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(54) Title: METHODS AND DEVICES FOR DETECTING GLOMERULONEPHRITIS AND ASSOCIATED DISORDERS

(57) Abstract: Methods and devices for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder in a mammal are described. In particular, methods and devices for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder using measured concentrations of a combination of three or more analytes in a test sample taken from the mammal are described.

METHODS AND DEVICES FOR DETECTING GLOMERULONEPHRITIS AND ASSOCIATED DISORDERS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority of US provisional application serial no. 61/327,389, filed April 23, 2010, and US provisional application serial no. 61/232,091, filed August 7, 2009, each of which is hereby incorporated by reference in its entirety and is related to U.S. Patent Application Nos. [Not Yet Assigned], entitled Methods and Devices for Detecting Obstructive Uropathy and Associated Disorders, Computer Methods and Devices for Detecting Kidney Damage, Methods and Devices for Detecting Kidney Damage, Devices for Detecting Renal Disorders, Methods and Devices for Detecting Kidney Transplant Rejection, Methods and Devices for Detecting Diabetic Nephropathy and Associated Disorders, Attorney Docket Nos. 060075- , filed on the same date as this application, the entire contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The invention encompasses methods and devices for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder in a mammal. In particular, the present invention provides methods and devices for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder using measured concentrations of a combination of three or more analytes in a test sample taken from the mammal.

BACKGROUND OF THE INVENTION

[0003] The urinary system, in particular the kidneys, perform several critical functions such as maintaining electrolyte balance and eliminating toxins from the bloodstream. In the human body, the pair of kidneys together process roughly 20% of the total cardiac output, amounting to about 1 L/min in a 70-kg adult male. Because compounds in circulation are concentrated in the kidney up to 1000-fold relative to the

plasma concentration, the kidney is especially vulnerable to injury due to exposure to toxic compounds.

[0004] Existing diagnostic tests such as BUN and serum creatine tests typically detect only advanced stages of kidney damage. Other diagnostic tests such as kidney tissue biopsies or CAT scans have the advantage of enhanced sensitivity to earlier stages of kidney damage, but these tests are also generally costly, slow, and/or invasive.

[0005] A need exists in the art for a fast, simple, reliable, and sensitive method of detecting glomerulonephritis or an associated disorder. In a clinical setting, the early detection of kidney damage would help medical practitioners to diagnose and treat kidney damage more quickly and effectively.

SUMMARY OF THE INVENTION

[0006] The present invention provides methods and devices for diagnosing, monitoring, or determining a renal disorder in a mammal. In particular, the present invention provides methods and devices for diagnosing, monitoring, or determining a renal disorder using measured concentrations of a combination of three or more analytes in a test sample taken from the mammal.

[0007] One aspect of the invention encompasses a method for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder in a mammal. The method typically comprises providing a test sample comprising a sample of bodily fluid taken from the mammal. Then, the method comprises determining a combination of sample concentrations for three or more sample analytes in the test sample, wherein the sample analytes are selected from the group consisting of alpha-1 microglobulin, beta-2 microglobulin, calbindin, clusterin, CTGF, creatinine, cystatin C, GST-alpha, KIM-1, microalbumin, NGAL, osteopontin, THP, TIMP-1, TFF-3, and VEGF. The combination of sample concentrations may be compared to a data set comprising at least one entry, wherein each entry of the data set comprises a list comprising three or more minimum diagnostic concentrations indicative of glomerulonephritis or an associated disorder. Each minimum diagnostic concentration comprises a maximum of a range of analyte concentrations for a healthy mammal. Next, the method comprises determining a

matching entry of the dataset in which all minimum diagnostic concentrations are less than the corresponding sample concentrations and identifying an indicated disorder comprising the particular disorder of the matching entry.

[0008] Another aspect of the invention encompasses a method for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder in a mammal. The method generally comprises providing a test sample comprising a sample of bodily fluid taken from the mammal. Then the method comprises determining the concentrations of three or more sample analytes in a panel of biomarkers in the test sample, wherein the sample analytes are selected from the group consisting of alpha-1 microglobulin, beta-2 microglobulin, calbindin, clusterin, CTGF, creatinine, cystatin C, GST-alpha, KIM-1, microalbumin, NGAL, osteopontin, THP, TIMP-1, TFF-3, and VEGF. Diagnostic analytes are identified in the test sample, wherein the diagnostic analytes are the sample analytes whose concentrations are statistically different from concentrations found in a control group of humans who do not suffer from glomerulonephritis or an associated disorder. The combination of diagnostic analytes is compared to a dataset comprising at least one entry, wherein each entry of the dataset comprises a combination of three or more diagnostic analytes reflective of glomerulonephritis or an associated disorder. The particular disorder having the combination of diagnostic analytes that essentially match the combination of sample analytes is then identified.

[0009] An additional aspect of the invention encompasses a method for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder in a mammal. The method usually comprises providing an analyte concentration measurement device comprising three or more detection antibodies. Each detection antibody comprises an antibody coupled to an indicator, wherein the antigenic determinants of the antibodies are sample analytes associated with glomerulonephritis or an associated disorder. The sample analytes are generally selected from the group consisting of alpha-1 microglobulin, beta-2 microglobulin, calbindin, clusterin, CTGF, creatinine, cystatin C, GST-alpha, KIM-1, microalbumin, NGAL, osteopontin, THP, TIMP-1, TFF-3, and VEGF. The method next comprises providing a test sample comprising three or more sample analytes and a bodily fluid taken from the mammal.

The test sample is contacted with the detection antibodies and the detection antibodies are allowed to bind to the sample analytes. The concentrations of the sample analytes are determined by detecting the indicators of the detection antibodies bound to the sample analytes in the test sample. The concentrations of each sample analyte correspond to a corresponding minimum diagnostic concentration reflective of glomerulonephritis or an associated disorder.

[0010] Other aspects and iterations of the invention are described in more detail below.

DESCRIPTION OF FIGURES

[0011] **FIG. 1** shows the four different disease groups from which samples were analyzed, and a plot of two different estimations on eGFR outlining the distribution within each group.

[0012] **FIG. 2** is a number of scatter plots of results on selected proteins in urine and plasma. The various groups are indicated as follows - control: blue, AA: red, DN: green, GN: yellow, OU: orange. **(A)** A1M in plasma, **(B)** cystatin C in plasma, **(C)** B2M in urine, **(D)** cystatin C in urine.

[0013] **FIG. 3** depicts the multivariate analysis of the disease groups and their respective matched controls using plasma results. Relative importance shown using the random forest model.

[0014] **FIG. 4** depicts three graphs showing the mean AUROC and its standard deviation **(A)** for plasma samples, and mean error rates **(B)** and mean AUROC **(C)** from urine samples for each classification method used to distinguish disease samples vs. normal samples. Disease encompasses analgesic abuse (AA), glomerulonephritis (GN), obstructive uropathy (OU), and diabetic nephropathy (DN). Normal = NL.

[0015] **FIG. 5** depicts three graphs showing the average importance of analytes and clinical variables from 100 bootstrap runs measured by random forest **(A and B)** or boosting **(C)** to distinguish disease (AA + GN + ON + DN) samples vs. normal samples from plasma **(A)** and urine **(B and C)**.

[0016] **FIG. 6** depicts three graphs showing the mean AUROC and its standard deviation (**A**) for plasma samples, and mean error rates (**B**) and mean AUROC (**C**) from urine samples for each classification method used to distinguish glomerulonephritis samples vs. normal samples. Abbreviations as in Figure 4.

[0017] **FIG. 7** depicts three graphs showing the average importance of analytes and clinical variables from 100 bootstrap runs measured by random forest (**A** and **B**) or boosting (**C**) to distinguish glomerulonephritis samples vs. normal samples from plasma (**A**) and urine (**B** and **C**).

[0018] **FIG. 8** depicts three graphs showing the mean AUROC and its standard deviation (**A**) for plasma samples, and mean error rates (**B**) and mean AUROC (**C**) from urine samples for each classification method used to distinguish analgesic abuse samples vs. glomerulonephritis samples. Abbreviations as in Figure 4.

[0019] **FIG. 9** depicts three graphs showing the average importance of analytes and clinical variables from 100 bootstrap runs measured by random forest (**A** and **B**) or boosting (**C**) to distinguish analgesic abuse samples vs. glomerulonephritis samples from plasma (**A**) and urine (**B** and **C**).

[0020] **FIG. 10** depicts three graphs showing the mean AUROC and its standard deviation (**A**) for plasma samples, and mean error rates (**B**) and mean AUROC (**C**) from urine samples for each classification method used to distinguish obstructive uropathy samples vs. glomerulonephritis samples. Abbreviations as in Figure 4.

[0021] **FIG. 11** depicts three graphs showing the average importance of analytes and clinical variables from 100 bootstrap runs measured by random forest (**A** and **B**) or boosting (**C**) to distinguish obstructive uropathy samples vs. glomerulonephritis samples from plasma (**A**) and urine (**B** and **C**).

[0022] **FIG. 12** depicts three graphs showing the mean AUROC and its standard deviation (**A**) for plasma samples, and mean error rates (**B**) and mean AUROC (**C**) from urine samples for each classification method used to distinguish diabetic nephropathy samples vs. glomerulonephritis samples. Abbreviations as in Figure 4.

[0023] **FIG. 13** depicts three graphs showing the average importance of analytes and clinical variables from 100 bootstrap runs measured by random forest (**A**

and **B**) or boosting (**C**) to distinguish diabetic nephropathy samples vs. glomerulonephritis samples from plasma (**A**) and urine (**B** and **C**).

DETAILED DESCRIPTION OF THE INVENTION

[0024] It has been discovered that a multiplexed panel of at least three, six, or preferably 16 biomarkers may be used to detect glomerulonephritis and associated disorders. As used herein, the term “glomerulonephritis” refers to a disorder characterized by inflammation of the glomeruli. The term may encompass chronic glomerulonephritis, acute glomerulonephritis, primary glomerulonephritis, or secondary glomerulonephritis. Additionally, the present invention encompasses biomarkers that may be used to detect a disorder associated with glomerulonephritis. As used herein, the phrase “a disorder associated with glomerulonephritis” refers to a disorder that stems from inflammation of the glomeruli. For instance, non-limiting examples of associated disorders may include nephritic syndrome, chronic kidney failure, and end-stage kidney disease.

[0025] The biomarkers included in a multiplexed panel of the invention are analytes known in the art that may be detected in the urine, serum, plasma and other bodily fluids of mammals. As such, the analytes of the multiplexed panel may be readily extracted from the mammal in a test sample of bodily fluid. The concentrations of the analytes within the test sample may be measured using known analytical techniques such as a multiplexed antibody-based immunological assay. The combination of concentrations of the analytes in the test sample may be compared to empirically determined combinations of minimum diagnostic concentrations and combinations of diagnostic concentration ranges associated with healthy kidney function or glomerulonephritis or an associated disorder to determine whether glomerulonephritis is indicated in the mammal.

[0026] One embodiment of the present invention provides a method for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder in a mammal that includes determining the presence or concentration of a combination of three or more sample analytes in a test sample containing the bodily fluid of the mammal. The measured concentrations of the combination of sample analytes is

compared to the entries of a dataset in which each entry contains the minimum diagnostic concentrations of a combination of three or more analytes reflective of glomerulonephritis or an associated disorder. Other embodiments provide computer-readable media encoded with applications containing executable modules, systems that include databases and processing devices containing executable modules configured to diagnose, monitor, or determine a renal disorder in a mammal. Still other embodiments provide antibody-based devices for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder in a mammal.

[0027] The analytes used as biomarkers in the multiplexed assay, methods of diagnosing, monitoring, or determining a renal disorder using measurements of the analytes, systems and applications used to analyze the multiplexed assay measurements, and antibody-based devices used to measure the analytes are described in detail below.

I. Analytes in Multiplexed Assay

[0028] One embodiment of the invention measures the concentrations of three, six, or preferably sixteen biomarker analytes within a test sample taken from a mammal and compares the measured analyte concentrations to minimum diagnostic concentrations to diagnose, monitor, or determine glomerulonephritis or an associated disorder in a mammal. In this aspect, the biomarker analytes are known in the art to occur in the urine, plasma, serum and other bodily fluids of mammals. The biomarker analytes are proteins that have known and documented associations with early renal damage in humans. As defined herein, the biomarker analytes include but are not limited to alpha-1 microglobulin, beta-2 microglobulin, calbindin, clusterin, CTGF, creatinine, cystatin C, GST-alpha, KIM-1, microalbumin, NGAL, osteopontin, THP, TIMP-1, TFF-3, and VEGF. A description of each biomarker analyte is given below.

(a) *Alpha-1 Microglobulin (A1M)*

[0029] Alpha-1 microglobulin (A1M, Swiss-Prot Accession Number P02760) is a 26 kDa glycoprotein synthesized by the liver and reabsorbed in the proximal tubules. Elevated levels of A1M in human urine are indicative of

glomerulotubular dysfunction. A1M is a member of the lipocalin super family and is found in all tissues. Alpha-1-microglobulin exists in blood in both a free form and complexed with immunoglobulin A (IgA) and heme. Half of plasma A1M exists in a free form, and the remainder exists in complexes with other molecules including prothrombin, albumin, immunoglobulin A and heme. Nearly all of the free A1M in human urine is reabsorbed by the megalin receptor in proximal tubular cells, where it is then catabolized. Small amounts of A1M are excreted in the urine of healthy humans. Increased A1M concentrations in human urine may be an early indicator of renal damage, primarily in the proximal tubule.

(b) *Beta-2 Microglobulin (B2M)*

[0030] Beta-2 microglobulin (B2M, Swiss-Prot Accession Number P61769) is a protein found on the surfaces of all nucleated cells and is shed into the blood, particularly by tumor cells and lymphocytes. Due to its small size, B2M passes through the glomerular membrane, but normally less than 1% is excreted due to reabsorption of B2M in the proximal tubules of the kidney. Therefore, high plasma levels of B2M occur as a result of renal failure, inflammation, and neoplasms, especially those associated with B-lymphocytes.

(c) *Calbindin*

[0031] Calbindin (Calbindin D-28K, Swiss-Prot Accession Number P05937) is a Ca-binding protein belonging to the troponin C superfamily. It is expressed in the kidney, pancreatic islets, and brain. Calbindin is found predominantly in subpopulations of central and peripheral nervous system neurons, in certain epithelial cells involved in Ca²⁺ transport such as distal tubular cells and cortical collecting tubules of the kidney, and in enteric neuroendocrine cells.

(d) *Clusterin*

[0032] Clusterin (Swiss-Prot Accession Number P10909) is a highly conserved protein that has been identified independently by many different laboratories and named SGP2, S35-S45, apolipoprotein J, SP-40, 40, ADHC-9, gp80, GPIII, and

testosterone-repressed prostate message (TRPM-2). An increase in clusterin levels has been consistently detected in apoptotic heart, brain, lung, liver, kidney, pancreas, and retinal tissue both *in vivo* and *in vitro*, establishing clusterin as a ubiquitous marker of apoptotic cell loss. However, clusterin protein has also been implicated in physiological processes that do not involve apoptosis, including the control of complement-mediated cell lysis, transport of beta-amyloid precursor protein, shuttling of aberrant beta-amyloid across the blood-brain barrier, lipid scavenging, membrane remodeling, cell aggregation, and protection from immune detection and tumor necrosis factor induced cell death.

(e) *Connective Tissue Growth Factor (CTGF)*

[0033] Connective tissue growth factor (CTGF, Swiss-Prot Accession Number P29279) is a 349–amino acid cysteine-rich polypeptide belonging to the CCN family. *In vitro* studies have shown that CTGF is mainly involved in extracellular matrix synthesis and fibrosis. Up-regulation of CTGF mRNA and increased CTGF levels have been observed in various diseases, including diabetic nephropathy and cardiomyopathy, fibrotic skin disorders, systemic sclerosis, biliary atresia, liver fibrosis and idiopathic pulmonary fibrosis, and nondiabetic acute and progressive glomerular and tubulointerstitial lesions of the kidney. A recent cross-sectional study found that urinary CTGF may act as a progression promoter in diabetic nephropathy.

(f) *Creatinine*

[0034] Creatinine is a metabolite of creatine phosphate in muscle tissue, and is typically produced at a relatively constant rate by the body. Creatinine is chiefly filtered out of the blood by the kidneys, though a small amount is actively secreted by the kidneys into the urine. Creatinine levels in blood and urine may be used to estimate the creatinine clearance, which is representative of the overall glomerular filtration rate (GFR), a standard measure of renal function. Variations in creatinine concentrations in the blood and urine, as well as variations in the ratio of urea to creatinine concentration in the blood, are common diagnostic measurements used to assess renal function.

(g) *Cystatin C (Cyst C)*

[0035] Cystatin C (Cyst C, Swiss-Prot Accession Number P01034) is a 13 kDa protein that is a potent inhibitor of the C1 family of cysteine proteases. It is the most abundant extracellular inhibitor of cysteine proteases in testis, epididymis, prostate, seminal vesicles and many other tissues. Cystatin C, which is normally expressed in vascular wall smooth muscle cells, is severely reduced in both atherosclerotic and aneurismal aortic lesions.

(h) *Glutathione S-Transferase alpha (GST-alpha)*

[0036] Glutathione S-transferase alpha (GST-alpha, Swiss-Prot Accession Number P08263) belongs to a family of enzymes that utilize glutathione in reactions contributing to the transformation of a wide range of compounds, including carcinogens, therapeutic drugs, and products of oxidative stress. These enzymes play a key role in the detoxification of such substances.

(i) *Kidney Injury Molecule-1 (KIM-1)*

[0037] Kidney injury molecule-1 (KIM-1, Swiss-Prot Accession Number Q96D42) is an immunoglobulin superfamily cell-surface protein highly upregulated on the surface of injured kidney epithelial cells. It is also known as TIM-1 (T-cell immunoglobulin mucin domain-1), as it is expressed at low levels by subpopulations of activated T-cells and hepatitis A virus cellular receptor-1 (HAVCR-1). KIM-1 is increased in expression more than any other protein in the injured kidney and is localized predominantly to the apical membrane of the surviving proximal epithelial cells.

(j) *Microalbumin*

[0038] Albumin is the most abundant plasma protein in humans and other mammals. Albumin is essential for maintaining the osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues. Healthy, normal kidneys typically filter out albumin from the urine. The presence of albumin in the urine may indicate damage to the kidneys. Albumin in the urine may also

occur in patients with long-standing diabetes, especially type 1 diabetes. The amount of albumin eliminated in the urine has been used to differentially diagnose various renal disorders. For example, nephrotic syndrome usually results in the excretion of about 3.0 to 3.5 grams of albumin in human urine every 24 hours. Microalbuminuria, in which less than 300mg of albumin is eliminated in the urine every 24 hours, may indicate the early stages of diabetic nephropathy.

(k) *Neutrophil Gelatinase-Associated Lipocalin (NGAL)*

[0039] Neutrophil gelatinase-associated lipocalin (NGAL, Swiss-Prot Accession Number P80188) forms a disulfide bond-linked heterodimer with MMP-9. It mediates an innate immune response to bacterial infection by sequestering iron. Lipocalins interact with many different molecules such as cell surface receptors and proteases, and play a role in a variety of processes such as the progression of cancer and allergic reactions.

(l) *Osteopontin (OPN)*

[0040] Osteopontin (OPN, Swiss-Prot Accession Number P10451) is a cytokine involved in enhancing production of interferon-gamma and IL-12, and inhibiting the production of IL-10. OPN is essential in the pathway that leads to type I immunity. OPN appears to form an integral part of the mineralized matrix. OPN is synthesized within the kidney and has been detected in human urine at levels that may effectively inhibit calcium oxalate crystallization. Decreased concentrations of OPN have been documented in urine from patients with renal stone disease compared with normal individuals.

(m) *Tamm-Horsfall Protein (THP)*

[0041] Tamm-Horsfall protein (THP, Swiss-Prot Accession Number P07911), also known as uromodulin, is the most abundant protein present in the urine of healthy subjects and has been shown to decrease in individuals with kidney stones. THP is secreted by the thick ascending limb of the loop of Henley. THP is a monomeric

glycoprotein of ~ 85 kDa with ~30% carbohydrate moiety that is heavily glycosylated. THP may act as a constitutive inhibitor of calcium crystallization in renal fluids.

(n) *Tissue Inhibitor of Metalloproteinase-1 (TIMP-1)*

[0042] Tissue inhibitor of metalloproteinase-1 (TIMP-1, Swiss-Prot Accession Number P01033) is a major regulator of extracellular matrix synthesis and degradation. A certain balance of MMPs and TIMPs is essential for tumor growth and health. Fibrosis results from an imbalance of fibrogenesis and fibrolysis, highlighting the importance of the role of the inhibition of matrix degradation role in renal disease.

(o) *Trefoil Factor 3 (TFF3)*

[0043] Trefoil factor 3 (TFF3, Swiss-Prot Accession Number Q07654), also known as intestinal trefoil factor, belongs to a small family of mucin-associated peptides that include TFF1, TFF2, and TFF3. TFF3 exists in a 60-amino acid monomeric form and a 118-amino acid dimeric form. Under normal conditions TFF3 is expressed by goblet cells of the intestine and the colon. TFF3 expression has also been observed in the human respiratory tract, in human goblet cells and in the human salivary gland. In addition, TFF3 has been detected in the human hypothalamus.

(p) *Vascular Endothelial Growth Factor (VEGF)*

[0044] Vascular endothelial growth factor (VEGF, Swiss-Prot Accession Number P15692) is an important factor in the pathophysiology of neuronal and other tumors, most likely functioning as a potent promoter of angiogenesis. VEGF may also be involved in regulating blood-brain-barrier functions under normal and pathological conditions. VEGF secreted from the stromal cells may be responsible for the endothelial cell proliferation observed in capillary hemangioblastomas, which are typically composed of abundant microvasculature and primitive angiogenic elements represented by stromal cells.

II. Combinations of Analytes Measured by Multiplexed Assay

[0045] The method for diagnosing, monitoring, or determining a renal disorder involves determining the presence or concentrations of a combination of sample analytes in a test sample. The combinations of sample analytes, as defined herein, are any group of three or more analytes selected from the biomarker analytes, including but not limited to alpha-1 microglobulin, beta-2 microglobulin, calbindin, clusterin, CTGF, creatinine, cystatin C, GST-alpha, KIM-1, microalbumin, NGAL, osteopontin, THP, TIMP-1, TFF-3, and VEGF. In one embodiment, the combination of analytes may be selected to provide a group of analytes associated with glomerulonephritis or an associated disorder.

[0046] In one embodiment, the combination of sample analytes may be any three of the biomarker analytes. In other embodiments, the combination of sample analytes may be any four, any five, any six, any seven, any eight, any nine, any ten, any eleven, any twelve, any thirteen, any fourteen, any fifteen, or all sixteen of the sixteen biomarker analytes. In some embodiments, the combination of sample analytes comprises alpha-1 microglobulin, beta-2 microglobulin, cystatin C, KIM-1, THP, and TIMP-1. In another embodiment, the combination of sample analytes may be a combination listed in **Table A**.

TABLE A

| | | |
|-----------------------|----------------------|--------------|
| alpha-1 microglobulin | beta-2 microglobulin | calbindin |
| alpha-1 microglobulin | beta-2 microglobulin | clusterin |
| alpha-1 microglobulin | beta-2 microglobulin | CTGF |
| alpha-1 microglobulin | beta-2 microglobulin | creatinine |
| alpha-1 microglobulin | beta-2 microglobulin | cystatin C |
| alpha-1 microglobulin | beta-2 microglobulin | GST-alpha |
| alpha-1 microglobulin | beta-2 microglobulin | KIM-1 |
| alpha-1 microglobulin | beta-2 microglobulin | microalbumin |
| alpha-1 microglobulin | beta-2 microglobulin | NGAL |
| alpha-1 microglobulin | beta-2 microglobulin | osteopontin |
| alpha-1 microglobulin | beta-2 microglobulin | THP |
| alpha-1 microglobulin | beta-2 microglobulin | TIMP-1 |
| alpha-1 microglobulin | beta-2 microglobulin | TFF-3 |
| alpha-1 microglobulin | beta-2 microglobulin | VEGF |

| | | |
|-----------------------|------------|--------------|
| alpha-1 microglobulin | calbindin | clusterin |
| alpha-1 microglobulin | calbindin | CTGF |
| alpha-1 microglobulin | calbindin | creatinine |
| alpha-1 microglobulin | calbindin | cystatin C |
| alpha-1 microglobulin | calbindin | GST-alpha |
| alpha-1 microglobulin | calbindin | KIM-1 |
| alpha-1 microglobulin | calbindin | microalbumin |
| alpha-1 microglobulin | calbindin | NGAL |
| alpha-1 microglobulin | calbindin | osteopontin |
| alpha-1 microglobulin | calbindin | THP |
| alpha-1 microglobulin | calbindin | TIMP-1 |
| alpha-1 microglobulin | calbindin | TFF-3 |
| alpha-1 microglobulin | calbindin | VEGF |
| alpha-1 microglobulin | clusterin | CTGF |
| alpha-1 microglobulin | clusterin | creatinine |
| alpha-1 microglobulin | clusterin | cystatin C |
| alpha-1 microglobulin | clusterin | GST-alpha |
| alpha-1 microglobulin | clusterin | KIM-1 |
| alpha-1 microglobulin | clusterin | microalbumin |
| alpha-1 microglobulin | clusterin | NGAL |
| alpha-1 microglobulin | clusterin | osteopontin |
| alpha-1 microglobulin | clusterin | THP |
| alpha-1 microglobulin | clusterin | TIMP-1 |
| alpha-1 microglobulin | clusterin | TFF-3 |
| alpha-1 microglobulin | clusterin | VEGF |
| alpha-1 microglobulin | CTGF | creatinine |
| alpha-1 microglobulin | CTGF | cystatin C |
| alpha-1 microglobulin | CTGF | GST-alpha |
| alpha-1 microglobulin | CTGF | KIM-1 |
| alpha-1 microglobulin | CTGF | microalbumin |
| alpha-1 microglobulin | CTGF | NGAL |
| alpha-1 microglobulin | CTGF | osteopontin |
| alpha-1 microglobulin | CTGF | THP |
| alpha-1 microglobulin | CTGF | TIMP-1 |
| alpha-1 microglobulin | CTGF | TFF-3 |
| alpha-1 microglobulin | CTGF | VEGF |
| alpha-1 microglobulin | creatinine | cystatin C |
| alpha-1 microglobulin | creatinine | GST-alpha |
| alpha-1 microglobulin | creatinine | KIM-1 |
| alpha-1 microglobulin | creatinine | microalbumin |
| alpha-1 microglobulin | creatinine | NGAL |
| alpha-1 microglobulin | creatinine | osteopontin |
| alpha-1 microglobulin | creatinine | THP |
| alpha-1 microglobulin | creatinine | TIMP-1 |
| alpha-1 microglobulin | creatinine | TFF-3 |
| alpha-1 microglobulin | creatinine | VEGF |
| alpha-1 microglobulin | cystatin C | GST-alpha |
| alpha-1 microglobulin | cystatin C | KIM-1 |

| | | |
|-----------------------|--------------|--------------|
| alpha-1 microglobulin | cystatin C | microalbumin |
| alpha-1 microglobulin | cystatin C | NGAL |
| alpha-1 microglobulin | cystatin C | osteopontin |
| alpha-1 microglobulin | cystatin C | THP |
| alpha-1 microglobulin | cystatin C | TIMP-1 |
| alpha-1 microglobulin | cystatin C | TFF-3 |
| alpha-1 microglobulin | cystatin C | VEGF |
| alpha-1 microglobulin | GST-alpha | KIM-1 |
| alpha-1 microglobulin | GST-alpha | microalbumin |
| alpha-1 microglobulin | GST-alpha | NGAL |
| alpha-1 microglobulin | GST-alpha | osteopontin |
| alpha-1 microglobulin | GST-alpha | THP |
| alpha-1 microglobulin | GST-alpha | TIMP-1 |
| alpha-1 microglobulin | GST-alpha | TFF-3 |
| alpha-1 microglobulin | GST-alpha | VEGF |
| alpha-1 microglobulin | KIM-1 | microalbumin |
| alpha-1 microglobulin | KIM-1 | NGAL |
| alpha-1 microglobulin | KIM-1 | osteopontin |
| alpha-1 microglobulin | KIM-1 | THP |
| alpha-1 microglobulin | KIM-1 | TIMP-1 |
| alpha-1 microglobulin | KIM-1 | TFF-3 |
| alpha-1 microglobulin | KIM-1 | VEGF |
| alpha-1 microglobulin | microalbumin | NGAL |
| alpha-1 microglobulin | microalbumin | osteopontin |
| alpha-1 microglobulin | microalbumin | THP |
| alpha-1 microglobulin | microalbumin | TIMP-1 |
| alpha-1 microglobulin | microalbumin | TFF-3 |
| alpha-1 microglobulin | microalbumin | VEGF |
| alpha-1 microglobulin | NGAL | osteopontin |
| alpha-1 microglobulin | NGAL | THP |
| alpha-1 microglobulin | NGAL | TIMP-1 |
| alpha-1 microglobulin | NGAL | TFF-3 |
| alpha-1 microglobulin | NGAL | VEGF |
| alpha-1 microglobulin | osteopontin | THP |
| alpha-1 microglobulin | osteopontin | TIMP-1 |
| alpha-1 microglobulin | osteopontin | TFF-3 |
| alpha-1 microglobulin | osteopontin | VEGF |
| alpha-1 microglobulin | THP | TIMP-1 |
| alpha-1 microglobulin | THP | TFF-3 |
| alpha-1 microglobulin | THP | VEGF |
| alpha-1 microglobulin | TIMP-1 | TFF-3 |
| alpha-1 microglobulin | TIMP-1 | VEGF |
| alpha-1 microglobulin | TFF-3 | VEGF |
| beta-2 microglobulin | calbindin | clusterin |
| beta-2 microglobulin | calbindin | CTGF |
| beta-2 microglobulin | calbindin | creatinine |
| beta-2 microglobulin | calbindin | cystatin C |
| beta-2 microglobulin | calbindin | GST-alpha |

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|----------------------|------------|--------------|
| beta-2 microglobulin | calbindin | KIM-1 |
| beta-2 microglobulin | calbindin | microalbumin |
| beta-2 microglobulin | calbindin | NGAL |
| beta-2 microglobulin | calbindin | osteopontin |
| beta-2 microglobulin | calbindin | THP |
| beta-2 microglobulin | calbindin | TIMP-1 |
| beta-2 microglobulin | calbindin | TFF-3 |
| beta-2 microglobulin | calbindin | VEGF |
| beta-2 microglobulin | clusterin | CTGF |
| beta-2 microglobulin | clusterin | creatinine |
| beta-2 microglobulin | clusterin | cystatin C |
| beta-2 microglobulin | clusterin | GST-alpha |
| beta-2 microglobulin | clusterin | KIM-1 |
| beta-2 microglobulin | clusterin | microalbumin |
| beta-2 microglobulin | clusterin | NGAL |
| beta-2 microglobulin | clusterin | osteopontin |
| beta-2 microglobulin | clusterin | THP |
| beta-2 microglobulin | clusterin | TIMP-1 |
| beta-2 microglobulin | clusterin | TFF-3 |
| beta-2 microglobulin | clusterin | VEGF |
| beta-2 microglobulin | CTGF | creatinine |
| beta-2 microglobulin | CTGF | cystatin C |
| beta-2 microglobulin | CTGF | GST-alpha |
| beta-2 microglobulin | CTGF | KIM-1 |
| beta-2 microglobulin | CTGF | microalbumin |
| beta-2 microglobulin | CTGF | NGAL |
| beta-2 microglobulin | CTGF | osteopontin |
| beta-2 microglobulin | CTGF | THP |
| beta-2 microglobulin | CTGF | TIMP-1 |
| beta-2 microglobulin | CTGF | TFF-3 |
| beta-2 microglobulin | CTGF | VEGF |
| beta-2 microglobulin | creatinine | cystatin C |
| beta-2 microglobulin | creatinine | GST-alpha |
| beta-2 microglobulin | creatinine | KIM-1 |
| beta-2 microglobulin | creatinine | microalbumin |
| beta-2 microglobulin | creatinine | NGAL |
| beta-2 microglobulin | creatinine | osteopontin |
| beta-2 microglobulin | creatinine | THP |
| beta-2 microglobulin | creatinine | TIMP-1 |
| beta-2 microglobulin | creatinine | TFF-3 |
| beta-2 microglobulin | creatinine | VEGF |
| beta-2 microglobulin | cystatin C | GST-alpha |
| beta-2 microglobulin | cystatin C | KIM-1 |
| beta-2 microglobulin | cystatin C | microalbumin |
| beta-2 microglobulin | cystatin C | NGAL |
| beta-2 microglobulin | cystatin C | osteopontin |
| beta-2 microglobulin | cystatin C | THP |
| beta-2 microglobulin | cystatin C | TIMP-1 |

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| beta-2 microglobulin | cystatin C | TFF-3 |
| beta-2 microglobulin | cystatin C | VEGF |
| beta-2 microglobulin | GST-alpha | KIM-1 |
| beta-2 microglobulin | GST-alpha | microalbumin |
| beta-2 microglobulin | GST-alpha | NGAL |
| beta-2 microglobulin | GST-alpha | osteopontin |
| beta-2 microglobulin | GST-alpha | THP |
| beta-2 microglobulin | GST-alpha | TIMP-1 |
| beta-2 microglobulin | GST-alpha | TFF-3 |
| beta-2 microglobulin | GST-alpha | VEGF |
| beta-2 microglobulin | KIM-1 | microalbumin |
| beta-2 microglobulin | KIM-1 | NGAL |
| beta-2 microglobulin | KIM-1 | osteopontin |
| beta-2 microglobulin | KIM-1 | THP |
| beta-2 microglobulin | KIM-1 | TIMP-1 |
| beta-2 microglobulin | KIM-1 | TFF-3 |
| beta-2 microglobulin | KIM-1 | VEGF |
| beta-2 microglobulin | microalbumin | NGAL |
| beta-2 microglobulin | microalbumin | osteopontin |
| beta-2 microglobulin | microalbumin | THP |
| beta-2 microglobulin | microalbumin | TIMP-1 |
| beta-2 microglobulin | microalbumin | TFF-3 |
| beta-2 microglobulin | microalbumin | VEGF |
| beta-2 microglobulin | NGAL | osteopontin |
| beta-2 microglobulin | NGAL | THP |
| beta-2 microglobulin | NGAL | TIMP-1 |
| beta-2 microglobulin | NGAL | TFF-3 |
| beta-2 microglobulin | NGAL | VEGF |
| beta-2 microglobulin | osteopontin | THP |
| beta-2 microglobulin | osteopontin | TIMP-1 |
| beta-2 microglobulin | osteopontin | TFF-3 |
| beta-2 microglobulin | osteopontin | VEGF |
| beta-2 microglobulin | THP | TIMP-1 |
| beta-2 microglobulin | THP | TFF-3 |
| beta-2 microglobulin | THP | VEGF |
| beta-2 microglobulin | TIMP-1 | TFF-3 |
| beta-2 microglobulin | TIMP-2 | VEGF |
| beta-2 microglobulin | TFF-3 | VEGF |
| calbindin | clusterin | CTGF |
| calbindin | clusterin | creatinine |
| calbindin | clusterin | cystatin C |
| calbindin | clusterin | GST-alpha |
| calbindin | clusterin | KIM-1 |
| calbindin | clusterin | microalbumin |
| calbindin | clusterin | NGAL |
| calbindin | clusterin | osteopontin |
| calbindin | clusterin | THP |
| calbindin | clusterin | TIMP-1 |

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| calbindin | clusterin | TFF-3 |
| calbindin | clusterin | VEGF |
| calbindin | CTGF | creatinine |
| calbindin | CTGF | cystatin C |
| calbindin | CTGF | GST-alpha |
| calbindin | CTGF | KIM-1 |
| calbindin | CTGF | microalbumin |
| calbindin | CTGF | NGAL |
| calbindin | CTGF | osteopontin |
| calbindin | CTGF | THP |
| calbindin | CTGF | TIMP-1 |
| calbindin | CTGF | TFF-3 |
| calbindin | CTGF | VEGF |
| calbindin | creatinine | cystatin C |
| calbindin | creatinine | GST-alpha |
| calbindin | creatinine | KIM-1 |
| calbindin | creatinine | microalbumin |
| calbindin | creatinine | NGAL |
| calbindin | creatinine | osteopontin |
| calbindin | creatinine | THP |
| calbindin | creatinine | TIMP-1 |
| calbindin | creatinine | TFF-3 |
| calbindin | creatinine | VEGF |
| calbindin | cystatin C | GST-alpha |
| calbindin | cystatin C | KIM-1 |
| calbindin | cystatin C | microalbumin |
| calbindin | cystatin C | NGAL |
| calbindin | cystatin C | osteopontin |
| calbindin | cystatin C | THP |
| calbindin | cystatin C | TIMP-1 |
| calbindin | cystatin C | TFF-3 |
| calbindin | cystatin C | VEGF |
| calbindin | GST-alpha | KIM-1 |
| calbindin | GST-alpha | microalbumin |
| calbindin | GST-alpha | NGAL |
| calbindin | GST-alpha | osteopontin |
| calbindin | GST-alpha | THP |
| calbindin | GST-alpha | TIMP-1 |
| calbindin | GST-alpha | TFF-3 |
| calbindin | GST-alpha | VEGF |
| calbindin | KIM-1 | microalbumin |
| calbindin | KIM-1 | NGAL |
| calbindin | KIM-1 | osteopontin |
| calbindin | KIM-1 | THP |
| calbindin | KIM-1 | TIMP-1 |
| calbindin | KIM-1 | TFF-3 |
| calbindin | KIM-1 | VEGF |
| calbindin | microalbumin | NGAL |

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| calbindin | microalbumin | osteopontin |
| calbindin | microalbumin | THP |
| calbindin | microalbumin | TIMP-1 |
| calbindin | microalbumin | TFF-3 |
| calbindin | microalbumin | VEGF |
| calbindin | NGAL | osteopontin |
| calbindin | NGAL | THP |
| calbindin | NGAL | TIMP-1 |
| calbindin | NGAL | TFF-3 |
| calbindin | NGAL | VEGF |
| calbindin | osteopontin | THP |
| calbindin | osteopontin | TIMP-1 |
| calbindin | osteopontin | TFF-3 |
| calbindin | osteopontin | VEGF |
| calbindin | THP | TIMP-1 |
| calbindin | THP | TFF-3 |
| calbindin | THP | VEGF |
| calbindin | TIMP-1 | TFF-3 |
| calbindin | TIMP-1 | VEGF |
| calbindin | TFF-3 | VEGF |
| clusterin | CTGF | creatinine |
| clusterin | CTGF | cystatin C |
| clusterin | CTGF | GST-alpha |
| clusterin | CTGF | KIM-1 |
| clusterin | CTGF | microalbumin |
| clusterin | CTGF | NGAL |
| clusterin | CTGF | osteopontin |
| clusterin | CTGF | THP |
| clusterin | CTGF | TIMP-1 |
| clusterin | CTGF | TFF-3 |
| clusterin | CTGF | VEGF |
| clusterin | creatinine | cystatin C |
| clusterin | creatinine | GST-alpha |
| clusterin | creatinine | KIM-1 |
| clusterin | creatinine | microalbumin |
| clusterin | creatinine | NGAL |
| clusterin | creatinine | osteopontin |
| clusterin | creatinine | THP |
| clusterin | creatinine | TIMP-1 |
| clusterin | creatinine | TFF-3 |
| clusterin | creatinine | VEGF |
| clusterin | cystatin C | GST-alpha |
| clusterin | cystatin C | KIM-1 |
| clusterin | cystatin C | microalbumin |
| clusterin | cystatin C | NGAL |
| clusterin | cystatin C | osteopontin |
| clusterin | cystatin C | THP |
| clusterin | cystatin C | TIMP-1 |

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| clusterin | cystatin C | TFF-3 |
| clusterin | cystatin C | VEGF |
| clusterin | GST-alpha | KIM-1 |
| clusterin | GST-alpha | microalbumin |
| clusterin | GST-alpha | NGAL |
| clusterin | GST-alpha | osteopontin |
| clusterin | GST-alpha | THP |
| clusterin | GST-alpha | TIMP-1 |
| clusterin | GST-alpha | TFF-3 |
| clusterin | GST-alpha | VEGF |
| clusterin | KIM-1 | microalbumin |
| clusterin | KIM-1 | NGAL |
| clusterin | KIM-1 | osteopontin |
| clusterin | KIM-1 | THP |
| clusterin | KIM-1 | TIMP-1 |
| clusterin | KIM-1 | TFF-3 |
| clusterin | KIM-1 | VEGF |
| clusterin | microalbumin | NGAL |
| clusterin | microalbumin | osteopontin |
| clusterin | microalbumin | THP |
| clusterin | microalbumin | TIMP-1 |
| clusterin | microalbumin | TFF-3 |
| clusterin | microalbumin | VEGF |
| clusterin | NGAL | osteopontin |
| clusterin | NGAL | THP |
| clusterin | NGAL | TIMP-1 |
| clusterin | NGAL | TFF-3 |
| clusterin | NGAL | VEGF |
| clusterin | osteopontin | THP |
| clusterin | osteopontin | TIMP-1 |
| clusterin | osteopontin | TFF-3 |
| clusterin | osteopontin | VEGF |
| clusterin | THP | TIMP-1 |
| clusterin | THP | TFF-3 |
| clusterin | THP | VEGF |
| clusterin | TIMP-1 | TFF-3 |
| clusterin | TIMP-1 | VEGF |
| clusterin | TFF-3 | VEGF |
| CTGF | creatinine | cystatin C |
| CTGF | creatinine | GST-alpha |
| CTGF | creatinine | KIM-1 |
| CTGF | creatinine | microalbumin |
| CTGF | creatinine | NGAL |
| CTGF | creatinine | osteopontin |
| CTGF | creatinine | THP |
| CTGF | creatinine | TIMP-1 |
| CTGF | creatinine | TFF-3 |
| CTGF | creatinine | VEGF |

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| CTGF | cystatin C | GST-alpha |
| CTGF | cystatin C | KIM-1 |
| CTGF | cystatin C | microalbumin |
| CTGF | cystatin C | NGAL |
| CTGF | cystatin C | osteopontin |
| CTGF | cystatin C | THP |
| CTGF | cystatin C | TIMP-1 |
| CTGF | cystatin C | TFF-3 |
| CTGF | cystatin C | VEGF |
| CTGF | GST-alpha | KIM-1 |
| CTGF | GST-alpha | microalbumin |
| CTGF | GST-alpha | NGAL |
| CTGF | GST-alpha | osteopontin |
| CTGF | GST-alpha | THP |
| CTGF | GST-alpha | TIMP-1 |
| CTGF | GST-alpha | TFF-3 |
| CTGF | GST-alpha | VEGF |
| CTGF | KIM-1 | microalbumin |
| CTGF | KIM-1 | NGAL |
| CTGF | KIM-1 | osteopontin |
| CTGF | KIM-1 | THP |
| CTGF | KIM-1 | TIMP-1 |
| CTGF | KIM-1 | TFF-3 |
| CTGF | KIM-1 | VEGF |
| CTGF | microalbumin | NGAL |
| CTGF | microalbumin | osteopontin |
| CTGF | microalbumin | THP |
| CTGF | microalbumin | TIMP-1 |
| CTGF | microalbumin | TFF-3 |
| CTGF | microalbumin | VEGF |
| CTGF | NGAL | osteopontin |
| CTGF | NGAL | THP |
| CTGF | NGAL | TIMP-1 |
| CTGF | NGAL | TFF-3 |
| CTGF | NGAL | VEGF |
| CTGF | osteopontin | THP |
| CTGF | osteopontin | TIMP-1 |
| CTGF | osteopontin | TFF-3 |
| CTGF | osteopontin | VEGF |
| CTGF | THP | TIMP-1 |
| CTGF | THP | TFF-3 |
| CTGF | THP | VEGF |
| CTGF | TIMP-1 | TFF-3 |
| CTGF | TIMP-1 | VEGF |
| CTGF | TFF-3 | VEGF |
| creatinine | cystatin C | GST-alpha |
| creatinine | cystatin C | KIM-1 |
| creatinine | cystatin C | microalbumin |

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| creatinine | cystatin C | NGAL |
| creatinine | cystatin C | osteopontin |
| creatinine | cystatin C | THP |
| creatinine | cystatin C | TIMP-1 |
| creatinine | cystatin C | TFF-3 |
| creatinine | cystatin C | VEGF |
| creatinine | GST-alpha | KIM-1 |
| creatinine | GST-alpha | microalbumin |
| creatinine | GST-alpha | NGAL |
| creatinine | GST-alpha | osteopontin |
| creatinine | GST-alpha | THP |
| creatinine | GST-alpha | TIMP-1 |
| creatinine | GST-alpha | TFF-3 |
| creatinine | GST-alpha | VEGF |
| creatinine | KIM-1 | microalbumin |
| creatinine | KIM-1 | NGAL |
| creatinine | KIM-1 | osteopontin |
| creatinine | KIM-1 | THP |
| creatinine | KIM-1 | TIMP-1 |
| creatinine | KIM-1 | TFF-3 |
| creatinine | KIM-1 | VEGF |
| creatinine | microalbumin | NGAL |
| creatinine | microalbumin | osteopontin |
| creatinine | microalbumin | THP |
| creatinine | microalbumin | TIMP-1 |
| creatinine | microalbumin | TFF-3 |
| creatinine | microalbumin | VEGF |
| creatinine | NGAL | osteopontin |
| creatinine | NGAL | THP |
| creatinine | NGAL | TIMP-1 |
| creatinine | NGAL | TFF-3 |
| creatinine | NGAL | VEGF |
| creatinine | osteopontin | THP |
| creatinine | osteopontin | TIMP-1 |
| creatinine | osteopontin | TFF-3 |
| creatinine | osteopontin | VEGF |
| creatinine | THP | TIMP-1 |
| creatinine | THP | TFF-3 |
| creatinine | THP | VEGF |
| creatinine | TIMP-1 | TFF-3 |
| creatinine | TIMP-1 | VEGF |
| creatinine | TFF-3 | VEGF |
| cystatin C | GST-alpha | KIM-1 |
| cystatin C | GST-alpha | microalbumin |
| cystatin C | GST-alpha | NGAL |
| cystatin C | GST-alpha | osteopontin |
| cystatin C | GST-alpha | THP |
| cystatin C | GST-alpha | TIMP-1 |

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| cystatin C | GST-alpha | TFF-3 |
| cystatin C | GST-alpha | VEGF |
| cystatin C | KIM-1 | microalbumin |
| cystatin C | KIM-1 | NGAL |
| cystatin C | KIM-1 | osteopontin |
| cystatin C | KIM-1 | THP |
| cystatin C | KIM-1 | TIMP-1 |
| cystatin C | KIM-1 | TFF-3 |
| cystatin C | KIM-1 | VEGF |
| cystatin C | microalbumin | NGAL |
| cystatin C | microalbumin | osteopontin |
| cystatin C | microalbumin | THP |
| cystatin C | microalbumin | TIMP-1 |
| cystatin C | microalbumin | TFF-3 |
| cystatin C | microalbumin | VEGF |
| cystatin C | NGAL | osteopontin |
| cystatin C | NGAL | THP |
| cystatin C | NGAL | TIMP-1 |
| cystatin C | NGAL | TFF-3 |
| cystatin C | NGAL | VEGF |
| cystatin C | osteopontin | THP |
| cystatin C | osteopontin | TIMP-1 |
| cystatin C | osteopontin | TFF-3 |
| cystatin C | osteopontin | VEGF |
| cystatin C | THP | TIMP-1 |
| cystatin C | THP | TFF-3 |
| cystatin C | THP | VEGF |
| cystatin C | TIMP-1 | TFF-3 |
| cystatin C | TIMP-1 | VEGF |
| cystatin C | TFF-3 | VEGF |
| GST-alpha | KIM-1 | microalbumin |
| GST-alpha | KIM-1 | NGAL |
| GST-alpha | KIM-1 | osteopontin |
| GST-alpha | KIM-1 | THP |
| GST-alpha | KIM-1 | TIMP-1 |
| GST-alpha | KIM-1 | TFF-3 |
| GST-alpha | KIM-1 | VEGF |
| GST-alpha | microalbumin | NGAL |
| GST-alpha | microalbumin | osteopontin |
| GST-alpha | microalbumin | THP |
| GST-alpha | microalbumin | TIMP-1 |
| GST-alpha | microalbumin | TFF-3 |
| GST-alpha | microalbumin | VEGF |
| GST-alpha | NGAL | osteopontin |
| GST-alpha | NGAL | THP |
| GST-alpha | NGAL | TIMP-1 |
| GST-alpha | NGAL | TFF-3 |
| GST-alpha | NGAL | VEGF |

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|--------------|--------------|-------------|
| GST-alpha | osteopontin | THP |
| GST-alpha | osteopontin | TIMP-1 |
| GST-alpha | osteopontin | TFF-3 |
| GST-alpha | osteopontin | VEGF |
| GST-alpha | THP | TIMP-1 |
| GST-alpha | THP | TFF-3 |
| GST-alpha | THP | VEGF |
| GST-alpha | TIMP-1 | TFF-3 |
| GST-alpha | TIMP-1 | VEGF |
| GST-alpha | TFF-3 | VEGF |
| KIM-1 | microalbumin | NGAL |
| KIM-1 | microalbumin | osteopontin |
| KIM-1 | microalbumin | THP |
| KIM-1 | microalbumin | TIMP-1 |
| KIM-1 | microalbumin | TFF-3 |
| KIM-1 | microalbumin | VEGF |
| KIM-1 | NGAL | osteopontin |
| KIM-1 | NGAL | THP |
| KIM-1 | NGAL | TIMP-1 |
| KIM-1 | NGAL | TFF-3 |
| KIM-1 | NGAL | VEGF |
| KIM-1 | osteopontin | THP |
| KIM-1 | osteopontin | TIMP-1 |
| KIM-1 | osteopontin | TFF-3 |
| KIM-1 | osteopontin | VEGF |
| KIM-1 | THP | TIMP-1 |
| KIM-1 | THP | TFF-3 |
| KIM-1 | THP | VEGF |
| KIM-1 | TIMP-1 | TFF-3 |
| KIM-1 | TIMP-1 | VEGF |
| KIM-1 | TFF-3 | VEGF |
| microalbumin | NGAL | osteopontin |
| microalbumin | NGAL | THP |
| microalbumin | NGAL | TIMP-1 |
| microalbumin | NGAL | TFF-3 |
| microalbumin | NGAL | VEGF |
| microalbumin | osteopontin | THP |
| microalbumin | osteopontin | TIMP-1 |
| microalbumin | osteopontin | TFF-3 |
| microalbumin | osteopontin | VEGF |
| microalbumin | THP | TIMP-1 |
| microalbumin | THP | TFF-3 |
| microalbumin | THP | VEGF |
| microalbumin | TIMP-1 | TFF-3 |
| microalbumin | TIMP-1 | VEGF |
| microalbumin | TFF-3 | VEGF |
| NGAL | osteopontin | THP |
| NGAL | osteopontin | TIMP-1 |

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|-------------|-------------|--------|
| NGAL | osteopontin | TFF-3 |
| NGAL | osteopontin | VEGF |
| NGAL | THP | TIMP-1 |
| NGAL | THP | TFF-3 |
| NGAL | THP | VEGF |
| NGAL | TIMP-1 | TFF-3 |
| NGAL | TIMP-1 | VEGF |
| NGAL | TFF-3 | VEGF |
| osteopontin | THP | TIMP-1 |
| osteopontin | THP | TFF-3 |
| osteopontin | THP | VEGF |
| osteopontin | TIMP-1 | TFF-3 |
| osteopontin | TIMP-1 | VEGF |
| osteopontin | TFF-3 | VEGF |
| THP | TIMP-1 | TFF-3 |
| THP | TIMP-1 | VEGF |
| THP | TFF-3 | VEGF |
| TIMP-1 | TFF-3 | VEGF |

[0047] In one exemplary embodiment, the combination of sample analytes may include creatinine, KIM-1, and THP. In another exemplary embodiment, the combination of sample analytes may include microalbumin, creatinine, and KIM-1. In yet another exemplary embodiment, the combination of sample analytes may include creatinine, KIM-1, and TIMP-1. In still another exemplary embodiment, the combination of sample analytes may include microalbumin, cystatin C, and creatinine. In an alternative exemplary embodiment, the combination of sample analytes may include creatinine, KIM-1, TIMP-1, A1M, THP, and osteopontin.

III. Test Sample

[0048] The method for diagnosing, monitoring, or determining a renal disorder involves determining the presence of sample analytes in a test sample. A test sample, as defined herein, is an amount of bodily fluid taken from a mammal. Non-limiting examples of bodily fluids include urine, blood, plasma, serum, saliva, semen, perspiration, tears, mucus, and tissue lysates. In an exemplary embodiment, the bodily fluid contained in the test sample is urine, plasma, or serum.

(a) Mammals

[0049] A mammal, as defined herein, is any organism that is a member of the class Mammalia. Non-limiting examples of mammals appropriate for the various embodiments may include humans, apes, monkeys, rats, mice, dogs, cats, pigs, and livestock including cattle and oxen. In an exemplary embodiment, the mammal is a human.

(b) *Devices and Methods of Taking Bodily Fluids from Mammals*

[0050] The bodily fluids of the test sample may be taken from the mammal using any known device or method so long as the analytes to be measured by the multiplexed assay are not rendered undetectable by the multiplexed assay. Non-limiting examples of devices or methods suitable for taking bodily fluid from a mammal include urine sample cups, urethral catheters, swabs, hypodermic needles, thin needle biopsies, hollow needle biopsies, punch biopsies, metabolic cages, and aspiration.

[0051] In order to adjust the expected concentrations of the sample analytes in the test sample to fall within the dynamic range of the multiplexed assay, the test sample may be diluted to reduce the concentration of the sample analytes prior to analysis. The degree of dilution may depend on a variety of factors including but not limited to the type of multiplexed assay used to measure the analytes, the reagents utilized in the multiplexed assay, and the type of bodily fluid contained in the test sample. In one embodiment, the test sample is diluted by adding a volume of diluent ranging from about $\frac{1}{2}$ of the original test sample volume to about 50,000 times the original test sample volume.

[0052] In one exemplary embodiment, if the test sample is human urine and the multiplexed assay is an antibody-based capture-sandwich assay, the test sample is diluted by adding a volume of diluent that is about 100 times the original test sample volume prior to analysis. In another exemplary embodiment, if the test sample is human serum and the multiplexed assay is an antibody-based capture-sandwich assay, the test sample is diluted by adding a volume of diluent that is about 5 times the original test sample volume prior to analysis. In yet another exemplary embodiment, if the test sample is human plasma and the multiplexed assay is an antibody-based capture-

sandwich assay, the test sample is diluted by adding a volume of diluent that is about 2,000 times the original test sample volume prior to analysis.

[0053] The diluent may be any fluid that does not interfere with the function of the multiplexed assay used to measure the concentration of the analytes in the test sample. Non-limiting examples of suitable diluents include deionized water, distilled water, saline solution, Ringer's solution, phosphate buffered saline solution, TRIS-buffered saline solution, standard saline citrate, and HEPES-buffered saline.

IV. Multiplexed Assay Device

[0054] In one embodiment, the concentration of a combination of sample analytes is measured using a multiplexed assay device capable of measuring the concentrations of up to sixteen of the biomarker analytes. A multiplexed assay device, as defined herein, is an assay capable of simultaneously determining the concentration of three or more different sample analytes using a single device and/or method. Any known method of measuring the concentration of the biomarker analytes may be used for the multiplexed assay device. Non-limiting examples of measurement methods suitable for the multiplexed assay device may include electrophoresis, mass spectrometry, protein microarrays, surface plasmon resonance and immunoassays including but not limited to western blot, immunohistochemical staining, enzyme-linked immunosorbent assay (ELISA) methods, and particle-based capture-sandwich immunoassays.

(a) Multiplexed Immunoassay Device

[0055] In one embodiment, the concentrations of the analytes in the test sample are measured using a multiplexed immunoassay device that utilizes capture antibodies marked with indicators to determine the concentration of the sample analytes.

(i) capture antibodies

[0056] In the same embodiment, the multiplexed immunoassay device includes three or more capture antibodies. Capture antibodies, as defined herein, are

antibodies in which the antigenic determinant is one of the biomarker analytes. Each of the at least three capture antibodies has a unique antigenic determinant that is one of the biomarker analytes. When contacted with the test sample, the capture antibodies form antigen-antibody complexes in which the analytes serve as antigens.

[0057] The term “antibody,” as used herein, encompasses a monoclonal ab, an antibody fragment, a chimeric antibody, and a single-chain antibody.

[0058] In some embodiments, the capture antibodies may be attached to a substrate in order to immobilize any analytes captured by the capture antibodies. Non-limiting examples of suitable substrates include paper, cellulose, glass, or plastic strips, beads, or surfaces, such as the inner surface of the well of a microtitration tray. Suitable beads may include polystyrene or latex microspheres.

(ii) indicators

[0059] In one embodiment of the multiplexed immunoassay device, an indicator is attached to each of the three or more capture antibodies. The indicator, as defined herein, is any compound that registers a measurable change to indicate the presence of one of the sample analytes when bound to one of the capture antibodies. Non-limiting examples of indicators include visual indicators and electrochemical indicators.

[0060] Visual indicators, as defined herein, are compounds that register a change by reflecting a limited subset of the wavelengths of light illuminating the indicator, by fluorescing light after being illuminated, or by emitting light via chemiluminescence. The change registered by visual indicators may be in the visible light spectrum, in the infrared spectrum, or in the ultraviolet spectrum. Non-limiting examples of visual indicators suitable for the multiplexed immunoassay device include nanoparticulate gold, organic particles such as polyurethane or latex microspheres loaded with dye compounds, carbon black, fluorophores, phycoerythrin, radioactive isotopes, nanoparticles, quantum dots, and enzymes such as horseradish peroxidase or alkaline phosphatase that react with a chemical substrate to form a colored or chemiluminescent product.

[0061] Electrochemical indicators, as defined herein, are compounds that register a change by altering an electrical property. The changes registered by electrochemical indicators may be an alteration in conductivity, resistance, capacitance, current conducted in response to an applied voltage, or voltage required to achieve a desired current. Non-limiting examples of electrochemical indicators include redox species such as ascorbate (vitamin C), vitamin E, glutathione, polyphenols, catechols, quercetin, phytoestrogens, penicillin, carbazole, murrans, phenols, carbonyls, benzoates, and trace metal ions such as nickel, copper, cadmium, iron and mercury.

[0062] In this same embodiment, the test sample containing a combination of three or more sample analytes is contacted with the capture antibodies and allowed to form antigen-antibody complexes in which the sample analytes serve as the antigens. After removing any uncomplexed capture antibodies, the concentrations of the three or more analytes are determined by measuring the change registered by the indicators attached to the capture antibodies.

[0063] In one exemplary embodiment, the indicators are polyurethane or latex microspheres loaded with dye compounds and phycoerythrin.

(b) Multiplexed Sandwich Immunoassay Device

[0064] In another embodiment, the multiplexed immunoassay device has a sandwich assay format. In this embodiment, the multiplexed sandwich immunoassay device includes three or more capture antibodies as previously described. However, in this embodiment, each of the capture antibodies is attached to a capture agent that includes an antigenic moiety. The antigenic moiety serves as the antigenic determinant of a detection antibody, also included in the multiplexed immunoassay device of this embodiment. In addition, an indicator is attached to the detection antibody.

[0065] In this same embodiment, the test sample is contacted with the capture antibodies and allowed to form antigen-antibody complexes in which the sample analytes serve as antigens. The detection antibodies are then contacted with the test sample and allowed to form antigen-antibody complexes in which the capture agent serves as the antigen for the detection antibody. After removing any uncomplexed

detection antibodies the concentration of the analytes are determined by measuring the changes registered by the indicators attached to the detection antibodies.

(c) Multiplexing Approaches

[0066] In the various embodiments of the multiplexed immunoassay devices, the concentrations of each of the sample analytes may be determined using any approach known in the art. In one embodiment, a single indicator compound is attached to each of the three or more antibodies. In addition, each of the capture antibodies having one of the sample analytes as an antigenic determinant is physically separated into a distinct region so that the concentration of each of the sample analytes may be determined by measuring the changes registered by the indicators in each physically separate region corresponding to each of the sample analytes.

[0067] In another embodiment, each antibody having one of the sample analytes as an antigenic determinant is marked with a unique indicator. In this manner, a unique indicator is attached to each antibody having a single sample analyte as its antigenic determinant. In this embodiment, all antibodies may occupy the same physical space. The concentration of each sample analyte is determined by measuring the change registered by the unique indicator attached to the antibody having the sample analyte as an antigenic determinant.

(d) Microsphere-Based Capture-Sandwich Immunoassay Device

[0068] In an exemplary embodiment, the multiplexed immunoassay device is a microsphere-based capture-sandwich immunoassay device. In this embodiment, the device includes a mixture of three or more capture-antibody microspheres, in which each capture-antibody microsphere corresponds to one of the biomarker analytes. Each capture-antibody microsphere includes a plurality of capture antibodies attached to the outer surface of the microsphere. In this same embodiment, the antigenic determinant of all of the capture antibodies attached to one microsphere is the same biomarker analyte.

[0069] In this embodiment of the device, the microsphere is a small polystyrene or latex sphere that is loaded with an indicator that is a dye compound. In

some embodiments, the microsphere may be between about 3 μm and about 5 μm in diameter. Each capture-antibody microsphere corresponding to one of the biomarker analytes is loaded with the same indicator. In this manner, each capture-antibody microsphere corresponding to a biomarker analyte is uniquely color-coded.

[0070] In this same exemplary embodiment, the multiplexed immunoassay device further includes three or more biotinylated detection antibodies in which the antigenic determinant of each biotinylated detection antibody is one of the biomarker analytes. The device further includes a plurality of streptavidin proteins complexed with a reporter compound. A reporter compound, as defined herein, is an indicator selected to register a change that is distinguishable from the indicators used to mark the capture-antibody microspheres.

[0071] The concentrations of the sample analytes may be determined by contacting the test sample with a mixture of capture-antigen microspheres corresponding to each sample analyte to be measured. The sample analytes are allowed to form antigen-antibody complexes in which a sample analyte serves as an antigen and a capture antibody attached to the microsphere serves as an antibody. In this manner, the sample analytes are immobilized onto the capture-antigen microspheres. The biotinylated detection antibodies are then added to the test sample and allowed to form antigen-antibody complexes in which the analyte serves as the antigen and the biotinylated detection antibody serves as the antibody. The streptavidin-reporter complex is then added to the test sample and allowed to bind to the biotin moieties of the biotinylated detection antibodies. The antigen-capture microspheres may then be rinsed and filtered.

[0072] In this embodiment, the concentration of each analyte is determined by first measuring the change registered by the indicator compound embedded in the capture-antigen microsphere in order to identify the particular analyte. For each microsphere corresponding to one of the biomarker analytes, the quantity of analyte immobilized on the microsphere is determined by measuring the change registered by the reporter compound attached to the microsphere.

[0073] For example, the indicator embedded in the microspheres associated with one sample analyte may register an emission of orange light, and the

reporter may register an emission of green light. In this example, a detector device may measure the intensity of orange light and green light separately. The measured intensity of the green light would determine the concentration of the analyte captured on the microsphere, and the intensity of the orange light would determine the specific analyte captured on the microsphere.

[0074] Any sensor device may be used to detect the changes registered by the indicators embedded in the microspheres and the changes registered by the reporter compound, so long as the sensor device is sufficiently sensitive to the changes registered by both indicator and reporter compound. Non-limiting examples of suitable sensor devices include spectrophotometers, photosensors, colorimeters, cyclic coulometry devices, and flow cytometers. In an exemplary embodiment, the sensor device is a flow cytometer.

V. Method for Diagnosing, Monitoring, or Determining a Renal Disorder

[0075] In one embodiment, a method is provided for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder that includes providing a test sample, determining the concentration of a combination of three or more sample analytes, comparing the measured concentrations of the combination of sample analytes to the entries of a dataset, and identifying glomerulonephritis or an associated disorder based on the comparison between the concentrations of the sample analytes and the minimum diagnostic concentrations contained within each entry of the dataset.

(a) Diagnostic Dataset

[0076] In an embodiment, the concentrations of the sample analytes are compared to the entries of a dataset. In this embodiment, each entry of the dataset includes a combination of three or more minimum diagnostic concentrations indicative of a particular renal disorder. A minimum diagnostic concentration, as defined herein, is the concentration of an analyte that defines the limit between the concentration range corresponding to normal, healthy renal function and the concentration reflective of a particular renal disorder. In one embodiment, each minimum diagnostic concentration is

the maximum concentration of the range of analyte concentrations for a healthy, normal individual. The minimum diagnostic concentration of an analyte depends on a number of factors including but not limited to the particular analyte and the type of bodily fluid contained in the test sample. As an illustrative example, **Table 1** lists the expected normal ranges of the biomarker analytes in human plasma, serum, and urine.

TABLE 1: Normal Concentration Ranges In Human Plasma, Serum, and Urine Samples

| Analyte | Units | Plasma | | Sera | | Urine | |
|--------------|-------|--------|-------|--------------------|--------|-------|---------|
| | | low | high | low | high | low | high |
| Calbindin | ng/ml | - | < 5.0 | - | < 2.6 | 4.2 | 233 |
| Clusterin | µg/ml | 86 | 134 | 37 | 152 | - | < 0.089 |
| CTGF | ng/ml | 2.8 | 7.5 | - | < 8.2 | - | < 0.90 |
| GST-alpha | ng/ml | 6.7 | 62 | 1.2 | 52 | - | < 26 |
| KIM-1 | ng/ml | 0.053 | 0.57 | - | < 0.35 | 0.023 | 0.67 |
| VEGF | pg/ml | 222 | 855 | 219 | 1630 | 69 | 517 |
| B2M | µg/ml | 0.68 | 2.2 | 1.00 | 2.6 | | < 0.17 |
| Cyst C | ng/ml | 608 | 1170 | 476 | 1250 | 3.9 | 79 |
| NGAL | ng/ml | 89 | 375 | 102 | 822 | 2.9 | 81 |
| OPN | ng/ml | 4.1 | 25 | 0.49 | 12 | 291 | 6130 |
| TIMP-1 | ng/ml | 50 | 131 | 100 | 246 | - | < 3.9 |
| A1M | µg/ml | 6.2 | 16 | 5.7 | 17 | - | < 4.2 |
| THP | µg/ml | 0.0084 | 0.052 | 0.007 ₉ | 0.053 | 0.39 | 2.6 |
| TFF3 | µg/ml | 0.040 | 0.49 | 0.021 | 0.17 | - | < 21 |
| Creatinine | mg/dL | - | - | - | - | 13 | 212 |
| Microalbumin | µg/ml | - | - | - | - | - | >16 |

[0077] In one embodiment, the high values shown for each of the biomarker analytes in **Table 1** for the analytic concentrations in human plasma, sera and urine are the minimum diagnostics values for the analytes in human plasma, sera,

and urine, respectively. In one exemplary embodiment, the minimum diagnostic concentration in human plasma of alpha-1 microglobulin is about 16 µg/ml, beta-2 microglobulin is about 2.2 µg/ml, calbindin is greater than about 5 ng/ml, clusterin is about 134 µg/ml, CTGF is about 16 ng/ml, cystatin C is about 1170 ng/ml, GST-alpha is about 62 ng/ml, KIM-1 is about 0.57 ng/ml, NGAL is about 375 ng/ml, osteopontin is about 25 ng/ml, THP is about 0.052 µg/ml, TIMP-1 is about 131 ng/ml, TFF-3 is about 0.49 µg/ml, and VEGF is about 855 pg/ml.

[0078] In another exemplary embodiment, the minimum diagnostic concentration in human sera of alpha-1 microglobulin is about 17 µg/ml, beta-2 microglobulin is about 2.6 µg/ml, calbindin is greater than about 2.6 ng/ml, clusterin is about 152 µg/ml, CTGF is greater than about 8.2 ng/ml, cystatin C is about 1250 ng/ml, GST-alpha is about 52 ng/ml, KIM-1 is greater than about 0.35 ng/ml, NGAL is about 822 ng/ml, osteopontin is about 12 ng/ml, THP is about 0.053 µg/ml, TIMP-1 is about 246 ng/ml, TFF-3 is about 0.17 µg/ml, and VEGF is about 1630 pg/ml.

[0079] In yet another exemplary embodiment, the minimum diagnostic concentration in human urine of alpha-1 microglobulin is about 233 µg/ml, beta-2 microglobulin is greater than about 0.17 µg/ml, calbindin is about 233 ng/ml, clusterin is greater than about 0.089 µg/ml, CTGF is greater than about 0.90 ng/ml, cystatin C is about 1170 ng/ml, GST-alpha is greater than about 26 ng/ml, KIM-1 is about 0.67 ng/ml, NGAL is about 81 ng/ml, osteopontin is about 6130 ng/ml, THP is about 2.6 µg/ml, TIMP-1 is greater than about 3.9 ng/ml, TFF-3 is greater than about 21 µg/ml, and VEGF is about 517 pg/ml.

[0080] In one embodiment, the minimum diagnostic concentrations represent the maximum level of analyte concentrations falling within an expected normal range. Glomerulonephritis or an associated disorder may be indicated if the concentration of an analyte is higher than the minimum diagnostic concentration for the analyte.

[0081] If diminished concentrations of a particular analyte are known to be associated with glomerulonephritis or an associated disorder, the minimum diagnostic concentration may not be an appropriate diagnostic criterion for identifying glomerulonephritis or an associated disorder indicated by the sample analyte

concentrations. In these cases, a maximum diagnostic concentration may define the limit between the expected normal concentration range for the analyte and a sample concentration reflective of glomerulonephritis or an associated disorder. In those cases in which a maximum diagnostic concentration is the appropriate diagnostic criterion, sample concentrations that fall below a maximum diagnostic concentration may indicate glomerulonephritis or an associated disorder.

[0082] A critical feature of the method of the multiplexed analyte panel is that a combination of sample analyte concentrations may be used to diagnose glomerulonephritis or an associated disorder. In addition to comparing subsets of the biomarker analyte concentrations to diagnostic criteria, the analytes may be algebraically combined and compared to corresponding diagnostic criteria. In one embodiment, two or more sample analyte concentrations may be added and/or subtracted to determine a combined analyte concentration. In another embodiment, two or more sample analyte concentrations may be multiplied and/or divided to determine a combined analyte concentration. To identify glomerulonephritis or an associated disorder, the combined analyte concentration may be compared to a diagnostic criterion in which the corresponding minimum or maximum diagnostic concentrations are combined using the same algebraic operations used to determine the combined analyte concentration.

[0083] In yet another embodiment, the analyte concentration measured from a test sample containing one type of body fluid may be algebraically combined with an analyte concentration measured from a second test sample containing a second type of body fluid to determine a combined analyte concentration. For example, the ratio of urine calbindin to plasma calbindin may be determined and compared to a corresponding minimum diagnostic urine: plasma calbindin ratio to identify a particular renal disorder.

[0084] A variety of methods known in the art may be used to define the diagnostic criteria used to identify glomerulonephritis or an associated disorder. In one embodiment, any sample concentration falling outside the expected normal range indicates glomerulonephritis or an associated disorder. In another embodiment, the multiplexed analyte panel may be used to evaluate the analyte concentrations in test

samples taken from a population of patients having glomerulonephritis or an associated disorder and compared to the normal expected analyte concentration ranges. In this same embodiment, any sample analyte concentrations that are significantly higher or lower than the expected normal concentration range may be used to define a minimum or maximum diagnostic concentration, respectively. A number of studies comparing the biomarker concentration ranges of a population of patients having a renal disorder to the corresponding analyte concentrations from a population of normal healthy subjects are described in the examples section below.

[0085] In an exemplary embodiment, an analyte value in a test sample higher than the minimum diagnostic value for the top 3 analytes of the particular sample type (e.g. plasma, urine, etc.), wherein the top 3 are determined by the random forest classification method may result in a diagnosis of glomerulonephritis.

VI. Automated Method for Diagnosing, Monitoring, or Determining a Renal Disorder

[0100] In one embodiment, a system for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder in a mammal is provided that includes a database to store a plurality of renal disorder database entries, and a processing device that includes the modules of a renal disorder determining application. In this embodiment, the modules are executable by the processing device, and include an analyte input module, a comparison module, and an analysis module.

[0101] The analyte input module receives three or more sample analyte concentrations that include the biomarker analytes. In one embodiment, the sample analyte concentrations are entered as input by a user of the application. In another embodiment, the sample analyte concentrations are transmitted directly to the analyte input module by the sensor device used to measure the sample analyte concentration via a data cable, infrared signal, wireless connection or other methods of data transmission known in the art.

[0102] The comparison module compares each sample analyte concentration to an entry of a renal disorder database. Each entry of the renal disorder database includes a list of minimum diagnostic concentrations reflective of a particular

renal disorder. The entries of the renal disorder database may further contain additional minimum diagnostic concentrations to further define diagnostic criteria including but not limited to minimum diagnostic concentrations for additional types of bodily fluids, additional types of mammals, and severities of a particular disorder.

[0103] The analysis module determines a most likely renal disorder by combining the particular renal disorders identified by the comparison module for all of the sample analyte concentrations. In one embodiment, the most likely renal disorder is the particular renal disorder from the database entry having the most minimum diagnostic concentrations that are less than the corresponding sample analyte concentrations. In another embodiment, the most likely renal disorder is the particular renal disorder from the database entry having minimum diagnostic concentrations that are all less than the corresponding sample analyte concentrations. In yet other embodiments, the analysis module combines the sample analyte concentrations algebraically to calculate a combined sample analyte concentration that is compared to a combined minimum diagnostic concentration calculated from the corresponding minimum diagnostic criteria using the same algebraic operations. Other combinations of sample analyte concentrations from within the same test sample, or combinations of sample analyte concentrations from two or more different test samples containing two or more different bodily fluids may be used to determine a particular renal disorder in still other embodiments.

[0104] The system includes one or more processors and volatile and/or nonvolatile memory and can be embodied by or in one or more distributed or integrated components or systems. The system may include computer readable media (CRM) on which one or more algorithms, software, modules, data, and/or firmware is loaded and/or operates and/or which operates on the one or more processors to implement the systems and methods identified herein. The computer readable media may include volatile media, nonvolatile media, removable media, non-removable media, and/or other media or mediums that can be accessed by a general purpose or special purpose computing device. For example, computer readable media may include computer storage media and communication media, including but not limited to computer readable media. Computer storage media further may include volatile, nonvolatile,

removable, and/or non-removable media implemented in a method or technology for storage of information, such as computer readable instructions, data structures, program modules, and/or other data. Communication media may, for example, embody computer readable instructions, data structures, program modules, algorithms, and/or other data, including but not limited to as or in a modulated data signal. The communication media may be embodied in a carrier wave or other transport mechanism and may include an information delivery method. The communication media may include wired and wireless connections and technologies and may be used to transmit and/or receive wired or wireless communications. Combinations and/or sub-combinations of the above and systems, components, modules, and methods and processes described herein may be made.

[0105] The following examples are included to demonstrate preferred embodiments of the invention.

EXAMPLES

[0106] The following examples illustrate various iterations of the invention.

Example 1: Least Detectable Dose and Lower Limit of Quantitation of Assay for Analytes Associated with Renal Disorders

[0107] To assess the least detectable doses (LDD) and lower limits of quantitation (LLOQ) of a variety of analytes associated with renal disorders, the following experiment was conducted. The analytes measured were alpha-1 microglobulin (A1M), beta-2 microglobulin (B2M), calbindin, clusterin, CTGF, cystatin C, GST-alpha, KIM-1, NGAL, osteopontin (OPN), THP, TIMP-1, TFF-3, and VEGF.

[0108] The concentrations of the analytes were measured using a capture-sandwich assay using antigen-specific antibodies. For each analyte, a range of standard sample dilutions ranging over about four orders of magnitude of analyte concentration were measured using the assay in order to obtain data used to construct a standard dose response curve. The dynamic range for each of the analytes, defined herein as the range of analyte concentrations measured to determine its dose response curve, is presented below.

[0109] To perform the assay, 5 μ L of a diluted mixture of capture-antibody microspheres were mixed with 5 μ L of blocker and 10 μ L of pre-diluted standard sample in each of the wells of a hard-bottom microtiter plate. After incubating the hard-bottom plate for 1 hour, 10 μ L of biotinylated detection antibody was added to each well, and then the hard-bottom plate was incubated for an additional hour. 10 μ L of diluted streptavidin-phycoerythrin was added to each well and then the hard-bottom plate was incubated for another 60 minutes.

[0110] A filter-membrane microtiter plate was pre-wetted by adding 100 μ L wash buffer, and then aspirated using a vacuum manifold device. The contents of the wells of the hard-bottom plate were then transferred to the corresponding wells of the filter-membrane plate. All wells of the hard-bottom plate were vacuum-aspirated and the contents were washed twice with 100 μ L of wash buffer. After the second wash, 100 μ L of wash buffer was added to each well, and then the washed microspheres were resuspended with thorough mixing. The plate was then analyzed using a Luminex 100 Analyzer (Luminex Corporation, Austin, Texas, USA). Dose response curves were constructed for each analyte by curve-fitting the median fluorescence intensity (MFI) measured from the assays of diluted standard samples containing a range of analyte concentrations.

[0111] The least detectable dose (LDD) was determined by adding three standard deviations to the average of the MFI signal measured for 20 replicate samples of blank standard solution (i.e. standard solution containing no analyte). The MFI signal was converted to an LDD concentration using the dose response curve and multiplied by a dilution factor of 2.

[0112] The lower limit of quantification (LLOQ), defined herein as the point at which the coefficient of variation (CV) for the analyte measured in the standard samples was 30%, was determined by the analysis of the measurements of increasingly diluted standard samples. For each analyte, the standard solution was diluted by 2 fold for 8 dilutions. At each stage of dilution, samples were assayed in triplicate, and the CV of the analyte concentration at each dilution was calculated and plotted as a function of analyte concentration. The LLOQ was interpolated from this plot and multiplied by a dilution factor of 2.

[0113] The LDD and LLOQ results for each analyte are summarized in **Table 2:**

TABLE 2: LDD, LLOQ, and Dynamic Range of Analyte Assay

| Analyte | Units | LDD | LLOQ | Dynamic Range | |
|--------------|------------|-------|-------|---------------|---------|
| | | | | minimum | maximum |
| Calbindin | ng/mL | 1.1 | 3.1 | 0.516 | 2580 |
| Clusterin | ng/mL | 2.4 | 2.3 | 0.676 | 3378 |
| CTGF | ng/mL | 1.3 | 3.8 | 0.0794 | 400 |
| GST-alpha | ng/mL | 1.4 | 3.6 | 0.24 | 1,200 |
| KIM-1 | ng/mL | 0.016 | 0.028 | 0.00478 | 24 |
| VEGF | pg/mL | 4.4 | 20 | 8.76 | 44,000 |
| β -2 M | μ g/mL | 0.012 | 0.018 | 0.0030 | 15 |
| Cystatin C | ng/mL | 2.8 | 3.7 | 0.60 | 3,000 |
| NGAL | ng/mL | 4.1 | 7.8 | 1.2 | 6,000 |
| Osteopontin | ng/mL | 29 | 52 | 3.9 | 19,500 |
| TIMP-1 | ng/mL | 0.71 | 1.1 | 0.073 | 365 |
| A-1 M | μ g/mL | 0.059 | 0.29 | 0.042 | 210 |
| THP | μ g/mL | 0.46 | 0.30 | 0.16 | 800 |
| TFF-3 | μ g/mL | 0.06 | 0.097 | 0.060 | 300 |

[0114] The results of this experiment characterized the least detectible dose and the lower limit of quantification for fourteen analytes associated with various renal disorders using a capture-sandwich assay.

Example 2: Precision of Assay for Analytes Associated with Renal Disorders

[0115] To assess the precision of an assay used to measure the concentration of analytes associated with renal disorders, the following experiment was conducted. The analytes measured were alpha-1 microglobulin (A1M), beta-2 microglobulin (B2M), calbindin, clusterin, CTGF, cystatin C, GST-alpha, KIM-1, NGAL, osteopontin (OPN), THP, TIMP-1, TFF-3, and VEGF. For each analyte, three

concentration levels of standard solution were measured in triplicate during three runs using the methods described in Example 1. The percent errors for each run at each concentration are presented in **Table 3** for all of the analytes tested:

TABLE 3: Precision of Analyte Assay

| Analyte | Average concentration (ng/mL) | Run 1 Error (%) | Run 2 Error (%) | Run 2 Error (%) | Interrun Error (%) |
|--------------|-------------------------------|-----------------|-----------------|-----------------|--------------------|
| Calbindin | 4.0 | 6 | 2 | 6 | 13 |
| | 36 | 5 | 3 | 2 | 7 |
| | 281 | 1 | 6 | 0 | 3 |
| Clusterin | 4.4 | 4 | 9 | 2 | 6 |
| | 39 | 5 | 1 | 6 | 8 |
| | 229 | 1 | 3 | 0 | 2 |
| CTGF | 1.2 | 10 | 17 | 4 | 14 |
| | 2.5 | 19 | 19 | 14 | 14 |
| | 18 | 7 | 5 | 13 | 9 |
| GST-alpha | 3.9 | 14 | 7 | 5 | 10 |
| | 16 | 13 | 7 | 10 | 11 |
| | 42 | 1 | 16 | 6 | 8 |
| KIM-1 | 0.035 | 2 | 0 | 5 | 13 |
| | 0.32 | 4 | 5 | 2 | 8 |
| | 2.9 | 0 | 5 | 7 | 4 |
| VEGF | 65 | 10 | 1 | 6 | 14 |
| | 534 | 9 | 2 | 12 | 7 |
| | 5,397 | 1 | 13 | 14 | 9 |
| β -2 M | 0.040 | 6 | 1 | 8 | 5 |
| | 0.43 | 2 | 2 | 0 | 10 |
| | 6.7 | 6 | 5 | 11 | 6 |
| Cystatin C | 10.5 | 4 | 1 | 7 | 13 |
| | 49 | 0 | 0 | 3 | 9 |

| Analyte | Average concentration (ng/mL) | Run 1 Error (%) | Run 2 Error (%) | Run 2 Error (%) | Interrun Error (%) |
|-------------|-------------------------------|-----------------|-----------------|-----------------|--------------------|
| | 424 | 2 | 6 | 2 | 5 |
| NGAL | 18.1 | 11 | 3 | 6 | 13 |
| | 147 | 0 | 0 | 6 | 5 |
| | 1,070 | 5 | 1 | 2 | 5 |
| Osteopontin | 44 | 1 | 10 | 2 | 11 |
| | 523 | 9 | 9 | 9 | 7 |
| | 8,930 | 4 | 10 | 1 | 10 |
| TIMP-1 | 2.2 | 13 | 6 | 3 | 13 |
| | 26 | 1 | 1 | 4 | 14 |
| | 130 | 1 | 3 | 1 | 4 |
| A-1 M | 1.7 | 11 | 7 | 7 | 14 |
| | 19 | 4 | 1 | 8 | 9 |
| | 45 | 3 | 5 | 2 | 4 |
| THP | 9.4 | 3 | 10 | 11 | 11 |
| | 15 | 3 | 7 | 8 | 6 |
| | 37 | 4 | 5 | 0 | 5 |
| TFF-3 | 0.3 | 13 | 3 | 11 | 12 |
| | 4.2 | 5 | 8 | 5 | 7 |
| | 1.2 | 3 | 7 | 0 | 13 |

[0116] The results of this experiment characterized the precision of a capture-sandwich assay for fourteen analytes associated with various renal disorders over a wide range of analyte concentrations. The precision of the assay varied between about 1% and about 15% error within a given run, and between about 5% and about 15% error between different runs. The percent errors summarized in Table 2 provide information concerning random error to be expected in an assay measurement caused by variations in technicians, measuring instruments, and times of measurement.

Example 3: Linearity of Assay for Analytes Associated with Renal Disorders

[0117] To assess the linearity of an assay used to measure the concentration of analytes associated with renal disorders, the following experiment was conducted. The analytes measured were alpha-1 microglobulin (A1M), beta-2 microglobulin (B2M), calbindin, clusterin, CTGF, cystatin C, GST-alpha, KIM-1, NGAL, osteopontin (OPN), THP, TIMP-1, TFF-3, and VEGF. For each analyte, three concentration levels of standard solution were measured in triplicate during three runs using the methods described in Example 1. Linearity of the assay used to measure each analyte was determined by measuring the concentrations of standard samples that were serially-diluted throughout the assay range. The % recovery was calculated as observed vs. expected concentration based on the dose-response curve. The results of the linearity analysis are summarized in **Table 4**.

TABLE 4: Linearity of Analyte Assay

| Analyte | Dilution | Expected concentration | Observed concentration | Recovery (%) |
|-------------------|----------|------------------------|------------------------|--------------|
| Calbindin (ng/mL) | 1:2 | 61 | 61 | 100 |
| | 1:4 | 30 | 32 | 106 |
| | 1:8 | 15 | 17 | 110 |
| Clusterin (ng/mL) | 1:2 | 41 | 41 | 100 |
| | 1:4 | 21 | 24 | 116 |
| | 1:8 | 10 | 11 | 111 |
| CTGF (ng/mL) | 1:2 | 1.7 | 1.7 | 100 |
| | 1:4 | 0.84 | 1.0 | 124 |
| | 1:8 | 0.42 | 0.51 | 122 |
| GST-alpha (ng/mL) | 1:2 | 25 | 25 | 100 |
| | 1:4 | 12 | 14 | 115 |
| | 1:8 | 6.2 | 8.0 | 129 |
| KIM-1 (ng/mL) | 1:2 | 0.87 | 0.87 | 100 |
| | 1:4 | 0.41 | 0.41 | 101 |
| | 1:8 | 0.21 | 0.19 | 93 |

| Analyte | Dilution | Expected concentration | Observed concentration | Recovery (%) |
|----------------------------|----------|------------------------|------------------------|--------------|
| VEGF (pg/mL) | 1:2 | 2,525 | 2,525 | 100 |
| | 1:4 | 1,263 | 1,340 | 106 |
| | 1:8 | 631 | 686 | 109 |
| β -2 M (μ g/mL) | 1:100 | 0.63 | 0.63 | 100 |
| | 1:200 | 0.31 | 0.34 | 106 |
| | 1:400 | 0.16 | 0.17 | 107 |
| Cystatin C (ng/mL) | 1:100 | 249 | 249 | 100 |
| | 1:200 | 125 | 122 | 102 |
| | 1:400 | 62 | 56 | 110 |
| NGAL (ng/mL) | 1:100 | 1,435 | 1,435 | 100 |
| | 1:200 | 718 | 775 | 108 |
| | 1:400 | 359 | 369 | 103 |
| Osteopontin (ng/mL) | 1:100 | 6,415 | 6,415 | 100 |
| | 1:200 | 3,208 | 3,275 | 102 |
| | 1:400 | 1,604 | 1,525 | 95 |
| TIMP-1 (ng/mL) | 1:100 | 35 | 35 | 100 |
| | 1:200 | 18 | 18 | 100 |
| | 1:400 | 8.8 | 8.8 | 100 |
| A-1 M (μ g/mL) | 1:2000 | 37 | 37 | 100 |
| | 1:4000 | 18 | 18 | 99 |
| | 1:8000 | 9.1 | 9.2 | 99 |
| THP (μ g/mL) | 1:2000 | 28 | 28 | 100 |
| | 1:4000 | 14 | 14 | 96 |
| | 1:8000 | 6.7 | 7.1 | 94 |
| TFF-3 (μ g/mL) | 1:2000 | 8.8 | 8.8 | 100 |
| | 1:4000 | 3.8 | 4.4 | 86 |
| | 1:8000 | 1.9 | 2.2 | 86 |

[0118] The results of this experiment demonstrated reasonably linear responses of the sandwich-capture assay to variations in the concentrations of the analytes in the tested samples.

Example 4: Spike Recovery of Analytes Associated with Renal Disorders

[0119] To assess the recovery of analytes spiked into urine, serum, and plasma samples by an assay used to measure the concentration of analytes associated with renal disorders, the following experiment was conducted. The analytes measured were alpha-1 microglobulin (A1M), beta-2 microglobulin (B2M), calbindin, clusterin, CTGF, cystatin C, GST-alpha, KIM-1, NGAL, osteopontin (OPN), THP, TIMP-1, TFF-3, and VEGF. For each analyte, three concentration levels of standard solution were spiked into known urine, serum, and plasma samples. Prior to analysis, all urine samples were diluted 1:2000 (sample: diluent), all plasma samples were diluted 1:5 (sample: diluent), and all serum samples were diluted 1:2000 (sample: diluent).

[0120] The concentrations of the analytes in the samples were measured using the methods described in Example 1. The average % recovery was calculated as the proportion of the measurement of analyte spiked into the urine, serum, or plasma sample (observed) to the measurement of analyte spiked into the standard solution (expected). The results of the spike recovery analysis are summarized in **Table 5**.

TABLE 5: Spike Recovery of Analyte Assay in Urine, Serum, and Plasma Samples

| Analyte | Spike Concentration | Recovery in Urine Sample (%) | Recovery in Serum Sample (%) | Recovery in Plasma Sample (%) |
|-------------------|----------------------------|-------------------------------------|-------------------------------------|--------------------------------------|
| Calbindin (ng/mL) | 66 | 76 | 82 | 83 |
| | 35 | 91 | 77 | 71 |
| | 18 | 80 | 82 | 73 |
| | average | 82 | 80 | 76 |
| Clusterin (ng/mL) | 80 | 72 | 73 | 75 |
| | 37 | 70 | 66 | 72 |
| | 20 | 90 | 73 | 70 |

| Analyte | Spike Concentration | Recovery in Urine Sample (%) | Recovery in Serum Sample (%) | Recovery in Plasma Sample (%) |
|----------------------------|---------------------|------------------------------|------------------------------|-------------------------------|
| | average | 77 | 70 | 72 |
| CTGF (ng/mL) | 8.4 | 91 | 80 | 79 |
| | 4.6 | 114 | 69 | 78 |
| | 2.4 | 76 | 80 | 69 |
| | average | 94 | 77 | 75 |
| GST-alpha (ng/mL) | 27 | 75 | 84 | 80 |
| | 15 | 90 | 75 | 81 |
| | 7.1 | 82 | 84 | 72 |
| | average | 83 | 81 | 78 |
| KIM-1 (ng/mL) | 0.63 | 87 | 80 | 83 |
| | .029 | 119 | 74 | 80 |
| | 0.14 | 117 | 80 | 78 |
| | average | 107 | 78 | 80 |
| VEGF (pg/mL) | 584 | 88 | 84 | 82 |
| | 287 | 101 | 77 | 86 |
| | 123 | 107 | 84 | 77 |
| | average | 99 | 82 | 82 |
| β -2 M (μ g/mL) | 0.97 | 117 | 98 | 98 |
| | 0.50 | 124 | 119 | 119 |
| | 0.24 | 104 | 107 | 107 |
| | average | 115 | 108 | 105 |
| Cystatin C (ng/mL) | 183 | 138 | 80 | 103 |
| | 90 | 136 | 97 | 103 |
| | 40 | 120 | 97 | 118 |
| | average | 131 | 91 | 108 |
| NGAL (ng/mL) | 426 | 120 | 105 | 111 |
| | 213 | 124 | 114 | 112 |
| | 103 | 90 | 99 | 113 |

| Analyte | Spike Concentration | Recovery in Urine Sample (%) | Recovery in Serum Sample (%) | Recovery in Plasma Sample (%) |
|---------------------|---------------------|------------------------------|------------------------------|-------------------------------|
| | average | 111 | 106 | 112 |
| Osteopontin (ng/mL) | 1,245 | 204 | 124 | 68 |
| | 636 | 153 | 112 | 69 |
| | 302 | 66 | 103 | 67 |
| | average | 108 | 113 | 68 |
| TIMP-1 (ng/mL) | 25 | 98 | 97 | 113 |
| | 12 | 114 | 89 | 103 |
| | 5.7 | 94 | 99 | 113 |
| | average | 102 | 95 | 110 |
| A-1 M (µg/mL) | 0.0028 | 100 | 101 | 79 |
| | 0.0012 | 125 | 80 | 81 |
| | 0.00060 | 118 | 101 | 82 |
| | Average | 114 | 94 | 81 |
| THP (µg/mL) | 0.0096 | 126 | 108 | 90 |
| | 0.0047 | 131 | 93 | 91 |
| | 0.0026 | 112 | 114 | 83 |
| | average | 123 | 105 | 88 |
| TFF-3 (µg/mL) | 0.0038 | 105 | 114 | 97 |
| | 0.0019 | 109 | 104 | 95 |
| | 0.0010 | 102 | 118 | 93 |
| | average | 105 | 112 | 95 |

[0121] The results of this experiment demonstrated that the sandwich-type assay is reasonably sensitive to the presence of all analytes measured, whether the analytes were measured in standard samples, urine samples, plasma samples, or serum samples.

Example 5: Matrix Interferences of Analytes Associated with Renal Disorders

[0122] To assess the matrix interference of hemoglobin, bilirubin, and triglycerides spiked into standard samples, the following experiment was conducted. The analytes measured were alpha-1 microglobulin (A1M), beta-2 microglobulin (B2M), calbindin, clusterin, CTGF, cystatin C, GST-alpha, KIM-1, NGAL, osteopontin (OPN), THP, TIMP-1, TFF-3, and VEGF. For each analyte, three concentration levels of standard solution were spiked into known urine, serum, and plasma samples. Matrix interference was assessed by spiking hemoglobin, bilirubin, and triglyceride into standard analyte samples and measuring analyte concentrations using the methods described in Example 1. A % recovery was determined by calculating the ratio of the analyte concentration measured from the spiked sample (observed) divided by the analyte concentration measured from the standard sample (expected). The results of the matrix interference analysis are summarized in **Table 6**.

TABLE 6: Matrix Interference of Hemoglobin, Bilirubin, and Triglyceride on the Measurement of Analytes

| Analyte | Matrix Compound Spiked into Sample | Maximum Spike Concentration | Overall Recovery (%) |
|-------------------|------------------------------------|-----------------------------|----------------------|
| Calbindin (mg/mL) | Hemoglobin | 500 | 110 |
| | Bilirubin | 20 | 98 |
| | Triglyceride | 500 | 117 |
| Clusterin (mg/mL) | Hemoglobin | 500 | 125 |
| | Bilirubin | 20 | 110 |
| | Triglyceride | 500 | 85 |
| CTGF (mg/mL) | Hemoglobin | 500 | 91 |
| | Bilirubin | 20 | 88 |
| | Triglyceride | 500 | 84 |
| GST-alpha (mg/mL) | Hemoglobin | 500 | 100 |
| | Bilirubin | 20 | 96 |
| | Triglyceride | 500 | 96 |
| KIM-1 | Hemoglobin | 500 | 108 |

| Analyte | Matrix Compound Spiked into Sample | Maximum Spike Concentration | Overall Recovery (%) |
|----------------------------|---|------------------------------------|-----------------------------|
| (mg/mL) | Bilirubin | 20 | 117 |
| | Triglyceride | 500 | 84 |
| VEGF (mg/mL) | Hemoglobin | 500 | 112 |
| | Bilirubin | 20 | 85 |
| | Triglyceride | 500 | 114 |
| β -2 M (μ g/mL) | Hemoglobin | 500 | 84 |
| | Bilirubin | 20 | 75 |
| | Triglyceride | 500 | 104 |
| Cystatin C (ng/mL) | Hemoglobin | 500 | 91 |
| | Bilirubin | 20 | 102 |
| | Triglyceride | 500 | 124 |
| NGAL (ng/mL) | Hemoglobin | 500 | 99 |
| | Bilirubin | 20 | 92 |
| | Triglyceride | 500 | 106 |
| Osteopontin (ng/mL) | Hemoglobin | 500 | 83 |
| | Bilirubin | 20 | 86 |
| | Triglyceride | 500 | 106 |
| TIMP-1 (ng/mL) | Hemoglobin | 500 | 87 |
| | Bilirubin | 20 | 86 |
| | Triglyceride | 500 | 93 |
| A-1 M (μ g/mL) | Hemoglobin | 500 | 103 |
| | Bilirubin | 20 | 110 |
| | Triglyceride | 500 | 112 |
| THP (μ g/mL) | Hemoglobin | 500 | 108 |
| | Bilirubin | 20 | 101 |
| | Triglyceride | 500 | 121 |
| TFF-3 | Hemoglobin | 500 | 101 |

| Analyte | Matrix Compound Spiked into Sample | Maximum Spike Concentration | Overall Recovery (%) |
|---------|------------------------------------|-----------------------------|----------------------|
| (µg/mL) | Bilirubin | 20 | 101 |
| | Triglyceride | 500 | 110 |

[0123] The results of this experiment demonstrated that hemoglobin, bilirubin, and triglycerides, three common compounds found in urine, plasma, and serum samples, did not significantly degrade the ability of the sandwich-capture assay to detect any of the analytes tested.

Example 6: Sample Stability of Analytes Associated with Renal Disorders

[0124] To assess the ability of analytes spiked into urine, serum, and plasma samples to tolerate freeze-thaw cycles, the following experiment was conducted. The analytes measured were alpha-1 microglobulin (A1M), beta-2 microglobulin (B2M), calbindin, clusterin, CTGF, cystatin C, GST-alpha, KIM-1, NGAL, osteopontin (OPN), THP, TIMP-1, TFF-3, and VEGF. Each analyte was spiked into known urine, serum, and plasma samples at a known analyte concentration. The concentrations of the analytes in the samples were measured using the methods described in Example 1 after the initial addition of the analyte, and after one, two and three cycles of freezing and thawing. In addition, analyte concentrations in urine, serum and plasma samples were measured immediately after the addition of the analyte to the samples as well as after storage at room temperature for two hours and four hours, and after storage at 4° C for 2 hours, four hours, and 24 hours.

[0125] The results of the freeze-thaw stability analysis are summarized in **Table 7**. The % recovery of each analyte was calculated as a percentage of the analyte measured in the sample prior to any freeze-thaw cycles.

TABLE 7: Freeze-Thaw Stability of the Analytes in Urine, Serum, and Plasma

| Analyte | Period and Temp | Urine Sample | | Serum Sample | | Plasma Sample | |
|----------------------------|-----------------|---------------|--------------|---------------|--------------|---------------|--------------|
| | | Concentration | Recovery (%) | Concentration | Recovery (%) | Concentration | Recovery (%) |
| Calbindin (ng/mL) | Control | 212 | 100 | 31 | 100 | 43 | 100 |
| | 1X | 221 | 104 | 30 | 96 | 41 | 94 |
| | 2X | 203 | 96 | 30 | 99 | 39 | 92 |
| | 3X | 234 | 110 | 30 | 97 | 40 | 93 |
| Clusterin (ng/mL) | 0 | 315 | 100 | 232 | 100 | 187 | 100 |
| | 1X | 329 | 104 | 227 | 98 | 177 | 95 |
| | 2X | 341 | 108 | 240 | 103 | 175 | 94 |
| | 3X | 379 | 120 | 248 | 107 | 183 | 98 |
| CTGF (ng/mL) | 0 | 6.7 | 100 | 1.5 | 100 | 1.2 | 100 |
| | 1X | 7.5 | 112 | 1.3 | 82 | 1.2 | 94 |
| | 2X | 6.8 | 101 | 1.4 | 90 | 1.2 | 100 |
| | 3X | 7.7 | 115 | 1.2 | 73 | 1.3 | 107 |
| GST-alpha (ng/mL) | 0 | 12 | 100 | 23 | 100 | 11 | 100 |
| | 1X | 13 | 104 | 24 | 105 | 11 | 101 |
| | 2X | 14 | 116 | 21 | 92 | 11 | 97 |
| | 3X | 14 | 111 | 23 | 100 | 12 | 108 |
| KIM-1 (ng/mL) | 0 | 1.7 | 100 | 0.24 | 100 | 0.24 | 100 |
| | 1X | 1.7 | 99 | 0.24 | 102 | 0.22 | 91 |
| | 2X | 1.7 | 99 | 0.22 | 94 | 0.19 | 78 |
| | 3X | 1.8 | 107 | 0.23 | 97 | 0.22 | 93 |
| VEGF (pg/mL) | 0 | 1,530 | 100 | 1,245 | 100 | 674 | 100 |
| | 1X | 1,575 | 103 | 1,205 | 97 | 652 | 97 |
| | 2X | 1,570 | 103 | 1,140 | 92 | 612 | 91 |
| | 3X | 1,700 | 111 | 1,185 | 95 | 670 | 99 |
| β -2 M (μ g/mL) | 0 | 0.0070 | 100 | 1.2 | 100 | 15 | 100 |
| | 1X | 0.0073 | 104 | 1.1 | 93 | 14 | 109 |
| | 2X | 0.0076 | 108 | 1.2 | 103 | 15 | 104 |

| Analyte | Period and Temp | Urine Sample | | Serum Sample | | Plasma Sample | |
|------------------------|-----------------|---------------|--------------|---------------|--------------|---------------|--------------|
| | | Concentration | Recovery (%) | Concentration | Recovery (%) | Concentration | Recovery (%) |
| | 3X | 0.0076 | 108 | 1.1 | 97 | 13 | 116 |
| Cystatin C (ng/mL) | 0 | 1,240 | 100 | 1,330 | 100 | 519 | 100 |
| | 1X | 1,280 | 103 | 1,470 | 111 | 584 | 113 |
| | 2X | 1,410 | 114 | 1,370 | 103 | 730 | 141 |
| | 3X | 1,420 | 115 | 1,380 | 104 | 589 | 113 |
| NGAL (ng/mL) | 0 | 45 | 100 | 245 | 100 | 84 | 100 |
| | 1X | 46 | 102 | 179 | 114 | 94 | 112 |
| | 2X | 47 | 104 | 276 | 113 | 91 | 108 |
| | 3X | 47 | 104 | 278 | 113 | 91 | 109 |
| Osteopontin (ng/mL) | 0 | 38 | 100 | 1.7 | 100 | 5.0 | 100 |
| | 1X | 42 | 110 | 1.8 | 102 | 5.5 | 110 |
| | 2X | 42 | 108 | 1.5 | 87 | 5.5 | 109 |
| | 3X | 42 | 110 | 1.3 | 77 | 5.4 | 107 |
| TIMP-1 (ng/mL) | 0 | 266 | 100 | 220 | 100 | 70 | 100 |
| | 1X | 265 | 100 | 220 | 10 | 75 | 108 |
| | 2X | 255 | 96 | 215 | 98 | 77 | 110 |
| | 3X | 295 | 111 | 228 | 104 | 76 | 109 |
| A-1 M (µg/mL) | 0 | 14 | 100 | 26 | 100 | 4.5 | 100 |
| | 1X | 13 | 92 | 25 | 96 | 4.2 | 94 |
| | 2X | 15 | 107 | 25 | 96 | 4.3 | 97 |
| | 3X | 16 | 116 | 23 | 88 | 4.0 | 90 |
| THP (µg/mL) | 0 | 4.6 | 100 | 31 | 100 | 9.2 | 100 |
| | 1X | 4.4 | 96 | 31 | 98 | 8.8 | 95 |
| | 2X | 5.0 | 110 | 31 | 100 | 9.2 | 100 |
| | 3X | 5.2 | 114 | 27 | 85 | 9.1 | 99 |
| TFF-3 (µg/mL) | 0 | 4.6 | 100 | 24 | 100 | 22 | 100 |
| | 1X | 4.4 | 96 | 23 | 98 | 22 | 103 |
| | 2X | 5.0 | 110 | 24 | 103 | 22 | 101 |

| Analyte | Period and Temp | Urine Sample | | Serum Sample | | Plasma Sample | |
|---------|-----------------|---------------|--------------|---------------|--------------|---------------|--------------|
| | | Concentration | Recovery (%) | Concentration | Recovery (%) | Concentration | Recovery (%) |
| | 3X | 5.2 | 114 | 19 | 82 | 22 | 102 |

[0126] The results of the short-term stability assessment are summarized in **Table 8**. The % recovery of each analyte was calculated as a percentage of the analyte measured in the sample prior to any short-term storage.

TABLE 8: Short-Term Stability of Analytes in Urine, Serum, and Plasma

| Analyte | Storage Time/Temp | Urine Sample | | Serum Sample | | Plasma Sample | |
|-------------------|-------------------|--------------|--------------|--------------|--------------|---------------|--------------|
| | | Sample Conc. | Recovery (%) | Sample Conc. | Recovery (%) | Sample Conc. | Recovery (%) |
| Calbindin (ng/mL) | Control | 226 | 100 | 33 | 100 | 7 | 100 |
| | 2 hr/ room temp | 242 | 107 | 30 | 90 | 6.3 | 90 |
| | 2 hr. @ 4° C | 228 | 101 | 29 | 89 | 6.5 | 93 |
| | 4 hr @ room temp | 240 | 106 | 28 | 84 | 5.6 | 79 |
| | 4 hr. @ 4° C | 202 | 89 | 29 | 86 | 5.5 | 79 |
| | 24 hr. @ 4° C | 199 | 88 | 26 | 78 | 7.1 | 101 |
| Clusterin (ng/mL) | Control | 185 | 100 | 224 | 100 | 171 | 100 |
| | 2 hr @ room temp | 173 | 94 | 237 | 106 | 180 | 105 |
| | 2 hr. @ 4° C | 146 | 79 | 225 | 100 | 171 | 100 |
| | 4 hr @ room temp | 166 | 89 | 214 | 96 | 160 | 94 |
| | 4 hr. @ 4° C | 157 | 85 | 198 | 88 | 143 | 84 |
| | 24 hr. @ 4° C | 185 | 100 | 207 | 92 | 162 | 94 |
| CTGF | Control | 1.9 | 100 | 8.8 | 100 | 1.2 | 100 |

| Analyte | Storage Time/ Temp | Urine Sample | | Serum Sample | | Plasma Sample | |
|----------------------|-----------------------|--------------|--------------|--------------|--------------|---------------|--------------|
| | | Sample Conc. | Recovery (%) | Sample Conc. | Recovery (%) | Sample Conc. | Recovery (%) |
| (ng/mL) | 2 hr @ room temp | 1.9 | 99 | 6.7 | 76 | 1 | 83 |
| | 2 hr. @ 4° C | 1.8 | 96 | 8.1 | 92 | 1.1 | 89 |
| | 4 hr @ room temp | 2.1 | 113 | 5.6 | 64 | 1 | 84 |
| | 4 hr. @ 4° C | 1.7 | 91 | 6.4 | 74 | 0.9 | 78 |
| | 24 hr. @ 4° C | 2.2 | 116 | 5.9 | 68 | 1.1 | 89 |
| GST-alpha (ng/mL) | Control | 14 | 100 | 21 | 100 | 11 | 100 |
| | 2 hr @ room temp | 11 | 75 | 23 | 107 | 11 | 103 |
| | 2 hr. @ 4° C | 13 | 93 | 22 | 104 | 9.4 | 90 |
| | 4 hr @ room temp | 11 | 79 | 21 | 100 | 11 | 109 |
| | 4 hr. @ 4° C | 12 | 89 | 21 | 98 | 11 | 100 |
| | 24 hr. @ 4° C | 13 | 90 | 22 | 103 | 14 | 129 |
| KIM-1 (ng/mL) | Control | 1.5 | 100 | 0.23 | 100 | 0.24 | 100 |
| | 2 hr @ room temp | 1.2 | 78 | 0.2 | 86 | 0.22 | 90 |
| | 2 hr. @ 4° C | 1.6 | 106 | 0.23 | 98 | 0.21 | 85 |
| | 4 hr @ room temp | 1.3 | 84 | 0.19 | 82 | 0.2 | 81 |
| | 4 hr. @ 4° C | 1.4 | 90 | 0.22 | 93 | 0.19 | 80 |
| | 24 hr. @ 4° C | 1.1 | 76 | 0.18 | 76 | 0.23 | 94 |
| VEGF | Control | 851 | 100 | 1215 | 100 | 670 | 100 |

| Analyte | Storage Time/ Temp | Urine Sample | | Serum Sample | | Plasma Sample | |
|----------------------------|-----------------------|--------------|--------------|--------------|--------------|---------------|--------------|
| | | Sample Conc. | Recovery (%) | Sample Conc. | Recovery (%) | Sample Conc. | Recovery (%) |
| (pg/mL) | 2 hr @ room temp | 793 | 93 | 1055 | 87 | 622 | 93 |
| | 2 hr. @ 4° C | 700 | 82 | 1065 | 88 | 629 | 94 |
| | 4 hr @ room temp | 704 | 83 | 1007 | 83 | 566 | 84 |
| | 4 hr. @ 4° C | 618 | 73 | 1135 | 93 | 544 | 81 |
| | 24 hr. @ 4° C | 653 | 77 | 1130 | 93 | 589 | 88 |
| β -2 M (μ g/mL) | Control | 0.064 | 100 | 2.6 | 100 | 1.2 | 100 |
| | 2 hr @ room temp | 0.062 | 97 | 2.4 | 92 | 1.1 | 93 |
| | 2 hr. @ 4° C | 0.058 | 91 | 2.2 | 85 | 1.2 | 94 |
| | 4 hr @ room temp | 0.064 | 101 | 2.2 | 83 | 1.2 | 94 |
| | 4 hr. @ 4° C | 0.057 | 90 | 2.2 | 85 | 1.2 | 98 |
| | 24 hr. @ 4° C | 0.06 | 94 | 2.5 | 97 | 1.3 | 103 |
| Cys-tatin C (ng/mL) | Control | 52 | 100 | 819 | 100 | 476 | 100 |
| | 2 hr @ room temp | 50 | 96 | 837 | 102 | 466 | 98 |
| | 2 hr. @ 4° C | 44 | 84 | 884 | 108 | 547 | 115 |
| | 4 hr @ room temp | 49 | 93 | 829 | 101 | 498 | 105 |
| | 4 hr. @ 4° C | 46 | 88 | 883 | 108 | 513 | 108 |
| | 24 hr. @ 4° C | 51 | 97 | 767 | 94 | 471 | 99 |
| NGAL | Control | 857 | 100 | 302 | 100 | 93 | 100 |

| Analyte | Storage Time/ Temp | Urine Sample | | Serum Sample | | Plasma Sample | |
|---------------------|-----------------------|--------------|--------------|--------------|--------------|---------------|--------------|
| | | Sample Conc. | Recovery (%) | Sample Conc. | Recovery (%) | Sample Conc. | Recovery (%) |
| (ng/mL) | 2 hr @ room temp | 888 | 104 | 287 | 95 | 96 | 104 |
| | 2 hr. @ 4° C | 923 | 108 | 275 | 91 | 92 | 100 |
| | 4 hr @ room temp | 861 | 101 | 269 | 89 | 88 | 95 |
| | 4 hr. @ 4° C | 842 | 98 | 283 | 94 | 94 | 101 |
| | 24 hr. @ 4° C | 960 | 112 | 245 | 81 | 88 | 95 |
| Osteopontin (ng/mL) | Control | 2243 | 100 | 6.4 | 100 | 5.2 | 100 |
| | 2 hr @ room temp | 2240 | 100 | 6.8 | 107 | 5.9 | 114 |
| | 2 hr. @ 4° C | 2140 | 95 | 6.4 | 101 | 6.2 | 120 |
| | 4 hr @ room temp | 2227 | 99 | 6.9 | 108 | 5.8 | 111 |
| | 4 hr. @ 4° C | 2120 | 95 | 7.7 | 120 | 5.2 | 101 |
| | 24 hr. @ 4° C | 2253 | 100 | 6.5 | 101 | 6 | 116 |
| TIMP-1 (ng/mL) | Control | 17 | 100 | 349 | 100 | 72 | 100 |
| | 2 hr @ room temp | 17 | 98 | 311 | 89 | 70 | 98 |
| | 2 hr. @ 4° C | 16 | 94 | 311 | 89 | 68 | 95 |
| | 4 hr @ room temp | 17 | 97 | 306 | 88 | 68 | 95 |
| | 4 hr. @ 4° C | 16 | 93 | 329 | 94 | 74 | 103 |
| | 24 hr. @ 4° C | 18 | 105 | 349 | 100 | 72 | 100 |
| A-1 M | Control | 3.6 | 100 | 2.2 | 100 | 1 | 100 |

| Analyte | Storage Time/ Temp | Urine Sample | | Serum Sample | | Plasma Sample | |
|------------------|-----------------------|--------------|--------------|--------------|--------------|---------------|--------------|
| | | Sample Conc. | Recovery (%) | Sample Conc. | Recovery (%) | Sample Conc. | Recovery (%) |
| (µg/mL) | 2 hr @ room temp | 3.5 | 95 | 2 | 92 | 1 | 105 |
| | 2 hr. @ 4° C | 3.4 | 92 | 2.1 | 97 | 0.99 | 99 |
| | 4 hr @ room temp | 3.2 | 88 | 2.2 | 101 | 0.99 | 96 |
| | 4 hr. @ 4° C | 3 | 82 | 2.2 | 99 | 0.97 | 98 |
| | 24 hr. @ 4° C | 3 | 83 | 2.2 | 100 | 1 | 101 |
| THP (µg/mL) | Control | 1.2 | 100 | 34 | 100 | 2.1 | 100 |
| | 2 hr @ room temp | 1.2 | 99 | 34 | 99 | 2 | 99 |
| | 2 hr. @ 4° C | 1.1 | 90 | 34 | 100 | 2 | 98 |
| | 4 hr @ room temp | 1.1 | 88 | 27 | 80 | 2 | 99 |
| | 4 hr. @ 4° C | 0.95 | 79 | 33 | 97 | 2 | 95 |
| | 24 hr. @ 4° C | 0.91 | 76 | 33 | 98 | 2.4 | 116 |
| TFF-3 (µg/mL) | Control | 1230 | 100 | 188 | 100 | 2240 | 100 |
| | 2 hr @ room temp | 1215 | 99 | 179 | 95 | 2200 | 98 |
| | 2 hr. @ 4° C | 1200 | 98 | 195 | 104 | 2263 | 101 |
| | 4 hr @ room temp | 1160 | 94 | 224 | 119 | 2097 | 94 |
| | 4 hr. @ 4° C | 1020 | 83 | 199 | 106 | 2317 | 103 |
| | 24 hr. @ 4° C | 1030 | 84 | 229 | 122 | 1940 | 87 |

[0127] The results of this experiment demonstrated that the analytes associated with renal disorders tested were suitably stable over several freeze/thaw cycles, and up to 24 hr of storage at a temperature of 4° C.

Example 8: Analysis of kidney biomarkers in plasma and urine from patients with renal injury.

[0128] A screen for potential protein biomarkers in relation to kidney toxicity/damage was performed using a panel of biomarkers, in a set of urine and plasma samples from patients with documented renal damage. The investigated patient groups included diabetic nephropathy (DN), obstructive uropathy (OU), analgesic abuse (AA) and glomerulonephritis (GN) along with age, gender and BMI matched control groups. Multiplexed immunoassays were applied in order to quantify the following protein analytes: Alpha-1 Microglobulin (α 1M), KIM-1, Microalbumin, Beta-2-Microglobulin (β 2M), Calbindin, Clusterin, CystatinC, TreFoilFactor-3 (TFF-3), CTGF, GST-alpha, VEGF, Calbindin, Osteopontin, Tamm-HorsfallProtein (THP), TIMP-1 and NGAL.

[0129] Li-Heparin plasma and mid-stream spot urine samples were collected from four different patient groups. Samples were also collected from age, gender and BMI matched control subjects. 20 subjects were included in each group resulting in a total number of 160 urine and plasma samples. All samples were stored at -80°C before use. Glomerular filtration rate for all samples was estimated using two different estimations (Modification of Diet in Renal Disease or MDRD, and the Chronic Kidney Disease Epidemiology Collaboration or CKD-EPI) to outline the eGFR (estimated glomerular filtration rate) distribution within each patient group (**FIG. 1**). Protein analytes were quantified in human plasma and urine using multiplexed immunoassays in the Luminex xMAP™ platform. The microsphere-based multiplex immunoassays consist of antigen-specific antibodies and optimized reagents in a capture-sandwich format. Output data was given as g/ml calculated from internal standard curves. Because urine creatinine (uCr) correlates with renal filtration rate, data analysis was performed without correction for uCr. Univariate and multivariate data

analysis was performed comparing all case vs. control samples as well as cases vs. control samples for the various disease groups.

[0130] The majority of the measured proteins showed a correlation to eGFR. Measured variables were correlated to eGFR using Pearson's correlations coefficient, and samples from healthy controls and all disease groups were included in the analysis. 11 and 7 proteins displayed *P*-values below 0.05 for plasma and urine (**Table 9**) respectively.

Table 9: Correlation analysis of eGFR and variables for all case samples

| Variable | URINE | | Variable | PLASMA | |
|-----------------------|-------------|-------------------|-----------------------|-------------|-------------------|
| | Pearson's r | P-Value | | Pearson's r | P-Value |
| Alpha-1-Microglobulin | -0.08 | 0.3 | Alpha-1-Microglobulin | -0.33 | <0.0001 |
| Beta-2-Microglobulin | -0.23 | <i>0.003</i> | Beta-2-Microglobulin | -0.39 | <0.0001 |
| Calbindin | -0.16 | 0.04 | Calbindin | -0.18 | <0.02 |
| Clusterin | -0.07 | 0.4 | Clusterin | -0.51 | <0.0001 |
| CTGF | -0.08 | 0.3 | CTGF | -0.05 | 0.5 |
| Creatinine | -0.32 | <0.0001 | Cystatin-C | -0.42 | <0.0001 |
| Cystatin-C | -0.24 | <i>0.002</i> | GST-alpha | -0.12 | 0.1 |
| GST-alpha | -0.11 | 0.2 | KIM-1 | -0.17 | 0.03 |
| KIM-1 | -0.08 | 0.3 | NGAL | -0.28 | <i><0.001</i> |
| Microalbumin_UR | -0.17 | 0.03 | Osteopontin | -0.33 | <0.0001 |
| NGAL | -0.15 | 0.07 | THP | -0.31 | <0.0001 |
| Osteopontin | -0.19 | 0.02 | TIMP-1 | -0.28 | <i><0.001</i> |
| THP | -0.05 | 0.6 | TFF3 | -0.38 | <0.0001 |
| TIMP-1 | -0.19 | 0.01 | VEGF | -0.14 | 0.08 |
| TFF2 | -0.09 | 0.3 | | | |
| VEGF | -0.07 | 0.4 | | | |

P values <0.0001 are shown in bold italics

P values <0.005 are shown in bold

P values <0.05 are shown in italics

[0131] For the various disease groups, univariate statistical analysis revealed that in a direct comparison (T-test) between cases and controls, a number of proteins were differentially expressed in both urine and plasma (**Table 10** and **FIG. 2**). In particular, clusterin showed a marked differential pattern in plasma.

Table 10: Differentially regulated proteins by

| univariate statistical analysis | | | |
|--|---------------|-----------------------|----------------|
| Group | Matrix | Protein | p-value |
| AA | Urine | Calbindin | 0.016 |
| AA | Urine | NGAL | 0.04 |
| AA | Urine | Osteopontin | 0.005 |
| AA | Urine | Creatinine | 0.001 |
| AA | Plasma | Calbindin | 0.05 |
| AA | Plasma | Clusterin | 0.003 |
| AA | Plasma | KIM-1 | 0.03 |
| AA | Plasma | THP | 0.001 |
| AA | Plasma | TIMP-1 | 0.02 |
| | | | |
| DN | Urine | Creatinine | 0.04 |
| DN | Plasma | Clusterin | 0.006 |
| DN | Plasma | KIM-1 | 0.01 |
| | | | |
| GN | Urine | Creatinine | 0.004 |
| GN | Urine | Microalbumin | 0.0003 |
| GN | Urine | NGAL | 0.05 |
| GN | Urine | Osteopontin | 0.05 |
| GN | Urine | TFF3 | 0.03 |
| GN | Plasma | Alpha 1 Microglobulin | 0.002 |
| GN | Plasma | Beta 2 Microglobulin | 0.03 |
| GN | Plasma | Clusterin | 0.00 |
| GN | Plasma | Cystatin C | 0.01 |
| GN | Plasma | KIM-1 | 0.003 |
| GN | Plasma | NGAL | 0.03 |
| GN | Plasma | THP | 0.001 |
| GN | Plasma | TIMP-1 | 0.003 |
| GN | Plasma | TFF3 | 0.01 |
| GN | Plasma | VEGF | 0.02 |
| | | | |
| OU | Urine | Clusterin | 0.02 |
| OU | Urine | Microalbumin | 0.007 |
| OU | Plasma | Clusterin | 0.00 |

[0132] Application of multivariate analysis yielded statistical models that predicted disease from control samples (**FIG. 3**).

[0133] In conclusion, these results form a valuable base for further studies on these biomarkers in urine and plasma both regarding baseline levels in normal populations and regarding the differential expression of the analytes in various disease

groups. Using this panel of analytes, error rates from adaboosting and/or random forest were low enough (<10%) to allow a prediction model to differentiate between control and disease patient samples. Several of the analytes showed a greater correlation to eGFR in plasma than in urine.

Example 9: Statistical analysis of kidney biomarkers in plasma and urine from patients with renal injury.

[0134] Urine and plasma samples were taken from 80 normal control group subjects and 20 subjects from each of four disorders: analgesic abuse, diabetic nephropathy, glomerulonephritis, and obstructive uropathy. The samples were analyzed for the quantity and presence of 16 different proteins (alpha-1 microglobulin (α 1M), beta-2 microglobulin (β 2M), calbindin, clusterin, CTGF, creatinine, cystatin C, GST-alpha, KIM-1, microalbumin, NGAL, osteopontin, THP, TIMP-1, TFF-3, and VEGF) as described in Example 1 above. The goal was to determine the analytes that distinguish between a normal sample and a diseased sample, a normal sample and an obstructive uropathy (OU) sample, and finally, an glomerulonephritis sample from the other disease samples (diabetic nephropathy (DN), analgesic abuse (AA), and glomerulonephritis (GN)).

[0135] From the above protein analysis data, bootstrap analysis was used to estimate the future performance of several classification algorithms. For each bootstrap run, training data and testing data was randomly generated. Then, the following algorithms were applied on the training data to generate models and then apply the models to the testing data to make predictions: automated Matthew's classification algorithm, classification and regression tree (CART), conditional inference tree, bagging, random forest, boosting, logistic regression, SVM, and Lasso. The accuracy rate and ROC areas were recorded for each method on the prediction of the testing data. The above was repeated 100 times. The mean and the standard deviation of the accuracy rates and of the ROC areas were calculated.

[0136] The mean error rates and AUROC were calculated from urine and AUROC was calculated from plasma for 100 runs of the above method for each of the following comparisons: disease (AA + GN + OU + DN) vs. normal (**FIG. 4, Table 11**),

GN vs. normal (**FIG. 6, Table 13**), GN vs. AA (**FIG. 8, Table 15**), OU vs. GN (**FIG. 10, Table 17**), and GN vs. DN (**FIG. 12, Table 19**).

[0137] The average relative importance of 16 different analytes (alpha-1 microglobulin, beta-2 microglobulin, calbindin, clusterin, CTGF, creatinine, cystatin C, GST-alpha, KIM-1, microalbumin, NGAL, osteopontin, THP, TIMP-1, TFF-3, and VEGF) and 4 different clinical variables (weight, BMI, age, and gender) from 100 runs were analyzed with two different statistical methods – random forest (plasma and urine samples) and boosting (urine samples) – for each of the following comparisons: disease (AA + GN + OU + DN) vs. normal (**FIG. 5, Table 12**), GN vs. normal (**FIG. 7, Table 14**), GN vs. AA (**FIG. 9, Table 16**), OU vs. GN (**FIG. 11, Table 18**), and GN vs. DN (**FIG. 13, Table 20**).

Table 11: Disease v. Normal

| method | Mean AUROC | Standard deviation AUROC |
|---------------------|------------|--------------------------|
| random forest | 0.931 | 0.039 |
| bagging | 0.919 | 0.045 |
| svm | 0.915 | 0.032 |
| boosting | 0.911 | 0.06 |
| lasso | 0.897 | 0.044 |
| logistic regression | 0.891 | 0.041 |
| ctree | 0.847 | 0.046 |
| cart | 0.842 | 0.032 |
| mat | 0.83 | 0.023 |

Table 12: Disease v. Normal

| analyte | relative importance |
|-----------------|---------------------|
| Creatinine | 11.606 |
| Kidney_Injury_M | 8.486 |
| Tamm_Horsfall_P | 8.191 |
| Total_Protein | 6.928 |
| Osteopontin | 6.798 |
| Neutrophil_Gela | 6.784 |
| Tissue_Inhibito | 6.765 |
| Vascular_Endoth | 6.716 |
| Trefoil_Factor_ | 6.703 |
| Cystatin_C | 6.482 |
| Alpha_1_Microgl | 6.418 |
| Beta_2_Microglo | 6.228 |
| Glutathione_S_T | 6.053 |
| clusterin | 5.842 |

Table 13: GN v. NL

| method | Mean AUROC | Standard deviation of AUROC |
|---------------|------------|-----------------------------|
| boosting | 0.958 | 0.057 |
| random forest | 0.958 | 0.053 |
| bagging | 0.951 | 0.061 |
| lasso | 0.951 | 0.083 |
| svm | 0.916 | 0.083 |

| | | |
|---------------------|-------|-------|
| matt | 0.887 | 0.096 |
| logistic regression | 0.853 | 0.113 |
| ctree | 0.836 | 0.117 |
| cart | 0.822 | 0.109 |

Table 14: GN v. NL

| analyte | Relative importance |
|-----------------|---------------------|
| Total_Protein | 13.122 |
| Creatinine | 12.358 |
| Kidney_Injury_M | 10.320 |
| Tissue_Inhibito | 8.399 |
| Alpha_1_Microgl | 7.087 |
| Tamm_Horsfall_P | 7.014 |
| Osteopontin | 6.563 |
| Vascular_Endoth | 5.609 |
| Neutrophil_Gela | 5.263 |
| Trefoil_Factor_ | 5.092 |
| Cystatin_C | 5.056 |
| clusterin | 4.868 |
| Beta_2_Microglo | 4.798 |
| Glutathione_S_T | 4.451 |

Table 15: GN v. AA

| method | Mean AUROC | Standard deviation of |
|--------|------------|-----------------------|
|--------|------------|-----------------------|

| | | AUROC |
|---------------------|-------|-------|
| svm | 0.689 | 0.11 |
| boosting | 0.675 | 0.102 |
| bagging | 0.674 | 0.106 |
| random forest | 0.66 | 0.096 |
| matt | 0.631 | 0.085 |
| cart | 0.626 | 0.089 |
| logistic regression | 0.614 | 0.091 |
| lasso | 0.606 | 0.102 |
| ctree | 0.53 | 0.061 |

Table 16: GN v. AA

| analyte | Relative importance |
|-----------------|---------------------|
| Creatinine | 10.780 |
| Alpha_1_Microgl | 8.847 |
| Kidney_Injury_M | 8.604 |
| clusterin | 8.109 |
| Total_Protein | 7.679 |
| Glutathione_S_T | 7.493 |
| Neutrophil_Gela | 6.721 |
| Vascular_Endoth | 6.461 |
| Cystatin_C | 6.444 |
| Beta_2_Microglo | 6.261 |
| Trefoil_Factor_ | 6.184 |

| | |
|-----------------|-------|
| Tamm_Horsfall_P | 5.872 |
| Tissue_Inhibito | 5.690 |
| Osteopontin | 4.855 |

Table 17: OU v. GN

| method | Mean AUROC | Standard deviation of AUROC |
|---------------------|------------|-----------------------------|
| random forest | 0.946 | 0.061 |
| boosting | 0.941 | 0.071 |
| bagging | 0.933 | 0.078 |
| svm | 0.932 | 0.07 |
| lasso | 0.888 | 0.092 |
| cart | 0.879 | 0.091 |
| ctree | 0.879 | 0.104 |
| mat | 0.872 | 0.099 |
| logistic regression | 0.869 | 0.134 |

Table 18: OU v. GN

| analyte | Relative importance |
|-----------------|---------------------|
| Total_Protein | 14.391 |
| Alpha_1_Microgl | 11.033 |
| Kidney_Injury_M | 8.453 |
| Tissue_Inhibito | 7.934 |
| Creatinine | 7.714 |

| | |
|-----------------|-------|
| clusterin | 7.353 |
| Osteopontin | 7.123 |
| Vascular_Endoth | 6.321 |
| Neutrophil_Gela | 6.244 |
| Cystatin_C | 5.632 |
| Beta_2_Microglo | 4.962 |
| Tamm_Horsfall_P | 4.829 |
| Trefoil_Factor_ | 4.594 |
| Glutathione_S_T | 3.418 |

Table 19: GN v. DN

| method | Mean AUROC | Standard deviation of AUROC |
|---------------------|------------|-----------------------------|
| lasso | 0.955 | 0.077 |
| random forest | 0.912 | 0.076 |
| bagging | 0.906 | 0.087 |
| boosting | 0.904 | 0.087 |
| svm | 0.887 | 0.089 |
| ctree | 0.824 | 0.095 |
| matt | 0.793 | 0.114 |
| logistic regression | 0.788 | 0.134 |
| cart | 0.768 | 0.1 |

Table 20: GN v. DN

| analyte | Relative importance |
|-----------------|---------------------|
| Total_Protein | 13.122 |
| Creatinine | 11.028 |
| Alpha_1_Microgl | 8.291 |
| Beta_2_Microglo | 7.856 |
| Tissue_Inhibito | 7.799 |
| Glutathione_S_T | 6.532 |
| Kidney_Injury_M | 6.489 |
| Osteopontin | 6.424 |
| Vascular_Endoth | 6.262 |
| Neutrophil_Gela | 5.418 |
| Trefoil_Factor_ | 5.382 |
| Cystatin_C | 5.339 |
| Tamm_Horsfall_P | 5.117 |
| clusterin | 4.940 |

[0138] It should be appreciated by those of skill in the art that the techniques disclosed in the examples above represent techniques discovered by the inventors to function well in the practice of the invention. Those of skill in the art should, however, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention, therefore all matter set forth or shown in the accompanying drawings is to be interpreted as illustrative and not in a limiting sense.

CLAIMS

What is claimed is:

1. A method for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder in a mammal, the method comprising:
 - a. providing a test sample comprising a sample of bodily fluid taken from the mammal;
 - b. determining sample concentrations for sample analytes in the test sample, wherein the sample analytes are creatinine, KIM-1, TIMP-1, A1M, THP and osteopontin;
 - c. comparing the combination of sample concentrations to a data set comprising at least one entry, wherein each entry of the data set comprises a list comprising three or more minimum diagnostic concentrations indicative of glomerulonephritis or an associated disorder, wherein each minimum diagnostic concentration comprises a maximum of a range of analyte concentrations for a healthy mammal;
 - d. determining a matching entry of the dataset in which all minimum diagnostic concentrations are less than the corresponding sample concentrations; and,
 - e. identifying an indicated disorder comprising the particular disorder of the matching entry.
2. A method for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder in a mammal, the method comprising:
 - a. providing a test sample comprising a sample of bodily fluid taken from the mammal;
 - b. determining a combination of sample concentrations for three or more sample analytes in the test sample, wherein the sample analytes are selected from the group consisting of alpha-1 microglobulin, beta-2 microglobulin, calbindin, clusterin, CTGF, creatinine, cystatin C, GST-alpha, KIM-1, microalbumin, NGAL, osteopontin, THP, TIMP-1, TFF-3, and VEGF;

- c. comparing the combination of sample concentrations to a data set comprising at least one entry, wherein each entry of the data set comprises a list comprising three or more minimum diagnostic concentrations indicative of glomerulonephritis or an associated disorder, wherein each minimum diagnostic concentration comprises a maximum of a range of analyte concentrations for a healthy mammal;
 - d. determining a matching entry of the dataset in which all minimum diagnostic concentrations are less than the corresponding sample concentrations; and,
 - e. identifying an indicated disorder comprising the particular disorder of the matching entry.
3. The method of claim 2, wherein the mammal is selected from the group consisting of humans, apes, monkeys, rats, mice, dogs, cats, pigs, and livestock including cattle and oxen.
4. The method of claim 2, wherein the bodily fluid is selected from the group consisting of urine, blood, plasma, serum, saliva, semen, and tissue lysates.
5. The method of claim 2, wherein the minimum diagnostic concentration in human plasma of alpha-1 microglobulin is about 16 µg/ml, beta-2 microglobulin is about 2.2 µg/ml, calbindin is greater than about 5 ng/ml, clusterin is about 134 µg/ml, CTGF is about 16 ng/ml, cystatin C is about 1170 ng/ml, GST-alpha is about 62 ng/ml, KIM-1 is about 0.57 ng/ml, NGAL is about 375 ng/ml, osteopontin is about 25 ng/ml, THP is about 0.052 µg/ml, TIMP-1 is about 131 ng/ml, TFF-3 is about 0.49 µg/ml, and VEGF is about 855 pg/ml.
6. The method of claim 2, wherein the minimum diagnostic concentration in human sera of alpha-1 microglobulin is about 17 µg/ml, beta-2 microglobulin is about 2.6 µg/ml, calbindin is greater than about 2.6 ng/ml, clusterin is about 152 µg/ml, CTGF is greater than about 8.2 ng/ml, cystatin C is about 1250 ng/ml, GST-alpha is about 52 ng/ml, KIM-1 is greater than about 0.35 ng/ml, NGAL is about 822 ng/ml, osteopontin is about 12 ng/ml, THP is about 0.053 µg/ml, TIMP-1 is about 246 ng/ml, TFF-3 is about 0.17 µg/ml, and VEGF is about 1630 pg/ml.

7. The method of claim 2, wherein the minimum diagnostic concentration in human urine of alpha-1 microglobulin is about 233 µg/ml, beta-2 microglobulin is greater than about 0.17 µg/ml, calbindin is about 233 ng/ml, clusterin is greater than about 0.089 µg/ml, CTGF is greater than about 0.90 ng/ml, cystatin C is about 1170 ng/ml, GST-alpha is greater than about 26 ng/ml, KIM-1 is about 0.67 ng/ml, NGAL is about 81 ng/ml, osteopontin is about 6130 ng/ml, THP is about 2.6 µg/ml, TIMP-1 is greater than about 3.9 ng/ml, TFF-3 is greater than about 21 µg/ml, and VEGF is about 517 pg/ml.
8. The method of claim 2, wherein a combination of sample concentrations for six or more sample analytes in the test sample are determined.
9. The method of claim 8, wherein sample concentrations are determined for the analytes selected from the group consisting of alpha-1 microglobulin, beta-2 microglobulin, cystatin C, KIM-1, THP, and TIMP-1.
10. The method of claim 2, wherein a combination of sample concentrations for sixteen sample analytes in the test sample are determined.
11. A method for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder in a mammal, the method comprising:
 - a. providing a test sample comprising a sample of bodily fluid taken from the mammal;
 - b. determining the concentrations of three or more sample analytes in a panel of biomarkers in the test sample, wherein the sample analytes are selected from the group consisting of alpha-1 microglobulin, beta-2 microglobulin, calbindin, clusterin, CTGF, creatinine, cystatin C, GST-alpha, KIM-1, microalbumin, NGAL, osteopontin, THP, TIMP-1, TFF-3, and VEGF;
 - c. identifying diagnostic analytes in the test sample, wherein the diagnostic analytes are the sample analytes whose concentrations are statistically different from concentrations found in a control group of humans who do not suffer from glomerulonephritis or an associated disorder;

- d. comparing the combination of diagnostic analytes to a dataset comprising at least one entry, wherein each entry of the dataset comprises a combination of three or more diagnostic analytes reflective of glomerulonephritis or an associated disorder; and,
 - e. identifying the particular disorder having the combination of diagnostic analytes that essentially match the combination of sample analytes.
12. The method of claim 11, wherein the mammal is selected from the group consisting of humans, apes, monkeys, rats, mice, dogs, cats, pigs, and livestock including cattle and oxen.
13. The method of claim 11, wherein the bodily fluid is selected from the group consisting of urine, blood, plasma, serum, saliva, semen, and tissue lysates.
14. The method of claim 11, wherein the test sample is plasma and the diagnostic analytes comprise creatinine, KIM-1 and THP or creatinine, KIM-1, and TIMP-1.
15. The method of claim 11, wherein the test sample is urine and the diagnostic analytes comprise microalbumin, creatinine, and KIM-1 or microalbumin, cystatin C, and creatinine.
16. The method of claim 11, wherein the diagnostic analytes comprise creatinine, KIM-1, TIMP-1, A1M, THP, and osteopontin.
17. A method for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder in a mammal, the method comprising:
- a. providing an analyte concentration measurement device comprising three or more detection antibodies, wherein each detection antibody comprises an antibody coupled to an indicator, wherein the antigenic determinants of the antibodies are sample analytes associated with glomerulonephritis or an associated disorder, and wherein the sample analytes are selected from the group consisting of alpha-1 microglobulin, beta-2 microglobulin, calbindin, clusterin, CTGF, creatinine, cystatin C, GST-alpha, KIM-1, microalbumin, NGAL, osteopontin, THP, TIMP-1, TFF-3, and VEGF;
 - b. providing a test sample comprising three or more sample analytes and a bodily fluid taken from the mammal;

- c. contacting the test sample with the detection antibodies and allowing the detection antibodies to bind to the sample analytes;
 - d. determining the concentrations of the sample analytes by detecting the indicators of the detection antibodies bound to the sample analytes in the test sample; and,
 - e. comparing the concentrations of each sample analyte to a corresponding minimum diagnostic concentration reflective of glomerulonephritis or an associated disorder.
18. The method of claim 17, wherein the bodily fluid is selected from the group consisting of urine, blood, plasma, serum, saliva, semen, and tissue lysates.
 19. The method of claim 17, wherein the analyte concentration measurement device comprises six or more detection antibodies.
 20. The method claim 17, wherein the analyte concentration measurement device comprises sixteen detection antibodies.
 21. The method of claim 17, wherein the sample analytes are selected from the group consisting of alpha-1 microglobulin, beta-2 microglobulin, cystatin C, KIM-1, THP, and TIMP-1.
22. A method for diagnosing, monitoring, or determining glomerulonephritis or an associated disorder in a mammal, the method comprising:
 - a. providing a test sample comprising a sample of bodily fluid taken from the mammal;
 - b. determining sample concentrations for sample analytes in the test sample, wherein the sample analytes are alpha-1 microglobulin, beta-2 microglobulin, calbindin, clusterin, CTGF, creatinine, cystatin C, GST-alpha, KIM-1, microalbumin, NGAL, osteopontin, THP, TIMP-1, TFF-3, and VEGF;
 - c. comparing the combination of sample concentrations to a data set comprising at least one entry, wherein each entry of the data set comprises a list comprising three or more minimum diagnostic

- concentrations indicative of glomerulonephritis or an associated disorder, wherein each minimum diagnostic concentration comprises a maximum of a range of analyte concentrations for a healthy mammal;
- d. determining a matching entry of the dataset in which all minimum diagnostic concentrations are less than the corresponding sample concentrations; and,
 - e. identifying an indicated disorder comprising the particular disorder of the matching entry.

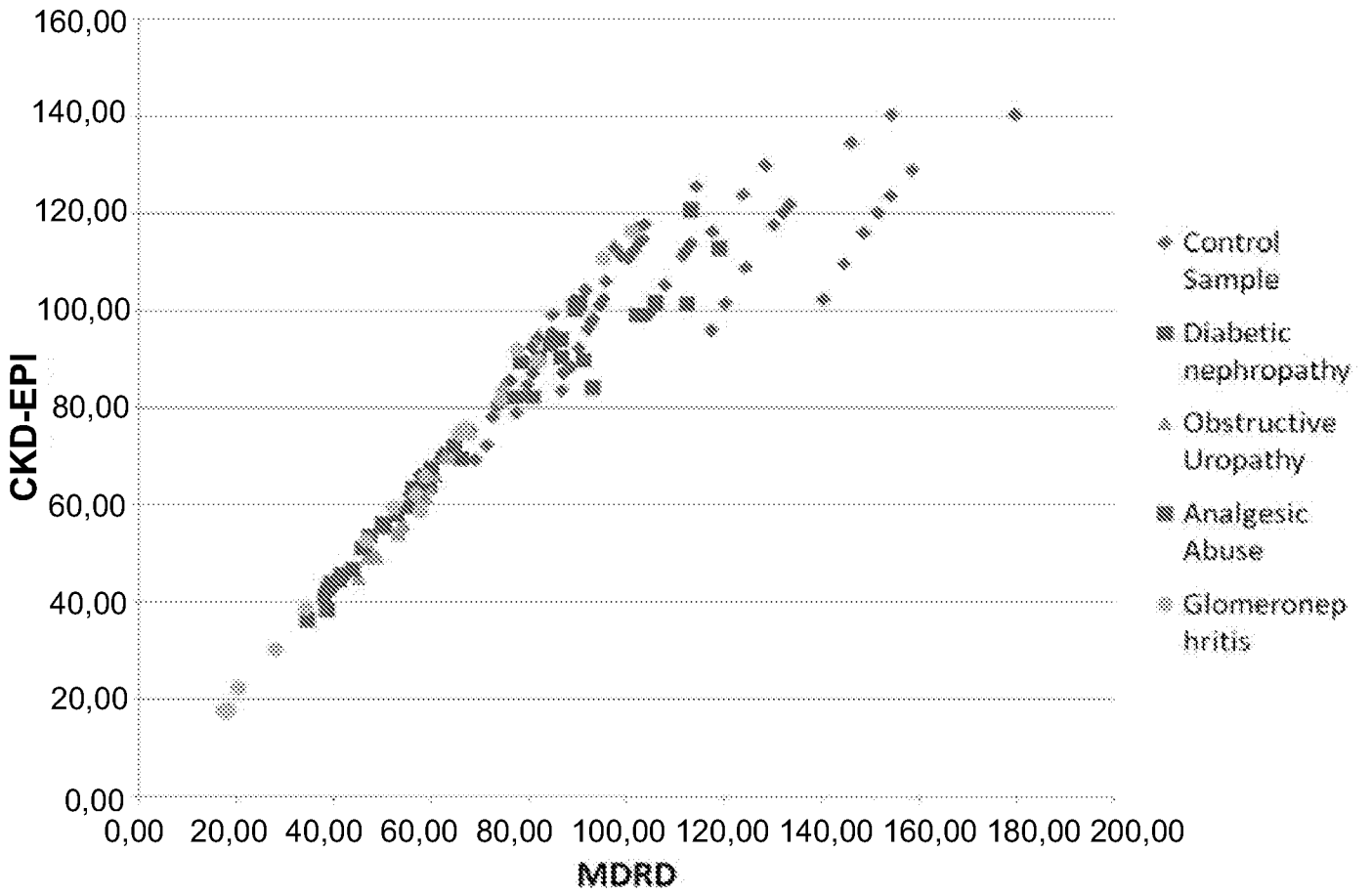
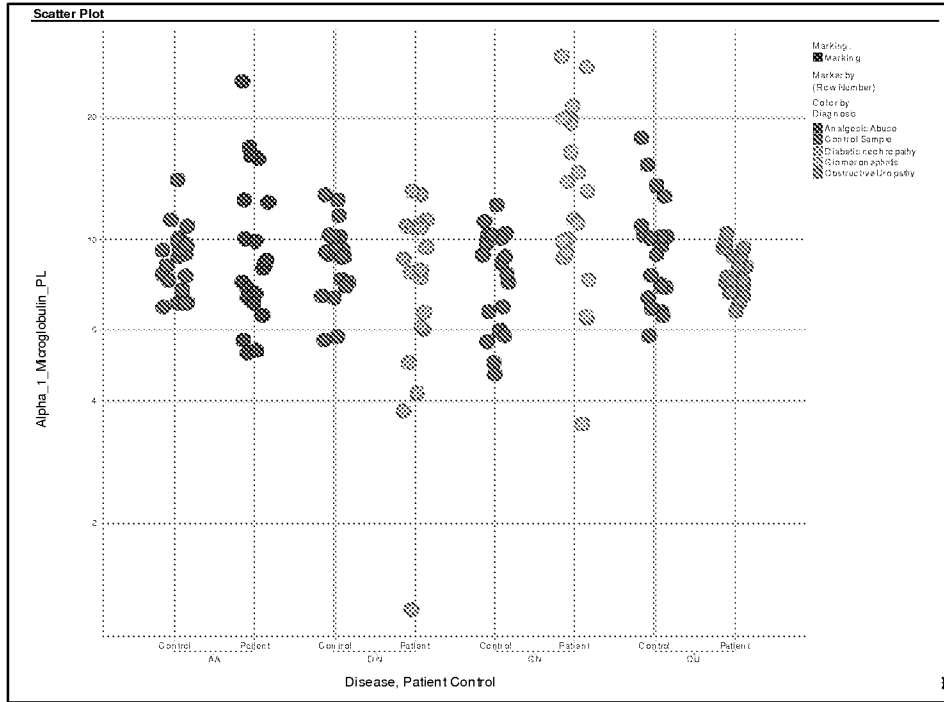


FIG. 1

A



B

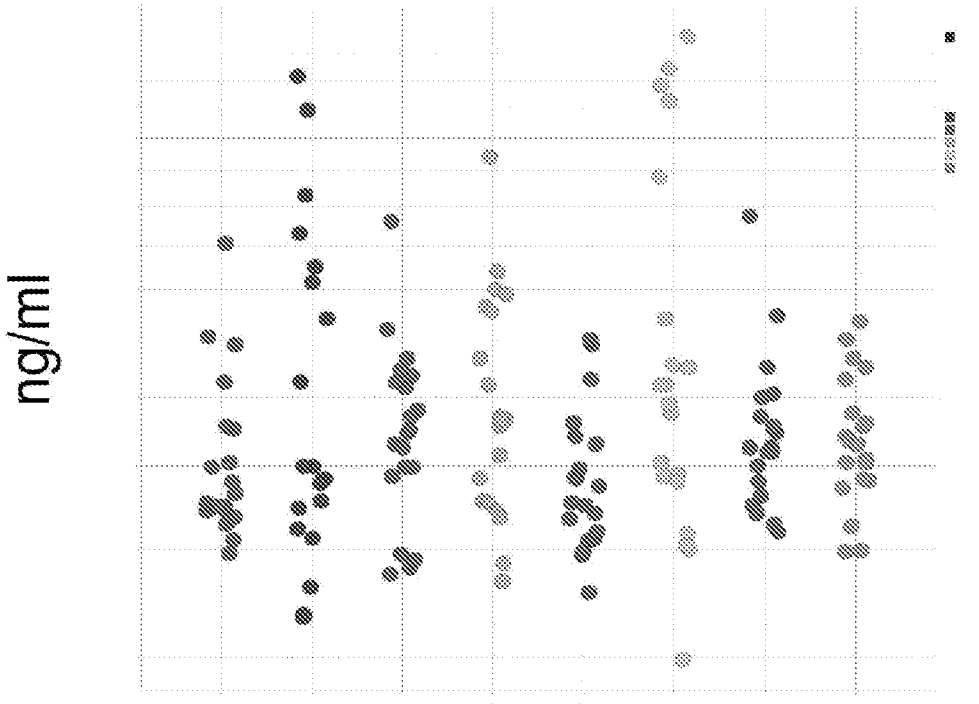
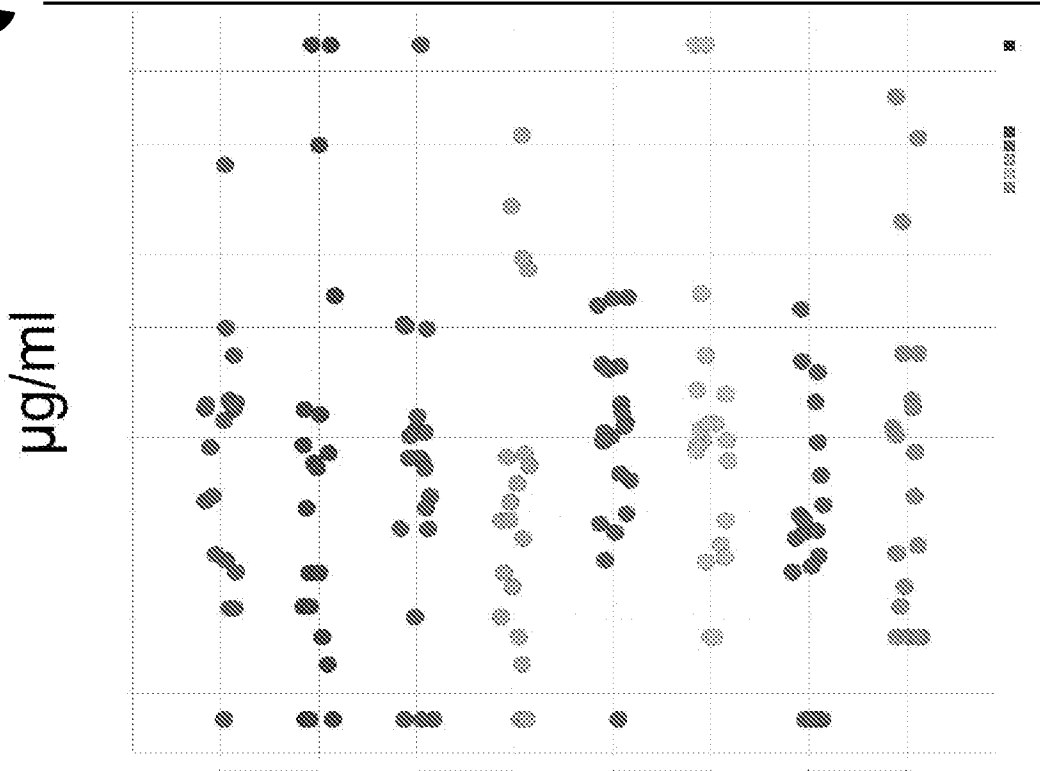


FIG. 2

C



D

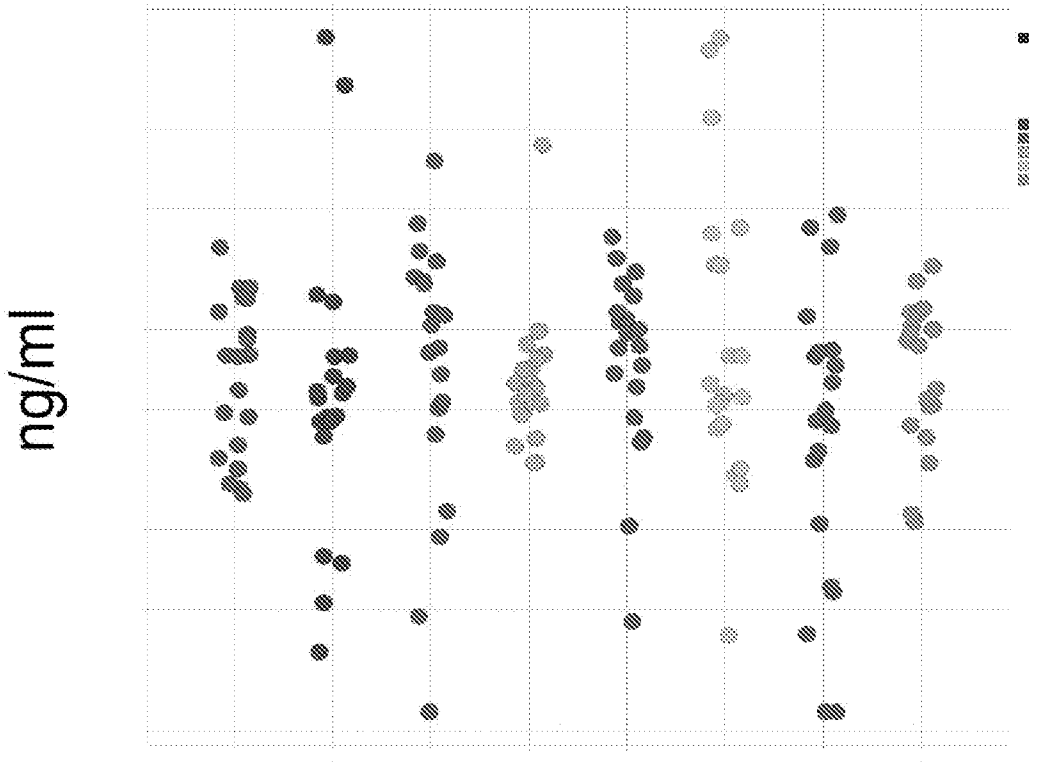


FIG. 2 CONT.

ABNORMAL vs. NORMAL kidney plasma

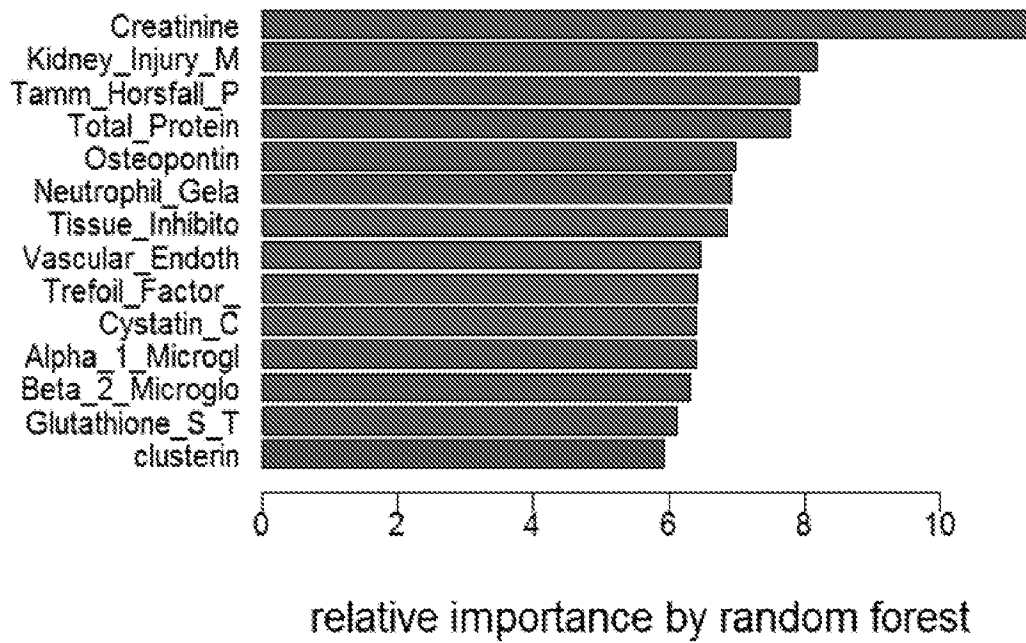


FIG. 3

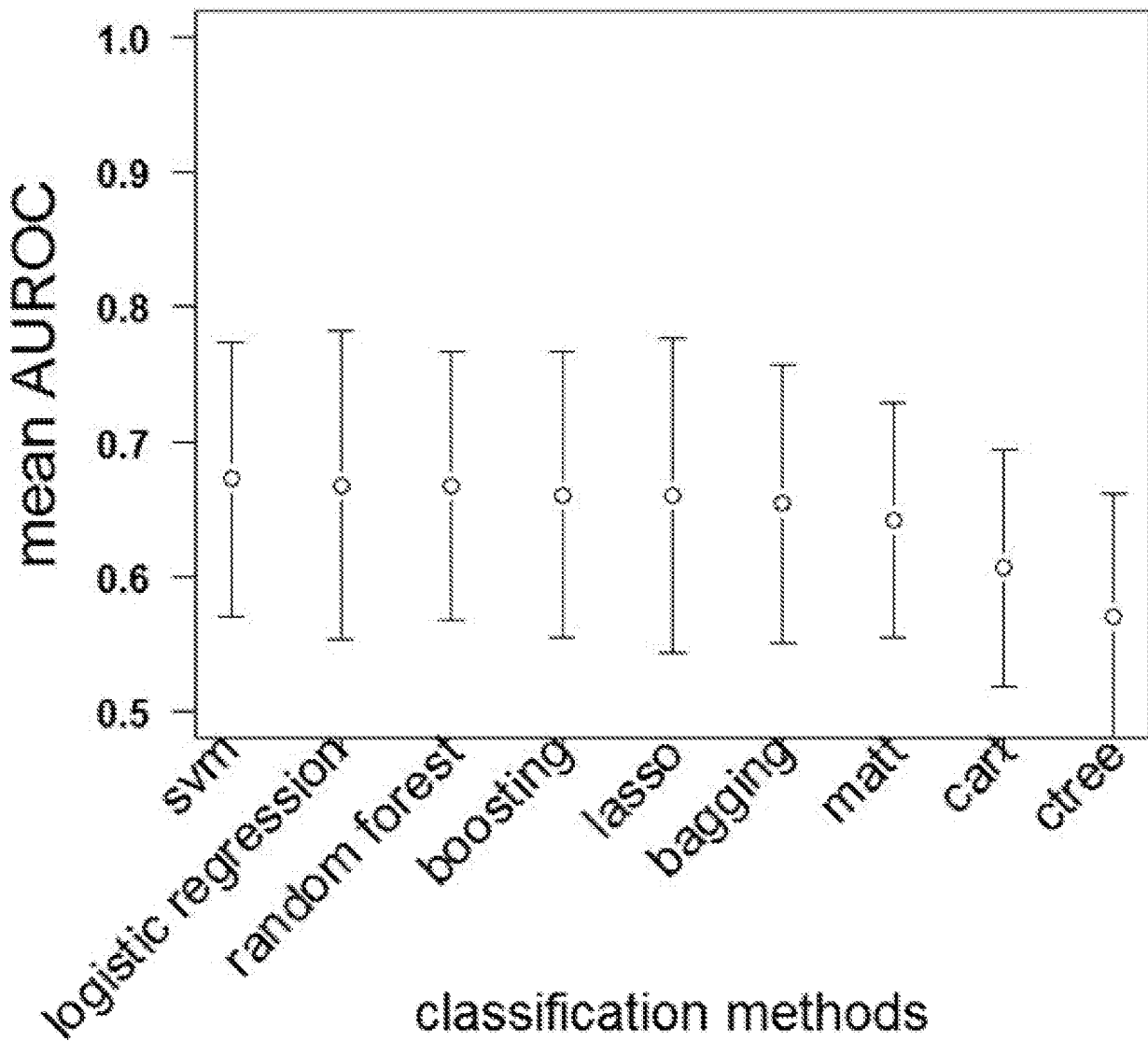


FIG. 4A

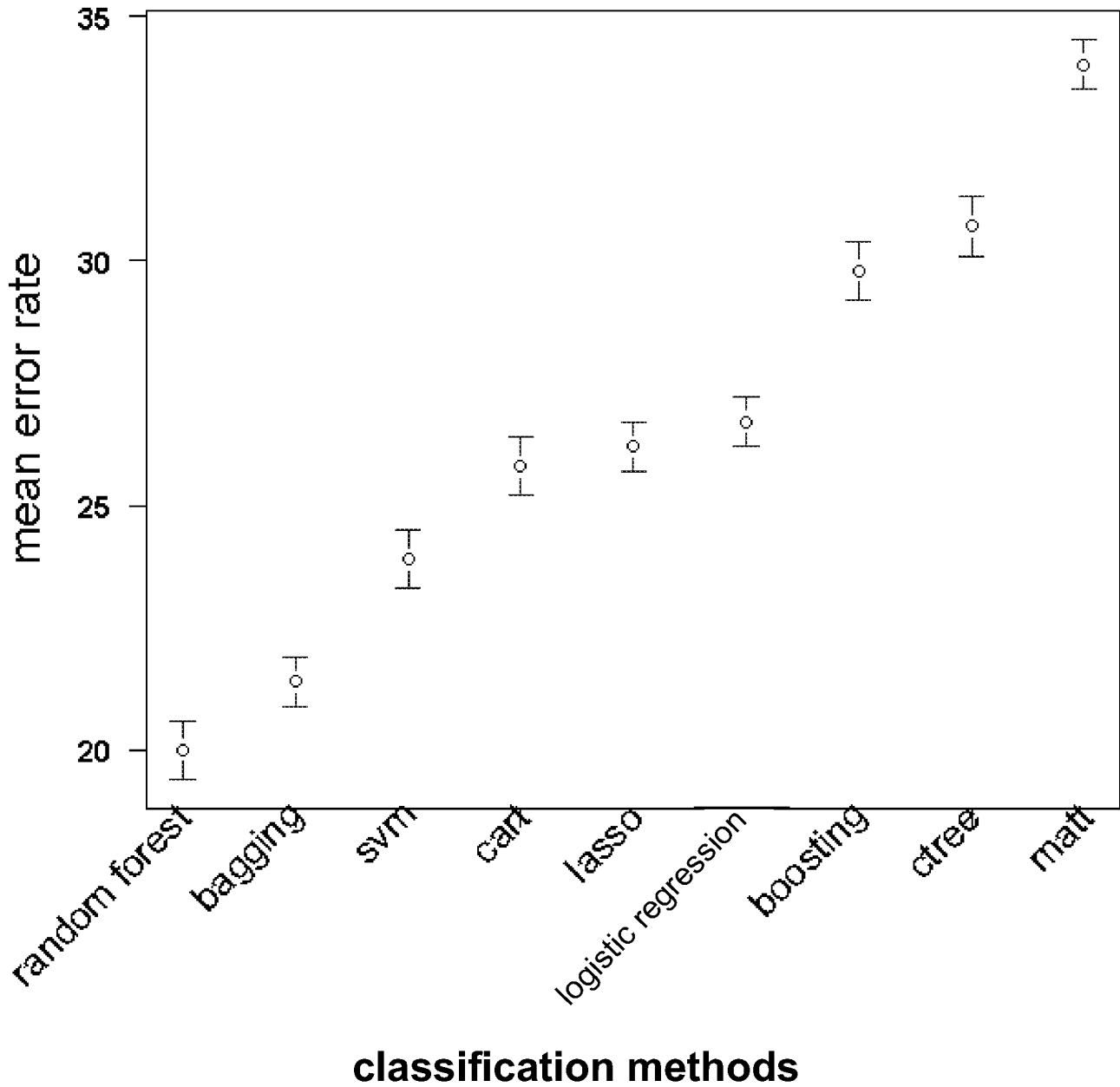


FIG. 4B

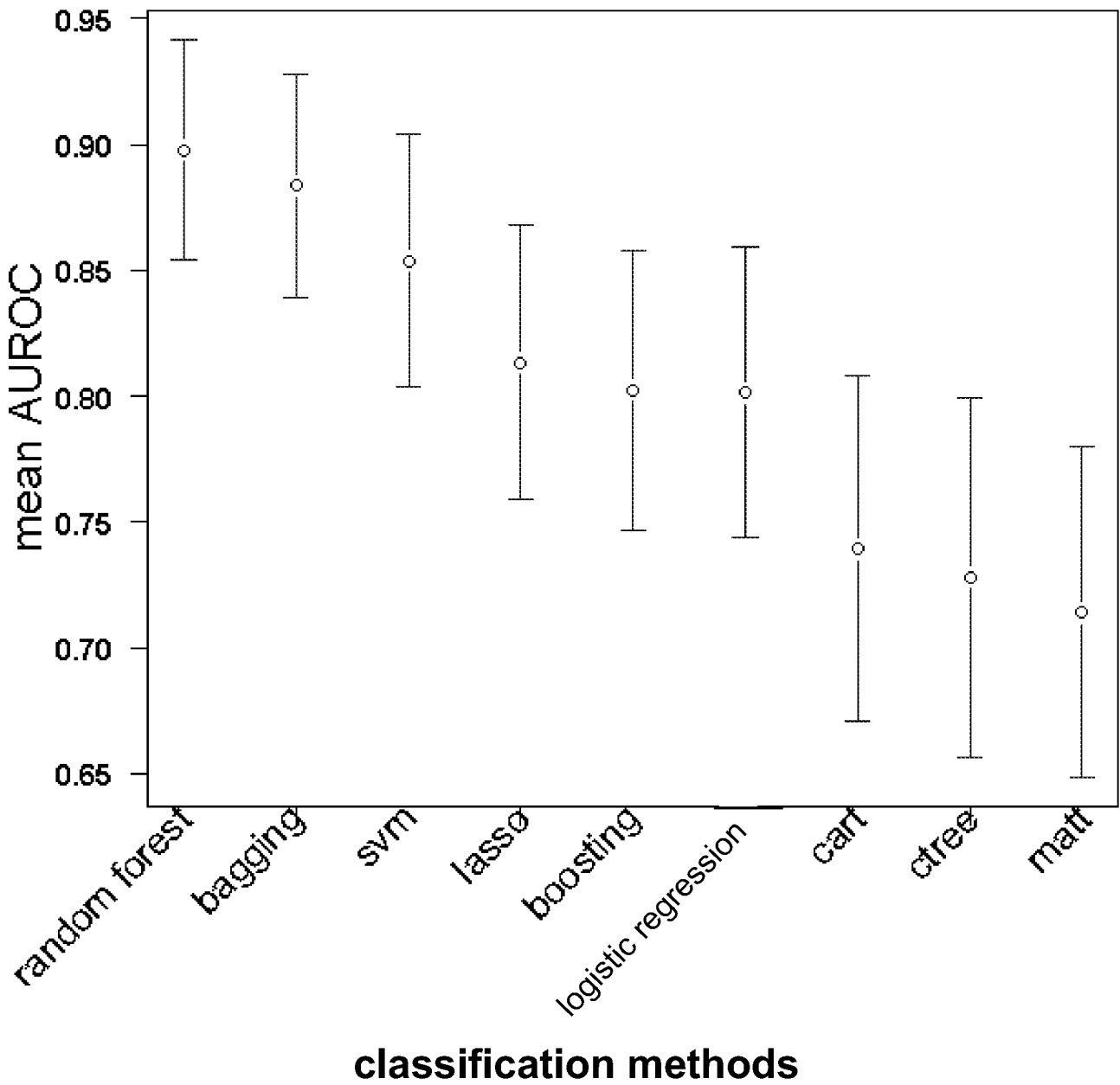


FIG. 4C

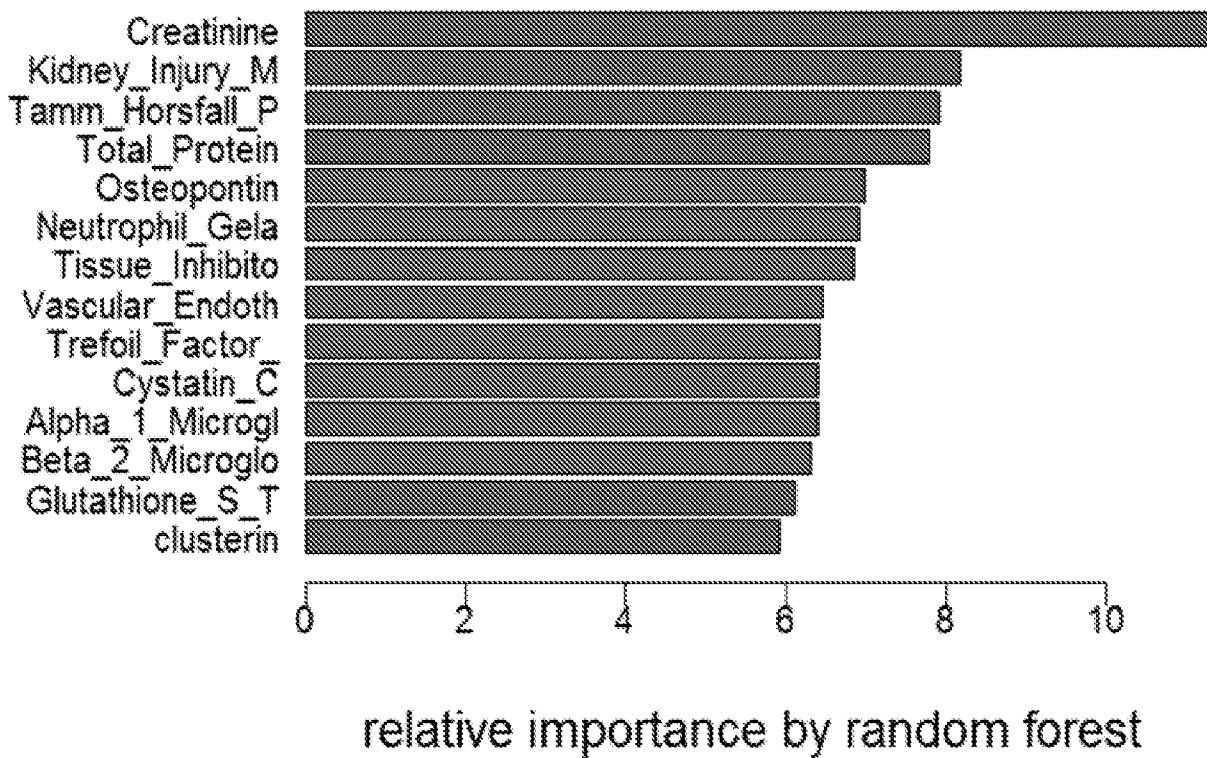
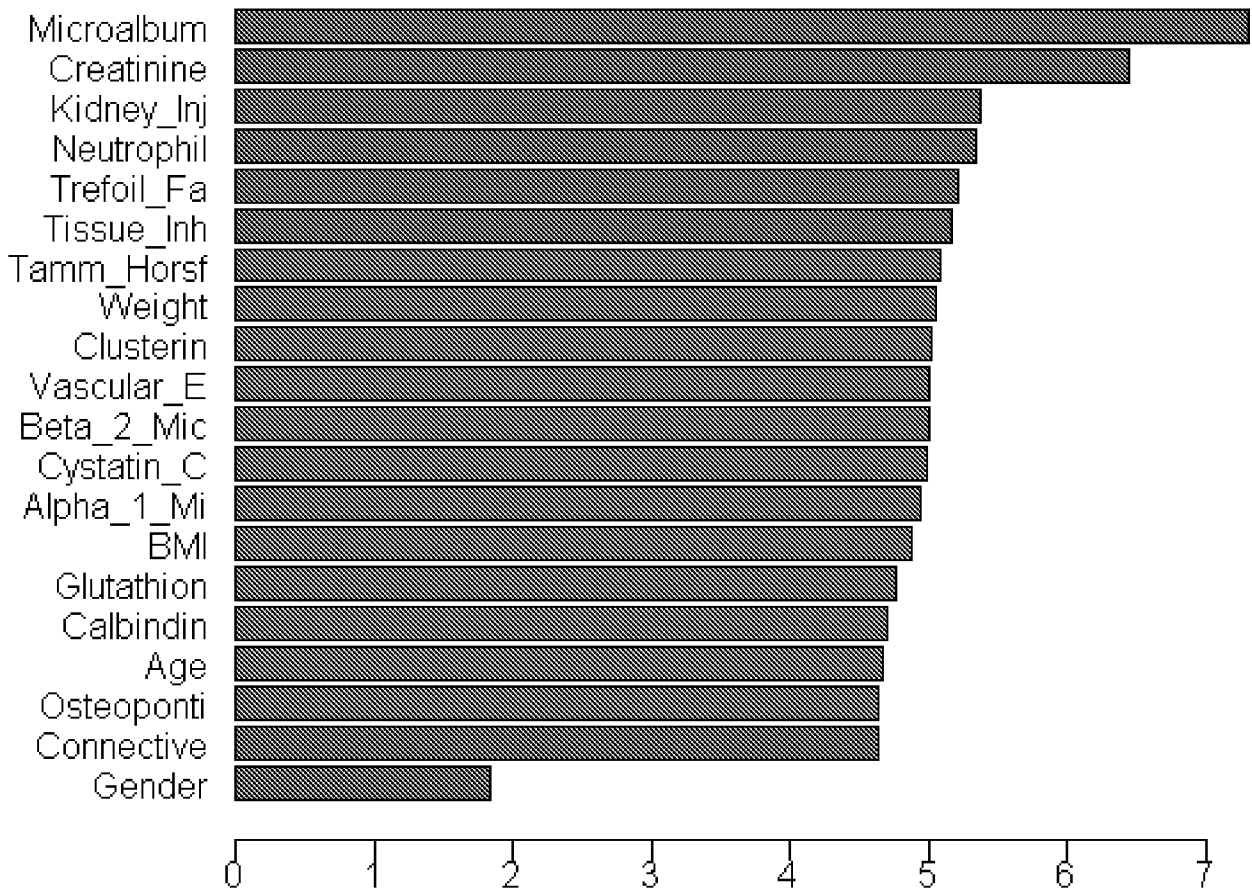


FIG. 5A



relative importance by random forest

FIG. 5B

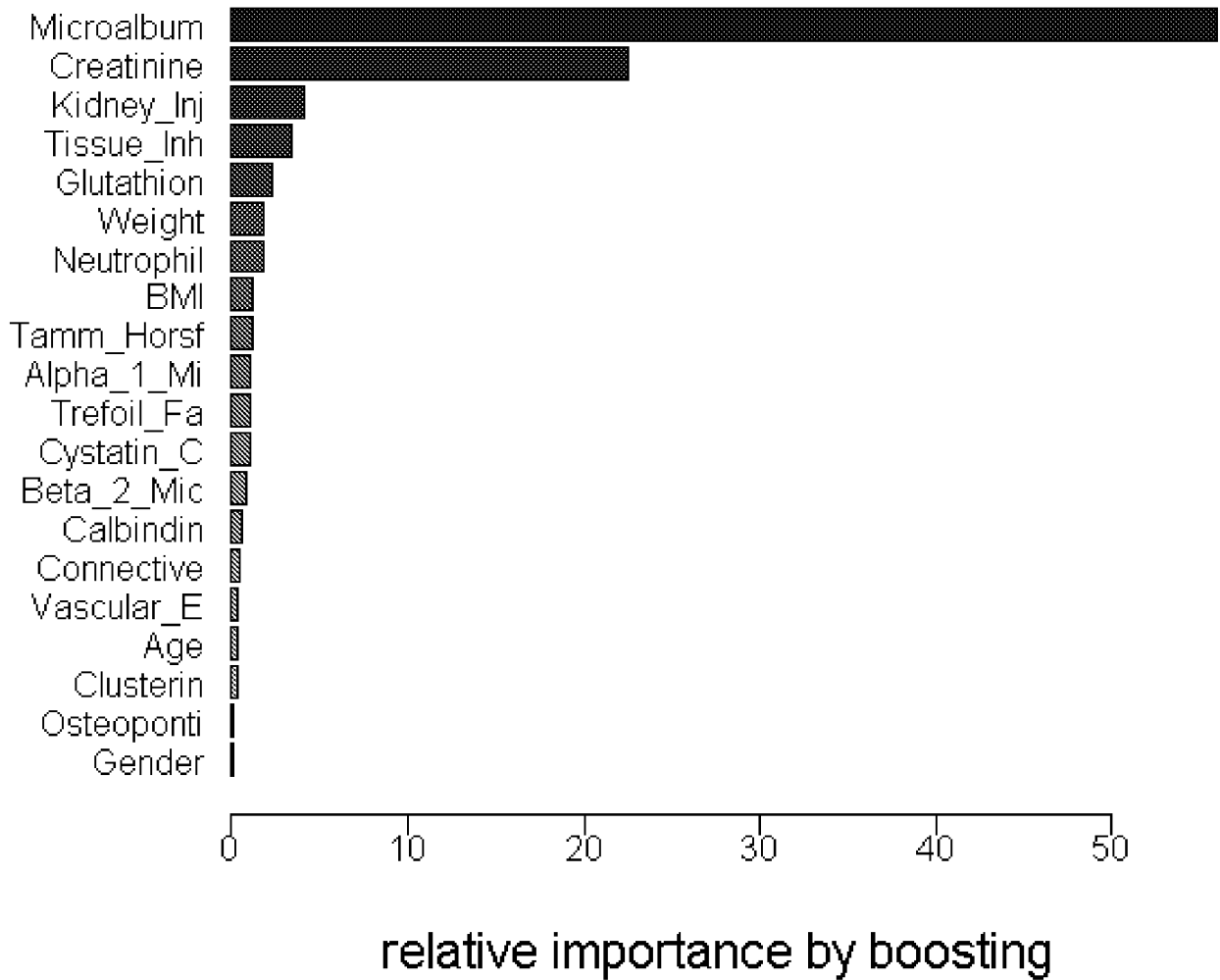


FIG. 5C

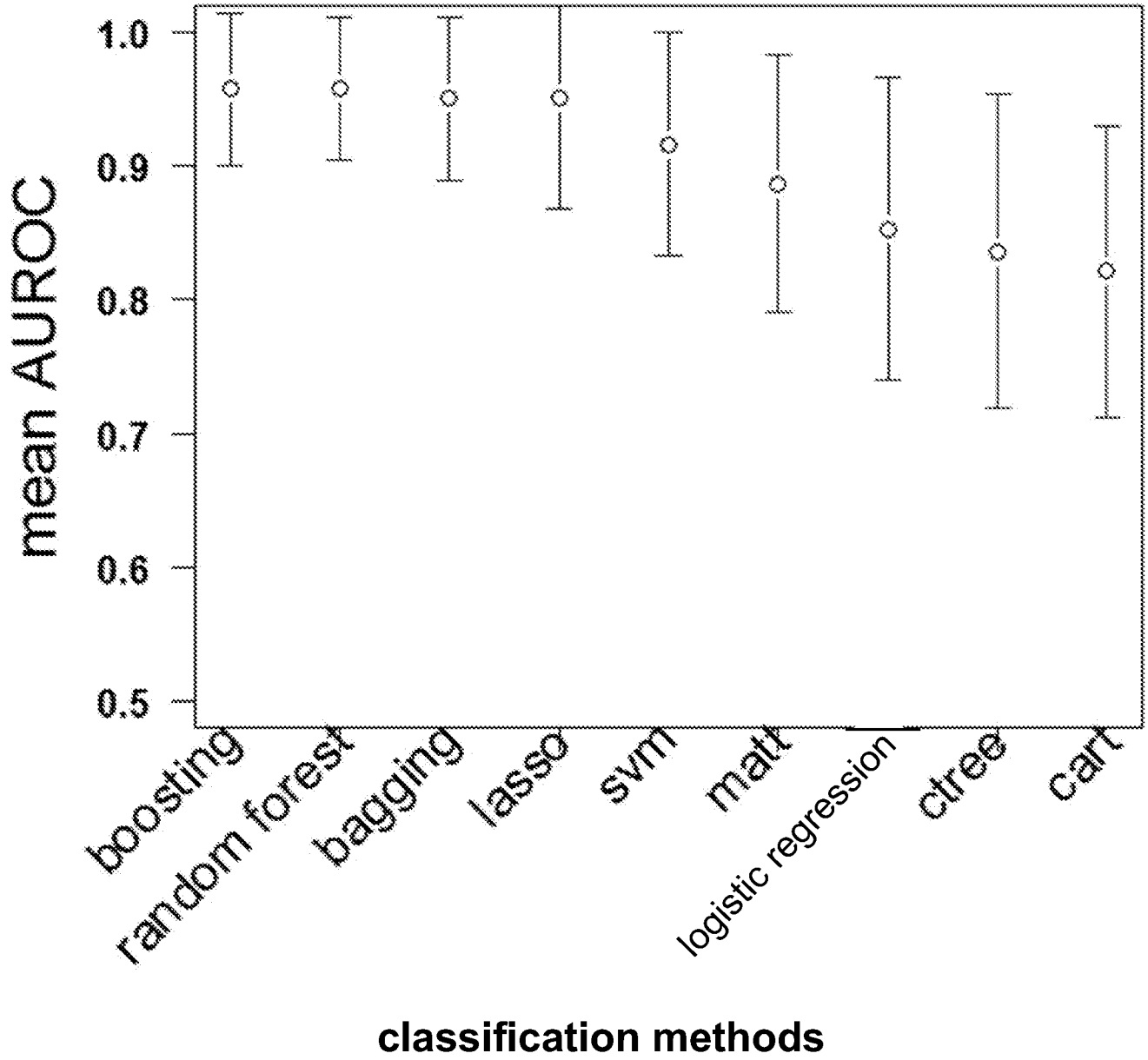


FIG. 6A

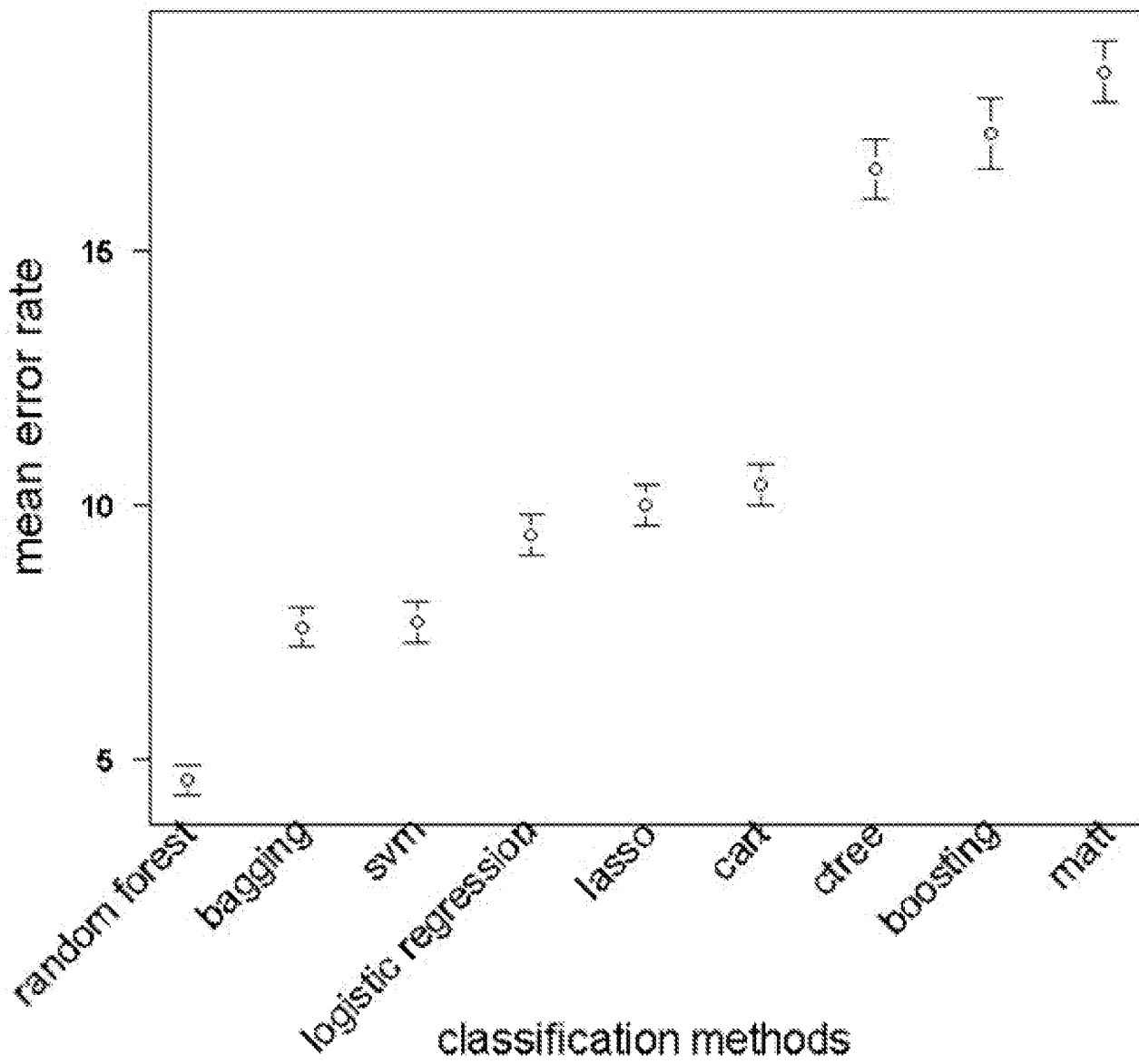


FIG. 6B

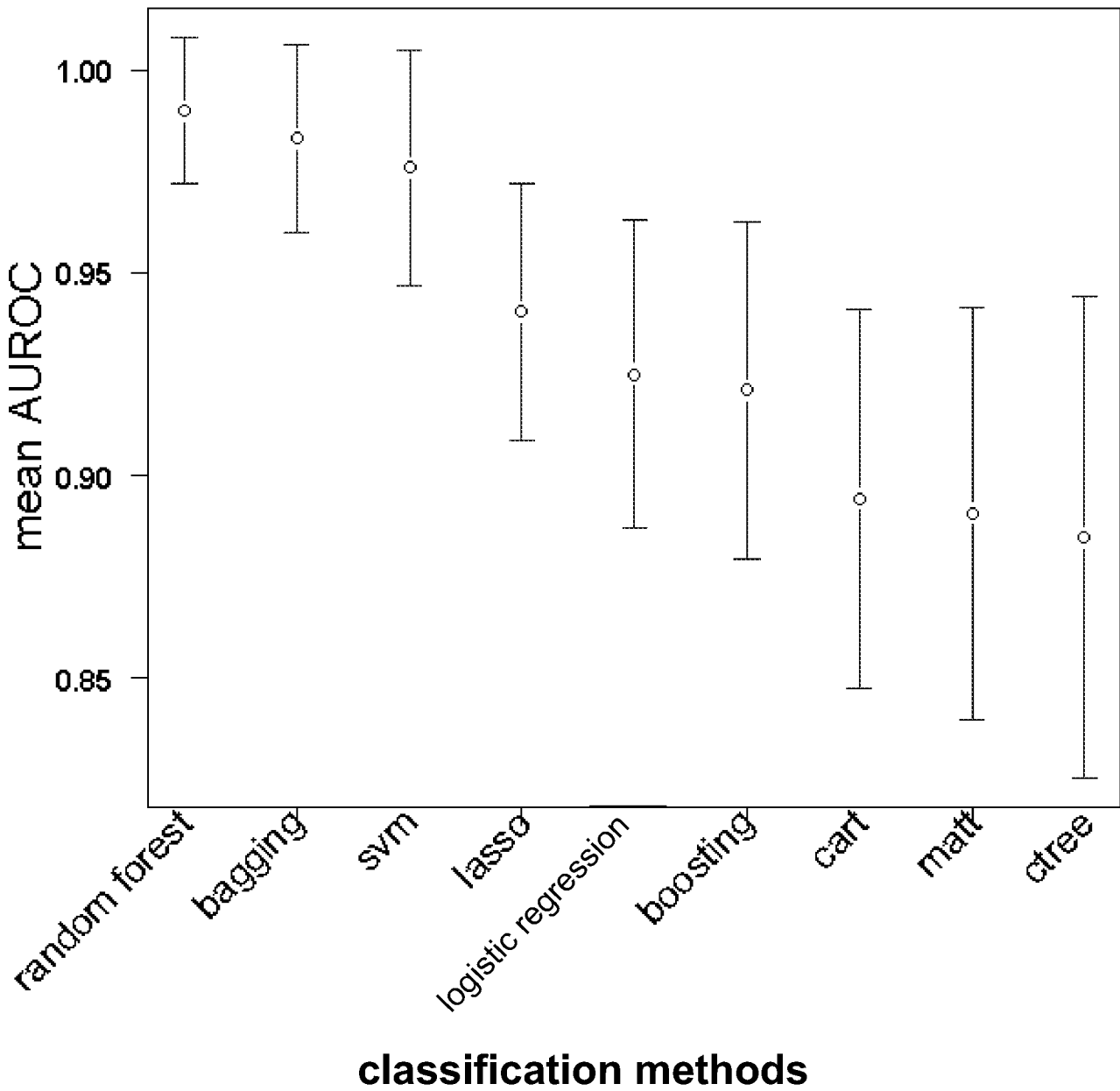
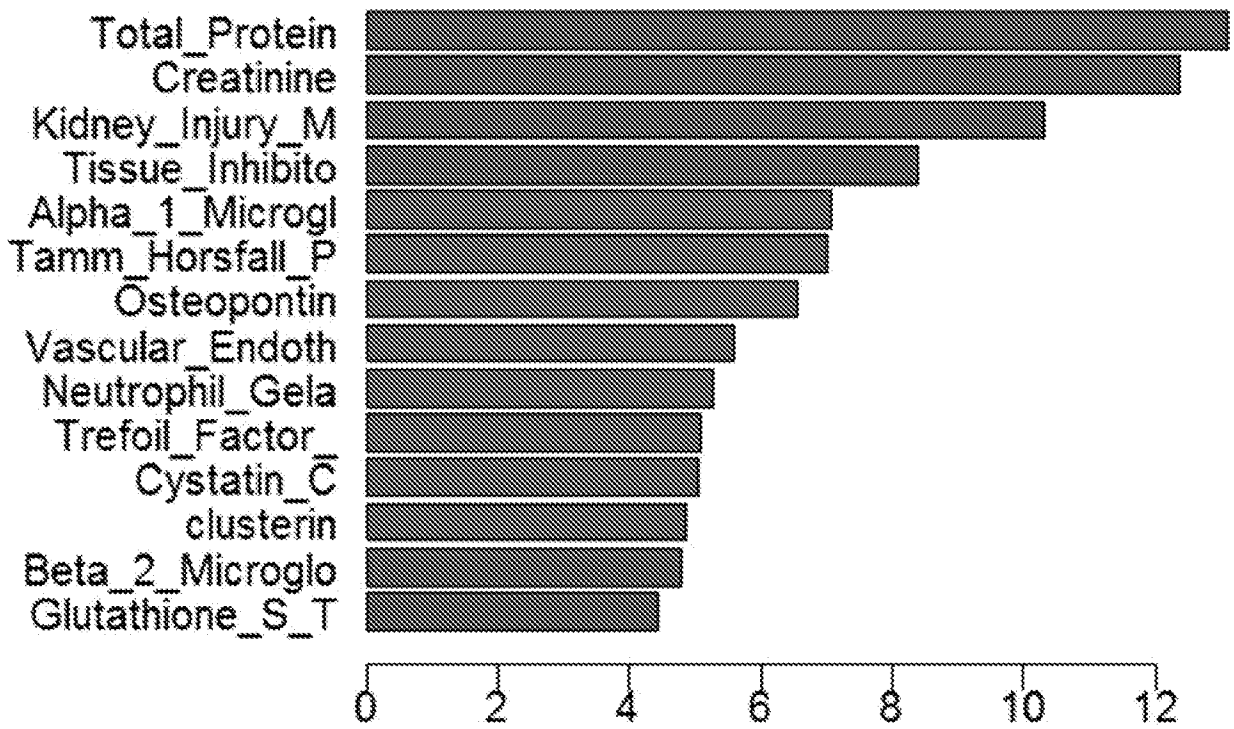
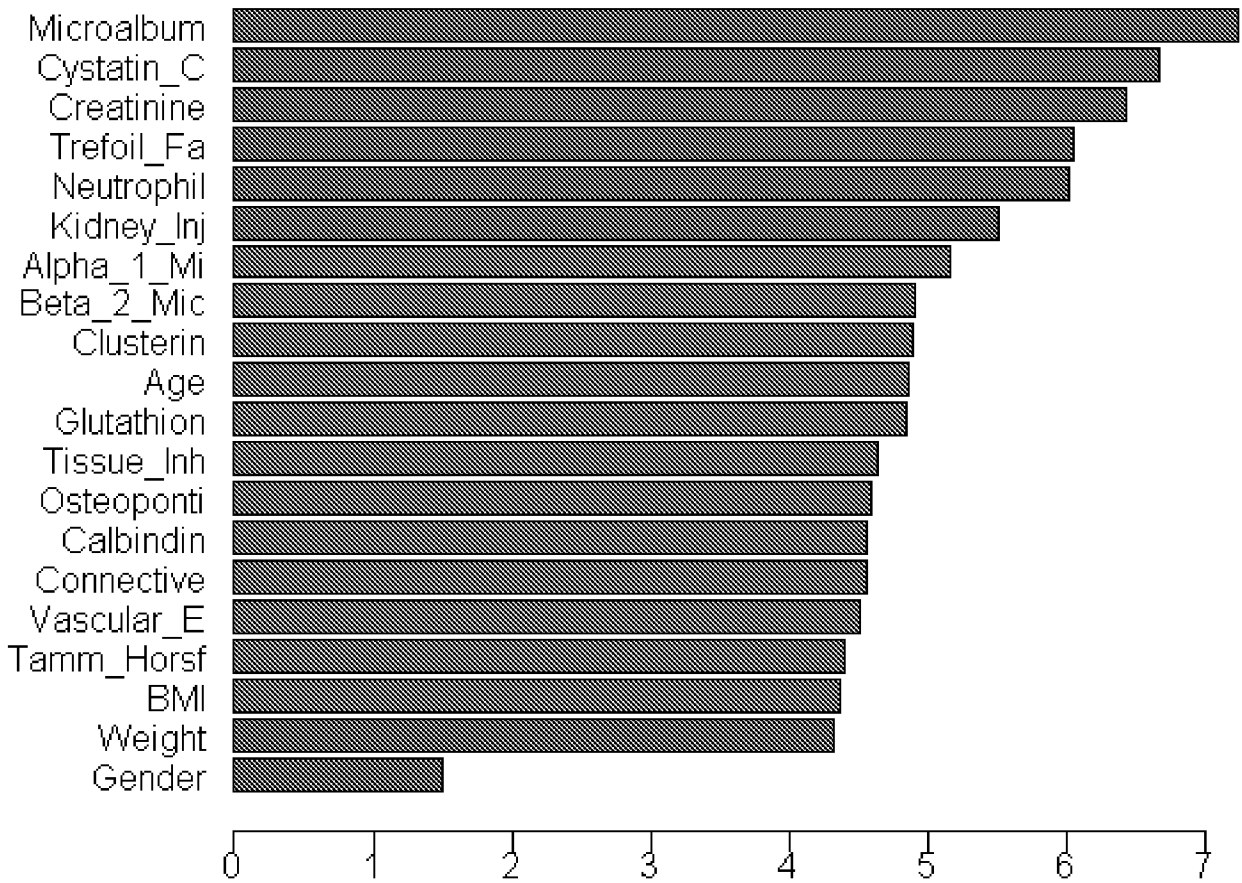


FIG. 6C



relative importance by random forest

FIG. 7A



relative importance by random forest

FIG. 7B

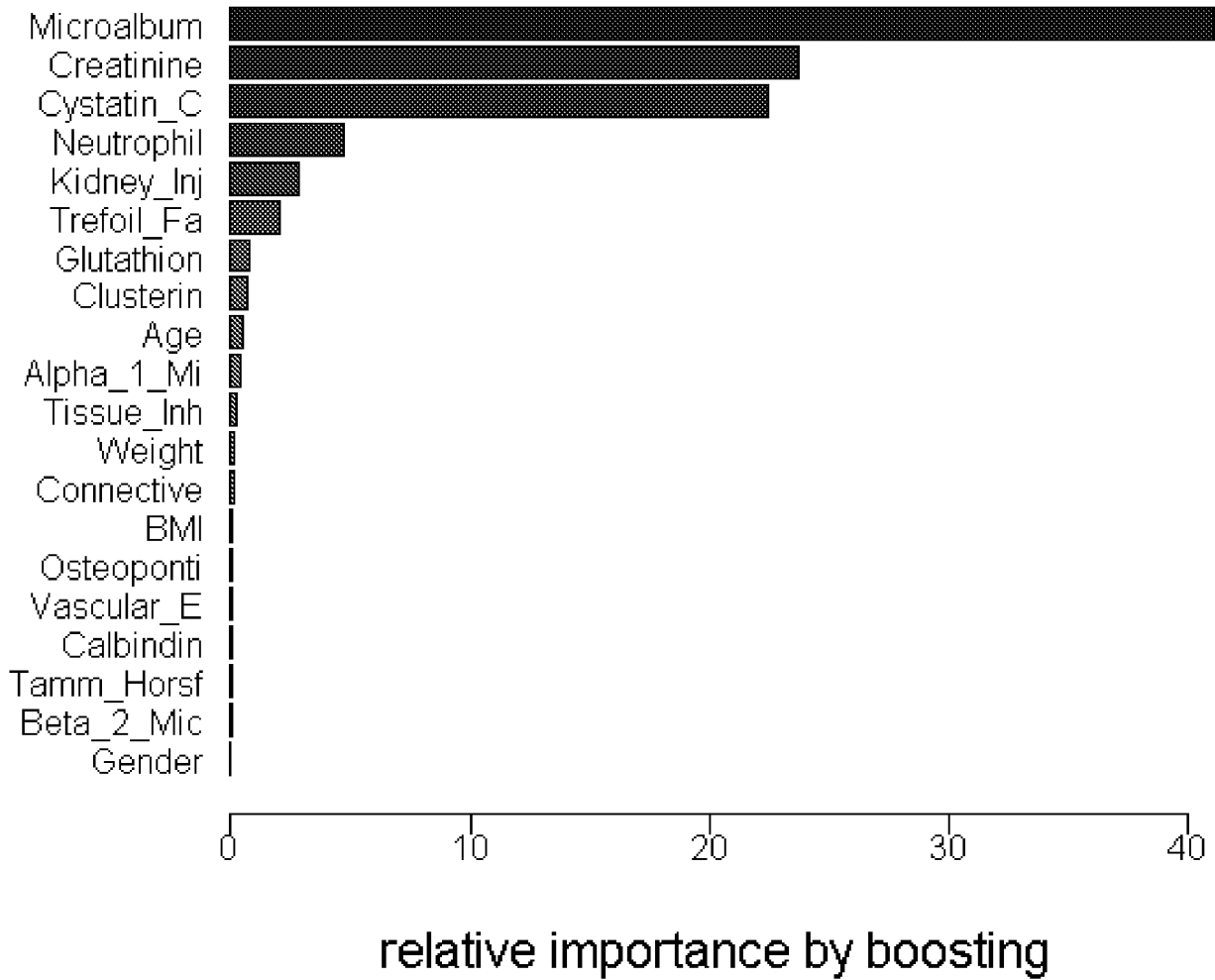


FIG. 7C

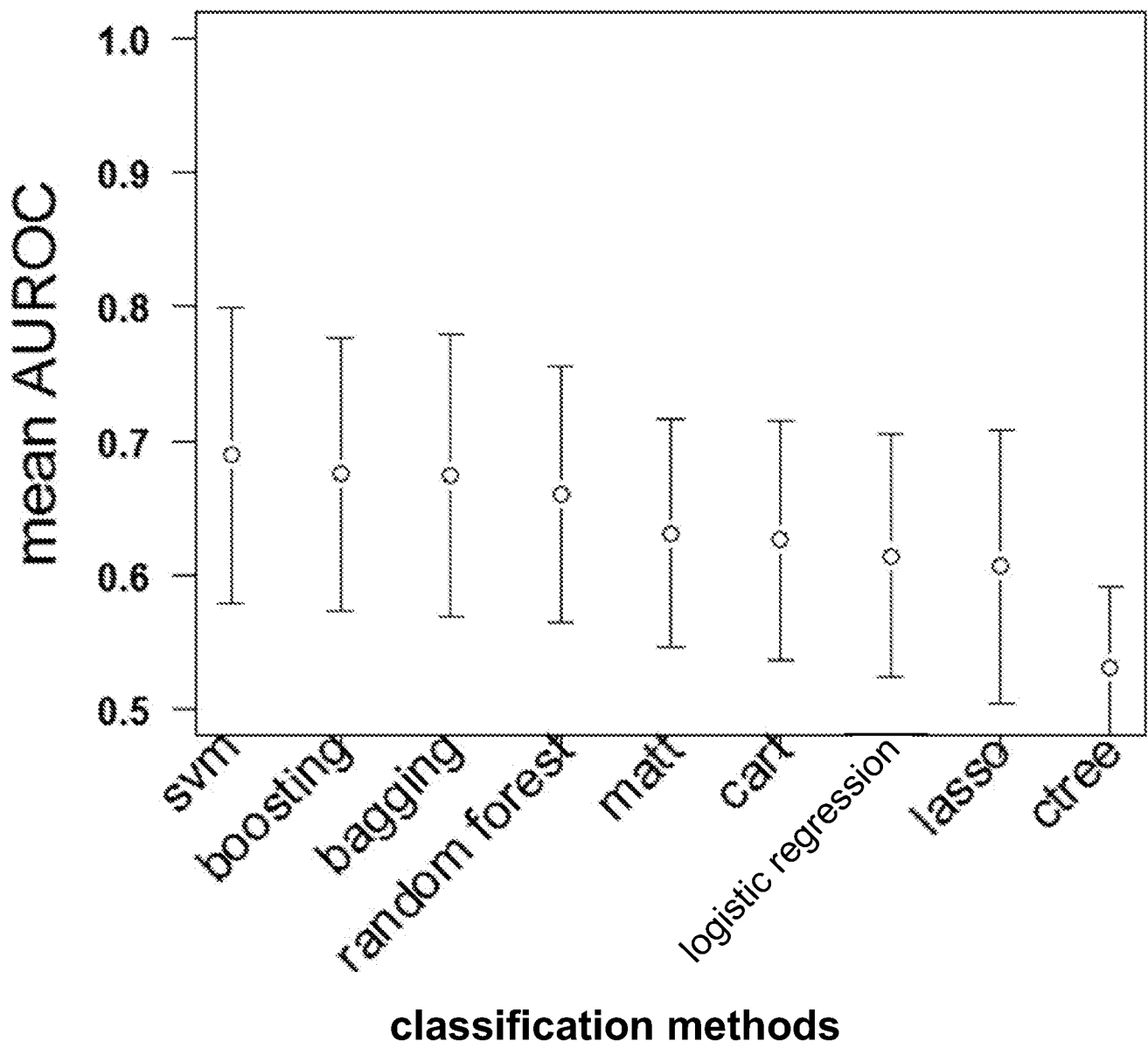


FIG. 8A

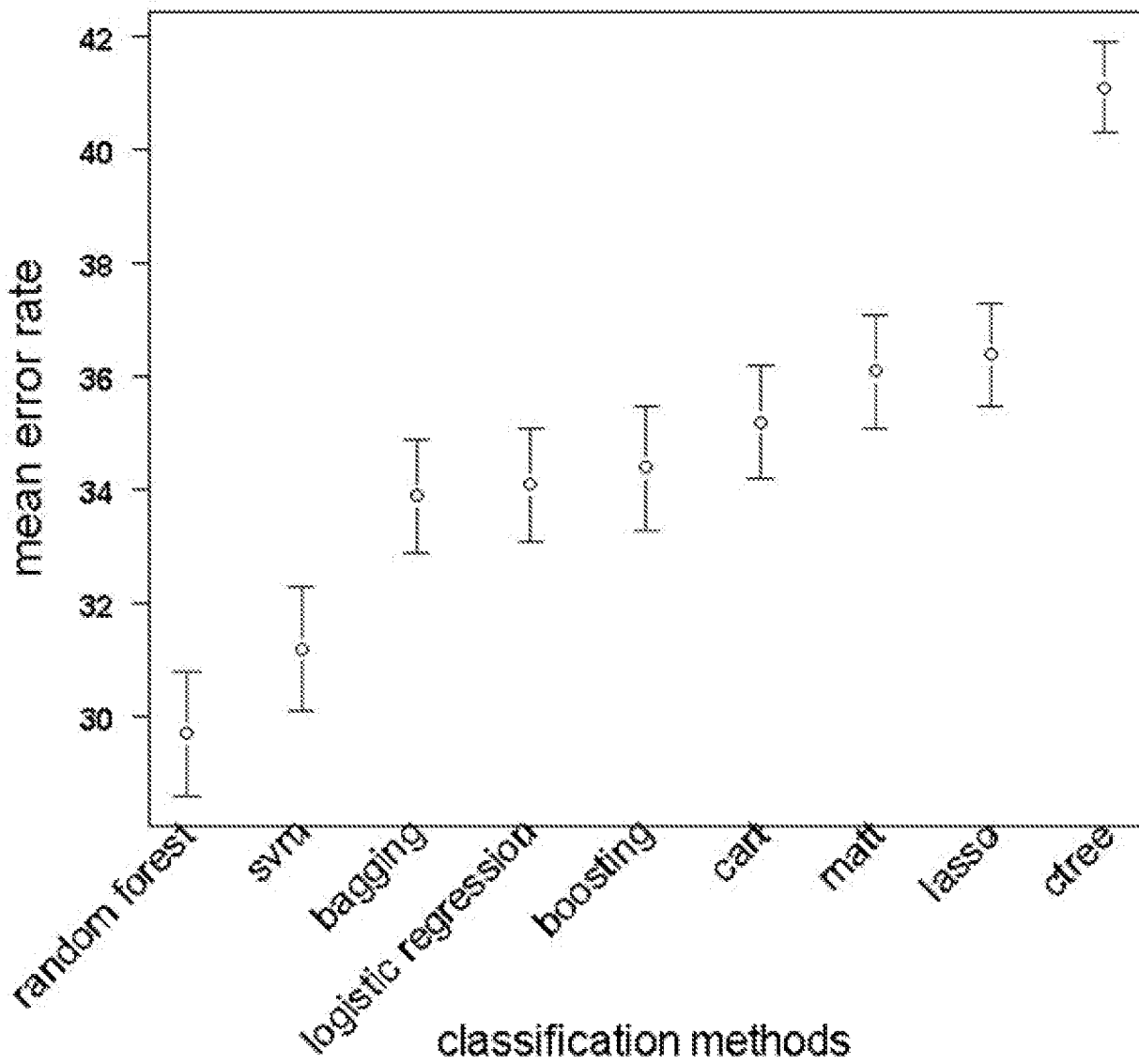


FIG. 8B

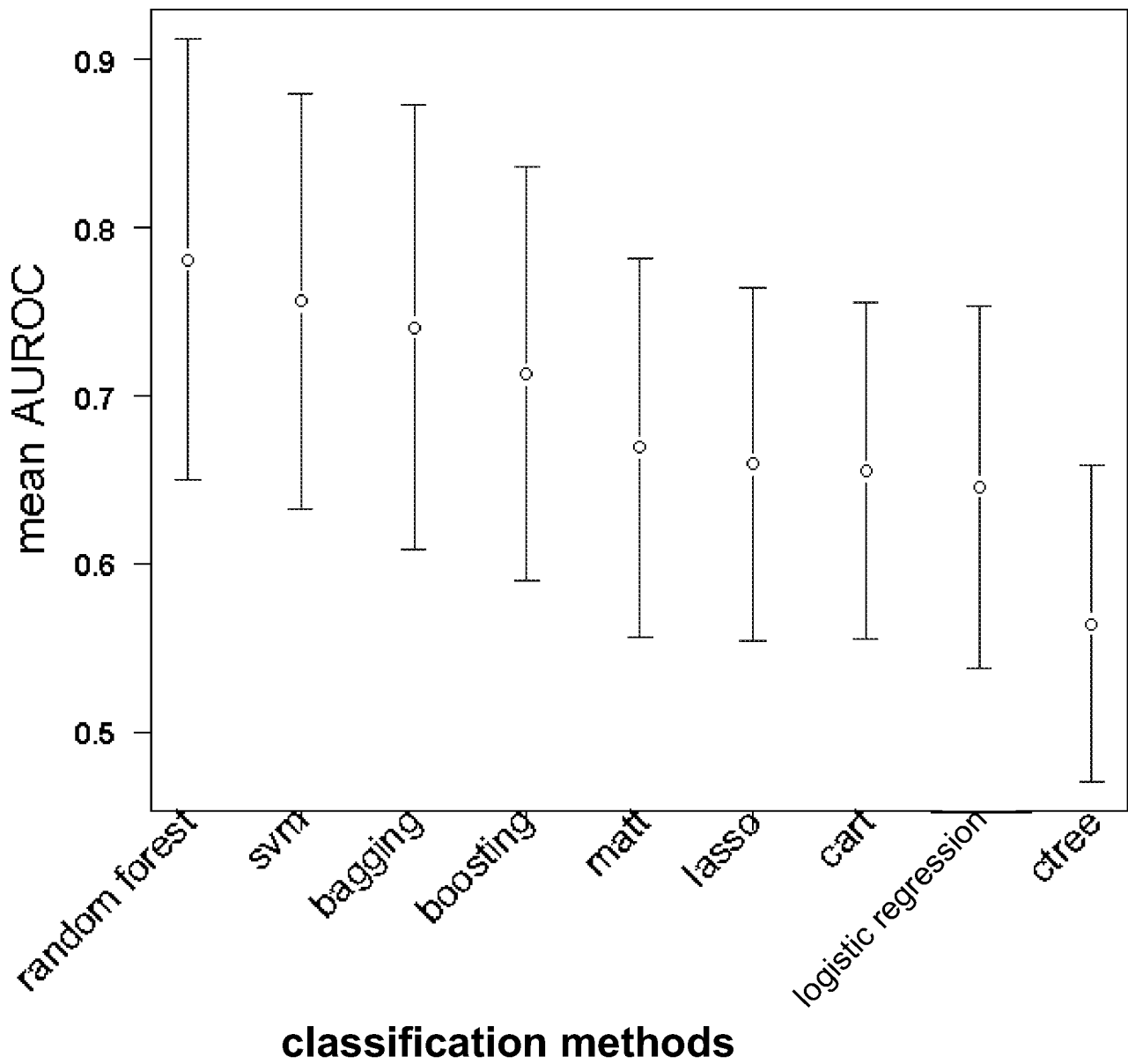
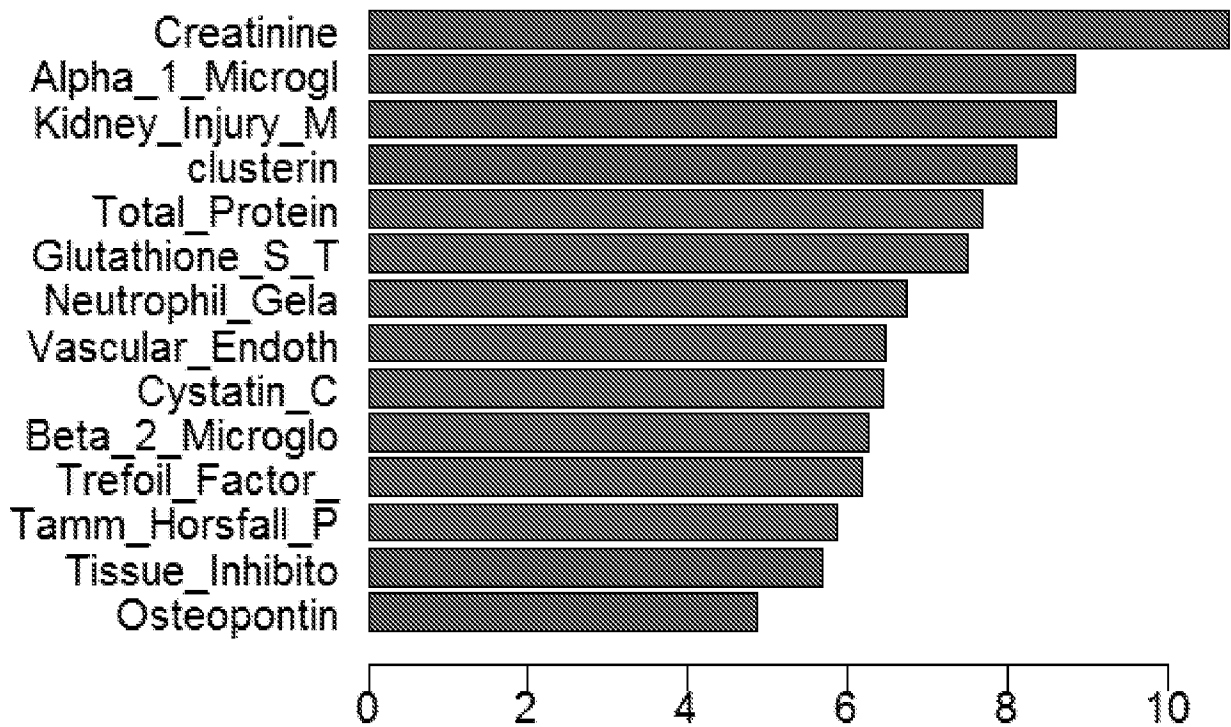
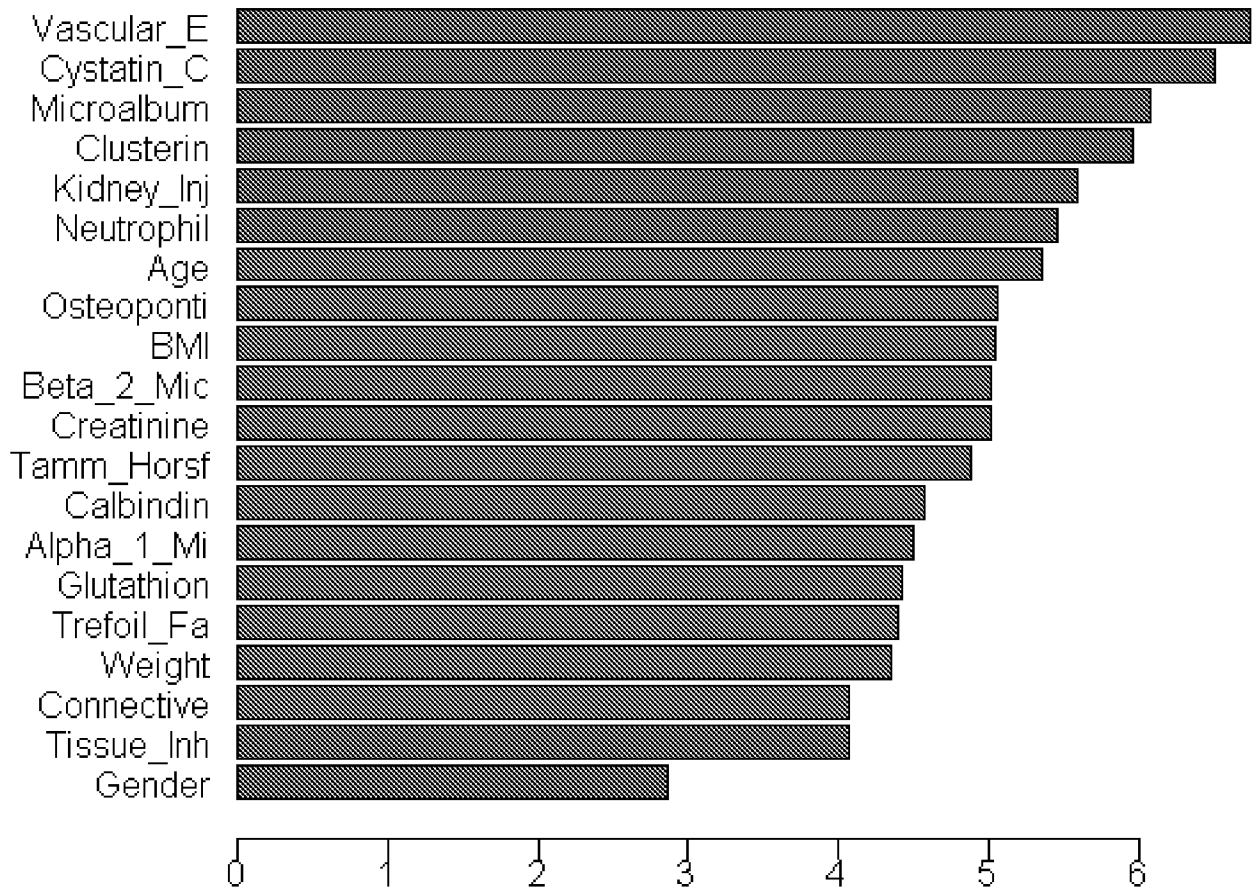


FIG. 8C



relative importance by random forest

FIG. 9A



relative importance by random forest

FIG. 9B

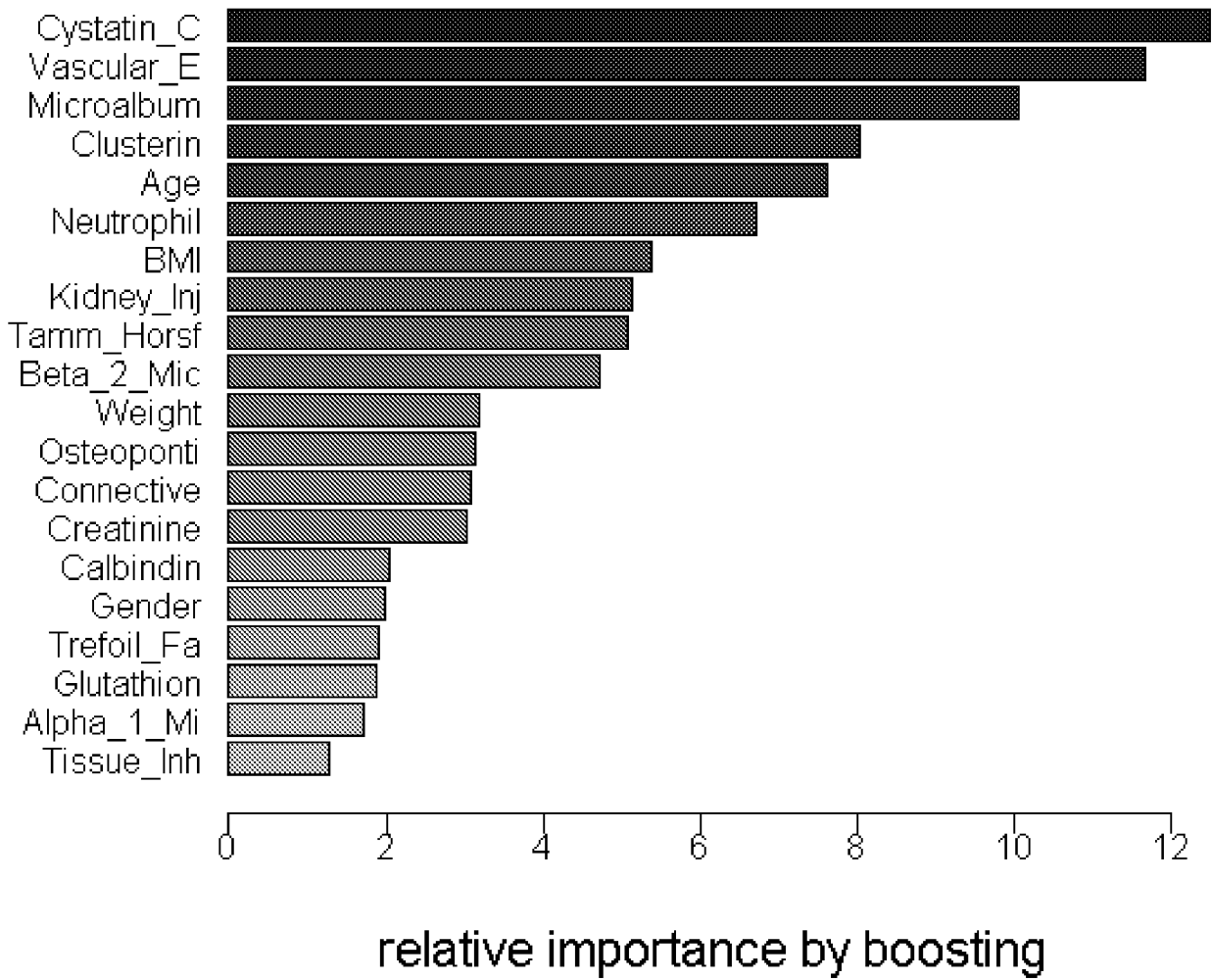


FIG. 9C

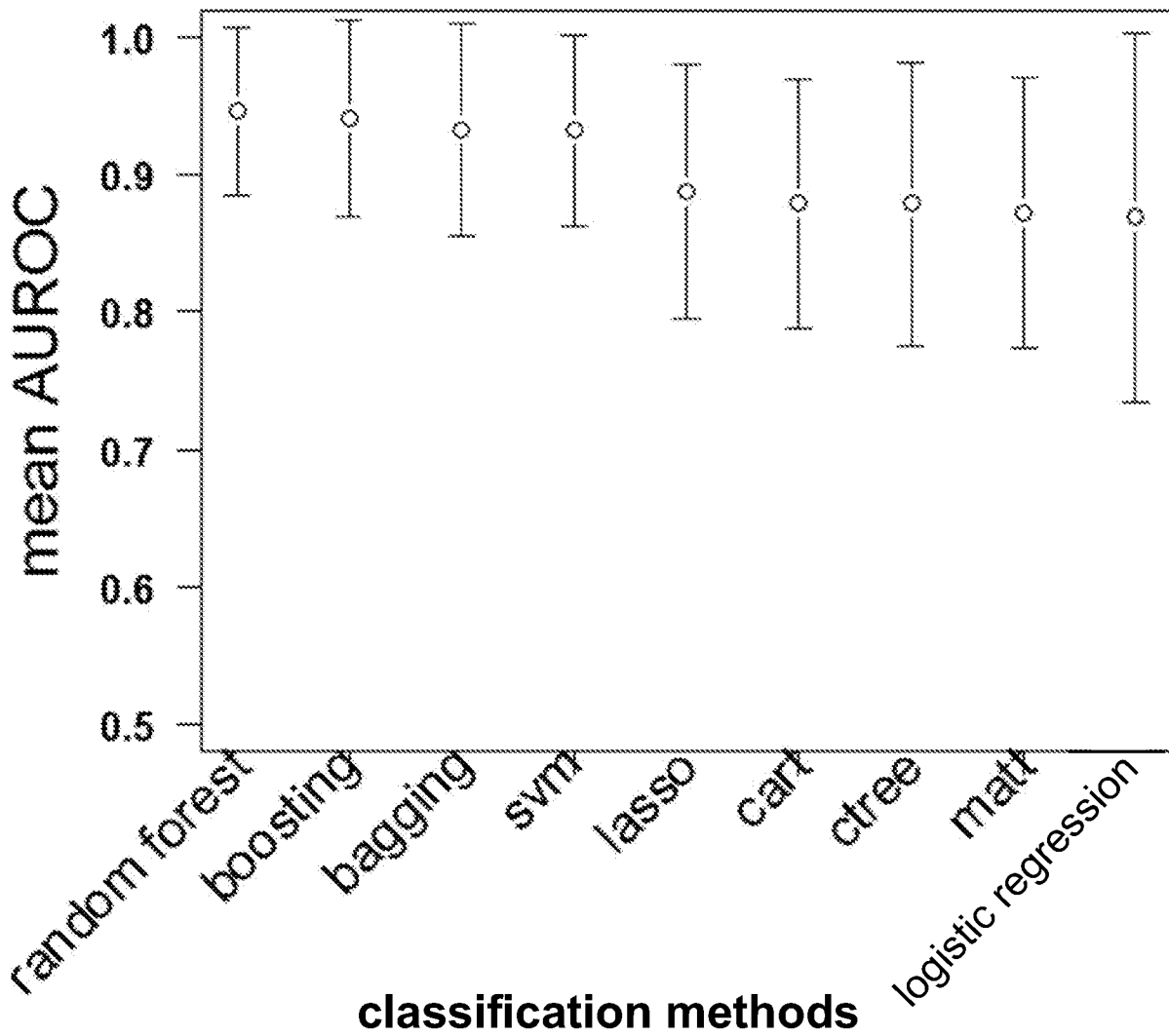


FIG. 10A

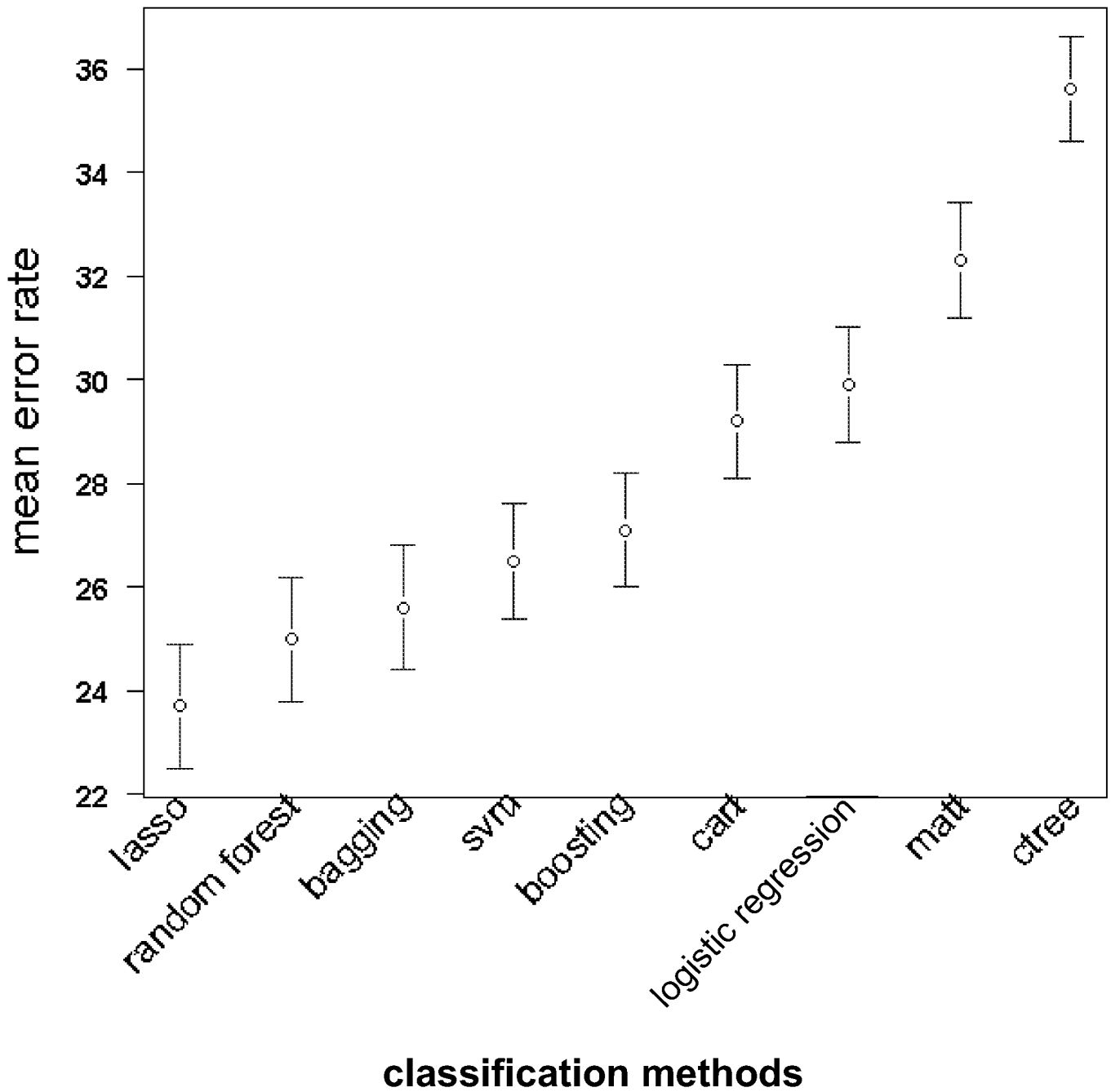


FIG. 10B

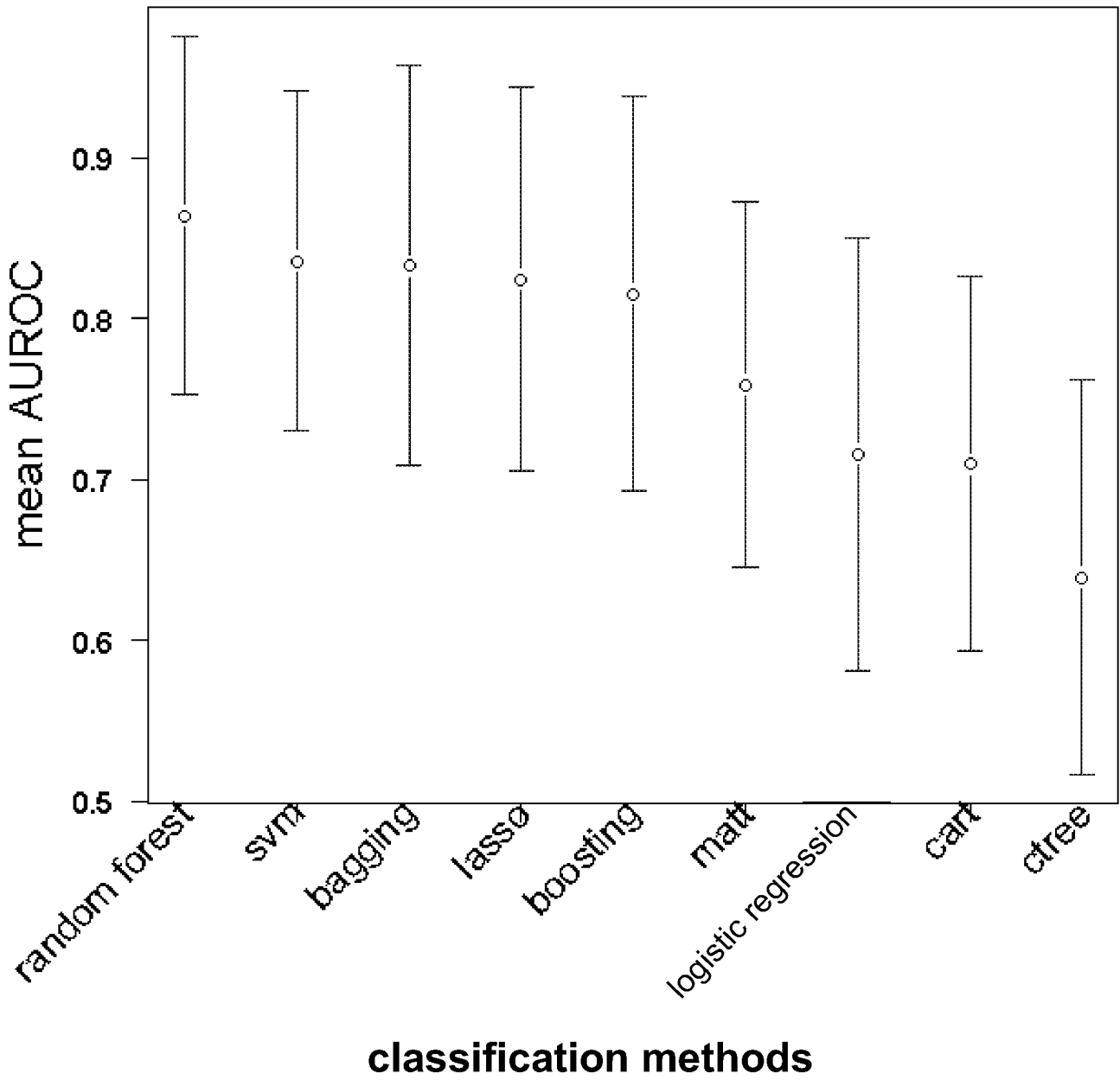


FIG. 10C

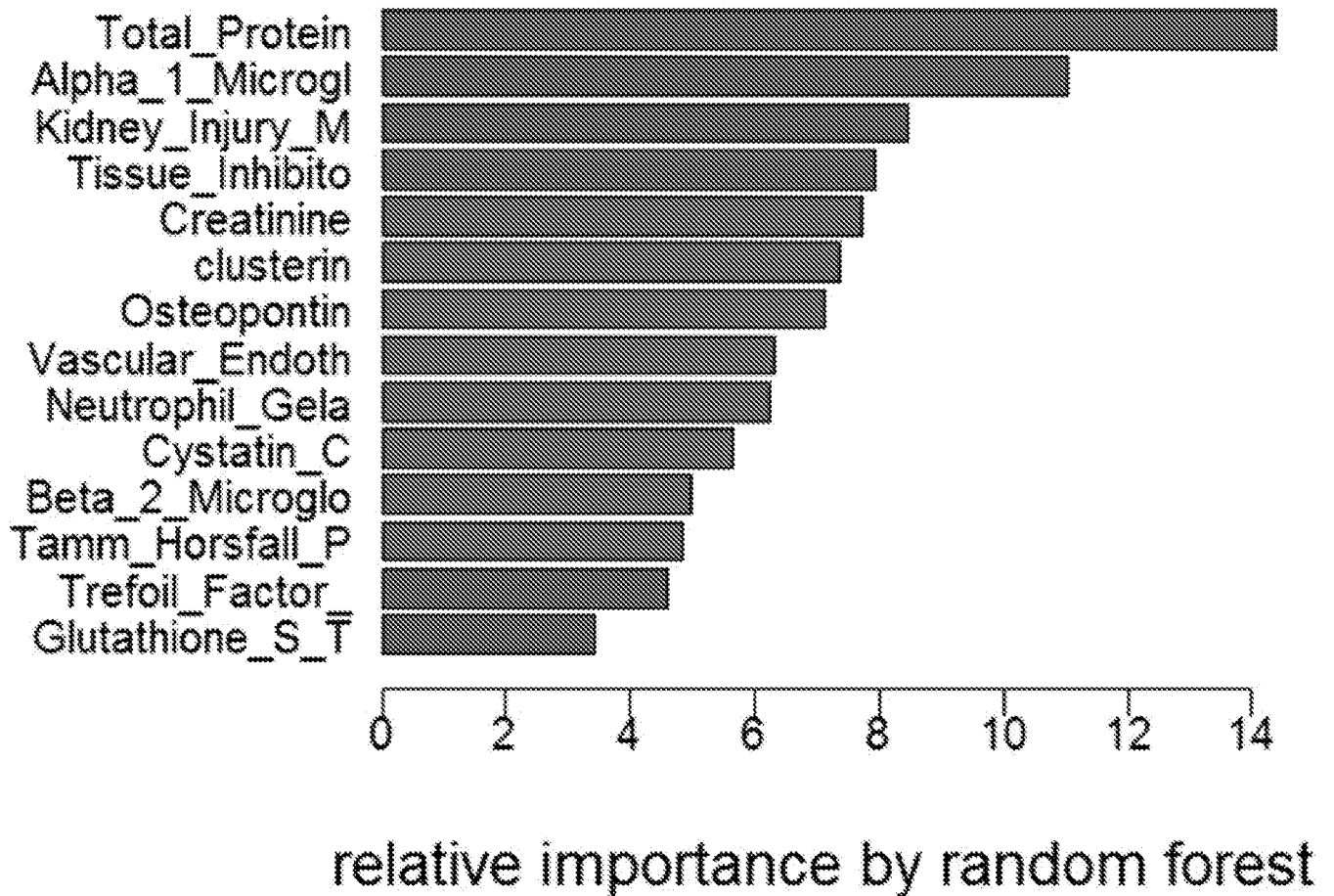
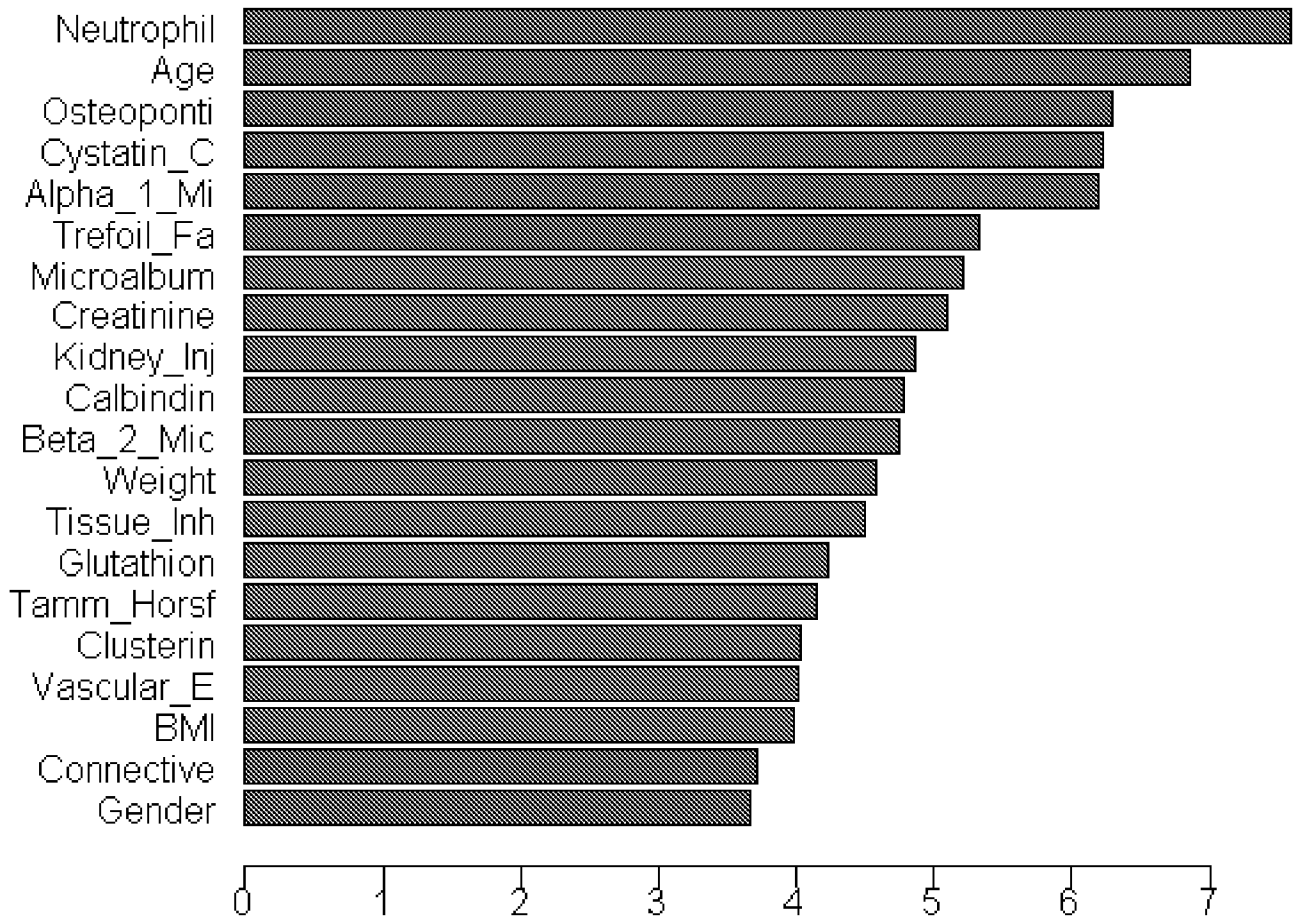


FIG. 11A



relative importance by random forest

FIG. 11B

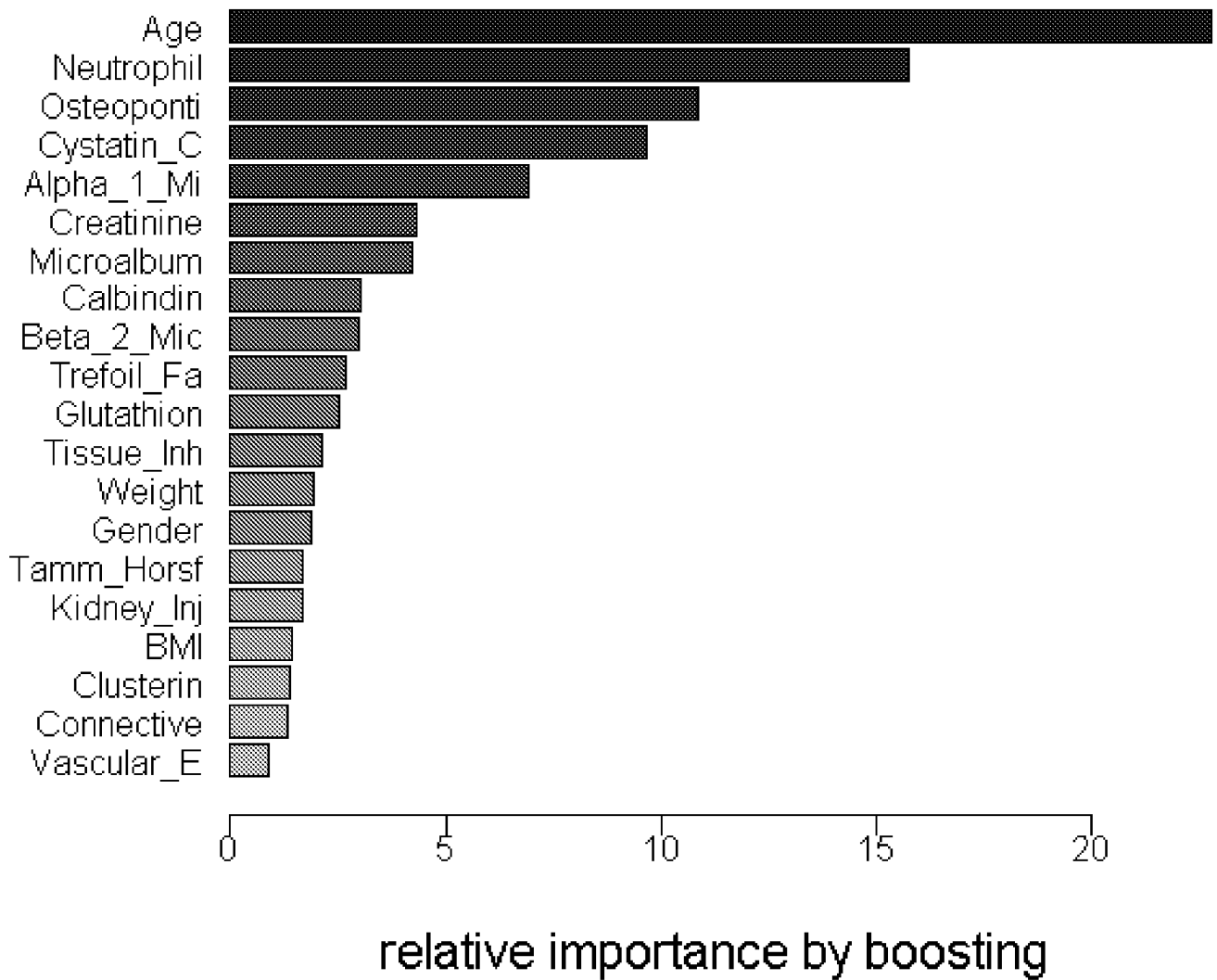


FIG. 11C

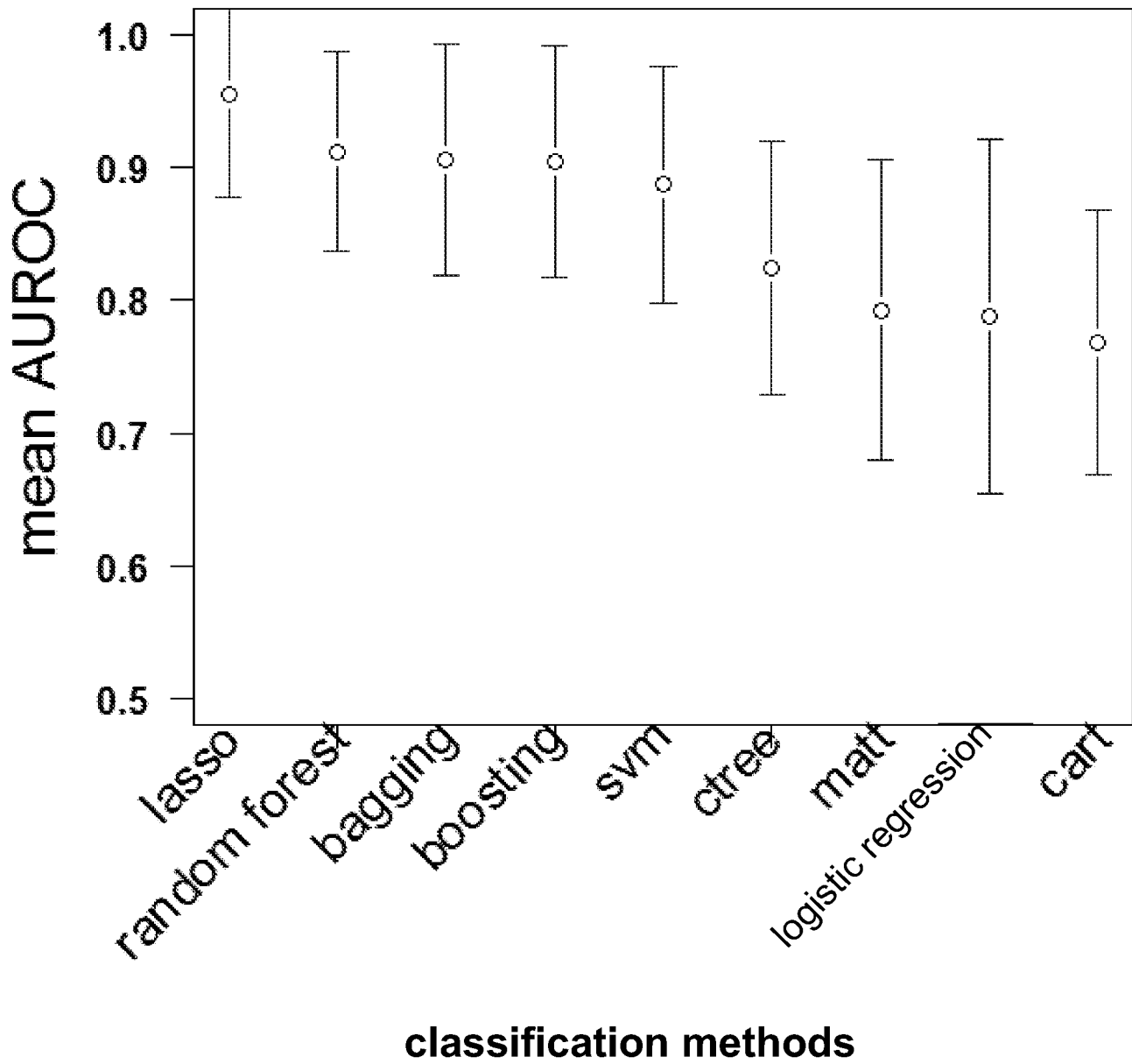


FIG. 12A

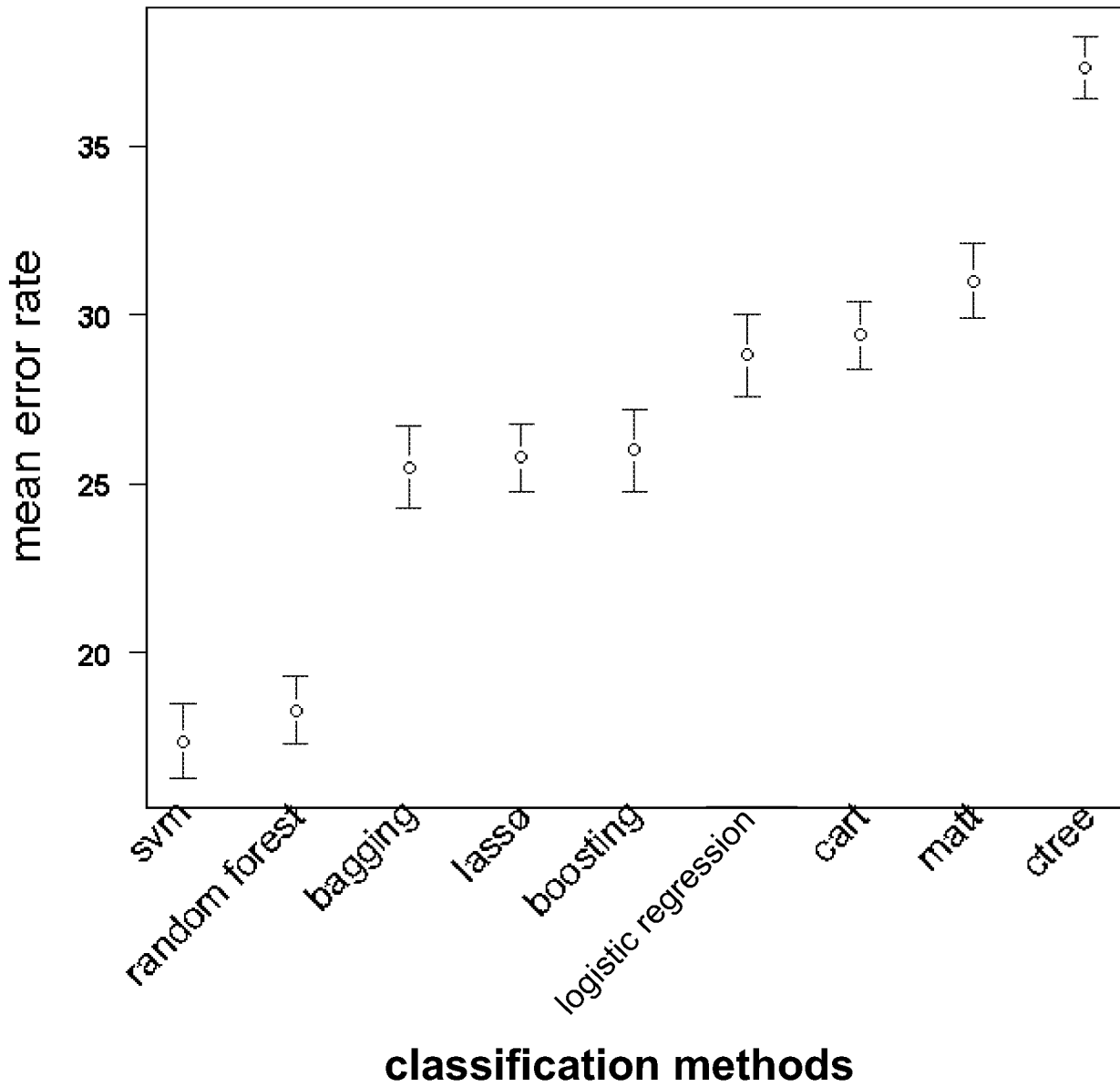


FIG. 12B

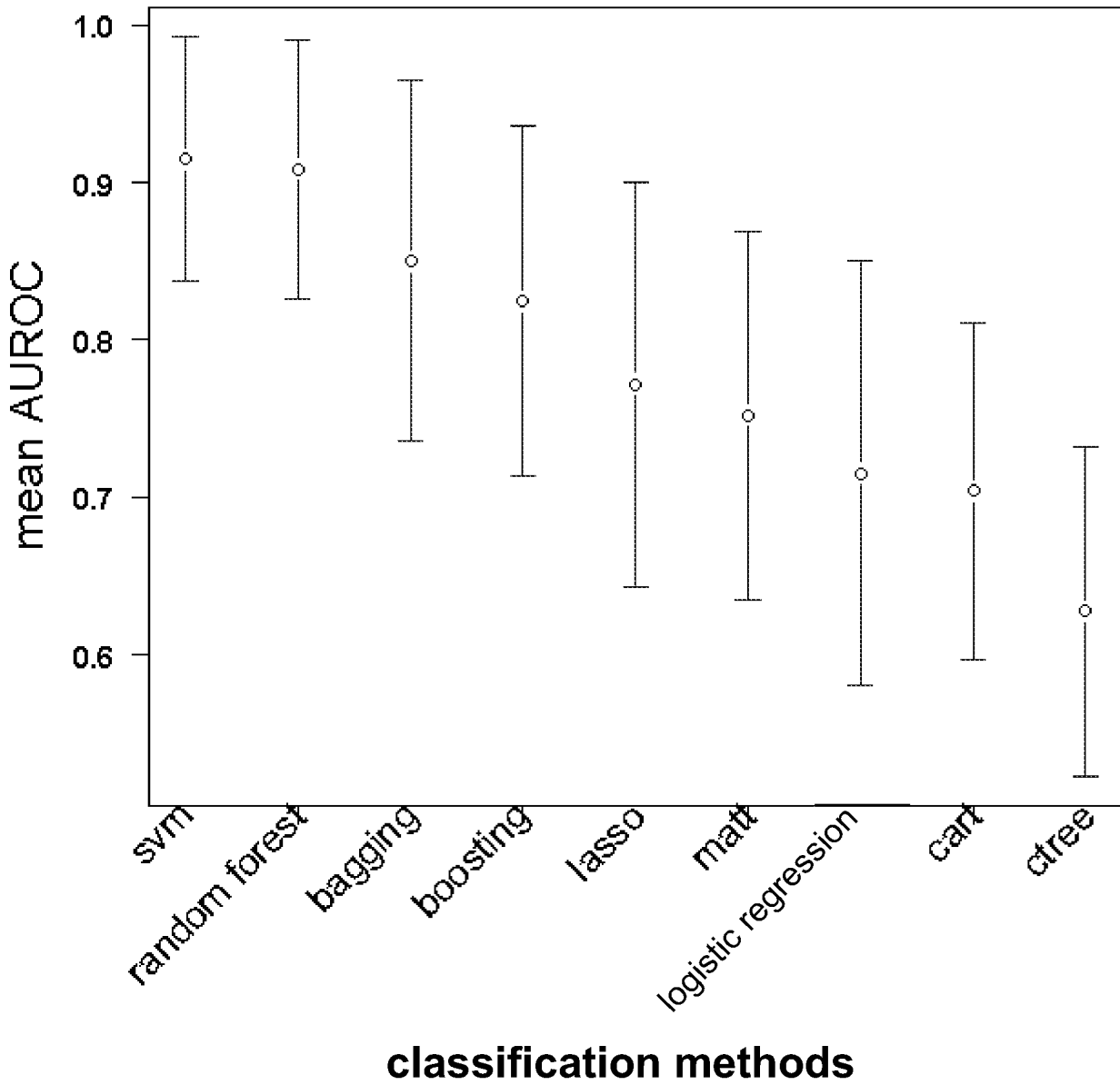
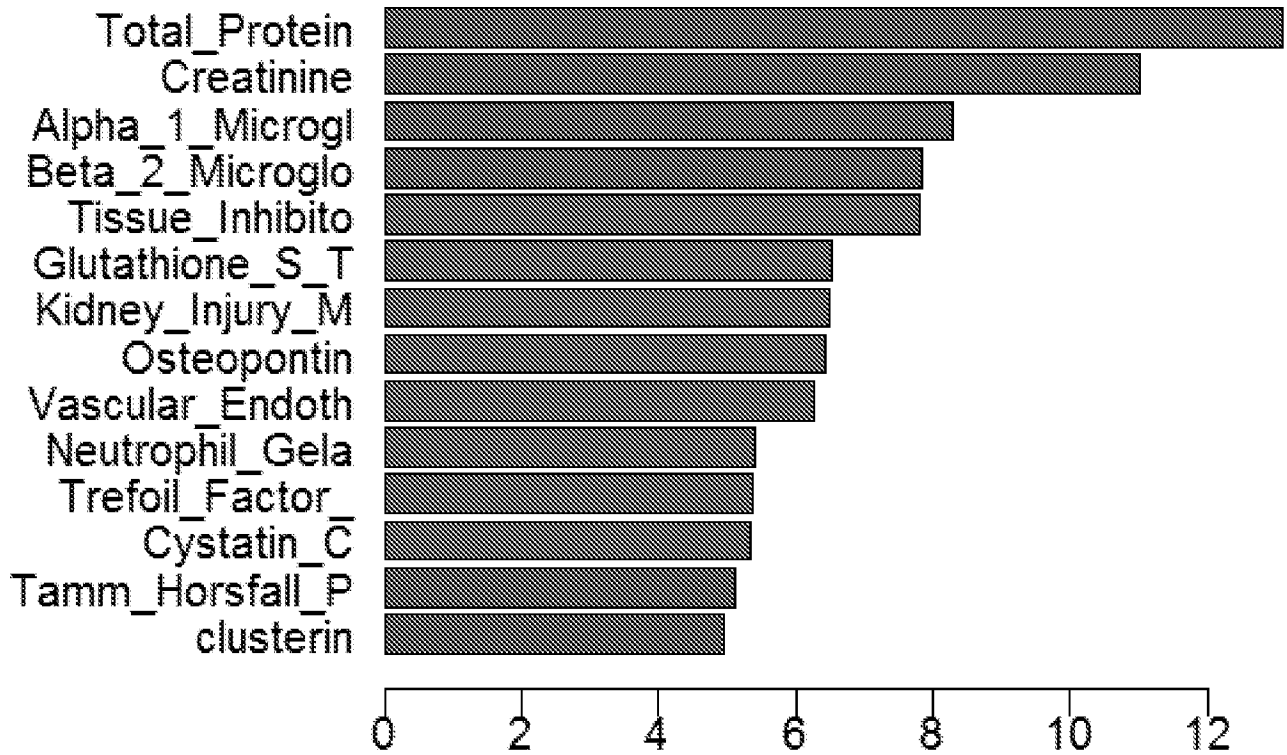
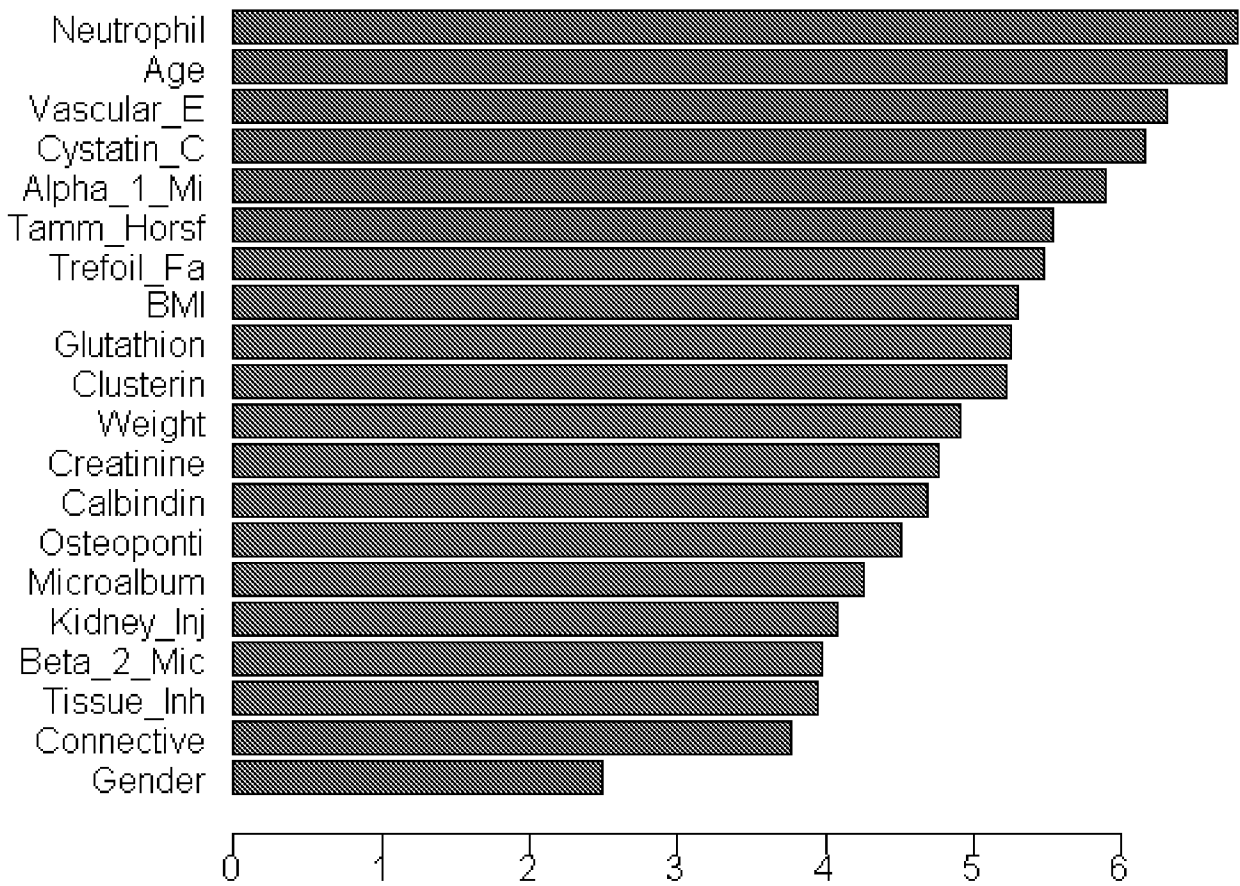


FIG. 12C



relative importance by random forest

FIG. 13A



relative importance by random forest

FIG. 13B

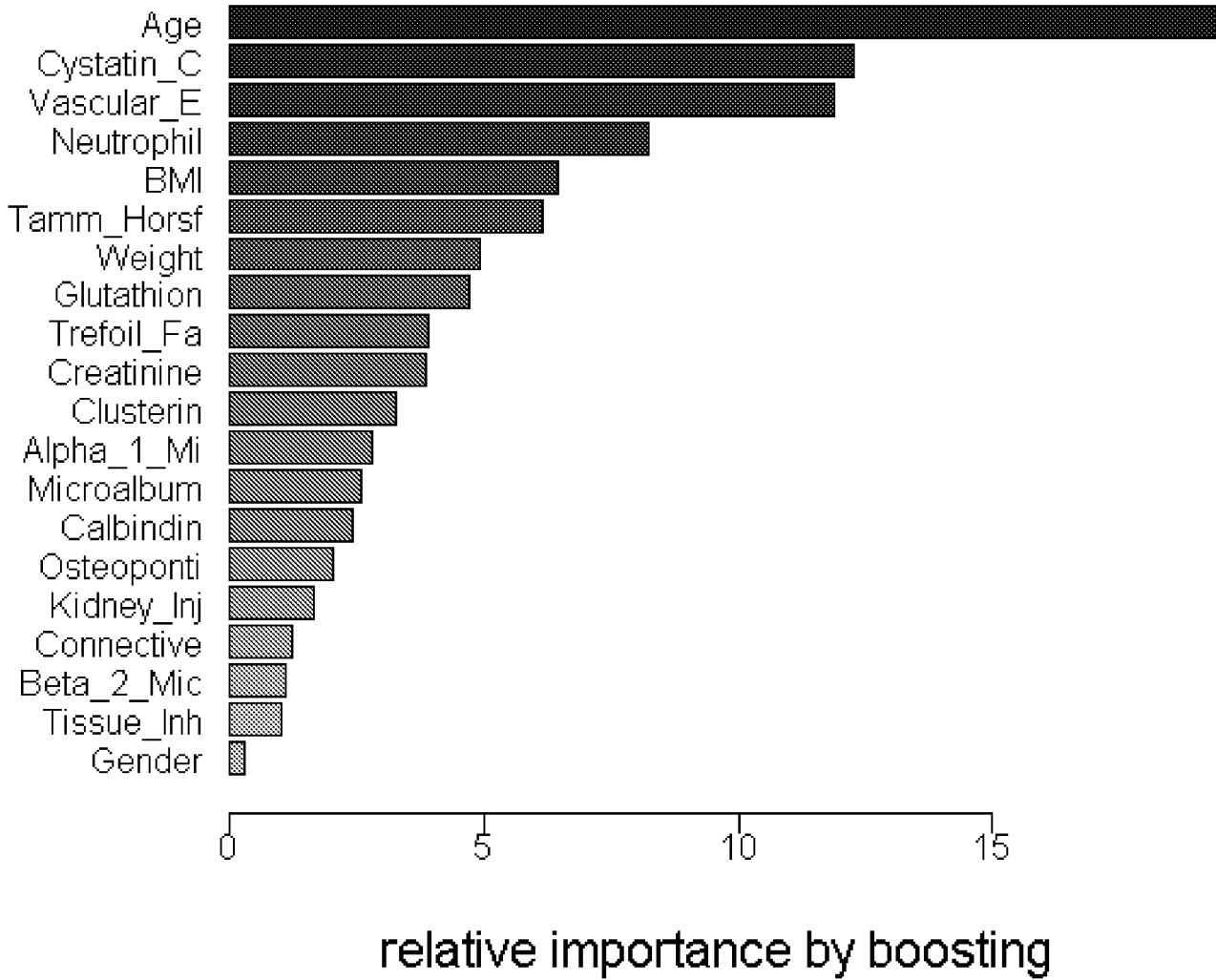


FIG. 13C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/44808

| <p>A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - G01N 33/53 (2010.01) USPC - 435/7.1 According to International Patent Classification (IPC) or to both national classification and IPC</p> | | | | | | | | | | | | | | | | | |
|--|---|--|--|---|---|--|--|--|--|---|--|---|--|---------|---|---|---------------|
| <p>B. FIELDS SEARCHED</p> <p>Minimum documentation searched (classification system followed by classification symbols) USPC: 435/7.1</p> <p>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC: 435/7.1, 4, 7.21, 7.92, 7.93, 7.94, 7.95, 7.5, 287.2</p> <p>Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PubWest (PGPB, USPT, EPAB, JPAB), Google Search Terms Used: (((alpha-1 OR BETA-2) NEAR microglobulin) OR calbindin OR "APOLIPOPROTEIN?J" OR "DAG PROTEIN" OR TRPM-2 OR SGP-2 OR KUB1 OR APOJ OR AAG4 OR clusterin OR CTGF OR CCN2 OR NOV2 OR creatinine OR CYSTATIN OR GST</p> | | | | | | | | | | | | | | | | | |
| <p>C. DOCUMENTS CONSIDERED TO BE RELEVANT</p> <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>Y</td> <td>US 2009/0093010 A1 (Nickerson et al.) 9 April 2009 (09.04.2009), the entire document, especially para[0001], para[0020], para[0052], para[0073], para[0078], para[0121], para[0129], para[0193].</td> <td>1-22</td> </tr> <tr> <td>Y</td> <td>Hepfer et al., Development and Validation of a Multi-Analyte Profile (MAP) of Putative Biomarkers of Human Kidney Damage, http://www.rulesbasedmedicine.com/scientific-literature/abstracts-posters.aspx, 13 March 2009 (13.03.2009), the entire article, especially pg 1 --Human KidneyMAP v1.0 Expected Ranges.</td> <td>1-22</td> </tr> <tr> <td>Y</td> <td>WO 2006/066587 A1 (Uttenenthal et al.) 29 June 2006 (29.06.2006), the entire document especially p 10, ln 11-15.</td> <td>5 and 6</td> </tr> <tr> <td>Y</td> <td>FERGUSON et al., Biomarkers of nephrotoxic acute kidney injury, Toxicology, Vol. 245 pp. 182-193 (2008), the entire article, especially p 183, Table 1.</td> <td>10, 15 and 20</td> </tr> </tbody> </table> | | | Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. | Y | US 2009/0093010 A1 (Nickerson et al.) 9 April 2009 (09.04.2009), the entire document, especially para[0001], para[0020], para[0052], para[0073], para[0078], para[0121], para[0129], para[0193]. | 1-22 | Y | Hepfer et al., Development and Validation of a Multi-Analyte Profile (MAP) of Putative Biomarkers of Human Kidney Damage, http://www.rulesbasedmedicine.com/scientific-literature/abstracts-posters.aspx , 13 March 2009 (13.03.2009), the entire article, especially pg 1 --Human KidneyMAP v1.0 Expected Ranges. | 1-22 | Y | WO 2006/066587 A1 (Uttenenthal et al.) 29 June 2006 (29.06.2006), the entire document especially p 10, ln 11-15. | 5 and 6 | Y | FERGUSON et al., Biomarkers of nephrotoxic acute kidney injury, Toxicology, Vol. 245 pp. 182-193 (2008), the entire article, especially p 183, Table 1. | 10, 15 and 20 |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. | | | | | | | | | | | | | | | |
| Y | US 2009/0093010 A1 (Nickerson et al.) 9 April 2009 (09.04.2009), the entire document, especially para[0001], para[0020], para[0052], para[0073], para[0078], para[0121], para[0129], para[0193]. | 1-22 | | | | | | | | | | | | | | | |
| Y | Hepfer et al., Development and Validation of a Multi-Analyte Profile (MAP) of Putative Biomarkers of Human Kidney Damage, http://www.rulesbasedmedicine.com/scientific-literature/abstracts-posters.aspx , 13 March 2009 (13.03.2009), the entire article, especially pg 1 --Human KidneyMAP v1.0 Expected Ranges. | 1-22 | | | | | | | | | | | | | | | |
| Y | WO 2006/066587 A1 (Uttenenthal et al.) 29 June 2006 (29.06.2006), the entire document especially p 10, ln 11-15. | 5 and 6 | | | | | | | | | | | | | | | |
| Y | FERGUSON et al., Biomarkers of nephrotoxic acute kidney injury, Toxicology, Vol. 245 pp. 182-193 (2008), the entire article, especially p 183, Table 1. | 10, 15 and 20 | | | | | | | | | | | | | | | |
| <p><input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/></p> | | | | | | | | | | | | | | | | | |
| <p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier application or patent but published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table> | | | "A" document defining the general state of the art which is not considered to be of particular relevance | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | "E" earlier application or patent but published on or after the international filing date | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone | "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art | "O" document referring to an oral disclosure, use, exhibition or other means | "&" document member of the same patent family | "P" document published prior to the international filing date but later than the priority date claimed | | | | | | |
| "A" document defining the general state of the art which is not considered to be of particular relevance | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | | | | | | | | | | | | | | | | |
| "E" earlier application or patent but published on or after the international filing date | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone | | | | | | | | | | | | | | | | |
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| "O" document referring to an oral disclosure, use, exhibition or other means | "&" document member of the same patent family | | | | | | | | | | | | | | | | |
| "P" document published prior to the international filing date but later than the priority date claimed | | | | | | | | | | | | | | | | | |
| <p>Date of the actual completion of the international search 18 September 2010 (18.09.2010)</p> | | <p>Date of mailing of the international search report 13 OCT 2010</p> | | | | | | | | | | | | | | | |
| <p>Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201</p> | | <p>Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774</p> | | | | | | | | | | | | | | | |

| | | | |
|---------------|--|---------|------------|
| 专利名称(译) | 用于检测肾小球肾炎和相关病症的方法和装置 | | |
| 公开(公告)号 | EP2462443A4 | 公开(公告)日 | 2013-01-30 |
| 申请号 | EP2010807272 | 申请日 | 2010-08-06 |
| 申请(专利权)人(译) | 规则为基础的医学, INC. | | |
| 当前申请(专利权)人(译) | 规则为基础的医学, INC. | | |
| [标]发明人 | SPAIN MICHAEL D MAPES JAMES P LABRIE SAMUEL T MCDADE RALPH L EISINGER DOMINIC BALLARD KARRI L | | |
| 发明人 | SPAIN, MICHAEL, D. MAPES, JAMES, P. LABRIE, SAMUEL, T. MCDADE, RALPH, L. EISINGER, DOMINIC BALLARD, KARRI, L. | | |
| IPC分类号 | G01N33/53 G01N33/68 | | |
| CPC分类号 | G01N33/6893 G01N33/5302 G01N33/566 G01N2333/47 G01N2333/4703 G01N2333/4706 G01N2333/4725 G01N2333/4727 G01N2333/475 G01N2333/52 G01N2333/70503 G01N2333/70539 G01N2333/765 G01N2333/775 G01N2333/8139 G01N2333/8146 G01N2333/82 G01N2333/91177 G01N2800/34 G01N2800/347 G01N2800/52 G01N2800/56 G01N2800/60 Y10T436/147777 | | |
| 代理机构(译) | 法思博事务所 | | |
| 优先权 | 61/232091 2009-08-07 US 61/327389 2010-04-23 US | | |
| 其他公开文献 | EP2462443A1 | | |
| 外部链接 | Espacenet | | |

摘要(译)

描述了用于诊断, 监测或确定哺乳动物中肾移植排斥或相关疾病的方法和装置。特别地, 描述了使用从哺乳动物获取的测试样品中的三种或更多种分析物的组合的测量浓度来诊断, 监测或确定肾移植排斥或相关疾病的方法和装置。