(12) STANDARD PATENT (11) Application No. **AU 2010210537 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Diagnosis and prognosis of renal injury and renal failure

(51) International Patent Classification(s)
C12Q 1/02 (2006.01)

(21) Application No: **2010210537** (22) Date of Filing: **2010.02.05**

(87) WIPO No: **WO10/091233**

(30) Priority Data

(31) Number	(32) Date	(33) Country
61/162,404	**2009.03.23**	**US**
61/150,376	**2009.02.06**	**US**
61/150,397	**2009.02.06**	**US**
61/166,337	**2009.04.03**	**US**
61/162,401	**2009.03.23**	**US**
61/162,410	**2009.03.23**	**US**

(43) Publication Date: **2010.08.12**
(44) Accepted Journal Date: **2015.06.18**

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(56) Related Art
CUCUIANU, M., et al., Revue Roumaine de Biochimie, 1991, vol. 28, pages 3-10
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 August 2010 (12.08.2010)

PCT

(10) International Publication Number
WO 2010/091233 A1

(51) **International Patent Classification**:
C12Q 1/02 (2006.01)

(21) **International Application Number**:
PCT/US2010/023294

(22) **International Filing Date**:
5 February 2010 (05.02.2010)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:

61/150,376	6 February 2009 (06.02.2009)	US
61/150,397	6 February 2009 (06.02.2009)	US
61/162,410	23 March 2009 (23.03.2009)	US
61/162,404	23 March 2009 (23.03.2009)	US
61/162,401	23 March 2009 (23.03.2009)	US
61/166,337	3 April 2009 (03.04.2009)	US

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

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

Published:

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

WO 2010/091233 A1

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

(57) **Abstract**: The present invention relates to methods and compositions for monitoring, diagnosis, prognosis, and determination of treatment regimens in subjects suffering from or suspected of having a renal injury. In particular, the invention relates to using assays that detect one or more markers selected from the group consisting of soluble Advanced glycosylation end product- specific receptor, Bactericidal permeability-increasing protein, Interleukin 12, Fibroblast growth factor 23, Vitamin K-dependent protein C, and Intestinal fatty acid-binding protein as diagnostic and prognostic biomarkers in renal injuries.

DIAGNOSIS AND PROGNOSIS OF RENAL INJURY AND RENAL FAILURE

[0001] The present invention claims priority from U.S. Provisional Patent Applications 61/150,376 filed February 6, 2009; 61/150,397 filed February 6, 2009; 61/162,404 filed March 23, 2009; 61/162,410 filed March 23, 2009; and 61/166,337 filed April 3, 2009, each of which is hereby incorporated in its entirety including all tables, figures, and claims.

BACKGROUND OF THE INVENTION

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

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

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

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

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

Postrenal	
Tubular precipitation	Uric acid (tumor lysis), sulfonamides, triamterene, acyclovir, indinavir, methotrexate, ethylene glycol ingestion, myeloma protein, myoglobin
Ureteral obstruction	Intrinsic: Calculi, clots, sloughed renal tissue, fungus ball, edema, malignancy, congenital defects; Extrinsic: Malignancy, retroperitoneal fibrosis, ureteral trauma during surgery or high impact injury
Bladder obstruction	Mechanical: Benign prostatic hyperplasia, prostate cancer, bladder cancer, urethral strictures, phimosis, paraphimosis, urethral valves, obstructed indwelling urinary catheter; Neurogenic: Anticholinergic drugs, upper or lower motor neuron lesion

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

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

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

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

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

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

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

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

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

And included two clinical outcomes:

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

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

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

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

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

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

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

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

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

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

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

BRIEF SUMMARY OF THE INVENTION

[0013] Described herein are methods and compositions for evaluating renal function in a subject. As described herein, measurement of one or more

markers selected from the group consisting of soluble Advanced glycosylation end product-specific receptor, Bactericidal permeability-increasing protein, Interleukin 12, Fibroblast growth factor 23, Vitamin K-dependent protein C, and Intestinal fatty acid-binding protein (collectively referred to herein as "kidney injury markers, and individually 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] These kidney injury markers 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 kidney injury markers of the present invention in a body fluid sample obtained from the subject. The assay result(s), for example a measured concentration of one or more markers selected from the group consisting of soluble Advanced glycosylation end product-specific receptor, Bactericidal permeability-increasing protein, Interleukin 12, Fibroblast growth factor 23, Vitamin K-dependent protein C, and Intestinal fatty acid-binding protein is/are then correlated to the renal status

of the subject. This correlation to renal status may include correlating the assay result(s) to one or more of risk stratification, diagnosis, prognosis, staging, classifying and monitoring of the subject as described herein. Thus, the present invention utilizes one or more kidney injury markers of the present invention for the evaluation of renal injury.

[0015A] In one aspect described herein is a method for evaluating renal status in a subject, comprising performing one or more assays configured to detect Vitamin K-dependent protein C in a body fluid sample obtained from the subject to provide one or more assay results; and correlating the assay result(s) to one or more of risk stratification, staging, prognosis, classifying and monitoring of the renal status of the subject, wherein said correlating comprises: (i) determining a measured concentration of Vitamin K-dependent protein C in the body fluid sample; (ii) comparing the measured concentration of Vitamin K-dependent protein C to a threshold concentration of Vitamin K-dependent protein C; and (iii) based on the assay results: (a) assigning a likelihood of a future event occurring or determining that an event has occurred when the measured concentration is above the threshold; or (b) assigning a likelihood of a future event not occurring or determining that an event has not occurred when the measured concentration is below the threshold.

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

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

[0018] In other preferred risk stratification embodiments, these methods comprise determining a subject's risk for future reduced renal function, and the assay result(s) is/are correlated to a likelihood of such reduced renal function. For example, the measured concentrations may each be compared to a threshold value. For a "positive

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

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

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

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

requirement for renal replacement therapy, a requirement for withdrawal of renal toxins, end stage renal disease, heart failure, stroke, myocardial infarction, progression to chronic kidney disease, *etc.*, is assigned to the subject when the measured concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold.

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

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

[0024] In other embodiments, the methods for evaluating renal status described herein are methods for diagnosing a renal injury in the subject; that is, assessing whether or not a subject has suffered from an injury to renal function, reduced renal function, or ARF. In these embodiments, the assay result(s), for example a measured concentration of one or more markers selected from the group consisting of soluble Advanced glycosylation end

product-specific receptor, Bactericidal permeability-increasing protein, Interleukin 12, Fibroblast growth factor 23, Vitamin K-dependent protein C, and Intestinal fatty acid-binding protein is/are correlated to the occurrence or nonoccurrence of a change in renal status. The following are preferred diagnostic embodiments.

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

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

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

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

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

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

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

[0030] In still other embodiments, the methods for evaluating renal status described herein are methods for monitoring a renal injury in the subject; that is, assessing whether or not renal function is improving or worsening in a subject who has suffered from an injury to renal function, reduced renal function, or ARF. In these embodiments, the assay result(s), for example a measured concentration of one or more markers selected from the group consisting of soluble Advanced glycosylation end product-specific receptor, Bactericidal permeability-increasing protein, Interleukin 12, Fibroblast growth factor 23, Vitamin K-dependent protein C, and Intestinal fatty acid-binding protein is/are correlated

to the occurrence or nonoccurrence of a change in renal status. The following are preferred monitoring embodiments.

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

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

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

the subject; alternatively, when the measured concentration is above the threshold, an improvement of renal function may be assigned to the subject.

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

[0035] In still other embodiments, the methods for evaluating renal status described herein are methods for classifying a renal injury in the subject; that is, determining whether a renal injury in a subject is prerenal, intrinsic renal, or postrenal; and/or further subdividing these classes into subclasses such as acute tubular injury, acute glomerulonephritis acute tubulointerstitial nephritis, acute vascular nephropathy, or infiltrative disease; and/or assigning a likelihood that a subject will progress to a particular RIFLE stage. In these embodiments, the assay result(s), for example a measured concentration of one or more markers selected from the group consisting of soluble Advanced glycosylation end product-specific receptor, Bactericidal permeability-increasing protein, Interleukin 12, Fibroblast growth factor 23, Vitamin K-dependent protein C, and Intestinal fatty acid-binding 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.

[001] 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.

[0038] 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.

[0039] 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.

[0040] 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.

[0041] 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.

[0042] The foregoing method steps should not be interpreted to mean that the kidney injury marker assay result(s) is/are used in isolation in the methods described herein. Rather, additional variables or other clinical indicia may be included in the methods described herein. For example, a risk stratification, diagnostic, classification, monitoring, etc. method may combine the assay result(s) with one or more variables measured for the subject selected from the group consisting of demographic information (e.g., weight, sex, age, race), medical history (e.g., family history, type of surgery, pre-existing disease such as aneurism, congestive heart failure, preeclampsia, eclampsia, diabetes mellitus,

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

[0043] 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.

[0044] 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.

[0044A] In a related aspect described herein is the use of one or more antibodies or antibody fragments that bind selectively to an Antileukoproteinase polypeptide or an epitope-containing fragment thereof in a method performed *ex vivo* to stratify risk of acute kidney injury or to diagnose, stage, predict, classify or monitor acute kidney injury in a subject, wherein said method is performed on a biological sample obtained from the subject.

[0045] 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.

[0046] 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.*).

[0047] 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.

BRIEF DESCRIPTION OF THE FIGURES

[0048] Fig. 1 provides data tables determined in accordance with Example 6 for the comparison of marker levels in urine samples collected for 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. Tables provide

descriptive statistics, AUC analysis, and sensitivity, specificity and odds ratio calculations at various threshold (cutoff) levels for the various markers.

[0049] Fig. 2 provides data tables determined in accordance with Example 7 for the comparison of marker levels in urine samples collected for 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. Tables provide descriptive statistics, AUC analysis, and sensitivity, specificity and odds ratio calculations at various threshold (cutoff) levels for the various markers.

[0050] Fig. 3 provides data tables determined in accordance with Example 8 for the comparison of marker levels in urine samples collected for Cohort 1 (patients that reached, but did not progress beyond, RIFLE stage 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. Tables provide descriptive statistics, AUC analysis, and sensitivity, specificity and odds ratio calculations at various threshold (cutoff) levels for the various markers.

[0051] Fig. 4 provides data tables determined in accordance with Example 9 for the comparison of marker levels in urine samples collected for 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 F in Cohort 2. Tables provide descriptive statistics, AUC analysis, and sensitivity, specificity and odds ratio calculations at various threshold (cutoff) levels for the various markers.

[0052] Fig. 5 provides data tables determined in accordance with Example 6 for the comparison of marker levels in plasma samples collected for Cohort 1 (patients that did not progress beyond RIFLE stage 0) and in plasma samples collected from subjects at 0, 24 hours, and 48 hours prior to reaching stage R, I or F in Cohort 2. Tables provide descriptive statistics, AUC analysis, and sensitivity, specificity and odds ratio calculations at various threshold (cutoff) levels for the various markers.

[0053] Fig. 6 provides data tables determined in accordance with Example 7 for the comparison of marker levels in plasma samples collected for Cohort 1 (patients that did not progress beyond RIFLE stage 0 or R) and in plasma samples collected from subjects at 0, 24 hours, and 48 hours prior to reaching stage I or F in Cohort 2. Tables provide descriptive statistics, AUC analysis, and sensitivity, specificity and odds ratio calculations at various threshold (cutoff) levels for the various markers.

[0054] Fig. 7 provides data tables determined in accordance with Example 8 for the comparison of marker levels in plasma samples collected for Cohort 1 (patients that reached, but did not progress beyond, RIFLE stage R) and in plasma samples collected from subjects at 0, 24 hours, and 48 hours prior to reaching stage I or F in Cohort 2. Tables provide descriptive statistics, AUC analysis, and sensitivity, specificity and odds ratio calculations at various threshold (cutoff) levels for the various markers.

[0055] Fig. 8 provides data tables determined in accordance with Example 9 for the comparison of marker levels in plasma samples collected for Cohort 1 (patients that did not progress beyond RIFLE stage 0) and in plasma samples collected from subjects at 0, 24 hours, and 48 hours prior to reaching stage F in Cohort 2. Tables provide descriptive statistics, AUC analysis, and sensitivity, specificity and odds ratio calculations at various threshold (cutoff) levels for the various markers.

DETAILED DESCRIPTION OF THE INVENTION

[0056] 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 markers selected from the group consisting of soluble Advanced glycosylation end product-specific receptor, Bactericidal permeability-increasing protein, Interleukin 12, Fibroblast growth factor 23, Vitamin K-dependent protein C, and Intestinal fatty acid-binding protein, or one or more markers related thereto, are correlated to the renal status of the subject.

[0057] For purposes of this document, the following definitions apply:

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

within 48 hours) measurable increase in a measure of renal function. Preferred methods for measuring and/or estimating GFR are described hereinafter.

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

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

[0058] 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.

[0059] As used herein, the term "soluble advanced glycosylation end product-specific receptor" refers to one or more non-membrane-bound polypeptides present in a biological sample that are derived from the advanced glycosylation end product-specific receptor precursor (Swiss-Prot Q15109 (SEQ ID NO: 1)).

```
        10         20         30         40         50         60
MAAGTAVGAW VLVLSLWGAV VGAQNITARI GEPLVLKCKG APKKPPQRLE WKLNTGRTEA

        70         80         90        100        110        120
WKVLSPQGGG PWDSVARVLP NGSLFLPAVG IQDEGIFRCQ AMNRNGKETK SNYRVRVYQI
```

```
       130        140        150        160        170        180
PGKPEIVDSA SELTAGVPNK VGTCVSEGSY PAGTLSWHLD GKPLVPNEKG VSVKEQTRRH

       190        200        210        220        230        240
PETGLFTLQS ELMVTPARGG DPRPTFSCSF SPGLPRHRAL RTAPIQPRVW EPVPLEEVQL

       250        260        270        280        290        300
VVEPEGGAVA PGGTVTLTCE VPAQPSPQIH WMKDGVPLPL PPSPVLILPE IGPQDQGTYS

       310        320        330        340        350        360
CVATHSSHGP QESRAVSISI IEPGEEGPTA GSVGGSGLGT LALALGILGG LGTAALLIGV

       370        380        390        400
ILWQRRQRRG EERKAPENQE EEEERAELNQ SEEPEAGESS TGGP
```

or the splice variant thereof (SEQ ID NO: 2)

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        10         20         30         40         50         60
MAAGTAVGAW VLVLSLWGAV VGAQNITARI GEPLVLKCKG APKKPPQRLE WKLGGGPWDS

        70         80         90        100        110        120
VARVLPNGSL FLPAVGIQDE GIFRCQAMNR NGKETKSNYR VRVYQIPGKP EIVDSASELT

       130        140        150        160        170        180
AGVPNKVGTC VSEGSYPAGT LSWHLDGKPL VPNEKGVSVK EQTRRHPETG LFTLQSELMV

       190        200        210        220        230        240
TPARGGDPRP TFSCSFSPGL PRHRALRTAP IQPRVWEPVP LEEVQLVVEP EGGAVAPGGT

       250        260        270        280        290        300
VTLTCEVPAQ PSPQIHWMKD VSDLERGAGR TRRGGANCRL CGRIRAGNSS PGPGDPGRPG

       310        320        330        340
DSRPAHWGHL VAKAATPRRG EEGPRKPGGR GGACRTESVG GT
```

[0060] Advanced glycosylation end product-specific receptor is a single-pass type I membrane protein having a large extracellular domain, some or all of which is present in soluble forms of advanced glycosylation end product-specific receptor generated either through alternative splicing event which deletes all or a portion of the transmembrane domain, or by proteolysis of the membrane-bound form. In the case of an immunoassay, one or more antibodies that bind to epitopes within this extracellular domain may be used to detect these soluble form(s). The following domains have been identified in advanced glycosylation end product-specific receptor:

Residues	Length	Domain ID
1-22	22	Signal sequence
23-404	382	Advanced glycosylation end product-specific receptor

23-342	230	Extracellular domain
343-363	21	Transmembrane domain
364-404	41	Cytoplasmic domain

[0061] As used herein, the term "Bactericidal permeability-increasing protein" refers to one or polypeptides present in a biological sample that are derived from the Bactericidal permeability-increasing protein precursor (Swiss-Prot P17213 (SEQ ID NO: 3)).

```
        10         20         30         40         50         60
MRENMARGPC NAPRWASLMV LVAIGTAVTA AVNPGVVVRI SQKGLDYASQ QGTAALQKEL

        70         80         90        100        110        120
KRIKIPDYSD SFKIKHLGKG HYSFYSMDIR EFQLPSSQIS MVPNVGLKFS ISNANIKISG

       130        140        150        160        170        180
KWKAQKRFLK MSGNFDLSIE GMSISADLKL GSNPTSGKPT ITCSSCSSHI NSVHVHISKS

       190        200        210        220        230        240
KVGWLIQLFH KKIESALRNK MNSQVCEKVT NSVSSELQPY FQTLPVMTKI DSVAGINYGL

       250        260        270        280        290        300
VAPPATTAET LDVQMKGEFY SENHHNPPPF APPVMEFPAA HDRMVYLGLS DYFFNTAGLV

       310        320        330        340        350        360
YQEAGVLKMT LRDDMIPKES KFRLTTKFFG TFLPEVAKKF PNMKIQIHVS ASTPPHLSVQ

       370        380        390        400        410        420
PTGLTFYPAV DVQAFAVLPN SSLASLFLIG MHTTGSMEVS AESNRLVGEL KLDRLLLELK

       430        440        450        460        470        480
HSNIGPFPVE LLQDIMNYIV PILVLPRVNE KLQKGFPLPT PARVQLYNVV LQPHQNFLLF

GADVVYK
```

[0062] The following domains have been identified in Bactericidal permeability-increasing protein:

Residues	Length	Domain ID
1-31	31	Signal sequence
32-487	456	Bactericidal permeability-increasing protein

[0063] Holo-interleukin 12 is a heterodimer comprising an alpha and beta subunit. As used herein, the term "Interleukin-12" refers to one or more polypeptides present in a

biological sample that are derived from an Interleukin-12 precursor (Swiss-Prot P29459 (alpha subunit) (SEQ ID NO: 4)):

```
        10         20         30         40         50         60
MCPARSLLLV ATLVLLDHLS LARNLPVATP DPGMFPCLHH SQNLLRAVSN MLQKARQTLE

        70         80         90        100        110        120
FYPCTSEEID HEDITKDKTS TVEACLPLEL TKNESCLNSR ETSFITNGSC LASRKTSFMM

       130        140        150        160        170        180
ALCLSSIYED LKMYQVEFKT MNAKLLMDPK RQIFLDQNML AVIDELMQAL NFNSETVPQK

       190        200        210
SSLEEPDFYK TKIKLCILLH AFRIRAVTID RVMSYLNAS
```

(and Swiss-Prot P29460 (beta subunit) (SEQ ID NO: 5)):

```
        10         20         30         40         50         60
MCHQQLVISW FSLVFLASPL VAIWELKKDV YVVELDWYPD APGEMVVLTC DTPEEDGITW

        70         80         90        100        110        120
TLDQSSEVLG SGKTLTIQVK EFGDAGQYTC HKGGEVLSHS LLLLHKKEDG IWSTDILKDQ

       130        140        150        160        170        180
KEPKNKTFLR CEAKNYSGRF TCWWLTTIST DLTFSVKSSR GSSDPQGVTC GAATLSAERV

       190        200        210        220        230        240
RGDNKEYEYS VECQEDSACP AAEESLPIEV MVDAVHKLKY ENYTSSFFIR DIIKPDPPKN

       250        260        270        280        290        300
LQLKPLKNSR QVEVSWEYPD TWSTPHSYFS LTFCVQVQGK SKREKKDRVF TDKTSATVIC

       310        320
RKNASISVRA QDRYYSSSWS EWASVPCS
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[0064] The following domains have been identified in Interleukin-12 alpha subunit:

Residues	Length	Domain ID
1-22	22	Signal peptide
23-219	197	Interleukin-12 alpha subunit

[0065] The following domains have been identified in Interleukin-12 beta subunit:

Residues	Length	Domain ID
1-22	22	Signal peptide
23-328	306	Interleukin-12 beta subunit

[0066] Thus, the term "interleukin 12" as used herein includes both the alpha and beta subunits in isolation, and holo-interleukin 12, which is a heterodimer comprising an alpha and beta subunit. 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. For example, a sandwich assay may be formulated with two antibodies that bind to alpha chain, two antibodies that bind to beta light chain, or one antibody that binds to the alpha chain and one that binds to the beta chain. While such assays may detect the full length holo-Interleukin-12 molecule and the assay result be expressed as a concentration of Interleukin-12, the signal from the assay is actually a result of all such "immunoreactive" polypeptides present in the sample.

[0067] As used herein, the term "Fibroblast growth factor 23" refers to one or polypeptides present in a biological sample that are derived from the Fibroblast growth factor 23 precursor (Swiss-Prot Q9GZV9 (SEQ ID NO: 6)).

```
        10          20          30          40          50          60
MLGARLRLWV CALCSVCSMS VLRAYPNASP LLGSSWGGLI HLYTATARNS YHLQIHKNGH

        70          80          90         100         110         120
VDGAPHQTIY SALMIRSEDA GFVVITGVMS RRYLCMDFRG NIFGSHYFDP ENCRFQHQTL

       130         140         150         160         170         180
ENGYDVYHSP QYHFLVSLGR AKRAFLPGMN PPPYSQFLSR RNEIPLIHFN TPIPRRHTRS

       190         200         210         220         230         240
AEDDSERDPL NVLKPRARMT PAPASCSQEL PSAEDNSPMA SDPLGVVRGG RVNTHAGGTG

       250
PEGCRPFAKF I
```

[0068] The following domains have been identified in Fibroblast growth factor 23:

Residues	Length	Domain ID
1-24	24	Signal sequence
25-251	227	Fibroblast growth factor 23
25-179	155	Fibroblast growth factor 23 N-terminal peptide
180-251	72	Fibroblast growth factor 23 C-terminal peptide

[0069] As used herein, the term "Intestinal fatty acid-binding protein" refers to one or more polypeptides present in a biological sample that are derived from the Intestinal fatty acid-binding protein precursor (Swiss-Prot P12104 (SEQ ID NO: 7)).

```
        10         20         30         40         50         60
MAFDSTWKVD RSENYDKFME KMGVNIVKRK LAAHDNLKLT ITQEGNKFTV KESSAFRNIE

        70         80         90        100        110        120
VVFELGVTFN YNLADGTELR GTWSLEGNKL IGKFKRTDNG NELNTVREII GDELVQTYVY

       130
EGVEAKRIFK KD
```

[0070] The following domains have been identified in Intestinal fatty acid-binding protein:

Residues	Length	Domain ID
1	1	Initiator methionine
2-132	131	Intestinal fatty acid-binding protein

[0071] As used herein, the term "Vitamin K-dependent protein C" refers to one or polypeptides present in a biological sample that are derived from the Vitamin K-dependent protein C precursor (Swiss-Prot P04070 (SEQ ID NO: 8)).

```
        10         20         30         40         50         60
MWQLTSLLLF VATWGISGTP APLDSVFSSS ERAHQVLRIR KRANSFLEEL RHSSLERECI

        70         80         90        100        110        120
EEICDFEEAK EIFQNVDDTL AFWSKHVDGD QCLVLPLEHP CASLCCGHGT CIDGIGSFSC

       130        140        150        160        170        180
DCRSGWEGRF CQREVSFLNC SLDNGGCTHY CLEEVGWRRC SCAPGYKLGD DLLQCHPAVK

       190        200        210        220        230        240
FPCGRPWKRM EKKRSHLKRD TEDQEDQVDP RLIDGKMTRR GDSPWQVVLL DSKKKLACGA

       250        260        270        280        290        300
VLIHPSWVLT AAHCMDESKK LLVRLGEYDL RRWEKWELDL DIKEVFVHPN YSKSTTDNDI

       310        320        330        340        350        360
ALLHLAQPAT LSQTIVPICL PDSGLAEREL NQAGQETLVT GWGYHSSREK EAKRNRTFVL

       370        380        390        400        410        420
NFIKIPVVPH NECSEVMSNM VSENMLCAGI LGDRQDACEG DSGGPMVASF HGTWFLVGLV

       430        440        450        460
SWGEGCGLLH NYGVYTKVSR YLDWIHGHIR DKEAPQKSWA P
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[0072] The following domains have been identified in Vitamin K-dependent protein C:

Residues	Length	Domain ID
1-32	32	Signal sequence
33-42	227	Propeptide
43-197	155	Vitamin K-dependent protein C light chain
200-461	262	Vitamin K-dependent protein C heavy chain
200-211	12	Activation peptide

[0073] As used herein, the term "relating a signal to the presence or amount" of an analyte reflects this understanding. Assay signals are typically related to the presence or amount of an analyte through the use of a standard curve calculated using known concentrations of the analyte of interest. As the term is used herein, an assay is "configured to detect" an analyte if an assay can generate a detectable signal indicative of the presence or amount of a physiologically relevant concentration of the analyte. Because an antibody epitope is on the order of 8 amino acids, an immunoassay configured to detect a marker of interest will also detect polypeptides related to the marker sequence, so long as those polypeptides contain the epitope(s) necessary to bind to the antibody or antibodies used in the assay. 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.*

[0074] 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.

[0075] 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.

[0076] 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.

[0077] 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.

[0078] 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.

[0079] 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.

[0080] Marker Assays

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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.).

[0085] 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.

[0086] 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.

[0087] Antibodies

[0088] 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."

[0089] 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} .

[0090] 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.

[0091] 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.

[0092] 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.

[0093] 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.

[0094] 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.

Assay Correlations

[0095] 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.

[0096] 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.

[0097] Suitable thresholds may be determined in a variety of ways. For example, one recommended diagnostic threshold for the diagnosis of acute myocardial infarction using cardiac troponin is the 97.5^{th} percentile of the concentration seen in a normal population. Another method may be to look at serial samples from the same patient, where a prior "baseline" result is used to monitor for temporal changes in a biomarker level.

[0098] 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.

[0099] 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.

[0100] 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.

[0101] 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.

[0102] 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

[0103] 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); Cathepsin B (P07858); Ceruloplasmin (P00450); Clusterin (P10909); Complement C3 (P01024); Cysteine-rich protein (CYR61, O00622); Cytochrome C (P99999); Epidermal growth factor (EGF, P01133); Endothelin-1 (P05305); Exosomal Fetuin-A (P02765);

Fatty acid-binding protein, heart (FABP3, P05413); Fatty acid-binding protein, liver (P07148); Ferritin (light chain, 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).

[0104] 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.

[0105] 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.

[0106] 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.

[0107] Diagnosis of Acute Renal Failure

[0117] 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.

[0118] One skilled in the art readily appreciates that the present invention is well adapted to carry out the embodiments 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.

[0119] Example 1: Contrast-induced nephropathy sample collection

[0120] The objective of this sample collection study is to collect samples of plasma and urine and clinical data from patients before and after 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.

[0112] 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:

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

[0113] 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:

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

[0114] 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.

[0115] 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).

[0116] Selecting a Treatment Regimen

[0117] 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.

[0118] 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.

[0119] Example 1: Contrast-induced nephropathy sample collection

[0120] The objective of this sample collection study is to collect samples of plasma and urine and clinical data from patients before and after 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.

[0121] 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.

[0122] 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).

[0123] 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%.

[0124] Example 2: Cardiac surgery sample collection

[0125] 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.

[0126] 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.

[0127] Example 3: Acutely ill subject sample collection

[0128] The objective of this study is to collect samples from acutely ill patients. Approximately 900 adults expected to be in the ICU for at least 48 hours will be enrolled. To be enrolled in the study, each patient must meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria

males and females 18 years of age or older;

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

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

sepsis;

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

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

contrast media exposure within 24 hours of enrollment;

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

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

Study population 3: approximately 300 patients

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

Exclusion Criteria

known pregnancy;

institutionalized individuals;

previous renal transplantation;

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

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

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

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

[0129] After providing informed consent, an EDTA anti-coagulated blood sample (10 mL) and a urine sample (25-30 mL) are collected from each patient. Blood and urine samples are then collected at 4 (± 0.5) and 8 (± 1) hours after contrast administration (if applicable); at 12 (± 1), 24 (± 2), and 48 (± 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Blood is collected via direct venipuncture or via other available venous access, such as an existing femoral sheath, central venous line, peripheral intravenous line or hep-lock. These study blood samples are processed to plasma at the clinical site, frozen and shipped to Astute Medical, Inc., San Diego, CA. The study urine samples are frozen and shipped to Astute Medical, Inc.

[0130] Example 4. Immunoassay format

[0131] Analytes are is 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.

[0132] Concentrations are expressed in the following examples as follows: soluble Advanced glycosylation end product-specific receptor – pg/mL, Bactericidal permeability-increasing protein – pg/mL, Interleukin 12 – pg/mL, Fibroblast growth factor 23 – ng/mL, Vitamin K-dependent protein C – % of amount measured in normal human serum (100% = about 1 IU/,L or 4-5 μg/mL), Intestinal fatty acid-binding protein – pg/mL.

[0133] Example 5. Apparently Healthy Donor and Chronic Disease Patient Samples

[0134] 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.

[0135] 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.

[0136] Example 6. Kidney injury markers for evaluating renal status in patients at RIFLE Stage 0

[0137] Patients from the intensive care unit (ICU) were 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.

[0138] Two cohorts were defined as (Cohort 1) patients that did not progress beyond stage 0, and (Cohort 2) patients that reached stage R, I, or F within 10 days. To address normal marker fluctuations that occur within patients at the ICU and thereby assess utility for monitoring AKI status, marker levels were measured in urine samples collected for Cohort 1. Marker concentrations were measured in urine samples collected from a subject at 0, 24 hours, and 48 hours prior to reaching stage R, I or F in Cohort 2. In the following tables, 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 for this example (0 vs R, I, F) 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).

[0139] Each marker was measured by standard immunoassay methods using commercially available assay reagents. A receiver operating characteristic (ROC) curve was generated for each marker and the area under each ROC curve (AUC) was determined. Patients in Cohort 2 were also separated according to the reason for adjudication to stage R, I, or F 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. That is, for those patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements alone, the stage 0 cohort may have included 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 have included 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, for those patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements or urine output, the adjudication method which yielded the most severe RIFLE stage was used.

[0140] The ability to distinguish cohort 1 (subjects remaining in RIFLE 0) from Cohort 2 (subjects progressing to RIFLE R, I or F) 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 were calculated as described in Hanley, J. A., and McNeil, B.J., The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology (1982) 143: 29-36; p values were calculated with a two-tailed Z-test. An AUC < 0.5 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.

[0141] Various threshold (or "cutoff") concentrations were selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 were determined. OR is the odds ratio calculated for the particular cutoff concentration, and 95% CI is the confidence interval for the odds ratio.

[0142] The results of these three analyses for various markers of the present invention are presented in Fig. 1.

[0143] Example 7. Kidney injury markers for evaluating renal status in patients at RIFLE Stages 0 and R

[0144] Patients were classified and analyzed as described in Example 6. However, patients that reached stage R but did not progress to stage I or F were grouped with patients from non-injury stage 0 in Cohort 1. Cohort 2 in this example included only patients that progressed to stage I or F. Marker concentrations in urine samples were included for Cohort 1. Marker concentrations in urine samples collected within 0, 24, and 48 hours of reaching stage I or F were included for Cohort 2.

[0145] The ability to distinguish cohort 1 (subjects remaining in RIFLE 0 or R) from Cohort 2 (subjects progressing to RIFLE I or F) was determined using ROC analysis.

[0146] Various threshold (or "cutoff") concentrations were selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 were determined. OR is the odds ratio calculated for the particular cutoff concentration, and 95% CI is the confidence interval for the odds ratio.

[0147] The results of these three analyses for various markers of the present invention are presented in Fig. 2.

[0148] Example 8. Kidney injury markers for evaluating renal status in patients progressing from Stage R to Stages I and F

[0149] Patients were classified and analyzed as described in Example 6, but only those patients that reached Stage R were included in this example. Cohort 1 contained patients that reached stage R but did not progress to stage I or F within 10 days, and Cohort 2 included only patients that progressed to stage I or F. Marker concentrations in urine samples collected within 12 hours of reaching stage R were included in the analysis for both Cohort 1 and 2.

[0150] The ability to distinguish cohort 1 (subjects remaining in RIFLE R) from Cohort 2 (subjects progressing to RIFLE I or F) was determined using ROC analysis.

[0151] Various threshold (or "cutoff") concentrations were selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 were determined. OR is the odds ratio calculated for the particular cutoff concentration, and 95% CI is the confidence interval for the odds ratio.

[0152] The results of these three analyses for various markers of the present invention are presented in Fig. 3.

[0153] Example 9. Kidney injury markers for evaluating renal status in patients at RIFLE Stage 0

[0154] Patients were classified and analyzed as described in Example 6. However, patients that reached stage R or I but did not progress to stage F were eliminated from the analysis. Patients from non-injury stage 0 are included in Cohort 1. Cohort 2 in this example included only patients that progressed to stage F. The maximum marker concentrations in urine samples were included for each patient in Cohort 1. The maximum marker concentrations in urine samples collected within 0, 24, and 48 hours of reaching stage F were included for each patient in Cohort 2.

[0155] The ability to distinguish cohort 1 (subjects remaining in RIFLE 0 or R) from Cohort 2 (subjects progressing to RIFLE I or F) was determined using ROC analysis.

[0156] Various threshold (or "cutoff") concentrations were selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 were

determined. OR is the odds ratio calculated for the particular cutoff concentration, and 95% CI is the confidence interval for the odds ratio.

[0157] The results of these three analyses for various markers of the present invention are presented in Fig. 4.

[0158] Example 10. Kidney injury markers for evaluating renal status in patients at RIFLE Stage 0

[0159] Patients from the intensive care unit (ICU) were 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.

[0160] Two cohorts were defined as (Cohort 1) patients that did not progress beyond stage 0, and (Cohort 2) patients that reached stage R, I, or F within 10 days. To address normal marker fluctuations that occur within patients at the ICU and thereby assess utility for monitoring AKI status, marker levels were measured in the plasma component of blood samples collected for Cohort 1. Marker concentrations were measured in the plasma component of blood samples collected from a subject at 0, 24 hours, and 48 hours prior to reaching stage R, I or F in Cohort 2. In the following tables, 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 for this example (0 vs R, I, F) 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).

[0161] Each marker was measured by standard immunoassay methods using commercially available assay reagents. A receiver operating characteristic (ROC) curve was generated for each marker and the area under each ROC curve (AUC) was determined. Patients in Cohort 2 were also separated according to the reason for adjudication to stage R, I, or F 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. That is, for those patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements alone, the stage 0 cohort may have included 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 have included 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, for those patients adjudicated to stage R, I, or F on the basis of serum creatinine measurements or urine output, the adjudication method which yielded the most severe RIFLE stage was used.

[0162] The ability to distinguish cohort 1 (subjects remaining in RIFLE 0) from Cohort 2 (subjects progressing to RIFLE R, I or F) 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 were calculated as described in Hanley, J. A., and McNeil, B.J., The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology (1982) 143: 29-36; p values were calculated with a two-tailed Z-test. An AUC < 0.5 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.

[0163] Various threshold (or "cutoff") concentrations were selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 were determined. OR is the odds ratio calculated for the particular cutoff concentration, and 95% CI is the confidence interval for the odds ratio.

[0164] The results of these three analyses for various markers of the present invention are presented in Fig. 5.

[0165] Example 11. Kidney injury markers for evaluating renal status in patients at RIFLE Stages 0 and R

[0166] Patients were classified and analyzed as described in Example 10. However, patients that reached stage R but did not progress to stage I or F were grouped with patients from non-injury stage 0 in Cohort 1. Cohort 2 in this example included only patients that progressed to stage I or F. Marker concentrations in the plasma component of blood samples were included for Cohort 1. Marker concentrations in the plasma component of blood samples collected within 0, 24, and 48 hours of reaching stage I or F were included for Cohort 2.

[0167] The ability to distinguish cohort 1 (subjects remaining in RIFLE 0 or R) from Cohort 2 (subjects progressing to RIFLE I or F) was determined using ROC analysis.

[0168] Various threshold (or "cutoff") concentrations were selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 were

determined. OR is the odds ratio calculated for the particular cutoff concentration, and 95% CI is the confidence interval for the odds ratio.

[0169] The results of these three analyses for various markers of the present invention are presented in Fig. 6.

[0170] Example 12. Kidney injury markers for evaluating renal status in patients progressing from Stage R to Stages I and F

[0171] Patients were classified and analyzed as described in Example 10, but only those patients that reached Stage R were included in this example. Cohort 1 contained patients that reached stage R but did not progress to stage I or F within 10 days, and Cohort 2 included only patients that progressed to stage I or F. Marker concentrations in the plasma component of blood samples collected within 12 hours of reaching stage R were included in the analysis for both Cohort 1 and 2.

[0172] The ability to distinguish cohort 1 (subjects remaining in RIFLE R) from Cohort 2 (subjects progressing to RIFLE I or F) was determined using ROC analysis.

[0173] Various threshold (or "cutoff") concentrations were selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 were determined. OR is the odds ratio calculated for the particular cutoff concentration, and 95% CI is the confidence interval for the odds ratio.

[0174] The results of these three analyses for various markers of the present invention are presented in Fig. 7.

[0175] Example 13. Kidney injury markers for evaluating renal status in patients at RIFLE Stage 0

[0176] Patients were classified and analyzed as described in Example 10. However, patients that reached stage R or I but did not progress to stage F were eliminated from the analysis. Patients from non-injury stage 0 are included in Cohort 1. Cohort 2 in this example included only patients that progressed to stage F. The maximum marker concentrations in the plasma component of blood samples were included from each patient in Cohort 1. The maximum marker concentrations in the plasma component of blood samples collected within 0, 24, and 48 hours of reaching stage F were included from each patient in Cohort 2.

[0177] The ability to distinguish cohort 1 (subjects remaining in RIFLE 0 or R) from Cohort 2 (subjects progressing to RIFLE I or F) was determined using ROC analysis.

[0178] Various threshold (or "cutoff") concentrations were selected, and the associated sensitivity and specificity for distinguishing cohort 1 from cohort 2 were determined. OR is the odds ratio calculated for the particular cutoff concentration, and 95% CI is the confidence interval for the odds ratio.

[0179] The results of these three analyses for various markers of the present invention are presented in Fig. 8.

[0180] 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.

[0181] 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.

[0182] 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.

[0183] 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.

[0184] Other embodiments are set forth within the following claims.

2010210537 22 May 2015

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

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

performing one or more assays configured to detect Vitamin K-dependent protein C in a body fluid sample obtained from the subject to provide one or more assay results; and

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

(i) determining a measured concentration of Vitamin K-dependent protein C in the body fluid sample;

(ii) comparing the measured concentration of Vitamin K-dependent protein C to a threshold concentration of Vitamin K-dependent protein C and;

(iii) based on the assay results:

(a) assigning a likelihood of a future event occurring or determining that an event has occurred when the measured concentration is above the threshold; or

(b) assigning a likelihood of a future event not occurring or determining that an event has not occurred when the measured concentration is below the threshold.

2. The method according to claim 1, wherein determining a measured concentration of Vitamin K-dependent protein C in the body fluid sample comprises contacting the body fluid sample with an antibody or antibody fragment that binds to Vitamin K-dependent protein C or part thereof, to thereby form a complex, and correlating the amount of the antibody or antibody fragment bound to the Vitamin K-dependent protein C or part thereof in the body fluid sample.

3. The method according to claim 2, wherein the assay result comprises an amount of the complex formed that is related to the amount of Vitamin K-dependent protein C in the body fluid sample such that the measured concentration of Vitamin K-dependent protein C is determined from the amount of complex formed.

4. The method according to claim 2 or 3, wherein an antibody or antibody fragment is bound to a solid support.

5. The method according to any one of claims 2 to 4, wherein an antibody or antibody fragment is detectably labelled.

6. The method according to any one of claims 1 to 5, wherein the body fluid sample comprises urine.

7. The method according to any one of claims 1 to 5, wherein the body fluid sample comprises blood, serum or plasma.

8. The method according to any one of claims 1 to 7, wherein the threshold concentration of Vitamin K-dependent protein C is a level of Vitamin K-dependent protein C in a comparable body fluid sample within the 75th percentile or 85th percentile or 90th percentile or 95th percentile or 99th percentile of a population of healthy subjects not having a pre-existing known risk factor for prerenal, intrinsic renal, or postrenal acute renal failure (ARF).

9. The method according to any one of claims 1 to 7, wherein the threshold concentration of Vitamin K-dependent protein C is determined by ROC analysis such that an area under a Receiver Operating Characteristic (ROC) curve is at least 0.5.

10. The method according to any one of claims 1 to 7, wherein the threshold concentration of Vitamin K-dependent protein C is a level of Vitamin K-dependent protein C that distinguishes subjects that are predisposed to prerenal, intrinsic renal, or postrenal acute renal failure (ARF) from subjects that are not predisposed to prerenal, intrinsic renal, or postrenal ARF, wherein said threshold value is determined by an odds ratio having a value greater than 1.0 and/or a specificity having a value greater than 0.5 and/or a sensitivity having a value greater than 0.5 and/or a positive likelihood ratio having a value greater than 1.0.

11. The method according to any one of claims 1 to 10, wherein correlating the assay result(s) to the renal status of the subject comprises assigning a likelihood of one or more future events occurring or not occurring based on the assay result.

12. The method according to claim 11, wherein a future event is selected from a future injury to renal function, a future reduced renal function, a future improvement in renal function, and a future acute renal failure (ARF).

13. The method according to claim 11, wherein a future event comprises a clinical outcome related to a renal injury suffered by the subject.

14. The method according to claim 11, wherein a future event comprises a worsening stage of acute kidney injury (AKI), mortality, a need for renal replacement therapy, a need for withdrawal of renal toxins, end stage renal disease, heart failure, stroke, myocardial infarction, or chronic kidney disease.

15. The method according to any one of claims 11 to 14, wherein the likelihood of the future event occurring or not occurring is a likelihood of the event occurring or not occurring within 30 days from the time at which the body fluid sample is obtained from the subject.

16. The method according to any one of claims 11 to 14, wherein the likelihood of the future event occurring or not occurring is a likelihood of the event occurring or not occurring within a period of time selected from the group consisting of 21 days or 14 days or 7 days or 5 days or 96 hours or 72 hours or 48 hours or 36 hours or 24 hours or 12 hours from the time at which the body fluid is obtained from the subject.

17. The method according to any one of claims 6 to 8, wherein the likelihood of the future event occurring or not occurring is a likelihood of the event occurring or not occurring within a period of time between 12 hours and 48 hours from the time at which the body fluid sample is obtained from the subject.

18. The method according to any one of claims 1 to 10, wherein correlating the assay result(s) to the renal status of the subject comprises assigning a diagnosis that an event has occurred or not occurred based on the assay result(s), and wherein the event comprises an event selected from an injury to renal function, reduced renal function, acute renal failure (ARF), a need for renal replacement therapy, a need for withdrawal of renal toxins, end stage renal disease, heart failure, stroke, myocardial infarction, or chronic kidney disease.

19. The method according to any one of claims 1 to 10, wherein the event is a change in renal status of a subject who has suffered from an injury to renal function, reduced renal function, or acute renal failure (ARF), and wherein correlating the assay result(s) to the renal status of the subject based on the assay result(s) comprises assigning a diagnosis of worsening renal function to the subject when the measured concentration of Vitamin K-dependent protein C in the body fluid sample is above the threshold, or assigning a diagnosis of improving renal function when the measured concentration of Vitamin K-dependent protein C in the body fluid sample is below the threshold.

20. The method according to any one of claims 1 to 19, wherein the subject has a known pre-existing known risk factor for one or more of prerenal ARF, intrinsic renal ARF, or postrenal ARF.

21. The method according to any one of claims 1 to 19, wherein the subject ha a pre-existing diagnosis when the body fluid sample is obtained for 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.

22. The method according to any one of claims 1 to 21 when used to diagnose acute renal failure or to predict a risk of future acute renal failure in a subject.

23. The method according to any one of claims 1 to 21 when used to diagnose a need or otherwise for renal replacement therapy or renal transplantation in a subject.

24. A kit when used in the method according to any one of claims 1 to 21, wherein the kit comprises an antibody or antibody fragment that binds specifically to Vitamin K-dependent protein C or a part thereof in said method.

25. The kit according to claim 24, wherein the antibody or antibody fragment is contained in a single assay device.

26. The kit according to claim 25, wherein the assay device is configured for performance of the assay as a sandwich binding assay or a competitive binding assay.

27. The kit according to any one of claims 24 to 26, wherein the antibody or antibody fragment is bound to a solid support.

28. The kit according to any one of claims 24 to 27, wherein the kit comprises a detectably-labelled antibody or antibody fragment that binds specifically to Vitamin K-dependent protein C or a part thereof.

Advanced glycosylation end product-specific receptor

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	6.855	8.139	6.855	7.753	6.855	6.478
average	12.110	13.167	12.110	14.243	12.110	14.333
stdev	17.736	15.457	17.736	15.931	17.736	16.247
p (t-test)		0.715		0.437		0.558
min	0.000	0.000	0.000	0.000	0.000	0.000
max	157.048	60.844	157.048	60.844	157.048	48.133
n (Samp)	117	50	117	59	117	26
n (Pat)	98	50	98	59	98	26

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	6.819	8.865	6.819	11.406	6.819	13.439
average	12.541	12.126	12.541	20.138	12.541	23.598
stdev	16.430	14.031	16.430	17.835	16.430	22.191
p (t-test)		0.919		0.036		0.017
min	0.000	0.000	0.000	0.000	0.000	0.000
max	157.048	54.823	157.048	57.901	157.048	63.671
n (Samp)	259	17	259	23	259	14
n (Pat)	159	17	159	23	159	14

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	6.855	7.434	6.855	6.819	6.855	5.203
average	11.550	13.606	11.550	12.589	11.550	13.740
stdev	17.850	16.217	17.850	15.335	17.850	15.764
p (t-test)		0.511		0.726		0.588
min	0.000	0.000	0.000	0.000	0.000	0.000
max	157.048	60.844	157.048	60.844	157.048	48.133
n (Samp)	106	44	106	49	106	23
n (Pat)	84	44	84	49	84	23

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.51	0.049	117	50	0.781
24 hours	0.54	0.046	117	59	0.433
48 hours	0.49	0.062	117	26	0.858

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.50	0.073	259	17	0.960
24 hours	0.65	0.064	259	23	0.022
48 hours	0.65	0.082	259	14	0.065

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.53	0.052	106	44	0.599
24 hours	0.50	0.050	106	49	0.934
48 hours	0.49	0.066	106	23	0.848

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	4.1060226	70%	26%	1			
	2.695557	84%	16%	2	0.7	0.4	1.1
	2.4004743	90%	12%	3	1.0	0.6	1.5
	12.537211	30%	73%	4	1.1	0.7	1.7
	18.202346	20%	80%				

FIG. 1 - 1

	25.74785	16%	91%				
24 hours	4.7647201	73%	37%	1			
	3.0449662	81%	25%	2	1.5	1.0	2.3
	1.1155724	92%	7%	3	1.4	0.9	2.1
	12.537211	29%	73%	4	1.5	1.0	2.3
	18.202346	24%	80%				
	25.74785	19%	91%				
48 hours	2.0823337	81%	8%	1			
	2.0823337	81%	8%	2	0.4	0.2	0.9
	0	100%	0%	3	0.5	0.2	1.0
	12.537211	35%	73%	4	0.9	0.5	1.6
	18.202346	27%	80%				
	25.74785	23%	91%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	3.8341457	71%	29%	1			
	2.4004743	82%	17%	2	0.4	0.1	1.6
	0	100%	0%	3	1.0	0.4	2.3
	12.537211	29%	71%	4	1.0	0.4	2.3
	18.676326	18%	81%				
	34.771676	6%	90%				
24 hours	7.114362	74%	54%	1			
	6.5017597	83%	47%	2	1.0	0.3	3.9
	2.0823337	91%	12%	3	2.9	1.1	7.5
	12.537211	39%	71%	4	3.2	1.3	8.2
	18.676326	39%	81%				
	34.771676	26%	90%				
48 hours	7.8431149	71%	56%	1			
	3.9993481	86%	29%	2	0.5	0.0	9.9
	0	100%	0%	3	2.1	0.4	9.5
	12.537211	50%	71%	4	3.7	1.0	14.0
	18.676326	43%	81%				
	34.771676	36%	90%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	4.3680939	70%	33%	1			
	2.695557	86%	19%	2	0.8	0.5	1.4
	2.3465455	91%	13%	3	0.9	0.5	1.5
	12.096245	34%	72%	4	1.2	0.8	2.0
	14.033515	23%	81%				
	23.570652	16%	91%				
24 hours	4.3680939	76%	33%	1			
	2.3465455	84%	13%	2	1.1	0.7	1.7
	0	100%	0%	3	0.9	0.5	1.4
	12.096245	27%	72%	4	1.1	0.7	1.7
	14.033515	22%	81%				
	23.570652	14%	91%				
48 hours	2.0823337	78%	9%	1			
	0	100%	0%	2	0.3	0.1	0.9
	0	100%	0%	3	0.4	0.2	1.1
	12.096245	39%	72%	4	1.0	0.5	2.0
	14.033515	35%	81%				
	23.570652	26%	91%				

FIG. 1 - 2

Bactericidal permeability-increasing protein

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	68.589	1.197	68.589	188.416	68.589	1.197
average	521.788	1639.124	521.788	1638.451	521.788	2364.029
stdev	1276.638	5720.117	1276.638	4950.622	1276.638	na
p (t-test)		0.188		0.135		na
min	1.197	1.197	1.197	1.197	1.197	2364.029
max	6951.049	26300.578	6951.049	23569.364	6951.049	2364.029
n (Samp)	51	21	51	22	51	1
n (Pat)	40	21	40	22	40	1

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	68.589	26.851	68.589	1.197	68.589	4645.281
average	1034.836	772.914	1034.836	237.731	1034.836	4645.281
stdev	3761.716	1530.053	3761.716	396.119	3761.716	4971.749
p (t-test)		0.866		0.578		0.184
min	1.197	1.197	1.197	1.197	1.197	1129.724
max	26300.578	3838.655	26300.578	1055.470	26300.578	8160.839
n (Samp)	91	6	91	7	91	2
n (Pat)	73	6	73	7	73	2

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	13.962	1.197	13.962	744.883	13.962	1318.997
average	531.640	1861.582	531.640	1879.756	531.640	1619.466
stdev	1372.041	6532.374	1372.041	5157.268	1372.041	1817.350
p (t-test)		0.211		0.117		0.147
min	1.197	1.197	1.197	1.197	1.197	1.214
max	6951.049	26300.578	6951.049	23569.364	6951.049	3838.655
n (Samp)	42	16	42	20	42	4
n (Pat)	33	16	33	20	33	4

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.47	0.075	51	21	0.689
24 hours	0.59	0.074	51	22	0.236
48 hours	0.96	0.137	51	1	0.001

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.52	0.123	91	6	0.894
24 hours	0.44	0.108	91	7	0.553
48 hours	0.91	0.143	91	2	0.004

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.46	0.084	42	16	0.595
24 hours	0.68	0.076	42	20	0.021
48 hours	0.79	0.139	42	4	0.036

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0	100%	0%	1			
	0	100%	0%	2	0.4	0.1	1.4
	0	100%	0%	3	0.8	0.3	2.2
	242.176	29%	71%	4	1.3	0.5	3.3
	435.64912	29%	80%				
	1476.2431	10%	90%				

FIG. 1 - 3

24 hours	0	100%	0%	1			
	0	100%	0%	2	17.0	1.4	209.4
	0	100%	0%	3	3.4	0.2	59.3
	242.176	45%	71%	4	15.3	1.3	184.4
	435.64912	45%	80%				
	1476.2431	18%	90%				
48 hours	2322.0168	100%	96%	1			
	2322.0168	100%	96%	2	na	na	na
	2322.0168	100%	96%	3	na	na	na
	242.176	100%	71%	4	na	na	na
	435.64912	100%	80%				
	1476.2431	100%	90%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0	100%	0%	1			
	0	100%	0%	2	na	na	na
	0	100%	0%	3	na	na	na
	260.49123	33%	70%	4	na	na	na
	860.99448	17%	80%				
	1837.9832	17%	90%				
24 hours	0	100%	0%	1			
	0	100%	0%	2	0.5	0.0	11.2
	0	100%	0%	3	1.6	0.3	9.6
	260.49123	29%	70%	4	0.5	0.0	11.2
	860.99448	14%	80%				
	1837.9832	0%	90%				
48 hours	1033.2103	100%	84%	1			
	1033.2103	100%	84%	2	na	na	na
	1033.2103	100%	84%	3	na	na	na
	260.49123	100%	70%	4	na	na	na
	860.99448	100%	80%				
	1837.9832	50%	90%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0	100%	0%	1			
	0	100%	0%	2	0.5	0.1	2.8
	0	100%	0%	3	2.4	0.7	7.9
	205.312	25%	71%	4	0.8	0.2	3.4
	435.64912	25%	81%				
	1476.2431	6%	90%				
24 hours	9.3090909	70%	50%	1			
	0	100%	0%	2	3.0	0.5	16.2
	0	100%	0%	3	2.4	0.4	14.3
	205.312	60%	71%	4	8.4	1.6	42.6
	435.64912	55%	81%				
	1476.2431	20%	90%				
48 hours	242.176	75%	76%	1			
	1.1968	100%	50%	2	na	na	na
	1.1968	100%	50%	3	na	na	na
	205.312	75%	71%	4	na	na	na
	435.64912	50%	81%				
	1476.2431	50%	90%				

FIG. 1 - 4

Fatty acid-binding protein, intestinal

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	128.000	254.072	128.000	313.435	128.000	1.009
average	268.455	336.124	268.455	412.012	268.455	293.398
stdev	382.350	418.596	382.350	384.452	382.350	na
p (t-test)		0.509		0.146		na
min	1.009	1.009	1.009	1.009	1.009	293.398
max	1831.799	1826.301	1831.799	1307.113	1831.799	293.398
n (Samp)	51	21	51	22	51	1
n (Pat)	40	21	40	22	40	1

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	133.949	115.223	133.949	721.311	133.949	287.698
average	302.158	173.388	302.158	659.143	302.158	287.698
stdev	404.635	165.169	404.635	477.563	404.635	375.731
p (t-test)		0.442		0.029		0.960
min	1.009	1.009	1.009	13.781	1.009	22.016
max	1831.799	452.414	1831.799	1430.126	1831.799	553.379
n (Samp)	91	6	91	7	91	2
n (Pat)	73	6	73	7	73	2

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	132.710	266.217	132.710	313.435	132.710	287.228
average	304.659	384.649	304.659	383.398	304.659	291.557
stdev	400.682	462.708	400.682	386.671	400.682	128.001
p (t-test)		0.518		0.467		0.949
min	1.009	1.009	1.009	1.009	1.009	139.358
max	1831.799	1826.301	1831.799	1307.113	1831.799	452.414
n (Samp)	42	16	42	20	42	4
n (Pat)	33	16	33	20	33	4

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.57	0.076	51	21	0.353
24 hours	0.62	0.074	51	22	0.106
48 hours	0.75	0.288	51	1	0.396

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.47	0.120	91	6	0.831
24 hours	0.74	0.111	91	7	0.029
48 hours	0.50	0.207	91	2	1.000

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.56	0.086	42	16	0.507
24 hours	0.55	0.079	42	20	0.559
48 hours	0.66	0.155	42	4	0.301

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	92.647619	71%	45%	1			
	29.696	81%	27%	2	1.0	0.3	3.5
	0	100%	0%	3	1.8	0.6	5.4
	251.85352	52%	71%	4	2.2	0.8	6.6
	464	19%	80%				
	697.70492	10%	90%				

FIG. 1 - 5

24 hours	47.36	73%	33%	1			
	29.696	82%	27%	2	1.0	0.3	3.5
	12.032	91%	24%	3	1.0	0.3	3.5
	251.85352	55%	71%	4	3.9	1.4	11.0
	464	45%	80%				
	697.70492	27%	90%				
48 hours	291.08434	100%	75%	1			
	291.08434	100%	75%	2	na	na	na
	291.08434	100%	75%	3	na	na	na
	251.85352	100%	71%	4	na	na	na
	464	0%	80%				
	697.70492	0%	90%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	65.828571	83%	40%	1			
	65.828571	83%	40%	2	2.2	0.1	49.0
	0	100%	0%	3	2.2	0.1	49.0
	317.68675	17%	70%	4	1.0	0.0	62.1
	593.10345	0%	80%				
	745.37931	0%	90%				
24 hours	601.37931	71%	81%	1			
	136.09639	86%	54%	2	0.0	0.0	na
	12.032	100%	19%	3	1.0	0.0	59.8
	317.68675	71%	70%	4	5.8	0.5	72.6
	593.10345	71%	80%				
	745.37931	29%	90%				
48 hours	19.712	100%	21%	1			
	19.712	100%	21%	2	0.0	0.0	na
	19.712	100%	21%	3	0.0	0.0	na
	317.68675	50%	70%	4	1.0	0.0	62.7
	593.10345	0%	80%				
	745.37931	0%	90%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	94.334247	75%	43%	1			
	28.928	81%	17%	2	0.4	0.1	2.4
	0	100%	0%	3	1.9	0.5	6.6
	356.41379	44%	71%	4	0.9	0.2	3.5
	601.37931	19%	81%				
	721.31148	13%	90%				
24 hours	47.36	70%	24%	1			
	28.928	80%	17%	2	0.2	0.0	1.1
	0	100%	0%	3	1.0	0.3	3.0
	356.41379	45%	71%	4	0.9	0.3	2.6
	601.37931	30%	81%				
	721.31148	20%	90%				
48 hours	236.70986	75%	67%	1			
	136.09639	100%	55%	2	na	na	na
	136.09639	100%	55%	3	na	na	na
	356.41379	25%	71%	4	na	na	na
	601.37931	0%	81%				
	721.31148	0%	90%				

FIG. 1 - 6

Fibroblast growth factor 23 (Intact; N and C-term peptides)

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	19.469	20.516	19.469	20.858	19.469	0.022
average	19.502	25.115	19.502	29.402	19.502	21.619
stdev	13.011	22.924	13.011	29.729	13.011	na
p (t-test)		0.214		0.052		na
min	0.022	5.036	0.022	6.581	0.022	21.619
max	97.051	108.680	97.051	113.238	97.051	21.619
n (Samp)	51	17	51	21	51	1
n (Pat)	40	17	40	21	40	1

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	18.770	36.648	18.770	25.702	18.770	18.857
average	19.825	56.259	19.825	36.980	19.825	18.857
stdev	15.251	39.997	15.251	34.400	15.251	4.567
p (t-test)		0.000		0.013		0.929
min	0.022	14.386	0.022	13.882	0.022	15.628
max	106.824	108.680	106.824	113.238	106.824	22.086
n (Samp)	87	5	87	7	87	2
n (Pat)	69	5	69	7	69	2

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	20.167	20.167	20.167	18.941	20.167	40.417
average	19.548	17.968	19.548	25.454	19.548	52.783
stdev	6.922	6.053	6.922	24.586	6.922	38.427
p (t-test)		0.464		0.159		0.000
min	0.022	5.036	0.022	6.581	0.022	21.619
max	33.907	24.998	33.907	106.824	33.907	108.680
n (Samp)	41	13	41	18	41	4
n (Pat)	33	13	33	18	33	4

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.58	0.082	51	17	0.359
24 hours	0.56	0.076	51	21	0.446
48 hours	0.73	0.293	51	1	0.442

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.85	0.111	87	5	0.002
24 hours	0.77	0.107	87	7	0.011
48 hours	0.56	0.213	87	2	0.788

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.46	0.091	41	13	0.628
24 hours	0.48	0.082	41	18	0.778
48 hours	0.93	0.092	41	4	0.000

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	17.548066	71%	45%	1			
	14.230939	82%	31%	2	1.4	0.3	6.0
	9.1679558	94%	14%	3	1.4	0.3	6.0
	21.38895	41%	73%	4	2.5	0.7	9.3
	23.113594	29%	80%				
	26.442656	18%	90%				
24 hours	15.103867	71%	33%	1			

FIG. 1 - 7

	11.149398	81%	18%	2	1.0	0.3	3.0
	9.5171271	90%	16%	3	0.7	0.2	2.4
	21.38895	43%	73%	4	1.7	0.6	4.5
	23.113594	33%	80%				
	26.442656	24%	90%				
48 hours	21.38895	100%	73%	1			
	21.38895	100%	73%	2	na	na	na
	21.38895	100%	73%	3	na	na	na
	21.38895	100%	73%	4	na	na	na
	23.113594	0%	80%				
	26.442656	0%	90%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	16.479518	77%	29%	1			
	9.2457831	85%	7%	2	1.6	0.3	7.6
	9.1679558	92%	7%	3	1.0	0.2	5.3
	21.38895	31%	71%	4	1.1	0.2	5.9
	24.221328	8%	80%				
	28.849095	0%	90%				
24 hours	13.624096	72%	20%	1			
	10.390055	83%	7%	2	1.4	0.4	4.8
	6.5807229	94%	5%	3	0.7	0.2	3.0
	21.38895	39%	71%	4	2.1	0.6	7.1
	24.221328	22%	80%				
	28.849095	17%	90%				
48 hours	33.90689	75%	100%	1			
	21.38895	100%	71%	2	na	na	na
	21.38895	100%	71%	3	na	na	na
	21.38895	100%	71%	4	na	na	na
	24.221328	75%	80%				
	28.849095	75%	90%				

FIG. 1 - 8

Interleukin-12

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.677	0.621	0.677	0.567	0.677	0.341
average	2.464	1.563	2.464	0.900	2.464	0.607
stdev	10.300	1.995	10.300	1.163	10.300	0.933
p (t-test)		0.537		0.243		0.361
min	0.000	0.000	0.000	0.000	0.000	0.000
max	110.665	7.954	110.665	5.530	110.665	3.407
n (Samp)	116	51	116	60	116	26
n (Pat)	98	51	98	60	98	26

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.599	0.629	0.599	0.629	0.599	0.383
average	1.720	1.793	1.720	0.882	1.720	0.665
stdev	6.985	1.993	6.985	1.144	6.985	0.933
p (t-test)		0.966		0.567		0.573
min	0.000	0.205	0.000	0.000	0.000	0.000
max	110.665	7.010	110.665	5.530	110.665	2.936
n (Samp)	260	17	260	23	260	14
n (Pat)	159	17	159	23	159	14

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.690	0.621	0.690	0.482	0.690	0.319
average	2.656	1.555	2.656	0.769	2.656	0.558
stdev	10.811	1.969	10.811	1.062	10.811	0.849
p (t-test)		0.499		0.221		0.356
min	0.000	0.000	0.000	0.000	0.000	0.000
max	110.665	7.954	110.665	4.552	110.665	3.407
n (Samp)	105	45	105	50	105	23
n (Pat)	84	45	84	50	84	23

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.50	0.049	116	51	0.970
24 hours	0.41	0.044	116	60	0.045
48 hours	0.30	0.051	116	26	0.000

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.59	0.075	260	17	0.243
24 hours	0.48	0.062	260	23	0.756
48 hours	0.35	0.068	260	14	0.032

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.49	0.051	105	45	0.871
24 hours	0.36	0.046	105	50	0.002
48 hours	0.29	0.054	105	23	0.000

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.4372089	71%	32%	1			
	0.3600036	80%	28%	2	2.7	1.7	4.3
	0.1556098	90%	17%	3	1.1	0.6	1.9
	1.7046286	29%	72%	4	1.8	1.1	2.9
	2.9495786	18%	81%				
	4.2705662	10%	91%				

FIG. 1 - 9

24 hours	0.313338	70%	26%	1			
	0.1884147	80%	19%	2	2.2	1.4	3.5
	0.0691154	90%	10%	3	2.4	1.5	3.9
	1.7046286	15%	72%	4	2.7	1.7	4.3
	2.9495786	8%	81%				
	4.2705662	5%	91%				
48 hours	0.1564596	77%	17%	1			
	0.1556098	81%	17%	2	0.3	0.0	5.0
	0.0102627	92%	6%	3	4.8	1.8	12.8
	1.7046286	12%	72%	4	5.0	1.9	13.3
	2.9495786	4%	81%				
	4.2705662	0%	91%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.4900236	71%	43%	1			
	0.2425495	82%	22%	2	0.5	0.1	2.2
	0.2049268	94%	18%	3	1.3	0.5	3.3
	1.0290954	41%	70%	4	1.5	0.6	3.7
	2.3964026	35%	80%				
	3.751011	12%	90%				
24 hours	0.419541	74%	37%	1			
	0.2759994	83%	24%	2	5.7	1.6	19.5
	0.0583286	91%	9%	3	3.2	0.8	12.5
	1.0290954	17%	70%	4	2.7	0.6	11.1
	2.3964026	4%	80%				
	3.751011	4%	90%				
48 hours	0.2130757	71%	19%	1			
	0.1556098	86%	16%	2	0.5	0.0	10.0
	0.0302445	93%	8%	3	2.6	0.6	11.0
	1.0290954	14%	70%	4	3.2	0.8	12.7
	2.3964026	14%	80%				
	3.751011	0%	90%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.4123067	71%	31%	1			
	0.3662131	80%	29%	2	1.0	0.6	1.7
	0.0691154	91%	10%	3	1.4	0.9	2.3
	2.200234	29%	70%	4	0.8	0.5	1.4
	3.275838	13%	80%				
	4.3476354	11%	90%				
24 hours	0.2132405	70%	19%	1			
	0.1187304	80%	12%	2	1.8	1.0	3.2
	0.0394811	90%	9%	3	2.9	1.6	5.0
	2.200234	12%	70%	4	3.7	2.1	6.4
	3.275838	4%	80%				
	4.3476354	4%	90%				
48 hours	0.1846528	74%	17%	1			
	0.1556098	83%	16%	2	1.0	0.1	8.1
	0.0039205	91%	6%	3	5.9	1.5	22.6
	2.200234	9%	70%	4	6.8	1.8	25.8
	3.275838	4%	80%				
	4.3476354	0%	90%				

FIG. 1 - 10

Vitamin K-dependent protein C

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	31.521	39.267	31.521	49.672	31.521	2.716
average	43.646	49.087	43.646	58.020	43.646	52.157
stdev	39.530	45.890	39.530	60.702	39.530	na
p (t-test)		0.614		0.228		na
min	2.716	4.432	2.716	2.222	2.716	52.157
max	230.426	193.805	230.426	303.398	230.426	52.157
n (Samp)	51	21	51	23	51	1
n (Pat)	39	21	39	23	39	1

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	41.596	17.455	41.596	26.908	41.596	115.226
average	50.593	20.693	50.593	37.821	50.593	115.226
stdev	44.776	18.107	44.776	41.014	44.776	111.128
p (t-test)		0.108		0.466		0.052
min	2.716	4.432	2.716	2.222	2.716	36.646
max	303.398	50.044	303.398	118.163	303.398	193.805
n (Samp)	93	6	93	7	93	2
n (Pat)	72	6	72	7	72	2

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	30.769	49.130	30.769	49.672	30.769	51.101
average	36.071	58.550	36.071	60.893	36.071	39.995
stdev	26.324	48.005	26.324	61.887	26.324	23.606
p (t-test)		0.026		0.029		0.776
min	3.085	5.871	3.085	14.294	3.085	4.640
max	92.826	193.805	92.826	303.398	92.826	53.141
n (Samp)	42	16	42	21	42	4
n (Pat)	32	16	32	21	32	4

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.52	0.076	51	21	0.781
24 hours	0.59	0.073	51	23	0.210
48 hours	0.71	0.297	51	1	0.488

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.23	0.082	93	6	0.001
24 hours	0.37	0.101	93	7	0.203
48 hours	0.72	0.208	93	2	0.302

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.64	0.085	42	16	0.092
24 hours	0.66	0.075	42	21	0.030
48 hours	0.58	0.157	42	4	0.621

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	22.369478	71%	33%	1			
	6.2271898	81%	8%	2	0.6	0.2	1.8
	4.6395985	90%	6%	3	0.6	0.2	1.8
	51.225194	38%	71%	4	1.3	0.5	3.3
	60.478608	29%	80%				
	85.540247	19%	90%				
24 hours	26.782895	74%	41%	1			

FIG. 1 - 11

	25.953947	83%	41%	2	1.3	0.4	4.0
	9.255	91%	12%	3	1.8	0.6	5.4
	51.225194	48%	71%	4	2.5	0.9	7.3
	60.478608	30%	80%				
	85.540247	13%	90%				
48 hours	51.225194	100%	71%	1			
	51.225194	100%	71%	2	na	na	na
	51.225194	100%	71%	3	na	na	na
	51.225194	100%	71%	4	na	na	na
	60.478608	0%	80%				
	85.540247	0%	90%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	4.4318182	83%	3%	1			
	4.4318182	83%	3%	2	na	na	na
	3.6845321	100%	3%	3	na	na	na
	60.047096	0%	71%	4	na	na	na
	77.621465	0%	81%				
	92.260597	0%	90%				
24 hours	9.6448339	71%	12%	1			
	3.6845321	86%	3%	2	2.1	0.1	46.6
	0	100%	0%	3	1.0	0.0	59.3
	60.047096	14%	71%	4	3.3	0.2	53.0
	77.621465	14%	81%				
	92.260597	14%	90%				
48 hours	33.468507	100%	45%	1			
	33.468507	100%	45%	2	na	na	na
	33.468507	100%	45%	3	na	na	na
	60.047096	50%	71%	4	na	na	na
	77.621465	50%	81%				
	92.260597	50%	90%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	28.638158	75%	50%	1			
	20.506579	81%	36%	2	0.9	0.2	4.8
	5.8713504	94%	12%	3	0.6	0.1	4.4
	49.879206	50%	71%	4	4.2	1.1	16.3
	55.211389	50%	81%				
	76.229152	38%	90%				
24 hours	27.389558	71%	48%	1			
	25.953947	81%	45%	2	14.0	1.1	186.6
	20.506579	90%	36%	3	3.2	0.2	59.0
	49.879206	48%	71%	4	18.0	1.3	241.8
	55.211389	48%	81%				
	76.229152	19%	90%				
48 hours	49.879206	75%	71%	1			
	4.5394737	100%	12%	2	0.0	0.0	na
	4.5394737	100%	12%	3	2.2	0.1	63.6
	49.879206	75%	71%	4	0.9	0.0	66.6
	55.211389	0%	81%				
	76.229152	0%	90%				

FIG. 1 - 12

Advanced glycosylation end product-specific receptor

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	6.855	8.969	6.855	9.957	6.855	11.258
average	11.202	18.986	11.202	17.013	11.202	16.544
stdev	14.889	20.560	14.889	17.851	14.889	16.754
p (t-test)		0.021		0.036		0.146
min	0.000	0.000	0.000	0.597	0.000	0.000
max	157.048	60.844	157.048	59.206	157.048	48.133
n (Samp)	247	23	247	35	247	18
n (Pat)	159	23	159	35	159	18

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	7.114	3.877	7.114	11.137	7.114	35.826
average	12.460	3.877	12.460	12.537	12.460	34.893
stdev	15.646	5.482	15.646	9.907	15.646	25.197
p (t-test)		0.439		0.989		0.000
min	0.000	0.000	0.000	2.082	0.000	0.000
max	157.048	7.753	157.048	33.284	157.048	63.671
n (Samp)	318	2	318	8	318	7
n (Pat)	188	2	188	8	188	7

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	6.819	8.969	6.819	9.828	6.819	9.176
average	11.169	19.212	11.169	17.323	11.169	15.306
stdev	15.689	20.370	15.689	18.776	15.689	16.841
p (t-test)		0.025		0.051		0.313
min	0.000	0.000	0.000	0.597	0.000	0.000
max	157.048	60.844	157.048	59.206	157.048	48.133
n (Samp)	213	23	213	30	213	16
n (Pat)	132	23	132	30	132	16

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.61	0.065	247	23	0.101
24 hours	0.60	0.053	247	35	0.054
48 hours	0.54	0.072	247	18	0.547

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.28	0.149	318	2	0.135
24 hours	0.57	0.107	318	8	0.496
48 hours	0.76	0.107	318	7	0.016

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.63	0.065	213	23	0.047
24 hours	0.61	0.058	213	30	0.055
48 hours	0.52	0.076	213	16	0.800

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	5.7935097	74%	43%	1			
	3.9993481	83%	27%	2	0.7	0.2	2.4
	0	100%	0%	3	2.1	1.0	4.7
	11.732728	39%	70%	4	2.1	0.9	4.7
	14.033515	35%	81%				

FIG. 2 - 1

	26.01374	30%	90%				
24 hours	6.5017597	71%	45%	1			
	3.0427772	83%	24%	2	0.7	0.3	1.4
	2.0823337	91%	11%	3	1.7	1.0	2.8
	11.732728	40%	70%	4	1.8	1.1	3.0
	14.033515	34%	81%				
	26.01374	23%	90%				
48 hours	2.695557	72%	20%	1			
	0	100%	0%	2	0.3	0.1	1.2
	0	100%	0%	3	0.2	0.0	1.6
	11.732728	50%	70%	4	1.6	0.8	2.9
	14.033515	50%	81%				
	26.01374	28%	90%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0	100%	0%	1			
	0	100%	0%	2	na	na	na
	0	100%	0%	3	na	na	na
	12.537211	0%	72%	4	na	na	na
	18.202346	0%	80%				
	33.59374	0%	90%				
24 hours	8.5254634	75%	56%	1			
	2.0823337	88%	11%	2	0.0	0.0	na
	1.9735564	100%	11%	3	1.5	0.3	8.2
	12.537211	38%	72%	4	1.5	0.3	8.1
	18.202346	13%	80%				
	33.59374	0%	90%				
48 hours	23.570652	71%	86%	1			
	7.8431149	86%	54%	2	0.0	0.0	na
	0	100%	0%	3	1.0	0.0	52.9
	12.537211	71%	72%	4	5.2	0.5	57.4
	18.202346	71%	80%				
	33.59374	57%	90%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	5.7935097	74%	44%	1			
	5.1390615	83%	40%	2	1.4	0.4	4.6
	2.695557	91%	23%	3	2.9	1.1	7.7
	11.406438	39%	70%	4	2.9	1.1	7.7
	14.033515	35%	82%				
	26.01374	30%	90%				
24 hours	6.5017597	73%	47%	1			
	3.0449662	80%	28%	2	1.5	0.6	3.7
	2.695557	90%	23%	3	3.1	1.5	6.5
	11.406438	33%	70%	4	2.4	1.1	5.3
	14.033515	30%	82%				
	26.01374	23%	90%				
48 hours	2.0823337	75%	14%	1			
	0	100%	0%	2	0.6	0.2	1.8
	0	100%	0%	3	0.0	0.0	na
	11.406438	50%	70%	4	1.7	0.8	3.4
	14.033515	50%	82%				
	26.01374	25%	90%				

FIG. 2 - 2

Fatty acid-binding protein, intestinal

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	161.154	1.009	161.154	58.391	161.154	1.009
average	327.995	54.356	327.995	254.317	327.995	139.358
stdev	394.112	na	394.112	392.157	394.112	na
p (t-test)		na		0.490		na
min	1.009	54.356	1.009	1.009	1.009	139.358
max	1831.799	54.356	1831.799	1307.113	1831.799	139.358
n (Samp)	96	1	96	16	96	1
n (Pat)	74	1	74	16	74	1

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	136.096	na	136.096	997.540	136.096	na
average	311.629	na	311.629	854.593	311.629	na
stdev	389.099	na	389.099	658.743	389.099	na
p (t-test)		na		0.021		na
min	1.009	na	1.009	136.113	1.009	na
max	1831.799	na	1831.799	1430.126	1831.799	na
n (Samp)	111	0	111	3	111	0
n (Pat)	87	0	87	3	87	0

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	200.867	1.009	200.867	35.072	200.867	568.449
average	349.682	54.356	349.682	214.175	349.682	568.449
stdev	405.291	na	405.291	350.184	405.291	606.827
p (t-test)		na		0.229		0.457
min	1.009	54.356	1.009	1.009	1.009	139.358
max	1831.799	54.356	1831.799	1307.113	1831.799	997.540
n (Samp)	79	1	79	15	79	2
n (Pat)	61	1	61	15	61	2

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.31	0.230	96	1	0.415
24 hours	0.42	0.074	96	16	0.282
48 hours	0.48	0.287	96	1	0.942

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	nd	nd	111	0	nd
24 hours	0.81	0.153	111	3	0.045
48 hours	nd	nd	111	0	nd

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.27	0.207	79	1	0.258
24 hours	0.36	0.072	79	15	0.049
48 hours	0.70	0.210	79	2	0.335

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	49.664	100%	31%	1			
	49.664	100%	31%	2	na	na	na
	49.664	100%	31%	3	na	na	na
	452.41379	0%	71%	4	na	na	na
	609.57377	0%	80%				
	809.08231	0%	91%				

FIG. 2 - 3

24 hours	29.696	75%	23%	1			
	19.712	81%	20%	2	1.0	0.2	4.3
	0	100%	0%	3	1.4	0.4	5.1
	452.41379	19%	71%	4	2.3	0.7	7.2
	609.57377	19%	80%				
	809.08231	13%	91%				
48 hours	136.09639	100%	48%	1			
	136.09639	100%	48%	2	na	na	na
	136.09639	100%	48%	3	na	na	na
	452.41379	0%	71%	4	na	na	na
	609.57377	0%	80%				
	809.08231	0%	91%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	49.664	100%	27%	1			
	49.664	100%	27%	2	na	na	na
	49.664	100%	27%	3	na	na	na
	456.91803	0%	71%	4	na	na	na
	647.34426	0%	81%				
	809.08231	0%	91%				
24 hours	28.928	73%	18%	1			
	19.712	80%	15%	2	1.7	0.3	10.2
	0	100%	0%	3	1.0	0.1	8.5
	456.91803	13%	71%	4	5.9	1.4	24.9
	647.34426	7%	81%				
	809.08231	7%	91%				
48 hours	136.09639	100%	46%	1			
	136.09639	100%	46%	2	na	na	na
	136.09639	100%	46%	3	na	na	na
	456.91803	50%	71%	4	na	na	na
	647.34426	50%	81%				
	809.08231	50%	91%				

FIG. 2 - 4

Interleukin-12

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.613	0.406	0.613	0.494	0.613	0.523
average	1.801	1.268	1.801	1.178	1.801	1.596
stdev	7.155	1.658	7.155	1.475	7.155	2.395
p (t-test)		0.722		0.609		0.904
min	0.000	0.030	0.000	0.000	0.000	0.000
max	110.665	6.548	110.665	5.530	110.665	7.860
n (Samp)	247	23	247	35	247	18
n (Pat)	159	23	159	35	159	18

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.608	0.445	0.608	0.435	0.608	0.486
average	1.704	0.445	1.704	1.580	1.704	1.741
stdev	6.348	0.340	6.348	2.169	6.348	2.630
p (t-test)		0.780		0.956		0.988
min	0.000	0.205	0.000	0.000	0.000	0.213
max	110.665	0.686	110.665	5.530	110.665	7.295
n (Samp)	319	2	319	8	319	7
n (Pat)	188	2	188	8	188	7

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.613	0.406	0.613	0.541	0.613	0.414
average	1.914	1.258	1.914	0.996	1.914	1.147
stdev	7.684	1.663	7.684	1.185	7.684	2.006
p (t-test)		0.684		0.515		0.691
min	0.000	0.030	0.000	0.000	0.000	0.000
max	110.665	6.548	110.665	4.424	110.665	7.860
n (Samp)	213	23	213	30	213	16
n (Pat)	132	23	132	30	132	16

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.47	0.062	247	23	0.663
24 hours	0.45	0.051	247	35	0.347
48 hours	0.47	0.069	247	18	0.638

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.37	0.179	319	2	0.466
24 hours	0.45	0.100	319	8	0.629
48 hours	0.50	0.110	319	7	0.972

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.46	0.062	213	23	0.536
24 hours	0.44	0.054	213	30	0.272
48 hours	0.40	0.069	213	16	0.158

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.3669232	74%	30%	1			
	0.3003591	83%	24%	2	0.7	0.3	1.6
	0.2130757	91%	19%	3	1.4	0.7	2.6
	1.4112931	26%	70%	4	0.8	0.4	1.8
	2.4995337	17%	80%				
	3.6984641	9%	90%				

FIG. 2 - 5

24 hours	0.2493054	71%	21%	1			
	0.1413584	80%	14%	2	0.9	0.5	1.6
	0.0117762	91%	7%	3	0.9	0.5	1.5
	1.4112931	29%	70%	4	1.8	1.1	2.8
	2.4995337	14%	80%				
	3.6984641	11%	90%				
48 hours	0.3538007	72%	28%	1			
	0.2130757	83%	19%	2	0.6	0.2	1.8
	0.1038116	94%	11%	3	1.0	0.4	2.4
	1.4112931	28%	70%	4	1.0	0.4	2.4
	2.4995337	22%	80%				
	3.6984641	11%	90%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.2027877	100%	18%	1			
	0.2027877	100%	18%	2	na	na	na
	0.2027877	100%	18%	3	na	na	na
	1.4443003	0%	70%	4	na	na	na
	2.6475288	0%	80%				
	3.8034446	0%	90%				
24 hours	0.2425495	75%	22%	1			
	0.0117762	88%	7%	2	0.5	0.0	9.8
	0	100%	0%	3	0.5	0.0	9.8
	1.4443003	38%	70%	4	2.1	0.5	9.5
	2.6475288	25%	80%				
	3.8034446	25%	90%				
48 hours	0.419541	71%	36%	1			
	0.2132405	86%	19%	2	0.5	0.0	9.9
	0.2130757	100%	19%	3	1.0	0.1	7.5
	1.4443003	29%	70%	4	1.0	0.1	7.6
	2.6475288	29%	80%				
	3.8034446	14%	90%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.3669232	74%	29%	1			
	0.3003591	83%	23%	2	0.8	0.3	2.0
	0.2130757	91%	18%	3	1.9	1.0	3.9
	1.517195	26%	70%	4	1.0	0.4	2.4
	2.8129052	17%	80%				
	3.9362227	9%	90%				
24 hours	0.2611924	70%	21%	1			
	0.131522	80%	13%	2	1.2	0.6	2.3
	0.0394811	90%	8%	3	1.2	0.6	2.3
	1.517195	23%	70%	4	1.8	1.0	3.3
	2.8129052	10%	80%				
	3.9362227	3%	90%				
48 hours	0.2130757	75%	18%	1			
	0.1986469	81%	17%	2	1.0	0.3	4.0
	0.0882454	94%	10%	3	1.4	0.4	4.7
	1.517195	19%	70%	4	2.2	0.8	6.2
	2.8129052	13%	80%				
	3.9362227	6%	90%				

FIG. 2 - 6

Fatty acid-binding protein, intestinal

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	254.072	63.767	254.072	63.767	254.072	63.767
average	341.320	177.476	341.320	177.476	341.320	177.476
stdev	404.050	299.755	404.050	299.755	404.050	299.755
p (t-test)		0.256		0.256		0.256
min	1.009	1.009	1.009	1.009	1.009	1.009
max	1826.301	997.540	1826.301	997.540	1826.301	997.540
n (Samp)	25	10	25	10	25	10
n (Pat)	25	10	25	10	25	10

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	94.334	281.059	94.334	281.059	94.334	281.059
average	195.549	471.571	195.549	471.571	195.549	471.571
stdev	220.967	461.232	220.967	461.232	220.967	461.232
p (t-test)		0.178		0.178		0.178
min	1.009	136.113	1.009	136.113	1.009	136.113
max	590.689	997.540	590.689	997.540	590.689	997.540
n (Samp)	9	3	9	3	9	3
n (Pat)	9	3	9	3	9	3

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	266.217	40.064	266.217	40.064	266.217	40.064
average	384.079	59.298	384.079	59.298	384.079	59.298
stdev	451.141	42.558	451.141	42.558	451.141	42.558
p (t-test)		0.097		0.097		0.097
min	1.009	27.392	1.009	27.392	1.009	27.392
max	1826.301	136.096	1826.301	136.096	1826.301	136.096
n (Samp)	18	6	18	6	18	6
n (Pat)	18	6	18	6	18	6

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.38	0.102	25	10	0.240
24 hours	0.38	0.102	25	10	0.240
48 hours	0.38	0.102	25	10	0.240

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.74	0.185	9	3	0.193
24 hours	0.74	0.185	9	3	0.193
48 hours	0.74	0.185	9	3	0.193

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.27	0.110	18	6	0.036
24 hours	0.27	0.110	18	6	0.036
48 hours	0.27	0.110	18	6	0.036

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	30.464	70%	24%	1			
	27.392	80%	24%	2	4.0	0.2	96.7
	22.784	90%	24%	3	6.4	0.3	140.2
	452.41379	10%	72%	4	2.7	0.1	89.3
	553.37931	10%	80%				
	727.60656	10%	92%				

FIG. 3 - 1

24 hours	30.464	70%	24%	1			
	27.392	80%	24%	2	4.0	0.2	96.7
	22.784	90%	24%	3	6.4	0.3	140.2
	452.41379	10%	72%	4	2.7	0.1	89.3
	553.37931	10%	80%				
	727.60656	10%	92%				
48 hours	30.464	70%	24%	1			
	27.392	80%	24%	2	4.0	0.2	96.7
	22.784	90%	24%	3	6.4	0.3	140.2
	452.41379	10%	72%	4	2.7	0.1	89.3
	553.37931	10%	80%				
	727.60656	10%	92%				

FIG. 3 - 2

Interleukin-12

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.697	0.747	0.697	0.747	0.697	0.747
average	1.543	1.260	1.543	1.260	1.543	1.260
stdev	1.760	1.802	1.760	1.802	1.760	1.802
p (t-test)		0.524		0.524		0.524
min	0.000	0.000	0.000	0.000	0.000	0.000
max	7.954	7.860	7.954	7.860	7.954	7.860
n (Samp)	54	23	54	23	54	23
n (Pat)	54	23	54	23	54	23

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.729	0.451	0.729	0.451	0.729	0.451
average	1.852	0.490	1.852	0.490	1.852	0.490
stdev	1.971	0.269	1.971	0.269	1.971	0.269
p (t-test)		0.189		0.189		0.189
min	0.000	0.249	0.000	0.249	0.000	0.249
max	7.010	0.809	7.010	0.809	7.010	0.809
n (Samp)	20	4	20	4	20	4
n (Pat)	20	4	20	4	20	4

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.740	0.716	0.740	0.716	0.740	0.716
average	1.640	1.191	1.640	1.191	1.640	1.191
stdev	1.749	1.805	1.749	1.805	1.749	1.805
p (t-test)		0.367		0.367		0.367
min	0.000	0.018	0.000	0.018	0.000	0.018
max	7.954	7.860	7.954	7.860	7.954	7.860
n (Samp)	44	18	44	18	44	18
n (Pat)	44	18	44	18	44	18

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.43	0.070	54	23	0.292
24 hours	0.43	0.070	54	23	0.292
48 hours	0.43	0.070	54	23	0.292

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.34	0.140	20	4	0.245
24 hours	0.34	0.140	20	4	0.245
48 hours	0.34	0.140	20	4	0.245

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.42	0.078	44	18	0.286
24 hours	0.42	0.078	44	18	0.286
48 hours	0.42	0.078	44	18	0.286

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.2391299	74%	9%	1			
	0.0779926	83%	6%	2	2.9	1.0	8.2
	1E-09	91%	6%	3	0.5	0.1	2.6
	2.2267257	22%	70%	4	3.6	1.3	10.0
	2.8403203	9%	81%				
	3.5263017	9%	91%				

FIG. 3 - 3

24 hours	0.2391299	74%	9%	1			
	0.0779926	83%	6%	2	2.9	1.0	8.2
	1E-09	91%	6%	3	0.5	0.1	2.6
	2.2267257	22%	70%	4	3.6	1.3	10.0
	2.8403203	9%	81%				
	3.5263017	9%	91%				
48 hours	0.2391299	74%	9%	1			
	0.0779926	83%	6%	2	2.9	1.0	8.2
	1E-09	91%	6%	3	0.5	0.1	2.6
	2.2267257	22%	70%	4	3.6	1.3	10.0
	2.8403203	9%	81%				
	3.5263017	9%	91%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.3473055	72%	16%	1			
	0.0877944	83%	7%	2	6.1	1.2	31.7
	0.0184271	94%	7%	3	1.6	0.2	11.1
	2.3964026	6%	70%	4	4.7	0.9	24.7
	2.9930363	6%	82%				
	3.6984641	6%	91%				
24 hours	0.3473055	72%	16%	1			
	0.0877944	83%	7%	2	6.1	1.2	31.7
	0.0184271	94%	7%	3	1.6	0.2	11.1
	2.3964026	6%	70%	4	4.7	0.9	24.7
	2.9930363	6%	82%				
	3.6984641	6%	91%				
48 hours	0.3473055	72%	16%	1			
	0.0877944	83%	7%	2	6.1	1.2	31.7
	0.0184271	94%	7%	3	1.6	0.2	11.1
	2.3964026	6%	70%	4	4.7	0.9	24.7
	2.9930363	6%	82%				
	3.6984641	6%	91%				

FIG. 3 - 4

Advanced glycosylation end product-specific receptor

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	7.459	40.375	7.459	40.375	7.459	40.375
average	13.447	39.857	13.447	38.051	13.447	38.611
stdev	19.034	20.741	19.034	21.955	19.034	22.334
p (t-test)		0.000		0.000		0.001
min	0.000	9.700	0.000	9.700	0.000	3.999
max	157.048	63.671	157.048	63.671	157.048	63.671
n (Samp)	98	12	98	12	98	8
n (Pat)	98	12	98	12	98	8

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	9.700	38.502	9.700	36.829	9.700	49.138
average	16.126	39.832	16.126	36.221	16.126	48.101
stdev	18.987	18.515	18.987	21.184	18.987	13.387
p (t-test)		0.003		0.012		0.001
min	0.000	9.957	0.000	9.957	0.000	33.284
max	157.048	60.844	157.048	60.844	157.048	60.844
n (Samp)	159	6	159	6	159	4
n (Pat)	159	6	159	6	159	4

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	7.779	57.141	7.779	57.141	7.779	48.378
average	13.106	44.754	13.106	44.754	13.106	39.205
stdev	19.659	22.654	19.659	22.654	19.659	26.299
p (t-test)		0.000		0.000		0.003
min	0.000	9.700	0.000	9.700	0.000	3.999
max	157.048	63.671	157.048	63.671	157.048	63.671
n (Samp)	84	8	84	8	84	6
n (Pat)	84	8	84	8	84	6

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.87	0.068	98	12	0.000
24 hours	0.85	0.071	98	12	0.000
48 hours	0.82	0.092	98	8	0.000

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.85	0.099	159	6	0.000
24 hours	0.82	0.106	159	6	0.003
48 hours	0.93	0.092	159	4	0.000

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.88	0.081	84	8	0.000
24 hours	0.88	0.081	84	8	0.000
48 hours	0.78	0.114	84	6	0.014

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	30.485273	75%	94%	1			
	9.8846294	92%	57%	2	na	na	na
	9.8846294	92%	57%	3	na	na	na
	12.935703	75%	71%	4	na	na	na
	20.394872	75%	82%				

FIG. 4 - 1

	27.310375	75%	91%				
24 hours	14.033515	75%	74%	1			
	9.8846294	92%	57%	2	na	na	na
	9.8846294	92%	57%	3	na	na	na
	12.935703	75%	71%	4	na	na	na
	20.394872	67%	82%				
	27.310375	67%	91%				
48 hours	30.485273	75%	94%	1			
	9.8846294	88%	57%	2	0.0	0.0	na
	3.0449662	100%	20%	3	1.0	0.0	59.0
	12.935703	75%	71%	4	7.1	0.6	83.5
	20.394872	75%	82%				
	27.310375	75%	91%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	35.826138	75%	96%	1			
	9.8846294	88%	58%	2	na	na	na
	8.8647429	100%	57%	3	na	na	na
	12.537211	75%	71%	4	na	na	na
	18.676326	75%	82%				
	25.74785	75%	90%				
24 hours	35.826138	75%	96%	1			
	9.8846294	88%	58%	2	na	na	na
	8.8647429	100%	57%	3	na	na	na
	12.537211	75%	71%	4	na	na	na
	18.676326	75%	82%				
	25.74785	75%	90%				
48 hours	9.8846294	83%	58%	1			
	9.8846294	83%	58%	2	0.0	0.0	na
	3.0449662	100%	19%	3	1.0	0.0	60.7
	12.537211	67%	71%	4	4.4	0.3	62.4
	18.676326	67%	82%				
	25.74785	67%	90%				

FIG. 4 - 2

Bactericidal permeability-increasing protein

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	71.809	222.914	71.809	222.914	71.809	1837.983
average	613.151	5695.969	613.151	5695.969	613.151	7183.388
stdev	1425.164	9914.356	1425.164	9914.356	1425.164	10888.018
p (t-test)		0.002		0.002		0.000
min	1.197	1.197	1.197	1.197	1.197	1.197
max	6951.049	23569.364	6951.049	23569.364	6951.049	19710.983
n (Samp)	40	8	40	8	40	3
n (Pat)	40	8	40	8	40	3

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	116.114	1.197	116.114	1.197	116.114	1.197
average	1261.104	112.055	1261.104	112.055	1261.104	1.197
stdev	4172.950	221.717	4172.950	221.717	4172.950	na
p (t-test)		0.586		0.586		na
min	1.197	1.197	1.197	1.197	1.197	1.197
max	26300.578	444.632	26300.578	444.632	26300.578	1.197
n (Samp)	73	4	73	4	73	1
n (Pat)	73	4	73	4	73	1

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	52.488	1837.983	52.488	1837.983	52.488	1837.983
average	630.249	9024.145	630.249	9024.145	630.249	7183.388
stdev	1531.179	11621.532	1531.179	11621.532	1531.179	10888.018
p (t-test)		0.000		0.000		0.001
min	1.197	1.197	1.197	1.197	1.197	1.197
max	6951.049	23569.364	6951.049	23569.364	6951.049	19710.983
n (Samp)	33	5	33	5	33	3
n (Pat)	33	5	33	5	33	3

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.57	0.115	40	8	0.568
24 hours	0.57	0.115	40	8	0.568
48 hours	0.70	0.175	40	3	0.242

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.32	0.121	73	4	0.133
24 hours	0.32	0.121	73	4	0.133
48 hours	0.19	0.163	73	1	0.059

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.67	0.141	33	5	0.220
24 hours	0.67	0.141	33	5	0.220
48 hours	0.71	0.175	33	3	0.225

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0	100%	0%	1			
	0	100%	0%	2	1.0	0.1	10.5
	0	100%	0%	3	0.5	0.0	12.5
	236.8	50%	70%	4	1.7	0.2	12.9
	747.8453	38%	80%				
	1543.4254	38%	90%				

FIG. 4 - 3

24 hours	0	100%	0%	1			
	0	100%	0%	2	1.0	0.1	10.5
	0	100%	0%	3	0.5	0.0	12.5
	236.8	50%	70%	4	1.7	0.2	12.9
	747.8453	38%	80%				
	1543.4254	38%	90%				
48 hours	0	100%	0%	1			
	0	100%	0%	2	na	na	na
	0	100%	0%	3	na	na	na
	236.8	67%	70%	4	na	na	na
	747.8453	67%	80%				
	1543.4254	67%	90%				

FIG. 4 - 4

Fibroblast growth factor 23 (N and C-term peptides)

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	20.516	18.334	20.516	18.334	20.516	15.628
average	21.306	38.197	21.306	38.197	21.306	16.151
stdev	13.769	37.886	13.769	37.886	13.769	4.649
p (t-test)		0.030		0.030		0.526
min	5.535	10.390	5.535	10.390	5.535	11.787
max	97.051	106.824	97.051	106.824	97.051	21.040
n (Samp)	40	8	40	8	40	3
n (Pat)	40	8	40	8	40	3

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	20.167	29.572	20.167	29.572	20.167	5.036
average	21.591	40.236	21.591	40.236	21.591	21.040
stdev	16.401	33.373	16.401	33.373	16.401	na
p (t-test)		0.041		0.041		na
min	5.036	13.882	5.036	13.882	5.036	21.040
max	106.824	87.918	106.824	87.918	106.824	21.040
n (Samp)	69	4	69	4	69	1
n (Pat)	69	4	69	4	69	1

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	20.691	15.628	20.691	15.628	20.691	15.628
average	20.655	33.134	20.655	33.134	20.655	16.151
stdev	6.513	41.400	6.513	41.400	6.513	4.649
p (t-test)		0.094		0.094		0.253
min	5.535	10.390	5.535	10.390	5.535	11.787
max	33.907	106.824	33.907	106.824	33.907	21.040
n (Samp)	33	5	33	5	33	3
n (Pat)	33	5	33	5	33	3

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.52	0.114	40	8	0.837
24 hours	0.52	0.114	40	8	0.837
48 hours	0.31	0.140	40	3	0.171

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.69	0.151	69	4	0.199
24 hours	0.69	0.151	69	4	0.199
48 hours	0.59	0.303	69	1	0.756

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.38	0.128	33	5	0.354
24 hours	0.38	0.128	33	5	0.354
48 hours	0.28	0.135	33	3	0.108

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	13.624096	75%	20%	1			
	11.149398	88%	13%	2	0.3	0.0	5.5
	9.1679558	100%	10%	3	0.3	0.0	5.5
	21.809639	38%	70%	4	1.0	0.2	5.7
	23.798883	38%	80%				
	28.081937	38%	90%				

FIG. 4 - 5

24 hours	13.624096	75%	20%	1			
	11.149398	88%	13%	2	0.3	0.0	5.5
	9.1679558	100%	10%	3	0.3	0.0	5.5
	21.809639	38%	70%	4	1.0	0.2	5.7
	23.798883	38%	80%				
	28.081937	38%	90%				
48 hours	11.149398	100%	13%	1			
	11.149398	100%	13%	2	na	na	na
	11.149398	100%	13%	3	na	na	na
	21.809639	0%	70%	4	na	na	na
	23.798883	0%	80%				
	28.081937	0%	90%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	20.865193	75%	59%	1			
	13.814458	100%	25%	2	0.0	0.0	na
	13.814458	100%	25%	3	1.0	0.0	63.5
	22.370221	50%	71%	4	2.0	0.1	47.6
	23.295775	50%	81%				
	28.663984	50%	91%				
24 hours	20.865193	75%	59%	1			
	13.814458	100%	25%	2	0.0	0.0	na
	13.814458	100%	25%	3	1.0	0.0	63.5
	22.370221	50%	71%	4	2.0	0.1	47.6
	23.295775	50%	81%				
	28.663984	50%	91%				
48 hours	20.865193	100%	59%	1			
	20.865193	100%	59%	2	na	na	na
	20.865193	100%	59%	3	na	na	na
	22.370221	0%	71%	4	na	na	na
	23.295775	0%	81%				
	28.663984	0%	91%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	11.149398	80%	9%	1			
	11.149398	80%	9%	2	1.1	0.0	90.1
	9.1679558	100%	6%	3	1.0	0.0	77.9
	23.113594	20%	73%	4	2.6	0.1	80.0
	26.442656	20%	82%				
	28.938547	20%	91%				
24 hours	11.149398	80%	9%	1			
	11.149398	80%	9%	2	1.1	0.0	90.1
	9.1679558	100%	6%	3	1.0	0.0	77.9
	23.113594	20%	73%	4	2.6	0.1	80.0
	26.442656	20%	82%				
	28.938547	20%	91%				
48 hours	11.149398	100%	9%	1			
	11.149398	100%	9%	2	na	na	na
	11.149398	100%	9%	3	na	na	na
	23.113594	0%	73%	4	na	na	na
	26.442656	0%	82%				
	28.938547	0%	91%				

FIG. 4 - 6

Interleukin-12

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.677	0.637	0.677	0.539	0.677	0.646
average	2.691	1.264	2.691	1.074	2.691	1.374
stdev	11.178	1.568	11.178	1.538	11.178	1.527
p (t-test)		0.661		0.619		0.741
min	0.000	0.000	0.000	0.000	0.000	0.030
max	110.665	4.424	110.665	4.424	110.665	4.167
n (Samp)	98	12	98	12	98	8
n (Pat)	98	12	98	12	98	8

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.703	0.802	0.703	0.555	0.703	0.802
average	2.289	1.471	2.289	1.097	2.289	1.564
stdev	8.849	1.577	8.849	1.542	8.849	1.751
p (t-test)		0.822		0.743		0.871
min	0.000	0.000	0.000	0.000	0.000	0.486
max	110.665	4.167	110.665	4.167	110.665	4.167
n (Samp)	159	6	159	6	159	4
n (Pat)	159	6	159	6	159	4

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.726	0.607	0.726	0.592	0.726	0.646
average	3.048	0.986	3.048	0.981	3.048	1.041
stdev	12.045	1.431	12.045	1.433	12.045	1.203
p (t-test)		0.631		0.631		0.686
min	0.000	0.000	0.000	0.000	0.000	0.030
max	110.665	4.424	110.665	4.424	110.665	3.407
n (Samp)	84	8	84	8	84	6
n (Pat)	84	8	84	8	84	6

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.46	0.086	98	12	0.630
24 hours	0.41	0.083	98	12	0.281
48 hours	0.51	0.107	98	8	0.905

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.50	0.121	159	6	0.979
24 hours	0.40	0.110	159	6	0.377
48 hours	0.56	0.150	159	4	0.715

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.41	0.099	84	8	0.342
24 hours	0.41	0.099	84	8	0.342
48 hours	0.44	0.118	84	6	0.625

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.4900236	75%	38%	1			
	0.025292	83%	8%	2	1.0	0.2	4.5
	0	100%	0%	3	1.0	0.2	4.3
	1.9973109	25%	70%	4	1.0	0.2	4.5
	3.275838	17%	81%				
	4.2705662	8%	91%				

FIG. 4 - 7

24 hours	0.2611924	75%	20%	1			
	0.025292	83%	8%	2	1.6	0.3	9.7
	0	100%	0%	3	1.6	0.3	9.3
	1.9973109	17%	70%	4	2.3	0.4	11.5
	3.275838	17%	81%				
	4.2705662	8%	91%				
48 hours	0.5807389	75%	40%	1			
	0.4900236	88%	38%	2	3.1	0.2	50.0
	0.025292	100%	8%	3	2.1	0.1	46.2
	1.9973109	25%	70%	4	2.0	0.1	44.3
	3.275838	25%	81%				
	4.2705662	0%	91%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.5000297	83%	34%	1			
	0.5000297	83%	34%	2	2.1	0.1	42.9
	0	100%	0%	3	1.0	0.0	55.6
	2.0262017	33%	70%	4	2.0	0.1	41.7
	2.9930363	17%	81%				
	4.3476354	0%	91%				
24 hours	0.2611924	83%	16%	1			
	0.2611924	83%	16%	2	1.0	0.0	56.9
	0	100%	0%	3	2.1	0.1	43.9
	2.0262017	17%	70%	4	2.1	0.1	43.9
	2.9930363	17%	81%				
	4.3476354	0%	91%				
48 hours	0.5807389	75%	38%	1			
	0.4626806	100%	33%	2	na	na	na
	0.4626806	100%	33%	3	na	na	na
	2.0262017	25%	70%	4	na	na	na
	2.9930363	25%	81%				
	4.3476354	0%	91%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.4853467	75%	36%	1			
	0.025292	88%	8%	2	1.0	0.0	60.2
	0	100%	0%	3	4.6	0.3	65.0
	2.4995337	13%	71%	4	2.1	0.1	47.6
	3.5539117	13%	81%				
	4.3476354	13%	90%				
24 hours	0.4853467	75%	36%	1			
	0.025292	88%	8%	2	1.0	0.0	60.2
	0	100%	0%	3	4.6	0.3	65.0
	2.4995337	13%	71%	4	2.1	0.1	47.6
	3.5539117	13%	81%				
	4.3476354	13%	90%				
48 hours	0.4853467	83%	36%	1			
	0.4853467	83%	36%	2	1.0	0.0	63.4
	0.025292	100%	8%	3	3.3	0.2	54.3
	2.4995337	17%	71%	4	1.0	0.0	63.4
	3.5539117	0%	81%				
	4.3476354	0%	90%				

FIG. 4 - 8

Advanced glycosylation end product-specific receptor

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	96.696	98.108	96.696	140.161	96.696	114.741
average	160.746	158.955	160.746	238.008	160.746	204.981
stdev	188.767	167.227	188.767	330.426	188.767	274.202
p (t-test)		0.966		0.159		0.447
min	6.382	28.725	6.382	20.588	6.382	44.866
max	1156.927	686.054	1156.927	1907.734	1156.927	1188.110
n						
(Samp)	54	29	54	38	54	18
n (Pat)	52	29	52	38	52	18

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	104.629	102.412	104.629	131.746	104.629	178.679
average	149.219	237.706	149.219	345.388	149.219	363.244
stdev	155.895	247.596	155.895	499.212	155.895	400.234
p (t-test)		0.101		0.000		0.001
min	6.382	32.456	6.382	34.323	6.382	51.120
max	1156.927	686.054	1156.927	1907.734	1156.927	1188.110
n						
(Samp)	131	10	131	21	131	8
n (Pat)	101	10	101	21	101	8

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	117.565	85.109	117.565	137.844	117.565	119.344
average	175.029	125.070	175.029	172.396	175.029	157.722
stdev	184.222	118.439	184.222	169.464	184.222	125.265
p (t-test)		0.236		0.948		0.750
min	21.871	28.725	21.871	20.588	21.871	44.866
max	1156.927	513.517	1156.927	914.925	1156.927	494.137
n						
(Samp)	53	23	53	31	53	13
n (Pat)	46	23	46	31	46	13

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.48	0.067	54	29	0.799
24 hours	0.61	0.061	54	38	0.082
48 hours	0.54	0.080	54	18	0.575

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.51	0.096	131	10	0.905
24 hours	0.60	0.070	131	21	0.136
48 hours	0.65	0.108	131	8	0.181

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.38	0.068	53	23	0.078
24 hours	0.52	0.066	53	31	0.789
48 hours	0.50	0.090	53	13	0.994

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	62.442721	72%	22%	1			
	48.353851	83%	17%	2	1.2	0.5	2.8
	32.455761	93%	6%	3	0.8	0.3	1.9
	147.73132	28%	70%	4	1.3	0.6	3.1

FIG. 5 - 1

	230.08968	17%	81%				
	298.72994	17%	91%				
24 hours	96.341234	71%	50%	1			
	57.739874	82%	20%	2	0.8	0.4	1.8
	38.058812	92%	7%	3	1.7	0.8	3.5
	147.73132	47%	70%	4	2.0	1.0	4.2
	230.08968	26%	81%				
	298.72994	16%	91%				
48 hours	73.406841	72%	31%	1			
	69.883004	83%	30%	2	1.9	0.5	7.2
	48.353851	94%	17%	3	1.9	0.5	7.2
	147.73132	33%	70%	4	1.9	0.5	7.2
	230.08968	22%	81%				
	298.72994	11%	91%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	44.865532	70%	11%	1			
	41.798964	80%	10%	2	0.2	0.0	3.0
	38.058812	90%	6%	3	0.2	0.0	3.0
	144.1759	40%	70%	4	1.0	0.3	2.9
	204.86321	40%	80%				
	267.89998	40%	90%				
24 hours	93.996537	71%	44%	1			
	67.304809	81%	27%	2	1.3	0.5	3.5
	41.798964	90%	10%	3	0.7	0.2	2.6
	144.1759	48%	70%	4	2.6	1.1	6.1
	204.86321	43%	80%				
	267.89998	29%	90%				
48 hours	70.919483	75%	31%	1			
	67.304809	88%	27%	2	2.0	0.1	42.6
	48.353851	100%	12%	3	1.0	0.0	55.0
	144.1759	63%	70%	4	4.3	0.3	55.8
	204.86321	50%	80%				
	267.89998	38%	90%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	54.03695	74%	13%	1			
	48.353851	83%	11%	2	1.9	0.5	7.0
	41.302309	91%	9%	3	2.5	0.7	8.6
	206.65619	13%	72%	4	4.8	1.5	15.8
	235.40816	9%	81%				
	344.787	9%	91%				
24 hours	89.278597	71%	40%	1			
	57.739874	81%	13%	2	0.7	0.3	1.5
	48.353851	90%	11%	3	1.2	0.6	2.7
	206.65619	26%	72%	4	1.0	0.5	2.2
	235.40816	19%	81%				
	344.787	6%	91%				
48 hours	73.756552	77%	25%	1			
	73.406841	85%	25%	2	1.6	0.4	6.6
	69.883004	92%	21%	3	1.0	0.2	4.9
	206.65619	23%	72%	4	1.1	0.2	5.3
	235.40816	23%	81%				
	344.787	8%	91%				

FIG. 5 - 2

Bactericidal permeability-increasing protein

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	8611.940	18896.301	8611.940	14468.657	8611.940	na
average	11995.009	22932.950	11995.009	15299.317	11995.009	na
stdev	11137.380	12052.661	11137.380	12183.608	11137.380	na
p (t-test)		0.023		0.365		na
min	2469.202	6659.701	2469.202	687.071	2469.202	na
max	53084.648	37991.510	53084.648	45853.659	53084.648	na
n						
(Samp)	26	8	26	17	26	0
n (Pat)	25	8	25	17	25	0

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	10797.015	24154.486	10797.015	11262.687	10797.015	687.071
average	15746.924	24154.486	15746.924	12002.168	15746.924	14182.090
stdev	15439.174	15040.279	15439.174	10008.818	15439.174	na
p (t-test)		0.454		0.489		na
min	687.071	13519.403	687.071	1742.818	687.071	14182.090
max	80631.277	34789.569	80631.277	35138.872	80631.277	14182.090
n						
(Samp)	47	2	47	9	47	1
n (Pat)	44	2	44	9	44	1

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	9113.433	18255.913	9113.433	19565.797	9113.433	2469.202
average	12923.892	21239.147	12923.892	18328.602	12923.892	3058.701
stdev	11671.440	11945.741	11671.440	13901.438	11671.440	na
p (t-test)		0.104		0.228		na
min	2469.202	6659.701	2469.202	687.071	2469.202	3058.701
max	53084.648	37991.510	53084.648	45853.659	53084.648	3058.701
n						
(Samp)	27	7	27	11	27	1
n (Pat)	25	7	25	11	25	1

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.80	0.101	26	8	0.003
24 hours	0.59	0.091	26	17	0.342
48 hours	nd	nd	26	0	nd

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.73	0.207	47	2	0.259
24 hours	0.44	0.102	47	9	0.571
48 hours	0.62	0.305	47	1	0.702

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.76	0.114	27	7	0.025
24 hours	0.60	0.105	27	11	0.344
48 hours	0.04	0.050	27	1	0.000

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	14253.731	75%	81%	1			
	13197.015	88%	73%	2	na	na	na
	5951.1688	100%	35%	3	na	na	na
	13197.015	88%	73%	4	na	na	na

FIG. 5 - 3

	14253.731	75%	81%				
	21312.31	38%	92%				
24 hours	7107.4627	71%	35%	1			
	4326.8275	82%	23%	2	0.6	0.1	3.1
	687.07097	94%	0%	3	0.9	0.2	4.2
	13197.015	53%	73%	4	1.8	0.4	8.4
	14253.731	53%	81%				
	21312.31	18%	92%				
48 hours	na	na	na	1			
	na	na	na	2	na	na	na
	na	na	na	3	na	na	na
	na	na	na	4	na	na	na
	na	na	na				
	na	na	na				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	13197.015	100%	60%	1			
	13197.015	100%	60%	2	na	na	na
	13197.015	100%	60%	3	na	na	na
	18255.913	50%	70%	4	na	na	na
	20060.643	50%	81%				
	37292.905	0%	91%				
24 hours	4326.8275	78%	19%	1			
	3742.6345	89%	13%	2	5.2	0.3	85.2
	687.07097	100%	2%	3	1.0	0.0	68.1
	18255.913	11%	70%	4	3.5	0.2	67.2
	20060.643	11%	81%				
	37292.905	0%	91%				
48 hours	13823.881	100%	62%	1			
	13823.881	100%	62%	2	na	na	na
	13823.881	100%	62%	3	na	na	na
	18255.913	0%	70%	4	na	na	na
	20060.643	0%	81%				
	37292.905	0%	91%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	13823.881	71%	78%	1			
	13197.015	86%	74%	2	na	na	na
	5951.1688	100%	30%	3	na	na	na
	12301.493	86%	70%	4	na	na	na
	20002.426	29%	81%				
	34673.135	29%	93%				
24 hours	7107.4627	73%	30%	1			
	5951.1688	82%	30%	2	0.9	0.1	10.5
	2469.202	91%	4%	3	1.8	0.2	16.4
	12301.493	64%	70%	4	2.3	0.3	18.6
	20002.426	36%	81%				
	34673.135	18%	93%				
48 hours	2469.202	100%	4%	1			
	2469.202	100%	4%	2	na	na	na
	2469.202	100%	4%	3	na	na	na
	12301.493	0%	70%	4	na	na	na
	20002.426	0%	81%				
	34673.135	0%	93%				

FIG. 5 - 4

Fatty acid-binding protein, intestinal

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	1.009	1.009	1.009	1.009	1.009	na
average	120.811	5.843	120.811	36.281	120.811	na
stdev	331.825	13.672	331.825	68.832	331.825	na
p (t-test)		0.340		0.308		na
min	1.009	1.009	1.009	1.009	1.009	na
max	1629.046	39.680	1629.046	224.865	1629.046	na
n						
(Samp)	26	8	26	17	26	0
n (Pat)	25	8	25	17	25	0

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	1.009	1.009	1.009	1.009	1.009	1.009
average	80.937	1.009	80.937	30.605	80.937	66.560
stdev	252.191	0.000	252.191	74.192	252.191	na
p (t-test)		0.659		0.558		na
min	1.009	1.009	1.009	1.009	1.009	66.560
max	1629.046	1.009	1629.046	224.865	1629.046	66.560
n						
(Samp)	47	2	47	9	47	1
n (Pat)	44	2	44	9	44	1

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	1.009	1.009	1.009	1.009	1.009	1.009
average	116.373	6.533	116.373	35.170	116.373	1.009
stdev	326.197	14.616	326.197	58.315	326.197	na
p (t-test)		0.385		0.421		na
min	1.009	1.009	1.009	1.009	1.009	1.009
max	1629.046	39.680	1629.046	148.757	1629.046	1.009
n						
(Samp)	27	7	27	11	27	1
n (Pat)	25	7	25	11	25	1

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.38	0.109	26	8	0.261
24 hours	0.46	0.090	26	17	0.670
48 hours	nd	nd	26	0	nd

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.34	0.177	47	2	0.367
24 hours	0.45	0.103	47	9	0.613
48 hours	0.81	0.267	47	1	0.247

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.39	0.116	27	7	0.348
24 hours	0.50	0.105	27	11	0.987
48 hours	0.33	0.248	27	1	0.502

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0	100%	0%	1			
	0	100%	0%	2	0.0	0.0	na
	0	100%	0%	3	10.0	0.5	219.1
	20.48	13%	73%	4	2.7	0.1	89.3

FIG. 5 - 5

	112.64	0%	81%				
	338.2069	0%	92%				
24 hours	0	100%	0%	1			
	0	100%	0%	2	0.2	0.0	3.3
	0	100%	0%	3	4.7	0.9	24.7
	20.48	29%	73%	4	1.2	0.2	5.7
	112.64	18%	81%				
	338.2069	0%	92%				
48 hours	na	na	na	1			
	na	na	na	2	na	na	na
	na	na	na	3	na	na	na
	na	na	na	4	na	na	na
	na	na	na				
	na	na	na				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0	100%	0%	1			
	0	100%	0%	2	na	na	na
	0	100%	0%	3	na	na	na
	7.9412245	0%	70%	4	na	na	na
	61.44	0%	81%				
	238.7027	0%	91%				
24 hours	0	100%	0%	1			
	0	100%	0%	2	0.0	0.0	na
	0	100%	0%	3	3.3	0.6	19.2
	7.9412245	22%	70%	4	1.0	0.1	9.8
	61.44	11%	81%				
	238.7027	0%	91%				
48 hours	61.44	100%	81%	1			
	61.44	100%	81%	2	na	na	na
	61.44	100%	81%	3	na	na	na
	7.9412245	100%	70%	4	na	na	na
	61.44	100%	81%				
	238.7027	0%	91%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0	100%	0%	1			
	0	100%	0%	2	0.0	0.0	na
	0	100%	0%	3	6.4	0.3	140.2
	7.9412245	14%	70%	4	2.7	0.1	89.3
	112.64	0%	81%				
	338.2069	0%	93%				
24 hours	0	100%	0%	1			
	0	100%	0%	2	0.3	0.0	6.7
	0	100%	0%	3	3.5	0.6	20.1
	7.9412245	36%	70%	4	0.3	0.0	6.7
	112.64	18%	81%				
	338.2069	0%	93%				
48 hours	0	100%	0%	1			
	0	100%	0%	2	na	na	na
	0	100%	0%	3	na	na	na
	7.9412245	0%	70%	4	na	na	na
	112.64	0%	81%				
	338.2069	0%	93%				

FIG. 5 - 6

Fibroblast growth factor 23 (N and C-term peptides)

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	46.454	39.784	46.454	75.726	46.454	na
average	68.812	86.481	68.812	130.930	68.812	na
stdev	70.733	119.680	70.733	178.147	70.733	na
p (t-test)		0.606		0.119		na
min	16.331	17.818	16.331	17.632	16.331	na
max	289.484	377.897	289.484	737.977	289.484	na
n (Samp)	26	8	26	16	26	0
n (Pat)	25	8	25	16	25	0

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	47.309	39.784	47.309	81.948	47.309	16.331
average	73.152	39.784	73.152	127.887	73.152	76.889
stdev	73.633	1.330	73.633	102.748	73.633	na
p (t-test)		0.529		0.072		na
min	16.331	38.844	16.331	43.632	16.331	76.889
max	377.897	40.725	377.897	310.918	377.897	76.889
n (Samp)	48	2	48	8	48	1
n (Pat)	46	2	46	8	46	1

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	47.223	38.844	47.223	91.343	47.223	16.331
average	95.821	93.017	95.821	159.611	95.821	662.425
stdev	132.091	127.717	132.091	200.219	132.091	na
p (t-test)		0.960		0.245		na
min	16.331	17.818	16.331	17.632	16.331	662.425
max	651.708	377.897	651.708	737.977	651.708	662.425
n (Samp)	27	7	27	12	27	1
n (Pat)	25	7	25	12	25	1

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.49	0.118	26	8	0.935
24 hours	0.66	0.089	26	16	0.078
48 hours	nd	nd	26	0	nd

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.39	0.189	48	2	0.544
24 hours	0.70	0.109	48	8	0.065
48 hours	0.73	0.293	48	1	0.434

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.46	0.122	27	7	0.745
24 hours	0.65	0.099	27	12	0.128
48 hours	1.00	0.000	27	1	nd

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	35.594796	75%	35%	1			
	28.241636	88%	35%	2	0.5	0.0	16.6
	17.445882	100%	12%	3	2.8	0.3	23.8
	59.364312	25%	73%	4	0.5	0.0	16.6
	74.064191	25%	81%				
	157.79987	13%	92%				

FIG. 5 - 7

24 hours	44.144981	75%	46%	1			
	41.066914	81%	42%	2	0.9	0.1	5.5
	18.561176	94%	15%	3	1.6	0.3	9.0
	59.364312	56%	73%	4	2.8	0.5	14.6
	74.064191	56%	81%				
	157.79987	19%	92%				
48 hours	na	na	na	1			
	na	na	na	2	na	na	na
	na	na	na	3	na	na	na
	na	na	na	4	na	na	na
	na	na	na				
	na	na	na				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	35.594796	71%	30%	1			
	28.241636	86%	30%	2	0.5	0.0	16.6
	17.445882	100%	11%	3	1.8	0.2	16.4
	67.743494	29%	70%	4	0.5	0.0	16.6
	149.6589	14%	81%				
	254.11922	14%	93%				
24 hours	74.064191	75%	74%	1			
	23.624535	83%	22%	2	0.0	0.0	na
	17.631765	92%	11%	3	2.0	0.3	11.7
	67.743494	75%	70%	4	1.3	0.2	8.0
	149.6589	25%	81%				
	254.11922	17%	93%				
48 hours	651.70797	100%	100%	1			
	651.70797	100%	100%	2	na	na	na
	651.70797	100%	100%	3	na	na	na
	67.743494	100%	70%	4	na	na	na
	149.6589	100%	81%				
	254.11922	100%	93%				

FIG. 5 - 8

Interleukin-12

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.878	0.653	0.878	0.701	0.878	0.532
average	56.836	3.146	56.836	9.686	56.836	12.455
stdev	420.619	8.471	420.619	40.126	420.619	56.720
p (t-test)		0.379		0.409		0.601
min	0.088	0.091	0.088	0.107	0.088	0.104
max	4026.625	38.947	4026.625	276.853	4026.625	284.609
n (Samp)	103	48	103	55	103	25
n (Pat)	98	48	98	55	98	25

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.697	0.805	0.697	0.699	0.697	0.511
average	64.271	97.076	64.271	4.464	64.271	7.631
stdev	574.260	377.091	574.260	10.905	574.260	23.856
p (t-test)		0.822		0.642		0.733
min	0.088	0.383	0.088	0.258	0.088	0.331
max	7807.975	1510.879	7807.975	44.220	7807.975	83.370
n (Samp)	240	16	240	20	240	12
n (Pat)	160	16	160	20	160	12

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.919	0.668	0.919	0.711	0.919	0.669
average	59.994	2.968	59.994	11.298	59.994	14.958
stdev	435.590	8.071	435.590	44.671	435.590	61.814
p (t-test)		0.410		0.462		0.638
min	0.258	0.091	0.258	0.107	0.258	0.104
max	4026.625	38.947	4026.625	276.853	4026.625	284.609
n (Samp)	96	40	96	44	96	21
n (Pat)	84	40	84	44	84	21

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.43	0.049	103	48	0.145
24 hours	0.45	0.048	103	55	0.312
48 hours	0.36	0.057	103	25	0.012

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.55	0.076	240	16	0.543
24 hours	0.50	0.067	240	20	0.962
48 hours	0.41	0.080	240	12	0.281

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.42	0.052	96	40	0.117
24 hours	0.44	0.052	96	44	0.269
48 hours	0.40	0.065	96	21	0.124

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.5257099	71%	20%	1			
	0.4438192	81%	13%	2	0.6	0.3	1.0
	0.3856638	92%	7%	3	1.1	0.7	1.8
	1.0901266	27%	71%	4	1.5	0.9	2.3
	1.3464175	17%	81%				
	4.5125034	8%	90%				

FIG. 5 - 9

24 hours	0.5377638	71%	21%	1			
	0.4568277	80%	13%	2	0.4	0.3	0.7
	0.3468788	91%	4%	3	1.0	0.7	1.5
	1.0901266	29%	71%	4	1.3	0.9	1.9
	1.3464175	24%	81%				
	4.5125034	11%	90%				
48 hours	0.4023484	72%	9%	1			
	0.3856638	80%	7%	2	0.8	0.3	2.1
	0.2729564	92%	3%	3	0.8	0.3	2.1
	1.0901266	20%	71%	4	3.2	1.6	6.7
	1.3464175	20%	81%				
	4.5125034	12%	90%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.618761	75%	39%	1			
	0.615415	81%	39%	2	2.6	0.6	11.1
	0.3856638	94%	9%	3	2.6	0.6	11.1
	1.0104446	25%	70%	4	2.1	0.4	9.6
	1.3691978	19%	80%				
	4.5125034	13%	90%				
24 hours	0.5521538	70%	33%	1			
	0.396639	80%	10%	2	0.4	0.1	1.1
	0.3468788	90%	7%	3	0.5	0.2	1.3
	1.0104446	35%	70%	4	0.8	0.4	1.7
	1.3691978	30%	80%				
	4.5125034	15%	90%				
48 hours	0.4326322	75%	14%	1			
	0.3899664	83%	9%	2	0.7	0.1	3.6
	0.3856638	92%	9%	3	0.3	0.0	4.7
	1.0104446	33%	70%	4	2.1	0.7	6.0
	1.3691978	25%	80%				
	4.5125034	8%	90%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.5026541	73%	14%	1			
	0.4438192	80%	9%	2	0.5	0.3	1.0
	0.4023484	90%	7%	3	1.0	0.6	1.7
	1.0968412	30%	71%	4	1.7	1.0	2.8
	1.3464175	20%	80%				
	4.2317175	10%	91%				
24 hours	0.5680016	70%	26%	1			
	0.4796977	82%	11%	2	0.5	0.3	1.0
	0.4301925	91%	9%	3	1.3	0.8	2.1
	1.0968412	27%	71%	4	1.3	0.8	2.1
	1.3464175	23%	80%				
	4.2317175	9%	91%				
48 hours	0.4275936	71%	8%	1			
	0.3753513	81%	3%	2	0.1	0.0	1.6
	0.3468788	90%	2%	3	0.8	0.3	2.0
	1.0968412	29%	71%	4	1.8	0.9	3.7
	1.3464175	29%	80%				
	4.2317175	19%	91%				

FIG. 5 - 10

Advanced glycosylation end product-specific receptor

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	101.449	166.585	101.449	142.399	101.449	116.586
average	160.281	257.966	160.281	190.954	160.281	215.554
stdev	193.202	247.346	193.202	172.657	193.202	255.034
p (t-test)		0.201		0.486		0.327
min	6.382	32.456	6.382	29.400	6.382	44.866
max	1570.287	686.054	1570.287	634.644	1570.287	914.925
n (Samp)	128	7	128	22	128	14
n (Pat)	102	7	102	22	102	14

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	106.019	513.517	106.019	154.847	106.019	70.011
average	158.412	410.675	158.412	265.918	158.412	183.183
stdev	184.414	338.718	184.414	258.703	184.414	243.886
p (t-test)		0.022		0.139		0.792
min	6.382	32.456	6.382	34.323	6.382	44.866
max	1570.287	686.054	1570.287	636.667	1570.287	547.846
n (Samp)	169	3	169	7	169	4
n (Pat)	124	3	124	7	124	4

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	105.324	153.545	105.324	140.622	105.324	123.035
average	176.889	143.434	176.889	187.946	176.889	214.919
stdev	226.774	71.153	226.774	160.307	226.774	261.063
p (t-test)		0.770		0.831		0.601
min	20.588	48.354	20.588	29.400	20.588	52.016
max	1570.287	218.291	1570.287	622.384	1570.287	914.925
n (Samp)	112	4	112	21	112	11
n (Pat)	87	4	87	21	87	11

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.61	0.116	128	7	0.322
24 hours	0.56	0.068	128	22	0.378
48 hours	0.55	0.083	128	14	0.577

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.66	0.174	169	3	0.349
24 hours	0.62	0.115	169	7	0.283
48 hours	0.41	0.136	169	4	0.523

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.57	0.152	112	4	0.664
24 hours	0.56	0.070	112	21	0.415
48 hours	0.56	0.094	112	11	0.536

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	139.70018	71%	67%	1			
	44.865532	86%	13%	2	0.0	0.0	na
	30.589903	100%	4%	3	1.0	0.1	7.8
	165.53499	57%	70%	4	1.5	0.3	8.7
	219.53809	29%	80%				

FIG. 6 - 1

	298.72994	29%	91%				
24 hours	68.207891	73%	27%	1			
	62.838294	82%	24%	2	0.4	0.1	1.3
	32.92071	91%	4%	3	0.8	0.3	1.9
	165.53499	45%	70%	4	1.4	0.7	2.8
	219.53809	32%	80%				
	298.72994	14%	91%				
48 hours	89.278597	71%	45%	1			
	51.120266	86%	14%	2	1.0	0.2	4.0
	48.353851	93%	14%	3	1.8	0.6	5.7
	165.53499	21%	70%	4	1.0	0.2	4.0
	219.53809	21%	80%				
	298.72994	21%	91%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	30.589903	100%	4%	1			
	30.589903	100%	4%	2	0.0	0.0	na
	30.589903	100%	4%	3	0.0	0.0	na
	163.92883	67%	70%	4	2.0	0.1	42.6
	218.29065	67%	80%				
	286.58446	67%	91%				
24 hours	108.88023	71%	51%	1			
	78.694673	86%	36%	2	1.0	0.0	55.2
	32.92071	100%	4%	3	2.0	0.1	42.5
	163.92883	43%	70%	4	3.1	0.2	47.1
	218.29065	29%	80%				
	286.58446	29%	91%				
48 hours	48.353851	75%	14%	1			
	43.479265	100%	12%	2	0.0	0.0	na
	43.479265	100%	12%	3	1.0	0.0	56.6
	163.92883	25%	70%	4	2.1	0.1	43.6
	218.29065	25%	80%				
	286.58446	25%	91%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	139.70018	75%	66%	1			
	44.865532	100%	12%	2	0.0	0.0	na
	44.865532	100%	12%	3	2.1	0.1	45.2
	168.60842	25%	71%	4	1.0	0.0	58.0
	220.4877	0%	80%				
	335.72774	0%	90%				
24 hours	78.694673	71%	33%	1			
	62.838294	86%	22%	2	0.6	0.2	1.8
	52.015694	90%	14%	3	1.2	0.5	2.9
	168.60842	43%	71%	4	1.5	0.6	3.3
	220.4877	33%	80%				
	335.72774	14%	90%				
48 hours	93.996537	73%	43%	1			
	89.278597	82%	42%	2	3.1	0.2	48.6
	78.694673	91%	33%	3	5.6	0.5	67.6
	168.60842	18%	71%	4	2.0	0.1	43.3
	220.4877	18%	80%				
	335.72774	18%	90%				

FIG. 6 - 2

Fatty acid-binding protein, intestinal

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	1.009	na	1.009	1.009	1.009	1.009
average	79.462	na	79.462	17.150	79.462	1.009
stdev	254.749	na	254.749	42.553	254.749	na
p (t-test)		na		0.386		na
min	1.009	na	1.009	1.009	1.009	1.009
max	1629.046	na	1629.046	145.297	1629.046	1.009
n (Samp)	46	0	46	13	46	1
n (Pat)	43	0	43	13	43	1

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	1.009	na	1.009	1.009	1.009	na
average	68.474	na	68.474	15.179	68.474	na
stdev	227.711	na	227.711	24.544	227.711	na
p (t-test)		na		0.689		na
min	1.009	na	1.009	1.009	1.009	na
max	1629.046	na	1629.046	43.520	1629.046	na
n (Samp)	59	0	59	3	59	0
n (Pat)	54	0	54	3	54	0

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	1.009	na	1.009	1.009	1.009	1.009
average	85.634	na	85.634	17.150	85.634	1.009
stdev	271.129	na	271.129	42.553	271.129	na
p (t-test)		na		0.372		na
min	1.009	na	1.009	1.009	1.009	1.009
max	1629.046	na	1629.046	145.297	1629.046	1.009
n (Samp)	40	0	40	13	40	1
n (Pat)	37	0	37	13	37	1

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	nd	nd	46	0	nd
24 hours	0.42	0.088	46	13	0.379
48 hours	0.35	0.249	46	1	0.542

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	nd	nd	59	0	nd
24 hours	0.51	0.173	59	3	0.948
48 hours	nd	nd	59	0	nd

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	nd	nd	40	0	nd
24 hours	0.41	0.089	40	13	0.335
48 hours	0.34	0.246	40	1	0.509

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	na	na	na	1			
	na	na	na	2	na	na	na
	na	na	na	3	na	na	na
	na	na	na	4	na	na	na
	na	na	na				
	na	na	na				

FIG. 6 - 3

24 hours	0	100%	0%	1			
	0	100%	0%	2	0.0	0.0	na
	0	100%	0%	3	13.0	2.3	72.5
	7.9412245	15%	72%	4	0.5	0.0	12.8
	43.52	15%	80%				
	238.7027	0%	91%				
48 hours	0	100%	0%	1			
	0	100%	0%	2	na	na	na
	0	100%	0%	3	na	na	na
	7.9412245	0%	72%	4	na	na	na
	43.52	0%	80%				
	238.7027	0%	91%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	na	na	na	1			
	na	na	na	2	na	na	na
	na	na	na	3	na	na	na
	na	na	na	4	na	na	na
	na	na	na				
	na	na	na				
24 hours	0	100%	0%	1			
	0	100%	0%	2	0.0	0.0	na
	0	100%	0%	3	13.5	2.1	86.0
	7.9412245	15%	70%	4	1.1	0.1	10.9
	43.52	15%	80%				
	238.7027	0%	90%				
48 hours	0	100%	0%	1			
	0	100%	0%	2	na	na	na
	0	100%	0%	3	na	na	na
	7.9412245	0%	70%	4	na	na	na
	43.52	0%	80%				
	238.7027	0%	90%				

FIG. 6 - 4

Interleukin-12

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.700	0.653	0.700	0.903	0.700	1.302
average	60.559	87.640	60.559	25.565	60.559	29.583
stdev	585.515	345.304	585.515	77.813	585.515	73.746
p (t-test)		0.843		0.753		0.833
min	0.088	0.221	0.088	0.325	0.088	0.331
max	7807.975	1510.879	7807.975	319.519	7807.975	284.609
n (Samp)	230	19	230	28	230	16
n (Pat)	158	19	158	28	158	16

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.700	757.859	0.700	0.715	0.700	1.422
average	52.764	757.859	52.764	7.755	52.764	17.442
stdev	518.334	1064.931	518.334	16.211	518.334	36.860
p (t-test)		0.058		0.819		0.879
min	0.088	4.838	0.088	0.393	0.088	0.331
max	7807.975	1510.879	7807.975	44.220	7807.975	83.370
n (Samp)	295	2	295	7	295	5
n (Pat)	187	2	187	7	187	5

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.690	0.653	0.690	1.276	0.690	1.302
average	69.301	566.256	69.301	193.967	69.301	135.648
stdev	630.805	2431.386	630.805	847.551	630.805	403.234
p (t-test)		0.027		0.365		0.699
min	0.091	0.221	0.091	0.325	0.091	0.444
max	7807.975	10606.241	7807.975	4330.706	7807.975	1510.879
n (Samp)	198	19	198	26	198	14
n (Pat)	132	19	132	26	132	14

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.51	0.069	230	19	0.867
24 hours	0.63	0.059	230	28	0.023
48 hours	0.62	0.077	230	16	0.113

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.95	0.109	295	2	0.000
24 hours	0.59	0.114	295	7	0.429
48 hours	0.55	0.134	295	5	0.684

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.52	0.070	198	19	0.814
24 hours	0.68	0.061	198	26	0.004
48 hours	0.65	0.082	198	14	0.063

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.4568277	74%	17%	1			
	0.4036447	84%	11%	2	0.5	0.2	1.3
	0.2729564	95%	4%	3	0.1	0.0	1.3
	0.9781035	37%	70%	4	1.0	0.5	1.8
	1.161229	37%	80%				
	2.9522188	26%	90%				

FIG. 6 - 5

24 hours	0.6523072	71%	44%	1			
	0.6115633	82%	37%	2	1.5	0.6	3.7
	0.3933013	93%	10%	3	1.3	0.5	3.3
	0.9781035	46%	70%	4	3.8	1.8	7.6
	1.161229	46%	80%				
	2.9522188	29%	90%				
48 hours	0.5257099	75%	27%	1			
	0.5223645	81%	25%	2	1.0	0.2	3.9
	0.4420384	94%	15%	3	0.3	0.0	4.7
	0.9781035	56%	70%	4	3.3	1.3	8.4
	1.161229	56%	80%				
	2.9522188	31%	90%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	4.5125034	100%	91%	1			
	4.5125034	100%	91%	2	na	na	na
	4.5125034	100%	91%	3	na	na	na
	1.00407	100%	70%	4	na	na	na
	1.3087344	100%	80%				
	4.2317175	100%	90%				
24 hours	0.6518573	71%	45%	1			
	0.6258399	86%	39%	2	2.0	0.1	39.9
	0.3907575	100%	10%	3	1.0	0.0	53.1
	1.00407	43%	70%	4	3.0	0.2	43.8
	1.3087344	43%	80%				
	4.2317175	29%	90%				
48 hours	0.3933013	80%	10%	1			
	0.3933013	80%	10%	2	0.0	0.0	na
	0.3245813	100%	5%	3	0.0	0.0	na
	1.00407	60%	70%	4	1.5	0.3	8.2
	1.3087344	60%	80%				
	4.2317175	20%	90%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.4568277	74%	17%	1			
	0.4036447	84%	11%	2	0.5	0.2	1.3
	0.2729564	95%	4%	3	0.1	0.0	1.3
	0.9692546	42%	70%	4	1.0	0.5	1.9
	1.1462567	37%	80%				
	2.8341725	26%	90%				
24 hours	0.8302281	73%	60%	1			
	0.6115633	81%	38%	2	0.7	0.2	2.5
	0.396639	92%	10%	3	1.3	0.5	3.3
	0.9692546	54%	70%	4	4.3	2.1	8.9
	1.1462567	54%	80%				
	2.8341725	35%	90%				
48 hours	0.5377638	71%	29%	1			
	0.5223645	86%	26%	2	1.5	0.3	8.5
	0.4961086	93%	21%	3	0.5	0.0	10.0
	0.9692546	57%	70%	4	4.5	1.2	16.8
	1.1462567	57%	80%				
	2.8341725	36%	90%				

FIG. 6 - 6

Fatty acid-binding protein, intestinal

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	1.009	1.009	1.009	1.009	1.009	1.009
average	6.533	13.043	6.533	13.043	6.533	13.043
stdev	14.616	34.037	14.616	34.037	14.616	34.037
p (t-test)		0.648		0.648		0.648
min	1.009	1.009	1.009	1.009	1.009	1.009
max	39.680	97.280	39.680	97.280	39.680	97.280
n (Samp)	7	8	7	8	7	8
n (Pat)	7	8	7	8	7	8

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	1.009	na	1.009	na	1.009	na
average	1.009	na	1.009	na	1.009	na
stdev	0.000	na	0.000	na	0.000	na
p (t-test)		na		na		na
min	1.009	na	1.009	na	1.009	na
max	1.009	na	1.009	na	1.009	na
n (Samp)	4	0	4	0	4	0
n (Pat)	4	0	4	0	4	0

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	1.009	1.009	1.009	1.009	1.009	1.009
average	8.743	14.762	8.743	14.762	8.743	14.762
stdev	17.294	36.387	17.294	36.387	17.294	36.387
p (t-test)		0.741		0.741		0.741
min	1.009	1.009	1.009	1.009	1.009	1.009
max	39.680	97.280	39.680	97.280	39.680	97.280
n (Samp)	5	7	5	7	5	7
n (Pat)	5	7	5	7	5	7

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.50	0.154	7	8	1.000
24 hours	0.50	0.154	7	8	1.000
48 hours	0.50	0.154	7	8	1.000

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	nd	nd	4	0	nd
24 hours	nd	nd	4	0	nd
48 hours	nd	nd	4	0	nd

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.49	0.176	5	7	0.935
24 hours	0.49	0.176	5	7	0.935
48 hours	0.49	0.176	5	7	0.935

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0	100%	0%	1			
	0	100%	0%	2	0.0	0.0	na
	0	100%	0%	3	na	na	na
	1.00864	13%	86%	4	na	na	na
	1.00864	13%	86%				
	39.68	13%	100%				

FIG. 7 - 1

24 hours	0	100%	0%	1			
	0	100%	0%	2	0.0	0.0	na
	0	100%	0%	3	na	na	na
	1.00864	13%	86%	4	na	na	na
	1.00864	13%	86%				
	39.68	13%	100%				
48 hours	0	100%	0%	1			
	0	100%	0%	2	0.0	0.0	na
	0	100%	0%	3	na	na	na
	1.00864	13%	86%	4	na	na	na
	1.00864	13%	86%				
	39.68	13%	100%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0	100%	0%	1			
	0	100%	0%	2	0.0	0.0	na
	0	100%	0%	3	na	na	na
	1.00864	14%	80%	4	na	na	na
	1.00864	14%	80%				
	39.68	14%	100%				
24 hours	0	100%	0%	1			
	0	100%	0%	2	0.0	0.0	na
	0	100%	0%	3	na	na	na
	1.00864	14%	80%	4	na	na	na
	1.00864	14%	80%				
	39.68	14%	100%				
48 hours	0	100%	0%	1			
	0	100%	0%	2	0.0	0.0	na
	0	100%	0%	3	na	na	na
	1.00864	14%	80%	4	na	na	na
	1.00864	14%	80%				
	39.68	14%	100%				

FIG. 7 - 2

Interleukin-12

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.646	0.933	0.646	0.933	0.646	0.933
average	152.957	24.266	152.957	24.266	152.957	24.266
stdev	1082.426	77.011	1082.426	77.011	1082.426	77.011
p (t-test)		0.628		0.628		0.628
min	0.091	0.348	0.091	0.348	0.091	0.348
max	7807.975	319.519	7807.975	319.519	7807.975	319.519
n (Samp)	52	17	52	17	52	17
n (Pat)	52	17	52	17	52	17

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.886	1.039	0.886	1.039	0.886	1.039
average	2.732	1.039	2.732	1.039	2.732	1.039
stdev	7.002	0.913	7.002	0.913	7.002	0.913
p (t-test)		0.742		0.742		0.742
min	0.348	0.393	0.348	0.393	0.348	0.393
max	31.198	1.684	31.198	1.684	31.198	1.684
n (Samp)	19	2	19	2	19	2
n (Pat)	19	2	19	2	19	2

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.641	1.061	0.641	1.061	0.641	1.061
average	192.983	29.286	192.983	29.286	192.983	29.286
stdev	1219.042	84.529	1219.042	84.529	1219.042	84.529
p (t-test)		0.620		0.620		0.620
min	0.091	0.444	0.091	0.444	0.091	0.444
max	7807.975	319.519	7807.975	319.519	7807.975	319.519
n (Samp)	41	14	41	14	41	14
n (Pat)	41	14	41	14	41	14

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.65	0.081	52	17	0.067
24 hours	0.65	0.081	52	17	0.067
48 hours	0.65	0.081	52	17	0.067

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.47	0.216	19	2	0.903
24 hours	0.47	0.216	19	2	0.903
48 hours	0.47	0.216	19	2	0.903

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.72	0.085	41	14	0.009
24 hours	0.72	0.085	41	14	0.009
48 hours	0.72	0.085	41	14	0.009

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.8142122	71%	63%	1			
	0.5945085	82%	40%	2	0.6	0.1	4.2
	0.4340774	94%	12%	3	1.9	0.5	7.5
	0.8876276	53%	71%	4	3.0	0.8	10.4
	1.125014	41%	81%				
	1.3952268	35%	90%				

FIG. 7 - 3

24 hours	0.8142122	71%	63%	1			
	0.5945085	82%	40%	2	0.6	0.1	4.2
	0.4340774	94%	12%	3	1.9	0.5	7.5
	0.8876276	53%	71%	4	3.0	0.8	10.4
	1.125014	41%	81%				
	1.3952268	35%	90%				
48 hours	0.8142122	71%	63%	1			
	0.5945085	82%	40%	2	0.6	0.1	4.2
	0.4340774	94%	12%	3	1.9	0.5	7.5
	0.8876276	53%	71%	4	3.0	0.8	10.4
	1.125014	41%	81%				
	1.3952268	35%	90%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.3833052	100%	11%	1			
	0.3833052	100%	11%	2	0.0	0.0	na
	0.3833052	100%	11%	3	0.0	0.0	na
	0.9386729	50%	74%	4	1.3	0.0	152.2
	1.161229	50%	84%				
	5.4715335	0%	95%				
24 hours	0.3833052	100%	11%	1			
	0.3833052	100%	11%	2	0.0	0.0	na
	0.3833052	100%	11%	3	0.0	0.0	na
	0.9386729	50%	74%	4	1.3	0.0	152.2
	1.161229	50%	84%				
	5.4715335	0%	95%				
48 hours	0.3833052	100%	11%	1			
	0.3833052	100%	11%	2	0.0	0.0	na
	0.3833052	100%	11%	3	0.0	0.0	na
	0.9386729	50%	74%	4	1.3	0.0	152.2
	1.161229	50%	84%				
	5.4715335	0%	95%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	0.8428186	71%	68%	1			
	0.595373	86%	46%	2	0.4	0.0	11.1
	0.4537941	93%	20%	3	2.2	0.3	13.9
	0.8738186	57%	71%	4	5.5	1.0	30.7
	0.97317	50%	80%				
	1.3758719	43%	90%				
24 hours	0.8428186	71%	68%	1			
	0.595373	86%	46%	2	0.4	0.0	11.1
	0.4537941	93%	20%	3	2.2	0.3	13.9
	0.8738186	57%	71%	4	5.5	1.0	30.7
	0.97317	50%	80%				
	1.3758719	43%	90%				
48 hours	0.8428186	71%	68%	1			
	0.595373	86%	46%	2	0.4	0.0	11.1
	0.4537941	93%	20%	3	2.2	0.3	13.9
	0.8738186	57%	71%	4	5.5	1.0	30.7
	0.97317	50%	80%				
	1.3758719	43%	90%				

FIG. 7 - 4

Advanced glycosylation end product-specific receptor

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	96.696	212.038	96.696	188.331	96.696	253.440
average	161.311	395.620	161.311	362.202	161.311	502.372
stdev	192.016	495.862	192.016	501.858	192.016	642.842
p (t-test)		0.008		0.023		0.003
min	6.382	34.323	6.382	34.323	6.382	110.138
max	1156.927	1907.734	1156.927	1907.734	1156.927	1907.734
n (Samp)	52	13	52	13	52	7
n (Pat)	52	13	52	13	52	7

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	110.138	212.038	110.138	154.847	110.138	287.833
average	159.245	460.018	159.245	397.955	159.245	802.535
stdev	171.155	656.615	171.155	671.088	171.155	957.880
p (t-test)		0.001		0.009		0.000
min	6.382	34.323	6.382	34.323	6.382	212.038
max	1156.927	1907.734	1156.927	1907.734	1156.927	1907.734
n (Samp)	101	7	101	7	101	3
n (Pat)	101	7	101	7	101	3

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	117.521	270.636	117.521	270.636	117.521	270.636
average	176.083	514.813	176.083	514.813	176.083	550.760
stdev	193.967	599.895	193.967	599.895	193.967	690.092
p (t-test)		0.003		0.003		0.004
min	21.871	110.138	21.871	110.138	21.871	110.138
max	1156.927	1907.734	1156.927	1907.734	1156.927	1907.734
n (Samp)	46	8	46	8	46	6
n (Pat)	46	8	46	8	46	6

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.74	0.085	52	13	0.004
24 hours	0.70	0.088	52	13	0.026
48 hours	0.80	0.104	52	7	0.004

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.70	0.114	101	7	0.077
24 hours	0.61	0.117	101	7	0.339
48 hours	0.90	0.120	101	3	0.001

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.79	0.100	46	8	0.005
24 hours	0.79	0.100	46	8	0.005
48 hours	0.77	0.117	46	6	0.020

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	110.13764	77%	56%	1			
	109.83449	85%	56%	2	2.1	0.1	53.1
	106.01902	92%	56%	3	5.0	0.3	77.7
	147.73132	69%	71%	4	8.2	0.6	109.7
	230.08968	46%	81%				

FIG. 8 - 1

	298.72994	31%	90%				
24 hours	109.83449	77%	56%	1			
	106.01902	85%	56%	2	2.1	0.1	53.1
	78.694673	92%	37%	3	5.0	0.3	77.7
	147.73132	62%	71%	4	8.2	0.6	109.7
	230.08968	38%	81%				
	298.72994	23%	90%				
48 hours	206.65619	71%	79%	1			
	117.47781	86%	58%	2	na	na	na
	106.01902	100%	56%	3	na	na	na
	147.73132	71%	71%	4	na	na	na
	230.08968	57%	81%				
	298.72994	29%	90%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	147.73132	71%	68%	1			
	106.01902	86%	50%	2	1.0	0.0	58.6
	32.92071	100%	6%	3	1.0	0.0	58.6
	163.92883	57%	70%	4	4.5	0.3	61.5
	218.29065	43%	80%				
	271.13449	43%	90%				
24 hours	106.01902	71%	50%	1			
	78.694673	86%	34%	2	2.1	0.1	45.9
	32.92071	100%	6%	3	1.0	0.0	58.6
	163.92883	43%	70%	4	3.3	0.2	51.9
	218.29065	29%	80%				
	271.13449	29%	90%				
48 hours	206.65619	100%	79%	1			
	206.65619	100%	79%	2	na	na	na
	206.65619	100%	79%	3	na	na	na
	163.92883	100%	70%	4	na	na	na
	218.29065	67%	80%				
	271.13449	67%	90%				

FIG. 8 - 2

Bactericidal permeability-increasing protein

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	9113.433	17613.903	9113.433	17613.903	9113.433	17613.903
average	12376.041	19796.406	12376.041	19796.406	12376.041	18094.219
stdev	11192.744	8560.581	11192.744	8560.581	11192.744	3941.757
p (t-test)		0.141		0.141		0.327
min	3400.668	11262.687	3400.668	11262.687	3400.668	14468.657
max	53084.648	35138.872	53084.648	35138.872	53084.648	22680.412
n						
(Samp)	25	6	25	6	25	4
n (Pat)	25	6	25	6	25	4

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	11191.045	22680.412	11191.045	22680.412	11191.045	18574.535
average	16187.781	24095.980	16187.781	24095.980	16187.781	18574.535
stdev	15750.612	10407.561	15750.612	10407.561	15750.612	5806.588
p (t-test)		0.399		0.399		0.833
min	687.071	14468.657	687.071	14468.657	687.071	14468.657
max	80631.277	35138.872	80631.277	35138.872	80631.277	22680.412
n						
(Samp)	44	3	44	3	44	2
n (Pat)	44	3	44	3	44	2

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	9632.836	17613.903	9632.836	17613.903	9632.836	20060.643
average	13518.139	17292.726	13518.139	17292.726	13518.139	19302.740
stdev	11909.876	5084.877	11909.876	5084.877	11909.876	3813.533
p (t-test)		0.542		0.542		0.418
min	3400.668	11262.687	3400.668	11262.687	3400.668	15167.164
max	53084.648	22680.412	53084.648	22680.412	53084.648	22680.412
n						
(Samp)	25	4	25	4	25	3
n (Pat)	25	4	25	4	25	3

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.83	0.110	25	6	0.003
24 hours	0.83	0.110	25	6	0.003
48 hours	0.84	0.129	25	4	0.008

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.78	0.162	44	3	0.084
24 hours	0.78	0.162	44	3	0.084
48 hours	0.74	0.207	44	2	0.248

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.75	0.149	25	4	0.094
24 hours	0.75	0.149	25	4	0.094
48 hours	0.81	0.156	25	3	0.045

FIG. 8 - 3

Fibroblast growth factor 23 (Intact; N and C-term peptides)

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	47.223	143.844	47.223	143.844	47.223	143.844
average	69.921	234.375	69.921	234.375	69.921	319.570
stdev	71.960	243.423	71.960	243.423	71.960	363.895
p (t-test)		0.005		0.005		0.003
min	16.331	18.933	16.331	18.933	16.331	76.889
max	289.484	737.977	289.484	737.977	289.484	737.977
n (Samp)	25	7	25	7	25	3
n (Pat)	25	7	25	7	25	3

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	47.309	315.204	47.309	315.204	47.309	16.331
average	74.353	430.944	74.353	430.944	74.353	737.977
stdev	75.010	268.568	75.010	268.568	75.010	na
p (t-test)		0.000		0.000		na
min	16.331	239.652	16.331	239.652	16.331	737.977
max	377.897	737.977	377.897	737.977	377.897	737.977
n (Samp)	46	3	46	3	46	1
n (Pat)	46	3	46	3	46	1

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	51.669	108.123	51.669	108.123	51.669	143.844
average	100.215	217.153	100.215	217.153	100.215	319.570
stdev	136.493	294.739	136.493	294.739	136.493	363.895
p (t-test)		0.168		0.168		0.039
min	16.331	18.933	16.331	18.933	16.331	76.889
max	651.708	737.977	651.708	737.977	651.708	737.977
n (Samp)	25	5	25	5	25	3
n (Pat)	25	5	25	5	25	3

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.79	0.110	25	7	0.009
24 hours	0.79	0.110	25	7	0.009
48 hours	0.88	0.132	25	3	0.004

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.97	0.069	46	3	0.000
24 hours	0.97	0.069	46	3	0.000
48 hours	1.00	0.000	46	1	nd

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.66	0.144	25	5	0.254
24 hours	0.66	0.144	25	5	0.254
48 hours	0.83	0.152	25	3	0.032

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	76.888609	71%	80%	1			
	74.064191	86%	80%	2	0.0	0.0	na
	18.561176	100%	16%	3	2.3	0.1	81.0
	59.364312	86%	72%	4	7.0	0.3	175.2
	74.064191	86%	80%				
	157.79987	43%	92%				

FIG. 8 - 4

24 hours	76.888609	71%	80%	1			
	74.064191	86%	80%	2	0.0	0.0	na
	18.561176	100%	16%	3	2.3	0.1	81.0
	59.364312	86%	72%	4	7.0	0.3	175.2
	74.064191	86%	80%				
	157.79987	43%	92%				
48 hours	74.064191	100%	80%	1			
	74.064191	100%	80%	2	na	na	na
	74.064191	100%	80%	3	na	na	na
	59.364312	100%	72%	4	na	na	na
	74.064191	100%	80%				
	157.79987	33%	92%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	74.064191	80%	72%	1			
	74.064191	80%	72%	2	0.0	0.0	na
	17.445882	100%	12%	3	2.4	0.1	93.1
	74.064191	80%	72%	4	2.0	0.1	72.7
	149.6589	20%	80%				
	254.11922	20%	92%				
24 hours	74.064191	80%	72%	1			
	74.064191	80%	72%	2	0.0	0.0	na
	17.445882	100%	12%	3	2.4	0.1	93.1
	74.064191	80%	72%	4	2.0	0.1	72.7
	149.6589	20%	80%				
	254.11922	20%	92%				
48 hours	74.064191	100%	72%	1			
	74.064191	100%	72%	2	na	na	na
	74.064191	100%	72%	3	na	na	na
	74.064191	100%	72%	4	na	na	na
	149.6589	33%	80%				
	254.11922	33%	92%				

FIG. 8 - 5

Interleukin-12

sCr or UO

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.879	1.901	0.879	1.711	0.879	1.901
average	59.703	44.712	59.703	48.507	59.703	68.047
stdev	431.126	126.899	431.126	133.106	431.126	158.182
p (t-test)		0.909		0.935		0.960
min	0.088	0.658	0.088	0.400	0.088	0.658
max	4026.625	425.413	4026.625	425.413	4026.625	425.413
n (Samp)	98	11	98	10	98	7
n (Pat)	98	11	98	10	98	7

sCr only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.839	1.901	0.839	1.672	0.839	1.901
average	91.743	3.250	91.743	2.563	91.743	2.846
stdev	701.723	2.655	701.723	2.501	701.723	3.030
p (t-test)		0.779		0.800		0.827
min	0.088	0.672	0.088	0.672	0.088	0.400
max	7807.975	6.236	7807.975	6.236	7807.975	6.236
n (Samp)	160	5	160	4	160	3
n (Pat)	160	5	160	4	160	3

UO only

	0 hr prior to AKI stage		24 hr prior to AKI stage		48 hr prior to AKI stage	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
median	0.955	1.764	0.955	1.711	0.955	1.849
average	68.469	59.769	68.469	59.674	68.469	78.348
stdev	465.391	148.501	465.391	148.543	465.391	170.688
p (t-test)		0.958		0.958		0.959
min	0.347	0.658	0.347	0.400	0.347	0.658
max	4026.625	425.413	4026.625	425.413	4026.625	425.413
n (Samp)	84	8	84	8	84	6
n (Pat)	84	8	84	8	84	6

sCr or UO

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.78	0.085	98	11	0.001
24 hours	0.74	0.093	98	10	0.010
48 hours	0.81	0.101	98	7	0.002

sCr only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.75	0.128	160	5	0.049
24 hours	0.72	0.147	160	4	0.139
48 hours	0.59	0.175	160	3	0.616

UO only

Time prior AKI stage	AUC	SE	nCohort 1	nCohort 2	p
0 hours	0.73	0.105	84	8	0.032
24 hours	0.69	0.108	84	8	0.080
48 hours	0.78	0.114	84	6	0.015

sCr or UO

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	1.3858432	73%	81%	1			
	1.3464175	82%	80%	2	na	na	na
	0.6626156	91%	36%	3	na	na	na
	1.0968412	82%	70%	4	na	na	na
	1.3858432	73%	81%				
	6.0450244	27%	91%				

FIG. 8 - 6

24 hours	1.3858432	70%	81%	1			
	1.3464175	80%	80%	2	1.0	0.0	58.6
	0.6518573	90%	34%	3	1.0	0.0	58.6
	1.0968412	80%	70%	4	9.1	0.8	101.7
	1.3858432	70%	81%				
	6.0450244	30%	91%				
48 hours	1.6357184	71%	85%	1			
	1.3464175	86%	80%	2	na	na	na
	0.6518573	100%	34%	3	na	na	na
	1.0968412	86%	70%	4	na	na	na
	1.3858432	71%	81%				
	6.0450244	43%	91%				

sCr only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	1.3952268	80%	78%	1			
	1.3952268	80%	78%	2	na	na	na
	0.6626156	100%	38%	3	na	na	na
	1.1089146	80%	70%	4	na	na	na
	1.5692833	60%	80%				
	6.3358452	0%	90%				
24 hours	1.3952268	75%	78%	1			
	0.6626156	100%	38%	2	na	na	na
	0.6626156	100%	38%	3	na	na	na
	1.1089146	75%	70%	4	na	na	na
	1.5692833	50%	80%				
	6.3358452	0%	90%				
48 hours	0.3856638	100%	5%	1			
	0.3856638	100%	5%	2	0.0	0.0	na
	0.3856638	100%	5%	3	0.0	0.0	na
	1.1089146	67%	70%	4	2.0	0.1	41.8
	1.5692833	67%	80%				
	6.3358452	0%	90%				

UO only

Time prior AKI stage	Cutoff value	sens	spec	Quartile	OR	95% CI of OR	
0 hours	1.3464175	75%	77%	1			
	0.6599437	88%	32%	2	na	na	na
	0.6518573	100%	31%	3	na	na	na
	1.1033338	75%	70%	4	na	na	na
	1.5692833	63%	81%				
	4.5125034	25%	90%				
24 hours	1.3464175	75%	77%	1			
	0.6518573	88%	31%	2	1.0	0.0	60.2
	0.3856638	100%	4%	3	1.0	0.0	60.2
	1.1033338	75%	70%	4	6.1	0.5	78.3
	1.5692833	63%	81%				
	4.5125034	25%	90%				
48 hours	1.3464175	83%	77%	1			
	1.3464175	83%	77%	2	na	na	na
	0.6518573	100%	31%	3	na	na	na
	1.1033338	83%	70%	4	na	na	na
	1.5692833	67%	81%				
	4.5125034	33%	90%				

FIG. 8 - 7

SEQUENCE LISTING

<110> ASTUTE MEDICAL, INC.
<120> METHODS AND COMPOSITIONS FOR DIAGNOSIS AND PROGNOSIS OF RENAL INJURY AND RENAL FAILURE

<130> 152169

<140> AU 2010210537
<141> 2010-02-05

<150> 61/166,337
<151> 2009-04-03

<150> 61/162,404
<151> 2009-03-23

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Leu Glu Trp Lys Leu Asn Thr Gly Arg Thr Glu Ala Trp Lys Val Leu
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Ser Pro Gln Gly Gly Gly Pro Trp Asp Ser Val Ala Arg Val Leu Pro
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Tyr Arg Val Arg Val Tyr Gln Ile Pro Gly Lys Pro Glu Ile Val Asp
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Val Ser Glu Gly Ser Tyr Pro Ala Gly Thr Leu Ser Trp His Leu Asp
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Met Val Thr Pro Ala Arg Gly Gly Asp Pro Arg Pro Thr Phe Ser Cys
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Ser Phe Ser Pro Gly Leu Pro Arg His Arg Ala Leu Arg Thr Ala Pro
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Ile Gln Pro Arg Val Trp Glu Pro Val Pro Leu Glu Glu Val Gln Leu
225                 230                 235                 240

Val Val Glu Pro Glu Gly Gly Ala Val Ala Pro Gly Gly Thr Val Thr
                245                 250                 255

Leu Thr Cys Glu Val Pro Ala Gln Pro Ser Pro Gln Ile His Trp Met
            260                 265                 270

Lys Asp Gly Val Pro Leu Pro Leu Pro Pro Ser Pro Val Leu Ile Leu
        275                 280                 285

Pro Glu Ile Gly Pro Gln Asp Gln Gly Thr Tyr Ser Cys Val Ala Thr
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His Ser Ser His Gly Pro Gln Glu Ser Arg Ala Val Ser Ile Ser Ile
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Ile Glu Pro Gly Glu Glu Gly Pro Thr Ala Gly Ser Val Gly Gly Ser
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Pro Leu Val Leu Lys Cys Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg
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Leu Glu Trp Lys Leu Gly Gly Gly Pro Trp Asp Ser Val Ala Arg Val
    50                  55                  60

Leu Pro Asn Gly Ser Leu Phe Leu Pro Ala Val Gly Ile Gln Asp Glu
65                  70                  75                  80

Gly Ile Phe Arg Cys Gln Ala Met Asn Arg Asn Gly Lys Glu Thr Lys
                85                  90                  95

Ser Asn Tyr Arg Val Arg Val Tyr Gln Ile Pro Gly Lys Pro Glu Ile
            100                 105                 110

Val Asp Ser Ala Ser Glu Leu Thr Ala Gly Val Pro Asn Lys Val Gly
        115                 120                 125

Thr Cys Val Ser Glu Gly Ser Tyr Pro Ala Gly Thr Leu Ser Trp His
    130                 135                 140

Leu Asp Gly Lys Pro Leu Val Pro Asn Glu Lys Gly Val Ser Val Lys
145                 150                 155                 160

Glu Gln Thr Arg Arg His Pro Glu Thr Gly Leu Phe Thr Leu Gln Ser
                165                 170                 175

Glu Leu Met Val Thr Pro Ala Arg Gly Gly Asp Pro Arg Pro Thr Phe
            180                 185                 190

Ser Cys Ser Phe Ser Pro Gly Leu Pro Arg His Arg Ala Leu Arg Thr
        195                 200                 205

Ala Pro Ile Gln Pro Arg Val Trp Glu Pro Val Pro Leu Glu Glu Val
    210                 215                 220

Gln Leu Val Val Glu Pro Glu Gly Gly Ala Val Ala Pro Gly Gly Thr
225                 230                 235                 240

Val Thr Leu Thr Cys Glu Val Pro Ala Gln Pro Ser Pro Gln Ile His
                245                 250                 255

Trp Met Lys Asp Val Ser Asp Leu Glu Arg Gly Ala Gly Arg Thr Arg
            260                 265                 270

Arg Gly Gly Ala Asn Cys Arg Leu Cys Gly Arg Ile Arg Ala Gly Asn
        275                 280                 285

Ser Ser Pro Gly Pro Gly Asp Pro Gly Arg Pro Gly Asp Ser Arg Pro
    290                 295                 300

Ala His Trp Gly His Leu Val Ala Lys Ala Ala Thr Pro Arg Arg Gly
305                 310                 315                 320

Glu Glu Gly Pro Arg Lys Pro Gly Gly Arg Gly Gly Ala Cys Arg Thr
                325                 330                 335

Glu Ser Val Gly Gly Thr
            340

<210> 3
<211> 487
<212> PRT
<213> Homo sapiens

<400> 3
Met Arg Glu Asn Met Ala Arg Gly Pro Cys Asn Ala Pro Arg Trp Ala
1               5                   10                  15
```

```
Ser Leu Met Val Leu Val Ala Ile Gly Thr Ala Val Thr Ala Ala Val
            20                  25                  30

Asn Pro Gly Val Val Val Arg Ile Ser Gln Lys Gly Leu Asp Tyr Ala
        35                  40                  45

Ser Gln Gln Gly Thr Ala Ala Leu Gln Lys Glu Leu Lys Arg Ile Lys
    50                  55                  60

Ile Pro Asp Tyr Ser Asp Ser Phe Lys Ile Lys His Leu Gly Lys Gly
65                  70                  75                  80

His Tyr Ser Phe Tyr Ser Met Asp Ile Arg Glu Phe Gln Leu Pro Ser
                85                  90                  95

Ser Gln Ile Ser Met Val Pro Asn Val Gly Leu Lys Phe Ser Ile Ser
            100                 105                 110

Asn Ala Asn Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe
        115                 120                 125

Leu Lys Met Ser Gly Asn Phe Asp Leu Ser Ile Glu Gly Met Ser Ile
    130                 135                 140

Ser Ala Asp Leu Lys Leu Gly Ser Asn Pro Thr Ser Gly Lys Pro Thr
145                 150                 155                 160

Ile Thr Cys Ser Ser Cys Ser Ser His Ile Asn Ser Val His Val His
                165                 170                 175

Ile Ser Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
            180                 185                 190

Ile Glu Ser Ala Leu Arg Asn Lys Met Asn Ser Gln Val Cys Glu Lys
        195                 200                 205

Val Thr Asn Ser Val Ser Ser Glu Leu Gln Pro Tyr Phe Gln Thr Leu
    210                 215                 220

Pro Val Met Thr Lys Ile Asp Ser Val Ala Gly Ile Asn Tyr Gly Leu
225                 230                 235                 240

Val Ala Pro Pro Ala Thr Thr Ala Glu Thr Leu Asp Val Gln Met Lys
                245                 250                 255

Gly Glu Phe Tyr Ser Glu Asn His His Asn Pro Pro Pro Phe Ala Pro
            260                 265                 270

Pro Val Met Glu Phe Pro Ala Ala His Asp Arg Met Val Tyr Leu Gly
        275                 280                 285

Leu Ser Asp Tyr Phe Phe Asn Thr Ala Gly Leu Val Tyr Gln Glu Ala
    290                 295                 300

Gly Val Leu Lys Met Thr Leu Arg Asp Asp Met Ile Pro Lys Glu Ser
305                 310                 315                 320

Lys Phe Arg Leu Thr Thr Lys Phe Phe Gly Thr Phe Leu Pro Glu Val
                325                 330                 335

Ala Lys Lys Phe Pro Asn Met Lys Ile Gln Ile His Val Ser Ala Ser
            340                 345                 350
```

```
Thr Pro Pro His Leu Ser Val Gln Pro Thr Gly Leu Thr Phe Tyr Pro
        355                 360                 365

Ala Val Asp Val Gln Ala Phe Ala Val Leu Pro Asn Ser Ser Leu Ala
    370                 375                 380

Ser Leu Phe Leu Ile Gly Met His Thr Thr Gly Ser Met Glu Val Ser
385                 390                 395                 400

Ala Glu Ser Asn Arg Leu Val Gly Glu Leu Lys Leu Asp Arg Leu Leu
                405                 410                 415

Leu Glu Leu Lys His Ser Asn Ile Gly Pro Phe Pro Val Glu Leu Leu
            420                 425                 430

Gln Asp Ile Met Asn Tyr Ile Val Pro Ile Leu Val Leu Pro Arg Val
        435                 440                 445

Asn Glu Lys Leu Gln Lys Gly Phe Pro Leu Pro Thr Pro Ala Arg Val
    450                 455                 460

Gln Leu Tyr Asn Val Val Leu Gln Pro His Gln Asn Phe Leu Leu Phe
465                 470                 475                 480

Gly Ala Asp Val Val Tyr Lys
                485
```

<210> 4
<211> 219
<212> PRT
<213> Homo sapiens

<400> 4

```
Met Cys Pro Ala Arg Ser Leu Leu Leu Val Ala Thr Leu Val Leu Leu
1               5                   10                  15

Asp His Leu Ser Leu Ala Arg Asn Leu Pro Val Ala Thr Pro Asp Pro
            20                  25                  30

Gly Met Phe Pro Cys Leu His His Ser Gln Asn Leu Leu Arg Ala Val
        35                  40                  45

Ser Asn Met Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys
    50                  55                  60

Thr Ser Glu Glu Ile Asp His Glu Asp Ile Thr Lys Asp Lys Thr Ser
65                  70                  75                  80

Thr Val Glu Ala Cys Leu Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys
                85                  90                  95

Leu Asn Ser Arg Glu Thr Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala
            100                 105                 110

Ser Arg Lys Thr Ser Phe Met Met Ala Leu Cys Leu Ser Ser Ile Tyr
        115                 120                 125

Glu Asp Leu Lys Met Tyr Gln Val Glu Phe Lys Thr Met Asn Ala Lys
    130                 135                 140

Leu Leu Met Asp Pro Lys Arg Gln Ile Phe Leu Asp Gln Asn Met Leu
145                 150                 155                 160

Ala Val Ile Asp Glu Leu Met Gln Ala Leu Asn Phe Asn Ser Glu Thr
                165                 170                 175
```

```
Val Pro Gln Lys Ser Ser Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys
            180                 185                 190

Ile Lys Leu Cys Ile Leu Leu His Ala Phe Arg Ile Arg Ala Val Thr
        195                 200                 205

Ile Asp Arg Val Met Ser Tyr Leu Asn Ala Ser
    210                 215

<210> 5
<211> 328
<212> PRT
<213> Homo sapiens

<400> 5
Met Cys His Gln Gln Leu Val Ile Ser Trp Phe Ser Leu Val Phe Leu
1               5                   10                  15

Ala Ser Pro Leu Val Ala Ile Trp Glu Leu Lys Lys Asp Val Tyr Val
            20                  25                  30

Val Glu Leu Asp Trp Tyr Pro Asp Ala Pro Gly Glu Met Val Val Leu
        35                  40                  45

Thr Cys Asp Thr Pro Glu Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln
    50                  55                  60

Ser Ser Glu Val Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys
65                  70                  75                  80

Glu Phe Gly Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val
                85                  90                  95

Leu Ser His Ser Leu Leu Leu Leu His Lys Lys Glu Asp Gly Ile Trp
            100                 105                 110

Ser Thr Asp Ile Leu Lys Asp Gln Lys Glu Pro Lys Asn Lys Thr Phe
        115                 120                 125

Leu Arg Cys Glu Ala Lys Asn Tyr Ser Gly Arg Phe Thr Cys Trp Trp
    130                 135                 140

Leu Thr Thr Ile Ser Thr Asp Leu Thr Phe Ser Val Lys Ser Ser Arg
145                 150                 155                 160

Gly Ser Ser Asp Pro Gln Gly Val Thr Cys Gly Ala Ala Thr Leu Ser
                165                 170                 175

Ala Glu Arg Val Arg Gly Asp Asn Lys Glu Tyr Glu Tyr Ser Val Glu
            180                 185                 190

Cys Gln Glu Asp Ser Ala Cys Pro Ala Ala Glu Glu Ser Leu Pro Ile
        195                 200                 205

Glu Val Met Val Asp Ala Val His Lys Leu Lys Tyr Glu Asn Tyr Thr
    210                 215                 220

Ser Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn
225                 230                 235                 240

Leu Gln Leu Lys Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser Trp
                245                 250                 255
```

```
Glu Tyr Pro Asp Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Thr
            260                 265                 270

Phe Cys Val Gln Val Gln Gly Lys Ser Lys Arg Glu Lys Lys Asp Arg
        275                 280                 285

Val Phe Thr Asp Lys Thr Ser Ala Thr Val Ile Cys Arg Lys Asn Ala
    290                 295                 300

Ser Ile Ser Val Arg Ala Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser
305                 310                 315                 320

Glu Trp Ala Ser Val Pro Cys Ser
                325

<210> 6
<211> 251
<212> PRT
<213> Homo sapiens

<400> 6
Met Leu Gly Ala Arg Leu Arg Leu Trp Val Cys Ala Leu Cys Ser Val
1               5                   10                  15

Cys Ser Met Ser Val Leu Arg Ala Tyr Pro Asn Ala Ser Pro Leu Leu
            20                  25                  30

Gly Ser Ser Trp Gly Gly Leu Ile His Leu Tyr Thr Ala Thr Ala Arg
        35                  40                  45

Asn Ser Tyr His Leu Gln Ile His Lys Asn Gly His Val Asp Gly Ala
    50                  55                  60

Pro His Gln Thr Ile Tyr Ser Ala Leu Met Ile Arg Ser Glu Asp Ala
65                  70                  75                  80

Gly Phe Val Val Ile Thr Gly Val Met Ser Arg Arg Tyr Leu Cys Met
                85                  90                  95

Asp Phe Arg Gly Asn Ile Phe Gly Ser His Tyr Phe Asp Pro Glu Asn
            100                 105                 110

Cys Arg Phe Gln His Gln Thr Leu Glu Asn Gly Tyr Asp Val Tyr His
        115                 120                 125

Ser Pro Gln Tyr His Phe Leu Val Ser Leu Gly Arg Ala Lys Arg Ala
    130                 135                 140

Phe Leu Pro Gly Met Asn Pro Pro Pro Tyr Ser Gln Phe Leu Ser Arg
145                 150                 155                 160

Arg Asn Glu Ile Pro Leu Ile His Phe Asn Thr Pro Ile Pro Arg Arg
                165                 170                 175

His Thr Arg Ser Ala Glu Asp Asp Ser Glu Arg Asp Pro Leu Asn Val
            180                 185                 190

Leu Lys Pro Arg Ala Arg Met Thr Pro Ala Pro Ala Ser Cys Ser Gln
        195                 200                 205

Glu Leu Pro Ser Ala Glu Asp Asn Ser Pro Met Ala Ser Asp Pro Leu
    210                 215                 220

Gly Val Val Arg Gly Gly Arg Val Asn Thr His Ala Gly Gly Thr Gly
225                 230                 235                 240
```

```
Pro Glu Gly Cys Arg Pro Phe Ala Lys Phe Ile
                245                 250

<210> 7
<211> 132
<212> PRT
<213> Homo sapiens

<400> 7
Met Ala Phe Asp Ser Thr Trp Lys Val Asp Arg Ser Glu Asn Tyr Asp
1               5                   10                  15

Lys Phe Met Glu Lys Met Gly Val Asn Ile Val Lys Arg Lys Leu Ala
            20                  25                  30

Ala His Asp Asn Leu Lys Leu Thr Ile Thr Gln Glu Gly Asn Lys Phe
        35                  40                  45

Thr Val Lys Glu Ser Ser Ala Phe Arg Asn Ile Glu Val Val Phe Glu
    50                  55                  60

Leu Gly Val Thr Phe Asn Tyr Asn Leu Ala Asp Gly Thr Glu Leu Arg
65                  70                  75                  80

Gly Thr Trp Ser Leu Glu Gly Asn Lys Leu Ile Gly Lys Phe Lys Arg
                85                  90                  95

Thr Asp Asn Gly Asn Glu Leu Asn Thr Val Arg Glu Ile Ile Gly Asp
            100                 105                 110

Glu Leu Val Gln Thr Tyr Val Tyr Glu Gly Val Glu Ala Lys Arg Ile
        115                 120                 125

Phe Lys Lys Asp
    130

<210> 8
<211> 461
<212> PRT
<213> Homo sapiens

<400> 8
Met Trp Gln Leu Thr Ser Leu Leu Leu Phe Val Ala Thr Trp Gly Ile
1               5                   10                  15

Ser Gly Thr Pro Ala Pro Leu Asp Ser Val Phe Ser Ser Ser Glu Arg
            20                  25                  30

Ala His Gln Val Leu Arg Ile Arg Lys Arg Ala Asn Ser Phe Leu Glu
        35                  40                  45

Glu Leu Arg His Ser Ser Leu Glu Arg Glu Cys Ile Glu Glu Ile Cys
    50                  55                  60

Asp Phe Glu Glu Ala Lys Glu Ile Phe Gln Asn Val Asp Asp Thr Leu
65                  70                  75                  80

Ala Phe Trp Ser Lys His Val Asp Gly Asp Gln Cys Leu Val Leu Pro
                85                  90                  95

Leu Glu His Pro Cys Ala Ser Leu Cys Cys Gly His Gly Thr Cys Ile
            100                 105                 110
```

```
Asp Gly Ile Gly Ser Phe Ser Cys Asp Cys Arg Ser Gly Trp Glu Gly
        115                 120                 125

Arg Phe Cys Gln Arg Glu Val Ser Phe Leu Asn Cys Ser Leu Asp Asn
    130                 135                 140

Gly Gly Cys Thr His Tyr Cys Leu Glu Glu Val Gly Trp Arg Arg Cys
145                 150                 155                 160

Ser Cys Ala Pro Gly Tyr Lys Leu Gly Asp Asp Leu Leu Gln Cys His
                165                 170                 175

Pro Ala Val Lys Phe Pro Cys Gly Arg Pro Trp Lys Arg Met Glu Lys
            180                 185                 190

Lys Arg Ser His Leu Lys Arg Asp Thr Glu Asp Gln Glu Asp Gln Val
        195                 200                 205

Asp Pro Arg Leu Ile Asp Gly Lys Met Thr Arg Arg Gly Asp Ser Pro
    210                 215                 220

Trp Gln Val Val Leu Leu Asp Ser Lys Lys Lys Leu Ala Cys Gly Ala
225                 230                 235                 240

Val Leu Ile His Pro Ser Trp Val Leu Thr Ala Ala His Cys Met Asp
                245                 250                 255

Glu Ser Lys Lys Leu Leu Val Arg Leu Gly Glu Tyr Asp Leu Arg Arg
            260                 265                 270

Trp Glu Lys Trp Glu Leu Asp Leu Asp Ile Lys Glu Val Phe Val His
        275                 280                 285

Pro Asn Tyr Ser Lys Ser Thr Thr Asp Asn Asp Ile Ala Leu Leu His
    290                 295                 300

Leu Ala Gln Pro Ala Thr Leu Ser Gln Thr Ile Val Pro Ile Cys Leu
305                 310                 315                 320

Pro Asp Ser Gly Leu Ala Glu Arg Glu Leu Asn Gln Ala Gly Gln Glu
                325                 330                 335

Thr Leu Val Thr Gly Trp Gly Tyr His Ser Ser Arg Glu Lys Glu Ala
            340                 345                 350

Lys Arg Asn Arg Thr Phe Val Leu Asn Phe Ile Lys Ile Pro Val Val
        355                 360                 365

Pro His Asn Glu Cys Ser Glu Val Met Ser Asn Met Val Ser Glu Asn
    370                 375                 380

Met Leu Cys Ala Gly Ile Leu Gly Asp Arg Gln Asp Ala Cys Glu Gly
385                 390                 395                 400

Asp Ser Gly Gly Pro Met Val Ala Ser Phe His Gly Thr Trp Phe Leu
                405                 410                 415

Val Gly Leu Val Ser Trp Gly Glu Gly Cys Gly Leu Leu His Asn Tyr
            420                 425                 430

Gly Val Tyr Thr Lys Val Ser Arg Tyr Leu Asp Trp Ile His Gly His
        435                 440                 445

Ile Arg Asp Lys Glu Ala Pro Gln Lys Ser Trp Ala Pro
    450                 455                 460
```