

Provided herein are methods of evaluating the risk of mortality for a subject (e.g., a subject having or diagnosed with heart failure) within a specific time period (e.g., within 3 months, 6 months, or a year or more (e.g., 2, 5, or 10 years)) that include determining a multimarker mortality risk score for a subject based upon the age of the subject; the level of ST2 in the subject, in combination with one or more of a natural logarithm of a level of a brain natriuretic peptide (BNP) in the subject; a level of troponin in the subject; a New York Heart Association (NYHA) score; a history of cardiovascular disease (CAD); a natural logarithm of a systolic blood pressure; a measure of renal function or a natural logarithm of a level of hemoglobin (Hgb), and age; and comparing the multimarker mortality risk score to a reference multimarker mortality risk score; wherein the presence of a multimarker mortality risk score that is at or above the reference multimarker mortality risk score indicates that the subject has an increased risk of mortality within the specific time period, and the presence of a multimarker mortality risk score that is below the reference multimarker mortality risk score indicates that the subject has a decreased risk of mortality within the specific time period (e.g., within one year).

In some examples, the risk score is determined using one of the following algorithms:

- (1) $AGE + ST2 + \ln_SBP + CAD + \ln_NTpro\text{-}BNP$
- (2) $AGE + ST2 + \ln_NTpro\text{-}BNP$
- (3) $AGE + ST2 + Troponin + NYHA$
- (4) $AGE + ST2 + [Troponin \text{ OR } NYHA]$
- (5) $AGE + ST2 + [Troponin \text{ AND/OR } NYHA] + \ln_Hgb$
- (6) $AGE + ST2 + [Troponin \text{ AND/OR } NYHA] + \ln_Hgb$
- (7) $AGE + ST2 + [Troponin \text{ AND/OR } NYHA] + \ln_Hgb + \ln_SBP$
- (8) $AGE + ST2 + [Troponin \text{ AND/OR } NYHA] + \ln_Hgb + \ln_SBP + \ln_NTpro\text{-}BNP$.

In some examples, the level of ST2 is determined and compared to a threshold and the presence of a level at or above the threshold is scored as “1” and the presence of a level below the threshold is scored as “0”. In some examples, the threshold level of ST2 is 35 or 50 ng/mL. In some examples, algorithm (1) or (2) is used and the threshold level of ST2 is 35 ng/mL. In some examples, algorithm

(3) or (4) is used and the threshold level of ST2 is 50 ng/mL. In some examples, the subject has been diagnosed with a cardiovascular disease (e.g., heart failure). In some examples, the reference multimarker mortality risk score represents a score corresponding to a low risk of death within a specific time period (e.g., within 3 months, 6 months, 1, 2, 5 or 10 years). In some examples, the sample contains serum, blood, plasma, urine, or body tissue.

In some examples, the subject has a BMI of 25-29, a BMI of ≥ 30 , or renal insufficiency. Some examples further include discharging the subject or treating the subject on an inpatient basis based on the presence of an increased risk of mortality determined using any of the methods described herein. For example, a subject identified as having an increased risk of mortality within the specific time period (e.g., within 3 months, 6 months, 1, 2, 5 or 10 years) is treated on an inpatient basis (e.g., newly admitted to a hospital or continued hospitalization) or a subject identified as having a decreased risk of mortality within the specific time period (e.g., within 3 months, 6 months, 1, 2, 5 or 10 years) is discharged from a hospital or continued to be treated on an outpatient basis. Some examples further include selecting and/or performing increased cardiac monitoring (e.g., any of the examples of increased cardiac monitoring described herein or known in the art) on a subject identified as having an increased risk of mortality within the specific time period (e.g., using any of the methods described herein), or selecting and/or performing low frequency monitoring (e.g., cardiac monitoring) on a subject (e.g., greater than 6 months between examinations, greater than 9 months between examinations, or one year or greater between examinations) identified as having a reduced risk of mortality within the specific time period (e.g., using any of the methods described herein). As described herein, increased cardiac monitoring can be, e.g., the monitoring of cardiac function in a subject (e.g., electrocardiogram (e.g., ambulatory electrocardiography), chest X-ray, echocardiography, stress testing, computer tomography, magnetic resonance imaging, positron emission tomography, and cardiac catheterization) or the monitoring of levels of soluble ST2 in the subject over time. Increased cardiac monitoring can also include increased frequency of clinical visits (e.g., about once every month, once every two months, once every three months, once every four months, once every five months, or once every six months). Also provided are methods of selecting a treatment for a subject receiving a treatment for a

cardiovascular disorder that include determining the subject's risk of mortality over a specific time period (e.g., within any of the time periods described herein, e.g., within 3 months, 6 months, 1, 2, 5 or 10 years) using any of the methods described herein, and selecting continuation of the treatment for a subject determined to have a reduced risk of mortality over the specific time period (e.g., using any of the methods described herein) or selecting a new (alternate) cardiovascular treatment for a subject determined to have an increased risk of mortality over the specific time period (e.g., using any of the methods described herein). In one aspect, there is provided an in vitro method for selecting a treatment for a subject receiving a treatment for a cardiovascular disorder, wherein the method comprises:

determining a multimarker mortality risk score using one of the following algorithms:

(1) the age of the subject (AGE) + the level of soluble ST2 in a biological sample from the subject (ST2) + a natural logarithm of a systolic blood pressure (ln_SBP) + a history of cardiovascular disease (CAD) + a natural logarithm of a level of a brain natriuretic peptide (BNP) in the subject (ln_NTpro-BNP);

(2) AGE + ST2 + ln_NTpro-BNP;

(3) AGE + ST2 + a level of troponin in the subject (Troponin) + a New York Heart Association (NYHA) score (NYHA);

(4) AGE + ST2 + [Troponin or NYHA];

(5) AGE + ST2 + [Troponin and/or NYHA] + a natural logarithm of a level of hemoglobin (ln_Hgb);

(6) AGE + ST2 + [Troponin and/or NYHA] + ln_Hgb + ln_SBP; and

(7) AGE + ST2 + [Troponin and/or NYHA] + ln_Hgb + ln_NTpro-BNP;

comparing the multimarker mortality risk score to a reference multimarker mortality risk score; and

selecting an alternate cardiovascular treatment for a subject having a multimarker mortality risk score that is elevated as compared to the reference multimarker mortality score, or

selecting continuation of the cardiovascular treatment previously administered for a subject having a multimarker mortality risk score that is decreased as compared to the reference multimarker mortality risk score, wherein:

age is measured in years;

ST2 is measured in ng/mL and compared to a threshold level of soluble ST2, where the presence of a level of ST2 at or above the threshold level of soluble ST2 is scored as "1" and the presence of a level of ST2 below the threshold level of soluble ST2 is scored as "0";

SBP is measured in mmHg;

CAD is either scored as "0" in the absence of CAD or scored as "1" in the presence of CAD;

NTpro-BNP is measured in pg/mL;

Hgb is measured in g/dL;

Troponin is measured in pg/mL; and

NYHA has a value of 1-4. As described herein, a new treatment can mean administration of a new combination therapeutic agents, administration of a new therapeutic agent, a different dosage of the previously administered therapeutic agent, a different frequency of administration of the previously administered therapeutic agent, or a different route of administration of the previously administered therapeutic agent. Some examples further include administering the selected treatment to a subject.

first multimarker risk score in a subject at a first time point (e.g., using any of the methods described herein), determining a second multimarker risk score in a subject at a second time point (e.g., using any of the methods described herein), where two or more doses of a treatment for a cardiovascular disorder (e.g., heart failure) are administered to the subject between the first and the second time points, comparing the second multimarker risk score to the first multimarker risk score, and identifying the treatment as effective in a subject having a decreased second multimarker risk score as compared to the first multimarker risk score, or identifying the treatment as not being effective in a subject having an elevated second multimarker risk score as compared to the first multimarker risk score. Some examples further include selecting the treatment identified as being effective in the subject, and/or continuing to administer the selected treatment to the subject.

Also provided are methods of selecting a treatment for a subject that include determining a first multimarker risk score for a subject at a first time point (e.g., using any of the methods described herein), determining a second multimarker risk score for a subject at a second time point (e.g., using any of the methods described herein), comparing the second multimarker risk score with the first multimarker risk score, and selecting inpatient treatment (e.g., initial hospital admission or continued inpatient treatment) for a subject having an elevated second multimarker risk score as compared to first multimarker risk score or selecting outpatient treatment (e.g., hospital discharge or continued outpatient treatment) for a subject having a decreased second multimarker risk score as compared to the first multimarker risk score. Some methods further include admitting the subject to the hospital, continuing inpatient treatment, discharging the subject, or continuing outpatient treatment based on the comparison of the second and first multimarker risk scores (e.g., as selected above).

Also provided are methods of selecting a treatment for a subject that include determining a first multimarker risk score for a subject at a first time point (e.g., using any of the methods described herein), determining a second multimarker risk score for a subject at a second time point (e.g., using any of the methods described herein), comparing the second multimarker risk score to the first multimarker risk score, and selecting increased cardiac monitoring for a subject having an elevated second multimarker risk score as compared to the first multimarker risk score or selecting low frequency monitoring (e.g., cardiac monitoring) (e.g., greater than 6 months

between examinations, greater than 9 months between examinations, or one year or greater between examinations) for a subject having a decreased second multimarker risk score as compared to the first multimarker risk score. As described herein, increased cardiac monitoring can be, e.g., the monitoring of cardiac function in a subject (e.g., electrocardiogram (e.g., ambulatory electrocardiography), chest X-ray, echocardiography, stress testing, computer tomography, magnetic resonance imaging, positron emission tomography, and cardiac catheterization) or the monitoring of the levels of soluble ST2 in the subject over time. Increased cardiac monitoring can also include increased frequency of clinical visits (e.g., about once every month, once every two months, once every three months, once every four months, once every five months, or once every six months). Some methods further include administering the selected treatment to the subject.

Also provided are methods of selecting a treatment for a subject that include determining a first multimarker risk score in a subject at a first time point (e.g., using any of the methods described herein), determining a second multimarker risk score in the subject at a second time point (e.g., using any of the methods described herein), where a subject has been administered at least two doses of treatment (e.g., a treatment of a cardiovascular disease) between the first time point and the second time point, comparing the first multimarker risk score to the second multimarker risk score, and selecting a new treatment for a subject having an elevated second multimarker risk score as compared to the first multimarker risk score or selecting the same treatment for a subject having a decreased second multimarker risk score compared to the first multimarker risk score. Some examples further include administering the selected treatment to the subject. As described herein, a new treatment can mean administration of a new combination therapeutic agents, administration of a new therapeutic agent, a different dosage of the previously administered therapeutic agent, a different frequency of administration of the previously administered therapeutic agent, or a different route of administration of the previously administered therapeutic agent.

Also provided are methods of selecting a subject for participation in a clinical study of a treatment for cardiovascular disease that include determining a first multimarker risk score in a subject at a first time point (e.g., using any of the methods described herein), determining a second multimarker risk score in the subject at a

second time point (e.g., using any of the methods described herein), and selecting a subject having an elevated second multimarker risk score as compared to first multimarker risk score for participation in a clinical study of a cardiovascular disease.

As used herein, a “sample” includes any bodily fluid or tissue, e.g., one or more of blood, serum, plasma, urine, and body tissue. In certain examples, a sample is a serum, plasma, or blood sample.

An antibody that “binds specifically to” an antigen, binds preferentially to the antigen in a sample containing other proteins.

The methods and kits described herein have a number of advantages. For example, the methods can be used to determine whether a patient should be admitted or held as an inpatient for further assessment, regardless of whether a definitive diagnosis has been made. For example, the methods can be used for risk stratification of a given subject, e.g., to make decisions regarding the level of aggressiveness of treatment that is appropriate for the subject, based on their multimarker risk score as determined by a method described herein. Better treatment decisions can lead to reduced morbidity and mortality, and better allocation of scarce health care resources. The methods described herein can be used to make general assessments as to whether a patient should be further tested to determine a specific diagnosis. The methods described herein can also be used for patient population risk stratification, e.g., to provide information about clinical performance or expected response to a therapeutic intervention. The methods described herein can be used regardless of the underlying cause or ultimate diagnosis, and therefore are not limited to specific indications.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting.

All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In addition, the present application incorporates by reference the entire contents of U.S. Patent Application No. 11/789,169, and international patent application nos. PCT/US2007/067626, PCT/US2007/067914, and PCT/US2007/068024.

WHAT IS CLAIMED IS:

1. An in vitro method for selecting a treatment for a subject receiving a treatment for a cardiovascular disorder, wherein the method comprises:

determining a multimarker mortality risk score using one of the following algorithms:

(1) the age of the subject (AGE) + the level of soluble ST2 in a biological sample from the subject (ST2) + a natural logarithm of a systolic blood pressure (ln_SBP) + a history of cardiovascular disease (CAD) + a natural logarithm of a level of a brain natriuretic peptide (BNP) in the subject (ln_NTpro-BNP);

(2) AGE + ST2 + ln_NTpro-BNP;

(3) AGE + ST2 + a level of troponin in the subject (Troponin) + a New York Heart Association (NYHA) score (NYHA);

(4) AGE + ST2 + [Troponin or NYHA];

(5) AGE + ST2 + [Troponin and/or NYHA] + a natural logarithm of a level of hemoglobin (ln_Hgb);

(6) AGE + ST2 + [Troponin and/or NYHA] + ln_Hgb + ln_SBP; and

(7) AGE + ST2 + [Troponin and/or NYHA] + ln_Hgb + ln_NTpro-BNP;

comparing the multimarker mortality risk score to a reference multimarker mortality risk score; and

selecting an alternate cardiovascular treatment for a subject having a multimarker mortality risk score that is elevated as compared to the reference multimarker mortality score, or

selecting continuation of the cardiovascular treatment previously administered for a subject having a multimarker mortality risk score that is decreased as compared to the reference multimarker mortality risk score, wherein:

age is measured in years;

ST2 is measured in ng/mL and compared to a threshold level of soluble ST2, where the presence of a level of ST2 at or above the threshold level of soluble ST2 is scored as “1” and the presence of a level of ST2 below the threshold level of soluble ST2 is scored as “0”;

SBP is measured in mmHg;

CAD is either scored as “0” in the absence of CAD or scored as “1” in the presence of CAD;

NTpro-BNP is measured in pg/mL;
Hgb is measured in g/dL;
Troponin is measured in pg/mL; and
NYHA has a value of 1-4.

2. The method of claim 1, wherein the threshold level of soluble ST2 is 35 or 50 ng/mL.
3. The method of claim 2, wherein the algorithm (1) or (2) is used and the threshold level of soluble ST2 is 35 ng/mL.
4. The method of claim 2, wherein algorithm (3) or (4) is used and the threshold level of soluble ST2 is 50 ng/mL.
5. The method of claim 1, wherein the subject has been diagnosed with heart failure.
6. The method of claim 1, wherein the reference multimarker mortality risk score represents a score corresponding to a low risk of death within one year or within five years.
7. The method of claim 1, wherein the reference multimarker mortality risk score represents a threshold level.
8. The method of claim 1, wherein the sample comprises blood, serum, or plasma.
9. The method of claim 1, wherein the subject has a BMI of 25-29.
10. The method of claim 1, wherein the subject has a BMI of ≥ 30 .
11. The method of claim 1, wherein the subject has renal insufficiency.