

MULTIMARKER RISK STRATIFICATION

CLAIM OF PRIORITY

5 This application claims priority to U.S. Provisional Patent Application Serial No. 61/691,706, filed on August 21, 2012. The entire contents of the foregoing are incorporated by reference herein.

TECHNICAL FIELD

10 The invention relates to methods for predicting risk of mortality, in subjects with cardiovascular disease, e.g., heart failure, based on multiple markers including a combination of biomarkers (e.g., ST2) and other clinical parameters (e.g., age).

BACKGROUND

15 Clinical evaluation for determination of risk of mortality due to heart failure may not always be straightforward. The decision whether to treat a subject aggressively or conservatively, or to admit the subject as an inpatient or to send them home, may sometimes be made solely on a physician's clinical assessment or "gut feeling" as to the individual's actual condition. A formula for determining a subject's likelihood of mortality would significantly enhance the physician's ability to make informed treatment decisions, improve patient care and reduce overall healthcare costs. A multi-marker approach for risk stratification has been
20 generally proposed for patients with acute coronary syndromes, see, e.g., Sabatine et al., Circulation 105(15):1760-3 (2002)), and methods for predicting risk of a major adverse cardiac event are describe in U.S. Patent No. 8090562.

SUMMARY

25 The present ~~invention~~-disclosure is based, at least in part, on the discovery that multiple markers, including serum levels of the biomarker ST2 (also known as Interleukin 1 Receptor Like 1 (IL1RL-1)), in combination with clinical parameters such as age and levels of at least one other biomarker, e.g., troponin or a natriuretic peptide (NP) such as the inactive N-terminal fragment of brain-type natriuretic peptide (NT-pro-BNP), can be used predict the likelihood of mortality due to CVD within a specific time period, e.g., 30 days, 3 or 6 months, or a year or
30 more (e.g., 2, 5 or 10 years).

In one aspect, there is provided an *in vitro* method for evaluating the risk of mortality for a subject within one year, the method comprising:

determining a multimarker mortality risk score for a subject based upon:

the age of the subject;

the level of ST2 in a biological sample from 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), 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 one year.

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 embodiments, the risk score is determined using one of the following algorithms:

(1) $AGE + ST2 + \ln_SBP + CAD + \ln_NTpro-BNP$

(2) $AGE + ST2 + \ln_NTpro-BNP$

(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) $\text{AGE} + \text{ST2} + [\text{Troponin AND/OR NYHA}] + \ln_H\text{gb} + \ln_S\text{BP}$

(8) $\text{AGE} + \text{ST2} + [\text{Troponin AND/OR NYHA}] + \ln_H\text{gb} + \ln_S\text{BP} + \ln_N\text{Tpro-BNP}$.

In some embodiments, 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 embodiments, the threshold level of ST2 is 35 or 50 ng/mL. In some embodiments, algorithm (1) or (2) is used and the threshold level of ST2 is 35 ng/mL. In some embodiments, algorithm

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 embodiments, 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 ~~invention~~ disclosure belongs. Methods and materials are described herein for use in the present ~~disclosureinvention~~; 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.

In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the ~~disclosure~~~~invention~~ will be apparent from the following detailed description and Figures, and from the claims.

DESCRIPTION OF DRAWINGS

5 Figure 1 shows the summary statistics for individual variables for 1 year-mortality.
 Figure 2 shows the summary statistics for individual variables for 5-year mortality.
 Figures 3-24 show linearity checks and cut-point evaluations performed for each variable.
 Figures 25 and 26 provide a summary of the results for each variable.

 Figures 27-34 show the results of several heuristic approaches used to identify the best
10 models for predicting risk of death, including backward, forward, and stepwise selection.
 Selection in each instance was made based on AIC (Akaike's Information Criteria) or BIC
 (Bayesian Information Criteria).

 Figure 35 shows the co-linearity analysis of several variables with risk of death.

 Figure 36 is a summary of the univariate performance of each variable.

15 Figures 37-49 show the results of linearity checks performed for each variable.

 Figure 50 provides a summary of the results for each variable.

 Figure 51 shows AIC-based marker selection.

 Figure 52 shows BIC-based marker selection.

 Figure 53 and 54 show a comparison of two models ($[Age + Ln_SBP + CAD + ST2$
20 $\geq 35 + LN_NTBNP]$ and $[Age + ST2 \geq 35 + LN_NTBNP]$).

 Figure 55 shows bootstrap AUC estimates for the 5-parameter and the 3-parameter
 model.

 Figure 56 is a graph showing the model calibration for the 5-parameter and the 3-
 parameter model.

25 Figure 57 is list of exemplary model parameters.

DETAILED DESCRIPTION

Clinical evaluation of patients, particularly patients with non-specific symptoms such as
dyspnea or chest pain, is often challenging. The results described

WHAT IS CLAIMED IS:

1. An *in vitro* method for evaluating the risk of mortality for a subject within one year, the method comprising:

determining a multimarker mortality risk score for a subject based upon:

the age of the subject;

the level of ST2 in a biological sample from 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), 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 one year.

2. The method of claim 1, wherein 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) $AGE + ST2 + [Troponin \text{ AND/OR } NYHA] + \ln_Hgb + \ln_SBP + \ln_NTpro\text{-}BNP$

3. The method of claim 2, wherein 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".

4. The method of claim 3, wherein the threshold is 35 or 50 ng/mL.