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(54) Title: METHODS AND COMPOSITIONS FOR DIAGNOSIS AND PROGNOSIS OF SEPSIS

(57) Abstract: The present disclosure relates to methods and compositions for monitoring, diagnosis, prognosis, and determination of treatment regimens in sepsis patients and in patients at risk for sepsis. In particular, the method relates to using assays that detect one or more of WAP four-disulfide core domain protein 2, Hepatitis A virus cellular receptor 1, Interleukin-1 receptor-like 1, and Proprotein convertase subtilisin/kexin type 9 as diagnostic and prognostic in such patients.



METHODS AND COMPOSITIONS FOR DIAGNOSIS AND PROGNOSIS OF SEPSIS

[0001] The present application claims priority to U.S. Provisional Patent Application 61/674,750, filed July 23, 2012, which is hereby incorporated by reference in its entirety including all tables, figures, and claims.

BACKGROUND OF THE INVENTION

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

[0003] The term “sepsis” has been used to describe a variety of clinical conditions related to systemic manifestations of inflammation accompanied by an infection. Because of clinical similarities to inflammatory responses secondary to non-infectious etiologies, identifying sepsis has been a particularly challenging diagnostic problem. Recently, the American College of Chest Physicians and the American Society of Critical Care Medicine (Bone et al., *Chest* 101: 1644-53, 1992) published definitions for “Systemic Inflammatory Response Syndrome” (or “SIRS”), which refers generally to a severe systemic response to an infectious or non-infectious insult, and for the related syndromes “sepsis,” “severe sepsis,” and “septic shock,” and extending to multiple organ dysfunction syndrome (“MODS”). These definitions, described below, are intended for each of these phrases for the purposes of the present application.

[0004] A systemic inflammatory response leading to a diagnosis of SIRS may be related to both infection and to numerous non-infective etiologies, including burns, pancreatitis, trauma, heat stroke, and neoplasia. While conceptually it may be relatively simple to distinguish between sepsis and non-septic SIRS, no diagnostic tools have been described to unambiguously distinguish these related conditions. *See, e.g.,* Llewelyn and Cohen, *Int. Care Med.* 27: S10-S32, 2001. For example, because more than 90% of sepsis cases involve bacterial infection, the “gold standard” for confirming infection has been microbial growth from blood, urine, pleural fluid, cerebrospinal fluid, peritoneal fluid, synovial fluid, sputum, or other tissue specimens. Such culture has been reported, however, to fail to confirm 50% or more of patients exhibiting strong clinical evidence of

sepsis. *See, e.g., Jaimes et al., Int. Care Med* 29: 1368-71, published electronically June 26, 2003.

[0005] Thus, despite improvements in the management of critically ill patients, sepsis remains the leading cause of death in such patients. This makes early determination diagnosis vital. The two biomarkers that have been most widely studied and used in patients with sepsis are C-reactive protein (CRP) and procalcitonin (PCT). Levels of both these biomarkers have been demonstrated to be raised in patients with sepsis, but because they lack specificity for sepsis and levels may be raised in other inflammatory diseases, these biomarkers are more useful for ruling out sepsis than for ruling it in, that is, a completely normal value makes a diagnosis of sepsis less likely, but an elevated level may not be due to infection.

[0006] Moreover, biomarkers are relevant in clinical practice not only for their ability to diagnose a pathological condition, but also for predicting morbidity and outcome. The ability to assign a severity of illness and outcome likelihood to a sepsis patient is equally vital for triaging of patients and guiding therapeutic decisions. By way of example, development of acute kidney injury (AKI) during sepsis increases patient morbidity, predicts higher mortality, has a significant effect on multiple organ functions, is associated with an increased length of stay in the intensive care unit, and hence consumes considerable healthcare resources. A biomarker able to stratify risk during the first days of admission could differ from one that provides a prediction later in the course of disease. A sequential determination of a biomarker may also be of use in following the acute response to treatment in patients with sepsis.

[0007] There remains in the art methods and compositions for evaluating sepsis in patients in order to identify onset of disease, and those most at risk for poor outcomes.

BRIEF SUMMARY OF THE INVENTION

[0008] It is an object of the invention to provide methods and compositions for evaluating a sepsis patient. As described herein, measurement of one or more biomarkers selected from the group consisting of WAP four-disulfide core domain protein 2, Hepatitis A virus cellular receptor 1, Interleukin-1 receptor-like 1, and Proprotein convertase subtilisin/kexin type 9 (each referred to herein as a “sepsis biomarker”) can be used for diagnosis, prognosis, risk stratification, staging, monitoring, categorizing and determination of further diagnosis and treatment regimens in sepsis patients.

[0009] The sepsis biomarkers of the present invention may be used, individually or in panels comprising a plurality of sepsis biomarkers, for identifying a subject suffering from SIRS, sepsis, severe sepsis, septic shock and/or MODS, for distinguishing amongst these conditions, for assigning a risk that a subject at risk for sepsis will progress to sepsis, severe sepsis, septic shock and/or MODS; or for assigning a prognosis to a subject suffering from one or more of these conditions, *etc.*

[0010] In a first aspect, the present invention relates to methods for evaluating a sepsis patient or a patient being evaluated for a possible sepsis diagnosis. These methods comprise performing an assay method that is configured to detect one or more biomarkers selected from the group consisting of WAP four-disulfide core domain protein 2, Hepatitis A virus cellular receptor 1, Interleukin-1 receptor-like 1, and Proprotein convertase subtilisin/kexin type 9, the results of which assay(s) is/are then correlated to the status of the patient. This correlation to status may include correlating the assay result(s) to one or more of diagnosis, risk stratification, prognosis, staging, classifying and monitoring of the sepsis patient as described herein. Thus, the present invention utilizes one or more sepsis biomarkers of the present invention for the evaluation of a patient.

[0011] In certain embodiments, the methods for evaluating a sepsis patient described herein are methods for risk stratification of the sepsis patient; that is, assigning a likelihood of one or more future changes in health status to the sepsis patient. In these embodiments, the assay result(s) is/are correlated to one or more such future changes. The following are preferred risk stratification embodiments.

[0012] In preferred risk stratification embodiments, these methods comprise determining a sepsis patient's risk for future progression to a worsening (or improving) stage within the definition of SIRS. By way of example, the method may comprise assigning a likelihood of progression from SIRS to sepsis; from sepsis to severe sepsis; from sepsis or severe sepsis to septic shock; from sepsis, severe sepsis, or septic shock to MODS. Alternatively, the method may comprise assigning a likelihood of progression from recovery from sepsis; from severe sepsis; from septic shock; from MODS. For example, the measured concentration(s) may each be compared to a threshold value. For a "positive going" sepsis biomarker, an increased likelihood of progression to a worsening stage is assigned to the sepsis patient when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold. For a "negative going" sepsis biomarker, an increased likelihood of

progressing to a worsening stage is assigned to the sepsis patient when the measured concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold.

[0013] In other preferred risk stratification embodiments, these methods comprise determining a sepsis patient's risk for future reduced renal function, and the assay result(s) is/are correlated to a likelihood of such reduced renal function. For example, the measured concentrations may each be compared to a threshold value. For a "positive going" sepsis biomarker, an increased likelihood of suffering a future reduced renal function is assigned to the sepsis patient when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold. For a "negative going" sepsis biomarker, an increased likelihood of future reduced renal function is assigned to the sepsis patient when the measured concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold.

[0014] In yet other preferred risk stratification embodiments, these methods comprise determining a sepsis patient's risk for progression to ARF, and the result(s) is/are correlated to a likelihood of such progression to ARF. For example, the measured concentration(s) may each be compared to a threshold value. For a "positive going" sepsis biomarker, an increased likelihood of progression to ARF is assigned to the sepsis patient when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold. For a "negative going" sepsis biomarker, an increased likelihood of progression to ARF is assigned to the sepsis patient when the measured concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold.

[0015] And in other preferred risk stratification embodiments, these methods comprise determining a sepsis patient's outcome risk, and the assay result(s) is/are correlated to a likelihood mortality by the sepsis patient. For example, the measured concentration(s) may each be compared to a threshold value. For a "positive going" sepsis biomarker, an increased likelihood of mortality is assigned to the sepsis patient when the measured concentration is above the threshold, relative to a likelihood assigned when the measured concentration is below the threshold. For a "negative going" sepsis biomarker, an increased likelihood of mortality is assigned to the sepsis patient when the measured

concentration is below the threshold, relative to a likelihood assigned when the measured concentration is above the threshold.

[0016] In such risk stratification embodiments, preferably the likelihood or risk assigned is that an event of interest is more or less likely to occur within 180 days of the time at which the body fluid sample is obtained from the sepsis patient. In particularly preferred embodiments, the likelihood or risk assigned relates to an event of interest occurring within a shorter time period such as 18 months, 120 days, 90 days, 60 days, 45 days, 30 days, 21 days, 14 days, 7 days, 5 days, 96 hours, 72 hours, 48 hours, 36 hours, 24 hours, 12 hours, or less. A risk at 0 hours of the time at which the body fluid sample is obtained from the sepsis patient is equivalent to diagnosis of a current condition.

[0017] In other embodiments, the methods for evaluating status described herein are methods for diagnosis, which refers to identifying a subject suffering from sepsis, severe sepsis, septic shock and/or MODS. In these embodiments, the assay result(s), for example measured concentration(s) of one or more biomarkers selected from the group consisting of WAP four-disulfide core domain protein 2, Hepatitis A virus cellular receptor 1, Interleukin-1 receptor-like 1, and Proprotein convertase subtilisin/kexin type 9 is/are correlated to the occurrence or nonoccurrence disease. The following are preferred diagnostic embodiments. In various embodiments, the methods comprise relating the assay result(s) to ruling in or out one or more of the following diagnoses: that the subject has at least sepsis; that the subject has at least severe sepsis; that the subject has at least septic shock; that the subject has MODS.

[0018] In preferred diagnostic embodiments, these methods comprise distinguishing among SIRS, sepsis, severe sepsis, septic shock and/or MODS. These methods comprise relating the assay result(s) to ruling in or out one or more of the following diagnoses: that the subject has SIRS, but not sepsis, severe sepsis, septic shock, or MODS; that the subject has sepsis, but not severe sepsis, septic shock, or MODS; that the subject has septic shock but not MODS; that the subject has MODS. For a positive going marker, an increased likelihood of the occurrence of a diagnosis is assigned to the patient when the measured concentration is above the threshold (relative to the likelihood assigned when the measured concentration is below the threshold); alternatively, when the measured concentration is below the threshold, an increased likelihood of the nonoccurrence of a diagnosis may be assigned to the patient (relative to the likelihood assigned when the measured concentration is above the threshold). For a negative going marker, an

increased likelihood of the occurrence of a diagnosis is assigned to the patient when the measured concentration is below the threshold (relative to the likelihood assigned when the measured concentration is above the threshold); alternatively, when the measured concentration is above the threshold, an increased likelihood of the nonoccurrence of a diagnosis may be assigned to the patient (relative to the likelihood assigned when the measured concentration is below the threshold).

[0019] A variety of methods may be used by the skilled artisan to arrive at a desired threshold value for use in these methods. For example, for a positive going marker the threshold value may be determined from a population of SIRS patients not having sepsis by selecting a concentration representing the 75th, 85th, 90th, 95th, or 99th percentile of a sepsis biomarker measured in such SIRS patients. Alternatively, the threshold value may be determined from a “diseased” population of sepsis patients by selecting a concentration representing the 75th, 85th, 90th, 95th, or 99th percentile of a sepsis biomarker measured in such sepsis patients.

[0020] Alternatively, the threshold value may be determined from a “diseased” population of sepsis patients having a predisposition for an outcome such as death, worsening disease, AKI, *etc.*), by selecting a concentration representing the 75th, 85th, 90th, 95th, or 99th percentile of a sepsis biomarker measured in such sepsis patients. In another alternative, the threshold value may be determined from a prior measurement of a sepsis biomarker in the same sepsis patient; that is, a temporal change in the level of a sepsis biomarker in the sepsis patient may be used to assign risk to the sepsis patient.

[0021] The foregoing discussion is not meant to imply, however, that the sepsis biomarkers of the present invention must be compared to corresponding individual thresholds. Methods for combining assay results can comprise the use of multivariate logistical regression, loglinear modeling, neural network analysis, n-of-m analysis, decision tree analysis, calculating ratios of markers, *etc.* This list is not meant to be limiting. In these methods, a composite result which is determined by combining individual markers may be treated as if it is itself a marker; that is, a threshold may be determined for the composite result as described herein for individual markers, and the composite result for an individual patient compared to this threshold.

[0022] The ability of a particular test to distinguish two populations can be established using ROC analysis. For example, ROC curves established from a “first”

subpopulation which has a particular disease (or which is predisposed to some outcome), and a “second” subpopulation which does not have the disease (or is not so predisposed) can be used to calculate a ROC curve, and the area under the curve provides a measure of the quality of the test. Preferably, the tests described herein provide a ROC curve area greater than 0.5, preferably at least 0.6, more preferably 0.7, still more preferably at least 0.8, even more preferably at least 0.9, and most preferably at least 0.95.

[0023] In certain aspects, the measured concentration of one or more sepsis biomarkers, or a composite of such markers, may be treated as continuous variables. For example, any particular concentration can be converted into a corresponding probability of existing disease, of a future outcome for the sepsis patient, or mortality, of a SIRS classification, etc. In yet another alternative, a threshold that can provide an acceptable level of specificity and sensitivity in separating a population of sepsis patients into “bins” such as a “first” subpopulation and a “second” subpopulation. A threshold value is selected to separate this first and second population by one or more of the following measures of test accuracy:

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

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

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

than about 0.7, still more preferably greater than about 0.8, more preferably greater than about 0.9, and most preferably greater than about 0.95;

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

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

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

[0024] The term “about” in the context of any of the above measurements refers to +/- 5% of a given measurement.

[0025] Multiple thresholds may also be used to assess a sepsis patient. For example, a “first” subpopulation identified by an existing disease, predisposition to a future outcome for the sepsis patient, predisposition to mortality, etc. , and a “second” subpopulation which is not so predisposed can be combined into a single group. This group is then subdivided into three or more equal parts (known as tertiles, quartiles, quintiles, etc., depending on the number of subdivisions). An odds ratio is assigned to sepsis patients based on which subdivision they fall into. If one considers a tertile, the lowest or highest tertile can be used as a reference for comparison of the other subdivisions. This reference subdivision is assigned an odds ratio of 1. The second tertile is assigned an odds ratio that is relative to that first tertile. That is, someone in the second tertile might be 3 times more likely to suffer one or more future changes in disease status in comparison to someone in the first tertile. The third tertile is also assigned an odds ratio that is relative to that first tertile.

[0026] In certain embodiments, the assay method is an immunoassay. Antibodies for use in such assays will specifically bind a full length sepsis biomarker of interest, and may also bind one or more polypeptides that are “related” thereto, as that term is defined hereinafter. Numerous immunoassay formats are known to those of skill in the art. Preferred body fluid samples are selected from the group consisting of urine, blood, serum, saliva, tears, and plasma.

[0027] The foregoing method steps should not be interpreted to mean that the sepsis biomarker assay result(s) is/are used in isolation in the methods described herein. Rather,

additional variables or other clinical indicia may be included in the methods described herein. For example, a risk stratification, diagnostic, classification, monitoring, etc. method may combine the assay result(s) with one or more variables measured for the sepsis patient selected from the group consisting of demographic information (e.g., weight, sex, age, race), medical history (e.g., family history, type of surgery, pre-existing disease such as aneurism, congestive heart failure, preeclampsia, eclampsia, diabetes mellitus, hypertension, coronary artery disease, proteinuria, or renal insufficiency, clinical variables (e.g., blood pressure, temperature, respiration rate), risk scores (APACHE score, PREDICT score, TIMI Risk Score for UA/NSTEMI, Framingham Risk Score).

[0028] When more than one marker is measured, the individual markers may be measured in samples obtained at the same time, or may be determined from samples obtained at different (e.g., an earlier or later) times. The individual markers may also be measured on the same or different body fluid samples. For example, one sepsis biomarker may be measured in a serum or plasma sample and another sepsis biomarker may be measured in a urine sample. In addition, assignment of a likelihood may combine an individual sepsis biomarker assay result with temporal changes in one or more additional variables.

[0029] In various related aspects, the present invention also relates to devices and kits for performing the methods described herein. Suitable kits comprise reagents sufficient for performing an assay for at least one of the described sepsis biomarkers, together with instructions for performing the described threshold comparisons.

[0030] In certain embodiments, reagents for performing such assays are provided in an assay device, and such assay devices may be included in such a kit. Preferred reagents can comprise one or more solid phase antibodies, the solid phase antibody comprising antibody that detects the intended biomarker target(s) bound to a solid support. In the case of sandwich immunoassays, such reagents can also include one or more detectably labeled antibodies, the detectably labeled antibody comprising antibody that detects the intended biomarker target(s) bound to a detectable label. Additional optional elements that may be provided as part of an assay device are described hereinafter.

[0031] Detectable labels may include molecules that are themselves detectable (e.g., fluorescent moieties, electrochemical labels, ecl (electrochemical luminescence) labels, metal chelates, colloidal metal particles, *etc.*) as well as molecules that may be indirectly

detected by production of a detectable reaction product (*e.g.*, enzymes such as horseradish peroxidase, alkaline phosphatase, *etc.*) or through the use of a specific binding molecule which itself may be detectable (*e.g.*, a labeled antibody that binds to the second antibody, biotin, digoxigenin, maltose, oligohistidine, 2,4-dinitrobenzene, phenylarsenate, ssDNA, dsDNA, *etc.*).

[0032] Generation of a signal from the signal development element can be performed using various optical, acoustical, and electrochemical methods well known in the art. Examples of detection modes include fluorescence, radiochemical detection, reflectance, absorbance, amperometry, conductance, impedance, interferometry, ellipsometry, *etc.* In certain of these methods, the solid phase antibody is coupled to a transducer (*e.g.*, a diffraction grating, electrochemical sensor, *etc.*) for generation of a signal, while in others, a signal is generated by a transducer that is spatially separate from the solid phase antibody (*e.g.*, a fluorometer that employs an excitation light source and an optical detector). This list is not meant to be limiting. Antibody-based biosensors may also be employed to determine the presence or amount of analytes that optionally eliminate the need for a labeled molecule.

DETAILED DESCRIPTION OF THE INVENTION

[0033] The present invention relates to methods and compositions for diagnosis, differential diagnosis, risk stratification, monitoring, classifying and determination of treatment regimens in patients diagnosed with, or at risk of, sepsis. In various embodiments, a measured concentration of one or more biomarkers selected from the group consisting of WAP four-disulfide core domain protein 2, Hepatitis A virus cellular receptor 1, Interleukin-1 receptor-like 1, and Proprotein convertase subtilisin/kexin type 9, or one or more markers related thereto, are correlated to the status of the sepsis patient. As described herein, measurement of one or more sepsis biomarkers of the present invention may be used, individually or in panels comprising a plurality of sepsis biomarkers, for identifying a subject suffering from SIRS, sepsis, severe sepsis, septic shock and/or MODS, for distinguishing amongst these conditions, or for assigning a prognosis to a subject suffering from one or more of these conditions, *etc.*

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

[0035] As used herein, “SIRS” refers to a condition that exhibits two or more of the following:

a temperature $> 38^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$;
a heart rate of > 90 beats per minute (tachycardia);
a respiratory rate of > 20 breaths per minute (tachypnea) or a $\text{PaCO}_2 < 4.3$ kPa; and
a white blood cell count $> 12,000$ per mm^3 , $< 4,000$ per mm^3 , or $> 10\%$ immature (band) forms.

[0036] As used herein, “Sepsis” refers to SIRS, further accompanied by a clinically evident or microbiologically confirmed infection. This infection may be bacterial, fungal, parasitic, or viral.

[0037] As used herein, “Severe sepsis” refers to a subset of sepsis patients, in which sepsis is further accompanied by organ hypoperfusion made evident by at least one sign of organ dysfunction such as hypoxemia, oliguria, metabolic acidosis, or altered cerebral function.

[0038] As used herein, “Septic shock” refers to a subset of severe sepsis patients, in which severe sepsis is further accompanied by hypotension, made evident by a systolic blood pressure < 90 mm Hg, or the requirement for pharmaceutical intervention to maintain blood pressure.

[0039] As used herein, MODS (multiple organ dysfunction syndrome) is the presence of altered organ function in a patient who is acutely ill such that homeostasis cannot be maintained without intervention. Primary MODS is the direct result of a well-defined insult in which organ dysfunction occurs early and can be directly attributable to the insult itself. Secondary MODS develops as a consequence of a host response and is identified within the context of SIRS.

[0040] As used herein, an “injury to renal function” is an abrupt (within 14 days, preferably within 7 days, more preferably within 72 hours, and still more preferably within 48 hours) measurable reduction in a measure of renal function. Such an injury may be identified, for example, by a decrease in glomerular filtration rate or estimated GFR, a reduction in urine output, an increase in serum creatinine, an increase in serum cystatin C, a requirement for renal replacement therapy, *etc.* “Improvement in Renal Function” is an abrupt (within 14 days, preferably within 7 days, more preferably within 72 hours, and still more preferably within 48 hours) measurable increase in a measure of renal function. Preferred methods for measuring and/or estimating GFR are described hereinafter.

[0041] As used herein, “reduced renal function” is an abrupt (within 14 days, preferably within 7 days, more preferably within 72 hours, and still more preferably within 48 hours) reduction in kidney function identified by an absolute increase in serum creatinine of greater than or equal to 0.1 mg/dL ($\geq 8.8 \mu\text{mol/L}$), a percentage increase in serum creatinine of greater than or equal to 20% (1.2-fold from baseline), or a reduction in urine output (documented oliguria of less than 0.5 ml/kg per hour).

[0042] As used herein, “acute renal failure” or “ARF” is an abrupt (within 14 days, preferably within 7 days, more preferably within 72 hours, and still more preferably within 48 hours) reduction in kidney function identified by an absolute increase in serum creatinine of greater than or equal to 0.3 mg/dl ($\geq 26.4 \mu\text{mol/l}$), a percentage increase in serum creatinine of greater than or equal to 50% (1.5-fold from baseline), or a reduction in urine output (documented oliguria of less than 0.5 ml/kg per hour for at least 6 hours). This term is synonymous with “acute kidney injury” or “AKI.”

[0043] As used herein, the term “relating a signal to the presence or amount” of an analyte reflects the following understanding. Assay signals are typically related to the presence or amount of an analyte through the use of a standard curve calculated using known concentrations of the analyte of interest. As the term is used herein, an assay is “configured to detect” an analyte if an assay can generate a detectable signal indicative of the presence or amount of a physiologically relevant concentration of the analyte. Because an antibody epitope is on the order of 8 amino acids, an immunoassay configured to detect a marker of interest will also detect polypeptides related to the marker sequence, so long as those polypeptides contain the epitope(s) necessary to bind to the antibody or antibodies used in the assay. The term “related marker” as used herein with regard to a biomarker such as one of the sepsis biomarkers described herein refers to one or more fragments, variants, etc., of a particular marker or its biosynthetic parent that may be detected as a surrogate for the marker itself or as independent biomarkers. The term also refers to one or more polypeptides present in a biological sample that are derived from the biomarker precursor complexed to additional species, such as binding proteins, receptors, heparin, lipids, sugars, *etc.*

[0044] In this regard, the skilled artisan will understand that the signals obtained from an immunoassay are a direct result of complexes formed between one or more antibodies and the target biomolecule (*i.e.*, the analyte) and polypeptides containing the necessary epitope(s) to which the antibodies bind. While such assays may detect the full length

biomarker and the assay result be expressed as a concentration of a biomarker of interest, the signal from the assay is actually a result of all such “immunoreactive” polypeptides present in the sample. Expression of biomarkers may also be determined by means other than immunoassays, including protein measurements (such as dot blots, western blots, chromatographic methods, mass spectrometry, *etc.*) and nucleic acid measurements (mRNA quantitation). This list is not meant to be limiting. With regard to biomarkers which exist in one form as type-I, type-II, or GPI-anchored membrane proteins, such membrane proteins typically comprise a substantial extracellular domain, some or all of which can be detected as soluble forms present in aqueous samples such as blood, serum, plasma, urine, *etc.*, either as cleavage products or as splice variants which delete an effective membrane spanning domain. Preferred assays detect soluble forms of these biomarkers.

[0045] The term “positive going” marker as that term is used herein refer to a marker that is determined to be elevated in sepsis patients suffering from a disease or condition, relative to sepsis patients not suffering from that disease or condition. The term “negative going” marker as that term is used herein refer to a marker that is determined to be reduced in sepsis patients suffering from a disease or condition, relative to sepsis patients not suffering from that disease or condition.

[0046] The term “subject” as used herein refers to a human or non-human organism. Thus, the methods and compositions described herein are applicable to both human and veterinary disease. Further, while a subject is preferably a living organism, the invention described herein may be used in post-mortem analysis as well. Preferred subjects are humans, and most preferably “patients,” which as used herein refers to living humans that are receiving medical care for a disease or condition. This includes persons with no defined illness who are being investigated for signs of pathology. A “sepsis patient” is a patient suffering from sepsis.

[0047] Preferably, an analyte such as a sepsis biomarker is measured in a sample. Such a sample may be obtained from a patient, such as a sepsis patient. Preferred samples are body fluid samples.

[0048] The term “body fluid sample” as used herein refers to a sample of bodily fluid obtained for the purpose of diagnosis, prognosis, classification or evaluation of a sepsis patient of interest, such as a patient or transplant donor. In certain embodiments, such a

sample may be obtained for the purpose of determining the outcome of an ongoing condition or the effect of a treatment regimen on a condition. Preferred body fluid samples include blood, serum, plasma, cerebrospinal fluid, urine, saliva, sputum, and pleural effusions. In addition, one of skill in the art would realize that certain body fluid samples would be more readily analyzed following a fractionation or purification procedure, for example, separation of whole blood into serum or plasma components.

[0049] The term “diagnosis” as used herein refers to methods by which the skilled artisan can estimate and/or determine the probability (“a likelihood”) of whether or not a patient is suffering from a given disease or condition. In the case of the present invention, “diagnosis” includes using the results of an assay, most preferably an immunoassay, for a sepsis biomarker of the present invention, optionally together with other clinical characteristics, to arrive at a diagnosis (that is, the occurrence or nonoccurrence) of a disease or condition. That such a diagnosis is “determined” is not meant to imply that the diagnosis is 100% accurate. Many biomarkers are indicative of multiple conditions. The skilled clinician does not use biomarker results in an informational vacuum, but rather test results are used together with other clinical indicia to arrive at a diagnosis. Thus, a measured biomarker level on one side of a predetermined diagnostic threshold indicates a greater likelihood of the occurrence of disease in the sepsis patient relative to a measured level on the other side of the predetermined diagnostic threshold.

[0050] Similarly, a prognostic risk signals a probability (“a likelihood”) that a given course or outcome will occur. A level or a change in level of a prognostic indicator, which in turn is associated with an increased probability of morbidity (e.g., worsening sepsis or death) is referred to as being “indicative of an increased likelihood” of an adverse outcome in a patient.

[0051] Sepsis Biomarkers

[0052] The following table provides a list of the sepsis biomarkers of the present invention, together with the Swiss-Prot entry number for the human precursor.

SwissProt Accession Preferred Name

Q14508	WAP four-disulfide core domain protein 2
Q96D42	Hepatitis A virus cellular receptor 1
Q01638	Interleukin-1 receptor-like 1

Q8NBP7 Proprotein convertase subtilisin/kexin type 9

[0053] Additional biomarkers having utility in the evaluation of sepsis may be used together with one or more of the sepsis biomarkers disclosed herein in multimarker panels. Examples of such biomarkers are provided in the following table:

<u>SwissProt Accession</u>	<u>Preferred Name</u>
P25942	Tumor necrosis factor receptor superfamily member 5
P29965	CD40 ligand
P01343	Insulin-like growth factor IA
P01133	Pro-epidermal growth factor
P00738	Haptoglobin
P08254	Stromelysin-1
P01033	Metalloproteinase inhibitor 1
P19320	Vascular cell adhesion protein 1
P15692	Vascular endothelial growth factor A
P18510	Interleukin-1 receptor antagonist protein
P80511	Protein S100-A12
P17174	Aspartate aminotransferase, cytoplasmic
P40933	Interleukin-15
P01374	Lymphotoxin-alpha
P29459;P29460	Interleukin-12
P47992	Lymphotoxin
P51671	Eotaxin
O00626	C-C motif chemokine 22
P13501	C-C motif chemokine 5
P42830	C-X-C motif chemokine 5
P08700	Interleukin-3
P21583	Kit ligand

P04141	Granulocyte-macrophage colony-stimulating factor
P22301	Interleukin-10
P01584	Interleukin-1 beta
P01584	Interleukin-1 beta
Q14005	Pro-interleukin-16
P01579	Interferon gamma
P60568	Interleukin-2
P05112	Interleukin-4
P01583	Interleukin-1 alpha
P09919	Granulocyte colony-stimulating factor
P01375	Tumor necrosis factor
Q14790	Caspase-8
P08246	Neutrophil elastase
P25445	Tumor necrosis factor receptor superfamily member 6
P48023	Tumor necrosis factor ligand superfamily member 6
P10144	Granzyme B
P13598	Intercellular adhesion molecule 2
P35228	Nitric oxide synthase, inducible
P16109	P-selectin
P09601	Heme oxygenase 1
P08107	Heat shock 70 kDa protein 1
P16284	Platelet endothelial cell adhesion molecule
Q92934	Bcl2 antagonist of cell death
P29466	Caspase-1
P16070	CD44 antigen
P78423	Fractalkine

P08887	Interleukin-6 receptor subunit alpha
P78380	Oxidized low-density lipoprotein receptor 1
P04637	Cellular tumor antigen p53
P17813	Endoglin
P16581	E-selectin
P08571	Monocyte differentiation antigen CD14
P01137	Transforming growth factor beta-1
P07996	Thrombospondin-1
P27930	Interleukin-1 receptor type II
P02751	Fibronectin
P10451	Osteopontin
P09341	Growth-regulated protein alpha
P05164	Myeloperoxidase
P09038	Heparin-binding growth factor 2
P05362	Intercellular adhesion molecule 1
P14780	Matrix metalloproteinase-9
P00797	Renin
P99999	Cytochrome c
P19875	C-X-C motif chemokine 2
P07148	Fatty acid-binding protein, liver
P02778	C-X-C motif chemokine 10
Q07325	C-X-C motif chemokine 9
P12277	Creatine kinase B-type
Q8WXI7	Mucin-16
P05113	Interleukin-5
P40225	Thrombopoietin

P13726	Tissue factor
P01258	Calcitonin
P02771	Alpha-fetoprotein
P02647	Apolipoprotein A-I
P23560	Brain-derived neurotrophic factor
P02741	C-reactive protein
P08709	Coagulation factor VII
P35225	Interleukin-13
P01308	Insulin
P02144	Myoglobin
P02671, P02675, P02679	Fibrinogen
P01241	Somatotropin
P13232	Interleukin-7
P61769	Beta-2-microglobulin
P05121	Plasminogen activator inhibitor 1
P20333	Tumor necrosis factor receptor superfamily member 1B
P05231	Interleukin-6
P10147	C-C motif chemokine 3
P13500	C-C motif chemokine 2
P13236	C-C motif chemokine 4
P10145	Interleukin-8
P08263	Glutathione S-transferase A1
P05305	Endothelin-1
P02794, P02792	Ferritin
na	Immunoglobulin A
P04275	von Willebrand Factor

P41159	Leptin
P19438	Tumor necrosis factor receptor superfamily member 1A
P01034	Cystatin-C
Q14116	Interleukin-18
P17213	Bactericidal permeability-increasing protein
P80188	Neutrophil gelatinase-associated lipocalin
P16860	Natriuretic peptides B
P27487	Dipeptidyl peptidase 4
P08833	Insulin-like growth factor-binding protein 1
P18065	Insulin-like growth factor-binding protein 2
Q03405	Urokinase plasminogen activator surface receptor
P05937	Calbindin
P04271	Protein S100-B
P14174	Macrophage migration inhibitory factor
P07858	Cathepsin B
Q16665	Hypoxia-inducible factor 1 alpha
P13591	Neural cell adhesion molecule 1
P10600	Transforming growth factor beta-3
P07204	Thrombomodulin
P08473	Neprilysin
P80098	C-C motif chemokine 7
P80075	C-C motif chemokine 8
P55773	C-C motif chemokine 23
P80162	C-X-C motif chemokine 6
P02775	Platelet basic protein
P01589	Interleukin-2 receptor alpha chain

P04070	Vitamin K-dependent protein C
Q99731	C-C motif chemokine 19
P50591	Tumor necrosis factor ligand superfamily member 10
na	Malondialdehyde-modified low-density lipoprotein
P32942	Intercellular adhesion molecule 3
Q99616	C-C motif chemokine 13
00300	Tumor necrosis factor receptor superfamily member 11B
P09237	Matrilysin
P01138	Beta-nerve growth factor
P40189	Interleukin-6 receptor subunit beta
P14778	Interleukin-1 receptor type I
P24394	Interleukin-4 receptor alpha chain
O43927	C-X-C motif chemokine 13
Q9Y258	C-C motif chemokine 26
Q9Y4X3	C-C motif chemokine 27
P15018	Leukemia inhibitory factor
P07339	Cathepsin D
Q16552	Interleukin-17A
P22692	Insulin-like growth factor-binding protein 4
P22894	Neutrophil collagenase
P09603	Macrophage colony-stimulating factor 1
P20809	Interleukin-11
P02735	Serum amyloid A protein
P19883	Follistatin
na	Hydrocortisone (Cortisol)
O14788	Tumor necrosis factor ligand superfamily member 11

O00253	Agouti-related protein
P02766	Transthyretin
P15514	Amphiregulin
Q9NSA1	Fibroblast growth factor 21
P10809	60 kDa heat shock protein, mitochondrial
P01137	Transforming growth factor beta-1

[0054] Marker Assays

[0055] In general, immunoassays involve contacting a sample containing or suspected of containing a biomarker of interest with at least one antibody that specifically binds to the biomarker. A signal is then generated indicative of the presence or amount of complexes formed by the binding of polypeptides in the sample to the antibody. The signal is then related to the presence or amount of the biomarker in the sample. Numerous methods and devices are well known to the skilled artisan for the detection and analysis of biomarkers. *See, e.g.*, U.S. Patents 6,143,576; 6,113,855; 6,019,944; 5,985,579; 5,947,124; 5,939,272; 5,922,615; 5,885,527; 5,851,776; 5,824,799; 5,679,526; 5,525,524; and 5,480,792, and *The Immunoassay Handbook*, David Wild, ed. Stockton Press, New York, 1994, each of which is hereby incorporated by reference in its entirety, including all tables, figures and claims.

[0056] The assay devices and methods known in the art can utilize labeled molecules in various sandwich, competitive, or non-competitive assay formats, to generate a signal that is related to the presence or amount of the biomarker of interest. Suitable assay formats also include chromatographic, mass spectrographic, and protein “blotting” methods. Additionally, certain methods and devices, such as biosensors and optical immunoassays, may be employed to determine the presence or amount of analytes without the need for a labeled molecule. *See, e.g.*, U.S. Patents 5,631,171; and 5,955,377, each of which is hereby incorporated by reference in its entirety, including all tables, figures and claims. One skilled in the art also recognizes that robotic instrumentation including but not limited to Beckman ACCESS®, Abbott AXSYM®, Roche ELECSYS®, Dade Behring STRATUS® systems are among the immunoassay analyzers that are capable of performing immunoassays. But any suitable immunoassay may be

utilized, for example, enzyme-linked immunoassays (ELISA), radioimmunoassays (RIAs), competitive binding assays, and the like.

[0057] Antibodies or other polypeptides may be immobilized onto a variety of solid supports for use in assays. Solid phases that may be used to immobilize specific binding members include those developed and/or used as solid phases in solid phase binding assays. Examples of suitable solid phases include membrane filters, cellulose-based papers, beads (including polymeric, latex and paramagnetic particles), glass, silicon wafers, microparticles, nanoparticles, TentaGels, AgroGels, PEGA gels, SPOCC gels, and multiple-well plates. An assay strip could be prepared by coating the antibody or a plurality of antibodies in an array on solid support. This strip could then be dipped into the test sample and then processed quickly through washes and detection steps to generate a measurable signal, such as a colored spot. Antibodies or other polypeptides may be bound to specific zones of assay devices either by conjugating directly to an assay device surface, or by indirect binding. In an example of the latter case, antibodies or other polypeptides may be immobilized on particles or other solid supports, and that solid support immobilized to the device surface.

[0058] Biological assays require methods for detection, and one of the most common methods for quantitation of results is to conjugate a detectable label to a protein or nucleic acid that has affinity for one of the components in the biological system being studied. Detectable labels may include molecules that are themselves detectable (*e.g.*, fluorescent moieties, electrochemical labels, metal chelates, *etc.*) as well as molecules that may be indirectly detected by production of a detectable reaction product (*e.g.*, enzymes such as horseradish peroxidase, alkaline phosphatase, *etc.*) or by a specific binding molecule which itself may be detectable (*e.g.*, biotin, digoxigenin, maltose, oligohistidine, 2,4-dinitrobenzene, phenylarsenate, ssDNA, dsDNA, *etc.*).

[0059] Preparation of solid phases and detectable label conjugates often comprise the use of chemical cross-linkers. Cross-linking reagents contain at least two reactive groups, and are divided generally into homofunctional cross-linkers (containing identical reactive groups) and heterofunctional cross-linkers (containing non-identical reactive groups). Homobifunctional cross-linkers that couple through amines, sulfhydryls or react non-specifically are available from many commercial sources. Maleimides, alkyl and aryl halides, alpha-haloacyls and pyridyl disulfides are thiol reactive groups. Maleimides, alkyl and aryl halides, and alpha-haloacyls react with sulfhydryls to form thiol ether

bonds, while pyridyl disulfides react with sulfhydryls to produce mixed disulfides. The pyridyl disulfide product is cleavable. Imidoesters are also very useful for protein-protein cross-links. A variety of heterobifunctional cross-linkers, each combining different attributes for successful conjugation, are commercially available.

[0060] In certain aspects, the present invention provides kits for the analysis of the described sepsis biomarkers. The kit comprises reagents for the analysis of at least one test sample which comprise at least one antibody that binds a sepsis biomarker. The kit can also include devices and instructions for performing one or more of the diagnostic and/or prognostic correlations described herein. Preferred kits will comprise an antibody pair for performing a sandwich assay, or a labeled species for performing a competitive assay, for the analyte. Preferably, an antibody pair comprises a first antibody conjugated to a solid phase and a second antibody conjugated to a detectable label, wherein each of the first and second antibodies bind a sepsis biomarker. Most preferably each of the antibodies are monoclonal antibodies. The instructions for use of the kit and performing the correlations can be in the form of labeling, which refers to any written or recorded material that is attached to, or otherwise accompanies a kit at any time during its manufacture, transport, sale or use. For example, the term labeling encompasses advertising leaflets and brochures, packaging materials, instructions, audio or video cassettes, computer discs, as well as writing imprinted directly on kits.

[0061] Antibodies

[0062] The term "antibody" as used herein refers to a peptide or polypeptide derived from, modeled after or substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, capable of specifically binding an antigen or epitope. See, e.g. *Fundamental Immunology*, 3rd Edition, W.E. Paul, ed., Raven Press, N.Y. (1993); Wilson (1994; *J. Immunol. Methods* 175:267-273; Yarmush (1992) *J. Biochem. Biophys. Methods* 25:85-97. The term antibody includes antigen-binding portions, i.e., "antigen binding sites," (e.g., fragments, subsequences, complementarity determining regions (CDRs)) that retain capacity to bind antigen, including (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) *Nature* 341:544-546), which consists of a VH domain;

and (vi) an isolated complementarity determining region (CDR). Single chain antibodies are also included by reference in the term "antibody."

[0063] Antibodies used in the immunoassays described herein preferably specifically bind to a sepsis biomarker of the present invention. The term "specifically binds" is not intended to indicate that an antibody binds exclusively to its intended target since, as noted above, an antibody binds to any polypeptide displaying the epitope(s) to which the antibody binds. Rather, an antibody "specifically binds" if its affinity for its intended target is about 5-fold greater when compared to its affinity for a non-target molecule which does not display the appropriate epitope(s). Preferably the affinity of the antibody will be at least about 5 fold, preferably 10 fold, more preferably 25-fold, even more preferably 50-fold, and most preferably 100-fold or more, greater for a target molecule than its affinity for a non-target molecule. In preferred embodiments, Preferred antibodies bind with affinities of at least about 10^7 M^{-1} , and preferably between about 10^8 M^{-1} to about 10^9 M^{-1} , about 10^9 M^{-1} to about 10^{10} M^{-1} , or about 10^{10} M^{-1} to about 10^{12} M^{-1} .

[0064] Affinity is calculated as $K_d = k_{\text{off}}/k_{\text{on}}$ (k_{off} is the dissociation rate constant, K_{on} is the association rate constant and K_d is the equilibrium constant). Affinity can be determined at equilibrium by measuring the fraction bound (r) of labeled ligand at various concentrations (c). The data are graphed using the Scatchard equation: $r/c = K(n-r)$: where r = moles of bound ligand/mole of receptor at equilibrium; c = free ligand concentration at equilibrium; K = equilibrium association constant; and n = number of ligand binding sites per receptor molecule. By graphical analysis, r/c is plotted on the Y-axis versus r on the X-axis, thus producing a Scatchard plot. Antibody affinity measurement by Scatchard analysis is well known in the art. *See, e.g., van Erp et al., J. Immunoassay* 12: 425-43, 1991; Nelson and Griswold, *Comput. Methods Programs Biomed.* 27: 65-8, 1988.

[0065] The term "epitope" refers to an antigenic determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[0066] Numerous publications discuss the use of phage display technology to produce and screen libraries of polypeptides for binding to a selected analyte. *See, e.g., Cwirla et*

al., *Proc. Natl. Acad. Sci. USA* 87, 6378-82, 1990; Devlin *et al.*, *Science* 249, 404-6, 1990, Scott and Smith, *Science* 249, 386-88, 1990; and Ladner *et al.*, U.S. Pat. No. 5,571,698. A basic concept of phage display methods is the establishment of a physical association between DNA encoding a polypeptide to be screened and the polypeptide. This physical association is provided by the phage particle, which displays a polypeptide as part of a capsid enclosing the phage genome which encodes the polypeptide. The establishment of a physical association between polypeptides and their genetic material allows simultaneous mass screening of very large numbers of phage bearing different polypeptides. Phage displaying a polypeptide with affinity to a target bind to the target and these phage are enriched by affinity screening to the target. The identity of polypeptides displayed from these phage can be determined from their respective genomes. Using these methods a polypeptide identified as having a binding affinity for a desired target can then be synthesized in bulk by conventional means. *See, e.g.*, U.S. Patent No. 6,057,098, which is hereby incorporated in its entirety, including all tables, figures, and claims.

[0067] The antibodies that are generated by these methods may then be selected by first screening for affinity and specificity with the purified polypeptide of interest and, if required, comparing the results to the affinity and specificity of the antibodies with polypeptides that are desired to be excluded from binding. The screening procedure can involve immobilization of the purified polypeptides in separate wells of microtiter plates. The solution containing a potential antibody or groups of antibodies is then placed into the respective microtiter wells and incubated for about 30 min to 2 h. The microtiter wells are then washed and a labeled secondary antibody (for example, an anti-mouse antibody conjugated to alkaline phosphatase if the raised antibodies are mouse antibodies) is added to the wells and incubated for about 30 min and then washed. Substrate is added to the wells and a color reaction will appear where antibody to the immobilized polypeptide(s) are present.

[0068] The antibodies so identified may then be further analyzed for affinity and specificity in the assay design selected. In the development of immunoassays for a target protein, the purified target protein acts as a standard with which to judge the sensitivity and specificity of the immunoassay using the antibodies that have been selected. Because the binding affinity of various antibodies may differ; certain antibody pairs (e.g., in sandwich assays) may interfere with one another sterically, etc., assay performance of an

antibody may be a more important measure than absolute affinity and specificity of an antibody.

[0069] While the present application describes antibody-based binding assays in detail, alternatives to antibodies as binding species in assays are well known in the art. These include receptors for a particular target, aptamers, etc. Aptamers are oligonucleic acid or peptide molecules that bind to a specific target molecule. Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist. High-affinity aptamers containing modified nucleotides can confer improved characteristics on the ligand, such as improved in vivo stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions, and may include amino acid side chain functionalities.

[0070] Assay Correlations

[0071] The term “correlating” as used herein in reference to the use of biomarkers refers to comparing the presence or amount of the biomarker(s) in a patient to its presence or amount in persons known to suffer from, or known to be at risk of, a given condition; or in persons known to be free of a given condition. Often, this takes the form of comparing an assay result in the form of a biomarker concentration to a predetermined threshold selected to be indicative of the occurrence or nonoccurrence of a disease or the likelihood of some future outcome.

[0072] Selecting a diagnostic threshold involves, among other things, consideration of the probability of disease, distribution of true and false diagnoses at different test thresholds, and estimates of the consequences of treatment (or a failure to treat) based on the diagnosis. For example, when considering administering a specific therapy which is highly efficacious and has a low level of risk, few tests are needed because clinicians can accept substantial diagnostic uncertainty. On the other hand, in situations where treatment options are less effective and more risky, clinicians often need a higher degree of diagnostic certainty. Thus, cost/benefit analysis is involved in selecting a diagnostic threshold.

[0073] Suitable thresholds may be determined in a variety of ways. For example, one recommended diagnostic threshold for the diagnosis of acute myocardial infarction using cardiac troponin is the 97.5th percentile of the concentration seen in a normal population.

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

[0074] Population studies may also be used to select a decision threshold. Receiver Operating Characteristic (“ROC”) arose from the field of signal detection theory developed during World War II for the analysis of radar images, and ROC analysis is often used to select a threshold able to best distinguish a “diseased” subpopulation from a “nondiseased” subpopulation. A false positive in this case occurs when the person tests positive, but actually does not have the disease. A false negative, on the other hand, occurs when the person tests negative, suggesting they are healthy, when they actually do have the disease. To draw a ROC curve, the true positive rate (TPR) and false positive rate (FPR) are determined as the decision threshold is varied continuously. Since TPR is equivalent with sensitivity and FPR is equal to 1 - specificity, the ROC graph is sometimes called the sensitivity vs (1 - specificity) plot. A perfect test will have an area under the ROC curve of 1.0; a random test will have an area of 0.5. A threshold is selected to provide an acceptable level of specificity and sensitivity.

[0075] In this context, “diseased” is meant to refer to a population having one characteristic (the presence of a disease or condition or the occurrence of some outcome) and “nondiseased” is meant to refer to a population lacking the characteristic. While a single decision threshold is the simplest application of such a method, multiple decision thresholds may be used. For example, below a first threshold, the absence of disease may be assigned with relatively high confidence, and above a second threshold the presence of disease may also be assigned with relatively high confidence. Between the two thresholds may be considered indeterminate. This is meant to be exemplary in nature only.

[0076] In addition to threshold comparisons, other methods for correlating assay results to a patient classification (occurrence or nonoccurrence of disease, likelihood of an outcome, etc.) include decision trees, rule sets, Bayesian methods, and neural network methods. These methods can produce probability values representing the degree to which a sepsis patient belongs to one classification out of a plurality of classifications.

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

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

[0077] As discussed above, suitable tests may exhibit one or more of the following results on these various measures: a specificity of greater than 0.5, preferably at least 0.6, more preferably at least 0.7, still more preferably at least 0.8, even more preferably at least 0.9 and most preferably at least 0.95, with a corresponding sensitivity greater than 0.2, preferably greater than 0.3, more preferably greater than 0.4, still more preferably at least 0.5, even more preferably 0.6, yet more preferably greater than 0.7, still more preferably greater than 0.8, more preferably greater than 0.9, and most preferably greater than 0.95; a sensitivity of greater than 0.5, preferably at least 0.6, more preferably at least 0.7, still more preferably at least 0.8, even more preferably at least 0.9 and most preferably at least 0.95, with a corresponding specificity greater than 0.2, preferably greater than 0.3, more preferably greater than 0.4, still more preferably at least 0.5, even more preferably 0.6, yet more preferably greater than 0.7, still more preferably greater than 0.8, more preferably greater than 0.9, and most preferably greater than 0.95; at least 75% sensitivity, combined with at least 75% specificity; a ROC curve area of greater than 0.5, preferably at least 0.6, more preferably 0.7, still more preferably at least 0.8, even more preferably at least 0.9, and most preferably at least 0.95; an odds ratio different from 1, preferably at least about 2 or more or about 0.5 or less, more preferably at least about 3 or more or about 0.33 or less, still more preferably at least about 4 or more or about 0.25 or less, even more preferably at least about 5 or more or about 0.2 or less, and most preferably at least about 10 or more or about 0.1 or less; a positive likelihood ratio (calculated as sensitivity/(1-specificity)) of greater than 1, at least 2, more preferably at least 3, still more preferably at least 5, and most preferably at least 10; and or a negative likelihood ratio (calculated as (1-sensitivity)/specificity) of less than 1, less than or equal to 0.5, more preferably less than or equal to 0.3, and most preferably less than or equal to 0.1

[0078] Additional clinical indicia may be combined with the sepsis biomarker assay result(s) of the present invention. Other clinical indicia which may be combined with the sepsis biomarker assay result(s) of the present invention includes demographic

information (e.g., weight, sex, age, race), medical history (e.g., family history, type of surgery, pre-existing disease such as aneurism, congestive heart failure, preeclampsia, eclampsia, diabetes mellitus, hypertension, coronary artery disease, proteinuria, or renal insufficiency), risk scores (APACHE score, PREDICT score, TIMI Risk Score for UA/NSTEMI, Framingham Risk Score), a urine total protein measurement, a glomerular filtration rate, an estimated glomerular filtration rate, a urine production rate, a serum or plasma creatinine concentration, a renal papillary antigen 1 (RPA1) measurement; a renal papillary antigen 2 (RPA2) measurement; a urine creatinine concentration, a fractional excretion of sodium, a urine sodium concentration, a urine creatinine to serum or plasma creatinine ratio, a urine specific gravity, a urine osmolality, a urine urea nitrogen to plasma urea nitrogen ratio, a plasma BUN to creatinine ratio, and/or a renal failure index calculated as $\text{urine sodium} / (\text{urine creatinine} / \text{plasma creatinine})$.

[0079] Combining assay results/clinical indicia in this manner can comprise the use of multivariate logistical regression, loglinear modeling, neural network analysis, n-of-m analysis, decision tree analysis, etc. This list is not meant to be limiting.

[0080] Selecting a Treatment Regimen

[0081] Once a diagnosis is obtained, the clinician can readily select a treatment regimen that is compatible with the diagnosis. The skilled artisan is aware of appropriate treatments for numerous diseases discussed in relation to the methods of diagnosis described herein. See, e.g., Merck Manual of Diagnosis and Therapy, 17th Ed. Merck Research Laboratories, Whitehouse Station, NJ, 1999. In addition, since the methods and compositions described herein provide prognostic information, the markers of the present invention may be used to monitor a course of treatment. For example, improved or worsened prognostic state may indicate that a particular treatment is or is not efficacious.

[0082] A number of medications are used in treating sepsis. They include:

Antibiotics. Treatment with antibiotics begins immediately — even before the infectious agent is identified. Initially broad-spectrum antibiotics, which are effective against a variety of bacteria, may be employed. The antibiotics are typically administered intravenously. After identification of the organism(s) involved, the patient may be switched to a different antibiotic that's more appropriate against the particular bacteria causing the infection. Intravenous antibiotics usually have to be given for 7 to 10 days. If the sepsis is caused by a

virus, antibiotics will not work. However, it is likely that antibiotics will be started anyway. This is because it would be too dangerous to delay antibiotic treatment until an accurate diagnosis is made. In some cases, antiviral medication may be given.

Vasopressors. If blood pressure remains too low even after receiving intravenous fluids, the patient may be given a vasopressor medication, which constricts blood vessels and helps to increase blood pressure.

Fluids. Often the body needs extra fluids to help keep the blood pressure from dropping dangerously low, throwing the patient into shock. Giving the fluids by IV allows the health care staff to track how much fluid is being administered and to control the type of fluid the patient is getting. Colloids that may be given by IV include albumin *and* dextran. Colloids do not dissolve as quickly as crystalloids. More crystalloid fluid is needed than colloid fluid to achieve the same goal of boosting body fluid volume, but crystalloids are less expensive.

Steroids. Until the late 1980s, physicians regularly gave high, anti-inflammatory doses of steroids to patients in septic shock. A number of smaller studies, as well as a meta-analysis that looked at many of the studies published since 1987, found that low-dose steroid regimens have consistently reported a beneficial effect for steroids.

[0083] In addition, supportive therapies such as kidney dialysis, mechanical ventilation, surgery to remove infected tissue, etc. may be employed.

[0084] One skilled in the art readily appreciates that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The examples provided herein are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention.

[0085] Example 1: Sepsis patient sample collection

[0086] The objective of this study is to collect samples from acutely ill patients. Approximately 1900 adults expected to be in the ICU for at least 48 hours will be enrolled. To be enrolled in the study, each patient must meet all of the following inclusion criteria and none of the following exclusion criteria:

Inclusion Criteria

males and females 18 years of age or older;

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

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

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

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

contrast media exposure within 24 hours of enrollment;

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

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

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

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

(i) respiratory SOFA score of ≥ 2 ($\text{PaO}_2/\text{FiO}_2 < 300$), (ii) cardiovascular SOFA score of ≥ 1 (MAP < 70 mm Hg and/or any vasopressor required).

Exclusion Criteria

known pregnancy;

institutionalized individuals;

previous renal transplantation;

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

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

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

meets any of the following:

- (i) active bleeding with an anticipated need for > 4 units PRBC in a day;
- (ii) hemoglobin < 7 g/dL;
- (iii) any other condition that in the physician's opinion would contraindicate drawing serial blood samples for clinical study purposes;

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

[0087] After obtaining informed consent, an EDTA anti-coagulated blood sample (10 mL) and a urine sample (25-50 mL) are collected from each patient. Blood and urine samples are then collected at 4 ($\pm$ 0.5) and 8 ($\pm$ 1) hours after contrast administration (if applicable); at 12 ($\pm$ 1), 24 ($\pm$ 2), 36 ($\pm$ 2), 48 ($\pm$ 2), 60 ($\pm$ 2), 72 ($\pm$ 2), and 84 ($\pm$ 2) hours after enrollment, and thereafter daily up to day 7 to day 14 while the subject is hospitalized. Blood is collected via direct venipuncture or via other available venous access, such as an existing femoral sheath, central venous line, peripheral intravenous line or hep-lock. These study blood samples are processed to plasma at the clinical site, frozen and shipped to Astute Medical, Inc., San Diego, CA. The study urine samples are frozen and shipped to Astute Medical, Inc.

[0088] Example 2. Immunoassay format

[0089] Analytes are measured using standard sandwich enzyme immunoassay techniques. A first antibody which binds the analyte is immobilized in wells of a 96 well polystyrene microplate. Analyte standards and test samples are pipetted into the appropriate wells and any analyte present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase-conjugated second antibody which binds the analyte is added to the wells, thereby forming sandwich complexes with the analyte (if present) and the first antibody. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution comprising

tetramethylbenzidine and hydrogen peroxide is added to the wells. Color develops in proportion to the amount of analyte present in the sample. The color development is stopped and the intensity of the color is measured at 540 nm or 570 nm. An analyte concentration is assigned to the test sample by comparison to a standard curve determined from the analyte standards.

[0090] Example 3. Use of analyte as a marker for sepsis

[0091] Patients from the intensive care unit (ICU) are classified as positive or negative for sepsis according to clinical diagnosis on each day from enrollment to 6 days after.

[0092] Two cohorts were defined as (Cohort 1) patients who had sepsis, and (Cohort 2) patients who did not have sepsis on any day from enrollment to 6 days after (7 days total). Both urine and plasma samples from each patient in Cohorts 1 and 2 were collected on the day of enrollment. The concentrations of the analytes in these samples were measured by standard immunoassay methods using commercially available assay reagents. A receiver operating characteristic (ROC) curve was generated using the concentrations, and the performance of the analyte is assessed by the area under the ROC curve (AUC). The two-tailed *p*-value of the AUC for the analyte was calculated, and if the *p*-value is less than 0.1, the AUC is considered statistically significant.

[0093] Table 1: plasma samples

SwissProt	Preferred Name	AUC	#ND	#D	p
Q01638	Interleukin-1 receptor-like 1	0.7265	35	21	0.0019
Q8NBP7	Proprotein convertase subtilisin/kexin type 9	0.3075	35	21	0.0107
Q96D42	Hepatitis A virus cellular receptor 1	0.6548	33	23	0.0412
Q14508	WAP four-disulfide core domain protein 2	0.6436	33	21	0.0686

[0094] Table 2: urine samples

SwissProt	Preferred Name	AUC	#ND	#D	p
Q01638	Interleukin-1 receptor-like 1	0.7066	38	20	0.0059
Q14508	WAP four-disulfide core domain protein 2	0.6292	36	20	0.1056
Q96D42	Hepatitis A virus cellular receptor 1	0.6053	296	177	0.0001
Q8NBP7	Proprotein convertase subtilisin/kexin type 9	0.5947	38	20	0.2375

[0095] Example 4. Use of analyte as a marker for progression to sepsis

[0096] Two cohorts are defined as (Cohort 1) patients who did not have sepsis during the 5 days prior to enrollment but had sepsis on any of the subsequent 7 days (enrollment to 6 days after), and (Cohort 2) patients who did not have sepsis on any of the 5 days prior to enrollment and on any of the subsequent 7 days. The urine and plasma sample collections and analyses using the measured analyte concentrations are the same as in Example 3 above.

[0097] Example 5. Use of analyte as a marker for septic shock

[0098] Two cohorts are defined as (Cohort 1) patients who had both sepsis and hypotension on the same day (septic shock), and (Cohort 2) patients who did not have septic shock on any day from enrollment to 6 days after. A patient is classified as having hypotension if his/her systolic blood pressure is below 90mm Hg. The urine and plasma sample collections and analyses using the measured analyte concentrations are the same as in Example 3 above.

[0099] Example 6. Use of analyte as a marker for progression to septic shock

[0100] Two cohorts are defined as (Cohort 1) patients who did not have septic shock (see Example 5 for definition) on any of the 5 days prior to enrollment but had septic shock on any of the subsequent 7 days (enrollment to 6 days after), and (Cohort 2) patients who did not have septic shock on any of the 5 days prior to enrollment and on any of the subsequent 7 days. The urine and plasma sample collections and analyses using the measured analyte concentrations are the same as in Example 3 above.

[0101] Example 7. Use of analyte as a marker for death with sepsis

[0102] Two cohorts are defined as (Cohort 1) patients who had sepsis on any day from enrollment to 6 days after and died within 30 days after enrollment, and (Cohort 2) patients who did not die and patients who died within 30 days after enrollment but did not have sepsis on any day from enrollment to 6 days after. The urine and plasma sample collections and analyses using the measured analyte concentrations are the same as in Example 3 above.

[0103] Example 8. Use of analyte as a marker for death with septic shock

[0104] Two cohorts are defined as (Cohort 1) patients who had septic shock (see Example 5 for definition) on any day from enrollment to 6 days after and died within 30 days after enrollment, and (Cohort 2) patients who did not die and patients who died within 30 days after enrollment but did not have septic shock on any day from enrollment to 6 days after. The urine and plasma sample collections and analyses using the measured analyte concentrations are the same as in Example 3 above.

[0105] While the invention has been described and exemplified in sufficient detail for those skilled in this art to make and use it, various alternatives, modifications, and improvements should be apparent without departing from the spirit and scope of the invention. The examples provided herein are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Modifications therein and other uses will occur to those skilled in the art. These modifications are encompassed within the spirit of the invention and are defined by the scope of the claims.

[0106] It will be readily apparent to a person skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

[0107] All patents and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

[0108] The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically

disclosed herein. Thus, for example, in each instance herein any of the terms “comprising”, “consisting essentially of” and “consisting of” may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[0109] Other embodiments are set forth within the following claims.

We claim:

1. A method of diagnosing SIRS, sepsis, severe sepsis, septic shock, or MODS in a subject, or assigning a prognostic risk for one or more clinical outcomes for a subject suffering from SIRS, sepsis, severe sepsis, septic shock, or MODS, the method comprising:

performing one or more assays configured to detect one or more biomarkers selected from the group consisting of WAP four-disulfide core domain protein 2, Hepatitis A virus cellular receptor 1, Interleukin-1 receptor-like 1, and Proprotein convertase subtilisin/kexin type 9 on a body fluid sample obtained from the sepsis patient to provide one or more assay result(s); and

relating the immunoassay result(s) to one or more diagnoses or prognoses selected from the group consisting of the presence or absence of SIRS, the presence or absence of sepsis, the presence or absence of severe sepsis, the presence or absence of septic shock, the presence or absence of MODS, and the prognostic risk of one or more clinical outcomes for the subject suffering from or believed to suffer from SIRS, sepsis, severe sepsis, septic shock, or MODS.

2. A method according to claim 1, wherein the relating step comprises relating the immunoassay result to a prognostic risk of mortality.

3. A method according to claim 1, wherein the relating step comprises relating the immunoassay result to a prognostic risk of one or more future changes in renal status.

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

5. A method according to claim 1, wherein said relating step comprises comparing each of the immunoassay result(s) to a corresponding predetermined threshold level selected to provide a sensitivity or specificity of at least 0.7 for the diagnosis of sepsis, compared to SIRS not progressed to sepsis.

6. A method according to claim 1, wherein said relating step comprises comparing each of the immunoassay result(s) to a corresponding predetermined threshold level selected to provide a sensitivity or specificity of at least 0.7 for the diagnosis of severe sepsis, compared to SIRS not progressed to severe sepsis.
7. A method according to claim 1, wherein said relating step comprises comparing each of the immunoassay result(s) to a corresponding predetermined threshold level selected to provide a sensitivity or specificity of at least 0.7 for the diagnosis of septic shock, compared to SIRS not progressed to septic shock.
8. A method according to claim 1, wherein said relating step comprises comparing each of the immunoassay result(s) to a corresponding predetermined threshold level selected to provide an odds ratio of at least 2 for the prognostic risk of mortality.
9. A method according to claim 1, wherein said relating step comprises comparing each of the immunoassay result(s) to a corresponding predetermined threshold level selected to provide an odds ratio of at least 2 for the prognostic risk of a worsening sepsis classification level selected from the group consisting of severe sepsis, septic shock, and MODS.
10. A method according to claim 1, wherein said relating step comprises comparing each of the immunoassay result(s) to a corresponding predetermined threshold level selected to provide an odds ratio of at least 2 for the prognostic risk of one or more of a future injury to renal function, future reduced renal function, future improvement in renal function, and future ARF.
11. A method according to claim 1, wherein the sample is selected from the group consisting of blood, serum, and plasma.
12. A method according to claim 1, wherein the method is a prognostic method, and the method differentiates between a risk of future sepsis and a risk of future severe sepsis or septic shock.
13. A method according to claim 1, wherein the method is a prognostic method, and the method differentiates between a risk of future sepsis or severe sepsis and a risk of future septic shock.

14. A method according to claim 1, wherein the method is a diagnostic method, and the method differentiates between a diagnosis of sepsis and a diagnosis of severe sepsis or septic shock.

15. A method according to claim 1, wherein the method is a diagnostic method, and the method differentiates between a diagnosis of sepsis or severe sepsis and a diagnosis of septic shock.

16. A method according to one of claims 1-15, further comprising treating the patient based on the diagnosis of SIRS, sepsis, severe sepsis, septic shock, or MODS or on the prognostic risk for one or more clinical outcomes.

17. A method for evaluating biomarker levels in a body fluid sample, comprising:
obtaining a body fluid sample from a subject selected for evaluation based on a determination that the subject is at risk of a future or current diagnosis of sepsis, severe sepsis, septic shock or MODS; and
performing one or more analyte binding assays configured to detect one or more biomarkers selected from the group consisting of WAP four-disulfide core domain protein 2, Hepatitis A virus cellular receptor 1, Interleukin-1 receptor-like 1, and Proprotein convertase subtilisin/kexin type 9 by introducing the body fluid sample obtained from the subject into an assay instrument which (i) contacts a plurality of reagents which specifically bind for detection the plurality of biomarkers with the urine sample, and (ii) generates one or more assay results indicative of binding of each biomarker which is assayed to a respective specific binding reagent in the plurality of reagents; and
displaying the one or more assay results from the assay instrument as a quantitative result in a human-readable form.

18. A method according to claim 17, wherein the subject is selected for evaluation based on a determination that the subject has sepsis and is at risk of a future diagnosis of severe sepsis, septic shock or MODS.

19. A method according to claim 17, wherein the subject is selected for evaluation based on a determination that the subject has sepsis or severe sepsis and is at risk of a future diagnosis of septic shock or MODS.

20. A method according to claim 17, wherein a plurality of assay results are combined using a function that converts said assay results into a single composite result.

21. A method according to claim 17, wherein the subject is selected for evaluation based on a determination that the subject is at risk of a future diagnosis of severe sepsis, septic shock or MODS within a period selected from the group consisting of 21 days, 14 days, 7 days, 5 days, 96 hours, 72 hours, 48 hours, 36 hours, 24 hours, and 12 hours.

22. A method according to claim 17, wherein the subject is selected for evaluation based on a determination that the subject is at risk of a future diagnosis of one or more of an injury to renal function, reduced renal function, improvement in renal function, and future ARF.

23. A method according to claim 17, wherein the plurality of assays are immunoassays performed by (i) introducing the urine sample into an assay device comprising a plurality of antibodies, at least one of which binds to each biomarker which is assayed, and (ii) generating an assay result indicative of binding of each biomarker to its respective antibody.

24. A method according to one of claims 17-23, further comprising treating the patient based on the diagnosis of SIRS, sepsis, severe sepsis, septic shock, or MODS or on the prognostic risk for one or more clinical outcomes.

25. A system for evaluating biomarker levels, comprising:

a plurality of reagents which specifically bind for detection the plurality of biomarkers selected from the group consisting of WAP four-disulfide core domain protein 2, Hepatitis A virus cellular receptor 1, Interleukin-1 receptor-like 1, and Proprotein convertase subtilisin/kexin type 9;

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

26. A system according to claim 25 wherein the reagents comprise a plurality of antibodies, at least one of which binds to each of the biomarkers which are assayed.

27. A system according to claim 25 wherein assay instrument comprises an assay device and an assay device reader, wherein the plurality of antibodies are immobilized at a plurality of predetermined locations within the assay device, wherein the assay device is configured to receive the urine sample such that the urine sample contacts the plurality of predetermined locations, and wherein the assay device reader interrogates the plurality of predetermined locations to generate the assay results.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 13/51546

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/00, G01N 33/53, G01N 33/566, C12M 1/34 (2013.01)

USPC - 435/7.94, 436/501, 435/7.1, 435/7.92, 435/287.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC - G01N 33/00, G01N 33/53, G01N 33/566, C12M 1/34 (2013.01)

USPC - 435/7.94, 436/501, 435/7.1, 435/7.92, 435/287.2

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

USPC - 435/7.94, 436/501, 435/7.1, 435/7.92, 435/287.2 (keyword limited - see terms below)

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

PatBase; Google Scholar, Search Terms: sepsis, septic shock, Systemic Inflammatory Response Syndrome, multiple organ dysfunction syndrome, MODS, SIRS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---	BRUNNER et al. "Increased levels of soluble ST2 protein and IgG1 production in patients with sepsis and trauma" 28 February 2004 (28.02.2004) Vol. 30, pp 1468?1473, entire document, especially pg 1469, col 2, para 1-2; pg 1469, col 2, para 4; para 1471, col 1, para 2; pg 1471, col 1, para 3; pg 1472, col 1, para 1; pg 1472, col 2, para 1	1-2, 11-15, 16/(1-2,11-15) ---
Y		3-10, 16/(3-10), 17-27
Y	WO 2011/162821 A1 (ANDERBERG et al.) 29 December 2011 (29.12.2011) entire documents, especially paras [0017], [0039], [0042], [0043], [0096], [0111], [0134], [0158]	3-10, 16/(3-10), 17-27
A	US 2012/0046181 A1 (HARB et al.) 23 February 2012 (23.02.2012) entire document, especially para [0047]	1-27

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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