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(54) Title: METHOD OF DETERMINING RISK OF AN ADVERSE CARDIAC EVENT

(57) Abstract: Methods of determining the risk of an adverse cardiovascular event or death in a mammal are provided which include determining in a biological sample obtained from the mammal the level of a combination of biomarkers selected from a glucose metabolism biomarker, a heart function biomarker, a renal function biomarker and at least one biomarker of cardiac injury. A score is allotted based on the level of each biomarker, and the cumulative score is indicative of the risk of an adverse cardiovascular event.

METHOD OF DETERMINING RISK OF AN ADVERSE CARDIAC EVENT

FIELD OF INVENTION

[0001] The present invention relates to a prognostic method in the field of cardiology, and in particular, to a laboratory score for use to confirm or rule-out an acute cardiovascular event in a patient, as well as for risk stratification (i.e. to identify risk in a patient for subsequent cardiovascular events).

BACKGROUND OF THE INVENTION

[0002] The optimum laboratory test to identify myocardial injury is cardiac troponin. For those patients presenting with chest pain to the emergency department (ED), a physician's decision to discharge a patient from the hospital or admit and treat them for myocardial infarction (MI or heart attack) is often based on the patient's cardiac troponin measurement. However, individuals may have detectable and elevated cardiac troponin measurements for several reasons other than due to MI. Thus, a laboratory test or laboratory score consisting of several biomarkers is needed to identify patients both in the ED and in the non-acute setting who are at risk of MI or another serious cardiac event (e.g. cardiac ischemia, heart failure, percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG), or death) within days to months. In short, a method providing a laboratory score to identify those at lowest risk for a subsequent cardiac event or death would be a tremendous tool for ED physicians wishing to discharge patients following receipt of results of a first blood collection. This would save considerable time and money in that patients with low risk of a cardiac event could be discharged immediately, as opposed to waiting the recommended 3-6 hours in the ED before being discharged. Moreover, cardiac injury and risk for subsequent cardiac events are now being tested in the community population, as well as in other patient populations at risk for cardiac injury (e.g., cancer patients). A laboratory score method would also be helpful in guiding the care and management of these patients.

[0003] Thus, it would be desirable to develop an accurate method, such as a method that yields a laboratory score for use in the risk stratification of patients with possible cardiac injury.

SUMMARY OF THE INVENTION

[0004] The present invention provides methods useful to determine if a mammal is at a low or high risk for an adverse cardiac event and/or death when they present with a possible cardiac injury. Briefly, methods are provided in which various parameters can be measured in a biological sample from the mammal, e.g. blood, plasma or serum sample, to assess the risk of an adverse cardiac event.

[0005] In one aspect, a method of determining the risk of an adverse cardiae event in a mammal is provided comprising the steps of:

i) determining in a biological sample obtained from the mammal the concentration of a glucose metabolism biomarker, and allotting a score of 1 when the concentration is greater than a normal level of the glucose metabolism marker;

ii) determining in the biological sample the level of a heart function biomarker, and allotting a score of 1 when the level is greater than a normal level of the heart function biomarker, or determining in the biological sample the level of a renal function biomarker and allotting a score of 1 when the estimated glomerular filtration rate (eGFR) which is based on the level of the renal function biomarker is less than the normal level of eGFR;

iii) determining in the biological sample the level of at least one biomarker of cardiac injury using a high sensitivity assay, and allotting a score of 1 if the level is greater than the level of an ambulatory population at risk for future cardiovascular events but less than the level at which analytical variation occurs, allotting a score of 2 if the level is greater than the level at which analytical variation occurs and less than the upper limit of normal of a general population, and allotting a score of 3 if the level is greater than the upper limit of normal of the general population;

iv) generating a total laboratory score based on the sum of the scores for each of the biomarkers of i), ii) and iii), wherein a laboratory score of greater than 4 indicates a risk of an adverse cardiac event in the mammal with a likelihood ratio of greater than 1; and

v) administering a treatment of the adverse cardiac event to the diagnosed mammal when there is a laboratory score of greater than 4.

[0006] In another aspect, a method of determining the risk of an adverse cardiac event in a patient is provided comprising the steps of:

i) determining in a biological sample obtained from the mammal the concentration of a glucose metabolism biomarker, and allotting a score of 1 when the concentration is greater than a normal level of the glucose metabolism marker;

ii) determining in the biological sample the level of a heart function biomarker, and allotting a score of 1 when the level is greater than a normal level of the heart function biomarker;

iii) determining in the biological sample the level of a renal function biomarker and allotting a score of 1 when the estimated glomerular filtration rate (eGFR) which is based on the level of the renal function biomarker is less than the normal level of eGFR;

iv) determining in the biological sample the level of at least one biomarker of cardiac injury using a sensitive assay, and allotting a score of 1 if the level is at about the limit of detection of the biomarker, a score of 2 is allotted if the biomarker level is greater than the limit of detection but less than the upper limit of normal of the biomarker level in a general population and a score of 3 is allotted when the biomarker level is greater than the upper limit of normal of the general population;

v) generating a total laboratory score based on the sum of the scores for each of the biomarkers of i), ii) and iii), wherein a laboratory score of greater than 5 indicates a risk of an adverse cardiac event in the mammal with a likelihood ratio of greater than 1; and

vi) administering a treatment of the adverse cardiac event to the diagnosed mammal when there is a laboratory score of greater than 5.

[0007] In another aspect, a method of determining the risk of an adverse cardiovascular event or death in a mammal is provided comprising the steps of:

i) determining in a biological sample obtained from the mammal the concentration of a glucose metabolism biomarker, and allotting a score of 1 when the concentration is greater than a normal level of the glucose metabolism marker;

ii) determining in the biological sample the level of a renal function biomarker and allotting a score of 1 when the estimated glomerular filtration rate (eGFR) which is based on the level of the renal function biomarker is less than the normal level of eGFR;

iii) determining in the biological sample the level of at least two biomarkers of cardiac injury using a sensitivity assay, and allotting a score of 1 if the level is at about the limit of detection of the biomarker, a score of 2 is allotted if the biomarker level is greater than the limit of detection but less than the upper limit of normal of the biomarker level in a general population and a score of 3 is allotted when the biomarker level is greater than the upper limit of normal of the general population;

iv) generating a total laboratory score based on the sum of the scores for each of the biomarkers of i), ii) and iii), wherein a laboratory score of greater than 7 indicates a risk of an adverse cardiac event in the mammal with a likelihood ratio of greater than 1; and

v) administering a treatment of the adverse cardiac event to the diagnosed mammal when there is a laboratory score of greater than 7.

[0008] In a further aspect, a kit for use in a method of determining the risk of an adverse cardiovascular event or death in a mammal is provided comprising a biomarker-specific reactant for one or more biomarkers selected from the group consisting of a glucose metabolism biomarker, a heart function biomarker, a renal function biomarker, and a cardiac injury biomarker, wherein the reactant is suitable for use to determine the level of the biomarker in a biological sample from the mammal, and guidelines indicating a score to be allotted based on the level of each target biomarker and indicating the relationship between a total score and risk of an adverse cardiovascular event.

[0009] These and other aspects of the invention will become apparent in the detailed description that follows by reference to non-limiting examples.

DETAILED DESCRIPTION OF THE INVENTION

[0010] A method for determining the risk of an acute or adverse cardiovascular event or death in a mammal based on the determination of the concentration or level of a combination of parameters, including parameters of cardiac injury such as cardiac troponin I and T, metabolism, e.g. glucose and related metabolites, renal function and heart function or hematology is provided. A laboratory score is allotted to each parameter based on the level of each of the parameters. The combined laboratory score is effective to determine if a patient is at risk of an acute or adverse cardiovascular event (e.g. myocardial infarction, cardiac ischemia, heart failure, percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG)), or death, including identifying that a patient is at low risk of a cardiovascular event or death and can be ruled-out for further treatment, and/or identifying a patient at high risk and ruled-in for further medical treatment and management.

[0011] The first step of the method is to obtain a biological sample from a mammal to assess risk for myocardial injury, e.g. mammals with symptoms of chest pain would be a reasonable group to test. The term "biological sample" is meant to encompass any mammalian sample that may contain glucose, RDW, creatinine, cardiac troponin, e.g. troponin T and troponin I, and related proteins. Suitable biological samples include, for example, blood, serum, plasma, urine and cerebrospinal fluid. The sample is obtained from the mammal in a manner well-established in the art.

[0012] The term "mammal" is used herein to refer to both human and non-human mammals.

[0013] Once a suitable biological sample is obtained, it is analyzed for the level or concentration of each of the selected parameters or biomarkers, including a combination of biomarkers selected from a metabolic biomarker, e.g. glucose, or a biomarker that reflects glucose metabolism such as glycated hemoglobin A1c (HbA1c); a biomarker that provides prognostic information related to heart function such as red cell distribution width (RDW), natriuretic peptides such as brain natriuretic peptide (BNP) and amino-terminal pro-brain natriuretic peptide (NT-proBNP); a biomarker associated with renal function such as creatinine or cystatin c; and at least one biomarker indicative of cardiac injury, such as cardiac troponin,

e.g. troponin T and/or troponin I, or other heart specific proteins such as heart-specific fatty acid binding protein. An amount of <1 mL of biological sample is generally used to conduct the determination of these biomarkers.

[0014] Methods used to determine the level or concentration of the biomarkers will exhibit an appropriate specificity for detection of the selected biomarker. Detection methods may vary from biomarker to biomarker, and may include photometric, electrochemical, enzymatic, or immunogenic methods of determination using an antibody directed to the target biomarker.

[0015] The determination of the level of a selected metabolic biomarker such as glucose may be conducted photometrically, for example, using the hexokinase method, or electrochemically using glucose-oxidase-based methods. Glycated hemoglobin levels may also be determined chromatographically, photometrically, e.g. based on peroxidase activity of a hemoglobin/haptoglobin complex formed from the glycated hemoglobin, and by immunoassay, including ELISA and chemiluminescent immunoassay (CLIA).

[0016] The determination of creatinine levels may also be determined by absorbance/photometrically (e.g. using the Jaffe method for creatinine) or enzymatic (i.e., creatininase methods for creatinine measurement). Cystatin c levels may be determined photometrically, chromatographically, or by immunoassay, e.g. using a particle enhanced nephelometric immunoassay or latex enhanced immunoturbidimetric method.

[0017] Red cell distribution width is a measure of the range of variation of red blood cell volume that is reported as part of a standard complete blood count. RDW is calculated using the formula: RDW (%) = (Standard deviation of mean corpuscular volume (MCV) ÷ mean MCV) × 100. RDW may be determined using an automated hematology analyzer. Elevated RDW is indicative of heart failure. RDW may be substituted with natriuretic peptides which may be detected by immunoassay, for example, including ELISA and chemiluminescent immunoassay.

[0018] Cardiac troponin levels, including troponin T and/or I, may be determined using immunoassay methodology (i.e., based on ELISA principles of sandwich or competitive immunoassays). These assays may be sensitive cardiac troponin (cTn) assays or highly sensitive

cardiac troponin (hs-cTn) assays. Hs-cTn assays are herein defined as assays able to detect cTn in the single digit range of nanograms per litre, e.g., measurable concentrations less than 10 ng/L, with a coefficient of variation (CV) of <10% of the 99th percentile of cTn concentration in reference subjects (the recommended upper reference limit [URL]), and measurable concentrations of cardiac troponin in greater than 50% of a general population. Sensitive-cTn assays exhibit a CV of <20% at the 99th percentile URL, and measureable concentrations of cardiac troponin in a general population of less than 50%. Heart-specific fatty acid binding protein may also be detected using immunoassay.

[0019] The term "antibody" is used herein to refer to monoclonal or polyclonal antibodies, or antigen-binding fragments thereof, e.g. an antibody fragment that retains specific binding affinity for the target biomarker. Antibodies to target biomarkers are generally commercially available, for example, from Abnova, Origene, Novus Biologicals and Lifespan BioSciences, Inc. As one of skill in the art will appreciate, antibodies to the target biomarkers may also be raised using techniques conventional in the art. For example, antibodies may be made by injecting a host animal, e.g. a mouse or rabbit, with the antigen (target biomarker), and then isolating antibody from a biological sample taken from the host animal.

[0020] Different types of immunoassay may be used to determine expression level of target biomarkers, including indirect immunoassay in which the biomarker is non-specifically immobilized on a surface; sandwich immunoassay in which the biomarker is specifically immobilized on a surface by linkage to a capture antibody bound to the surface; competitive binding immunoassay in which a sample is first combined with a known quantity of biomarker antibody to bind biomarker in the sample, and then the sample is exposed to immobilized biomarker which competes with the sample to bind any unbound antibody. To the immobilized biomarker/antibody is added a detectably-labeled secondary antibody that detects the amount of immobilized primary antibody, thereby revealing the inverse of the amount of biomarker in the sample.

[0021] A preferred immunoassay for use to determine expression levels of protein biomarkers is an ELISA (Enzyme Linked ImmunoSorbent Assay) or Enzyme ImmunoAssay (EIA). To determine the level or concentration of the biomarker using ELISA, the biomarker to

be analyzed is generally immobilized, for example, on a solid adherent support, such as a microtiter plate, polystyrene beads, nitrocellulose, cellulose acetate, glass fibers and other suitable porous polymers, which is pretreated with an appropriate ligand for the target biomarker, and then complexed with a specific reactant or ligand such as an antibody which is itself linked (either before or following formation of the complex) to an indicator, such as an enzyme. Detection may then be accomplished by incubating this enzyme-complex with a substrate for the enzyme that yields a detectable product. The indicator may be linked directly to the reactant (e.g. antibody) or may be linked via another entity, such as a secondary antibody that recognizes the first or primary antibody. Alternatively, the linker may be a protein such as streptavidin if the primary antibody is biotin-labeled. Examples of suitable enzymes for use as an indicator include, but are not limited to, horseradish peroxidase (HRP), alkaline phosphatase (AP), ß-galactosidase, acetylcholincsterase and catalase. A large selection of substrates is available for performing the ELISA with these indicator enzymes. As one of skill in the art will appreciate, the substrate will vary with the enzyme utilized. Useful substrates also depend on the level of detection required and the detection instrumentation used, e.g. spectrophotometer, fluorometer or luminometer. Substrates for HRP include 3,3',5,5'-Tetramethylbenzidine (TMB), 3,3'-Diaminobenzidine (DAB) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). Substrates for AP include para-Nitrophenylphosphates. Substrates for ß-galactosidase include β-galactosides; the substrate for acetylcholinesterase is acetylcholine, and the substrate for catalase is hydrogen peroxide.

[0022] Following biomarker determinations, a laboratory score is allotted to each biomarker based on the concentration or level of that biomarker. For the metabolic biomarker, a determination of a concentration that is greater than a normal level, i.e. greater than the upper limit of the biomarker in a healthy individual, e.g. a non-diabetic individual, yields a score of 1. A level below this concentration yields a score of 0. For glucose, a concentration which is greater than the guideline recommended cutoff for glucose (e.g. >5.5 mmol/L for glucose as per the American Diabetes Association (ADA) guidelines), yields a score of 1. If HbA1c level is determined instead of glucose, a level of HbA1c of ≥ 6.5% from total hemoglobin in the system (e.g. the cutoff used by the ADA guidelines to diagnose diabetes using NGSP, or ≥ 48 mmol/mol using IFCC (International Federation of Clinical Chemistry) units) yields a score of 1. If a

patient is known to be diabetic, then the metabolic biomarker determination may not be required and a score of 1 may be assigned.

[0023] For the biomarker related to heart function, a determination of the % of RDW which is greater than the prognostic RDW clinical cutoff (e.g. >13.3%) yields a score of 1. Alternatively, if natriuretic peptides are detected in place of RDW, then a determination of a level that is greater than the upper limit of the biomarker in a healthy individual (e.g. upper limit of normal) yields a score of 1 (i.e. BNP > 35 pg/ml or NT-proBNP >125 pg/mL yield a score of 1). When % RDW is less than the cutoff, or natriuretic peptides levels are lower than the upper limit of normal, a score of 0 is allotted. If a patient has undergone a recent blood transfusion or has a history of heart failure, then determination of the heart function biomarker may not be required, and a score of 1 may be assigned.

[0024] For the biomarker related to renal (kidney) function, a determination of estimated glomerular filtration rate (eGFR) of less than the accepted normal value, e.g. < 90 milliliters per minute per 1.73 m^2 (as determined by measuring creatinine or cystatin c, and calculated by CKD-EPI equation as described in *Greenslade et al. Ann Emerg Med. 2013;62:38-46*) yields a score of 1. Otherwise, a score of 0 is allotted.

[0025] With respect to determination of a biomarker associated with cardiac injury, when cardiac troponin concentration is determined using a high-sensitivity assay (hs-cTn), then scores are allotted as follows:

a) if the hs-cTn concentration (first concentration) is greater than the cutoff of an ambulatory population at risk for future cardiovascular events, e.g. patients with established cardiovascular disease (secondary prevention) and patients at high risk of cardiovascular disease who have not yet established the disease (primary prevention), e.g. who have current symptoms of heart disease (such as have suffered a heart attack, suffer angina, or have received coronary revascularisation), those with symptoms of other arterial disease (such as stroke, transient ischaemic attack, or peripheral vascular disease), or the elderly, but less than the concentration where analytical variation has been reported (e.g. about 10 ng/L, thus, hs-cTn concentration range = cutoff to cutoff +10 ng/L), then a score of 1 is assigned. The cut-off may vary somewhat based on the high sensitivity cTn assay used, but will generally be less than 10 ng/L. Thus, in

one embodiment, a score of 1 applies when the cTn concentration is less than about 20 ng/L. In other embodiments, for example, using a hs-cTnI assay of Abbott Laboratories, a concentration between 4 ng/L to 14 ng/L yields a score of 1, for a hs-cTnI assay of Beckman Coulter, Inc., a concentration between 6 ng/L to 16 ng/L yields a score of 1, and for Roche Molecular Diagnostics' hs-cTnT assay, a concentration between 8 ng/L to 18 ng/L yields a score of 1;

b) if the hs-cTn concentration is greater than the first concentration in (a) but below the upper limit of normal (ULN) of a general population (e.g. the general population refers to all individuals without reference to any specific characteristic, comprising individuals of various ages and wellness), then a score of 2 is assigned. The cut-off may vary somewhat based on the high sensitivity cTn assay used. For example, using a hs-cTnI assay of Abbott Laboratories, a concentration between 15 ng/L to 30 ng/L yields a score of 2, for a hs-cTnI assay of Beckman Coulter, Inc., a concentration between 17 ng/L to 40 ng/L yields a score of 2, and for Roche Molecular Diagnostics' hs-cTnT assay, a concentration between 19 ng/L to 30 ng/L yields a score of 2;

c) if the hs-cTn concentration is greater than the upper limit of normal of a general population, then a score of 3 is assigned. The cut-off may vary somewhat based on the high sensitivity cTn assay used. For example, using a hs-cTnI assay of Abbott Laboratories, a concentration of greater than 30 ng/L yields a score of 3, for a hs-cTnI assay of Beckman Coulter, Inc., a concentration of greater than 40 ng/L yields a score of 3, and for Roche Molecular Diagnostics' hs-cTnT assay, a concentration of greater than 30 ng/L yields a score of 3.

[0026] When cardiac troponin concentration is determined using a sensitive assay (s-cTn), then scores are allotted as follows. For cardiac troponin I (cTnI), determination of a concentration of less than 0.01 ug/L yields a score of 0; determination of a concentration of 0.01 ug/L yields a score of 1; determination of a concentration of 0.02-0.03 ug/L yields a score of 2, and determination of a concentration of greater than 0.03 ug/L yields a score of 3. For cardiac troponin T (cTnT), determination of a concentration of less than 20 ng/L yields a score of 0; determination of a concentration of 20-30 ng/L yields a score of 1; determination of a

concentration of 31-50 ng/L yields a score of 2, and determination of a concentration of greater than 50 ng/L yields a score of 3.

[0027] When heart-specific fatty acid binding protein (hFABP) is determined, scores are allotted as follows. If hFABP concentration is a first concentration greater than the cutoff that defines an ambulatory population at risk for future cardiovascular events, but less than the concentration where analytical variation has been reported, then a score of 1 is assigned. Levels that are less than this yield a score of 0. If hFABP concentration is greater than the first concentration but below the upper limit of normal (ULN) of a general population, then a score of 2 is assigned. If the hFABP concentration is greater than the upper limit of normal of the general population, then a score of 3 is assigned.

[0028] Thus, in one embodiment, the method of determining risk of an adverse cardiac event comprises the determination of the level of a metabolic marker (e.g. glucose/glycated hemoglobin), a biomarker indicative of heart function (e.g. RDW or natriuretie peptides) and a biomarker indicative of cardiac injury using a high sensitivity assay, e.g. hs-cTn. The score for each marker based on the levels of each is combined to yield a cumulative overall laboratory score. The maximum score is 5, and the minimum score is 0.

[0029] In another embodiment, the method of determining risk of an adverse cardiac event comprises the determination of the level of a metabolic marker (e.g. glucose/glycated hemoglobin), a biomarker indicative of renal failure (e.g. creatinine or cystatin c) and a biomarker indicative of cardiac injury using a high sensitivity assay, e.g. hs-cTn. The score for each marker based on the levels of each is combined to yield a cumulative overall laboratory score. The maximum score is 5, and the minimum score is 0.

[0030] In another embodiment, the method of determining risk of an adverse cardiac event comprises the determination of the level of a metabolic marker (e.g. glucose/glycated hemoglobin), a biomarker indicative of heart funetion (e.g. RDW or natriuretic peptides), a biomarker indicative of renal failure (e.g. creatinine or cystatin c) and a biomarker indicative of cardiac injury using a high sensitivity assay, e.g. hs-cTn. The score for each marker based on the levels of each is combined to yield a cumulative overall laboratory score. The maximum score is 6, and the minimum score is 0.

[0031] In another embodiment, the method of determining risk of an adverse cardiac event comprises the determination of the level of a metabolic marker (e.g. glucose/glycated hemoglobin), a biomarker indicative of heart function (e.g. RDW or natriuretic peptides), a biomarker indicative of renal failure (e.g. creatinine or cystatin c) and a biomarker indicative of cardiac injury using a sensitive assay, e.g. cTnI or cTnT. The score for each marker based on the levels of each is combined to yield a cumulative overall laboratory score. The maximum score is 6, and the minimum score is 0.

[0032] In another embodiment, the method of determining risk of an adverse cardiac event comprises the determination of the level of a metabolic marker (e.g. glucose/glycated hemoglobin), a biomarker indicative of renal failure (e.g. creatinine or cystatin c) and biomarkers indicative of cardiac injury using a sensitive assay, e.g. cTnI and cTnT. The score for each marker based on the levels of each is combined to yield a cumulative overall laboratory score. The maximum score is 8, and the minimum score is 0.

[0033] For laboratory scores close to 0, e.g. less than 2, less than 1 or 0, a patient is confirmed to be at low risk of an adverse cardiac event. For laboratory scores of greater than or equal to 4 wherein the maximum score is 5, or greater than or equal to 5 where the maximum score is 6, or greater than or equal to 7 wherein the maximum score is 8, the patient is confirmed to be at high risk of an adverse cardiac event, or death. The scores are indicative of the Liklihood Ratio (LR), i.e. the likelihood that a given test result would be expected in a patient that will experience an adverse cardiac event or death compared to the likelihood that that same result would be expected in a patient that will not experience an adverse cardiac event or death. Positive LR, i.e. the LR for positive results (a patient will experience an adverse cardiac event or death) is determined using the formula: sensitivity/1-specificity (of the method). Negative LR, i.e. the LR for negative results (a patient will not experience an adverse cardiac event or death) is determined using the formula: 1-sensitivity/specificity (of the method). Generally, a positive likelihood (LR+) ratio of greater than 1, preferably greater than 5, or greater than 10, indicates the test result is associated with an adverse cardiac event/death. A negative likelihood (LR-) ratio of less than 1, preferably less than 0.01, indicates that the result is associated with absence of an adverse cardiac event/death.

[0034] Following determination of a laboratory score for a given patient, the patient may then treated based on the laboratory score. For example, for patients confirmed to be at low risk of an adverse cardiac event or death (laboratory score of less than 2; LR- of less than 1), treatment is not required and the patient may be released from the hospital. For patients confirmed to be at high risk of an adverse cardiac event or death (laboratory score of greater than 4, 5 or ≥7; LR+ of greater than 1), the patient is administered an appropriate treatment, which may include one or more treatments selected from: administration of anticoagulants (e.g. warfarin, heparin, Dabigatran and low dose aspirin (75 mg daily)), thrombolytics (e.g. alteplase, reteplase, streptokinase, tenecteplase), nitroglycerin, antiplatelet drugs (e.g. clopidogrel, prasugrel or ticagrelor), beta blockers (e.g. propranolol, atenolol and bisoprolol), combined alpha and beta-blockers, calcium channel blockers (e.g. Amlodipine, Bepridil, Diltiazem, Felodipine, Nicardipine, Nifedipine, Nisoldipine, Verapamil), angiotensin-converting enzyme (ACE) inhibitors (**e.g.** Benazepril, Captopril, Enalapril, Fosinopril, Lisinopril, Moexipril, Perindopril, Quinapril, Ramipril, Trandolapril), angiotensin-receptor blockers (e.g. Candesartan, Eprosartan, Irbesartan, Losartan, Olmesartan, Telmisartan, Valsartan), angiotensin-receptor neprilysin inhibitors, cholesterol-lowering medications such as statins, vasodilators (e.g. hydralazine, hydralazine and isosorbide dinitrate), and/or diuretics (e.g. furosemide, bumetanide, torsemide, hydrochlorothiazide, metolazone, spironolactone); angioplasty and stenting; and coronary bypass surgery.

[0035] In another aspect of the invention, an article of manufacture or kit is provided that is useful to conduct the present method(s). The kit comprises one or more biomarker-specific reactant/reagents suitable for use to determine the level of biomarkers of the selected method in a biological sample from a mammal. For glucose, determination may be conducted using the hexokinase photometric method. Reagents may include: hexokinase, Glucose-6-phosphate dehydrogenase, adenosine triphosphate, nicotinamide adenine dinucleotide and/or (NAD). Creatinine determination may be conducted using the Jaffe method with the reagent, alkaline picrate, or using enzymatic reactions with enzyme reagents such as creatininase, creatine amidinohydrolase (creatinase) and sarcosine oxidase. For determination of glycated hemoglobin, as well as natriuretic peptides such as BNP and NT-proBNP, cystatin c, cardiac troponin I, cardiac troponin T and hFABP as biomarkers, determination may be conducted by immunoassay, utilizing antibodies (monoclonal or polyclonal) directed to the target biomarker(s). The kit may

also include detectable markers which may be linked to the antibody or other component of the assay. Detectable markers may include radioactive, fluorescent, phosphorescent and luminescent (e.g. chemiluminescent or bioluminescent) compounds, dyes (e.g. leuco dyes), particles such as colloidal gold and enzyme indicators or labels. Additional reagents for inclusion in the kit may include distilled water, buffers, substrate for enzyme indicators and linkers as herein described.

[0036] The kit may additionally include instructions for conducting the method, as described herein, as well as instructions with respect to laboratory scoring based on the determined levels of biomarkers within a given sample. Further, the kit may include materials for conducting assays to determine levels of the biomarkers, such as test-tubes, cuvettes, other sampling containers and the like.

[0037] Embodiments of the invention are described by reference to the following specific examples, which is not to be construed as limiting.

EXAMPLE 1

[0038] For a period of 5 weeks within Hamilton Health Sciences, ED nurses collected blood samples from patients (n=100, convenient sampling) being evaluated for acute coronary syndrome (ACS). Nurses collected blood at ED presentation: one blood sample for clinical measurements for cardiac troponin T (cTnT), complete blood count (CBC; which includes RDW) and glucose and another blood sample (serum separator tube - SST; gold top) for measurement of other cardiac biomarkers. A serum SST tube was used, as this is the preferred tube for many biomarkers; and allowed the serum specimens to be processed by the medical laboratory assistants without compromising the integrity of the sample (this type of specimen is preferred if testing is delayed). Following processing by the medical laboratory assistants, the separated serum specimens were transported to the Clinical Research and Clinical Trials Laboratory (CRCTL) at the Hamilton General Hospital for storage (below -80°C).

[0039] An aliquot from the ED presentation sample (n=100) was thawed (8 months after collection) and measured using the following immunoassays (analyte/platform/company): 1) high-sensitivity cardiac troponin I (hs-cTnI/ Access II instrument/Beckman Coulter) and 2) high-

sensitivity cardiac troponin T (hs-cTnT/ Elecsys 2010 instrument/Roche Diagnostics). The laboratory score (L5HF algorithm) was determined for hs-cTnI and hs-cTnT separately.

[0040] Briefly, the laboratory score was calculated as follows:

1. If the concentration of glucose was > 5.5 mmol/L, then a score of 1 was assigned;

2. If red cell distribution width (RDW) was > 13.3%, then a score of 1 was assigned; and

3. If a high-sensitivity cardiac troponin (hs-cTn) concentration was:

a) greater than (>) the cutoff that defines an ambulatory population at risk for future cardiovascular events but less than the concentration where analytical variation has been reported (e.g., hs-cTn concentration range = cutoff to cutoff +10 ng/L), then a score of 1 was assigned, (e.g. for Beckman measurements of hs-cTnI, a concentration between 6 ng/L to 16 ng/L; for Roche measurements of hs-cTnT, a concentration between 8 ng/L to 18 ng/L).

b) greater than (>) concentration (a) but below the upper limit of normal of a reference population then a score of 2 was assigned (e.g. for Beckman measurements, an hs-cTnI concentration between 17 ng/L to 40 ng/L; for Roche measurements, an hs-cTnT concentration between 19 ng/L to 30 ng/L);

c) greater than (>) the upper limit of normal of a reference population then a score of 3 was assigned (e.g. for Beckman measurements, an hs-cTnI concentration > 40 ng/L; for Roche measurements, an hs-cTnT concentration > 30 ng/L).

[0041] Biomarker levels not within the ranges indicated above were given a score of zero. The sum of the scores from 1 to 3 above was obtained to determine the overall score (maximum score is 5, minimum score is 0) for hs-cTnI and hs-cTnT, separately.

[0042] Each patient was contacted up to 72 hours after their ED presentation to determine if a serious cardiac outcome, e.g. PCI, CABG, refractory ischemic symptoms, heart failure, myocardial infarction, stroke, cardiac arrest, or death, occurred within this timeframe. These outcomes were adjudicated by an emergency physician blinded to the exploratory biomarkers tested in the study population.

RESULTS

[0043] There were 14 individuals from the 100 patients that experienced a serious cardiac outcome (+ Feature) within the first 72 hours. A laboratory score of 0 in the presentation sample determined either with the hs-cTnI or hs-cTnT assay yielded a likelihood ratio (LR) of 0 for an acute cardiovascular event (i.e. patients could be ruled-out). A laboratory score of 5 yielded the highest LR for a serious cardiac outcome within the first 72 hours (see TABLE 1 for laboratory score using hs-cTnI and TABLE 2 for laboratory score using hs-cTnT).

TABLE 1 - Laboratory score determination using hs-cTnI assay (Beckman)

Lab Score	+ Feature	- Feature	Likelihood Ratio	95% CI (Koopman)
0	0	5	0	0 to 4.077487
1	0	19	0	0 to 1.005021
2	4	20	1.228571	0.471874 to 2.696732
3	1	31	0.198157	0.034793 to 0.906138
4	5	9	3.412698	1.30257 to 8.063795
5	4	2	12.285714	2.775149 to 52.796373

TABLE 2 - Laboratory Score using hs-cTnT assay (Roche)

Lab Score	+ Feature	- Feature	Likelihood Ratio	95% CI (Koopman)
0	0	6	0	0 to 3.361013
1	2	22	0.558442	0.150915 to 1.696226
2	1	18	0.34127	0.058975 to 1.61906
3	3	21	0.877551	0.294353 to 2.175058
4	3	10	1.842857	0.576192 to 5.132779
5	5	9	3.412698	1.30257 to 8.063795

EXAMPLE 2

[0044] Briefly, this was an all-comer ED population that consisted of all consecutive ED patients from two EDs over a period of 3 months who had glucose, RDW, and Abbott Laboratories hs-cTnI measurements (i2000 analyzer) available at presentation (n=4313). The outcome for this prospective observational study was hospital death.

[0045] In this Example, the laboratory score (L5HF algorithm) was determined in the same manner as in Example 1. Cardiac troponin was measured using an immunoassay by Abbott Laboratories. Briefly, the laboratory score was calculated as follows:

1. If glucose was > 5.5 mmol/L then a score of 1 was assigned

2. If red cell distribution width (RDW) > 13.3% then a score of 1 was assigned

3. If a high-sensitivity cardiac troponin (hs-cTn) concentration was:

a) greater than (>) the cutoff that defines an ambulatory population at risk for future cardiovascular events but less than the concentration where analytical variation has been reported (e.g. hs-cTn concentration range = cutoff to cutoff +10 ng/L), then a score of 1 was assigned (e.g. for Abbott hs-cTnI measurements, a concentration between 4 ng/L to 14 ng/L);

b) greater than (>) the concentration of (a) but below the upper limit of normal of a reference population, then a score of 2 was assigned (e.g. for Abbott hs-cTnI measurements, a concentration between 15 ng/L to 30 ng/L); or

c) greater than (>) the upper limit of normal of a reference population, then a score of 3 was assigned (e.g. for Abbott hs-cTnI measurements, a concentration of > 30 ng/L).

[0046] The sum of the scores from 1 to 3 above was obtained to determine the overall score (maximum score is 5, minimum score is 0).

[0047] Also, as previously done in this population, the following was performed to assess LR for hospital death as described in *Shorrt et al. Clin Biochem 2015;48:282-7.* Specifically, a dual panel testing was conducted in which Abbott hs-cTnI measurement <4 ng/L and glucose <5.6 mmol/L was a negative panel result, and either hs-cTnI or glucose concentration above these cutoffs (hs-cTnI >=4 or glucose >=5.6) yielded a positive result. A limit of detection (LoD) analysis was also conducted where the LoD was defined as <1 ng/L for a negative result, with >=1 ng/L defined as a positive result.

RESULTS

[0048] There were 214 hospital deaths from the 4313 patients. The laboratory score of 0 in the presentation sample yielded a LR of 0 (no patients died with a score of 0) (see Table 3A), whereas both the dual testing (see Table 4) and LoD test (see Table 5) missed a patient. Combining laboratory scores of 0 & 1 produced a LR of 0.019 (95%CI:0.003-0.108), lower than that of either the Dual Testing or LoD (see Table 3B). While combining laboratory scores of 4&5 yielded a significantly higher LR of 2.7 (95%CI:2.4-3.0) as compared to dual testing (LR=1.12; 95%CI:1.10-1.14) or LoD testing (LR=1.05; 95%CI:1.03-1.06) (see Table 3B).

TABLE 3 - Laboratory Score and Liklihood Ratio using Abbott hs-cTnI assay

A) Likelihood Ratios for each laboratory score

Lab Score	+ Feature	- Feature	Likelihood Ratio	95% CI (Koopman)
0	0	248	0	0 to 0.291536
1	1	735	0.02606	0.004599 to 0.145069
2	26	1128	0.441498	0.305255 to 0.627535
3	47	984	0.914886	0.701709 to 1.17283
4	57	562	1.942686	1.525054 to 2.436129
5	83	442	3.59683	2.95537 to 4.318031

B) Likelihood Ratios for grouped laboratory scores

Lab Score	+ Feature	- Feature	Likelihood Ratio	95% CI (Koopman)
0&1	1	983	0.019485	0.00344 to 0.108441
2&3	73	2112	0.662054	0.544007 to 0.79189
4&5	140	1004	2.670905	2.373192 to 2.965946

TABLE 4 - Likelihood ratios using Abbott Dual Test (Glucose > 5.5 or hsTnI >4 is positive)

Dual Test	+ Feature	- Feature	Likelihood Ratio	95% CI (Koopman)
negative	1	464	0.041281	0.007282 to 0.22994
positive	213	3635	1.122378	1.097257 to 1.136853

TABLE 5 - Likelihood ratios using Abbott hs-cTnI LoD test (hs-cTnI concentration >=LoD is positive

[hs-cTnI]	+ Feature	- Feature	Likelihood Ratio	95% CI (Koopman)
<LoD	1	208	0.092088	0.016213 to 0.51401
>=LoD	213	3891	1.048534	1.025681 to 1.058207

EXAMPLE 3

[0049] Briefly, this study was based on an all-comer population that consisted of all consecutive ED patients from 2 EDs over a period of the first 2 months after clinically reporting hs-cTnI. The study population consisted of ED patients using only the first result of glucose, RDW, and Abbott Laboratories hs-cTnI measurements (i2000 analyzer) who also had an ED discharge home or hospital admission recorded (n=4444). The primary outcome for this prospective observational study was whether patients were eventually discharged from the ED or admitted as well as the length of stay in the ED with respect to different laboratory scores.

[0050] The laboratory score (L5HF algorithm) was determined in the same manner as in Example 2.

RESULTS

[0051] There were 25 ED deaths in the population with all 25 having a laboratory score of 1 or higher (average laboratory score of 4). Those patients that were discharged home with a laboratory score of 0 in the earliest sample had a significantly shorter ED length of stay (median = 5.6 hours) as compared to those with a score of 1 (median = 6.0 hours); score of 2 (median = 6.2 hours); score of 3 (median = 6.8 hours); score of 4 (median = 7.5 hours); or score of 5 (median = 9.1 hours) ($p<0.05$, by Kruskal-Wallis: all pairwise comparisons by Conover-Inman).

[0052] Therefore, the lower the laboratory score, the higher the likelihood that patients would be discharged home (e.g., a laboratory score of 0 yielded a 95% upper confidence limit exceeding 10) with a laboratory score of 5 indicating a group of high risk patients that might not be discharged home (e.g., a laboratory score of 5 yielded a 95% lower confidence limit of 0.10) (see TABLE 6).

TABLE 6 - Laboratory Score using Abbott hs-cTnI test clinically assessing the Likelihood ratios in ED patients that were discharged home or admitted to hospital

Lab Score	Home	Admit	Likelihood Ratio	95% CI (Koopman)
0	230	30	7.5189	5.1785 to 10.931964
1	634	185	3.360975	2.886821 to 3.917328
2	712	466	1.498449	1.355143 to 1.65773
3	465	618	0.737925	0.664373 to 0.819366
4	131	452	0.284237	0.2361 to 0.341836
5	59	437	0.132409	0.101564 to 0.172388

EXAMPLE 4

[0053] In this study adults presenting to the ED with symptoms of and investigated for acute coronary syndrome (ACS) were enrolled. Patients were excluded if they met any of the following exclusion criteria prior to cTnI testing: death, ST-elevation myocardial infarction (STEMI) and serious ventricular cardiac dysrhythmia or they had experienced one of the following conditions within the last month: cardiac surgery/manipulation, STEMI or NSTEMI (non-ST-elevation myocardial infarction); diagnosis of pulmonary embolus; malignancy; sepsis; or who were previously enrolled or transferred from another primary care facility. Patients were included in the analysis if they had presentation cTnI, glucose, hs-cTnI, hs-cTnT, eGFR and RDW values. The primary outcome was MI or all cause death at 30 days after presentation.

[0054] The laboratory score (L5HF algorithm) was determined in the same manner as in Examples 1 and 2.

[0055] The L5HF algorithm score consists of the following 3 tests (1 cardiac injury, 1 metabolic, and 1 heart function/hematology test used in patients with heart conditions) with the following values assigned to determine the overall score:

1. If glucose concentration (measurement via Hexokinase method or another method with acceptable agreement to this method) is >5.5 mmol/L, then assign a score of 1;

2. If red cell distribution width (RDW) (reported as RDW-CV = 1SD/MCV X100 to yield a %, calculated from Beckman Coulter LH750 or another method with acceptable agreement) is > 13.3% then assign a score of 1;

3. If a high-sensitivity cardiac troponin (hs-cTn) concentration is:

a) Greater than (>) the cutoff that defines an ambulatory population at risk for future cardiovascular event but less than the concentration where analytical variation has been reported (e.g., hs-Tn concentration range = cutoff to cutoff +10 ng/L), then assign a score of 1 (e.g., for Abbott hs-cTnI measurements, concentration was between 4 ng/L to 14 ng/L; for Roche hs-cTnT measurements, concentration was between 8 ng/L to 18 ng/L);

b) Greater than (>) concentration (a) but below the upper limit of normal of a reference population then assign a score of 2 (e.g., for Abbott hs-cTnI measurments, concentration was between 15 ng/L to 30 ng/L; for Roche hs-cTnT measurements, concentration was between 19 ng/L to 30 ng/L); or

c) Is greater than the upper limit of normal of a reference population than assign a score of 3 (e.g., for Abbott hs-cTnI measurement, concentration was > 30 ng/L; and for Roche hs-cTnT measurements, concentration was > 30 ng/L).

[0056] Sum scores from 1 to 3 above to determine the overall score (maximum score is 5, minimum score is 0). Laboratory scores closer to 0 (e.g. less than 2) represent low risk patients that may be ruled out for a cardiac event, whereas laboratory scores closer to 5 (e.g. 4 or greater) represent high risk patients and would be ruled-in for further medical treatment and management.

[0057] The laboratory score was also was also determined using the following 3 tests (1 cardiac injury, 1 metabolic, and 1 renal test (e.g. the L5HR algorithm) as follows:

1. If glucose concentration (measurement via Hexokinase method or another method with acceptable agreement to this method) is >5.5 mmol/L then assign a score of 1

2. If estimated glomerular filtration rate (eGFR) (obtained by measuring creatinine and used in an equation such as CKD-EPI [chronic kidney disease epidemiologic collaboration equation] to derive the eGFR) is <90 milliliters per minute per 1.73 m^2 then assign a score of 1

3. If a high-sensitivity cardiac troponin (hs-cTn) concentration is:

a) Greater than (>) the cutoff that defines an ambulatory population at risk for future cardiovascular event but less than the concentration where analytical variation has been reported (e.g., hs-Tn concentration range = cutoff to cutoff +10 ng/L), then assign a score of 1 (e.g., for Abbott hs-cTnI measurements, concentration was between 4 ng/L to 14 ng/L; for Roche hs-cTnT measurements, concentration was between 8 ng/L to 18 ng/L);

b) Greater than (>) concentration (a) but below the upper limit of normal of a general reference population then assign a score of 2 (e.g., for Abbott hs-cTnI measurments,

concentration was between 15 ng/L to 30 ng/L; for Roche hs-cTnT measurements, concentration was between 19 ng/L to 30 ng/L); or

c) Is greater than the upper limit of normal of a general reference population than assign a score of 3 (e.g., for Abbott hs-cTnI measurement, concentration was > 30 ng/L; and for Roche hs-cTnT measurements, concentration was > 30 ng/L).

[0058] Sum scores from each test to determine the overall laboratory score (maximum score is 5, minimum score is 0). Laboratory scores closer to 0 (e.g. less than 2) represent low risk patients that may be ruled out for a cardiac event whereas laboratory scores closer to 5 (e.g. 4 or greater) represent high risk patients and would be ruled-in for further medical treatment and management.

[0059] The laboratory score was also determined using the following tests, e.g. determination of cardiac injury, 1 metabolic, 1 renal test and 1 heart function/hematology (e.g. the L6HHR algorithm) as follows:

1. If glucose concentration (measurement via Hexokinase method or another method with acceptable agreement to this method) is >5.5 mmol/L then assign a score of 1;

2. If red cell distribution width (RDW) (reported as RDW-CV = 1SD/MCV X100 to yield a %, calculated from Beckman Coulter LH750 or another method with acceptable agreement) is > 13.3% then assign a score of 1;

3. If estimated glomerular filtration rate (eGFR) (obtained by measuring creatinine and used in an equation such as CKD-EPI [chronic kidney disease epidemiologic collaboration equation] to derive the eGFR) is <90 milliliters per minute per 1.73 m^2 then assign a score of 1

4. If a high-sensitivity cardiac troponin (hs-cTn) concentration is as described above, then assign a score of 1, 2 or 3.

[0060] Sum scores from 1 to 4 above to determine the overall score (maximum score is 6, minimum score is 0). Laboratory scores closer to 0, e.g. less than 2, represent low risk patients that may be ruled out for a cardiac event, whereas laboratory scores closer to 6, e.g. 5 or more, represent high risk patients and would be ruled-in for further medical treatment and management.

[0061] The laboratory score was also determined using the following tests: 1 cardiac injury, 1 metabolic, 1 renal and 1 heart function/hematology test used in patients with heart conditions (L6SHR algorithm) as follows:

1. If glucose concentration (measurement via Hexokinase method or another method with acceptable agreement to this method) is >5.5 mmol/L then assign a score of 1;

2. If red cell distribution width (RDW) (reported as RDW-CV = 1SD/MCV X100 to yield a %, calculated from Beckman Coulter LH750 or another method with acceptable agreement) is > 13.3% then assign a score of 1;

3. If estimated glomerular filtration rate (eGFR) (obtained by measuring creatinine and used in an equation such as CKD-EPI [chronic kidney disease epidemiologic collaboration equation] to derive the eGFR) is <90 milliliters per minute per 1.73 m^2 then assign a score of 1;

4. If a sensitive cardiac troponin I (cTnI) concentration is:

a) Greater than (≥) than the limit of detection (e.g., cTnI is measurable) then assign a score of 1 (e.g., using Abbott cTnI measurements the limit of detection is 0.01 ug/L (as <0.01 ug/L is undetectable and below the limit of detection);

b) Greater than (>) concentration (a) but below the upper limit of normal of a reference population then assign a score of 2 (e.g., using Abbott cTnI measurement, the concentration is between 0.02 ug/L to 0.03 ug/L);

c) Is greater than the upper limit of normal of a reference population than assign a score of 3 (e.g., using Abbott cTnI measurement, the concentration > 0.03 ug/L.

[0062] Sum scores from 1 to 4 above to determine the overall score (maximum score is 6, minimum score is 0). Laboratory scores closer to 0, e.g. less than 2, represent low risk patients that may be ruled out for a cardiac event whereas laboratory scores closer to 6, e.g. 5 or more, represent high risk patients and would be ruled-in for further medical treatment and management.

[0063] The laboratory score was also determined using the following tests: 1 cardiac troponin I, 1 cardiac troponin T, 1 metabolic and 1 renal test (Labscore 8 algorithm) as follows:

1. If glucose concentration (measurement via Hexokinase method or another method with acceptable agreement to this method) is >5.5 mmol/L then assign a score of 1;

2. If estimated glomerular filtration rate (eGFR) (obtained by measuring creatinine and used in an equation such as CKD-EPI [chronic kidney disease epidemiologic collaboration equation] to derive the eGFR) is <90 milliliters per minute per 1.73 m^2 then assign a score of 1;

3. If cardiac troponin I concentration using a sensitive assay is as described above, then assign a score of 1, 2 or 3, as applicable.

4. If the concentration of cardiac troponin T using a sensitive assay is greater than or equal to 20 to 30 ng/L, assign a score of 1; if cardiac troponin T concentration is greater than 20 to 30 ng/L and below the upper limit of normal of a reference population, assign a score of 2; and if the cardiac troponin T concentration is greater than the upper limit of a reference population, assign a score of 3.

[0064] Sum scores from 1 to 4 above to determine the overall score (maximum score is 8, minimum score is 0). Laboratory scores closer to 0, e.g. 1 or less, represent low risk patients that may be ruled out for a cardiac event whereas laboratory scores closer to 8, e.g. 7 or greater, represent high risk patients and would be ruled-in for further medical treatment and management.

[0065] Thus, for each of the L5HF, L5HR, L6HHR, and L6SHR algorithms, a score <2 indicate a patient is at low risk that may be ruled out for a cardiac event, while a score >4 for L5HF and L5HR algorithms, or >5 for L6HHR and L6SHR algorithms, indicate a patient at high risk that would be ruled-in for further medical treatment and management. For the Labscore 8 algorithm, rule-out (low risk patient) was a score <1 and rule-in (high risk patient) was a score ≥7. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) was determined for all 5 algorithms using these cutoffs.

RESULTS

[0066] There were 1095 patients who were evaluated by all 5 algorithms. The NPV and sensitivity were approximately 99% indicating that these algorithms can rule-out patients at presentation for MI or death within the next 30 days. The specificity and PPV were also high

suggesting that these algorithms can also rule-in patients at ED presentation. TABLE 7 shows the diagnostic performance of all 5 algorithms (L6HHR, L6SHR, Labscore 8, L5HF, and L5HR) for ruling-out/-in at ED presentation for 30 day MI or death in 1095 patients who present to the ED with symptoms suggestive of acute coronary syndrome.

Table 7.

Test	Liklihood Ratio (LR)	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Rule-out	**Negative LR**				
L6HHR with hsTnI as the assay Labscore <2	0.00	1.000	0.177	0.183	1.000
L6SHR with cTnI as the assay Labscore <2	0.05	0.988	0.232	0.191	0.991
Labscore 8 point Labscore <1	0.00	1.000	0.121	0.173	1.000
L5HF with hsTnI as the assay Labscore <2	0.04	0.988	0.299	0.206	0.993
L5HR with hsTnI as the assay Labscore <2	0.04	0.988	0.308	0.208	0.993
L6HHR with hsTnT as the assay Labscore <2	0.03	0.994	0.177	0.182	0.994
L5HF with hsTnT as the assay Labscore <2	0.02	0.994	0.283	0.203	0.996
L5HR with hsTnT Labscore <2	0.04	0.988	0.294	0.205	0.993
Rule-in	**Positive LR**				
L6HHR with hsTnI as the assay Labscore >5	11.21	0.400	0.964	0.673	0.897
L6SHR with cTnI as the assay Labscore >5	12.14	0.341	0.972	0.690	0.889
Labscore 8 point Labscore >6	10.42	0.529	0.949	0.657	0.916
L5HF with hsTnI as the assay Labscore >4	11.64	0.453	0.961	0.681	0.905
L5HR with hsTnI as the assay Labscore >4	10.88	0.459	0.958	0.667	0.906
L6HHR with hsTnT as the assay Labscore >5	5.37	0.435	0.919	0.497	0.899
L5HF with hsTnT as the assay Labscore >4	5.86	0.494	0.916	0.519	0.908
L5HR with hsTnT Labscore >4	5.25	0.476	0.909	0.491	0.904

DISCUSSION

[0067] Described are methods useful to determine risk in a patient of a cardiac event. The method includes determining levels of various biomarkers in a patient to yield a cumulative laboratory score. Scores close to 0 identify patients at low risk for an adverse cardiac event or death, who can be discharged after their initial blood work from the ED (thereby decreasing ED length of stay). The present methods are also useful to identify patients at high risk/high likelihood for an acute cardiovascular event or death. The determination of both low and high risks patients leads to the appropriate treatments/care/management to each patient, including care/treatment to the acute (e.g., ED setting), and non-acute (ambulatory or community) populations, as well as to patients at risk for cardiac injury.

[0068] The present study data indicates that a laboratory score of 0 can rule-out patients in the ED at presentation for an acute cardiovascular event or death (likelihood ratio = 0, sensitivity = 100% for laboratory score >0). This has not previously been possible using prior determinations of parameters such as high-sensitivity cardiac troponin limit of detection, either alone or in combination with glucose.

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CLAIMS

We Claim

1. A method of determining the risk of an adverse cardiovascular event or death in a mammal comprising the steps of:

i) determining in a biological sample obtained from the mammal the concentration of a glucose metabolism biomarker, and allotting a score of 1 when the concentration is greater than a normal level of the glucose metabolism marker;

ii) determining in the biological sample the level of a heart function biomarker, and allotting a score of 1 when the level is greater than a normal level of the heart function biomarker, or determining in the biological sample the level of a renal function biomarker and allotting a score of 1 when the estimated glomerular filtration rate (eGFR) which is based on the level of the renal function biomarker is less than the normal level of eGFR;

iii) determining in the biological sample the level of at least one biomarker of cardiac injury using a high sensitivity assay, and allotting a score of 1 if the level is greater than the level of an ambulatory population at risk for future cardiovascular events but less than the level at which analytical variation occurs, allotting a score of 2 if the level is greater than the level at which analytical variation occurs and less than the upper limit of normal of a general population, and allotting a score of 3 if the level is greater than the upper limit of normal of the general population;

iv) generating a total laboratory score based on the sum of the scores for each of the biomarkers of i), ii) and iii), wherein a laboratory score of less than 1 indicates low risk of an adverse cardiac event in the mammal with a negative likelihood ratio of less than 1, and a laboratory score of greater than 4 indicates a risk of an adverse cardiac event in the mammal with a positive likelihood ratio of greater than 1; and

v) optionally administering a treatment of the adverse cardiac event to the diagnosed mammal when there is a laboratory score of greater than 4.

2. The method of claim 1, wherein the glucose metabolism marker is selected from glucose or glycated hemoglobin A1c (HbA1c), the heart function biomarker is selected from red cell distribution width (RDW) and a natriuretic peptide, the renal function biomarker is selected from creatinine or cystatin c, and the cardiac injury biomarker is selected from troponin T, troponin I and heart-specific fatty acid binding protein.

3. The method of claim 2, wherein a score of 1 is allotted when the concentration of glucose as the glucose metabolism marker is greater than 5.5 mmol/L, or when the concentration of glycated hemoglobin as the glucose metabolism marker is greater than or equal to 48 mmol/mol.

4. The method of claim 2, wherein a score of 1 is allotted when the level of RDW as the heart function biomarker is greater than 13.3%, when the level of brain natriuretic peptide (BNP) as the natriuretic peptide is greater than 35 pg/ml or the level of amino-terminal pro-brain natriuretic peptide (NT-proBNP) as the natriuretic peptide is greater than 125 pg/mL.

5. The method of claim 2, wherein a score of 1 is allotted when the eGFR is less than 90 milliliters per minute per 1.73 m^2.

6. The method of claim 1, wherein the level of the heart function biomarker and renal function biomarker are determined, and a laboratory score of greater than 5 indicates a risk of an adverse cardiac event in the mammal with a likelihood ratio of greater than 1.

7. The method of claim 1, wherein the biological sample is blood, serum, plasma, urine, and cerebrospinal fluid.

8. A method of determining the risk of an adverse cardiovascular event or death in a mammal comprising the steps of:

i) determining in a biological sample obtained from the mammal the concentration of a glucose metabolism biomarker, and allotting a score of 1 when the concentration is greater than a normal level of the glucose metabolism marker;

ii) determining in the biological sample the level of a heart function biomarker, and allotting a score of 1 when the level is greater than a normal level of the heart function biomarker;

iii) determining in the biological sample the level of a renal function biomarker and allotting a score of 1 when the estimated glomerular filtration rate (eGFR) which is based on the level of the renal function biomarker is less than the normal level of eGFR;

iv) determining in the biological sample the level of at least one biomarker of cardiac injury using a sensitive assay, and allotting a score of 1 if the level is at about the limit of detection of the biomarker, a score of 2 is allotted if the biomarker level is greater than the limit of detection but less than the upper limit of normal of the biomarker level in a general population and a score of 3 is allotted when the biomarker level is greater than the upper limit of normal of the general population;

v) generating a total laboratory score based on the sum of the scores for each of the biomarkers of i), ii) and iii), wherein a laboratory score of greater than 5 indicates a risk of an adverse cardiac event in the mammal with a positive likelihood ratio of greater than 1; and

vi) optionally administering a treatment of the adverse cardiac event to the diagnosed mammal when there is a laboratory score of greater than 5.

9. The method of claim 8, wherein the glucose metabolism marker is selected from glucose or glycated hemoglobin A1c (HbA1c), the heart function biomarker is selected from red cell distribution width (RDW) and a natriuretic peptide, the renal function biomarker is selected from creatinine or cystatin c, and the cardiac injury biomarker is selected from troponin T, troponin I and heart-specific fatty acid binding protein.

10. The method of claim 9, wherein a score of 1 is allotted when the concentration of glucose as the glucose metabolism marker is greater than 5.5 mmol/L, or when the concentration of glycated hemoglobin as the glucose metabolism marker is greater than or equal to 48 mmol/mol.

11. The method of claim 9, wherein a score of 1 is allotted when the level of RDW as the heart function biomarker is greater than 13.3%, when the level of BNP as the natriuretic peptide

is greater than 35 pg/ml or when the level of NT-proBNP as the natriuretic peptide is greater than 125 pg/mL.

12. The method of claim 8, wherein a score of 1 is allotted when the eGFR is less than 90 milliliters per minute per 1.73 m^2.

13. The method of claim 9, wherein when the cardiac injury biomarker is troponin I, determination of a concentration of less than 0.01 ug/L yields a score of 0, determination of a concentration of 0.01 ug/L yields a score of 1, determination of a concentration of 0.02-0.03 ug/L yields a score of 2, and determination of a concentration of greater than 0.03 ug/L yields a score of 3.

14. The method of claim 9, wherein when the cardiac injury biomarker is troponin T, determination of a concentration of less than 20 ng/L yields a score of 0, determination of a concentration of 20-30 ng/L yields a score of 1, determination of a concentration of 31-50 ng/L yields a score of 2, and determination of a concentration of greater than 50 ng/L yields a score of 3.

15. A method of determining the risk of an adverse cardiovascular event or death in a mammal comprising the steps of:

i) determining in a biological sample obtained from the mammal the concentration of a glucose metabolism biomarker, and allotting a score of 1 when the concentration is greater than a normal level of the glucose metabolism marker;

ii) determining in the biological sample the level of a renal function biomarker and allotting a score of 1 when the estimated glomerular filtration rate (eGFR) which is based on the level of the renal function biomarker is less than the normal level of eGFR;

iii) determining in the biological sample the level of at least two biomarkers of cardiac injury using a sensitivity assay, and allotting a score of 1 if the level is at about the limit of detection of the biomarker, a score of 2 is allotted if the biomarker level is greater than the limit of detection but less than the upper limit of normal of the biomarker level in a general population

and a score of 3 is allotted when the biomarker level is greater than the upper limit of normal of the general population;

iv) generating a total laboratory score based on the sum of the scores for each of the biomarkers of i), ii) and iii), wherein a laboratory score of greater than or equal to 7 indicates a risk of an adverse cardiac event in the mammal with a likelihood ratio of greater than 1; and

v) optionally administering a treatment of the adverse cardiac event to the diagnosed mammal when there is a laboratory score of greater than or equal to 7.

16. The method of claim 15, wherein the glucose metabolism marker is selected from glucose or glycated hemoglobin A1c (HbA1c), the renal function biomarker is selected from creatinine or cystatin c, and the cardiac injury biomarkers are selected from troponin T, troponin I and heart-specific fatty acid binding protein.

17. The method of claim 16, wherein:

i) a score of 1 is allotted when the concentration of glucose as the glucose metabolism marker is greater than 5.5 mmol/L, or when the concentration of glycated hemoglobin as the glucose metabolism marker is greater than or equal to 48 mmol/mol;

ii) a score of 1 is allotted when the eGFR is less than 90 milliliters per minute per 1.73 m^2;

iii) a first cardiac injury biomarker is troponin I, and determination of a concentration of less than 0.01 ug/L yields a score of 0, determination of a concentration of 0.01 ug/L yields a score of 1, determination of a concentration of 0.02-0.03 ug/L yields a score of 2, and determination of a concentration of greater than 0.03 ug/L yields a score of 3; and

iv) a second cardiac injury biomarker is troponin T, and determination of a concentration of less than 20 ng/L yields a score of 0, determination of a concentration of 20-30 ng/L yields a score of 1, determination of a concentration of 31-50 ng/L yields a score of 2, and determination of a concentration of greater than 50 ng/L yields a score of 3.

18. A kit for use in a method of determining the risk of an adverse cardiovascular event or

death in a mammal comprising a biomarker-specific reactant for one or more biomarkers selected from the group consisting of a glucose metabolism biomarker, a heart function biomarker, a renal function biomarker, and a cardiac injury biomarker, wherein the reactant is suitable for use to determine the level of the biomarker in a biological sample from the mammal, and guidelines indicating a score to be allotted based on the level of each target biomarker and indicating the relationship between a total score and risk of an adverse cardiovascular event.

19. The kit of claim 18, wherein the glucose metabolism biomarker is selected from glucose or glycated hemoglobin A1c (HbA1c), the heart function biomarker is selected from red cell distribution width (RDW) and a natriuretic peptide, the renal function biomarker is selected from creatinine or cystatin c, and the cardiac injury biomarker is selected from troponin T, troponin I and heart-specific fatty acid binding protein.

20. The kit of claim 19, wherein the guidelines indicate a score of 1 is allotted when the concentration of glucose as the glucose metabolism marker is greater than 5.5 mmol/L, or when the concentration of glycated hemoglobin as the glucose metabolism marker is greater than or equal to 48 mmol/mol; a score of 1 is allotted when the level of RDW as the heart function biomarker is greater than 13.3%, when the level of brain natriuretic peptide (BNP) as the natriuretic peptide is greater than 35 pg/ml or the level of amino-terminal pro-brain natriuretic peptide (NT-proBNP) as the natriuretic peptide is greater than 125 pg/mL; a score of 1 is allotted when the eGFR is less than 90 milliliters per minute per 1.73 m^2; for troponin I determined using a sensitive assay, a determination of a concentration of less than 0.01 ug/L yields a score of 0, determination of a concentration of 0.01 ug/L yields a score of 1, determination of a concentration of 0.02-0.03 ug/L yields a score of 2, and determination of a concentration of greater than 0.03 ug/L yields a score of 3; for troponin T determined using a sensitive assay, and determination of a concentration of less than 20 ng/L yields a score of 0, determination of a concentration of 20-30 ng/L yields a score of 1, determination of a concentration of 31-50 ng/L yields a score of 2, and determination of a concentration of greater than 50 ng/L yields a score of 3; for troponin T or I determined using a high sensitivity assay, a score of 1 is allotted if the level of troponin T or I is greater than the troponin T or I level of an ambulatory population at risk for a cardiovascular event but less than the troponin T or I level at which analytical variation occurs,

a score of 2 is allotted if the troponin T or I level is greater than the troponin T or I level at which analytical variation occurs and less than the upper limit of normal of a general population, and a score of 3 is allotted if the troponin T or I level is greater than the upper limit of normal of the general population.

21. The kit of claim 20, wherein the guidelines indicate that a total score of less than 1 indicates that the mammal is at low risk of an adverse cardiac event with a negative likelihood ratio of less than 1, while a total score of > 4, >5 or ≥ 7 indicates that the mammal is at high risk of an adverse cardiac event with a positive likelihood ratio of greater than 1.

22. The method of claim 1, wherein the adverse cardiac event is selected from the group consisting of myocardial infarction, cardiac ischemia, heart failure, percutaneous coronary intervention, coronary artery bypass graft and death.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2016/051011

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***G01N 33/48*** (2006.01), ***C12Q 1/48*** (2006.01), ***G01N 33/53*** (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ***G01N 33/48*** (2006.01), ***C12Q 1/48*** (2006.01), ***G01N 33/53*** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Questel-Orbit, CIPO Library Discovery Tool, Scopus, PubMed, Intellect, Google

Search terms: glucose glycated hemoglobin HbA1c troponin heart specific fatty acid binding protein red cell distribution width RDW natriuretic adverse cardiac cardiovascular event glucose metabolism

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SHORTT, C. et al., "*An approach to rule-out an acute cardiovascular event or death in emergency department patients using outcome-based cutoffs for high-sensitivity cardiac troponin assays and glucose*". Clinical Biochemistry, 03 March 2015 (03-03-2015), Vol. 48(4-5): 282-287, see entire document	1-14, 18-21
X / Y	EP 1884777 A1 (WOLLERT et al.) 06 February 2008 (06-02-2008), see entire document	18, 19, 22 / 1, 6-8, 12, 20, 21
X / Y	US 2011/0053191 A1 (HESS et al.) 03 March 2011 (03-03-2011), see entire document	18, 19, 22 / 1, 7, 20, 21
Y	HONG et al., "*GDF15 is a novel biomarker for impaired fasting glucose*". Diabetes and Metabolism Journal, December 2014 (12-2014), Vol. 36(6), pp. 472-479, see entire document	1, 6-8, 12, 20, 21

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

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

Date of the actual completion of the international search 01 November 2016 (01-11-2016)	Date of mailing of the international search report 10 November 2016 (10-11-2016)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Kathryn Blackwell (819) 639-5049

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2016/051011

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

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

1. ☑ Claim Nos.: 1, 8
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 1, 8 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under PCT Rule 39.1(iv). However, as the step of treatment is merely optional, this Authority has carried out a search based on the subject matter of these claims without the final optional treatment step.

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

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

See extra sheet

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

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

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

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

1-14, 18-21 (partially), wherein the biomarkers include a glucose metabolism biomarker, a heart function biomarker and a cardiac injury biomarker

Remark on Protest

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

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

☑ No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CENGIZ et al., "*Placental growth factor as a new marker for predicting abnormal glucose challenge test results*". Gynecological Endocrinology, 10 July 2013 (10-07-2013), Vol. 29(10), pp. 909-911, see entire document	1, 7, 20, 21
X	DAMMAN et al., "*Multiple biomarkers at admission significantly improve the prediction of mortality in patients undergoing primary percutaneous coronary intervention for acute ST-segment elevation myocardial infarction*". Journal of the American College of Cardiology, 28 December 2010 (28-12-2010), Vol. 57(1), pp. 29-36, see entire document	1-14, 18-22
X	ALMEIDA et al., "*The value of NT-proBNP in early risk stratification of acute coronary syndromes*". Revista Portuguesa de Cardiologia, 2006, Vol. 25(1), pp. 71-75, see entire document	1-4, 7, 18-22
X	BEATTIE et al., "*Perioperative cardiac biomarkers: the utility and timing*". Current Opinion in Critical Care, August 2013 (08-2013), Vol. 19(4), pp. 334-341, see entire document	1-4, 7, 18-22

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
EP1884777A1	06 February 2008 (06-02-2008)	EP1884777A1	06 February 2008 (06-02-2008)
		AT511656T	15 June 2011 (15-06-2011)
		AU2007280413A1	07 February 2008 (07-02-2008)
		AU2007280413B2	26 August 2010 (26-08-2010)
		BRPI0715126A2	04 June 2013 (04-06-2013)
		CA2660691A1	07 February 2008 (07-02-2008)
		CN101517415A	26 August 2009 (26-08-2009)
		CN101517415B	20 April 2016 (20-04-2016)
		CN106018820A	12 October 2016 (12-10-2016)
		EP2047275A2	15 April 2009 (15-04-2009)
		EP2047275B1	01 June 2011 (01-06-2011)
		EP2315034A2	27 April 2011 (27-04-2011)
		EP2315034A3	17 August 2011 (17-08-2011)
		ES2365176T3	23 September 2011 (23-09-2011)
		JP2009545735A	24 December 2009 (24-12-2009)
		JP5309026B2	09 October 2013 (09-10-2013)
		KR20090047451A	12 May 2009 (12-05-2009)
		KR101245877B1	20 March 2013 (20-03-2013)
		MX2009000914A	18 June 2009 (18-06-2009)
		US2011065204A1	17 March 2011 (17-03-2011)
		US8951742B2	10 February 2015 (10-02-2015)
		US2015362511A1	17 December 2015 (17-12-2015)
		WO2008015254A2	07 February 2008 (07-02-2008)
		WO2008015254A3	05 June 2008 (05-06-2008)
US2011053191A1	03 March 2011 (03-03-2011)	US2011053191A1	03 March 2011 (03-03-2011)
		EP2294426A1	06 March 2011 (16-03-2011)
		WO2009144229A1	03 December 2009 (03-12-2009)

Continuation of Box No. III

The claims are directed to a plurality of inventive concepts as follows:

Group A - Claims 1-14, 18-21 (partially) are directed to a method of determining the risk of an adverse cardiovascular event or death in a mammal comprising determining a glucose metabolism biomarker, a heart function biomarker and a cardiac injury biomarker; and a kit therefor; and

Group B - Claims 1-14, 18-21 (partially); 15-17 (completely) are directed to a method of determining the risk of an adverse cardiovascular event or death in a mammal comprising determining a glucose metabolism biomarker, a renal function biomarker and a cardiac injury biomarker; and a kit therefor.

The claims must be limited to one inventive concept as set out in PCT Rule 13. The common concept linking the two groups is the use of a glucose metabolism biomarker and a cardiac injury biomarker to determine the risk of an adverse cardiovascular event or death in a mammal. However, D1 discloses that a combination of measurements of a high-sensitivity cardiac troponin (hs-cTn; a cardiac injury biomarker) with glucose (i.e. a glucose metabolism biomarker) is useful in determining the risk of an adverse cardiovascular event or death in a mammal. Thus the special technical feature of group A is the further inclusion of a heart function biomarker, while the special technical feature of group B is the further inclusion of a renal function biomarker. As such, the requirement of unity of invention is not met.

专利名称(译)	确定不良心脏事件风险的方法		
公开(公告)号	EP3341723A4	公开(公告)日	2019-05-01
申请号	EP2016840458	申请日	2016-08-26
申请(专利权)人(译)	麦克马斯特大学		
当前申请(专利权)人(译)	麦克马斯特大学		
[标]发明人	KAVSAK PETER WORSTER ANDREW		
发明人	KAVSAK, PETER WORSTER, ANDREW		
IPC分类号	G01N33/48 C12Q1/48 G01N33/53		
CPC分类号	G01N33/66 G01N33/6887 G01N33/70 G01N33/723 G01N33/80 G01N33/92 G01N2333/4712 G01N2333/58 G01N2333/8139 G01N2800/32 G01N2800/50 G01N2800/56		
优先权	62/211074 2015-08-28 US		
其他公开文献	EP3341723A1		
外部链接	Espacenet		

摘要(译)

提供了确定哺乳动物中不良心血管事件或死亡风险的方法，其包括在从哺乳动物获得的生物样品中确定选自葡萄糖代谢生物标志物，心脏功能生物标志物，肾功能的生物标志物的组合水平。生物标志物和至少一种心脏损伤的生物标志物。基于每种生物标志物的水平分配分数，并且累积分数指示不良心血管事件的风险。