sample. In addition, assignment of a likelihood may combine an individual kidney injury marker assay result with temporal changes in one or more additional variables.

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

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

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

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

DETAILED DESCRIPTION OF THE INVENTION

[0049] The present invention relates to methods and compositions for diagnosis, differential diagnosis, risk stratification, monitoring, classifying and determination of treatment regimens in subjects suffering or at risk of suffering from injury to renal function, reduced renal function and/or acute renal failure through measurement of one or

more kidney injury markers. In various embodiments, a measured concentration of one or more biomarkers selected from the group consisting of Heat shock protein beta-1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein or one or more markers related thereto, are correlated to the renal status of the subject.

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

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

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

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

[0054] As used herein, the term "Heat shock protein beta-1" refers to one or more polypeptides present in a biological sample that are derived from the Heat shock protein beta-1 precursor (human precursor Swiss-Prot P04792 (SEQ ID NO: 1)).

10 20 30 40 50 60

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MTERRVPFSL LRGPSWDPFR DWYPHSRLFD QAFGLPRLPE EWSQWLGGSS WPGYVRPLPP

         70          80          90         100         110         120
AAIESPAVAA PAYSRALSRQ LSSGVSEIRH TADRWRVSLD VNHFAPDELT VKTKDGVVEI

       130         140         150         160         170         180
TGKHEERQDE HGYISRCFTR KYTLPPGVDP TQVSSSLSPE GTLTVEAPMP KLATQSNEIT

       190         200
IPVTFESRAQ LGGPEAAKSD ETAAK
```

[0055] In certain embodiments, the Heat shock protein beta-1 polypeptide measured comprises one or more phopsoserine residues, and the assay distinguishes phosphorylated fron non-phosphorylated forms. In preferred embodiments, the polypeptide measured comprises phosposerine residues at residues 78 and/or 82.

[0056] As used herein, the terms "WAP four-disulfide core domain protein 2" "WAP4C" and "HE4" refer to one or polypeptides present in a biological sample that are derived from a WAP four-disulfide core domain protein 2 precursor (human precursor Swiss-Prot entry Q14508) (SEQ ID NO: 2)):

```
         10          20          30          40          50          60
MPACRLGPLA AALLLSLLLF GFTLVSGTGA EKTGVCPELQ ADQNCTQECV SDSECADNLK

         70          80          90         100         110         120
CCSAGCATFC SLPNDKEGSC PQVNINFPQL GLCRDQCQVD SQCPGQMKCC RNGCGKVSCV
```

[0057] The following domains have been identified in WAP four-disulfide core domain protein 2:

Residues	Length	Domain ID
1-30	30	signal sequence
31-124	94	WAP four-disulfide core domain protein 2

And the following alternative forms derived from the WAP four-disulfide core domain protein 2 precursor have been described:

2-23	22	→ LQVQVNLPVSPLPTYPYSFF YP (SEQ ID NO: 3) in isoform 2.
24-74	51	Missing in isoform 2.
27-74	48	Missing in isoform 3.

71-79	9	→ LLCPNGQLAE (SEQ ID NO: 4) in isoform 4.
75-102	28	→ ALFHWHLKTRRLWEISGPRP RRPTWDSS (SEQ ID NO: 5) in isoform 5.
80-124	45	Missing in isoform 4.
103-124	22	Missing in isoform 5.

[0058] As used herein, the term "Choriogonadotropin subunit beta" refers to one or polypeptides present in a biological sample that are derived from a Choriogonadotropin subunit beta precursor (human precursor Swiss-Prot entry P01233) (SEQ ID NO: 6)):

```
        10         20         30         40         50         60
MEMFQGLLLL LLLSMGGTWA SKEPLRPRCR PINATLAVEK EGCPVCITVN TTICAGYCPT
        70         80         90        100        110        120
MTRVLQGVLP ALPQVVCNYR DVRFESIRLP GCPRGVNPVV SYAVALSCQC ALCRRSTTDC
       130        140        150        160
GGPKDHPLTC DDPRFQDSSS SKAPPPSLPS PSRLPGPSDT PILPQ
```

[0059] The following domains have been identified in Choriogonadotropin subunit beta:

Residues	Length	Domain ID
1-20	20	signal sequence
21-165	145	Choriogonadotropin subunit beta

And the following alternative form derived from the Choriogonadotropin subunit beta precursor has been described:

1-4	→ MGRPGLGAAVSDPGEAVSLS (SEQ ID NO: 7) in isoform 2.

[0060] As used herein, the term "Mitochondrial 60 kDa heat shock protein" refers to one or polypeptides present in a biological sample that are derived from a Mitochondrial 60 kDa heat shock protein precursor (human precursor Swiss-Prot entry P10809) (SEQ ID NO: 7)):

```
        10         20         30         40         50         60
MLRLPTVFRQ MRPVSRVLAP HLTRAYAKDV KFGADARALM LQGVDLLADA VAVTMGPKGR
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        70          80          90         100         110         120
TVIIEQSWGS PKVTKDGVTV AKSIDLKDKY KNIGAKLVQD VANNTNEEAG DGTTTATVLA

       130         140         150         160         170         180
RSIAKEGFEK ISKGANPVEI RRGVMLAVDA VIAELKKQSK PVTTPEEIAQ VATISANGDK

       190         200         210         220         230         240
EIGNIISDAM KKVGRKGVIT VKDGKTLNDE LEIIEGMKFD RGYISPYFIN TSKGQKCEFQ

       250         260         270         280         290         300
DAYVLLSEKK ISSIQSIVPA LEIANAHRKP LVIIAEDVDG EALSTLVLNR LKVGLQVVAV

       310         320         330         340         350         360
KAPGFGDNRK NQLKDMAIAT GGAVFGEEGL TLNLEDVQPH DLGKVGEVIV TKDDAMLLKG

       370         380         390         400         410         420
KGDKAQIEKR IQEIIEQLDV TTSEYEKEKL NERLAKLSDG VAVLKVGGTS DVEVNEKKDR

       430         440         450         460         470         480
VTDALNATRA AVEEGIVLGG GCALLRCIPA LDSLTPANED QKIGIEIIKR TLKIPAMTIA

       490         500         510         520         530         540
KNAGVEGSLI VEKIMQSSSE VGYDAMAGDF VNMVEKGIID PTKVVRTALL DAAGVASLLT

       550         560         570
TAEVVVTEIP KEEKDPGMGA MGGMGGGMGG GMF
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[0061] The following domains have been identified in Mitochondrial 60 kDa heat shock protein:

Residues	Length	Domain ID
1-26	26	Mitochondrial transit peptide
27-573	145	Mitochondrial 60 kDa heat shock protein

[0062] As used herein, the term “Placenta growth factor” refers to one or polypeptides present in a biological sample that are derived from a Placenta growth factor precursor (human precursor Swiss-Prot entry P49763) (SEQ ID NO: 8)):

```
        10          20          30          40          50          60
MPVMRLFPCF LQLLAGLALP AVPPQQWALS AGNGSSEVEV VPFQEVWGRS YCRALERLVD

        70          80          90         100         110         120
VVSEYPSEVE HMFSPSCVSL LRCTGCCGDE NLHCVPVETA NVTMQLLKIR SGDRPSYVEL

       130         140         150         160         170         180
TFSQHVRCEC RHSPGRQSPD MPGDFRADAP SFLPPRRSLP MLFRMEWGCA LTGSQSAVWP

       190         200         210         220
SSPVPEEIPR MHPGRNGKKQ QRKPLREKMK PERCGDAVPR R
```

[0063] The following domains have been identified in Placenta growth factor:

Residues	Length	Domain ID
1-18	18	signal sequence
19-221	203	Placenta growth factor

And the following alternative forms derived from the Placenta growth factor precursor has been described:

132-203	missing in isoforms PLGF-1 and PLGF-2
213	→ RRRPKGRGKRRREKQRPTDCHL (SEQ ID NO: 9) in isoform PLGF-2.

[0064] As used herein, the term "relating a signal to the presence or amount" of an analyte reflects the following understanding. Assay signals are typically related to the presence or amount of an analyte through the use of a standard curve calculated using known concentrations of the analyte of interest. As the term is used herein, an assay is "configured to detect" an analyte if an assay can generate a detectable signal indicative of the presence or amount of a physiologically relevant concentration of the analyte. Because an antibody epitope is on the order of 8 amino acids, an immunoassay configured to detect a marker of interest will also detect polypeptides related to the marker sequence, so long as those polypeptides contain the epitope(s) necessary to bind to the antibody or antibodies used in the assay. The term "related marker" as used herein with regard to a biomarker such as one of the kidney injury markers described herein refers to one or more fragments, variants, etc., of a particular marker or its biosynthetic parent that may be detected as a surrogate for the marker itself or as independent biomarkers. The term also refers to one or more polypeptides present in a biological sample that are derived from the biomarker precursor complexed to additional species, such as binding proteins, receptors, heparin, lipids, sugars, etc.

[0065] In this regard, the skilled artisan will understand that the signals obtained from an immunoassay are a direct result of complexes formed between one or more antibodies and the target biomolecule (*i.e.*, the analyte) and polypeptides containing the necessary epitope(s) to which the antibodies bind. While such assays may detect the full length biomarker and the assay result be expressed as a concentration of a biomarker of interest, the signal from the assay is actually a result of all such "immunoreactive" polypeptides

present in the sample. Expression of biomarkers may also be determined by means other than immunoassays, including protein measurements (such as dot blots, western blots, chromatographic methods, mass spectrometry, *etc*.) and nucleic acid measurements (mRNA quatitation). This list is not meant to be limiting.

[0066] The term "positive going" marker as that term is used herein refer to a marker that is determined to be elevated in subjects suffering from a disease or condition, relative to subjects not suffering from that disease or condition. The term "negative going" marker as that term is used herein refer to a marker that is determined to be reduced in subjects suffering from a disease or condition, relative to subjects not suffering from that disease or condition.

[0067] 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.

[0068] 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.

[0069] 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.

[0070] 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.

[0071] 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.

[0072] Marker Assays

[0073] In general, immunoassays involve contacting a sample containing or suspected of containing a biomarker of interest with at least one antibody that specifically binds to the biomarker. A signal is then generated indicative of the presence or amount of complexes formed by the binding of polypeptides in the sample to the antibody. The signal is then related to the presence or amount of the biomarker in the sample. Numerous methods and devices are well known to the skilled artisan for the detection and analysis of biomarkers. *See, e.g.*, U.S. Patents 6,143,576; 6,113,855; 6,019,944; 5,985,579; 5,947,124; 5,939,272; 5,922,615; 5,885,527; 5,851,776; 5,824,799; 5,679,526; 5,525,524; and 5,480,792, and *The Immunoassay Handbook*, David Wild, ed. Stockton Press, New York, 1994, each of which is hereby incorporated by reference in its entirety, including all tables, figures and claims.

[0074] The assay devices and methods known in the art can utilize labeled molecules in various sandwich, competitive, or non-competitive assay formats, to generate a signal that is related to the presence or amount of the biomarker of interest. Suitable assay formats also include chromatographic, mass spectrographic, and protein "blotting" methods. Additionally, certain methods and devices, such as biosensors and optical immunoassays, may be employed to determine the presence or amount of analytes without the need for a labeled molecule. *See, e.g.*, U.S. Patents 5,631,171; and 5,955,377, each of which is hereby incorporated by reference in its entirety, including all tables, figures and claims. One skilled in the art also recognizes that robotic instrumentation including but not limited to Beckman ACCESS®, Abbott AXSYM®, Roche ELECSYS®, Dade Behring STRATUS® systems are among the immunoassay analyzers that are capable of performing immunoassays. But any suitable immunoassay may be utilized, for example, enzyme-linked immunoassays (ELISA), radioimmunoassays (RIAs), competitive binding assays, and the like.

[0075] 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.

[0076] Biological assays require methods for detection, and one of the most common methods for quantitation of results is to conjugate a detectable label to a protein or nucleic acid that has affinity for one of the components in the biological system being studied. Detectable labels may include molecules that are themselves detectable (*e.g.*, fluorescent moieties, electrochemical labels, metal chelates, *etc.*) as well as molecules that may be

indirectly detected by production of a detectable reaction product (*e.g.*, enzymes such as horseradish peroxidase, alkaline phosphatase, *etc.*) or by a specific binding molecule which itself may be detectable (e.g., biotin, digoxigenin, maltose, oligohistidine, 2,4-dintrobenzene, phenylarsenate, ssDNA, dsDNA, etc.).

[0077] 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.

[0078] In certain aspects, the present invention provides kits for the analysis of the described kidney injury markers. The kit comprises reagents for the analysis of at least one test sample which comprise at least one antibody that a kidney injury marker. The kit can also include devices and instructions for performing one or more of the diagnostic and/or prognostic correlations described herein. Preferred kits will comprise an antibody pair for performing a sandwich assay, or a labeled species for performing a competitive assay, for the analyte. Preferably, an antibody pair comprises a first antibody conjugated to a solid phase and a second antibody conjugated to a detectable label, wherein each of the first and second antibodies that bind a kidney injury marker. Most preferably each of the antibodies are monoclonal antibodies. The instructions for use of the kit and performing the correlations can be in the form of labeling, which refers to any written or recorded material that is attached to, or otherwise accompanies a kit at any time during its manufacture, transport, sale or use. For example, the term labeling encompasses advertising leaflets and brochures, packaging materials, instructions, audio or video cassettes, computer discs, as well as writing imprinted directly on kits.

[0079] Antibodies

[0080] 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."

[0081] 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} .

[0082] 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.

[0100] 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.

[0101] 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.

[0102] 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.

[0103] 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.

[0104] 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.

[0105] Assay Correlations

[0106] 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.

[0107] 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.

[0108] 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.

[0109] 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.

[0110] 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.

[0111] 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.

[0112] 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.

[0113] 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

[0114] Additional clinical indicia may be combined with the kidney injury marker assay result(s) of the present invention. These include other biomarkers related to renal status. Examples include the following, which recite the common biomarker name, followed by the Swiss-Prot entry number for that biomarker or its parent: Actin (P68133); Adenosine deaminase binding protein (DPP4, P27487); Alpha-1-acid glycoprotein 1 (P02763); Alpha-1-microglobulin (P02760); Albumin (P02768); Angiotensinogenase (Renin, P00797); Annexin A2 (P07355); Beta-glucuronidase (P08236); B-2-microglobulin (P61679); Beta-galactosidase (P16278); BMP-7 (P18075); Brain natriuretic peptide (proBNP, BNP-32, NTproBNP; P16860); Calcium-binding protein Beta (S100-beta, P04271); Carbonic anhydrase (Q16790); Casein Kinase 2 (P68400); Ceruloplasmin (P00450); Clusterin (P10909); Complement C3 (P01024); Cysteine-rich protein (CYR61, O00622); Cytochrome C (P99999); Epidermal growth factor (EGF, P01133); Endothelin-1 (P05305); Exosomal Fetuin-A (P02765); Fatty acid-binding protein, heart (FABP3, P05413); Fatty acid-binding protein, liver (P07148); Ferritin (light chain, P02793; heavy chain P02794); Fructose-1,6-biphosphatase (P09467); GRO-alpha (CXCL1, (P09341); Growth Hormone (P01241); Hepatocyte growth factor (P14210); Insulin-like growth factor I (P01343); Immunoglobulin G; Immunoglobulin Light Chains (Kappa and Lambda); Interferon gamma (P01308); Lysozyme (P61626); Interleukin-1alpha (P01583); Interleukin-2 (P60568); Interleukin-4 (P60568); Interleukin-9 (P15248); Interleukin-12p40 (P29460); Interleukin-13 (P35225); Interleukin-16 (Q14005); L1 cell adhesion molecule (P32004); Lactate dehydrogenase (P00338); Leucine Aminopeptidase (P28838); Meprin A-alpha subunit (Q16819); Meprin A-beta subunit (Q16820); Midkine (P21741); MIP2-alpha (CXCL2, P19875); MMP-2 (P08253); MMP-9 (P14780); Netrin-1 (O95631); Neutral endopeptidase (P08473); Osteopontin (P10451); Renal papillary antigen 1 (RPA1); Renal papillary antigen 2 (RPA2); Retinol binding protein (P09455);

Ribonuclease; S100 calcium-binding protein A6 (P06703); Serum Amyloid P Component (P02743); Sodium/Hydrogen exchanger isoform (NHE3, P48764); Spermidine/spermine N1-acetyltransferase (P21673); TGF-Beta1 (P01137); Transferrin (P02787); Trefoil factor 3 (TFF3, Q07654); Toll-Like protein 4 (O00206); Total protein; Tubulointerstitial nephritis antigen (Q9UJW2); Uromodulin (Tamm-Horsfall protein, P07911).

[0115] For purposes of risk stratification, Adiponectin (Q15848); Alkaline phosphatase (P05186); Aminopeptidase N (P15144); CalbindinD28k (P05937); Cystatin C (P01034); 8 subunit of F1FO ATPase (P03928); Gamma-glutamyltransferase (P19440); GSTa (alpha-glutathione-S-transferase, P08263); GSTpi (Glutathione-S-transferase P; GST class-pi; P09211); IGFBP-1 (P08833); IGFBP-2 (P18065); IGFBP-6 (P24592); Integral membrane protein 1 (Itm1, P46977); Interleukin-6 (P05231); Interleukin-8 (P10145); Interleukin-18 (Q14116); IP-10 (10 kDa interferon-gamma-induced protein, P02778); IRPR (IFRD1, O00458); Isovaleryl-CoA dehydrogenase (IVD, P26440); I-TAC/CXCL11 (O14625); Keratin 19 (P08727); Kim-1 (Hepatitis A virus cellular receptor 1, O43656); L-arginine:glycine amidinotransferase (P50440); Leptin (P41159); Lipocalin2 (NGAL, P80188); MCP-1 (P13500); MIG (Gamma-interferon-induced monokine Q07325); MIP-1a (P10147); MIP-3a (P78556); MIP-1beta (P13236); MIP-1d (Q16663); NAG (N-acetyl-beta-D-glucosaminidase, P54802); Organic ion transporter (OCT2, O15244); Osteoprotegerin (O14788); P8 protein (O60356); Plasminogen activator inhibitor 1 (PAI-1, P05121); ProANP(1-98) (P01160); Protein phosphatase 1-beta (PPI-beta, P62140); Rab GDI-beta (P50395); Renal kallikrein (Q86U61); RT1.B-1 (alpha) chain of the integral membrane protein (Q5Y7A8); Soluble tumor necrosis factor receptor superfamily member 1A (sTNFR-I, P19438); Soluble tumor necrosis factor receptor superfamily member 1B (sTNFR-II, P20333); Tissue inhibitor of metalloproteinases 3 (TIMP-3, P35625); uPAR (Q03405) may be combined with the kidney injury marker assay result(s) of the present invention.

[0116] Other clinical indicia which may be combined with the kidney injury marker assay result(s) of the present invention includes demographic information (e.g., weight, sex, age, race), medical history (e.g., family history, type of surgery, pre-existing disease such as aneurism, congestive heart failure, preeclampsia, eclampsia, diabetes mellitus, hypertension, coronary artery disease, proteinuria, renal insufficiency, or sepsis, type of toxin exposure such as NSAIDs, cyclosporines, tacrolimus, aminoglycosides, foscarnet, ethylene glycol, hemoglobin, myoglobin, ifosfamide, heavy metals, methotrexate,

radiopaque contrast agents, or streptozotocin), clinical variables (*e.g.*, blood pressure, temperature, respiration rate), risk scores (APACHE score, PREDICT score, TIMI Risk Score for UA/NSTEMI, Framingham Risk Score), a urine total protein measurement, a glomerular filtration rate, an estimated glomerular filtration rate, a urine production rate, a serum or plasma creatinine concentration, a renal papillary antigen 1 (RPA1) measurement; a renal papillary antigen 2 (RPA2) measurement; a urine creatinine concentration, a fractional excretion of sodium, a urine sodium concentration, a urine creatinine to serum or plasma creatinine ratio, a urine specific gravity, a urine osmolality, a urine urea nitrogen to plasma urea nitrogen ratio, a plasma BUN to creatnine ratio, and/or a renal failure index calculated as urine sodium / (urine creatinine / plasma creatinine). Other measures of renal function which may be combined with the kidney injury marker assay result(s) are described hereinafter and in Harrison's Principles of Internal Medicine, 17th Ed., McGraw Hill, New York, pages 1741-1830, and Current Medical Diagnosis & Treatment 2008, 47th Ed, McGraw Hill, New York, pages 785-815, each of which are hereby incorporated by reference in their entirety.

[0117] 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.

[0118] Diagnosis of Acute Renal Failure

[0119] 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:

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

[0120] 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.

[0121] 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.

[0122] 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:

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

[0123] 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.

[0124] 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).

[0125] Selecting a Treatment Regimen

[0126] 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.

[0127] 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.

[0128] Example 1: Contrast-induced nephropathy sample collection

[0129] The objective of this sample collection study is to collect samples of plasma and urine and clinical data from patients before and after receiving intravascular contrast media. Approximately 250 adults undergoing radiographic/angiographic procedures involving intravascular administration of iodinated contrast media are enrolled. To be enrolled in the study, each patient must meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria

males and females 18 years of age or older;

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

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

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

Exclusion Criteria

renal transplant recipients;

acutely worsening renal function prior to the contrast procedure;

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

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

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

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

[0130] Immediately prior to the first contrast administration (and after any pre-procedure hydration), an EDTA anti-coagulated blood sample (10 mL) and a urine

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

[0131] Serum creatinine is assessed at the site immediately prior to the first contrast administration (after any pre-procedure hydration) and at 4 (±0.5), 8 (±1), 24 (±2) and 48 (±2)), and 72 (±2) hours following the last administration of contrast (ideally at the same time as the study samples are obtained). In addition, each patient's status is evaluated through day 30 with regard to additional serum and urine creatinine measurements, a need for dialysis, hospitalization status, and adverse clinical outcomes (including mortality).

[0132] Prior to contrast administration, each patient is assigned a risk based on the following assessment: systolic blood pressure <80 mm Hg = 5 points; intra-arterial balloon pump = 5 points; congestive heart failure (Class III-IV or history of pulmonary edema) = 5 points; age >75 yrs = 4 points; hematocrit level <39% for men, <35% for women = 3 points; diabetes = 3 points; contrast media volume = 1 point for each 100 mL; serum creatinine level >1.5 g/dL = 4 points OR estimated GFR 40–60 mL/min/1.73 m^2 = 2 points, 20–40 mL/min/1.73 m^2 = 4 points, < 20 mL/min/1.73 m^2 = 6 points. The risks assigned are as follows: risk for CIN and dialysis: 5 or less total points = risk of CIN - 7.5%, risk of dialysis - 0.04%; 6–10 total points = risk of CIN - 14%, risk of dialysis - 0.12%; 11–16 total points = risk of CIN - 26.1%, risk of dialysis - 1.09%; >16 total points = risk of CIN - 57.3%, risk of dialysis - 12.8%.

[0133] Example 2: Cardiac surgery sample collection

[0134] The objective of this sample collection study is to collect samples of plasma and urine and clinical data from patients before and after undergoing cardiovascular surgery, a procedure known to be potentially damaging to kidney function. Approximately 900 adults undergoing such surgery are enrolled. To be enrolled in the study, each patient must meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria

males and females 18 years of age or older;

undergoing cardiovascular surgery;

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

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

Exclusion Criteria

known pregnancy;

previous renal transplantation;

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

RIFLE criteria);

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

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

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

[0135] Within 3 hours prior to the first incision (and after any pre-procedure hydration), an EDTA anti-coagulated blood sample (10 mL), whole blood (3 mL), and a urine sample (35 mL) are collected from each patient. Blood and urine samples are then collected at 3 (±0.5), 6 (±0.5), 12 (±1), 24 (±2) and 48 (±2) hrs following the procedure and then daily on days 3 through 7 if the subject remains in the hospital. Blood is collected via direct venipuncture or via other available venous access, such as an existing femoral sheath, central venous line, peripheral intravenous line or hep-lock. These study blood samples are frozen and shipped to Astute Medical, Inc., San Diego, CA. The study urine samples are frozen and shipped to Astute Medical, Inc.

[0136] Example 3: Acutely ill subject sample collection

[0137] The objective of this study is to collect samples from acutely ill patients. Approximately 1900 adults expected to be in the ICU for at least 48 hours will be enrolled. To be enrolled in the study, each patient must meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria

males and females 18 years of age or older;

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

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

sepsis;

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

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

contrast media exposure within 24 hours of enrollment;

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

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

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

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

(i) respiratory SOFA score of ≥ 2 (PaO_2/FiO_2 <300), (ii) cardiovascular SOFA score of ≥ 1 (MAP < 70 mm Hg and/or any vasopressor required).

Exclusion Criteria

known pregnancy;

institutionalized individuals;

previous renal transplantation;

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

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

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

meets any of the following:

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

(ii) hemoglobin < 7 g/dL;

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

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

[0138] After obtaining informed consent, an EDTA anti-coagulated blood sample (10 mL) and a urine sample (25-50 mL) are collected from each patient. Blood and urine samples are then collected at 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), 36 (± 2), 48 (± 2), 60 (± 2), 72 (± 2), and 84 (± 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Blood is collected via direct venipuncture or via other available venous access, such as an existing femoral sheath, central venous line, peripheral intravenous line or hep-lock. These study blood samples are processed to plasma at the clinical site, frozen and shipped to Astute Medical, Inc., San Diego, CA. The study urine samples are frozen and shipped to Astute Medical, Inc.

[0139] Example 4. Immunoassay format

[0140] Analytes are measured using standard sandwich enzyme immunoassay techniques. A first antibody which binds the analyte is immobilized in wells of a 96 well

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

[0141] Units for the concentrations reported in the following data tables are as follows: Heat shock protein beta-1 – pg/mL, WAP four-disulfide core domain protein 2 – pg/mL, Choriogonadotropin subunit beta – mU/mL, Placenta growth factor – pg/mL, and Mitochondrial 60 kDa heat shock protein – pg/mL. In the case of those kidney injury markers which are membrane proteins as described herein, the assays used in these examples detect soluble forms thereof.

[0142] Example 5. Apparently Healthy Donor and Chronic Disease Patient Samples

[0143] Human urine samples from donors with no known chronic or acute disease ("Apparently Healthy Donors") were purchased from two vendors (Golden West Biologicals, Inc., 27625 Commerce Center Dr., Temecula, CA 92590 and Virginia Medical Research, Inc., 915 First Colonial Rd., Virginia Beach, VA 23454). The urine samples were shipped and stored frozen at less than -20° C. The vendors supplied demographic information for the individual donors including gender, race (Black /White), smoking status and age.

[0144] Human urine samples from donors with various chronic diseases ("Chronic Disease Patients") including congestive heart failure, coronary artery disease, chronic kidney disease, chronic obstructive pulmonary disease, diabetes mellitus and hypertension were purchased from Virginia Medical Research, Inc., 915 First Colonial Rd., Virginia Beach, VA 23454. The urine samples were shipped and stored frozen at less than -20 degrees centigrade. The vendor provided a case report form for each individual

donor with age, gender, race (Black/White), smoking status and alcohol use, height, weight, chronic disease(s) diagnosis, current medications and previous surgeries.

[0145] Example 6. Use of Kidney Injury Markers for evaluating renal status in patients

[0146] Patients from the intensive care unit (ICU) were enrolled in the following study. Each patient was classified by kidney status as non-injury (0), risk of injury (R), injury (I), and failure (F) according to the maximum stage reached within 7 days of enrollment as determined by the RIFLE criteria. EDTA anti-coagulated blood samples (10 mL) and a urine samples (25-30 mL) were collected from each patient at enrollment, 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), and 48 (± 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Markers were each measured by standard immunoassay methods using commercially available assay reagents in the urine samples and the plasma component of the blood samples collected.

[0147] Two cohorts were defined to represent a "diseased" and a "normal" population. While these terms are used for convenience, "diseased" and "normal" simply represent two cohorts for comparison (say RIFLE 0 vs RIFLE R, I and F; RIFLE 0 vs RIFLE R; RIFLE 0 and R vs RIFLE I and F; etc.). The time "prior max stage" represents the time at which a sample is collected, relative to the time a particular patient reaches the lowest disease stage as defined for that cohort, binned into three groups which are +/- 12 hours. For example, "24 hr prior" which uses 0 vs R, I, F as the two cohorts would mean 24 hr (+/- 12 hours) prior to reaching stage R (or I if no sample at R, or F if no sample at R or I).

[0148] A receiver operating characteristic (ROC) curve was generated for each biomarker measured and the area under each ROC curve (AUC) is determined. Patients in Cohort 2 were also separated according to the reason for adjudication to cohort 2 as being based on serum creatinine measurements (sCr), being based on urine output (UO), or being based on either serum creatinine measurements or urine output. Using the same example discussed above (0 vs R, I, F), for those patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements alone, the stage 0 cohort may include patients adjudicated to stage R, I, or F on the basis of urine output; for those patients adjudicated to stage R, I, or F on the basis of urine output alone, the stage 0 cohort may

include patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements; and for those patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements or urine output, the stage 0 cohort contains only patients in stage 0 for both serum creatinine measurements and urine output. Also, in the data for patients adjudicated on the basis of serum creatinine measurements or urine output, the adjudication method which yielded the most severe RIFLE stage is used.

[0149] The ability to distinguish cohort 1 from Cohort 2 was determined using ROC analysis. SE is the standard error of the AUC, n is the number of sample or individual patients ("pts," as indicated). Standard errors are calculated as described in Hanley, J. A., and McNeil, B.J., The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology (1982) 143: 29-36; p values are calculated with a two-tailed Z-test. An AUC < 0.5 is indicative of a negative going marker for the comparison, and an AUC > 0.5 is indicative of a positive going marker for the comparison.

[0150] Various threshold (or "cutoff") concentrations were selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 are determined. OR is the odds ratio calculated for the particular cutoff concentration, and 95% CI is the confidence interval for the odds ratio.

[0151] Table 1: Comparison of marker levels in urine samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0) and in urine samples collected from subjects at 0, 24 hours, and 48 hours prior to reaching stage R, I or F in Cohort 2.

Placenta growth factor

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	44.7	51.5	44.7	50.4	44.7	50.4
Average	57.1	67.4	57.1	106	57.1	67.5
Stdev	42.4	65.2	42.4	361	42.4	62.8
p(t-test)		0.057		0.030		0.20
Min	4.82	6.04	4.82	6.50	4.82	10.3
Max	218	418	218	3660	218	301
n (Samp)	268	137	268	103	268	35
n (Patient)	148	137	148	103	148	35

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	51.5	29.4	51.5	33.0	51.5	29.7
Average	69.4	46.0	69.4	52.1	69.4	52.7

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Stdev	152	51.0	152	42.1	152	60.2
p(t-test)		0.34		0.49		0.60
Min	2.74	4.57	2.74	8.39	2.74	6.50
Max	3660	291	3660	201	3660	231
n (Samp)	660	38	660	37	660	23
n (Patient)	287	38	287	37	287	23

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	39.8	52.8	39.8	47.2	39.8	56.5
Average	55.1	72.8	55.1	106	55.1	65.9
Stdev	44.6	76.7	44.6	365	44.6	57.8
p(t-test)		0.0027		0.016		0.20
Min	4.82	7.83	4.82	6.50	4.82	10.3
Max	310	496	310	3660	310	301
n (Samp)	313	126	313	101	313	32
n (Patient)	152	126	152	101	152	32

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.52	0.36	0.56	0.54	0.43	0.56	0.53	0.38	0.57
SE	0.030	0.050	0.031	0.034	0.050	0.033	0.053	0.063	0.055
p	0.41	0.0043	0.044	0.24	0.16	0.090	0.54	0.068	0.22
nCohort 1	268	660	313	268	660	313	268	660	313
nCohort 2	137	38	126	103	37	101	35	23	32
Cutoff 1	29.2	21.3	32.8	31.9	24.9	33.8	29.7	24.2	31.5
Sens 1	70%	71%	71%	71%	70%	70%	71%	74%	72%
Spec 1	29%	18%	39%	33%	23%	40%	30%	22%	38%
Cutoff 2	21.7	16.3	21.6	28.4	21.3	28.8	25.9	16.1	25.6
Sens 2	80%	82%	80%	81%	81%	80%	80%	83%	81%
Spec 2	20%	11%	21%	28%	18%	33%	25%	11%	28%
Cutoff 3	16.1	8.53	15.7	18.8	17.2	18.4	14.4	10.1	14.4
Sens 3	91%	92%	90%	90%	92%	90%	91%	91%	91%
Spec 3	11%	3%	12%	15%	12%	15%	10%	4%	11%
Cutoff 4	70.5	72.1	65.2	70.5	72.1	65.2	70.5	72.1	65.2
Sens 4	33%	21%	43%	31%	22%	34%	34%	17%	38%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	81.6	87.9	81.1	81.6	87.9	81.1	81.6	87.9	81.1
Sens 5	26%	11%	27%	26%	22%	25%	20%	9%	19%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	113	124	112	113	124	112	113	124	112
Sens 6	15%	3%	17%	15%	3%	15%	9%	9%	9%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	0.66	0.56	0.47	1.5	0.88	1.5	1.3	0.49	0.82
p Value	0.17	0.37	0.024	0.26	0.80	0.25	0.62	0.42	0.76
95% CI of	0.36	0.16	0.25	0.76	0.31	0.76	0.46	0.089	0.24
OR Quart2	1.2	2.0	0.90	2.9	2.5	2.9	3.7	2.7	2.8

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
OR Quart 3	0.96	1.8	1.2	1.4	1.3	1.4	1.1	2.0	1.8
p Value	0.88	0.24	0.50	0.33	0.62	0.31	0.81	0.25	0.30
95% CI of	0.54	0.68	0.69	0.71	0.49	0.72	0.39	0.61	0.61
OR Quart3	1.7	4.6	2.2	2.7	3.3	2.8	3.3	6.9	5.1
OR Quart 4	1.1	2.3	1.3	1.7	1.5	1.9	1.6	2.3	1.9
p Value	0.70	0.083	0.34	0.11	0.35	0.058	0.33	0.17	0.22
95% CI of	0.63	0.90	0.75	0.89	0.62	0.98	0.60	0.70	0.68
OR Quart4	2.0	5.7	2.3	3.3	3.9	3.6	4.5	7.7	5.5

60 kDa heat shock protein, mitochondrial

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	143	235	143	168	143	379
Average	526	390	526	536	526	876
Stdev	1290	458	1290	930	1290	1120
p(t-test)		0.67		0.97		0.65
Min	2.53	2.53	2.53	2.53	2.53	91.0
Max	8920	1430	8920	3910	8920	2160
n (Samp)	51	18	51	18	51	3
n (Patient)	41	18	41	18	41	3

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	143	398	143	1060	143	192
Average	498	276	498	1370	498	192
Stdev	1060	223	1060	1480	1060	143
p(t-test)		0.64		0.083		0.69
Min	2.53	37.1	2.53	37.1	2.53	91.0
Max	8920	509	8920	3910	8920	294
n (Samp)	90	5	90	5	90	2
n (Patient)	71	5	71	5	71	2

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	91.0	235	91.0	161	91.0	398
Average	504	440	504	524	504	915
Stdev	1370	503	1370	939	1370	787
p(t-test)		0.87		0.95		0.52
Min	2.53	2.53	2.53	2.53	2.53	379
Max	8920	1430	8920	4070	8920	2160
n (Samp)	45	14	45	19	45	5
n (Patient)	35	14	35	19	35	5

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.49	0.52	0.55	0.50	0.74	0.56	0.69	0.51	0.84

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
SE	0.080	0.14	0.090	0.080	0.13	0.080	0.17	0.21	0.11
p	0.92	0.86	0.61	0.98	0.064	0.42	0.28	0.96	0.0025
nCohort 1	51	90	45	51	90	45	51	90	45
nCohort 2	18	5	14	18	5	19	3	2	5
Cutoff 1	2.53	2.53	2.53	2.53	668	2.53	37.1	37.1	379
Sens 1	94%	100%	93%	89%	80%	89%	100%	100%	80%
Spec 1	6%	7%	7%	6%	79%	7%	29%	36%	78%
Cutoff 2	2.53	2.53	2.53	2.53	668	2.53	37.1	37.1	379
Sens 2	94%	100%	93%	89%	80%	89%	100%	100%	80%
Spec 2	6%	7%	7%	6%	79%	7%	29%	36%	78%
Cutoff 3	2.53	2.53	2.53	0	2.53	0	37.1	37.1	294
Sens 3	94%	100%	93%	100%	100%	100%	100%	100%	100%
Spec 3	6%	7%	7%	0%	7%	0%	29%	36%	73%
Cutoff 4	379	379	193	379	379	193	379	379	193
Sens 4	44%	60%	50%	28%	80%	42%	33%	0%	100%
Spec 4	73%	71%	71%	73%	71%	71%	73%	71%	71%
Cutoff 5	629	894	453	629	894	453	629	894	453
Sens 5	22%	0%	36%	28%	60%	26%	33%	0%	40%
Spec 5	80%	83%	80%	80%	83%	80%	80%	83%	80%
Cutoff 6	1180	1180	1180	1180	1180	1180	1180	1180	1180
Sens 6	6%	0%	7%	11%	20%	11%	33%	0%	40%
Spec 6	90%	90%	91%	90%	90%	91%	90%	90%	91%
OR Quart 2	0.80	0	1.3	1.1	0	2.0	>1.0	>1.0	>0
p Value	0.77	na	0.74	0.91	na	0.42	<1.0	<0.98	<na
95% CI of	0.17	na	0.24	0.25	na	0.38	>0.056	>0.062	>na
OR Quart2	3.7	na	7.4	4.7	na	10	na	na	na
OR Quart 3	0.35	0.95	0.56	1.1	0	2.0	>1.1	>1.0	>2.4
p Value	0.25	0.96	0.57	0.91	na	0.42	<0.96	<0.98	<0.50
95% CI of	0.057	0.12	0.079	0.25	na	0.38	>0.061	>0.062	>0.19
OR Quart3	2.1	7.4	4.0	4.7	na	10	na	na	na
OR Quart 4	1.8	0.46	1.8	0.56	4.4	2.6	>1.0	>0	>3.6
p Value	0.41	0.53	0.48	0.48	0.20	0.25	<1.0	<na	<0.30
95% CI of	0.44	0.039	0.35	0.11	0.45	0.52	>0.056	>na	>0.32
OR Quart4	7.5	5.4	9.7	2.8	43	13	na	na	na

Heat shock protein beta-1 (phospho SER78 / phospho SER82)

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.00335	0.00191	0.00335	0.00335	0.00335	0.0235
Average	0.0615	0.0127	0.0615	0.647	0.0615	0.471
Stdev	0.233	0.0442	0.233	1.65	0.233	0.789
p(t-test)		0.38		0.015		0.016
Min	0.00191	0.00191	0.00191	0.00191	0.00191	0.00738
Max	1.50	0.190	1.50	6.52	1.50	1.38
n (Samp)	51	18	51	18	51	3
n (Patient)	41	18	41	18	41	3

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.00335	0.00335	0.00335	0.00335	0.00335	0.0134
Average	0.147	0.00277	0.147	0.908	0.147	0.0134
Stdev	0.731	0.000788	0.731	1.31	0.731	0.0143
p(t-test)		0.66		0.033		0.80
Min	0.00191	0.00191	0.00191	0.00191	0.00191	0.00335
Max	6.52	0.00335	6.52	2.88	6.52	0.0235
n (Samp)	90	5	90	5	90	2
n (Patient)	71	5	71	5	71	2

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.00335	0.00191	0.00335	0.00335	0.00335	0.00738
Average	0.134	0.0156	0.134	0.375	0.134	0.517
Stdev	0.487	0.0501	0.487	1.49	0.487	0.704
p(t-test)		0.37		0.33		0.12
Min	0.00191	0.00191	0.00191	0.00191	0.00191	0.00335
Max	2.88	0.190	2.88	6.52	2.88	1.38
n (Samp)	45	14	45	19	45	5
n (Patient)	35	14	35	19	35	5

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.35	0.50	0.37	0.53	0.71	0.50	0.92	0.77	0.80
SE	0.079	0.13	0.089	0.080	0.13	0.080	0.11	0.20	0.12
p	0.059	0.97	0.14	0.72	0.12	0.96	2.3E-4	0.18	0.013
nCohort 1	51	90	45	51	90	45	51	90	45
nCohort 2	18	5	14	18	5	19	3	2	5
Cutoff 1	0	0	0	0	0.00191	0	0.00335	0.00191	0.00191
Sens 1	100%	100%	100%	100%	80%	100%	100%	100%	100%
Spec 1	0%	0%	0%	0%	48%	0%	88%	48%	47%
Cutoff 2	0	0	0	0	0.00191	0	0.00335	0.00191	0.00191
Sens 2	100%	100%	100%	100%	80%	100%	100%	100%	100%
Spec 2	0%	0%	0%	0%	48%	0%	88%	48%	47%
Cutoff 3	0	0	0	0	0	0	0.00335	0.00191	0.00191
Sens 3	100%	100%	100%	100%	100%	100%	100%	100%	100%
Spec 3	0%	0%	0%	0%	0%	0%	88%	48%	47%
Cutoff 4	0.00335	0.00335	0.00335	0.00335	0.00335	0.00335	0.00335	0.00335	0.00335
Sens 4	6%	0%	7%	28%	40%	16%	100%	50%	60%
Spec 4	88%	86%	82%	88%	86%	82%	88%	86%	82%
Cutoff 5	0.00335	0.00335	0.00335	0.00335	0.00335	0.00335	0.00335	0.00335	0.00335
Sens 5	6%	0%	7%	28%	40%	16%	100%	50%	60%
Spec 5	88%	86%	82%	88%	86%	82%	88%	86%	82%
Cutoff 6	0.106	0.182	0.182	0.106	0.182	0.182	0.106	0.182	0.182
Sens 6	6%	0%	7%	28%	40%	16%	33%	0%	40%
Spec 6	90%	90%	91%	90%	90%	91%	90%	90%	91%
OR Quart 2	3.6	>3.4	3.5	>55	>3.3	2.0	>0	>1.0	>2.2
p Value	0.29	<0.30	0.30	<6.5E-4	<0.32	0.42	<na	<0.98	<0.55

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
95% CI of	0.34	>0.33	0.32	>5.5	>0.32	0.38	>na	>0.062	>0.17
OR Quart2	39	na	38	na	na	10	na	na	na
OR Quart 3	31	>1.0	16	>0	>0	3.4	>0	>0	>0
p Value	0.0027	<0.98	0.017	<na	<na	0.14	<na	<na	<na
95% CI of	3.3	>0.062	1.7	>na	>na	0.68	>na	>na	>na
OR Quart3	300	na	150	na	na	17	na	na	na
OR Quart 4	3.6	>1.1	2.3	>6.5	>2.1	1.4	>3.5	>1.0	>3.6
p Value	0.29	<0.95	0.51	<0.10	<0.56	0.67	<0.30	<0.98	<0.30
95% CI of	0.34	>0.064	0.19	>0.68	>0.18	0.27	>0.32	>0.062	>0.32
OR Quart4	39	na	29	na	na	7.8	na	na	na

WAP four-disulfide core domain protein 2

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	369000	477000	369000	1040000	369000	643000
Average	746000	1440000	746000	1610000	746000	743000
Stdev	993000	1860000	993000	1990000	993000	213000
p(t-test)		0.046		0.017		1.00
Min	23500	165000	23500	44300	23500	599000
Max	5640000	7500000	5640000	7500000	5640000	988000
n (Samp)	52	19	52	19	52	3
n (Patient)	41	19	41	19	41	3

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	599000	440000	599000	560000	599000	705000
Average	1090000	525000	1090000	580000	1090000	705000
Stdev	1460000	335000	1460000	454000	1460000	88300
p(t-test)		0.39		0.49		0.71
Min	23500	213000	23500	44300	23500	643000
Max	7500000	936000	7500000	1150000	7500000	768000
n (Samp)	93	5	93	4	93	2
n (Patient)	73	5	73	4	73	2

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	355000	949000	355000	1260000	355000	936000
Average	537000	1710000	537000	1940000	537000	814000
Stdev	464000	2020000	464000	2140000	464000	426000
p(t-test)		6.2E-4		1.0E-4		0.21
Min	23500	165000	23500	117000	23500	213000
Max	1650000	7500000	1650000	7500000	1650000	1340000
n (Samp)	44	15	44	20	44	5
n (Patient)	34	15	34	20	34	5

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

	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.64	0.42	0.71	0.69	0.42	0.79	0.67	0.53	0.69
SE	0.077	0.14	0.083	0.075	0.15	0.066	0.18	0.21	0.14
p	0.071	0.57	0.012	0.011	0.62	9.7E-6	0.35	0.88	0.18
nCohort 1	52	93	44	52	93	44	52	93	44
nCohort 2	19	5	15	19	4	20	3	2	5
Cutoff 1	321000	213000	378000	491000	491000	866000	595000	608000	578000
Sens 1	74%	80%	73%	74%	75%	70%	100%	100%	80%
Spec 1	46%	24%	57%	60%	47%	80%	63%	52%	66%
Cutoff 2	213000	213000	323000	303000	43800	645000	595000	608000	578000
Sens 2	84%	80%	80%	84%	100%	80%	100%	100%	80%
Spec 2	33%	24%	48%	44%	3%	70%	63%	52%	66%
Cutoff 3	178000	211000	178000	116000	43800	303000	595000	608000	209000
Sens 3	95%	100%	93%	95%	100%	90%	100%	100%	100%
Spec 3	31%	24%	25%	19%	3%	43%	63%	52%	27%
Cutoff 4	862000	1070000	645000	862000	1070000	645000	862000	1070000	645000
Sens 4	47%	0%	53%	58%	25%	80%	33%	0%	60%
Spec 4	71%	71%	70%	71%	71%	70%	71%	71%	70%
Cutoff 5	1130000	1460000	991000	1130000	1460000	991000	1130000	1460000	991000
Sens 5	32%	0%	47%	47%	0%	60%	0%	0%	20%
Spec 5	81%	81%	82%	81%	81%	82%	81%	81%	82%
Cutoff 6	1650000	3030000	1320000	1650000	3030000	1320000	1650000	3030000	1320000
Sens 6	26%	0%	33%	26%	0%	50%	0%	0%	20%
Spec 6	90%	90%	91%	90%	90%	91%	90%	90%	91%
OR Quart 2	8.0	>2.3	0.92	0.94	>1.1	1.0	>0	>0	>1.1
p Value	0.070	<0.51	0.94	0.95	<0.95	1.0	<na	<na	<0.95
95% CI of	0.85	>0.19	0.11	0.12	>0.064	0.12	>na	>na	>0.061
OR Quart2	76	na	7.6	7.5	na	8.1	na	na	na
OR Quart 3	8.0	>1.0	2.2	4.8	>2.3	3.2	>3.5	>2.1	>1.1
p Value	0.070	<0.98	0.42	0.081	<0.51	0.21	<0.30	<0.56	<0.95
95% CI of	0.85	>0.062	0.33	0.83	>0.19	0.52	>0.32	>0.18	>0.061
OR Quart3	76	na	14	28	na	20	na	na	na
OR Quart 4	8.0	>2.3	5.2	6.0	>1.1	15	>0	>0	>3.6
p Value	0.070	<0.51	0.072	0.044	<0.95	0.0032	<na	<na	<0.30
95% CI of	0.85	>0.19	0.86	1.0	>0.064	2.5	>na	>na	>0.32
OR Quart4	76	na	32	34	na	95	na	na	na

[0152] Table 2: Comparison of marker levels in urine samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0 or R) and in urine samples collected from subjects at 0, 24 hours, and 48 hours prior to reaching stage I or F in Cohort 2.

Placenta growth factor

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	45.0	57.1	45.0	47.7	45.0	30.9
Average	60.8	75.3	60.8	105	60.8	52.6
Stdev	57.1	84.2	57.1	415	57.1	62.5

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
p(t-test)		0.059		0.014		0.39
Min	4.57	2.74	4.57	9.16	4.57	2.18
Max	524	516	524	3660	524	312
n (Samp)	597	69	597	76	597	38
n (Patient)	279	69	279	76	279	38

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	47.9	25.6	47.9	44.3	47.9	26.8
Average	66.8	61.1	66.8	55.6	66.8	35.8
Stdev	139	90.2	139	39.2	139	28.6
p(t-test)		0.90		0.74		0.40
Min	2.74	8.93	2.74	15.0	2.74	8.53
Max	3660	291	3660	145	3660	109
n (Samp)	827	9	827	17	827	14
n (Patient)	352	9	352	17	352	14

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	44.3	57.4	44.3	48.0	44.3	32.3
Average	60.5	77.5	60.5	109	60.5	55.7
Stdev	57.8	84.0	57.8	427	57.8	64.3
p(t-test)		0.032		0.0092		0.63
Min	4.57	2.74	4.57	8.07	4.57	2.18
Max	524	516	524	3660	524	312
n (Samp)	604	66	604	72	604	35
n (Patient)	263	66	263	72	263	35

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.55	0.36	0.57	0.51	0.49	0.52	0.40	0.32	0.43
SE	0.037	0.10	0.038	0.035	0.071	0.036	0.050	0.080	0.052
p	0.18	0.17	0.058	0.69	0.85	0.58	0.052	0.025	0.18
nCohort 1	597	827	604	597	827	604	597	827	604
nCohort 2	69	9	66	76	17	72	38	14	35
Cutoff 1	33.5	13.4	37.7	30.7	29.5	30.7	18.8	21.6	19.5
Sens 1	71%	78%	71%	71%	71%	71%	71%	71%	71%
Spec 1	35%	6%	41%	33%	29%	34%	15%	19%	16%
Cutoff 2	22.1	12.7	24.2	24.0	20.6	21.6	13.6	14.0	14.0
Sens 2	81%	89%	80%	80%	82%	81%	82%	86%	80%
Spec 2	20%	6%	25%	24%	18%	20%	6%	7%	7%
Cutoff 3	12.3	8.63	14.0	18.2	18.4	14.5	10.1	10.5	10.1
Sens 3	91%	100%	91%	91%	94%	90%	92%	93%	91%
Spec 3	6%	3%	8%	14%	14%	9%	4%	4%	4%
Cutoff 4	66.8	70.0	66.4	66.8	70.0	66.4	66.8	70.0	66.4
Sens 4	39%	22%	41%	34%	29%	38%	26%	14%	29%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Cutoff 5	83.7	86.0	82.9	83.7	86.0	82.9	83.7	86.0	82.9
Sens 5	29%	11%	32%	18%	18%	22%	11%	7%	17%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	124	124	124	124	124	124	124	124	124
Sens 6	10%	11%	11%	7%	6%	7%	5%	0%	6%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	0.73	0.50	0.77	1.3	1.0	0.94	0.65	0.50	0.65
p Value	0.42	0.57	0.52	0.48	1.0	0.85	0.43	0.57	0.43
95% CI of	0.33	0.045	0.34	0.64	0.25	0.46	0.23	0.045	0.23
OR Quart2	1.6	5.5	1.7	2.6	4.1	1.9	1.9	5.6	1.9
OR Quart 3	1.1	1.0	1.2	1.3	1.3	1.1	0.88	2.0	0.88
p Value	0.85	1.0	0.57	0.48	0.74	0.86	0.80	0.42	0.80
95% CI of	0.52	0.14	0.59	0.64	0.33	0.53	0.33	0.37	0.33
OR Quart3	2.2	7.2	2.6	2.6	4.7	2.1	2.3	11	2.3
OR Quart 4	1.6	2.0	1.8	1.3	1.0	1.3	1.7	3.6	1.4
p Value	0.19	0.42	0.092	0.49	1.0	0.49	0.20	0.11	0.49
95% CI of	0.80	0.37	0.91	0.64	0.25	0.64	0.74	0.74	0.56
OR Quart4	3.1	11	3.7	2.6	4.1	2.5	4.1	18	3.3

60 kDa heat shock protein, mitochondrial

sCr or UO	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	91.0	401
Average	509	686
Stdev	1100	1060
p(t-test)		0.57
Min	2.53	2.53
Max	8920	4070
n (Samp)	95	14
n (Patient)	73	14

sCr only	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	91.0	1060
Average	533	887
Stdev	1100	328
p(t-test)		0.58
Min	2.53	509
Max	8920	1090
n (Samp)	107	3
n (Patient)	83	3

UO only	24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	91.0	193	91.0	1160
Average	479	619	479	1160
Stdev	1110	1100	1110	105

UO only	24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
p(t-test)		0.67		0.39
Min	2.53	2.53	2.53	1090
Max	8920	4070	8920	1240
n (Samp)	82	13	82	2
n (Patient)	62	13	62	2

	24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.57	0.81	0.53	nd	nd	0.89
SE	0.085	0.15	0.088	nd	nd	0.15
p	0.39	0.040	0.76	nd	nd	0.0093
nCohort 1	95	107	82	nd	nd	82
nCohort 2	14	3	13	nd	nd	2
Cutoff 1	37.1	453	2.53	nd	nd	1060
Sens 1	71%	100%	85%	nd	nd	100%
Spec 1	36%	74%	6%	nd	nd	85%
Cutoff 2	2.53	453	2.53	nd	nd	1060
Sens 2	86%	100%	85%	nd	nd	100%
Spec 2	5%	74%	6%	nd	nd	85%
Cutoff 3	0	453	0	nd	nd	1060
Sens 3	100%	100%	100%	nd	nd	100%
Spec 3	0%	74%	0%	nd	nd	85%
Cutoff 4	379	379	379	nd	nd	379
Sens 4	50%	100%	38%	nd	nd	100%
Spec 4	72%	70%	73%	nd	nd	73%
Cutoff 5	760	894	760	nd	nd	760
Sens 5	29%	67%	23%	nd	nd	100%
Spec 5	80%	82%	80%	nd	nd	80%
Cutoff 6	1240	1240	1180	nd	nd	1180
Sens 6	7%	0%	8%	nd	nd	50%
Spec 6	92%	92%	90%	nd	nd	90%
OR Quart 2	1.6	>0	2.1	nd	nd	>0
p Value	0.64	<na	0.42	nd	nd	<na
95% CI of	0.24	>na	0.35	nd	nd	>na
OR Quart2	10	na	13	nd	nd	na
OR Quart 3	2.2	>1.0	0.95	nd	nd	>0
p Value	0.40	<0.98	0.96	nd	nd	<na
95% CI of	0.36	>0.062	0.12	nd	nd	>na
OR Quart3	13	na	7.4	nd	nd	na
OR Quart 4	2.7	>2.1	2.8	nd	nd	>2.2
p Value	0.26	<0.56	0.26	nd	nd	<0.53
95% CI of	0.48	>0.18	0.48	nd	nd	>0.19
OR Quart4	15	na	16	nd	nd	na

WAP four-disulfide core domain protein 2

sCr or UO	24hr prior to AKI stage	
	Cohort 1	Cohort 2

sCr or UO	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	565000	1040000
Average	934000	2020000
Stdev	1220000	2220000
p(t-test)		0.0057
Min	23500	47600
Max	7500000	7500000
n (Samp)	97	15
n (Patient)	74	15

sCr only	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	603000	851000
Average	1070000	851000
Stdev	1450000	49900
p(t-test)		0.83
Min	23500	816000
Max	7500000	886000
n (Samp)	110	2
n (Patient)	85	2

UO only	24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	528000	1290000	528000	1110000
Average	865000	2160000	865000	1110000
Stdev	1140000	2260000	1140000	318000
p(t-test)		0.0013		0.76
Min	23500	47600	23500	886000
Max	7500000	7500000	7500000	1340000
n (Samp)	82	14	82	2
n (Patient)	62	14	62	2

	24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.69	0.61	0.72	nd	nd	0.75
SE	0.080	0.21	0.081	nd	nd	0.20
p	0.017	0.60	0.0062	nd	nd	0.22
nCohort 1	97	110	82	nd	nd	82
nCohort 2	15	2	14	nd	nd	2
Cutoff 1	768000	804000	768000	nd	nd	871000
Sens 1	73%	100%	71%	nd	nd	100%
Spec 1	59%	60%	59%	nd	nd	66%
Cutoff 2	755000	804000	685000	nd	nd	871000
Sens 2	80%	100%	86%	nd	nd	100%
Spec 2	59%	60%	59%	nd	nd	66%
Cutoff 3	145000	804000	145000	nd	nd	871000
Sens 3	93%	100%	93%	nd	nd	100%
Spec 3	16%	60%	15%	nd	nd	66%

	24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Cutoff 4	991000	1050000	988000	nd	nd	988000
Sens 4	53%	0%	64%	nd	nd	50%
Spec 4	70%	70%	71%	nd	nd	71%
Cutoff 5	1290000	1370000	1180000	nd	nd	1180000
Sens 5	40%	0%	50%	nd	nd	50%
Spec 5	80%	80%	80%	nd	nd	80%
Cutoff 6	1710000	2910000	1550000	nd	nd	1550000
Sens 6	33%	0%	43%	nd	nd	0%
Spec 6	91%	90%	90%	nd	nd	90%
OR Quart 2	0	>0	0	nd	nd	>0
p Value	na	<na	na	nd	nd	<na
95% CI of	na	>na	na	nd	nd	>na
OR Quart2	na	na	na	nd	nd	na
OR Quart 3	4.3	>2.2	2.9	nd	nd	>1.0
p Value	0.086	<0.54	0.23	nd	nd	<0.97
95% CI of	0.81	>0.18	0.50	nd	nd	>0.061
OR Quart3	23	na	17	nd	nd	na
OR Quart 4	3.5	>0	4.5	nd	nd	>1.0
p Value	0.14	<na	0.081	nd	nd	<0.97
95% CI of	0.65	>na	0.83	nd	nd	>0.061
OR Quart4	19	na	25	nd	nd	na

Choriogonadotropin subunit beta

sCr or UO	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	0.323	0.280
Average	0.838	0.676
Stdev	2.63	1.03
p(t-test)		0.81
Min	0.0484	0.140
Max	24.9	4.13
n (Samp)	100	15
n (Patient)	77	15

sCr only	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	0.293	0.825
Average	0.789	1.81
Stdev	2.48	2.01
p(t-test)		0.48
Min	0.0484	0.486
Max	24.9	4.13
n (Samp)	113	3
n (Patient)	88	3

UO only	24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2

UO only	24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.305	0.267	0.305	2.17
Average	0.612	0.394	0.612	2.17
Stdev	1.08	0.386	1.08	2.77
p(t-test)		0.46		0.054
Min	0.0484	0.140	0.0484	0.213
Max	6.45	1.62	6.45	4.13
n (Samp)	85	14	85	2
n (Patient)	65	14	65	2

	24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.53	0.84	0.49	nd	nd	0.68
SE	0.081	0.14	0.084	nd	nd	0.21
p	0.69	0.018	0.88	nd	nd	0.41
nCohort 1	100	113	85	nd	nd	85
nCohort 2	15	3	14	nd	nd	2
Cutoff 1	0.234	0.481	0.224	nd	nd	0.204
Sens 1	73%	100%	71%	nd	nd	100%
Spec 1	42%	71%	42%	nd	nd	39%
Cutoff 2	0.184	0.481	0.162	nd	nd	0.204
Sens 2	80%	100%	93%	nd	nd	100%
Spec 2	30%	71%	25%	nd	nd	39%
Cutoff 3	0.162	0.481	0.162	nd	nd	0.204
Sens 3	93%	100%	93%	nd	nd	100%
Spec 3	24%	71%	25%	nd	nd	39%
Cutoff 4	0.481	0.481	0.463	nd	nd	0.463
Sens 4	27%	100%	14%	nd	nd	50%
Spec 4	70%	71%	71%	nd	nd	71%
Cutoff 5	0.663	0.709	0.633	nd	nd	0.633
Sens 5	27%	67%	14%	nd	nd	50%
Spec 5	80%	81%	80%	nd	nd	80%
Cutoff 6	1.28	1.28	1.31	nd	nd	1.31
Sens 6	13%	33%	7%	nd	nd	50%
Spec 6	90%	90%	91%	nd	nd	91%
OR Quart 2	3.4	>0	2.2	nd	nd	>1.0
p Value	0.16	<na	0.39	nd	nd	<1.0
95% CI of	0.62	>na	0.36	nd	nd	>0.059
OR Quart2	18	na	13	nd	nd	na
OR Quart 3	1.5	>1.0	3.6	nd	nd	>0
p Value	0.67	<0.98	0.14	nd	nd	<na
95% CI of	0.23	>0.062	0.66	nd	nd	>na
OR Quart3	9.7	na	20	nd	nd	na
OR Quart 4	2.1	>2.1	1.0	nd	nd	>1.0
p Value	0.42	<0.54	0.97	nd	nd	<1.0
95% CI of	0.35	>0.18	0.14	nd	nd	>0.059
OR Quart4	12	na	8.1	nd	nd	na

[0153] Table 3: Comparison of the maximum marker levels in urine samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0) and the maximum values in urine samples collected from subjects between enrollment and 0, 24 hours, and 48 hours prior to reaching stage F in Cohort 2.

Placenta growth factor

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	60.1	53.9	60.1	52.9	60.1	52.9
Average	68.4	251	68.4	258	68.4	87.4
Stdev	46.4	725	46.4	738	46.4	90.1
p(t-test)		0.0025		0.0021		0.16
Min	4.82	4.49	4.82	4.49	4.82	14.0
Max	218	3660	218	3660	218	310
n (Samp)	148	28	148	27	148	17
n (Patient)	148	28	148	27	148	17

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	65.9	51.1	65.9	51.1	65.9	42.8
Average	95.3	77.2	95.3	77.2	95.3	77.8
Stdev	222	77.3	222	77.3	222	85.3
p(t-test)		0.75		0.75		0.79
Min	4.82	4.49	4.82	4.49	4.82	16.4
Max	3660	310	3660	310	3660	310
n (Samp)	287	15	287	15	287	12
n (Patient)	287	15	287	15	287	12

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	58.8	56.0	58.8	55.0	58.8	44.3
Average	69.7	341	69.7	356	69.7	75.5
Stdev	51.4	899	51.4	924	51.4	83.5
p(t-test)		2.4E-4		1.6E-4		0.74
Min	4.82	14.0	4.82	14.0	4.82	14.0
Max	310	3660	310	3660	310	291
n (Samp)	152	18	152	17	152	10
n (Patient)	152	18	152	17	152	10

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.51	0.43	0.52	0.51	0.43	0.51	0.49	0.40	0.45
SE	0.060	0.079	0.073	0.061	0.079	0.074	0.074	0.088	0.097
p	0.82	0.36	0.82	0.88	0.36	0.92	0.91	0.26	0.61
nCohort 1	148	287	152	148	287	152	148	287	152
nCohort 2	28	15	18	27	15	17	17	12	10
Cutoff 1	37.9	31.4	37.9	35.1	31.4	35.8	30.7	27.9	27.9

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Sens 1	71%	73%	72%	70%	73%	71%	71%	75%	70%
Spec 1	30%	17%	32%	26%	17%	28%	22%	14%	18%
Cutoff 2	31.4	29.8	32.7	31.4	29.8	32.7	22.7	23.1	22.7
Sens 2	82%	80%	83%	81%	80%	82%	82%	83%	80%
Spec 2	23%	15%	26%	23%	15%	26%	14%	11%	14%
Cutoff 3	16.1	16.1	16.1	16.1	16.1	16.1	16.1	18.8	16.1
Sens 3	93%	93%	94%	93%	93%	94%	94%	92%	90%
Spec 3	7%	6%	9%	7%	6%	9%	7%	8%	9%
Cutoff 4	81.1	90.9	81.5	81.1	90.9	81.5	81.1	90.9	81.5
Sens 4	36%	27%	33%	37%	27%	35%	35%	25%	30%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	97.5	117	102	97.5	117	102	97.5	117	102
Sens 5	32%	27%	28%	33%	27%	29%	29%	25%	20%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	143	161	145	143	161	145	143	161	145
Sens 6	14%	7%	11%	15%	7%	12%	18%	8%	10%
Spec 6	91%	90%	90%	91%	90%	90%	91%	90%	90%
OR Quart 2	1.4	0.24	2.5	1.3	0.24	2.6	0.38	0.32	0.32
p Value	0.58	0.21	0.20	0.62	0.21	0.19	0.26	0.33	0.34
95% CI of	0.46	0.027	0.61	0.44	0.027	0.62	0.069	0.033	0.032
OR Quart2	4.0	2.2	11	3.9	2.2	11	2.1	3.2	3.3
OR Quart 3	0.39	1.3	0.65	0.24	1.3	0.32	0.80	0.66	0.65
p Value	0.19	0.73	0.65	0.091	0.73	0.33	0.75	0.65	0.65
95% CI of	0.093	0.33	0.10	0.048	0.33	0.032	0.20	0.11	0.10
OR Quart3	1.6	4.9	4.1	1.3	4.9	3.2	3.2	4.1	4.1
OR Quart 4	1.4	1.3	2.1	1.3	1.3	2.1	1.3	2.1	1.4
p Value	0.58	0.72	0.32	0.62	0.72	0.32	0.71	0.30	0.67
95% CI of	0.46	0.33	0.49	0.44	0.33	0.49	0.35	0.51	0.29
OR Quart4	4.0	5.0	9.1	3.9	5.0	9.1	4.5	8.8	6.7

60 kDa heat shock protein, mitochondrial

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	143	509	143	509	143	294
Average	615	549	615	549	615	330
Stdev	1430	422	1430	422	1430	347
p(t-test)		0.91		0.91		0.73
Min	2.53	2.53	2.53	2.53	2.53	2.53
Max	8920	1090	8920	1090	8920	693
n (Samp)	41	7	41	7	41	3
n (Patient)	41	7	41	7	41	3

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	193	786	193	786	nd	nd
Average	594	666	594	666	nd	nd
Stdev	1180	517	1180	517	nd	nd

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
p(t-test)		0.90		0.90	nd	nd
Min	2.53	2.53	2.53	2.53	nd	nd
Max	8920	1090	8920	1090	nd	nd
n (Samp)	71	4	71	4	nd	nd
n (Patient)	71	4	71	4	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	91.0	244	91.0	244	91.0	294
Average	624	296	624	296	624	330
Stdev	1540	291	1540	291	1540	347
p(t-test)		0.68		0.68		0.75
Min	2.53	2.53	2.53	2.53	2.53	2.53
Max	8920	693	8920	693	8920	693
n (Samp)	35	4	35	4	35	3
n (Patient)	35	4	35	4	35	3

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.63	0.59	0.51	0.63	0.59	0.51	0.47	nd	0.48
SE	0.12	0.15	0.16	0.12	0.15	0.16	0.18	nd	0.18
p	0.30	0.56	0.93	0.30	0.56	0.93	0.87	nd	0.91
nCohort 1	41	71	35	41	71	35	41	nd	35
nCohort 2	7	4	4	7	4	4	3	nd	3
Cutoff 1	243	453	143	243	453	143	0	nd	0
Sens 1	71%	75%	75%	71%	75%	75%	100%	nd	100%
Spec 1	61%	68%	57%	61%	68%	57%	0%	nd	0%
Cutoff 2	143	0	0	143	0	0	0	nd	0
Sens 2	86%	100%	100%	86%	100%	100%	100%	nd	100%
Spec 2	51%	0%	0%	51%	0%	0%	0%	nd	0%
Cutoff 3	0	0	0	0	0	0	0	nd	0
Sens 3	100%	100%	100%	100%	100%	100%	100%	nd	100%
Spec 3	0%	0%	0%	0%	0%	0%	0%	nd	0%
Cutoff 4	453	509	379	453	509	379	453	nd	379
Sens 4	57%	50%	25%	57%	50%	25%	33%	nd	33%
Spec 4	71%	70%	71%	71%	70%	71%	71%	nd	71%
Cutoff 5	894	904	894	894	904	894	894	nd	894
Sens 5	29%	50%	0%	29%	50%	0%	0%	nd	0%
Spec 5	83%	80%	80%	83%	80%	80%	83%	nd	80%
Cutoff 6	1240	1240	1240	1240	1240	1240	1240	nd	1240
Sens 6	0%	0%	0%	0%	0%	0%	0%	nd	0%
Spec 6	90%	90%	91%	90%	90%	91%	90%	nd	91%
OR Quart 2	0	0	0	0	0	0	1.0	nd	1.1
p Value	na	na	na	na	na	na	1.0	nd	0.94
95% CI of	na	na	na	na	na	na	0.055	nd	0.060
OR Quart2	na	na	na	na	na	na	18	nd	21
OR Quart 3	5.5	0.94	2.0	5.5	0.94	2.0	0	nd	0

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
p Value	0.16	0.97	0.60	0.16	0.97	0.60	na	nd	na
95% CI of	0.51	0.055	0.15	0.51	0.055	0.15	na	nd	na
OR Quart3	59	16	27	59	16	27	na	nd	na
OR Quart 4	2.2	2.0	0.89	2.2	2.0	0.89	1.0	nd	1.1
p Value	0.54	0.59	0.94	0.54	0.59	0.94	1.0	nd	0.94
95% CI of	0.17	0.17	0.047	0.17	0.17	0.047	0.055	nd	0.060
OR Quart4	28	24	17	28	24	17	18	nd	21

WAP four-disulfide core domain protein 2

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	378000	1040000	378000	1040000	378000	1040000
Average	841000	1440000	841000	1440000	841000	1080000
Stdev	1080000	886000	1080000	886000	1080000	333000
p(t-test)		0.18		0.18		0.71
Min	23500	768000	23500	768000	23500	768000
Max	5640000	3230000	5640000	3230000	5640000	1430000
n (Samp)	41	7	41	7	41	3
n (Patient)	41	7	41	7	41	3

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	803000	886000	803000	886000	nd	nd
Average	1250000	913000	1250000	913000	nd	nd
Stdev	1580000	113000	1580000	113000	nd	nd
p(t-test)		0.71		0.71	nd	nd
Min	23500	816000	23500	816000	nd	nd
Max	7500000	1040000	7500000	1040000	nd	nd
n (Samp)	73	3	73	3	nd	nd
n (Patient)	73	3	73	3	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	428000	1430000	428000	1430000	428000	1040000
Average	604000	1670000	604000	1670000	604000	1080000
Stdev	490000	968000	490000	968000	490000	333000
p(t-test)		3.2E-4		3.2E-4		0.11
Min	23500	768000	23500	768000	23500	768000
Max	1650000	3230000	1650000	3230000	1650000	1430000
n (Samp)	34	5	34	5	34	3
n (Patient)	34	5	34	5	34	3

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.77	0.57	0.86	0.77	0.57	0.86	0.72	nd	0.77
SE	0.11	0.18	0.11	0.11	0.18	0.11	0.17	nd	0.16

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
p	0.014	0.69	6.9E-4	0.014	0.69	6.9E-4	0.21	nd	0.095
nCohort 1	41	73	34	41	73	34	41	nd	34
nCohort 2	7	3	5	7	3	5	3	nd	3
Cutoff 1	866000	804000	1020000	866000	804000	1020000	645000	nd	645000
Sens 1	71%	100%	80%	71%	100%	80%	100%	nd	100%
Spec 1	71%	52%	79%	71%	52%	79%	61%	nd	65%
Cutoff 2	804000	804000	1020000	804000	804000	1020000	645000	nd	645000
Sens 2	86%	100%	80%	86%	100%	80%	100%	nd	100%
Spec 2	66%	52%	79%	66%	52%	79%	61%	nd	65%
Cutoff 3	645000	804000	645000	645000	804000	645000	645000	nd	645000
Sens 3	100%	100%	100%	100%	100%	100%	100%	nd	100%
Spec 3	61%	52%	65%	61%	52%	65%	61%	nd	65%
Cutoff 4	866000	1290000	804000	866000	1290000	804000	866000	nd	804000
Sens 4	71%	0%	80%	71%	0%	80%	67%	nd	67%
Spec 4	71%	71%	71%	71%	71%	71%	71%	nd	71%
Cutoff 5	1320000	1650000	1050000	1320000	1650000	1050000	1320000	nd	1050000
Sens 5	43%	0%	60%	43%	0%	60%	33%	nd	33%
Spec 5	80%	81%	82%	80%	81%	82%	80%	nd	82%
Cutoff 6	1690000	3080000	1470000	1690000	3080000	1470000	1690000	nd	1470000
Sens 6	29%	0%	40%	29%	0%	40%	0%	nd	0%
Spec 6	90%	90%	91%	90%	90%	91%	90%	nd	91%
OR Quart 2	>0	>0	>0	>0	>0	>0	>0	nd	>0
p Value	<na	<na	<na	<na	<na	<na	<na	nd	<na
95% CI of	>na	>na	>na	>na	>na	>na	>na	nd	>na
OR Quart2	na	na	na	na	na	na	na	nd	na
OR Quart 3	>6.0	>3.6	>2.2	>6.0	>3.6	>2.2	>2.4	nd	>1.1
p Value	<0.14	<0.29	<0.54	<0.14	<0.29	<0.54	<0.49	nd	<0.94
95% CI of	>0.56	>0.34	>0.17	>0.56	>0.34	>0.17	>0.19	nd	>0.060
OR Quart3	na	na	na	na	na	na	na	nd	na
OR Quart 4	>4.0	>0	>3.9	>4.0	>0	>3.9	>1.1	nd	>2.2
p Value	<0.26	<na	<0.28	<0.26	<na	<0.28	<0.95	nd	<0.54
95% CI of	>0.35	>na	>0.33	>0.35	>na	>0.33	>0.060	nd	>0.17
OR Quart4	na	na	na	na	na	na	na	nd	na

Choriogonadotropin subunit beta

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.288	0.413	0.288	0.413	0.288	0.327
Average	1.03	0.903	1.03	0.903	1.03	0.287
Stdev	3.71	1.32	3.71	1.32	3.71	0.0828
p(t-test)		0.93		0.93		0.73
Min	0.0754	0.168	0.0754	0.168	0.0754	0.191
Max	24.9	4.13	24.9	4.13	24.9	0.341
n (Samp)	44	8	44	8	44	3
n (Patient)	44	8	44	8	44	3

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

	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.321	0.655	0.321	0.655	nd	nd
Average	0.831	1.44	0.831	1.44	nd	nd
Stdev	2.87	1.80	2.87	1.80	nd	nd
p(t-test)		0.67		0.67	nd	nd
Min	0.0754	0.341	0.0754	0.341	nd	nd
Max	24.9	4.13	24.9	4.13	nd	nd
n (Samp)	76	4	76	4	nd	nd
n (Patient)	76	4	76	4	nd	nd

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.271	0.327	0.271	0.327	0.271	0.327
Average	0.620	0.357	0.620	0.357	0.620	0.287
Stdev	1.09	0.237	1.09	0.237	1.09	0.0828
p(t-test)		0.60		0.60		0.60
Min	0.0754	0.168	0.0754	0.168	0.0754	0.191
Max	6.45	0.758	6.45	0.758	6.45	0.341
n (Samp)	37	5	37	5	37	3
n (Patient)	37	5	37	5	37	3

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.62	0.76	0.51	0.62	0.76	0.51	0.46	nd	0.48
SE	0.11	0.14	0.14	0.11	0.14	0.14	0.18	nd	0.18
p	0.28	0.067	0.92	0.28	0.067	0.92	0.83	nd	0.90
nCohort 1	44	76	37	44	76	37	44	nd	37
nCohort 2	8	4	5	8	4	5	3	nd	3
Cutoff 1	0.305	0.481	0.180	0.305	0.481	0.180	0.180	nd	0.180
Sens 1	75%	75%	80%	75%	75%	80%	100%	nd	100%
Spec 1	52%	71%	32%	52%	71%	32%	32%	nd	32%
Cutoff 2	0.180	0.337	0.180	0.180	0.337	0.180	0.180	nd	0.180
Sens 2	88%	100%	80%	88%	100%	80%	100%	nd	100%
Spec 2	32%	51%	32%	32%	51%	32%	32%	nd	32%
Cutoff 3	0.156	0.337	0.156	0.156	0.337	0.156	0.180	nd	0.180
Sens 3	100%	100%	100%	100%	100%	100%	100%	nd	100%
Spec 3	27%	51%	30%	27%	51%	30%	32%	nd	32%
Cutoff 4	0.481	0.481	0.437	0.481	0.481	0.437	0.481	nd	0.437
Sens 4	50%	75%	20%	50%	75%	20%	0%	nd	0%
Spec 4	70%	71%	70%	70%	71%	70%	70%	nd	70%
Cutoff 5	0.709	0.752	0.642	0.709	0.752	0.642	0.709	nd	0.642
Sens 5	38%	50%	20%	38%	50%	20%	0%	nd	0%
Spec 5	82%	80%	81%	82%	80%	81%	82%	nd	81%
Cutoff 6	1.32	1.31	1.34	1.32	1.31	1.34	1.32	nd	1.34
Sens 6	12%	25%	0%	12%	25%	0%	0%	nd	0%
Spec 6	91%	91%	92%	91%	91%	92%	91%	nd	92%
OR Quart 2	>3.9	>0	>2.2	>3.9	>0	>2.2	>2.4	nd	>2.5
p Value	<0.27	<na	<0.54	<0.27	<na	<0.54	<0.50	nd	<0.49
95% CI of	>0.35	>na	>0.17	>0.35	>na	>0.17	>0.19	nd	>0.19

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
OR Quart2	na	na	na	na	na	na	na	nd	na
OR Quart 3	>2.4	>2.2	>2.5	>2.4	>2.2	>2.5	>1.1	nd	>1.1
p Value	<0.51	<0.53	<0.49	<0.51	<0.53	<0.49	<0.95	nd	<0.94
95% CI of	>0.19	>0.19	>0.19	>0.19	>0.19	>0.19	>0.061	nd	>0.060
OR Quart3	na	na	na	na	na	na	na	nd	na
OR Quart 4	>3.9	>2.2	>1.0	>3.9	>2.2	>1.0	>0	nd	>0
p Value	<0.27	<0.53	<1.0	<0.27	<0.53	<1.0	<na	nd	<na
95% CI of	>0.35	>0.19	>0.055	>0.35	>0.19	>0.055	>na	nd	>na
OR Quart4	na	na	na	na	na	na	na	nd	na

[0154] Table 4: Comparison of marker levels in EDTA samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0) and in EDTA samples collected from subjects at 0, 24 hours, and 48 hours prior to reaching stage R, I or F in Cohort 2.

Placenta growth factor

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	9.39	11.0	9.39	11.7	9.39	9.53
Average	12.7	12.8	12.7	13.8	12.7	11.1
Stdev	12.9	7.53	12.9	12.1	12.9	6.42
p(t-test)		0.97		0.57		0.64
Min	1.63	2.26	1.63	1.38	1.63	2.93
Max	144	42.0	144	77.3	144	26.3
n (Samp)	156	70	156	54	156	15
n (Patient)	87	70	87	54	87	15

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	10.0	12.6	10.0	10.6	10.0	16.1
Average	12.0	15.2	12.0	13.5	12.0	16.3
Stdev	10.4	10.2	10.4	9.98	10.4	4.49
p(t-test)		0.21		0.65		0.28
Min	0.000223	3.42	0.000223	1.38	0.000223	11.1
Max	144	42.0	144	37.5	144	25.3
n (Samp)	373	18	373	11	373	7
n (Patient)	174	18	174	11	174	7

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	10.7	10.7	10.7	11.7	10.7	10.3
Average	14.2	11.9	14.2	13.2	14.2	12.2
Stdev	14.0	7.10	14.0	11.3	14.0	7.18
p(t-test)		0.22		0.63		0.56
Min	1.63	2.26	1.63	1.38	1.63	2.93

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Max	144	42.0	144	77.3	144	26.3
n (Samp)	181	63	181	59	181	18
n (Patient)	88	63	88	59	88	18

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.54	0.60	0.48	0.54	0.54	0.49	0.48	0.75	0.48
SE	0.042	0.072	0.043	0.046	0.090	0.043	0.079	0.11	0.072
p	0.35	0.16	0.58	0.41	0.62	0.88	0.78	0.020	0.78
nCohort 1	156	373	181	156	373	181	156	373	181
nCohort 2	70	18	63	54	11	59	15	7	18
Cutoff 1	8.42	9.29	7.11	8.93	7.24	7.57	6.68	14.4	6.68
Sens 1	70%	72%	71%	70%	73%	71%	73%	71%	72%
Spec 1	42%	47%	29%	47%	32%	31%	26%	72%	25%
Cutoff 2	6.23	6.79	5.92	5.92	5.67	5.92	4.74	13.2	4.74
Sens 2	80%	83%	81%	81%	82%	81%	80%	86%	83%
Spec 2	21%	29%	17%	18%	20%	17%	10%	66%	9%
Cutoff 3	4.49	4.74	4.49	3.90	5.37	3.50	3.90	10.9	3.90
Sens 3	91%	94%	90%	91%	91%	92%	93%	100%	94%
Spec 3	9%	14%	8%	8%	18%	7%	8%	55%	8%
Cutoff 4	14.4	14.2	15.8	14.4	14.2	15.8	14.4	14.2	15.8
Sens 4	34%	50%	22%	26%	36%	24%	33%	71%	39%
Spec 4	71%	70%	70%	71%	70%	70%	71%	70%	70%
Cutoff 5	18.0	16.7	19.1	18.0	16.7	19.1	18.0	16.7	19.1
Sens 5	17%	39%	11%	20%	27%	14%	7%	29%	22%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	22.0	21.0	25.0	22.0	21.0	25.0	22.0	21.0	25.0
Sens 6	11%	22%	5%	13%	18%	7%	7%	14%	6%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	0.74	1.7	2.2	0.68	0.33	2.6	1.8	>0	0.78
p Value	0.48	0.48	0.064	0.44	0.34	0.027	0.46	<na	0.73
95% CI of	0.32	0.39	0.95	0.26	0.033	1.1	0.39	>na	0.20
OR Quart2	1.7	7.3	5.2	1.8	3.2	6.0	7.9	na	3.1
OR Quart 3	1.9	0.99	1.6	2.1	1.0	1.1	1.0	>3.1	0.78
p Value	0.12	0.99	0.28	0.092	1.0	0.82	1.0	<0.33	0.73
95% CI of	0.86	0.19	0.68	0.89	0.20	0.45	0.19	>0.32	0.20
OR Quart3	4.1	5.0	3.8	4.9	5.1	2.8	5.3	na	3.1
OR Quart 4	1.1	2.4	1.6	1.1	1.3	1.4	1.4	>4.2	1.0
p Value	0.88	0.21	0.28	0.86	0.70	0.50	0.67	<0.20	0.97
95% CI of	0.47	0.60	0.68	0.44	0.29	0.56	0.29	>0.46	0.28
OR Quart4	2.4	9.6	3.8	2.7	6.2	3.3	6.7	na	3.8

60 kDa heat shock protein, mitochondrial

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1240	1550	1240	1460	1240	838
Average	2080	9240	2080	3190	2080	1040

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Stdev	2850	28900	2850	4990	2850	579
p(t-test)		0.073		0.22		0.28
Min	35.1	128	35.1	300	35.1	221
Max	15000	110000	15000	24700	15000	1920
n (Samp)	54	14	54	24	54	9
n (Patient)	53	14	53	24	53	9

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1120	1640	1120	1020	1120	1020
Average	2960	1800	2960	1020	2960	896
Stdev	10700	1160	10700	132	10700	474
p(t-test)		0.85		0.80		0.74
Min	2.11	727	2.11	930	2.11	371
Max	110000	3020	110000	1120	110000	1290
n (Samp)	111	3	111	2	111	3
n (Patient)	93	3	93	2	93	3

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1330	1790	1330	1640	1330	838
Average	2110	11100	2110	3980	2110	1040
Stdev	2980	31100	2980	6280	2980	579
p(t-test)		0.047		0.088		0.29
Min	35.1	128	35.1	300	35.1	221
Max	15000	110000	15000	24700	15000	1920
n (Samp)	48	12	48	25	48	9
n (Patient)	44	12	44	25	44	9

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.51	0.59	0.54	0.59	0.47	0.61	0.38	0.38	0.38
SE	0.088	0.18	0.095	0.071	0.21	0.071	0.11	0.18	0.11
p	0.90	0.61	0.69	0.21	0.87	0.13	0.28	0.51	0.28
nCohort 1	54	111	48	54	111	48	54	111	48
nCohort 2	14	3	12	24	2	25	9	3	9
Cutoff 1	618	618	558	838	838	838	727	300	727
Sens 1	71%	100%	75%	71%	100%	72%	78%	100%	78%
Spec 1	22%	25%	21%	39%	43%	38%	30%	12%	33%
Cutoff 2	221	618	221	831	838	831	221	300	221
Sens 2	86%	100%	83%	83%	100%	84%	89%	100%	89%
Spec 2	6%	25%	2%	31%	43%	33%	6%	12%	2%
Cutoff 3	128	618	128	618	838	618	35.1	300	35.1
Sens 3	93%	100%	92%	92%	100%	92%	100%	100%	100%
Spec 3	4%	25%	2%	22%	43%	25%	4%	12%	2%
Cutoff 4	1960	1960	1960	1960	1960	1960	1960	1960	1960
Sens 4	43%	33%	50%	42%	0%	44%	0%	0%	0%

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Spec 4	70%	73%	73%	70%	73%	73%	70%	73%	73%
Cutoff 5	2780	2520	2460	2780	2520	2460	2780	2520	2460
Sens 5	21%	33%	42%	25%	0%	44%	0%	0%	0%
Spec 5	81%	82%	81%	81%	82%	81%	81%	82%	81%
Cutoff 6	3480	3360	3480	3480	3360	3480	3480	3360	3480
Sens 6	7%	0%	17%	21%	0%	24%	0%	0%	0%
Spec 6	91%	90%	92%	91%	90%	92%	91%	90%	92%
OR Quart 2	0.43	>1.0	0.62	4.4	>1.1	5.0	>5.3	>1.1	>6.0
p Value	0.38	<1.0	0.63	0.057	<0.96	0.041	<0.16	<0.96	<0.13
95% CI of	0.068	>0.060	0.087	0.96	>0.064	1.1	>0.53	>0.064	>0.58
OR Quart2	2.8	na	4.3	20	na	23	na	na	na
OR Quart 3	0.70	>1.0	0.62	1.4	>1.1	1.0	>3.7	>1.0	>4.1
p Value	0.67	<0.98	0.63	0.68	<0.96	1.0	<0.28	<0.98	<0.25
95% CI of	0.13	>0.062	0.087	0.27	>0.064	0.17	>0.34	>0.062	>0.37
OR Quart3	3.7	na	4.3	7.4	na	5.8	na	na	na
OR Quart 4	1.4	>1.0	2.0	3.6	>0	5.6	>2.5	>1.1	>2.5
p Value	0.70	<1.0	0.41	0.10	<na	0.028	<0.48	<0.96	<0.48
95% CI of	0.29	>0.060	0.38	0.77	>na	1.2	>0.20	>0.064	>0.20
OR Quart4	6.3	na	11	16	na	26	na	na	na

Heat shock protein beta-1 (phospho SER78 / phospho SER82)

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	18.5	40.7	18.5	30.4	18.5	52.4
Average	46.1	61.1	46.1	64.3	46.1	56.5
Stdev	70.6	67.2	70.6	74.6	70.6	52.7
p(t-test)		0.48		0.31		0.68
Min	0.00141	0.00632	0.00141	0.00632	0.00141	0.193
Max	311	233	311	264	311	164
n (Samp)	54	14	54	24	54	9
n (Patient)	53	14	53	24	53	9

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	21.9	42.7	21.9	46.9	21.9	61.7
Average	47.6	80.2	47.6	46.9	47.6	92.7
Stdev	65.4	68.5	65.4	30.0	65.4	89.6
p(t-test)		0.40		0.99		0.24
Min	0.00141	38.7	0.00141	25.7	0.00141	22.7
Max	311	159	311	68.1	311	194
n (Samp)	111	3	111	2	111	3
n (Patient)	93	3	93	2	93	3

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	17.9	35.3	17.9	29.3	17.9	22.7
Average	46.6	54.5	46.6	62.3	46.6	49.6

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Stdev	73.2	66.3	73.2	73.7	73.2	55.8
p(t-test)		0.74		0.39		0.91
Min	0.00141	0.00632	0.00141	0.00632	0.00141	0.00141
Max	311	233	311	264	311	164
n (Samp)	48	12	48	25	48	9
n (Patient)	44	12	44	25	44	9

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.61	0.76	0.58	0.62	0.65	0.63	0.62	0.74	0.55
SE	0.088	0.16	0.095	0.071	0.21	0.071	0.11	0.17	0.11
p	0.19	0.11	0.39	0.084	0.47	0.073	0.26	0.15	0.67
nCohort 1	54	111	48	54	111	48	54	111	48
nCohort 2	14	3	12	24	2	25	9	3	9
Cutoff 1	26.7	38.1	17.7	21.3	24.1	20.2	13.6	22.2	12.4
Sens 1	71%	100%	75%	71%	100%	72%	78%	100%	78%
Spec 1	61%	67%	50%	54%	54%	58%	43%	52%	42%
Cutoff 2	6.11	38.1	6.11	9.68	24.1	15.0	12.4	22.2	0.00141
Sens 2	86%	100%	83%	83%	100%	80%	89%	100%	89%
Spec 2	24%	67%	27%	35%	54%	48%	39%	52%	2%
Cutoff 3	3.81	38.1	3.81	7.77	24.1	7.77	0.00632	22.2	0
Sens 3	93%	100%	92%	92%	100%	92%	100%	100%	100%
Spec 3	17%	67%	17%	28%	54%	31%	4%	52%	0%
Cutoff 4	38.9	51.3	55.6	38.9	51.3	55.6	38.9	51.3	55.6
Sens 4	50%	33%	25%	38%	50%	28%	56%	67%	33%
Spec 4	70%	70%	71%	70%	70%	71%	70%	70%	71%
Cutoff 5	69.8	71.7	69.8	69.8	71.7	69.8	69.8	71.7	69.8
Sens 5	29%	33%	25%	25%	0%	24%	33%	33%	33%
Spec 5	81%	80%	81%	81%	80%	81%	81%	80%	81%
Cutoff 6	102	122	102	102	122	102	102	122	102
Sens 6	21%	33%	17%	21%	0%	20%	22%	33%	22%
Spec 6	91%	90%	92%	91%	90%	92%	91%	90%	92%
OR Quart 2	0.29	>0	1.0	1.8	>0	1.9	2.0	>0	1.0
p Value	0.31	<na	1.0	0.48	<na	0.43	0.59	<na	1.0
95% CI of	0.027	>na	0.12	0.36	>na	0.38	0.16	>na	0.12
OR Quart2	3.1	na	8.2	8.8	na	9.6	25	na	8.3
OR Quart 3	2.5	>2.2	3.2	4.8	>1.0	6.2	3.2	>2.2	1.0
p Value	0.25	<0.54	0.21	0.044	<0.98	0.020	0.34	<0.54	1.0
95% CI of	0.52	>0.18	0.52	1.0	>0.062	1.3	0.30	>0.18	0.12
OR Quart3	13	na	20	22	na	29	35	na	8.3
OR Quart 4	1.4	>1.0	1.6	2.9	>1.0	2.9	3.2	>1.0	1.5
p Value	0.67	<1.0	0.63	0.18	<1.0	0.18	0.34	<1.0	0.69
95% CI of	0.27	>0.060	0.23	0.62	>0.060	0.62	0.30	>0.060	0.21
OR Quart4	7.7	na	11	13	na	14	35	na	11

Choriogonadotropin subunit beta

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

	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.254	0.239	0.254	0.209	0.254	0.241
Average	0.279	0.219	0.279	0.205	0.279	0.236
Stdev	0.153	0.0768	0.153	0.0718	0.153	0.0779
p(t-test)		0.16		0.025		0.41
Min	3.21E-5	0.0146	3.21E-5	0.0425	3.21E-5	0.0891
Max	0.958	0.311	0.958	0.325	0.958	0.368
n (Samp)	54	14	54	24	54	9
n (Patient)	53	14	53	24	53	9

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.243	0.132	0.243	0.237	0.243	0.210
Average	0.254	0.144	0.254	0.237	0.254	0.207
Stdev	0.121	0.136	0.121	0.124	0.121	0.163
p(t-test)		0.12		0.85		0.51
Min	3.21E-5	0.0146	3.21E-5	0.149	3.21E-5	0.0425
Max	0.958	0.285	0.958	0.325	0.958	0.368
n (Samp)	111	3	111	2	111	3
n (Patient)	93	3	93	2	93	3

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.243	0.239	0.243	0.211	0.243	0.241
Average	0.273	0.239	0.273	0.212	0.273	0.238
Stdev	0.153	0.0410	0.153	0.0787	0.153	0.0774
p(t-test)		0.45		0.066		0.51
Min	3.21E-5	0.152	3.21E-5	0.0425	3.21E-5	0.0891
Max	0.958	0.311	0.958	0.382	0.958	0.368
n (Samp)	48	12	48	25	48	9
n (Patient)	44	12	44	25	44	9

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.42	0.29	0.49	0.34	0.50	0.38	0.44	0.41	0.48
SE	0.088	0.17	0.094	0.070	0.21	0.071	0.11	0.18	0.11
p	0.39	0.22	0.91	0.022	1.0	0.094	0.58	0.62	0.84
nCohort 1	54	111	48	54	111	48	54	111	48
nCohort 2	14	3	12	24	2	25	9	3	9
Cutoff 1	0.210	3.21E-5	0.216	0.162	0.142	0.162	0.202	0.0357	0.202
Sens 1	71%	100%	75%	71%	100%	72%	78%	100%	78%
Spec 1	33%	1%	40%	15%	13%	15%	22%	2%	27%
Cutoff 2	0.142	3.21E-5	0.210	0.131	0.142	0.142	0.162	0.0357	0.162
Sens 2	86%	100%	83%	83%	100%	80%	89%	100%	89%
Spec 2	13%	1%	35%	11%	13%	12%	15%	2%	15%
Cutoff 3	0.131	3.21E-5	0.189	0.101	0.142	0.101	3.21E-5	0.0357	3.21E-5
Sens 3	93%	100%	92%	92%	100%	92%	100%	100%	100%
Spec 3	11%	1%	21%	4%	13%	2%	2%	2%	2%
Cutoff 4	0.289	0.266	0.281	0.289	0.266	0.281	0.289	0.266	0.281

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Sens 4	7%	33%	8%	12%	50%	16%	22%	33%	22%
Spec 4	72%	70%	71%	72%	70%	71%	72%	70%	71%
Cutoff 5	0.354	0.296	0.296	0.354	0.296	0.296	0.354	0.296	0.296
Sens 5	0%	0%	8%	0%	50%	16%	11%	33%	11%
Spec 5	81%	81%	81%	81%	81%	81%	81%	81%	81%
Cutoff 6	0.429	0.373	0.438	0.429	0.373	0.438	0.429	0.373	0.438
Sens 6	0%	0%	0%	0%	0%	0%	0%	0%	0%
Spec 6	91%	90%	92%	91%	90%	92%	91%	90%	92%
OR Quart 2	6.7	0	7.0	2.0	0	1.1	1.0	0	1.1
p Value	0.10	na	0.097	0.39	na	0.92	1.0	na	0.94
95% CI of	0.69	na	0.71	0.41	na	0.25	0.12	na	0.13
OR Quart2	65	na	69	10.0	na	4.6	8.1	na	8.9
OR Quart 3	4.9	0	5.1	3.1	0	1.4	1.6	1.0	1.8
p Value	0.18	na	0.17	0.15	na	0.64	0.63	1.0	0.57
95% CI of	0.49	na	0.50	0.66	na	0.34	0.23	0.060	0.25
OR Quart3	50	na	52	14	na	5.8	11	17	13
OR Quart 4	4.9	2.2	2.2	5.1	1.0	2.8	1.1	1.0	1.1
p Value	0.18	0.54	0.55	0.036	0.98	0.14	0.94	0.98	0.94
95% CI of	0.49	0.18	0.17	1.1	0.062	0.71	0.13	0.062	0.13
OR Quart4	50	25	27	23	17	11	8.8	17	8.9

WAP four-disulfide core domain protein 2

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	5290	4540	5290	5990	5290	6710
Average	8940	8500	8940	14400	8940	12400
Stdev	8910	9550	8910	17700	8910	12400
p(t-test)		0.87		0.072		0.31
Min	1830	1530	1830	1070	1830	4320
Max	41700	37800	41700	63700	41700	34700
n (Samp)	54	14	54	24	54	9
n (Patient)	53	14	53	24	53	9

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	5630	3730	5630	7230	5630	4630
Average	10900	3240	10900	7230	10900	3850
Stdev	12000	1530	12000	1060	12000	2480
p(t-test)		0.27		0.66		0.31
Min	1530	1530	1530	6480	1530	1070
Max	63700	4470	63700	7980	63700	5840
n (Samp)	111	3	111	2	111	3
n (Patient)	93	3	93	2	93	3

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	4890	7060	4890	6480	4890	6710

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Average	8240	10300	8240	14300	8240	12400
Stdev	7900	9980	7900	17300	7900	12400
p(t-test)		0.44		0.042		0.20
Min	1540	2420	1540	1070	1540	4260
Max	36700	37800	36700	63700	36700	34700
n (Samp)	48	12	48	25	48	9
n (Patient)	44	12	44	25	44	9

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.46	0.21	0.56	0.58	0.57	0.60	0.63	0.31	0.64
SE	0.088	0.16	0.095	0.072	0.21	0.071	0.11	0.17	0.11
p	0.68	0.068	0.54	0.28	0.75	0.16	0.22	0.26	0.18
nCohort 1	54	111	48	54	111	48	54	111	48
nCohort 2	14	3	12	24	2	25	9	3	9
Cutoff 1	3400	0	3400	4190	6380	4190	4630	0	4630
Sens 1	71%	100%	75%	75%	100%	76%	78%	100%	78%
Spec 1	24%	0%	23%	41%	53%	42%	48%	0%	50%
Cutoff 2	2900	0	3310	4000	6380	4020	4560	0	4260
Sens 2	86%	100%	83%	83%	100%	80%	89%	100%	89%
Spec 2	17%	0%	21%	37%	53%	38%	48%	0%	42%
Cutoff 3	2330	0	2850	2150	6380	2150	4300	0	4190
Sens 3	93%	100%	92%	92%	100%	92%	100%	100%	100%
Spec 3	6%	0%	12%	4%	53%	4%	43%	0%	42%
Cutoff 4	9940	10700	8200	9940	10700	8200	9940	10700	8200
Sens 4	21%	0%	50%	33%	0%	40%	22%	0%	33%
Spec 4	70%	70%	71%	70%	70%	71%	70%	70%	71%
Cutoff 5	11900	16100	10700	11900	16100	10700	11900	16100	10700
Sens 5	21%	0%	33%	33%	0%	36%	22%	0%	22%
Spec 5	81%	80%	81%	81%	80%	81%	81%	80%	81%
Cutoff 6	19100	26500	19100	19100	26500	19100	19100	26500	19100
Sens 6	7%	0%	8%	21%	0%	20%	22%	0%	22%
Spec 6	91%	90%	92%	91%	90%	92%	91%	90%	92%
OR Quart 2	1.0	>0	0.20	3.6	>0	3.2	>3.5	>1.1	>3.8
p Value	1.0	<na	0.17	0.10	<na	0.15	<0.31	<0.96	<0.27
95% CI of	0.17	>na	0.019	0.77	>na	0.67	>0.32	>0.064	>0.35
OR Quart2	5.8	na	2.0	16	na	15	na	na	na
OR Quart 3	1.4	>2.1	0.69	1.9	>2.2	2.5	>5.0	>1.0	>5.6
p Value	0.67	<0.54	0.67	0.43	<0.54	0.26	<0.17	<0.98	<0.15
95% CI of	0.27	>0.18	0.12	0.38	>0.18	0.51	>0.49	>0.062	>0.54
OR Quart3	7.7	na	3.8	9.4	na	12	na	na	na
OR Quart 4	1.4	>1.1	1.0	3.6	>0	4.5	>2.1	>1.1	>2.2
p Value	0.67	<0.96	1.0	0.10	<na	0.054	<0.55	<0.96	<0.55
95% CI of	0.27	>0.064	0.20	0.77	>na	0.97	>0.17	>0.064	>0.17
OR Quart4	7.7	na	5.0	16	na	21	na	na	na

[0155] Table 5: Comparison of marker levels in EDTA samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0 or R) and in EDTA samples collected from subjects at 0, 24 hours, and 48 hours prior to reaching stage I or F in Cohort 2.

Placenta growth factor

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	10.7	9.14	10.7	11.7	10.7	12.8
Average	13.3	11.5	13.3	14.5	13.3	13.1
Stdev	11.5	9.76	11.5	15.0	11.5	8.19
p(t-test)		0.42		0.59		0.95
Min	0.313	3.31	0.313	3.85	0.313	1.38
Max	144	54.3	144	77.3	144	26.8
n (Samp)	352	28	352	33	352	22
n (Patient)	174	28	174	33	174	22

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	10.7	13.7	10.7	9.33	10.7	13.3
Average	13.2	13.7	13.2	8.11	13.2	13.8
Stdev	11.5	1.12	11.5	2.60	11.5	8.04
p(t-test)		0.95		0.45		0.90
Min	0.000223	12.9	0.000223	5.12	0.000223	3.42
Max	144	14.5	144	9.87	144	25.3
n (Samp)	474	2	474	3	474	5
n (Patient)	213	2	213	3	213	5

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	10.8	8.99	10.8	11.8	10.8	12.0
Average	13.3	11.3	13.3	15.0	13.3	12.5
Stdev	11.6	9.75	11.6	15.1	11.6	8.13
p(t-test)		0.38		0.42		0.77
Min	0.313	3.31	0.313	3.85	0.313	1.38
Max	144	54.3	144	77.3	144	26.8
n (Samp)	343	28	343	34	343	20
n (Patient)	160	28	160	34	160	20

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.43	0.65	0.42	0.49	0.34	0.51	0.52	0.58	0.50
SE	0.058	0.21	0.058	0.053	0.17	0.052	0.064	0.13	0.067
p	0.23	0.48	0.19	0.86	0.36	0.91	0.73	0.57	0.99
nCohort 1	352	474	343	352	474	343	352	474	343
nCohort 2	28	2	28	33	3	34	22	5	20
Cutoff 1	6.23	12.9	6.23	6.72	5.01	6.74	6.68	10.5	6.68

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Sens 1	71%	100%	71%	73%	100%	71%	73%	80%	70%
Spec 1	21%	62%	21%	24%	14%	24%	23%	49%	24%
Cutoff 2	5.37	12.9	5.37	5.92	5.01	5.92	4.38	10.5	4.20
Sens 2	82%	100%	82%	82%	100%	82%	82%	80%	80%
Spec 2	14%	62%	15%	18%	14%	18%	9%	49%	10%
Cutoff 3	3.60	12.9	3.60	4.74	5.01	4.74	3.35	3.41	3.35
Sens 3	93%	100%	93%	91%	100%	91%	91%	100%	90%
Spec 3	7%	62%	8%	11%	14%	12%	7%	6%	7%
Cutoff 4	15.8	15.0	15.7	15.8	15.0	15.7	15.8	15.0	15.7
Sens 4	21%	0%	21%	27%	0%	29%	36%	40%	35%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	18.1	18.0	18.1	18.1	18.0	18.1	18.1	18.0	18.1
Sens 5	7%	0%	7%	21%	0%	24%	27%	20%	25%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	23.1	22.8	23.1	23.1	22.8	23.1	23.1	22.8	23.1
Sens 6	4%	0%	4%	6%	0%	9%	14%	20%	10%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	2.1	>0	1.8	1.7	>0	0.47	0.13	0.99	1.0
p Value	0.24	<na	0.36	0.31	<na	0.19	0.061	1.00	1.0
95% CI of	0.61	>na	0.51	0.62	>na	0.15	0.016	0.061	0.31
OR Quart2	7.2	na	6.4	4.5	na	1.4	1.1	16	3.2
OR Quart 3	1.3	>2.0	1.5	0.56	>2.1	1.1	1.0	0.99	0.16
p Value	0.73	<0.56	0.52	0.37	<0.56	0.82	1.0	1.00	0.090
95% CI of	0.33	>0.18	0.42	0.16	>0.18	0.45	0.34	0.061	0.019
OR Quart3	4.9	na	5.6	2.0	na	2.8	3.0	16	1.3
OR Quart 4	3.0	>0	3.0	1.7	>1.0	0.77	0.99	2.0	1.2
p Value	0.070	<na	0.067	0.31	<0.99	0.60	0.98	0.57	0.76
95% CI of	0.91	>na	0.93	0.62	>0.063	0.29	0.33	0.18	0.39
OR Quart4	9.7	na	9.9	4.5	na	2.1	2.9	22	3.7

60 kDa heat shock protein, mitochondrial

sCr or UO	24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1120	1640	1120	1070
Average	3100	1960	3100	1860
Stdev	10800	2170	10800	2340
p(t-test)		0.75		0.78
Min	2.11	128	2.11	221
Max	110000	7440	110000	6570
n (Samp)	113	9	113	6
n (Patient)	92	9	92	6

UO only	24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1210	1640	1210	1120
Average	3320	1960	3320	2020
Stdev	11500	2170	11500	2570

UO only	24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
p(t-test)		0.72		0.80
Min	2.11	128	2.11	221
Max	110000	7440	110000	6570
n (Samp)	99	9	99	5
n (Patient)	77	9	77	5

	24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.53	nd	0.53	0.49	nd	0.49
SE	0.10	nd	0.10	0.12	nd	0.13
p	0.79	nd	0.78	0.92	nd	0.92
nCohort 1	113	nd	99	113	nd	99
nCohort 2	9	nd	9	6	nd	5
Cutoff 1	780	nd	780	838	nd	838
Sens 1	78%	nd	78%	83%	nd	80%
Spec 1	32%	nd	33%	43%	nd	43%
Cutoff 2	618	nd	618	838	nd	838
Sens 2	89%	nd	89%	83%	nd	80%
Spec 2	27%	nd	28%	43%	nd	43%
Cutoff 3	35.1	nd	35.1	128	nd	128
Sens 3	100%	nd	100%	100%	nd	100%
Spec 3	4%	nd	4%	6%	nd	6%
Cutoff 4	1960	nd	1960	1960	nd	1960
Sens 4	22%	nd	22%	17%	nd	20%
Spec 4	73%	nd	74%	73%	nd	74%
Cutoff 5	2520	nd	2520	2520	nd	2520
Sens 5	11%	nd	11%	17%	nd	20%
Spec 5	81%	nd	83%	81%	nd	83%
Cutoff 6	3360	nd	3480	3360	nd	3480
Sens 6	11%	nd	11%	17%	nd	20%
Spec 6	90%	nd	91%	90%	nd	91%
OR Quart 2	3.1	nd	3.2	1.0	nd	1.0
p Value	0.34	nd	0.32	1.0	nd	1.0
95% CI of	0.30	nd	0.32	0.060	nd	0.059
OR Quart2	32	nd	33	17	nd	17
OR Quart 3	3.2	nd	3.2	3.2	nd	2.1
p Value	0.32	nd	0.32	0.32	nd	0.56
95% CI of	0.32	nd	0.32	0.32	nd	0.18
OR Quart3	33	nd	33	33	nd	25
OR Quart 4	2.0	nd	2.1	1.0	nd	1.0
p Value	0.58	nd	0.56	0.98	nd	1.0
95% CI of	0.17	nd	0.18	0.062	nd	0.059
OR Quart4	23	nd	24	17	nd	17

Choriogonadotropin subunit beta

sCr or UO	24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2

sCr or UO	24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.249	0.220	0.249	0.158
Average	0.259	0.218	0.259	0.163
Stdev	0.124	0.0580	0.124	0.0818
p(t-test)		0.33		0.067
Min	3.21E-5	0.101	3.21E-5	0.0425
Max	0.958	0.311	0.958	0.281
n (Samp)	113	9	113	6
n (Patient)	92	9	92	6

UO only	24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.243	0.220	0.243	0.167
Average	0.257	0.218	0.257	0.188
Stdev	0.121	0.0580	0.121	0.0630
p(t-test)		0.35		0.21
Min	3.21E-5	0.101	3.21E-5	0.122
Max	0.958	0.311	0.958	0.281
n (Samp)	99	9	99	5
n (Patient)	77	9	77	5

	24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.39	nd	0.39	0.25	nd	0.29
SE	0.10	nd	0.10	0.12	nd	0.13
p	0.27	nd	0.28	0.032	nd	0.11
nCohort 1	113	nd	99	113	nd	99
nCohort 2	9	nd	9	6	nd	5
Cutoff 1	0.198	nd	0.198	0.111	nd	0.142
Sens 1	78%	nd	78%	83%	nd	80%
Spec 1	22%	nd	22%	8%	nd	12%
Cutoff 2	0.185	nd	0.185	0.111	nd	0.142
Sens 2	89%	nd	89%	83%	nd	80%
Spec 2	19%	nd	19%	8%	nd	12%
Cutoff 3	0.0954	nd	0.0954	0.0357	nd	0.111
Sens 3	100%	nd	100%	100%	nd	100%
Spec 3	4%	nd	4%	3%	nd	7%
Cutoff 4	0.285	nd	0.273	0.285	nd	0.273
Sens 4	11%	nd	11%	0%	nd	20%
Spec 4	72%	nd	71%	72%	nd	71%
Cutoff 5	0.304	nd	0.296	0.304	nd	0.296
Sens 5	11%	nd	11%	0%	nd	0%
Spec 5	81%	nd	81%	81%	nd	81%
Cutoff 6	0.384	nd	0.382	0.384	nd	0.382
Sens 6	0%	nd	0%	0%	nd	0%
Spec 6	90%	nd	91%	90%	nd	91%
OR Quart 2	1.0	nd	1.0	>1.0	nd	>1.0
p Value	0.98	nd	1.0	<0.98	nd	<0.98

	24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
95% CI of	0.062	nd	0.059	>0.062	nd	>0.062
OR Quart2	17	nd	17	na	nd	na
OR Quart 3	4.4	nd	4.5	>1.0	nd	>1.0
p Value	0.19	nd	0.19	<0.98	nd	<0.98
95% CI of	0.47	nd	0.47	>0.062	nd	>0.062
OR Quart3	42	nd	43	na	nd	na
OR Quart 4	3.3	nd	3.2	>4.8	nd	>3.4
p Value	0.31	nd	0.32	<0.17	nd	<0.30
95% CI of	0.33	nd	0.32	>0.50	nd	>0.33
OR Quart4	34	nd	33	na	nd	na

[0156] Table 6: Comparison of the maximum marker levels in EDTA samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0) and the maximum values in EDTA samples collected from subjects between enrollment and 0, 24 hours, and 48 hours prior to reaching stage F in Cohort 2.

Placenta growth factor

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	11.2	19.0	11.2	19.0	11.2	19.0
Average	14.7	28.2	14.7	28.2	14.7	21.1
Stdev	16.2	21.6	16.2	21.6	16.2	8.32
p(t-test)		0.014		0.014		0.31
Min	1.69	7.46	1.69	7.46	1.69	9.38
Max	144	77.3	144	77.3	144	32.8
n (Samp)	87	11	87	11	87	7
n (Patient)	87	11	87	11	87	7

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	12.8	19.0	12.8	19.0	12.8	19.0
Average	15.0	20.0	15.0	20.0	15.0	20.0
Stdev	13.6	7.64	13.6	7.64	13.6	7.64
p(t-test)		0.41		0.41		0.41
Min	0.313	9.38	0.313	9.38	0.313	9.38
Max	144	27.9	144	27.9	144	27.9
n (Samp)	174	5	174	5	174	5
n (Patient)	174	5	174	5	174	5

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	12.3	19.0	12.3	19.0	12.3	19.0
Average	17.1	32.8	17.1	32.8	17.1	22.3
Stdev	18.1	26.0	18.1	26.0	18.1	9.35
p(t-test)		0.035		0.035		0.62

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Min	1.69	7.46	1.69	7.46	1.69	15.0
Max	144	77.3	144	77.3	144	32.8
n (Samp)	88	7	88	7	88	3
n (Patient)	88	7	88	7	88	3

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.75	0.72	0.73	0.75	0.72	0.73	0.75	0.72	0.73
SE	0.088	0.13	0.11	0.088	0.13	0.11	0.11	0.13	0.17
p	0.0044	0.091	0.041	0.0044	0.091	0.041	0.022	0.091	0.17
nCohort 1	87	174	88	87	174	88	87	174	88
nCohort 2	11	5	7	11	5	7	7	5	3
Cutoff 1	16.4	16.6	18.3	16.4	16.6	18.3	16.4	16.6	14.5
Sens 1	73%	80%	71%	73%	80%	71%	71%	80%	100%
Spec 1	67%	66%	70%	67%	66%	70%	67%	66%	57%
Cutoff 2	14.5	16.6	14.5	14.5	16.6	14.5	14.5	16.6	14.5
Sens 2	82%	80%	86%	82%	80%	86%	86%	80%	100%
Spec 2	62%	66%	57%	62%	66%	57%	62%	66%	57%
Cutoff 3	9.13	9.13	6.89	9.13	9.13	6.89	9.13	9.13	14.5
Sens 3	91%	100%	100%	91%	100%	100%	100%	100%	100%
Spec 3	44%	34%	25%	44%	34%	25%	44%	34%	57%
Cutoff 4	16.8	17.1	18.3	16.8	17.1	18.3	16.8	17.1	18.3
Sens 4	64%	60%	71%	64%	60%	71%	57%	60%	67%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	19.4	19.4	22.7	19.4	19.4	22.7	19.4	19.4	22.7
Sens 5	45%	40%	43%	45%	40%	43%	43%	40%	33%
Spec 5	80%	80%	81%	80%	80%	81%	80%	80%	81%
Cutoff 6	25.0	24.5	31.4	25.0	24.5	31.4	25.0	24.5	31.4
Sens 6	45%	40%	43%	45%	40%	43%	43%	40%	33%
Spec 6	91%	90%	91%	91%	90%	91%	91%	90%	91%
OR Quart 2	>2.1	>1.0	0	>2.1	>1.0	0	>1.0	>1.0	>0
p Value	<0.56	<1.0	na	<0.56	<1.0	na	<1.0	<1.0	<na
95% CI of	>0.18	>0.061	na	>0.18	>0.061	na	>0.059	>0.061	>na
OR Quart2	na	na	na	na	na	na	na	na	na
OR Quart 3	>4.8	>1.0	3.1	>4.8	>1.0	3.1	>3.4	>1.0	>2.1
p Value	<0.18	<1.0	0.34	<0.18	<1.0	0.34	<0.30	<1.0	<0.56
95% CI of	>0.50	>0.061	0.30	>0.50	>0.061	0.30	>0.33	>0.061	>0.18
OR Quart3	na	na	33	na	na	33	na	na	na
OR Quart 4	>6.0	>3.1	3.1	>6.0	>3.1	3.1	>3.3	>3.1	>1.0
p Value	<0.11	<0.33	0.34	<0.11	<0.33	0.34	<0.32	<0.33	<1.0
95% CI of	>0.65	>0.31	0.30	>0.65	>0.31	0.30	>0.32	>0.31	>0.059
OR Quart4	na	na	33	na	na	33	na	na	na

60 kDa heat shock protein, mitochondrial

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1210	4300	1210	4300

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Average	2090	4750	2090	4750
Stdev	2870	2490	2870	2490
p(t-test)		0.12		0.12
Min	35.1	2520	35.1	2520
Max	15000	7440	15000	7440
n (Samp)	53	3	53	3
n (Patient)	53	3	53	3

UO only	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	1420	4980	1420	4980
Average	2230	4980	2230	4980
Stdev	3080	3480	3080	3480
p(t-test)		0.22		0.22
Min	35.1	2520	35.1	2520
Max	15000	7440	15000	7440
n (Samp)	44	2	44	2
n (Patient)	44	2	44	2

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.89	nd	0.88	0.89	nd	0.88
SE	0.13	nd	0.16	0.13	nd	0.16
p	0.0024	nd	0.021	0.0024	nd	0.021
nCohort 1	53	nd	44	53	nd	44
nCohort 2	3	nd	2	3	nd	2
Cutoff 1	2460	nd	2460	2460	nd	2460
Sens 1	100%	nd	100%	100%	nd	100%
Spec 1	77%	nd	80%	77%	nd	80%
Cutoff 2	2460	nd	2460	2460	nd	2460
Sens 2	100%	nd	100%	100%	nd	100%
Spec 2	77%	nd	80%	77%	nd	80%
Cutoff 3	2460	nd	2460	2460	nd	2460
Sens 3	100%	nd	100%	100%	nd	100%
Spec 3	77%	nd	80%	77%	nd	80%
Cutoff 4	2250	nd	1960	2250	nd	1960
Sens 4	100%	nd	100%	100%	nd	100%
Spec 4	75%	nd	70%	75%	nd	70%
Cutoff 5	2780	nd	2780	2780	nd	2780
Sens 5	67%	nd	50%	67%	nd	50%
Spec 5	81%	nd	84%	81%	nd	84%
Cutoff 6	3480	nd	3480	3480	nd	3480
Sens 6	67%	nd	50%	67%	nd	50%
Spec 6	91%	nd	91%	91%	nd	91%
OR Quart 2	>0	nd	>0	>0	nd	>0
p Value	<na	nd	<na	<na	nd	<na
95% CI of	>na	nd	>na	>na	nd	>na

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
OR Quart2	na	nd	na	na	nd	na
OR Quart 3	>1.1	nd	>0	>1.1	nd	>0
p Value	<0.96	nd	<na	<0.96	nd	<na
95% CI of	>0.061	nd	>na	>0.061	nd	>na
OR Quart3	na	nd	na	na	nd	na
OR Quart 4	>2.3	nd	>2.2	>2.3	nd	>2.2
p Value	<0.51	nd	<0.54	<0.51	nd	<0.54
95% CI of	>0.19	nd	>0.17	>0.19	nd	>0.17
OR Quart4	na	nd	na	na	nd	na

WAP four-disulfide core domain protein 2

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	5150	16100	5150	16100
Average	8610	24000	8610	24000
Stdev	8650	16400	8650	16400
p(t-test)		0.0061		0.0061
Min	1830	13000	1830	13000
Max	41700	42800	41700	42800
n (Samp)	53	3	53	3
n (Patient)	53	3	53	3

UO only	0hr prior to AKI stage		24hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	5170	29400	5170	29400
Average	8160	29400	8160	29400
Stdev	7650	18900	7650	18900
p(t-test)		7.2E-4		7.2E-4
Min	1830	16100	1830	16100
Max	36700	42800	36700	42800
n (Samp)	44	2	44	2
n (Patient)	44	2	44	2

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.90	nd	0.94	0.90	nd	0.94
SE	0.12	nd	0.12	0.12	nd	0.12
p	9.8E-4	nd	1.3E-4	9.8E-4	nd	1.3E-4
nCohort 1	53	nd	44	53	nd	44
nCohort 2	3	nd	2	3	nd	2
Cutoff 1	11900	nd	15600	11900	nd	15600
Sens 1	100%	nd	100%	100%	nd	100%
Spec 1	83%	nd	89%	83%	nd	89%
Cutoff 2	11900	nd	15600	11900	nd	15600
Sens 2	100%	nd	100%	100%	nd	100%
Spec 2	83%	nd	89%	83%	nd	89%
Cutoff 3	11900	nd	15600	11900	nd	15600

	0hr prior to AKI stage			24hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Sens 3	100%	nd	100%	100%	nd	100%
Spec 3	83%	nd	89%	83%	nd	89%
Cutoff 4	9940	nd	8200	9940	nd	8200
Sens 4	100%	nd	100%	100%	nd	100%
Spec 4	72%	nd	70%	72%	nd	70%
Cutoff 5	11700	nd	10700	11700	nd	10700
Sens 5	100%	nd	100%	100%	nd	100%
Spec 5	81%	nd	82%	81%	nd	82%
Cutoff 6	18500	nd	16800	18500	nd	16800
Sens 6	33%	nd	50%	33%	nd	50%
Spec 6	91%	nd	91%	91%	nd	91%
OR Quart 2	>0	nd	>0	>0	nd	>0
p Value	<na	nd	<na	<na	nd	<na
95% CI of	>na	nd	>na	>na	nd	>na
OR Quart2	na	nd	na	na	nd	na
OR Quart 3	>0	nd	>0	>0	nd	>0
p Value	<na	nd	<na	<na	nd	<na
95% CI of	>na	nd	>na	>na	nd	>na
OR Quart3	na	nd	na	na	nd	na
OR Quart 4	>3.8	nd	>2.2	>3.8	nd	>2.2
p Value	<0.27	nd	<0.54	<0.27	nd	<0.54
95% CI of	>0.35	nd	>0.17	>0.35	nd	>0.17
OR Quart4	na	nd	na	na	nd	na

[0157] Table 7: Comparison of marker levels in urine samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0, R, or I) and in urine samples collected from Cohort 2 (subjects who progress to RIFLE stage F) at 0, 24 hours, and 48 hours prior to the subject reaching RIFLE stage I.

Placenta growth factor

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	47.3	28.0	47.3	39.6	47.3	31.4
Average	61.8	74.1	61.8	249	61.8	43.3
Stdev	59.5	97.1	59.5	852	59.5	37.3
p(t-test)		0.42		2.8E-9		0.33
Min	2.74	4.49	2.74	9.16	2.74	2.18
Max	524	310	524	3660	524	112
n (Samp)	884	16	884	18	884	10
n (Patient)	367	16	367	18	367	10

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	47.6	11.9	47.6	52.9	47.6	34.4
Average	67.5	84.5	67.5	65.6	67.5	40.3
Stdev	142	150	142	45.2	142	22.1

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
p(t-test)		0.81		0.97		0.61
Min	2.74	4.49	2.74	18.5	2.74	18.4
Max	3660	310	3660	145	3660	82.7
n (Samp)	916	4	916	8	916	7
n (Patient)	380	4	380	8	380	7

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	47.6	28.0	47.6	46.4	47.6	46.4
Average	62.0	60.9	62.0	311	62.0	53.7
Stdev	60.1	77.7	60.1	965	60.1	50.8
p(t-test)		0.95		3.6E-12		0.78
Min	2.18	8.39	2.18	8.07	2.18	10.3
Max	524	258	524	3660	524	112
n (Samp)	879	10	879	14	879	4
n (Patient)	342	10	342	14	342	4

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.42	0.28	0.40	0.48	0.56	0.50	0.39	0.40	0.44
SE	0.075	0.15	0.095	0.070	0.11	0.078	0.095	0.11	0.15
p	0.28	0.13	0.30	0.72	0.57	0.95	0.24	0.39	0.68
nCohort 1	884	916	879	884	916	879	884	916	879
nCohort 2	16	4	10	18	8	14	10	7	4
Cutoff 1	12.3	8.91	14.0	29.8	31.6	26.5	18.4	28.6	11.9
Sens 1	75%	75%	70%	72%	75%	71%	70%	71%	75%
Spec 1	6%	3%	8%	31%	34%	27%	15%	29%	6%
Cutoff 2	9.16	4.47	12.3	18.5	29.8	11.1	11.9	24.2	10.1
Sens 2	81%	100%	80%	83%	88%	86%	80%	86%	100%
Spec 2	3%	0%	6%	15%	31%	5%	6%	24%	4%
Cutoff 3	8.28	4.47	9.16	11.1	18.5	9.16	10.1	18.4	10.1
Sens 3	94%	100%	90%	94%	100%	93%	90%	100%	100%
Spec 3	3%	0%	3%	5%	15%	3%	4%	15%	4%
Cutoff 4	67.9	69.0	68.2	67.9	69.0	68.2	67.9	69.0	68.2
Sens 4	31%	25%	30%	28%	38%	36%	30%	14%	50%
Spec 4	70%	70%	70%	70%	70%	70%	70%	70%	70%
Cutoff 5	84.9	85.4	84.9	84.9	85.4	84.9	84.9	85.4	84.9
Sens 5	31%	25%	20%	22%	25%	36%	10%	0%	25%
Spec 5	80%	80%	80%	80%	80%	80%	80%	80%	80%
Cutoff 6	121	122	121	121	122	121	121	122	121
Sens 6	12%	25%	10%	6%	12%	7%	0%	0%	0%
Spec 6	90%	90%	90%	90%	90%	90%	90%	90%	90%
OR Quart 2	0.39	0	1.0	0.40	3.0	0.40	0.33	1.0	0
p Value	0.27	na	1.00	0.27	0.34	0.27	0.34	1.0	na
95% CI of	0.076	na	0.14	0.076	0.31	0.076	0.034	0.062	na
OR Quart2	2.1	na	7.2	2.1	29	2.1	3.2	16	na
OR Quart 3	0.20	0	0.50	1.4	2.0	0.60	0.33	3.0	0

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
p Value	0.14	na	0.57	0.56	0.57	0.48	0.34	0.34	na
95% CI of	0.023	na	0.045	0.44	0.18	0.14	0.034	0.31	na
OR Quart3	1.7	na	5.6	4.5	22	2.5	3.2	29	na
OR Quart 4	1.6	3.0	2.5	0.80	2.0	0.80	1.7	2.0	1.0
p Value	0.40	0.34	0.27	0.74	0.57	0.74	0.48	0.57	1.00
95% CI of	0.52	0.31	0.49	0.21	0.18	0.21	0.40	0.18	0.14
OR Quart4	5.0	29	13	3.0	22	3.0	7.2	22	7.2

60 kDa heat shock protein, mitochondrial

sCr or UO	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	91.0	401
Average	519	464
Stdev	1080	390
p(t-test)		0.90
Min	2.53	2.53
Max	8920	1090
n (Samp)	111	6
n (Patient)	86	6

sCr only	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	91.0	1060
Average	512	887
Stdev	1060	328
p(t-test)		0.54
Min	2.53	509
Max	8920	1090
n (Samp)	115	3
n (Patient)	89	3

UO only	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	91.0	244
Average	511	296
Stdev	1100	291
p(t-test)		0.70
Min	2.53	2.53
Max	8920	693
n (Samp)	96	4
n (Patient)	74	4

	24hr prior to AKI stage		
	sCr or UO	sCr only	UO only
AUC	0.60	0.82	0.50
SE	0.13	0.15	0.15

	24hr prior to AKI stage		
	sCr or UO	sCr only	UO only
p	0.41	0.030	0.98
nCohort 1	111	115	96
nCohort 2	6	3	4
Cutoff 1	161	453	161
Sens 1	83%	100%	75%
Spec 1	56%	75%	57%
Cutoff 2	161	453	0
Sens 2	83%	100%	100%
Spec 2	56%	75%	0%
Cutoff 3	0	453	0
Sens 3	100%	100%	100%
Spec 3	0%	75%	0%
Cutoff 4	379	379	379
Sens 4	50%	100%	25%
Spec 4	70%	70%	71%
Cutoff 5	894	760	894
Sens 5	17%	67%	0%
Spec 5	83%	80%	81%
Cutoff 6	1240	1240	1240
Sens 6	0%	0%	0%
Spec 6	92%	92%	93%
OR Quart 2	0	>0	0
p Value	na	<na	na
95% CI of	na	>na	na
OR Quart2	na	na	na
OR Quart 3	3.2	>1.0	2.1
p Value	0.32	<0.98	0.56
95% CI of	0.32	>0.062	0.18
OR Quart3	33	na	25
OR Quart 4	2.0	>2.1	1.0
p Value	0.58	<0.56	1.0
95% CI of	0.17	>0.18	0.059
OR Quart4	23	na	17

WAP four-disulfide core domain protein 2

sCr or UO	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	595000	1040000
Average	1030000	1440000
Stdev	1420000	886000
p(t-test)		0.45
Min	23500	768000
Max	7500000	3230000
n (Samp)	113	7
n (Patient)	87	7

sCr only	24hr prior to AKI stage

	Cohort 1	Cohort 2
Median	603000	851000
Average	1050000	851000
Stdev	1410000	49900
p(t-test)		0.85
Min	23500	816000
Max	7500000	886000
n (Samp)	118	2
n (Patient)	91	2

UO only	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	626000	1430000
Average	1010000	1670000
Stdev	1400000	968000
p(t-test)		0.30
Min	23500	768000
Max	7500000	3230000
n (Samp)	96	5
n (Patient)	74	5

	24hr prior to AKI stage		
	sCr or UO	sCr only	UO only
AUC	0.74	0.61	0.80
SE	0.11	0.21	0.12
p	0.028	0.59	0.015
nCohort 1	113	118	96
nCohort 2	7	2	5
Cutoff 1	871000	804000	1020000
Sens 1	71%	100%	80%
Spec 1	64%	60%	72%
Cutoff 2	804000	804000	1020000
Sens 2	86%	100%	80%
Spec 2	61%	60%	72%
Cutoff 3	755000	804000	690000
Sens 3	100%	100%	100%
Spec 3	58%	60%	55%
Cutoff 4	1020000	1050000	1010000
Sens 4	57%	0%	80%
Spec 4	71%	70%	71%
Cutoff 5	1340000	1410000	1290000
Sens 5	43%	0%	60%
Spec 5	81%	81%	80%
Cutoff 6	2150000	2910000	1650000
Sens 6	14%	0%	40%
Spec 6	90%	91%	91%
OR Quart 2	>0	>0	>0
p Value	<na	<na	<na
95% CI of	>na	>na	>na

	24hr prior to AKI stage		
	sCr or UO	sCr only	UO only
OR Quart2	na	na	na
OR Quart 3	>4.6	>2.1	>2.2
p Value	<0.18	<0.54	<0.54
95% CI of	>0.48	>0.18	>0.18
OR Quart3	na	na	na
OR Quart 4	>3.3	>0	>3.3
p Value	<0.31	<na	<0.32
95% CI of	>0.33	>na	>0.32
OR Quart4	na	na	na

Choriogonadotropin subunit beta

sCr or UO	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	0.287	0.341
Average	0.770	0.962
Stdev	2.45	1.42
p(t-test)		0.84
Min	0.0484	0.168
Max	24.9	4.13
n (Samp)	116	7
n (Patient)	90	7

sCr only	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	0.280	0.825
Average	0.751	1.81
Stdev	2.40	2.01
p(t-test)		0.45
Min	0.0484	0.486
Max	24.9	4.13
n (Samp)	121	3
n (Patient)	94	3

UO only	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	0.293	0.327
Average	0.619	0.357
Stdev	1.07	0.237
p(t-test)		0.59
Min	0.0484	0.168
Max	6.45	0.758
n (Samp)	99	5
n (Patient)	77	5

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

	24hr prior to AKI stage		
	sCr or UO	sCr only	UO only
AUC	0.62	0.85	0.49
SE	0.12	0.14	0.13
p	0.31	0.012	0.96
nCohort 1	116	121	99
nCohort 2	7	3	5
Cutoff 1	0.326	0.481	0.184
Sens 1	71%	100%	80%
Spec 1	53%	73%	29%
Cutoff 2	0.184	0.481	0.184
Sens 2	86%	100%	80%
Spec 2	31%	73%	29%
Cutoff 3	0.162	0.481	0.162
Sens 3	100%	100%	100%
Spec 3	25%	73%	23%
Cutoff 4	0.463	0.461	0.463
Sens 4	43%	100%	20%
Spec 4	72%	70%	72%
Cutoff 5	0.642	0.642	0.642
Sens 5	43%	67%	20%
Spec 5	80%	80%	81%
Cutoff 6	1.28	1.25	1.31
Sens 6	14%	33%	0%
Spec 6	91%	90%	91%
OR Quart 2	>2.1	>0	2.1
p Value	<0.56	<na	0.56
95% CI of	>0.18	>na	0.18
OR Quart2	na	na	25
OR Quart 3	>2.1	>1.0	1.0
p Value	<0.56	<0.98	1.0
95% CI of	>0.18	>0.062	0.059
OR Quart3	na	na	17
OR Quart 4	>3.2	>2.1	1.0
p Value	<0.32	<0.54	1.0
95% CI of	>0.32	>0.18	0.059
OR Quart4	na	na	17

[0158] Table 8: Comparison of marker levels in EDTA samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0, R, or I) and in EDTA samples collected from Cohort 2 (subjects who progress to RIFLE stage F) at 0, 24 hours, and 48 hours prior to the subject reaching RIFLE stage I.

Placenta growth factor

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	10.5	14.5	10.5	19.6	10.5	15.0
Average	12.7	19.2	12.7	33.3	12.7	16.3

sCr or UO	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Stdev	10.6	20.5	10.6	28.3	10.6	9.09
p(t-test)		0.18		6.3E-6		0.45
Min	0.000223	3.31	0.000223	5.12	0.000223	3.42
Max	144	54.3	144	77.3	144	26.8
n (Samp)	482	5	482	6	482	5
n (Patient)	217	5	217	6	217	5

sCr only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	nd	nd	10.6	7.49	10.6	13.3
Average	nd	nd	13.1	7.49	13.1	11.1
Stdev	nd	nd	11.4	3.36	11.4	6.87
p(t-test)	nd	nd		0.49		0.77
Min	nd	nd	0.000223	5.12	0.000223	3.42
Max	nd	nd	144	9.87	144	16.7
n (Samp)	nd	nd	496	2	496	3
n (Patient)	nd	nd	223	2	223	3

UO only	0hr prior to AKI stage		24hr prior to AKI stage		48hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	10.7	7.54	10.7	32.8	nd	nd
Average	12.8	18.2	12.8	41.4	nd	nd
Stdev	10.7	24.2	10.7	26.1	nd	nd
p(t-test)		0.32		8.8E-9	nd	nd
Min	0.000223	3.31	0.000223	18.4	nd	nd
Max	144	54.3	144	77.3	nd	nd
n (Samp)	482	4	482	5	nd	nd
n (Patient)	203	4	203	5	nd	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
AUC	0.55	nd	0.42	0.78	0.30	0.92	0.65	0.49	nd
SE	0.13	nd	0.15	0.11	0.21	0.083	0.13	0.17	nd
p	0.68	nd	0.59	0.015	0.34	3.6E-7	0.25	0.95	nd
nCohort 1	482	nd	482	482	496	482	482	496	nd
nCohort 2	5	nd	4	6	2	5	5	3	nd
Cutoff 1	6.02	nd	6.02	18.3	5.01	19.0	13.2	3.41	nd
Sens 1	80%	nd	75%	83%	100%	80%	80%	100%	nd
Spec 1	22%	nd	21%	82%	14%	83%	64%	7%	nd
Cutoff 2	6.02	nd	3.21	18.3	5.01	19.0	13.2	3.41	nd
Sens 2	80%	nd	100%	83%	100%	80%	80%	100%	nd
Spec 2	22%	nd	6%	82%	14%	83%	64%	7%	nd
Cutoff 3	3.21	nd	3.21	5.01	5.01	18.3	3.41	3.41	nd
Sens 3	100%	nd	100%	100%	100%	100%	100%	100%	nd
Spec 3	6%	nd	6%	15%	14%	82%	7%	7%	nd
Cutoff 4	14.5	nd	15.0	14.5	14.9	15.0	14.5	14.9	nd
Sens 4	40%	nd	25%	83%	0%	100%	60%	33%	nd

	0hr prior to AKI stage			24hr prior to AKI stage			48hr prior to AKI stage		
	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only	sCr or UO	sCr only	UO only
Spec 4	70%	nd	70%	70%	70%	70%	70%	70%	nd
Cutoff 5	17.3	nd	17.4	17.3	17.9	17.4	17.3	17.9	nd
Sens 5	40%	nd	25%	83%	0%	100%	40%	0%	nd
Spec 5	80%	nd	80%	80%	80%	80%	80%	80%	nd
Cutoff 6	22.5	nd	22.6	22.5	22.8	22.6	22.5	22.8	nd
Sens 6	20%	nd	25%	33%	0%	60%	40%	0%	nd
Spec 6	90%	nd	90%	90%	90%	90%	90%	90%	nd
OR Quart 2	0	nd	0	0	>0	>0	0	1.0	nd
p Value	na	nd	na	na	<na	<na	na	1.0	nd
95% CI of	na	nd	na	na	>na	>na	na	0.062	nd
OR Quart2	na	nd	na	na	na	na	na	16	nd
OR Quart 3	0.49	nd	1.0	0	>1.0	>0	2.0	0	nd
p Value	0.56	nd	1.0	na	<1.00	<na	0.57	na	nd
95% CI of	0.044	nd	0.062	na	>0.062	>na	0.18	na	nd
OR Quart3	5.5	nd	16	na	na	na	22	na	nd
OR Quart 4	0.99	nd	2.0	5.2	>1.0	>5.2	2.0	1.0	nd
p Value	0.99	nd	0.56	0.14	<0.99	<0.14	0.57	1.00	nd
95% CI of	0.14	nd	0.18	0.60	>0.063	>0.60	0.18	0.062	nd
OR Quart4	7.2	nd	23	45	na	na	22	16	nd

60 kDa heat shock protein, mitochondrial

sCr or UO	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	1120	4980
Average	2880	4980
Stdev	10100	3480
p(t-test)		0.77
Min	2.11	2520
Max	110000	7440
n (Samp)	129	2
n (Patient)	106	2

UO only	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	1120	4980
Average	3080	4980
Stdev	10800	3480
p(t-test)		0.80
Min	2.11	2520
Max	110000	7440
n (Samp)	113	2
n (Patient)	90	2

	24hr prior to AKI stage		
	sCr or UO	sCr only	UO only
AUC	0.88	nd	0.88
SE	0.16	nd	0.16

	24hr prior to AKI stage		
	sCr or UO	sCr only	UO only
p	0.014	nd	0.013
nCohort 1	129	nd	113
nCohort 2	2	nd	2
Cutoff 1	2460	nd	2460
Sens 1	100%	nd	100%
Spec 1	80%	nd	81%
Cutoff 2	2460	nd	2460
Sens 2	100%	nd	100%
Spec 2	80%	nd	81%
Cutoff 3	2460	nd	2460
Sens 3	100%	nd	100%
Spec 3	80%	nd	81%
Cutoff 4	1770	nd	1770
Sens 4	100%	nd	100%
Spec 4	71%	nd	71%
Cutoff 5	2520	nd	2460
Sens 5	50%	nd	100%
Spec 5	83%	nd	81%
Cutoff 6	3360	nd	3360
Sens 6	50%	nd	50%
Spec 6	91%	nd	90%
OR Quart 2	>0	nd	>0
p Value	<na	nd	<na
95% CI of	>na	nd	>na
OR Quart2	na	nd	na
OR Quart 3	>0	nd	>0
p Value	<na	nd	<na
95% CI of	>na	nd	>na
OR Quart3	na	nd	na
OR Quart 4	>2.1	nd	>2.1
p Value	<0.56	nd	<0.56
95% CI of	>0.18	nd	>0.18
OR Quart4	na	nd	na

WAP four-disulfide core domain protein 2

sCr or UO	24hr prior to AKI stage	
	Cohort 1	Cohort 2
Median	5420	29400
Average	9820	29400
Stdev	11000	18900
p(t-test)		0.014
Min	1070	16100
Max	63700	42800
n (Samp)	129	2
n (Patient)	106	2

UO only	24hr prior to AKI stage

	Cohort 1	Cohort 2
Median	5500	29400
Average	10100	29400
Stdev	11100	18900
p(t-test)		0.017
Min	1070	16100
Max	63700	42800
n (Samp)	113	2
n (Patient)	90	2

	24hr prior to AKI stage		
	sCr or UO	sCr only	UO only
AUC	0.91	nd	0.90
SE	0.14	nd	0.15
p	0.0042	nd	0.0056
nCohort 1	129	nd	113
nCohort 2	2	nd	2
Cutoff 1	15600	nd	15600
Sens 1	100%	nd	100%
Spec 1	83%	nd	82%
Cutoff 2	15600	nd	15600
Sens 2	100%	nd	100%
Spec 2	83%	nd	82%
Cutoff 3	15600	nd	15600
Sens 3	100%	nd	100%
Spec 3	83%	nd	82%
Cutoff 4	9790	nd	9970
Sens 4	100%	nd	100%
Spec 4	71%	nd	71%
Cutoff 5	14500	nd	14600
Sens 5	100%	nd	100%
Spec 5	81%	nd	81%
Cutoff 6	20100	nd	20100
Sens 6	50%	nd	50%
Spec 6	91%	nd	90%
OR Quart 2	>0	nd	>0
p Value	<na	nd	<na
95% CI of	>na	nd	>na
OR Quart2	na	nd	na
OR Quart 3	>0	nd	>0
p Value	<na	nd	<na
95% CI of	>na	nd	>na
OR Quart3	na	nd	na
OR Quart 4	>2.1	nd	>2.1
p Value	<0.56	nd	<0.56
95% CI of	>0.18	nd	>0.18
OR Quart4	na	nd	na

[0159] Table 9: Comparison of marker levels in enroll urine samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0 or R within 48hrs) and in enroll urine samples collected from Cohort 2 (subjects reaching RIFLE stage I or F within 48hrs). Enroll samples from patients already at RIFLE stage I or F were included in Cohort 2.

60 kDa heat shock protein, mitochondrial

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	91.0	443	91.0	1060	91.0	193
Average	438	551	436	782	378	474
Stdev	811	528	785	510	657	519
p(t-test)		0.71		0.46		0.71
Min	2.53	2.53	2.53	193	2.53	2.53
Max	3910	1240	3910	1090	3170	1240
n (Samp)	46	8	51	3	41	7
n (Patient)	46	8	51	3	41	7

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.62	0.81	0.59
SE	0.11	0.16	0.12
p	0.31	0.048	0.48
nCohort 1	46	51	41
nCohort 2	8	3	7
Cutoff 1	37.1	161	37.1
Sens 1	75%	100%	71%
Spec 1	41%	67%	44%
Cutoff 2	2.53	161	2.53
Sens 2	88%	100%	86%
Spec 2	7%	67%	7%
Cutoff 3	0	161	0
Sens 3	100%	100%	100%
Spec 3	0%	67%	0%
Cutoff 4	379	379	193
Sens 4	50%	67%	43%
Spec 4	76%	75%	71%
Cutoff 5	668	693	668
Sens 5	50%	67%	43%
Spec 5	80%	80%	80%
Cutoff 6	1090	1090	1090
Sens 6	12%	0%	14%
Spec 6	91%	90%	93%
OR Quart 2	2.0	>0	0.45
p Value	0.59	<na	0.54
95% CI of	0.16	>na	0.036
OR Quart2	25	na	5.8
OR Quart 3	1.0	>1.1	0.45

	At Enrollment		
	sCr or UO	sCr only	UO only
p Value	1.0	<0.96	0.54
95% CI of	0.056	>0.061	0.036
OR Quart3	18	na	5.8
OR Quart 4	4.8	>2.2	1.7
p Value	0.19	<0.55	0.62
95% CI of	0.46	>0.17	0.22
OR Quart4	50	na	12

Heat shock protein beta-1 (phospho SER78 / phospho SER82)

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	0.00191	0.00335	0.00335	0.00335	0.00191	0.00335
Average	0.173	0.322	0.180	0.459	0.154	0.368
Stdev	0.557	0.593	0.550	0.791	0.540	0.625
p(t-test)		0.49		0.41		0.35
Min	0.00191	0.00191	0.00191	0.00191	0.00191	0.00191
Max	2.88	1.37	2.88	1.37	2.88	1.37
n (Samp)	46	8	51	3	41	7
n (Patient)	46	8	51	3	41	7

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.64	0.61	0.72
SE	0.11	0.18	0.12
p	0.21	0.52	0.063
nCohort 1	46	51	41
nCohort 2	8	3	7
Cutoff 1	0.00191	0	0.00191
Sens 1	75%	100%	86%
Spec 1	52%	0%	56%
Cutoff 2	0	0	0.00191
Sens 2	100%	100%	86%
Spec 2	0%	0%	56%
Cutoff 3	0	0	0
Sens 3	100%	100%	100%
Spec 3	0%	0%	0%
Cutoff 4	0.00335	0.00335	0.00335
Sens 4	25%	33%	29%
Spec 4	87%	86%	88%
Cutoff 5	0.00335	0.00335	0.00335
Sens 5	25%	33%	29%
Spec 5	87%	86%	88%
Cutoff 6	0.333	0.333	0.106
Sens 6	25%	33%	29%
Spec 6	91%	90%	90%
OR Quart 2	0.92	0	0
p Value	0.96	na	na

	At Enrollment		
	sCr or UO	sCr only	UO only
95% CI of	0.052	na	na
OR Quart2	16	na	na
OR Quart 3	5.3	1.0	5.5
p Value	0.16	1.0	0.16
95% CI of	0.51	0.056	0.51
OR Quart3	56	18	59
OR Quart 4	2.0	0.92	2.2
p Value	0.59	0.96	0.54
95% CI of	0.16	0.052	0.17
OR Quart4	25	16	28

WAP four-disulfide core domain protein 2

	sCr or UO		sCr only		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	587000	1090000	713000	895000	645000	1150000
Average	760000	1340000	841000	895000	716000	1410000
Stdev	644000	794000	704000	13100	490000	834000
p(t-test)		0.025		0.91		0.0033
Min	38100	778000	38100	886000	44300	778000
Max	3080000	3230000	3230000	905000	1710000	3230000
n (Samp)	48	8	54	2	41	7
n (Patient)	48	8	54	2	41	7

	At Enrollment		
	sCr or UO	sCr only	UO only
AUC	0.75	0.61	0.78
SE	0.10	0.22	0.11
p	0.017	0.61	0.0095
nCohort 1	48	54	41
nCohort 2	8	2	7
Cutoff 1	886000	871000	1020000
Sens 1	75%	100%	71%
Spec 1	67%	61%	73%
Cutoff 2	871000	871000	886000
Sens 2	88%	100%	86%
Spec 2	67%	61%	68%
Cutoff 3	647000	871000	647000
Sens 3	100%	100%	100%
Spec 3	56%	61%	54%
Cutoff 4	1020000	1070000	962000
Sens 4	62%	0%	71%
Spec 4	71%	70%	71%
Cutoff 5	1290000	1410000	1150000
Sens 5	38%	0%	43%
Spec 5	81%	81%	80%
Cutoff 6	1650000	1650000	1460000
Sens 6	12%	0%	14%

	At Enrollment		
	sCr or UO	sCr only	UO only
Spec 6	92%	91%	90%
OR Quart 2	>1.1	>0	>1.1
p Value	<0.96	<na	<0.95
95% CI of	>0.061	>na	>0.061
OR Quart2	na	na	na
OR Quart 3	>5.6	>2.3	>4.0
p Value	<0.15	<0.51	<0.26
95% CI of	>0.54	>0.19	>0.35
OR Quart3	na	na	na
OR Quart 4	>3.8	>0	>4.0
p Value	<0.27	<na	<0.26
95% CI of	>0.35	>na	>0.35
OR Quart4	na	na	na

[0160] Table 10: Comparison of marker levels in enroll EDTA samples collected from Cohort 1 (patients that did not progress beyond RIFLE stage 0 or R within 48hrs) and in enroll EDTA samples collected from Cohort 2 (subjects reaching RIFLE stage I or F within 48hrs). Enroll samples from patients already at stage I or F were included in Cohort 2.

60 kDa heat shock protein, mitochondrial

	sCr or UO		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	954	1640	930	1640
Average	2500	2000	2660	2000
Stdev	5110	1800	5450	1800
p(t-test)		0.77		0.72
Min	2.11	727	2.11	727
Max	24700	6570	24700	6570
n (Samp)	46	9	40	9
n (Patient)	46	9	40	9

	At Enrollment	
	sCr or UO	UO only
AUC	0.63	0.63
SE	0.11	0.11
p	0.23	0.21
nCohort 1	46	40
nCohort 2	9	9
Cutoff 1	1020	1020
Sens 1	78%	78%
Spec 1	54%	55%
Cutoff 2	780	780
Sens 2	89%	89%
Spec 2	33%	35%

	At Enrollment	
	sCr or UO	UO only
Cutoff 3	618	618
Sens 3	100%	100%
Spec 3	30%	32%
Cutoff 4	1640	1640
Sens 4	22%	22%
Spec 4	74%	75%
Cutoff 5	2250	1960
Sens 5	22%	22%
Spec 5	83%	80%
Cutoff 6	3360	3360
Sens 6	11%	11%
Spec 6	91%	90%
OR Quart 2	>2.2	>2.4
p Value	<0.55	<0.50
95% CI of	>0.17	>0.19
OR Quart2	na	na
OR Quart 3	>7.2	>8.6
p Value	<0.093	<0.072
95% CI of	>0.72	>0.83
OR Quart3	na	na
OR Quart 4	>2.2	>2.2
p Value	<0.55	<0.55
95% CI of	>0.17	>0.17
OR Quart4	na	na

Heat shock protein beta-1 (phospho SER78 / phospho SER82)

	sCr or UO		UO only	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Median	18.9	29.3	17.3	29.3
Average	39.8	42.5	40.8	42.5
Stdev	63.9	45.5	67.6	45.5
p(t-test)		0.91		0.95
Min	0.00141	0.00632	0.00141	0.00632
Max	311	148	311	148
n (Samp)	46	9	40	9
n (Patient)	46	9	40	9

	At Enrollment	
	sCr or UO	UO only
AUC	0.59	0.59
SE	0.11	0.11
p	0.43	0.39
nCohort 1	46	40
nCohort 2	9	9
Cutoff 1	17.7	17.7
Sens 1	78%	78%
Spec 1	50%	52%

	At Enrollment	
	sCr or UO	UO only
Cutoff 2	5.15	5.15
Sens 2	89%	89%
Spec 2	30%	30%
Cutoff 3	0.00141	0.00141
Sens 3	100%	100%
Spec 3	4%	5%
Cutoff 4	36.6	33.1
Sens 4	33%	33%
Spec 4	72%	70%
Cutoff 5	68.1	68.1
Sens 5	22%	22%
Spec 5	80%	80%
Cutoff 6	93.2	93.2
Sens 6	11%	11%
Spec 6	91%	90%
OR Quart 2	2.0	2.2
p Value	0.59	0.54
95% CI of	0.16	0.17
OR Quart2	25	28
OR Quart 3	3.3	3.7
p Value	0.33	0.29
95% CI of	0.29	0.32
OR Quart3	36	42
OR Quart 4	3.3	3.3
p Value	0.33	0.33
95% CI of	0.29	0.29
OR Quart4	36	37

[0161] 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.

[0162] 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.

[0163] 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.

[0164] 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.

[0165] Other embodiments are set forth within the following claims.

We claim:

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

performing one or more assays configured to detect one or more biomarkers selected from the group consisting of Heat shock protein beta-1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein on a body fluid sample obtained from the subject to provide an assay result; and

correlating the assay result(s) to the renal status of the subject.

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

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

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

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

a measured concentration of Heat shock protein beta-1,

a measured concentration of WAP four-disulfide core domain protein 2,

a measured concentration of Choriogonadotropin subunit beta,

a measured concentration of Placenta growth factor, and

a measured concentration of Mitochondrial 60 kDa heat shock protein.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

sample is obtained, and (iii) has not experienced an increase of 0.3 mg/dL or greater in serum creatinine over a baseline value determined prior to the time at which the body fluid sample is obtained.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

100. A method according to one of claims 1-99, wherein said method comprises performing assays that detect one, two or three, or more of Heat shock protein beta-1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein.

101. Measurement of one or more biomarkers selected from the group consisting of Heat shock protein beta-1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein for the evaluation of renal injury.

102. Measurement of one or more biomarkers selected from the group consisting of Heat shock protein beta-1, WAP four-disulfide core domain protein 2,

Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein for the evaluation of acute renal injury.

103. A kit, comprising:

reagents for performing one or more assays configured to detect one or more kidney injury markers selected from the group consisting of Heat shock protein beta-1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein.

104. A kit according to claim 103, wherein said reagents comprise one or more binding reagents, each of which specifically binds one of said of kidney injury markers.

105. A kit according to claim 104, wherein a plurality of binding reagents are contained in a single assay device.

106. A kit according to claim 103, wherein at least one of said assays is configured as a sandwich binding assay.

107. A kit according to claim 103, wherein at least one of said assays is configured as a competitive binding assay.

108. A kit according to one of claims 103-107, wherein said one or more assays comprise assays that detect one, two or three, or more of Heat shock protein beta-1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein.

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

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

performing a plurality of analyte binding assays configured to detect a plurality of biomarkers, one or more of which is selected from the group consisting of Heat shock protein beta-1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein by introducing the urine sample obtained from the subject into an assay instrument which (i) contacts a plurality of reagents which specifically bind for detection the plurality of biomarkers with the urine sample, and (ii) generates one or more assay results indicative of binding of each biomarker which is assayed to a respective specific binding reagent in the plurality of reagents.

110. A method according to claim 109, wherein the subject is selected for evaluation based on a determination that the subject is in need of risk stratification, diagnosis, staging, prognosis, classifying or monitoring of the renal status of the subject.

111. A method according to claim 109, wherein the subject is selected for evaluation based on a determination that the subject is at risk of a future acute renal injury.

112. A method according to claim 111, wherein the subject is selected for evaluation based on a determination that the subject is at risk of a future injury to renal function, future reduced renal function, future improvement in renal function, and future acute renal failure (ARF).

113. A method according to claim 111, wherein the subject is selected for evaluation based on a determination that the subject is at risk of a future acute renal injury within 30 days of the time at which the urine sample is obtained from the subject.

114. A method according to claim 113, wherein the subject is selected for evaluation based on a determination that the subject is at risk of a future acute renal injury within a period selected from the group consisting of 21 days, 14 days, 7 days, 5 days, 96 hours, 72 hours, 48 hours, 36 hours, 24 hours, and 12 hours.

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

116. A method according to claim 109, wherein the subject is selected for evaluation based on an existing diagnosis of one or more of congestive heart failure, preeclampsia, eclampsia, diabetes mellitus, hypertension, coronary artery disease, proteinuria, renal insufficiency, glomerular filtration below the normal range, cirrhosis, serum creatinine above the normal range, sepsis, injury to renal function, reduced renal function, or ARF, or based on undergoing or having undergone major vascular surgery, coronary artery bypass, or other cardiac surgery, or based on exposure to NSAIDs, cyclosporines, tacrolimus, aminoglycosides, foscarnet, ethylene glycol, hemoglobin, myoglobin, ifosfamide, heavy metals, methotrexate, radiopaque contrast agents, or streptozotocin.

117. A method according to claim 109, wherein the plurality of assays are immunoassays performed by (i) introducing the urine sample into an assay device comprising a plurality of antibodies, at least one of which binds to each biomarker which

is assayed, and (ii) generating an assay result indicative of binding of each biomarker to its respective antibody.

118. A method according to claim 109, wherein the subject is selected for evaluation based on a determination that the subject is at risk of one or more future changes in renal status selected from the group consisting of a future injury to renal function, future reduced renal function, future improvement in renal function, and future acute renal failure (ARF) within 72 hours of the time at which the urine sample is obtained.

119. A method according to claim 109, wherein the subject is selected for evaluation based on a determination that the subject is at risk of one or more future changes in renal status selected from the group consisting of a future injury to renal function, future reduced renal function, future improvement in renal function, and future acute renal failure (ARF) within 48 hours of the time at which the urine sample is obtained.

120. A method according to claim 109, wherein the subject is selected for evaluation based on a determination that the subject is at risk of one or more future changes in renal status selected from the group consisting of a future injury to renal function, future reduced renal function, future improvement in renal function, and future acute renal failure (ARF) within 24 hours of the time at which the urine sample is obtained.

121. A method according to claim 109, wherein the subject is in RIFLE stage 0 or R.

122 A method according to claim 109, wherein the subject is in RIFLE stage 0, R, or I.

123. A method according to claim 109, wherein at least one assay result is a measured concentration of Heat shock protein beta-1, a measured concentration of WAP four-disulfide core domain protein 2, a measured concentration of Choriogonadotropin subunit beta, a measured concentration of Placenta growth factor, and a measured concentration of Mitochondrial 60 kDa heat shock protein.

124. A system for evaluating biomarker levels, comprising:

a plurality of reagents which specifically bind for detection the plurality of biomarkers, one or more of which is selected from the group consisting of Heat shock protein beta-1, WAP four-disulfide core domain protein 2, Choriogonadotropin subunit beta, Placenta growth factor, and Mitochondrial 60 kDa heat shock protein;

an assay instrument configured to receive a urine sample and contact the plurality of reagents with the urine sample and to generate one or more assay results indicative of

binding of each biomarker which is assayed to a respective specific binding reagent in the plurality of reagents.

125. A system according to claim 124, wherein the reagents comprise a plurality of antibodies, at least one of which binds to each of the biomarkers which are assayed.

126. A system according to claim 125, wherein assay instrument comprises an assay device and an assay device reader, wherein the plurality of antibodies are immobilized at a plurality of predetermined locations within the assay device, wherein the assay device is configured to receive the urine sample such that the urine sample contacts the plurality of predetermined locations, and wherein the assay device reader interrogates the plurality of predetermined locations to generate the assay results.

127. A system according to claim 126, wherein the plurality of reagents comprises reagents for performing at least one assay selected from the group consisting of a Heat shock protein beta-1 assay, a WAP four-disulfide core domain protein 2 assay, a Choriogonadotropin subunit beta assay, a Placenta growth factor assay, and a Mitochondrial 60 kDa heat shock protein assay.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/45583

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/50 (2012.01)
USPC - 436/63
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - G01N 33/50 (2012.01)
USPC - 436/63

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest (PGPB,USPT,USOC,EPAB,JPAB); PubMed (MEDLINE)
renal, kidney, neph, marker, biomarker, Hsp27, Hsp60, WFDC2, WFDC, WAP, choriogonadotropin, placental growth factor, failure, injury, function

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2006/057066 A1 (NATSOULIS et al.) 16 March 2006 (16.03.2006) para [0017], [0154], [0174], [0120], [0125], [0154], [0117], [0021], [0098]-[0100], [0096], [0042]	1-4, 7, 101-112, 115-117, 123-127 --- 5, 8-9, 113-114, 118-122
Y	US 2011/0104726 A1 (VALKIRS et al.) 05 May 2011 (05.05.2011) para [0029], [0042], [0048]	8-9, 113-114, 118-120
Y	BARRERA-CHIMAL et al. "Hsp72 is an early and sensitive biomarker to detect acute kidney injury". EMBO Mol. Med.; January 2011, published online 14 December 2010; Vol. 3, No. 1, pp 5-20; pg 6, col 1, para 3; pg 11, col 2, para 2; pg 16, col 1, para 1	121-122
Y	LANG et al. "Heat Shock Protein 60 Is Released in Immune-Mediated Glomerulonephritis and Aggravates Disease: In Vivo Evidence for an Immunologic Danger Signal" JASN; 1 February 2005; Vol. 16, No. 2; pg. 383-391. Especially abstract.	5
A	US 2007/0087387 A1 (DEVARAJAN et al.) 19 April 2007 (19.04.2007) entire document	1-4, 7-9, 101-127
X,P	US 2011/0195429 A1 (ANDERBERG et al.) 11 August 2011 (11.08.2011) entire document	1-4, 7-9, 101-127

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
11 September 2012 (11.09.2012)	21 SEP 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/45583

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

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

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

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

3. ☒ Claims Nos.: 6, 10-100
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.