

Embodiment 61. The method of embodiment 59, wherein the abnormal stress test is a treadmill or nuclear medicine based test.

Embodiment 62. The method of embodiment 59, wherein the imaging suggesting coronary heart disease is an angiogram showing coronary artery stenosis of 50% or greater.

Embodiment 63. The method of any one of embodiments 27-62, comprising determining the risk of the subject having a secondary cardiovascular event within four years from the date the sample was taken from the subject.

Embodiment 64. The method of embodiment 63, wherein the risk of the subject having a secondary cardiovascular event is within one, two, three, or four years from the date that the sample was taken from the subject.

Embodiment 65. The method of embodiment 63 or 64, wherein the secondary cardiovascular event is myocardial infarction, stroke, trans-ischemic attack, hospitalization for heart failure, or death.

Embodiment 66. The method of any one of embodiments 63-65, wherein the risk is determined as a quantitative probability.

Embodiment 67. The method of any one of embodiments 63-65, wherein the risk is determined as a qualitative level of risk.

Embodiment 68. The method of embodiment 67, wherein the qualitative level of risk is low, moderate, or high.

[0019] In some embodiments, methods for screening a subject for the risk of a cardiovascular (CV) event are provided. In some such embodiments, a method comprises detecting, in a sample from the subject, the level of each biomarker of a biomarker panel comprising N protein biomarkers, wherein the biomarker panel comprises CHSTC, and protein biomarkers selected from N-terminal pro-BNP, sTREM-1, MMP-12, Antithrombin III, GPR56, Gelsolin, ST4S6, FSH, IL-1 sRII, PLXB2, SAP, and TFPI, wherein N is an integer from 3 to 13; and comparing the detected level of each of the biomarkers in the biomarker panel and identifying biomarkers that are differentially expressed relative to control levels of the corresponding biomarkers to identify the subject at risk of a CV event.

[0020] In some embodiments, a method for screening a subject for the risk of a cardiovascular event (CV) event comprises

(a) forming a biomarker panel comprising N protein biomarkers selected from BNP, sTREM-1, MMP-12, SVEP1, ARL11, ANTR2, CA125, GOLM1, PPR1A, ERBB3, suPAR, GDF-11/8, JAM-B, ATS13, Spondin-1, NCAM-120,

TFF3, SIRT2, ANP, NELL1, LRP11, NDST1, PTPRJ, CILP2, CA2D3, ITI heavy chain H2, and IGDC4, wherein N is an integer from 8 to 27; and

(b) detecting the level of each of the N biomarkers of the panel in a sample from the subject.

[0021] In some embodiments, methods for predicting the likelihood that a subject will have a CV event are provided. In some such embodiments, a method comprises detecting, in a sample from the subject, the level of each biomarker of a biomarker panel comprising N protein biomarkers, wherein the biomarker panel comprises CHSTC, and protein biomarkers selected from N-terminal pro-BNP, sTREM-1, MMP-12, Antithrombin III, GPR56, Gelsolin, ST4S6, FSH, IL-1 sRII, PLXB2, SAP, and TFPI, wherein N is an integer from 3 to 13; and comparing the detected level of each of the biomarkers in the biomarker panel and identifying biomarkers that are differentially expressed relative to control levels of the corresponding biomarkers to identify the subject at risk of a CV event.

[0022] In some embodiments, a method for predicting the likelihood that a subject will have a CV event comprises

- (a) forming a biomarker panel comprising N protein biomarkers selected from BNP, sTREM-1, MMP-12, SVEP1, ARL11, ANTR2, CA125, GOLM1, PPR1A, ERBB3, suPAR, GDF-11/8, JAM-B, ATS13, Spondin-1, NCAM-120, TFF3, SIRT2, ANP, NELL1, LRP11, NDST1, PTPRJ, CILP2, CA2D3, ITI heavy chain H2, and IGDC4, wherein N is an integer from 8 to 27; and
- (b) detecting the level of each of the N biomarkers of the panel in a sample from the subject.

[0023] In some embodiments, methods for screening a subject for the risk or likelihood of a cardiovascular event (CV) event are provided, comprising detecting the level of at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least

22, at least 23, at least 24, at least 25, at least 26, or 27 of the biomarkers in the panel comprising N biomarkers.

[0024] In some embodiments, the risk or likelihood of the subject having a CV event within 4 years is high if the levels of at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, or at least 13 biomarkers of the set of biomarkers are each abnormal relative to a control level of the respective biomarker.

What is claimed is:

1. A method for screening a subject for the risk of a cardiovascular (CV) event comprising:
detecting, in a sample from the subject, the level of each biomarker of a biomarker panel comprising N protein biomarkers, wherein the biomarker panel comprises CHSTC, and protein biomarkers selected from N-terminal pro-BNP, sTREM-1, MMP-12, Antithrombin III, GPR56, Gelsolin, ST4S6, FSH, IL-1 sRII, PLXB2, SAP, and TFPI, wherein N is an integer from 3 to 13; and
comparing the detected level of each of the biomarkers in the biomarker panel and identifying biomarkers that are differentially expressed relative to control levels of the corresponding biomarkers to identify the subject at risk of a CV event.
2. A method for predicting the likelihood that a subject will have a CV event comprising
detecting, in a sample from the subject, the level of each biomarker of a biomarker panel comprising N protein biomarkers, wherein the biomarker panel comprises CHSTC, and protein biomarkers selected from N-terminal pro-BNP, sTREM-1, MMP-12, Antithrombin III, GPR56, Gelsolin, ST4S6, FSH, IL-1 sRII, PLXB2, SAP, and TFPI, wherein N is an integer from 3 to 13; and
comparing the detected level of each of the biomarkers in the biomarker panel and identifying biomarkers that are differentially expressed relative to control levels of the corresponding biomarkers to identify the subject at risk of a CV event.
3. The method of claim 1 or 2, wherein N is at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, or 13.
4. The method of any one of claims 1-3, wherein the risk or likelihood of the subject having a CV event within 4 years is high if the levels of at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, or at least 13 biomarkers of the panel of biomarkers are each over-expressed or under-expressed relative to a control level of the respective biomarker.
5. The method of any one of claims 1-4, wherein the CV event is myocardial infarction, stroke, trans-ischemic attack, hospitalization for heart failure, or death.
6. The method of any one of claims 1-5, wherein the subject does not have a history of CV events.
7. The method of any one of the preceding claims, wherein the sample is selected from a blood sample, a serum sample, a plasma sample, and a urine sample.
8. The method of claim 7, wherein the sample is a blood sample.
9. The method of any one of the preceding claims, wherein the method is performed *in vitro*.

10. The method of any one of claims 1-9, wherein the method comprises contacting biomarkers of the sample from the subject with a set of capture reagents, wherein each capture reagent of the set of capture reagents specifically binds to a different biomarker being detected.
11. The method of claim 10, wherein each capture reagent is an antibody or an aptamer.
12. The method of claim 11, wherein each biomarker capture reagent is an aptamer.
13. The method of claim 12, wherein at least one aptamer is a slow off-rate aptamer, wherein each slow off-rate aptamer binds to its target protein with an off rate ($t_{1/2}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.
14. The method of claim 13, wherein at least one slow off-rate aptamer comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications.
15. The method of any one of claims 10-14, wherein one capture reagent specifically binds to sTREM1.
16. The method of any one of claims 10-15, wherein one capture reagent specifically binds to MMP-12.
17. The method of any one of claims 10-16, wherein one capture reagent specifically binds to N-terminal pro-BNP.
18. The method of any one of claims 10-17, wherein one capture reagent specifically binds to Antithrombin III.
19. The method of any one of claims 10-18, wherein one capture reagent specifically binds to GPR56.
20. The method of any one of claims 10-19, wherein one capture reagent specifically binds to Gelsolin.
21. The method of any one of claims 10-20, wherein one capture reagent specifically binds to ST4S6.
22. The method of any one of claims 10-21, wherein one capture reagent specifically binds to FSH.
23. The method of any one of claims 10-22, wherein one capture reagent specifically binds to IL-1 sRII.
24. The method of any one of claims 10-23, wherein one capture reagent specifically binds to PLXB2.
25. The method of any one of claims 10-24, wherein one capture reagent specifically binds to SAP.

26. The method of any one of claims 10-25, wherein one capture reagent specifically binds to TFPI.

27. The method of any one of claims 1-26, wherein the panel of biomarkers comprises at least 13 biomarkers.

28. The method of any one of claims 1-26, wherein the panel of biomarkers consists of 13 biomarkers.

29. The method of any one of claims 1-28, wherein the subject is at least 40 years old.

30. The method of any one of claims 1-29, comprising determining the risk or likelihood of the subject having a cardiovascular event within four years from the date the sample was taken from the subject.

31. The method of claim 30, wherein the risk or likelihood of the subject having a cardiovascular event is within one, two, three, or four years from the date that sample was taken from the subject.

32. The method of claim 30 or 31, wherein the cardiovascular event is myocardial infarction, stroke, trans-ischemic attack, hospitalization for heart failure, or death due to cardiovascular disease.

33. The method of any one of claims 30-32, wherein the risk is determined as a quantitative probability.

34. The method of any one of claims 30-32, wherein the risk is determined as a qualitative level of risk.

35. The method of claim 34, wherein the qualitative level of risk is low, moderate, or high.

36. The method of any one of the preceding claims, wherein the risk or likelihood of a CV event is based on the biomarker levels and at least one item of additional biomedical information selected from

- a) information corresponding to the presence of cardiovascular risk factors selected from the group consisting of prior myocardial infarction, angiographic evidence of greater than 50% stenosis in one or more coronary vessels, exercise-induced ischemia by treadmill or nuclear testing or prior coronary revascularization,
- b) information corresponding to physical descriptors of the subject,
- c) information corresponding to a change in weight of the subject,
- d) information corresponding to the ethnicity of the subject,
- e) information corresponding to the gender of the subject,
- f) information corresponding to the subject's smoking history,
- g) information corresponding to the subject's alcohol use history,
- h) information corresponding to the subject's occupational history,

- i) information corresponding to the subject's family history of cardiovascular disease or other circulatory system conditions,
- j) information corresponding to the presence or absence in the subject of at least one genetic marker correlating with a higher risk of cardiovascular disease in the subject or a family member of the subject,
- k) information corresponding to clinical symptoms of the subject,
- l) information corresponding to other laboratory tests,
- m) information corresponding to gene expression values of the subject, and
- n) information corresponding to the subject's consumption of known cardiovascular risk factors such as diet high in saturated fats, high salt, high cholesterol,
- o) information corresponding to the subject's imaging results obtained by techniques selected from the group consisting of electrocardiogram, echocardiography, carotid ultrasound for intima-media thickness, flow mediated dilation, pulse wave velocity, ankle-brachial index, stress echocardiography, myocardial perfusion imaging, coronary calcium by CT, high resolution CT angiography, MRI imaging, and other imaging modalities,
- p) information regarding the subject's medications,
- q) information corresponding to the age of the subject, and
- r) information regarding the subject's kidney function.

37. The method of claim 36, wherein the at least one item of additional biomedical information is information corresponding to the age of the subject.

38. The method of any one of the preceding claims, wherein the method comprises determining the risk or likelihood of a CV event for the purpose of determining a medical insurance premium or life insurance premium.

39. The method of claim 38, wherein the method further comprises determining coverage or premium for medical insurance or life insurance.

40. The method of any one of claims 1-39, wherein the method further comprises using information resulting from the method to predict and/or manage the utilization of medical resources.

41. The method of any one of claims 1-40, wherein the method further comprises using information resulting from the method to enable a decision to acquire or purchase a medical practice, hospital, or company.