US 20210113544A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2021/0113544 A1**

Breton (43) **Pub. Date: Apr. 22, 2021**

(54) **METHODS OF MONITORING, TREATING, AND PREVENTING RENAL INFLAMMATION ASSOCIATED WITH INFECTION**

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(21) Appl. No.: **17/047,829**

(22) PCT Filed: **Apr. 29, 2019**

(86) PCT No.: **PCT/US2019/029577**

§ 371 (c)(1),
(2) Date: **Oct. 15, 2020**

Related U.S. Application Data

(60) Provisional application No. 62/664,464, filed on Apr. 30, 2018.

Publication Classification

(51) **Int. Cl.**

A61K 31/451	(2006.01)
A61K 45/06	(2006.01)
A61K 35/14	(2006.01)
A61K 31/192	(2006.01)
A61K 31/56	(2006.01)
G01N 33/53	(2006.01)
A61P 13/12	(2006.01)
A61P 29/00	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 31/451*** (2013.01); ***A61K 45/06*** (2013.01); ***A61K 35/14*** (2013.01); ***A61K 31/192*** (2013.01); ***A61K 31/56*** (2013.01); *G01N 2800/50* (2013.01); ***A61P 13/12*** (2018.01); ***A61P 29/00*** (2018.01); *G01N 2400/00* (2013.01); *G01N 2800/7095* (2013.01); ***G01N 33/5308*** (2013.01)

(57) **ABSTRACT**

The invention provides methods of monitoring development of renal inflammation in a subject who has an infection by analyzing levels of one or more UDP-hexoses, such as UDP-glucose, UDP-galactose, UDP-glucuronic acid, N-acetyl-UDP-glucosamine or N-acetyl-UDP-galactosamine, in a sample from the subject. The invention also provides methods of treating or preventing renal inflammation in a subject who has an infection by providing a P2Y14 receptor antagonist to the subject.

METHODS OF MONITORING, TREATING, AND PREVENTING RENAL INFLAMMATION ASSOCIATED WITH INFECTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of, and priority to, U.S. Provisional Application No. 62/664,464, filed Apr. 30, 2018, the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The invention relates generally to methods of monitoring, treating, and preventing renal inflammation associated with infection.

BACKGROUND

[0003] Each year more than a million people in the United States develop sepsis, and up to 30% of those individuals die from it. Sepsis occurs when the body produces an extreme immune response to infection, and widespread inflammation leads to blood clots and leaks in blood vessels that contribute to organ failure. One organ that is particularly susceptible to damage during sepsis is the kidney, where inadequate oxygenation causes acute kidney injury (AKI). Patients with sepsis who develop AKI are at increased risk of death.

SUMMARY

[0004] The invention is based on a discovery that a person's immune response in sepsis relates to more than a response to an invading pathogen. It is believed that an invading pathogen triggers an initial immune response, which may be treated by various different medicines. During this initial treatment period, UDP-hexoses (such as UDP-glucose, UDP-galactose, UDP-glucuronic acid, N-acetyl-UDP-glucosamine or N-acetyl-UDP-galactosamine) are released from tissues damaged by the pathogen. Those UDP-hexoses bind to P2Y14 receptors and trigger a secondary inflammatory response. Accordingly, even if the pathogenic infection is treated, the secondary inflammatory response caused by the binding of UDP-hexoses to P2Y14 has already been initiated and that secondary inflammatory response is not controlled by the medicines used to eradicate the invading pathogen. In that manner, the secondary inflammatory response cascades until the patient dies from the extreme immune response to the secondary, now uncontrollable, inflammatory conditions in the patient.

[0005] The invention recognizes that controlling the secondary inflammatory response in sepsis will return the patient to a normal state, thereby avoiding septic shock and death. The secondary inflammatory response is controlled by reducing or preventing the binding of UDP-hexoses to P2Y14. Accordingly, the invention provides methods of monitoring for inflammation associated with an infection in a subject by monitoring for elevated UPG-hexoses in the subject. The invention also provides method of treating or preventing inflammation associated with an infection in a subject by providing a P2Y14 antagonist to a subject that has an infection. In that manner, the invention provides methods that allow physicians to monitor and protect organs (such as kidney function) during sepsis.

[0006] In certain embodiments, aspects of the invention are accomplished by monitoring renal inflammation in patients with infections by analyzing levels of a UDP-hexose, such as UDP-glucose, UDP-galactose, UDP-glucuronic acid, N-acetyl-UDP-glucosamine or N-acetyl-UDP-galactosamine. The methods may involve obtaining a sample from a subject who has an infection, conducting an assay on the sample to measure the level of one or more UDP-hexoses in the sample, and comparing the level from the sample with a reference level. Based on the comparing step, the methods of the invention then allow for determining whether the subject is at risk of developing or has developed renal inflammation. For example, if the level of a UDP-hexose in the sample is elevated compared to a reference level, the patient is at increased risk of developing renal inflammation or is in an early stage of developing renal inflammation that may progress to AKI.

[0007] The infection may be an infection associated with sepsis in a subject. For example, the infection may cause or be likely to cause sepsis in the subject. The infection may reside in an organ in which infection causes or is likely to cause sepsis. For example, the infection may be in the lung, abdomen, skin, uterus, cerebrospinal fluid, blood, or urinary tract of the subject. The infection may be from a pathogenic organism. For example, the infection may be bacterial, viral, or fungal. The infection may be from a gram-positive or gram-negative bacterium.

[0008] The subject may display one or more symptoms associated with sepsis. For example, the subject may display altered body temperature, e.g., increased or decreased body temperature, altered consciousness, altered white blood cell count, e.g., increased or decreased white blood cell count, bandemia, decreased blood pressure, decreased partial pressure of carbon dioxide, metabolic acidosis, increased heart rate, increased number of immature neutrophils, or increased respiratory rate.

[0009] The subject may be a human of any age, e.g., a pediatric, a newborn, a neonate, an infant, a child, an adolescent, a pre-teen, a teenager, an adult, or an elderly patient. The subject may be in critical care, intensive care, neonatal intensive care, pediatric intensive care, coronary care, cardiothoracic care, surgical intensive care, medical intensive care, long-term intensive care, an operating room, an ambulance, a field hospital, or an out-of-hospital field setting.

[0010] The invention encompasses numerous methods for measuring and comparing UDP-hexose levels. In certain embodiments, a reference level is obtained or used. The reference level may be an average UDP-hexose level in a population of healthy subjects. The reference level may be a range of one standard deviation, two standard deviations, or three standard deviations from an average UDP-hexose level in a population of healthy subjects. A healthy subject may be a subject that does not have an infection, a subject that has not developed sepsis, or a subject that has not developed AKI.

[0011] The reference level may be the subject's own UDP-hexose level prior to infection or prior to diagnosis with an infection. The reference level may be a range of one standard deviation, two standard deviations, or three standard deviations from the subject's own UDP-hexose level prior to infection or prior to diagnosis with an infection. The reference level may be a cut-off concentration or range of concentrations below or above which the subject's risk of

developing or having renal inflammation that may lead to AKI may be estimated or determined.

[0012] The methods of the invention can be conducted using various types of samples that contain or may contain a UDP-hexose, such as UDP-glucose, UDP-galactose, UDP-glucuronic acid, N-acetyl-UDP-glucosamine, or N-acetyl-UDP-galactosamine. For example, the UDP-hexose may be UDP-glucose. UDP-glucose is present in many different body fluid samples. Exemplary body fluids that may contain UDP-glucose include urine, blood, plasma, serum, sweat, saliva, semen, feces, or phlegm. Preferably, the body fluid is blood, plasma, or urine. UDP-glucose is also found in tissue. Accordingly, the sample may also be a tissue sample.

[0013] The methods may include monitoring renal inflammation in the subject over time. In certain embodiments, monitoring may be accomplished by performing the method steps at multiple time points. For example, samples may be obtained at more than one time point, an assay may be conducted at each time point, and UDP-hexose levels may be compared to reference levels at each time point. UDP-hexose levels in sample taken from the subject at different time points may be compared to each other. The methods may include determining that the subject is at risk of developing or has developed renal inflammation if the levels of UDP-hexose in samples taken at different time points increase over time. The methods may include determining that renal inflammation may progress to AKI in the subject, or determining the likelihood that renal inflammation will progress to AKI in the subject.

[0014] The methods may include providing a P2Y14 antagonist to the subject if the subject has an elevated level of UDP-glucose. The P2Y14 antagonist may be a substituted 2-naphthoic acid. The P2Y14 antagonist may be 4-((piperidin-4-yl)-phenyl)-(7-(4-(trifluoromethyl)-phenyl)-2-naphthoic acid (PPTN).

[0015] In another aspect, the invention provides methods of treating or preventing renal inflammation in a subject who has an infection or who is suspected of having an infection by providing a P2Y14 antagonist to the subject.

[0016] Any of the above-mentioned elements, such as those concerning the subject, infection, and P2Y14 antagonist, described in relation to methods for monitoring renal inflammation by measuring and comparing UDP-hexose levels may apply to the methods of treating or preventing renal inflammation by providing a P2Y14 antagonist.

[0017] The methods may include providing an additional therapeutic agent. For example, the methods may include providing an antibiotic, antifungal, blood product, intravenous fluid, oxygen, steroid, and vasopressor.

DETAILED DESCRIPTION

[0018] The invention provides methods of monitoring, treating, and preventing renal inflammation associated with infection. Infection of certain tissues can lead to sepsis, an overwhelming immune response that triggers systemic inflammation. Inflammation of the kidneys contributes to the development and progression of acute kidney injury (AKI), and AKI in sepsis patients is associated with increased risk of death. Effective treatment of AKI requires immediate therapeutic intervention. However, providing expeditious treatment in patients with sepsis is challenging because both sepsis and AKI can be difficult to diagnose due the non-specificity of their symptoms. The invention addresses this problem by providing rapid and specific methods for detecting, treating, and preventing renal inflammation in patients with infections. Consequently, methods of the invention enable physicians to prevent AKI and preserve kidney function in patients who have, or are at risk of developing, sepsis.

[0019] Recent reports have identified the purinergic receptor P2Y14, also called GPR105, as a key mediator of renal inflammation. The gene and protein for human P2Y14 are described in, for example, Entrez Gene ID no. 9934, GenBank ID no. D13626, RefSeq ID no. NM_014879, and UniProt ID no. NM_01487, the contents of which are incorporated herein by reference. P2Y14 is a G protein-coupled receptor expressed on the surface of intercalated cells (ICs) in the collecting duct system of the kidney. P2Y14 binds uridine diphosphate glucose (UDP-glucose), an ester of pyrophosphoric acid with the nucleoside uridine, and other UDP-hexoses, such as UDP-galactose, UDP-glucuronic acid, N-acetyl-UDP-glucosamine, and N-acetyl-UDP-galactosamine. Abbracchio et al., Characterization of the UDP-glucose receptor (re-named here the P2Y14 receptor) adds diversity to the P2Y receptor family, Trends Pharmacol Sci. 2003 February; 24(2):52-5, DOI: 10.1016/S0165-6147(02)00038-X, the contents of which are incorporated herein by reference. UDP-glucose is released into extracellular fluids from damaged cells and in a regulated manner from intact cells. Binding of UDP-glucose to P2Y14 triggers ICs to produce chemokines that lead to infiltration of neutrophils into the renal medulla. See Azroyan et al., Renal Intercalated Cells Sense and Mediate Inflammation via the P2Y14 Receptor, PLoS ONE 10(3): e0121419 (2015), doi:10.1371/journal.pone.0121419. Thus, high levels of UDP-glucose activate P2Y14 to cause renal inflammation.

[0020] The invention addresses the difficulty of diagnosing and treating AKI in patients with infections by measuring the levels of one or more UDP-hexoses, such as UDP-glucose, UDP-galactose, UDP-glucuronic acid, N-acetyl-UDP-glucosamine, and N-acetyl-UDP-galactosamine, to monitor renal inflammation in such patients. By measuring UDP-hexose levels in infected patients, renal inflammation can be detected in a timely manner. The invention recognizes that because renal inflammation can be detected before the patient has developed AKI, AKI can be treated more effectively. The invention further recognizes that providing a P2Y14 antagonist to infected patients who display elevated levels of one or more UDP-hexoses attenuates renal inflammation. Consequently, the invention provides methods that allow treatment of AKI in its incipient stages or prevention of AKI altogether in patients who have, or are at risk of developing, sepsis.

Sepsis and Infection

[0021] Sepsis results from the body's excessive immune response to infection of a tissue. In response to the infection, the immune system releases signaling molecules into the bloodstream that trigger inflammation throughout the body, i.e., the inflammation is not confined to the infected tissue. This first phase is sometimes called the "cytokine storm." The burst of excessive inflammation may be followed by a prolonged period of decreased function of the immune system. Either phase of sepsis may be fatal. The cytokine storm causes blood clots and leaky blood vessels that can lead to failure of one or more organs, such as the kidneys,

lungs, brain, liver, and heart. In particular, renal inflammation can lead to multiple organ failure, a serious and often deadly condition.

[0022] Various criteria may be used to diagnose sepsis. One established set of symptoms is the systemic inflammatory response syndrome (SIRS) criteria, which include body temperature, heart rate, respiratory rate, and white blood cell count. See "American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis" (PDF). Critical Care Medicine. 20 (6): 864-74. 1992, doi:10.1097/00003246-199206000-00025, PMID 1597042, the contents of which are incorporated herein by reference. Under the SIRS criteria, adult patients are considered to have sepsis when they meet two or more of the following criteria: body temperature of less than 36° C. (96.8° F.) or greater than 38° C. (100.4° F.); heart rate greater than 90 beats per minute; high respiratory rate, defined as either (1) greater than 20 breaths per minute or (2) an arterial partial pressure of carbon dioxide less than 4.3 kPa (32 mmHg); and abnormal white blood cell count, defined as (1) less than 4000 cells/mm^3 (4×10^9 cells/L), (2) greater than 12,000 cells/mm^3 (12×10^9 cells/L), or (3) the presence of greater than 10% immature neutrophils (band forms). Band forms greater than 3% are called bandemia or a "left-shift." Under the SIRS criteria, pediatric patients are considered to have sepsis when they meet two or more of the following criteria: abnormal heart defined as (1) heart rate greater than 2 standard deviations above normal for age in the absence of stimuli such as pain and drug administration, (2) unexplained persistent elevation for greater than 30 minutes to 4 hours, or (3) for infants, heart rate less than 10th percentile for age in the absence of vagal stimuli, beta-blockers, or congenital heart disease or unexplained persistent depression for greater than 30 minutes; body temperature obtained orally, rectally, from Foley catheter probe, or from central venous catheter probe of less than 36° C. or greater than 38.5° C.; abnormal respiratory rate defined as (1) respiratory rate greater than 2 standard deviations above normal for age or (2) the requirement for mechanical ventilation not related to neuromuscular disease or the administration of anesthesia; and abnormal white blood cell count defined as (1) white blood cell count elevated or depressed for age not related to chemotherapy or (2) greater than 10% bands plus other immature forms.

[0023] Another standard for diagnosing sepsis is the sepsis-related organ failure assessment (SOFA), also called the sequential organ failure assessment score. See, e.g., Vincent J L, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, Reinhart C K, Suter P M, Thijs L G. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure, On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 1996 July; 22(7):707-10, PMID 8844239, the contents of which are incorporated herein by reference. The SOFA score is a composite score based on individual scores for six different organ systems: respiratory, neurological, cardiovascular, hepatic, coagulation, renal, systems. SOFA scores for individual organ systems are indicated in Tables 1-6.

TABLE 1

SOFA score for respiratory system

PaO_2/FiO_2 (mmHg)	SOFA score
≥400	0
<400	+1
<300	+2
<200 and mechanically ventilated	+3
<100 and mechanically ventilated	+4

TABLE 2

SOFA score for nervous system

Glasgow coma scale	SOFA score
15	0
13-14	+1
10-12	+2
6-9	+3
<6	+4

TABLE 3

SOFA score for cardiovascular system

Mean arterial pressure OR administration of vasopressors required	SOFA score
MAP ≥ 70 mm/Hg	0
MAP < 70 mm/Hg	+1
dopamine ≤ 5 μg/kg/min or dobutamine (any dose)	+2
dopamine > 5 μg/kg/min OR epinephrine ≤ 0.1 μg/kg/min OR norepinephrine ≤ 0.1 μg/kg/min	+3
dopamine > 15 μg/kg/min OR epinephrine > 0.1 μg/kg/min OR norepinephrine > 0.1 μg/kg/min	+4

TABLE 4

SOFA score for liver

Bilirubin (mg/dl) [μmol/L]	SOFA score
<1.2 [<20]	0
1.2-1.9 [20-32]	+1
2.0-5.9 [33-101]	+2
6.0-11.9 [102-204]	+3
>12.0 [>204]	+4

TABLE 5

SOFA score for coagulation system

Platelets × $10^3/\mu l$	SOFA score
≥150	0
<150	+1
<100	+2
<50	+3
<20	+4

TABLE 6

SOFA score for kidneys	
Creatinine (mg/dl) [μmol/L] (or urine output)	SOFA score
<1.2 [<110]	0
1.2-1.9 [110-170]	+1
2.0-3.4 [171-299]	+2
3.5-4.9 [300-440] (or <500 ml/d)	+3
>5.0 [>440] (or <200 ml/d)	+4

The Quick SOFA (qSOFA) score is a simplified version of the SOFA score that can be used to identify patients at high risk for poor outcome from an infection. See Angus, Derek C.; Seymour, Christopher W.; Coopersmith, Craig M.; Deutschman, Clifford S.; Klompas, Michael; Levy, Mitchell M.; Martin, Gregory S.; Osborn, Tiffany M.; Rhee, Chanu, "A Framework for the Development and Interpretation of Different Sepsis Definitions and Clinical Criteria", Critical Care Medicine, 44 (3): e113-e121. doi:10.1097/ccm.0000000000001730, the contents of which are incorporated herein by reference. Under the qSOFA criteria, a patient is deemed to have sepsis when two or more of the following criteria are met: low blood pressure, defined as SBP≤100 mmHg; high respiratory rate, defined as ≥22 breaths/min; and altered mentation, defined as GCS<13.

[0024] In addition to the symptoms described above, patients with sepsis may experience other symptoms, such as metabolic acidosis, chills, dizziness, fatigue, shivering, facial flushing, shortness of breath, low urine production, skin discoloration, dysfunction of one or more organs, shock, and sleepiness.

[0025] Sepsis may arise from infection of various tissues. Commonly, the infection occurs in the lungs, abdomen, uterus, cerebrospinal fluid, blood, skin, or urinary tract. In many cases, however, the source of the infection cannot be determined.

[0026] The infection may be from any type of pathogen. Infections that lead to sepsis are usually bacterial but may be fungal or viral. Among bacteria, both gram-positive and gram-negative bacterial infections can induce sepsis, and common bacterial species that cause sepsis include Staphylococci species, *Klebsiella* species, *Streptococcus pyogenes, Escherichia coli*, and *Pseudomonas aeruginosa. Candida* species are the most common cause of fungal sepsis.

Measuring UDP-Hexose Levels

[0027] The level of one or more UDP-hexoses, such as UDP-glucose, UDP-galactose, UDP-glucuronic acid, N-acetyl-UDP-glucosamine, or N-acetyl-UDP-galactosamine, may be measured by any suitable method. Examples below are discussed in the context of UDP-glucose. However, any method for measuring UDP-glucose is applicable to measuring any UDP-hexose. Preferably, the methods involve measuring the level of a UDP-hexose, such as UDP-glucose, UDP-galactose, UDP-glucuronic acid, N-acetyl-UDP-glucosamine or N-acetyl-UDP-galactosamine, that stimulates activity of P2Y14 in a subject.

[0028] UDP-glucose may be measured by coupling a reaction converting UDP-glucose to a byproduct with the stoichiometric production of NADH or UDP as described in WO 2017/165665, the contents of which are incorporated herein in their entirety. UDP-glucose levels may also be measured using the protocols described in Barrett et al., Molec. Pharmacol., 2013, 84, 41-49, the contents of which are incorporated herein by reference in their entirety. Alternatively or additionally, UDP-glucose levels may be measured by using an anti-UDP-glucose antibody.

[0029] In certain embodiments that entail measurement of NADH as the readout molecule, the assay may include pre-processing steps remove proteins that interact with NADH production and/or endogenous NADH from the sample. High levels (e.g., >2 μM) of endogenous NADH from the sample can inhibit the assay. Alternatively or additionally, a control reaction lacking exogenous enzyme may be performed to measure the amount of pre-existing NADH in the sample, and this value can be subtracted from the value obtained from a reaction that receives exogenous enzyme. Next, the liquid sample is buffered to pH 8-9, for example, pH 8.0. The enzyme UDP-glucose dehydrogenase is added to the reaction along with the co-factor NAD^+. During the reaction UDP-glucose is converted to UDP-glucuronic acid, and a stoichiometric amount of NAD^+ is converted to NADH. NADH is then measured, and its concentration is used to deduce the starting UDP-glucose concentration. The amount of substrate and/or the reaction rate may be optimized so that the reaction occurs substantially in a substantially linear portion of the Michaelis-Menten graph.

[0030] In some embodiments, excess NAD^+ is added to the reaction, along with enzyme in excess, such that UDP-glucose is limiting. For example, NAD^+ can be added to a concentration of 2 mM per well, and 0.04 units of enzyme added per well to achieve an excess of both. One unit of enzyme is the amount of UDP-glucose dehydrogenase required to oxidize 1.0 μmole of UDP-glucose to UDP-glucuronic acid per minute at pH 8.7 at 25° C.

[0031] Alternatively, the complete reaction curve can be determined for each sample and the data fit to a non-linear rate equation (e.g., "progress-curve analysis"). This is particularly useful when the slope of the linear region of the Michaelis-Menten kinetics curve for a desired enzyme is very steep (e.g., when the initial rate is too fast to measure accurately) or when an excess of substrate (e.g., NAD^+) is used in the reaction mix

[0032] The methods may include lateral flow assays adapted for use in the detection of NADH or UDP. Such lateral flow assays permit the flow of a liquid sample, applied to the sample application zone, to deliver the sample/reactants to a test region (e.g., a reaction zone) of the lateral strip or device, and then the sample with a generated byproduct is delivered to a detection zone, which provides a readout (e.g., visual, optical, fluorescent, etc.). As one example, an assay may use reduction of nitro blue tetrazolium (NBT) by NADH to generate a colored product at a test region. As samples with generated NADH flow over a region with NBT (no color), the NBT is reduced to the blue form, which is visible on a strip.

[0033] In some embodiments in which NBT is used to generate a detectable product, a reductase may be immobilized on the dipstick or test strip. The reductase may be a diaphorase, and it may be immobilized via adsorption or via immunocapture. As the NADH-containing solution flows through the region with the reductase enzyme, the NADH is oxidized and would reduce the NBT to the colored precipitate NBTH.

[0034] In some embodiments, the level of NADH or UDP in a sample is detected by a lateral flow assay test (LFA), or

strip test. LFAs detect the presence or absence of an analyte, e.g. NADH or UDP, in a liquid sample. With a lateral flow method, a spatial separation is defined in the strips between the sample application zone and detection region. Most conventional lateral flow strips are designed for test samples that are readily available in large quantities (e.g., urine). Lateral flow immunoassays are described below, but lateral flow assays may also be adapted for the measurement of an analyte without the use of antibody. Both lateral flow immunoassays (e.g., using a UDP-glucose antibody) and lateral flow analyte assays (e.g., detection of NADH to measure UDP-glucose levels) are contemplated for use herein.

[0035] In LFAs the test sample flows along a solid substrate via capillary action. After the sample is applied to the lateral flow strip, it encounters a test region where an enzymatic reaction coupled to NADH or UDP production occurs and continues to a region comprising a detection reagent that permits detection of NADH or UDP. The liquid may go through one or more different regions on the lateral flow strip following the test region and prior to the detection region.

[0036] LFAs are adapted to operate along a single axis to suit the test strip format or a dipstick format. Typically, LFAs proceed from sample application to readout without additional steps by the user, so sample application generally leads to an assay result with the further user input. Other lateral flow configurations may include one or more steps by the user after sample application, e.g., insertion into a detector device (e.g., a luminometer, fluorescence detector, etc.) or addition of another reagent. Strip tests are extremely versatile and can be easily modified by one skilled in the art for detecting an enormous range of antigens or analytes from fluid samples such as urine, blood, water samples etc. Strip tests are also known as "dipstick tests," the name bearing from the literal action of "dipping" the test strip into a fluid sample to be tested. LFA strip tests are easy to use, require minimum training and can easily be included as components of point-of-care test (POCT) diagnostics to be used on site in the field.

[0037] A typical test strip may comprise one or more of following components: (1) sample application zone comprising e.g., an absorbent pad (i.e., the matrix or material) onto which the test sample is applied; (2) test region comprising immobilized enzyme; (3) a test results area comprising a detection reagent or reaction membrane—such as a hydrophobic nitrocellulose or cellulose acetate membrane onto which, for example, a detection reagent is immobilized in a line across the membrane as a capture zone or test line (a control zone may also be present, containing NADH or another reducing agent, for example, that reduces NBT to generate a blue color) or an antibody reagent; and (4) optional wick or waste reservoir—a further absorbent pad designed to draw the sample across the detection reagent zone or reaction membrane by capillary action and collect it. In addition, lateral flow strips as described herein may further comprise one or more of the following: a region comprising a strong base or a region comprising immobilized NAD^+ nucleosidase to degrade unreacted NAD^+.

[0038] The components of the strip may be fixed to an inert backing material and may be presented in a simple dipstick format or within a plastic casing with a sample port and reaction window showing the test readout/capture and control zones. The test may incorporate a second, coated line which contains an antibody or other reagent that picks up free readout substrate (e.g., free latex or gold particles) in order to confirm the test has operated correctly.

[0039] The use of "dip sticks" or LFA test strips and other solid supports has been described in the art in the context of an immunoassay for a number of antigen biomarkers. U.S. Pat. Nos. 4,943,522; 6,485,982; 6,187,598; 5,770,460; 5,622,871; and 6,565,808, and U.S. patent application Ser. Nos. 10/278,676; 09/579,673; and Ser. No. 10/717,082, which are incorporated herein by reference in their entirety, are non-limiting examples of such lateral flow test devices. Examples of patents that describe the use of "dip stick" technology to detect soluble antigens via immunochemical assays include, but are not limited to, U.S. Pat. Nos. 4,444,880; 4,305,924; and 4,135,884; which are incorporated by reference herein in their entireties. The apparatuses and methods of these three patents broadly describe a first component fixed to a solid surface on a "dip stick" which is exposed to a solution containing a soluble antigen that binds to the component fixed upon the "dip stick," prior to detection of the component-antigen complex upon the stick. Given the reaction description and considerations described herein, it is within the skill of one in the art to modify the teachings regarding "dip stick" technology for the detection of NADH or UDP using e.g., dye, luciferin or fluorescent reagents as described herein.

[0040] In some embodiments, the reaction to generate a stoichiometric amount of NADH from the reaction of UDP-glucose with UDP-glucose dehydrogenase is incubated for a matter of minutes, e.g., 5 or 10 minutes, in the liquid assay format in order to generate sufficient amounts of NADH for detection. This extended time is not as readily achieved in the dipstick or lateral flow format. However, options to overcome this include performing the first enzymatic reaction in an assay well for a prescribed period of time before inserting a dipstick or applying sample to a test strip. Alternatively, if all reactions took place on the dipstick or test strip, a shorter incubation should not present a problem because most of the enzyme reaction actually takes place within the first minute, although the reaction continues to remain linear after a 5-minute incubation, after the initial linear velocity for low (physiological) concentrations of UDP-glucose (up to 100 μM).

[0041] A urine dipstick is a colorimetric chemical assay comprising a reagent stick-pad. The dipstick is typically immersed in a fresh urine specimen and then withdrawn. Alternatively, the urine sample may be applied directly to the sample application zone by the subject (e.g., analogous to a pregnancy test). After predetermined times the colors of the reagent pad are compared to standardized reference charts. The urine dipstick offers an inexpensive and fast method to perform screening urinalyses, which helps in identifying the presence of various diseases or health problems. A urine dipstick provides a simple and clear diagnostic guideline and may be used in the methods and kits as described herein. Accordingly, one aspect of the present technology relates to a method for detecting NADH or UDP using a device, such as a dipstick, as described herein. When the sample is not clear, a centrifugation or filtration step to render a clear sample may be applied so as to avoid pigment or other entities from fouling the optical readout.

[0042] In some cases, the lateral flow strip may also comprise a control that gives a signal to the user that the assay is performing properly. For instance, the control zone

may contain an immobilized receptive material that is generally capable of forming a chemical and/or physical bond with probes or with the receptive material immobilized on the probes. Some examples of such receptive materials include, but are not limited to, antigens, haptens, antibodies, protein A or G, avidin, streptavidin, secondary antibodies, and complexes thereof. In addition, it may also be desired to utilize various non-biological materials for the control zone receptive material. For instance, in some embodiments, the control zone receptive material may also include a polyelectrolyte that may bind to uncaptured probes. Because the receptive material at the control zone is only specific for probes, a signal forms regardless of whether the analyte is present. The control zone may be positioned at any location along the test strip, but is preferably positioned downstream from the detection zone.

[0043] In some embodiments, detection involves reduction of nitro blue tetrazolium by NADH present and/or generated during the assay. In such embodiments, the control line may include a line of NBT spatially downstream of the test line and immediately downstream of a line or zone of dried reducing agent. Flow of sample past the test line will liberate the reducing agent and carry it to the control line of NBT, which will be reduced to generate a control line indicating the sample reactants have successfully reacted at that point.

[0044] Qualitative, semi-quantitative, and quantitative results may be obtained with the lateral flow assays described herein. For example, when it is desired to semi-quantitatively or quantitatively detect an analyte, the intensity of any signals produced at the region comprising a detection reagent may be measured with e.g., an optical reader. The actual configuration and structure of the optical reader may generally vary as is readily understood by those skilled in the art. For example, optical detection techniques that may be utilized include, but are not limited to, luminescence (e.g., fluorescence, phosphorescence, etc.), absorbance (e.g., fluorescent or non-fluorescent), diffraction, etc. Further optical methods include but are not limited to, measurement of light scattering or simple reflectance, e.g., using a luminometer or photomultiplier tube; radioactivity, e.g., using a Geiger counter; electrical conductivity or dielectric capacitance; and release of electroactive agents, such as indium, bismuth, gallium or tellurium ions.

[0045] Once the amount of detection agent has been quantified, the amount may then be mapped onto another measurement scale. For example, while the result of the assay may be measured as a density of reflectance (Dr), the result reported may be more meaningful in other units, such as RI (intensity relative to that of a control zone or background level). Results may also be expressed as the number of copies of analyte present in the measurement volume.

[0046] The methods may include lateral flow immunoassays (LFIAs), in which antibodies that bind a target analyte are used in a competitive or sandwich immunoassay adapted to the lateral flow format. Conventional sandwich LFIAs are similar to sandwich ELISAs. The sample first encounters and mobilizes colored particles which are labeled with antibodies raised to the target antigen. The test line will also contain antibodies to the same target, although it may bind to a different epitope on the antigen. The test line will show as a colored band in positive samples, resulting from the accumulation or capture of antibody-bearing colored particles. In some embodiments, the lateral flow immunoassay may be a double antibody sandwich assay, a competitive assay, a quantitative assay or variations thereof. Conventional competitive LFIAs are similar to competitive ELISA. The sample first encounters colored particles which are labeled with the target antigen or an analogue. The test line contains antibodies to the target/its analogue. Unlabeled antigen in the sample will block the binding sites on the antibodies preventing capture of the colored particles at the test line. The test line will show as a colored band in negative samples. There are a number of variations on lateral flow technology. It is also possible to apply multiple capture zones to create a multiplex test.

[0047] Any substance generally capable of producing a signal that is detectable visually or by an instrumental device may be used as a detection reagent. Suitable detectable substances may include, for instance, luminescent compounds (e.g., fluorescent, phosphorescent, etc.); radioactive compounds; visual compounds (e.g., colored dye or metallic substance, such as gold); liposomes or other vesicles containing signal-producing substances; enzymes and/or substrates, and so forth. Other suitable detectable substances are described in U.S. Pat. Nos. 5,670,381 and 5,252,459, which are incorporated herein in their entirety by reference. If the detectable substance is colored, the ideal electromagnetic radiation is light of a complementary wavelength. For instance, blue detection probes strongly absorb red light.

[0048] In some embodiments, the detectable substance may be a luminescent compound that produces an optically detectable signal. For example, suitable fluorescent molecules may include, but are not limited to, fluorescein, europium chelates, phycobiliprotein, rhodamine, and their derivatives and analogs. Other suitable fluorescent compounds are semiconductor nanocrystals commonly referred to as "quantum dots."

[0049] In another embodiment, the detection agent is a particle. Examples of particles useful in the methods, assays and kits described herein include, but are not limited to, colloidal gold particles; colloidal sulphur particles; colloidal selenium particles; colloidal barium sulfate particles; colloidal iron sulfate particles; metal iodate particles; silver halide particles; silica particles; colloidal metal (hydrous) oxide particles; colloidal metal sulfide particles; colloidal lead selenide particles; colloidal cadmium selenide particles; colloidal metal phosphate particles; colloidal metal ferrite particles; any of the above-mentioned colloidal particles coated with organic or inorganic layers; protein or peptide molecules; liposomes; or organic polymer latex particles, such as polystyrene latex beads.

[0050] Further, suitable phosphorescent compounds include metal complexes of one or more metals, such as ruthenium, osmium, rhenium, iridium, rhodium, platinum, indium, palladium, molybdenum, technetium, copper, iron, chromium, tungsten, zinc, and so forth. Especially preferred are ruthenium, rhenium, osmium, platinum, and palladium. The metal complex may contain one or more ligands that facilitate the solubility of the complex in an aqueous or non-aqueous environment. For example, some suitable examples of ligands include, but are not limited to, pyridine; pyrazine; isonicotinamide; imidazole; bipyridine; terpyridine; phenanthroline; dipyridophenazine; porphyrin, porphine, and derivatives thereof. Such ligands may be, for instance, substituted with alkyl, substituted alkyl, aryl, substituted aryl, aralkyl, substituted aralkyl, carboxylate, carboxaldehyde, carboxamide, cyano, amino, hydroxy, imino,

hydroxycarbonyl, aminocarbonyl, amidine, guanidinium, ureide, sulfur-containing groups, phosphorus containing groups, and the carboxylate ester of N-hydroxy-succinimide.

[0051] Porphyrins and porphine metal complexes possess pyrrole groups coupled together with methylene bridges to form cyclic structures with metal chelating inner cavities. Many of these molecules exhibit strong phosphorescence properties at room temperature in suitable solvents (e.g., water) and an oxygen-free environment. Some suitable porphyrin complexes that are capable of exhibiting phosphorescent properties include, but are not limited to, platinum (II) coproporphyrin-I and II, palladium (II) coproporphyrin, ruthenium coproporphyrin, zinc(II)-coproporphyrin-I, derivatives thereof, and so forth. Similarly, some suitable porphine complexes that are capable of exhibiting phosphorescent properties include, but not limited to, platinum(II) tetra-meso-fluorophenylporphine and palladium(II) tetra-meso-fluorophenylporphine. Still other suitable porphyrin and/or porphine complexes are described in U.S. Pat. Nos. 4,614,723; 5,464,741; 5,518,883; 5,922,537; 6,004,530; and 6,582,930, which are incorporated herein in their entirety by reference.

[0052] Bipyridine metal complexes may also be utilized as phosphorescent compounds. Some examples of suitable bipyridine complexes include, but are not limited to, bis[(4,4'-carbomethoxy)-2,2'-bipyridine]2-[3-(4-methyl-2,2'-bipyridine-4-yl)propyl]-1,3-dioxolane ruthenium (II); bis(2,2'bipyridine)[4-(butan-1-al)-4'-methyl-2,2'-bi-pyridine] ruthenium (II); bis(2,2'-bipyridine)[4-(4'-methyl-2,2'-bipyridine-4'-yl)-butyric acid] ruthenium (II); tris(2,2'bipyridine)ruthenium (II); (2,2'-bipyridine)[bis-bis(1,2-diphenylphosphino)ethylene]2-[3-(4-methyl-2,2'-bipyridine-4'-yl)propyl]-1,3-dioxolane osmium (II); bis(2,2'-bipyridine)[4-(4'-methyl-2,2'-bipyridine)-butylamine] ruthenium (II); bis(2,2'-bipyridine)[1-bromo-4(4'-methyl-2,2'-bipyridine-4-yl)butane]ruthenium (II); bis(2,2'-bipyridine)maleimidohexanoic acid, 4-methyl-2,2'-bipyridine-4'-butylamide ruthenium (II), and so forth.

[0053] An immunoassay measures the concentration of a substance in a sample, typically a fluid sample, using the interaction of an antibody or antibodies to its antigen. The assay takes advantage of the highly specific binding of an antibody with its antigen. In some embodiments, specific binding of a UDP molecule with an anti-UDP antibody forms a UDP-antibody complex. The complex may then be detected by a variety of methods known in the art. An immunoassay also often involves the use of a detection antibody. Antibodies contemplated for use with the methods and assays described herein include an anti-UDP-glucose antibody, an anti-UDP antibody, and anti-UDP-glucuronic acid antibody. Such antibodies may be designed and generated using methods known in the art and/or described herein.

[0054] In one embodiment, the antibody is detectably labeled or capable of generating a detectable signal. In one embodiment, the antibody is fluorescently labeled.

[0055] In some embodiments, levels of a desired biomarker or analyte (e.g., UDP-glucose or other UDP-hexose) are measured by ELISA, also called enzyme immunoassay or EIA. ELISA is a biochemical technique that detects the presence of an antibody or an antigen in a sample.

[0056] In one embodiment, an ELISA involving at least one antibody with specificity for the particular desired antigen may be performed. A known amount of sample and/or antigen is immobilized on a solid support (e.g., a polystyrene micro titer plate). Immobilization may be either non-specific (e.g., by adsorption to the surface) or specific (e.g., where another antibody immobilized on the surface is used to capture antigen or a primary antibody). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody may be covalently linked to an enzyme, or may itself be detected by a secondary antibody which is linked to an enzyme through bio-conjugation. Between each step the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample.

[0057] Older ELISAs utilize chromogenic substrates, though newer assays employ fluorogenic substrates with much higher sensitivity.

[0058] In one embodiment, a sandwich ELISA is used, where two antibodies specific for the target may be used. There are other different forms of ELISA, which are well known to those skilled in the art. Standard techniques known in the art for ELISA are described in "Methods in Immunodiagnosis", 2nd Edition, Rose and Bigazzi, eds. John Wiley & Sons, 1980; and Oellerich, M. 1984, J. Clin. Chem. Clin. Biochem. 22: 895-904.

[0059] Antibodies or portions thereof may be used in immunoassays. For example, the immunoassay may use a complete immunoglobulin, antigen-binding fragment (Fab), Fab_2, variable domain (Fv), single chain variable fragment (scFv), third-generation (3G) antibody. The antibodies may be natural monoclonal antibodies or synthetic antibodies, such as recombinant antibodies, non-immunoglobulin derived synthetic antibodies, or affimer proteins. Methods of making monoclonal antibodies are known in the art and described in, for example, Antibodies: A Laboratory Manual, Second edition, edited by Greenfield, Cold Spring Harbor Laboratory Press (2014) ISBN 978-1-936113-81-1. Methods of making synthetic antibodies are described in, for example, US 2014/0221253; US 2016/0237142; and Miersch and Sidhu, Synthetic antibodies: concepts, potential and practical considerations, Methods. 2012 August; 57(4): 486-98. doi: 10.1016/j.ymeth.2012.06.012, the contents of each of which are incorporated herein by reference.

[0060] In some embodiments, UDP-glucose or another molecule that serves as an indicator of UDP-glucose is detected by liquid chromatography, optionally in combination with mass spectrometry. Molecules may be ionized for mass spectrometry by any method known in the art, such as ambient ionization, chemical ionization (CI), desorption electrospray ionization (DESI), electron impact (EI), electrospray ionization (ESI), fast-atom bombardment (FAB), field ionization, laser ionization (LIMS), matrix-assisted laser desorption ionization (MALDI), paper spray ionization, plasma and glow discharge, plasma-desorption ionization (PD), resonance ionization (RIMS), secondary ionization (SIMS), spark source, or thermal ionization (TIMS). Methods of mass spectrometry are known in the art and described in, for example, U.S. Pat. Nos. 8,895,918; 9,546,979; 9,761,426; Hoffman and Stroobant, Mass Spectrometry: Principles and Applications (2nd ed.). John Wiley and Sons (2001), ISBN 0-471-48566-7; Dass, Principles and practice of biological mass spectrometry, New York: John Wiley (2001) ISBN 0-471-33053-1; and Lee, ed., Mass

Spectrometry Handbook, John Wiley and Sons, (2012) ISBN: 978-0-470-53673-5, the contents of each of which are incorporated herein by reference.

[0061] In certain embodiments, a sample can be directly ionized without the need for use of a separation system. In other embodiments, mass spectrometry is performed in conjunction with a method for resolving and identifying ionic species. Suitable methods include chromatography, capillary electrophoresis-mass spectrometry, and ion mobility. Chromatographic methods include gas chromatography, liquid chromatography (LC), high-pressure liquid chromatography (HPLC), and reversed-phase liquid chromatography (RPLC). In a preferred embodiment, liquid chromatography-mass spectrometry (LC-MS) is used. Methods of coupling chromatography and mass spectrometry are known in the art and described in, for example, Holcapek and Brydwell, eds. Handbook of Advanced Chromatography/Mass Spectrometry Techniques, Academic Press and AOCS Press (2017), ISBN 9780128117323; Pitt, Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry, The Clinical Biochemist Reviews. 30(1): 19-34 (2017) ISSN 0159-8090; Niessen, Liquid Chromatography-Mass Spectrometry, Third Edition. Boca Raton: CRC Taylor & Francis. pp. 50-90. (2006) ISBN 9780824740825; Ohnesorge et al., Quantitation in capillary electrophoresis-mass spectrometry, Electrophoresis. 26 (21): 3973-87 (2005) doi:10.1002/elps.200500398; Kolch et al., Capillary electrophoresis-mass spectrometry as a powerful tool in clinical diagnosis and biomarker discovery, Mass Spectrom Rev. 24 (6): 959-77. (2005) doi:10.1002/mas.20051; Kanu et al., Ion mobility-mass spectrometry, Journal of Mass Spectrometry, 43 (1): 1-22 (2008) doi:10.1002/jms.1383, the contents of which are incorporated herein by reference.

[0062] The assays described herein may be adapted to be performed on an automated device platform that is programmed to automatically add, transfer and optionally, mix liquid samples or reaction mixtures, for example, in wells of a multiwell plate. The wells may include reagents as necessary, either added in liquid/solution form or, for example, dried or immobilized on a surface within the wells. Automated platforms that include liquid handling modules as well as detection (e.g., fluorescence, luminescence, absorbance, reflectance, etc.) modules are known to those of skill in the art. As but one non-limiting example, one might use, e.g., a Beckman Coulter AU5800 device. When adapted to an automated design, multiwell plates may include, in addition to test wells for assaying an unknown test sample, control wells including, e.g., blanks lacking enzyme or other reagents, to permit, among other things, the determination of background levels of, e.g., intermediate or surrogate indicator NADH. Other controls may include, e.g., positive control wells including a known amount of UDP-glucose; a set of separate positive control wells may include varying known amounts of UDP-glucose to establish a standard curve, e.g., over one or a plurality of orders of magnitude, that is read by the device and used to calculate amounts of UDP-glucose in the unknown test samples.

Samples

[0063] A sample may be obtained from any organ or tissue in the individual to be tested, provided that the sample is obtained in a liquid form or can be pre-treated to take a liquid form. For example and without limitation, the sample may be a blood sample, a urine sample, a serum sample, a semen sample, a sputum sample, a lymphatic fluid sample, a cerebrospinal fluid sample, a plasma sample, a pus sample, an amniotic fluid sample, a bodily fluid sample, a stool sample, a biopsy sample, a needle aspiration biopsy sample, a swab sample, a mouthwash sample, a cancer sample, a tumor sample, a tissue sample, a cell sample, a synovial fluid sample, a phlegm sample, a saliva sample, a sweat sample, or a combination of such samples. The sample may also be a solid or semi-solid sample, such as a tissue sample, feces sample, or stool sample, that has been treated to take a liquid form by, for example, homogenization, sonication, pipette trituration, cell lysis etc. For the methods described herein, it is preferred that a sample is from urine, serum, whole blood, or sputum.

[0064] In some embodiments, a sample is treated to remove cells or other biological particulates. Methods for removing cells from a blood or other sample are well known in the art and may include e.g., centrifugation, sedimentation, ultrafiltration, immune selection, etc.

[0065] The subject may be an animal (such as a mammal, such as a human). The subject may be a pediatric, a newborn, a neonate, an infant, a child, an adolescent, a pre-teen, a teenager, an adult, or an elderly patient. The subject may be in critical care, intensive care, neonatal intensive care, pediatric intensive care, coronary care, cardiothoracic care, surgical intensive care, medical intensive care, long-term intensive care, an operating room, an ambulance, a field hospital, or an out-of-hospital field setting.

[0066] The sample may be obtained from an individual who is known or suspected to have an infection. The infection may be bacterial, viral, or fungal.

Reference Levels

[0067] The reference level may be defined based on clinical trials that determine the concentration of one or more UDP-hexoses, such as UDP-glucose, UDP-galactose, UDP-glucuronic acid, N-acetyl-UDP-glucosamine, or N-acetyl-UDP-galactosamine, that optimally defines a cut-off point above which the likelihood of occurrence of AKI is high (the sensitivity) and below which the likelihood of occurrence of AKI is low (the specificity).

[0068] The reference level of UDP-hexose may be defined by a statistic describing the distribution of levels in normal healthy subjects. For example, the reference level may be an average level of UDP-hexose in a sample from a normal healthy subject or a population of normal healthy subjects. The reference level of UDP-hexose may be an average level of UDP-hexose in a sample from one or more subjects who do not have an infection, from one or more subjects who have an infection but who have not developed sepsis, or from one or more subjects who have an infection but have not developed AKI.

[0069] The reference level may be above the highest observed level of UDP-hexose in a sample from a normal healthy subject or a population of normal healthy subjects. Any level above the reference level may be deemed to be significantly different from the average level of UDP-hexose in a sample from a normal healthy subject or a population of normal healthy subjects. The reference level may be greater than 95% of the levels observed in samples from a normal healthy subject or a population of normal healthy subjects, or it may be above the lower limit of the highest decile,

quartile or tertile of the levels observed in samples from a normal healthy subject or a population of normal healthy subjects.

[0070] The reference level may be at least one standard deviation, at least two standard deviations, or at least three standard deviations above the average level of UDP-hexose in a sample from a normal healthy subject or a population of normal healthy subjects. Any level above the reference level may be deemed to be significantly different from the average level of UDP-hexose in a sample from a normal healthy subject or a population of normal healthy subjects.

[0071] The reference level may be at least one standard deviation, at least two standard deviations, or at least three standard deviations above the average level of UDP-hexose in a sample from one or more subjects who do not have an infection, from one or more subjects who have an infection but who have not developed sepsis, or from one or more subjects who have an infection but have not developed AKI.

[0072] The reference level may be a level of UDP-hexose in a control sample, a pooled sample of control individuals, or a numeric value or range of values based on the same. It is also contemplated that a set of standards may be established with reference levels providing thresholds indicative of the severity of renal inflammation.

[0073] The reference level may be a level of UDP-hexose in a sample of the same subject measured at an earlier time point. The reference level may be a level of UDP-hexose in a sample obtained from the same subject before the subject was known to have an infection or was suspected of having an infection.

[0074] The reference level may be at least one standard deviation, at least two standard deviations, or at least three standard deviations above a level of UDP-hexose in a sample obtained from the same subject at an earlier time point. Any level above the reference level may be deemed to be significantly different from the level in the earlier sample.

[0075] In some embodiments, the level of UDP-hexose measured in a sample from a subject identified as having renal inflammation may be at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 150%, at least 200%, or at least 300% higher than the reference level.

[0076] The reference level may be adjusted to account for variables such as sample type, gender, age, weight, and ethnicity. Thus, reference levels accounting for these and other variables may provide added accuracy for the methods described herein.

[0077] The methods may include making a diagnosis, prediction, or prognostication regarding the subject based on a comparison of a measured level of UDP-hexose to a reference level. The prediction or prognostication may include a probability, e.g., a statistical value. The diagnosis, prediction, or prognostication may indicate the presence of, or likelihood of developing, a condition. The condition may be renal inflammation, acute kidney injury, or a stage or category of renal inflammation or acute kidney injury.

Computer Systems for Measuring UDP-Hexose Levels

[0078] In some embodiments of the assays and/or methods described herein, the assay/method comprises or consists essentially of a system for determining (e.g. transforming and measuring) the level of UDP-hexose as described herein and comparing it to a reference level. If the comparison system, which may be a computer implemented system, indicates that the amount of the measured level of UDP-hexose is statistically higher than that of the reference amount, the subject from which the sample is collected may be identified as having renal inflammation.

[0079] In one embodiment, provided herein is a system comprising: (a) at least one memory containing at least one computer program adapted to control the operation of the computer system to implement a method that includes (i) a determination module configured to measure the level of UDP-hexose in a test sample obtained from a subject; (ii) a storage module configured to store output data from the determination module; (iii) a computing module adapted to identify from the output data whether the measured level of UDP-hexose in the test sample obtained from the subject is higher, by a statistically significant amount, than a reference level, and to provide a retrieved content; (iv) a display module for displaying for retrieved content (e.g., the amount of the measured level of UDP-hexose, or whether the measured level of UDP-hexose is higher than the reference level); and (b) at least one processor for executing the computer program.

[0080] Embodiments may be described through functional modules, which are defined by computer executable instructions recorded on computer readable media and which cause a computer to perform method steps when executed. The modules are segregated by function for the sake of clarity. However, it should be understood that the modules/systems need not correspond to discreet blocks of code and the described functions may be carried out by the execution of various code portions stored on various media and executed at various times. Furthermore, it should be appreciated that the modules may perform other functions, thus the modules are not limited to having any particular functions or set of functions.

[0081] The computer-readable storage media may be any available tangible media that can be accessed by a computer. Computer readable storage media includes volatile and nonvolatile, removable and non-removable tangible media implemented in any method or technology for storage of information such as computer readable instructions, data structures, program modules or other data. Computer readable storage media includes, but is not limited to, RAM (random access memory), ROM (read only memory), EPROM (erasable programmable read only memory), EEPROM (electrically erasable programmable read only memory), flash memory or other memory technology, CD-ROM (compact disc read only memory), DVDs (digital versatile disks) or other optical storage media, magnetic cassettes, magnetic tape, magnetic disk storage or other magnetic storage media, other types of volatile and non-volatile memory, and any other tangible medium which can be used to store the desired information and which can accessed by a computer including and any suitable combination of the foregoing.

[0082] Computer-readable data embodied on one or more computer-readable media may define instructions, for example, as part of one or more programs that, as a result of being executed by a computer, instruct the computer to perform one or more of the functions described herein, and/or various embodiments, variations and combinations thereof. Such instructions may be written in any of a plurality of programming languages, for example, Java, J#, Visual Basic, C, C#, C++, Fortran, Pascal, Eiffel, Basic,

COBOL assembly language, and the like, or any of a variety of combinations thereof. The computer-readable media on which such instructions are embodied may reside on one or more of the components of either of a system, or a computer readable storage medium described herein, may be distributed across one or more of such components.

[0083] The computer-readable media may be transportable such that the instructions stored thereon may be loaded onto any computer resource to implement the aspects of the technology discussed herein. In addition, it should be appreciated that the instructions stored on the computer-readable medium, described above, are not limited to instructions embodied as part of an application program running on a host computer. Rather, the instructions may be embodied as any type of computer code (e.g., software or microcode) that can be employed to program a computer to implement aspects of the technology described herein. The computer executable instructions may be written in a suitable computer language or combination of several languages. Basic computational biology methods are known to those of ordinary skill in the art and are described in, for example, Setubal and Meidanis et al., Introduction to Computational Biology Methods (PWS Publishing Company, Boston, 1997); Salzberg, Searles, Kasif, (Ed.), Computational Methods in Molecular Biology, (Elsevier, Amsterdam, 1998); Rashidi and Buehler, Bioinformatics Basics: Application in Biological Science and Medicine (CRC Press, London, 2000) and Ouelette and Bzevanis Bioinformatics: A Practical Guide for Analysis of Gene and Proteins (Wiley & Sons, Inc., 2nd ed., 2001).

[0084] The functional modules of certain embodiments may include at minimum a determination module, a storage module, a computing module, and a display module. The functional modules may be executed on one, or multiple, computers, or by using one, or multiple, computer networks. The determination module has computer executable instructions to provide e.g., levels of expression products etc in computer readable form.

[0085] The determination module may comprise any system for detecting a signal resulting from the detection of UDP-hexose in a biological sample. In some embodiments, such systems may include an instrument, e.g., a plate reader for measuring absorbance. In some embodiments, such systems may include an instrument, e.g., the Cell Biosciences NANOPRO 1000™ System (Protein Simple; Santa Clara, Calif.) for quantitative measurement of proteins.

[0086] The information determined in the determination system may be read by the storage module. As used herein the "storage module" is intended to include any suitable computing or processing apparatus or other device configured or adapted for storing data or information. Examples of electronic apparatus suitable for use with the technology described herein include stand-alone computing apparatus, data telecommunications networks, including local area networks (LAN), wide area networks (WAN), Internet, Intranet, and Extranet, and local and distributed computer processing systems. Storage modules also include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage media, magnetic tape, optical storage media such as CD-ROM, DVD, electronic storage media such as RAM, ROM, EPROM, EEPROM and the like, general hard disks and hybrids of these categories such as magnetic/optical storage media. The storage module is adapted or configured for having recorded thereon, for example, sample name, patient name, and numerical value of the level of UDP-hexose. Such information may be provided in digital form that may be transmitted and read electronically, e.g., via the Internet, on diskette, via USB (universal serial bus) or via any other suitable mode of communication. Those skilled in the art can readily adopt any of the presently known methods for recording information on known media to generate manufactures comprising expression level information.

[0087] In one embodiment of any of the systems described herein, the storage module stores the output data from the determination module. In additional embodiments, the storage module stores the reference information such as levels of UDP-hexose in healthy subjects. In some embodiments, the storage module stores the information such as levels of UDP-hexose measured from the same subject in earlier time points.

[0088] The computing module may use a variety of available software programs and formats for computing the levels of UDP-hexose. Such algorithms are well established in the art. A skilled artisan is readily able to determine the appropriate algorithms based on the size and quality of the sample and type of data. The data analysis may be implemented in the computing module. In one embodiment, the computing module further comprises a comparison module, which compares the level of UDP-hexose in the test sample obtained from a subject as described herein with the reference level. For example, when the level of UDP-hexose in the test sample obtained from a subject is measured, a comparison module may compare or match the output data, e.g. with the reference level. In certain embodiments, the reference level has been pre-stored in the storage module. During the comparison or matching process, the comparison module may determine whether the level of UDP-hexose in the test sample obtained from a subject is higher than the reference level to a statistically significant degree. In various embodiments, the comparison module may be configured using existing commercially-available or freely-available software for comparison purpose, and may be optimized for particular data comparisons that are conducted.

[0089] The computing and/or comparison module, or any other module, may include an operating system (e.g., UNIX) on which runs a relational database management system, a World Wide Web application, and a World Wide Web server. World Wide Web application includes the executable code necessary for generation of database language statements (e.g., Structured Query Language (SQL) statements). Generally, the executables will include embedded SQL statements. In addition, the World Wide Web application may include a configuration file which contains pointers and addresses to the various software entities that comprise the server as well as the various external and internal databases which must be accessed to service user requests. The Configuration file also directs requests for server resources to the appropriate hardware, as may be necessary should the server be distributed over two or more separate computers. In one embodiment, the World Wide Web server supports a TCP/IP protocol. Local networks such as this are sometimes referred to as "Intranets." An advantage of such Intranets is that they allow easy communication with public domain databases residing on the World Wide Web (e.g., the GenBank or Swiss Pro World Wide Web site). Thus, in a particular preferred embodiment, users can directly access data (via Hypertext

links for example) residing on Internet databases using a HTML interface provided by Web browsers and Web servers.

[0090] The computing and/or comparison module provides a computer readable comparison result that can be processed in computer readable form by predefined criteria, or criteria defined by a user, to provide content based in part on the comparison result that may be stored and output as requested by a user using an output module, e.g., a display module.

[0091] In some embodiments, the content displayed on the display module may be the relative levels of UDP-hexose in the test sample obtained from a subject as compared to a reference level. In certain embodiments, the content displayed on the display module may indicate whether the levels of UDP-hexose are found to be statistically significantly higher in the test sample obtained from a subject as compared to a reference level. In some embodiments, the content displayed on the display module may show the levels of UDP-hexose from the subject measured at multiple time points, e.g., in the form of a graph. In some embodiments, the content displayed on the display module may indicate whether the subject has renal inflammation. In certain embodiments, the content displayed on the display module may indicate whether the subject is in need of a treatment for renal inflammation.

[0092] In one embodiment, the content based on the computing and/or comparison result is displayed on a computer monitor. In one embodiment, the content based on the computing and/or comparison result is displayed through printable media. The display module may be any suitable device configured to receive from a computer and display computer readable information to a user. Non-limiting examples include, for example, general-purpose computers such as those based on Intel PENTIUM-type processor, Motorola PowerPC, Sun UltraSPARC, Hewlett-Packard PA-RISC processors, any of a variety of processors available from Advanced Micro Devices (AMD) of Sunnyvale, Calif., or any other type of processor, visual display devices such as flat panel displays, cathode ray tubes and the like, as well as computer printers of various types.

[0093] In one embodiment, a World Wide Web browser is used for providing a user interface for display of the content based on the computing/comparison result. It should be understood that other modules may be adapted to have a web browser interface. Through the Web browser, a user can construct requests for retrieving data from the computing/comparison module. Thus, the user will typically point and click to user interface elements such as buttons, pull down menus, scroll bars and the like conventionally employed in graphical user interfaces.

[0094] Systems and computer readable media described herein are merely illustrative embodiments of the technology relating to determining the levels of UDP-hexose, and therefore are not intended to limit the scope of the invention. Variations of the systems and computer readable media described herein are possible and are intended to fall within the scope of the invention.

[0095] The modules of the machine, or those used in the computer readable medium, may assume numerous configurations. For example, function may be provided on a single machine or distributed over multiple machines.

Methods of Providing a P2Y14 Receptor Antagonist

[0096] The invention provides methods of treating or preventing renal inflammation in a subject with an infection by providing a purinergic P2Y14 receptor antagonist, referred to simply as a P2Y14 antagonist. Providing the P2Y14 antagonist to the subject may include administering it to the subject. The P2Y14 antagonist may be administered by any suitable means. For example and without limitation, the P2Y14 antagonist may be administered orally, intravenously, enterally, parenterally, dermally, buccally, topically, transdermally, by injection, intravenously, subcutaneously, nasally, pulmonarily, or with or on an implantable medical device (e.g., stent or drug-eluting stent or balloon equivalents).

[0097] The P2Y14 antagonist may be provided at any suitable dosage. For example and without limitation, the P2Y14 antagonist may be provided at from 0.001 mg/kg body weight to 5 g/kg body weight. In some embodiments, the dosage range is from 0.001 mg/kg body weight to 1 g/kg body weight, from 0.001 mg/kg body weight to 0.5 g/kg body weight, from 0.001 mg/kg body weight to 0.1 g/kg body weight, from 0.001 mg/kg body weight to 50 mg/kg body weight, from 0.001 mg/kg body weight to 25 mg/kg body weight, from 0.001 mg/kg body weight to 10 mg/kg body weight, from 0.001 mg/kg body weight to 5 mg/kg body weight, from 0.001 mg/kg body weight to 1 mg/kg body weight, from 0.001 mg/kg body weight to 0.1 mg/kg body weight, or from 0.001 mg/kg body weight to 0.005 mg/kg body weight. Alternatively, in some embodiments the dosage range is from 0.1 g/kg body weight to 5 g/kg body weight, from 0.5 g/kg body weight to 5 g/kg body weight, from 1 g/kg body weight to 5 g/kg body weight, from 1.5 g/kg body weight to 5 g/kg body weight, from 2 g/kg body weight to 5 g/kg body weight, from 2.5 g/kg body weight to 5 g/kg body weight, from 3 g/kg body weight to 5 g/kg body weight, from 3.5 g/kg body weight to 5 g/kg body weight, from 4 g/kg body weight to 5 g/kg body weight, or from 4.5 g/kg body weight to 5 g/kg body weight. These doses may be administered at a single time or multiple times each day, or may be administered continuously, for example by continuous intravenous infusion. For example and without limitation, the P2Y14 antagonist may be provided at from 0.001 mg/kg body weight/day (mg/kg/day) to 5 g/kg body weight/day. In some embodiments, the dosage range is from 0.001 mg/kg body weight/day to 1 g/kg body weight/day, from 0.001 mg/kg body weight/day to 0.5 g/kg body weight/day, from 0.001 mg/kg body weight/day to 0.1 g/kg body weight/day, from 0.001 mg/kg body weight/day to 50 mg/kg body weight/day, from 0.001 mg/kg body weight/day to 25 mg/kg body weight/day, from 0.001 mg/kg body weight/day to 10 mg/kg body weight/day, from 0.001 mg/kg body weight/day to 5 mg/kg body weight/day, from 0.001 mg/kg body weight/day to 1 mg/kg body weight/day, from 0.001 mg/kg body weight/day to 0.1 mg/kg body weight/day, or from 0.001 mg/kg body weight/day to 0.005 mg/kg body weight/day. Alternatively, in some embodiments the dosage range is from 0.1 g/kg body weight/day to 5 g/kg body weight/day, from 0.5 g/kg body weight/day to 5 g/kg body weight/day, from 1 g/kg body weight/day to 5 g/kg body weight/day, from 1.5 g/kg body weight/day to 5 g/kg body weight/day, from 2 g/kg body weight/day to 5 g/kg body weight/day, from 2.5 g/kg body weight/day to 5 g/kg body weight/day, from 3 g/kg body weight/day to 5 g/kg body weight/day, from 3.5 g/kg body weight/day to 5 g/kg body

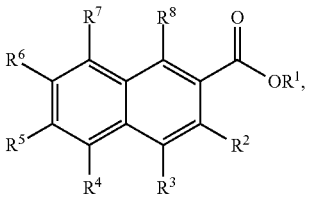
weight/day, from 4 g/kg body weight/day to 5 g/kg body weight/day, or from 4.5 g/kg body weight/day to 5 g/kg body weight/day. Effective doses may be estimated from dose-response relationships derived from in vitro or animal model test bioassays or systems or from clinical trials of the P2Y14 antagonist. The dosage should not be so large as to cause unacceptable adverse side effects.

[0098] The methods may include providing a P2Y14 antagonist in combination with another therapeutic agent. The other therapeutic agent may be provided in the same formulation as the P2Y14 antagonist, or it may be provided in a separate formulation. The P2Y14 antagonist and other therapeutic agent may be provided according to the same dosing schedule, or they may be provided according to different dosing schedules.

[0099] The other therapeutic agent may be an agent that ameliorates or may ameliorate infection, sepsis, or AKI. Several classes of therapeutic agents to treat sepsis are known in the art to be useful for treating sepsis. For example, antibiotics, including broad-spectrum antibiotics, such as aminoglycosides (except for streptomycin), amoxicillin, amoxicillin/clavulanic acid, i.e., augmentin, ampicillin, carbapenems, e.g. imipenem, chloramphenicol, piperacillin/tazobactam, quinolones, e.g. ciprofloxacin, teicoplanin, tetracyclines, ticarcillin, and trimethoprim/sulfamethoxazole, e.g., bactrim, and vancomycin, may be used. Antifungal agents, such as caspofungin, echinocandins, fluconazole, and itraconazole, micafungin, or triazole, may be used for fungal agents, and antiviral agents may be used for viral infections. Intravenous fluids are also useful for treating sepsis. Blood products, such as red blood cells, erythropoietin, plasma, or platelets, may be provided. Other therapeutic agents for treating sepsis include oxygen, steroids, such as glucocorticoids and hydrocortisone, and vasopressors, such as amezinium, dobutamine, dopamine, ephedrine hydrochloride, epinephrine, midodrine, noradrenaline hydrotartrate, and phenylephrine.

P2Y14 Antagonists

[0100] The P2Y14 antagonist may be any entity that interferes with ligand-binding, activation, or signaling by P2Y14. The P2Y14 antagonist may be a small or large organic or inorganic molecule. Preferably, the P2Y14 antagonist is a 4,7-disubstituted naphthoic acid derivative, such as one of the compounds described in U.S. Publication No. 2010/0298347, the contents of which are incorporated herein by reference. Such compounds may be represented by formula (I):



(I)

wherein:

[0101] R^1 is selected from the group consisting of hydrogen, C_{3-6} cycloalkyl, benzyl, and C_{1-6} alkyl wherein alkyl is optionally substituted with hydroxy, amino, C_{1-4} alkylamino, di-(C_{1-4} alkyl)amino, aminocarbonyl, C_{1-4} alkylaminocarbonyl, di-(C_{1-4}alkyl)aminocarbonyl, C_{1-4} alkylcarbonyloxy, C_{1-4} alkyloxy, or one to five fluorines;

[0102] R^2 is hydrogen, fluorine, or hydroxy;

[0103] R^3 is selected from the group consisting of: —$(CH_2)_m$aryl, —$(CH_2)_m$heteroaryl, —OCH_2-aryl, —OCH_2-heteroaryl, —$(S)_rCH_2$-aryl, —$(S)_rCH_2$-heteroaryl, —CH_2O-aryl, —CH_2O-heteroaryl, —$CH_2(S)_r$-aryl, and —$CH_2(S)_r$-heteroaryl;

wherein any methylene (CH_2) carbon atom in R3 is optionally substituted with one to two groups independently selected from fluorine, hydroxy, and C_{1-4} alkyl optionally substituted with one to three fluorines; or two substituents when on the same methylene (CH_2) group are taken together with the carbon atom to which they are attached to form a cyclopropyl group; and wherein aryl and heteroaryl are optionally substituted with one to three R^c substituents independently selected from the group consisting of:

[0104] halogen,

[0105] cyano,

[0106] nitro,

[0107] C_{1-6} alkoxy, wherein alkoxy is optionally substituted with one to five substituents independently selected from fluorine, hydroxy, and C_{1-3} alkoxy,

[0108] C_{1-6} alkyl, wherein alkyl is optionally substituted with one to five substituents independently selected from fluorine, hydroxy, and C_{1-3} alkoxy,

[0109] C_{2-6} alkenyl, wherein alkenyl is optionally substituted with one to five substituents independently selected from fluorine, hydroxy, and C_{1-3} alkoxy,

[0110] $(CH_2)_n$-aryl,

[0111] $(CH_2)_n$-heteroaryl,

[0112] $(CH_2)_n$-heterocyclyl,

[0113] $(CH_2)_n$—C_{3-6} cycloalkyl,

[0114] $(CH_2)_n$—OR^9,

[0115] $(CH_2)_n$—CO_2R^9,

[0116] $(CH_2)_n$—$N(R^9)_2$,

[0117] $(CH_2)_n$—$CON(R^9)_2$,

[0118] $(CH_2)_n$—$OCON(R^9)_2$,

[0119] $(CH_2)_n$—$SO_2N(R^9)_2$,

[0120] $(CH_2)_n$—$SO_2N(R^9)C(O)R^9$,

[0121] $(CH_2)_n$—$C(O)_n(R^9)SO_2R^{10}$,

[0122] $(CH_2)_n$—$S(O)_2R^{10}$,

[0123] $(CH_2)_n$—$NR^{11}SO_2R^{10}$;

[0124] $(CH_2)_n$—$NR^{11}CON(R^9)_2$,

[0125] $(CH_2)_n$—$NR^{11}COR^9$, and

[0126] $(CH_2)_n$—$NR^{11}CO_2R^{10}$;

wherein aryl, heteroaryl, cycloalkyl, and heterocyclyl are optionally substituted with one to three substituents independently selected from halogen, hydroxy, C_{1-4} alkyl, trifluoromethyl, and C_{1-4} alkoxy; and wherein any methylene (CH_2) carbon atom in R^c is optionally substituted with one to two groups independently selected from fluorine, hydroxy, and C_{1-4} alkyl optionally substituted with one to three fluorines; or two substituents when on the same methylene (CH_2) group are taken together with the carbon atom to which they are attached to form a cyclopropyl group;

[0127] R^4, R^5, R^7, and R^8 are each independently selected from the group consisting of:

[0128] hydrogen,

[0129] halogen,

[0130] C_{1-4} alkyl, optionally substituted with one to five fluorines,

[0131] C_{1-4} alkoxy, optionally substituted with one to five fluorines, and

[0132] C_{1-4} alkylthio, optionally substituted with one to five fluorines;

[0133] R6 is selected from the group consisting of:

[0134] —$(CH_2)_m$-aryl,

[0135] —$(CH_2)_m$-heteroaryl,

[0136] —OCH_2-aryl,

[0137] —OCH_2-heteroaryl,

[0138] —$(S)_rCH_2$-aryl,

[0139] —$(S)_rCH_2$-heteroaryl,

[0140] —CH_2O-aryl,

[0141] —CH_2O-heteroaryl,

[0142] —$CH_2(S)_r$-aryl, and

[0143] —$CH_2(S)_r$-heteroaryl;

wherein any methylene (CH_2) carbon atom in R6 is optionally substituted with one to two groups independently selected from fluorine, hydroxy, and C_{1-4} alkyl optionally substituted with one to three fluorines; or two substituents when on the same methylene (CH_2) group are taken together with the carbon atom to which they are attached to form a cyclopropyl group and wherein aryl and heteroaryl are optionally substituted with one to three Rd substituents independently selected from the group consisting of:

[0144] halogen,

[0145] cyano,

[0146] C_{1-4} alkyl, optionally substituted with one to five fluorines,

[0147] C_{1-4} alkoxy, optionally substituted with one to five fluorines,

[0148] C_{1-4} alkylthio, optionally substituted with one to five fluorines, and

[0149] C_{1-4} alkylsulfonyl, optionally substituted with one to five fluorines;

each R^9 is independently selected from the group consisting of hydrogen,

[0150] C_{1-6} alkyl,

[0151] $(CH_2)_m$-aryl,

[0152] $(CH_2)_m$-heteroaryl, and

[0153] $(CH_2)_mC_{3-6}$ cycloalkyl;

wherein any individual methylene (CH_2) carbon atom in $(CH_2)_m$ is optionally substituted with one to two substituents independently selected from fluorine, hydroxy, C_{1-4} alkyl, and C_{1-4} alkoxy, wherein alkyl and alkoxy are optionally substituted with one to five fluorines; or two substituents when on the same methylene (CH_2) group are taken together with the carbon atom to which they are attached to form a cyclopropyl group; and wherein alkyl, aryl, heteroaryl, and cycloalkyl are optionally substituted with one to three substituents independently selected from the group consisting of halogen, C_{1-4} alkyl, and C_{1-4} alkoxy; or two R^9 groups substituents together with the nitrogen atom to which they are attached form a heterocyclic ring selected from azetidine, pyrrolidine, piperidine, piperazine, and morpholine wherein said heterocyclic ring is optionally substituted with one to three substituents independently selected from the group consisting of halogen, hydroxy, C_{1-6}alkyl, and C_{1-6} alkoxy, wherein alkyl and alkoxy are optionally substituted with one to five fluorines;

each R^{10} is independently C_{1-6} alkyl, wherein alkyl is optionally substituted with one to five substituents independently selected from fluorine and hydroxy;

R^{11} is hydrogen or R^{10};

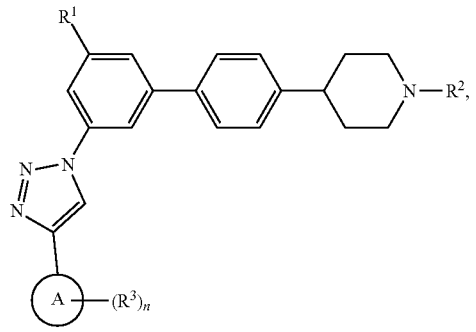
each n is independently an integer from 0 to 3;

each m is independently an integer from 0 to 2; and

each r is an integer from 0 to 2.

[0154] The P2Y14 antagonist may be a triazole derivative, such as one of the compounds described in WO 2017/053769, the contents of which are incorporated herein by reference. Such compounds may be represented by the formula (XI):

(XI)



wherein

[0155] ring A is aryl, heteroaryl, or cycloalkyl;

[0156] R^1 is —CO_2H, —CO_2(C_1-C_8 alkyl), or a bioisostere of carboxylate;

[0157] R^2 is H, C_1-C_8 alkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylalkyl, hydroxyalkyl, C_1-C_8 haloalkyl, cyanoalkyl, aryl, heteroaryl, heterocycloalkyl, —$(CH_2)_m$aryl, —$(CH_2)_m$heteroaryl, or —$(CH_2)_m$heterocycloalkyl;

[0158] each R^3 is the same or different and each is C_1-C_8 alkyl, C_2-C_8 alkenyl, C_3-C_6 cycloalkyl, hydroxy, hydroxyalkyl, C_1-C_8 alkoxy, C_3-C_6 cycloalkyloxy, aryloxy, halo, C_1-C_8 haloalkyl, C_1-C_8 haloalkoxy, —CN, —NO_2, —NR^5R^6, —$C(O)R^4$, —CO_2R^4, —$C(O)NR^5R^6$, —$NR^5C(O)R^4$, —$(CH_2)_m$aryl, —$(CH_2)_m$heteroaryl, or —$(CH_2)_m$heterocycloalkyl;

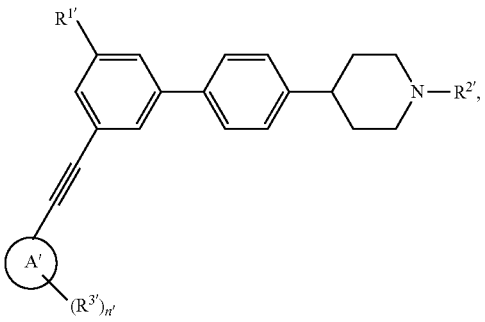
[0159] R^4, R^5, and R^6 are the same or different and each is H or C_1-C_8 alkyl; and

[0160] m and n are the same or different and each is 0 or an integer from 1-5;

or a pharmaceutically acceptable salt thereof.

[0161] Other compounds described in WO 2017/053769 that may be used as P2Y14 antagonists are represented by the formula (XII):

(XII)



wherein

[0162] ring A' is aryl, heteroaryl, or cycloalkyl;

[0163] $R^{1'}$ is —CO_2H, —CO_2(C_1-C_8 alkyl), or a bioisostere of carboxylate;

[0164] $R^{2'}$ is H, C_1-C_8 alkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, C_3-C_6 cycloalkyl, C_3-C_6 cycloalkylalkyl, hydroxyalkyl, C_1-C_8 haloalkyl, cyanoalkyl, aryl, heteroaryl, heterocycloalkyl, —$(CH_2)_m$aryl, —$(CH_2)_m$heteroaryl, or —$(CH_2)_m$heterocycloalkyl;

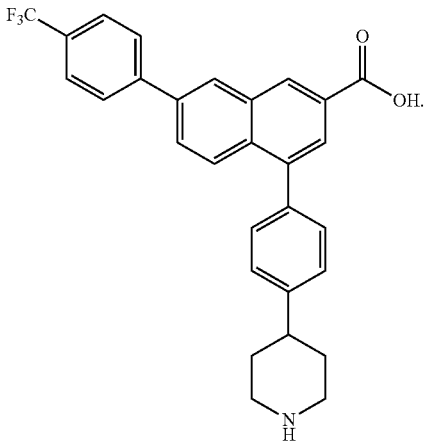
[0165] each $R^{3'}$ is the same or different and each is C_1-C_8 alkyl, C_2-C_8 alkenyl, C_3-C_6 cycloalkyl, hydroxy, hydroxyalkyl, C_1-C_8 alkoxy, C_3-C_6 cycloalkyloxy, aryloxy, halo, C_1-C_8 haloalkyl, C_1-C_8 haloalkoxy, —CN, —NO_2, —$NR^{5'}R^{6'}$, —$C(O)R^{4'}$, —$CO_2R^{4'}$, —$C(O)NR^{5'}R^{6'}$, —$NR^{5'}C(O)R^{4'}$, —$(CH_2)_m$aryl, —$(CH_2)_m$heteroaryl, or —$(CH_2)_m$heterocycloalkyl;

[0166] $R^{4'}$, $R^{5'}$, and $R^{6'}$ are the same or different and each is H or C_1-C_8 alkyl; and

[0167] m' and n' are the same or different and each is 0 or an integer from 1-5;

or a pharmaceutically acceptable salt thereof.

[0168] The P2Y14 antagonist may be 4-[4-(piperidin-4-yl)phenyl]-7-[4-(trifluoromethyl)phenyl]-2-naphthoic acid (PPTN), a compound having the structure:



The P2Y14 antagonist may be a prodrug, analog, derivative, or pharmaceutically acceptable salt of PPTN or of any other active compound that inhibits P2Y14.

[0169] Any of the compounds described above may be provided as a pharmaceutically acceptable salt. For example and without limitation, the pharmaceutically acceptable salt may include one or more of acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methanesulfonate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, N-methylglucamine ammonium salt, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, phosphate, diphosphate, polygalacturonate, salicylate, stearate, sulfate, subacetate, succinate, tamiate, tartrate, teoclate, tosylate, triethiodide, and valerate.

[0170] The P2Y14 antagonist may be provided in a pharmaceutical composition. A pharmaceutical composition may be in a form suitable for oral use, for example, as tablets, troches, lozenges, fast-melts, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs. Compositions intended for oral use may be prepared according to any method known in the art for the manufacture of pharmaceutical compositions, and such compositions may contain one or more agents selected from sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the compounds in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as lactose; granulating and disintegrating agents, for example corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc.

[0171] The tablets may be uncoated or they may be coated by known techniques to delay disintegration in the stomach and absorption lower down in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the techniques described in U.S. Pat. Nos. 4,256,108, 4,166,452 and 4,265,874, to form osmotic therapeutic tablets for control release. Preparation and administration of compounds is discussed in U.S. Pat. No. 6,214,841 and U.S. Pub. 2003/0232877, which are incorporated by reference herein in their entirety.

[0172] Formulations for oral use may also be presented as hard gelatin capsules in which the compounds are mixed with an inert solid diluent, such as kaolin. The formulations may be presented as soft gelatin capsules in which the compounds are mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

[0173] An alternative oral formulation, where control of gastrointestinal tract hydrolysis of the compound is sought, can be achieved using a controlled-release formulation, where a compound of the invention is encapsulated in an enteric coating.

[0174] Aqueous suspensions may contain the compounds in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents such as a naturally occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example, polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such a polyoxyethylene with partial esters derived from fatty acids and hexitol anhydrides, for example polyoxyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

[0175] Oily suspensions may be formulated by suspending the compounds in a vegetable oil, for example, arachis oil,

olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

[0176] Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the compounds in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified, for example sweetening, flavoring and coloring agents, may also be present.

[0177] The pharmaceutical compositions may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally occurring phosphatides, for example soya bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

[0178] Syrups and elixirs may be formulated with sweetening agents, such as glycerol, propylene glycol, sorbitol, or sucrose. Such formulations may also contain a demulcent, a preservative, and agents for flavoring and/or coloring. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be in a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

[0179] The pharmaceutical composition may be formulated for intravenous injection or subcutaneous administration. For example, the compositions may be dissolved, suspended or emulsified. The compositions may also be lyophilized, and the lyophilized material may be used to prepare a formulation for injection. Suitable solvents for injectable formulations include, for example and without limitation, water, physiological saline solution, alcohols, e.g. ethanol, propanol, glycerol, sugar solutions, such as hexose or mannitol solutions, and mixtures of the aforementioned solvents. The injectable solutions or suspensions may be formulated according to known art, using suitable non-toxic, parenterally-acceptable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution or isotonic sodium chloride solution, or suitable dispersing or wetting and suspending agents, such as sterile, bland, fixed oils, including synthetic monoglycerides or diglycerides, and fatty acids, including oleic acid.

[0180] The pharmaceutical composition may be formulated for delivery of a compound that is insoluble or poorly soluble in water. Examples of such formulations include nanoparticles, microparticles, nanosuspensions, phospholipid-coated microcrystals, emulsions, and stable aqueous formulations. Formulations for delivery of insoluble or poorly soluble compounds are known in the art and described in, for example, U.S. Pat. Nos. 5,091,187; 5,858,410; 8,313,777; 9,308,180; U.S. Publication No. 2002/0012704; U.S. Publication No. 2003/0027858; U.S. Publication No. 2008/0166411; U.S. Publication No. 2010/0093872; U.S. Publication No. 2013/0115165; International Publication No. WO 2014/165660; Pace S. et al., "Novel injectable formulations of insoluble drugs", Pharm. Tech, 1999, 23:116-134; and Panagiotou T. et al., "Production of stable nanosuspensions using microfluidics reaction technology", Nanotech. 2007, 4:246-249, ISBN 1420063766, the contents of each of which are incorporated herein by reference.

[0181] Pharmaceutical compositions may include other pharmaceutically acceptable carriers, such as sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin (glycerol), erythritol, xylitol, sorbitol, mannitol and polyethylene glycol; esters, such asethyl oleate and ethyllaurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in pharmaceutical formulations.

[0182] The P2Y14 antagonist may be provided as one or more pharmaceutically acceptable salts, such as nontoxic acid addition salts, which are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. In some embodiments, pharmaceutically acceptable salts include, but are not limited to, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphor sulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. In some embodiments, a pharmaceutically acceptable

salt is an alkali salt. In some embodiments, a pharmaceutically acceptable salt is a sodium salt. In some embodiments, a pharmaceutically acceptable salt is an alkaline earth metal salt. In some embodiments, pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counter ions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl having from 1 to 6 carbon atoms, sulfonate and aryl sulfonate.

[0183] The pharmaceutical composition or formulation may include one or more agents that increase the solubility of a P2Y14 antagonist, such as PPTN, in an aqueous medium. Examples of suitable agents include sulfobutyl ether beta-cyclodextrin (SBECD) or α-tocopherol polyethylene glycol succinate (TPGS).

[0184] The presence of the agent may allow the formulation to contain a certain concentration of the P2Y14 antagonist. Thus, the formulation, including the P2Y14 antagonist and the agent, may contain the P2Y14 antagonist at ≥0.001 μg/ml, ≥0.002 μg/ml, ≥0.005 μg/ml, ≥0.01 μg/ml, ≥0.02 μg/ml, ≥0.05 μg/ml, ≥0.1 μg/ml, ≥0.2 μg/ml, ≥0.5 μg/ml, ≥1 μg/ml, ≥2 μg/ml, ≥5 μg/ml, ≥10 μg/ml, ≥20 μg/ml, ≥50 μg/ml, ≥100 μg/ml, ≥200 μg/ml, ≥500 μg/ml, ≥1 mg/ml, ≥2 mg/ml, ≥5 mg/ml, ≥10 mg/ml, from about 1 μg/ml to about 20 mg/ml, from about 2 μg/ml to about 20 mg/ml, from about 5 μg/ml to about 20 mg/ml, from about 10 μg/ml to about 20 mg/ml, from about 20 μg/ml to about 20 mg/ml, from about 50 μg/ml to about 20 mg/ml, from about 100 μg/ml to about 20 mg/ml, from about 200 μg/ml to about 20 mg/ml, from about 500 μg/ml to about 20 mg/ml, from about 1 mg/ml to about 20 mg/ml, from about 2 mg/ml to about 20 mg/ml, from about 5 mg/ml to about 20 mg/ml, from about 1 μg/ml to about 10 mg/ml, from about 2 μg/ml to about 10 mg/ml, from about 5 μg/ml to about 10 mg/ml, from about 10 μg/ml to about 10 mg/ml, from about 20 μg/ml to about 10 mg/ml, from about 50 μg/ml to about 10 mg/ml, from about 100 μg/ml to about 10 mg/ml, from about 200 μg/ml to about 10 mg/ml, from about 500 μg/ml to about 10 mg/ml, from about 1 mg/ml to about 10 mg/ml, from about 2 mg/ml to about 10 mg/ml, from about 5 mg/ml to about 10 mg/ml, from about 1 μg/ml to about 5 mg/ml, from about 2 μg/ml to about 5 mg/ml, from about 5 μg/ml to about 5 mg/ml, from about 10 μg/ml to about 5 mg/ml, from about 20 μg/ml to about 5 mg/ml, from about 50 μg/ml to about 5 mg/ml, from about 100 μg/ml to about 5 mg/ml, from about 200 μg/ml to about 5 mg/ml, from about 500 μg/ml to about 5 mg/ml, from about 1 mg/ml to about 5 mg/ml, from about 2 mg/ml to about 5 mg/ml, from about 1 μg/ml to about 2 mg/ml, from about 2 μg/ml to about 2 mg/ml, from about 5 μg/ml to about 2 mg/ml, from about 10 μg/ml to about 2 mg/ml, from about 20 μg/ml to about 2 mg/ml, from about 50 μg/ml to about 2 mg/ml, from about 100 μg/ml to about 2 mg/ml, from about 200 μg/ml to about 2 mg/ml, from about 500 μg/ml to about 2 mg/ml, or from about 1 mg/ml to about 2 mg/ml.

[0185] The agent may promote solubility of the P2Y14 antagonist at a near-neutral pH. Thus, the formulation, including the P2Y14 antagonist and the agent, may have a pH of >4.0, >4.5, >5.0, >5.5, >6.0, >6.5, >7.0, >7.5, >8.0, from about 4.0 to about 9.0, from about 5.0 to about 9.0, from about 6.0 to about 9.0, from about 7.0 to about 9.0, from about 4.0 to about 8.0, from about 5.0 to about 8.0, from about 6.0 to about 8.0, from about 7.0 to about 8.0, from about 4.0 to about 7.0, from about 5.0 to about 7.0, from about 6.0 to about 7.0, about 5.0, about 5.5, about 6.0, about 6.5, about 7.0, about 7.5, or about 8.0.

[0186] The agent may be present in the formulation at a certain concentration. For example, the agent may be present in the formulation at less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 2%, less than about 1%, less than about 0.5%, less than about 0.2%, less than about 0.1%, less than about 0.05%, less than about 0.02%, less than about 0.01%, less than about 0.005%, less than about 0.002%, less than about 0.001%, from about 0.001% to about 0.01%, from about 0.003% to about 0.03%, from about 0.01% to about 0.1%, from about 0.03% to about 0.3%, from about 0.1% to about 1%, from about 0.3% to about 3%, from about 1% to about 10%, from about 2% to about 10%, from about 3% to about 10%, from about 5% to about 10%, from about 5% to about 12%, from about 5% to about 15%, from about 5% to about 20%, from about 7.5% to about 10%, from about 7.5% to about 12%, from about 7.5% to about 15%, from about 7.5% to about 20%, from about 10% to about 12%, from about 10% to about 15%, or from about 10% to about 20%.

[0187] The agent may improve the stability of the P2Y14 antagonist. For example, the agent may increase the half-life of the P2Y14 antagonist by about 10%, about 25%, about 50%, about 100%, about 200%, about 500%, about 1000%, or more.

[0188] The formulation may contain dimethyl sulfoxide (DMSO). The formulation may contain DMSO at or below a certain concentration. For example, DMSO may be present in the formulation at less than about 10%, less than about 5%, less than about 3%, less than about 2%, less than about 1%, less than about 0.5%, less than about 0.3%, less than about 0.2%, or less than about 0.1%.

[0189] The formulation may be substantially free of solvents or other chemicals that are not suitable for administration to a subject. For example, the formulation may be substantially free of dimethylacetamide (DMAc), ethanol, N-methylpyrrolidone (NMP), and/or polyethylene glycol (PEG).

[0190] Formulations that contain P2Y14 antagonists are described in, for example, co-owned, co-pending U.S. Patent Application No. 62/834,517, the contents of which are incorporated herein in their entirety.

Renal Inflammation Associated with Acute Kidney Injury (AKI)

[0191] The methods may treat or prevent renal inflammation associated with AKI. AKI may be assessed by any suitable standard. Several standards for acute kidney injury are known in the art, such as the criteria provided by the Acute Kidney Injury Network (AKIN); Kidney Disease Improving Global Outcomes (KDIGO); and Risk, Injury, Failure, Loss, and End-stage Kidney (RIFLE). AKI may be categorized or staged according to the AKI, KDIGO, or RIFLE criteria. For example, a subject may be deemed to have stage 1, stage 2, or stage 3 AKI, or a subject may be deemed to have risk, injury, failure, or loss. The standard may apply to an adult, pediatric, newborn, neonatal, infant, child, adolescent, pre-teen, teenage, or elderly subject.

[0192] Standards typically include measurements of serum creatinine (SCr) concentrations, urine output, or glomerular filtration rate (GFR). Standards may include multiple parameters, e.g., combinations of the aforemen-

tioned standards. A subject may be deemed to have AKI, or a stage or category thereof, when she has abnormally high SCr concentration, abnormally low urine output, abnormally low GFR, or any combination thereof. Standards may be absolute, e.g., they may require a value above or below a defined threshold value. Alternatively, standards may be relative, e.g., they may require an increase or decrease relative to a baseline value. Standards for different parameters, e.g., abnormally high SCr concentration abnormally low urine output, or abnormally low GFR, may independently be absolute or relative.

[0193] Standards for acute kidney injury may include a temporal component. For example, a subject may be deemed to have AKI when an elevated SCr concentration is measured at some interval following a preceding event. The preceding event may be an infection, sepsis, admission to a hospital, clinic, medical facility, or any unit thereof. The interval may be 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, 24 hours, 36 hours, 48 hours, or 72 hours. A subject may be deemed to have AKI when urine output is measured across some interval, such as 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, 24 hours, 36 hours, 48 hours, or 72 hours.

[0194] For example and without limitation, a standard for reduced urine output associated with AKI may be less than 0.5 mL/kg/h for 6-12 hours, less than 0.5 mL/kg/h for at least 12 hours, or less than 0.3 mL/kg/h for 24 hours, or anuria for at least 12 hours.

[0195] For example and without limitation, a standard for elevated SCr concentration associated with AKI may be a SCr concentration of at least 0.3 mg/dl, a SCr concentration of at least 1 mg/dl, a SCr concentration of at least 4 mg/dl, a SCr concentration of at least 26.5 μmol/l, or a SCr concentration of at least 353.6 μmol/l. For example and without limitation, a standard for elevated SCr concentration associated with AKI may be an increase of 50% over baseline, an increase of 100% over baseline, or an increase of 200% over baseline.

[0196] For example and without limitation, a standard for GFR associated with AKI may be a GFR of less than 35 ml/min per 1.73 mm2. For example and without limitation, a standard for GFR associated with AKI may be a decrease of at least at least 25% relative to a baseline, a decrease of at least at least 50% relative to a baseline, or a decrease of at least at least 75% relative to a baseline.

Improvement of Renal Function

[0197] Providing a P2Y14 antagonist may improve renal function. For example, renal function may be improved by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 200%, or at least 300%. Measurable markers of renal function, are well known in the medical and veterinary literature and to those of skill in the art, and include, but are not limited to, blood urea nitrogen or "BUN" levels (both static measurements and measurements of rates of increase or decrease in BUN levels), serum creatinine levels (both static measurements and measurements of rates of increase or decrease in serum creatinine levels), measurements of the BUN/creatinine ratio (static measurements of measurements of the rate of change of the BUN/creatinine ratio), urine/plasma ratios for creatinine, urine/plasma ratios for urea, glomerular filtration rates (GFR), serum concentrations of sodium (Na+), urine osmolarity, daily urine output, albuminuria, proteinuria, and the like. Of the above, measurements of the plasma concentrations of creatinine and/or urea or BUN are particularly important and useful readouts of renal function.

INCORPORATION BY REFERENCE

[0198] References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made throughout this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes.

EQUIVALENTS

[0199] Various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including references to the scientific and patent literature cited herein. The subject matter herein contains important information, exemplification and guidance that can be adapted to the practice of this invention in its various embodiments and equivalents thereof.

What is claimed is:

1. A method of monitoring for renal inflammation associated with an infection in a subject, the method comprising:
- obtaining a sample from a subject who has an infection;
- conducting an assay on the sample to measure a level of a UDP-hexose in the sample; and
- comparing the level of the UDP-hexose from the sample with a reference level of UDP-hexose, wherein an elevated level of the UDP-hexose indicates that the subject is at risk of developing or has developed renal inflammation.

2. The method of claim **1**, wherein the infection causes or may cause sepsis in the subject.

3. The method of claim **1**, wherein the subject displays at least one symptom selected from the group consisting of altered body temperature, altered consciousness, altered white blood cell count, bandemia, decreased blood pressure, decreased partial pressure of carbon dioxide, metabolic acidosis, increased heart rate, increased number of immature neutrophils, and increased respiratory rate.

4. The method of claim **1**, wherein the infection is in a lung, abdomen, skin, cerebrospinal fluid, limb, or urinary tract of the subject.

5. The method of claim **1**, wherein the infection is bacterial, viral, or fungal.

6. The method of claim **1**, wherein the reference level is an average UDP-hexose level in a population of healthy subjects.

7. The method of claim **1**, wherein the sample is a body fluid sample.

8. The method of claim **1**, further comprising providing a P2Y14 antagonist to the subject if the subject has an elevated level of UDP-hexose.

9. The method of claim **8**, wherein the P2Y14 antagonist is a substituted 2-naphthoic acid.

10. The method of claim **8**, wherein the P2Y14 antagonist is 4-((piperidin-4-yl)-phenyl)-(7-(4-(trifluoromethyl)-phenyl)-2-naphthoic acid (PPTN).

11. The method of claim **8**, further comprising repeating the obtaining, conducting, and comparing steps after the

subject has been provided the P2Y14 antagonist to thereby monitor the subject over time.

12. The method of claim **1**, wherein the UDP-hexose is at least one compound selected from the group consisting of UDP-glucose, UDP-galactose, UDP-glucuronic acid, N-acetyl-UDP-glucosamine, N-acetyl-UDP-galactosamine, and combinations thereof.

13. A method of treating or preventing renal inflammation associated with an infection in a subject, the method comprising: providing a P2Y14 antagonist to a subject who has an infection.

14. The method of claim **13**, wherein the infection causes or may cause sepsis in the subject.

15. The method of claim **13**, wherein the subject displays at least one symptom selected from the group consisting of altered body temperature, altered consciousness, altered white blood cell count, bandemia, decreased blood pressure, decreased partial pressure of carbon dioxide, metabolic acidosis, increased heart rate, increased number of immature neutrophils, and increased respiratory rate.

16. The method of claim **13**, wherein the infection is in a lung, abdomen, skin, cerebrospinal fluid, limb, or urinary tract of the subject.

17. The method of claim **13**, wherein the infection is bacterial, viral, or fungal.

18. The method of claim **13**, wherein the P2Y14 antagonist is a substituted 2-naphthoic acid.

19. The method of claim **18**, wherein the P2Y14 antagonist is 4-((piperidin-4-yl)-phenyl)-(7-(4-(trifluoromethyl)-phenyl)-2-naphthoic acid (PPTN).

20. The method of claim **13**, further comprising providing at least one selected from the group consisting of an antibiotic, antifungal, blood product, intravenous fluid, oxygen, steroid, and vasopressor.

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