

CLAIMS

1. A method of determining whether a subject has hepatic steatosis, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least one, and wherein at least one of the N biomarker proteins is selected from PTGR1, INHBC, and BPIB1.
2. The method of claim 1, comprising determining whether the subject has NASH with hepatic steatosis.
3. The method of claim 1 or 2, wherein N is 1 to 12, or N is 2 to 12, or N is 3 to 12, or N is 4 to 12, or N is 5 to 12, or N is 1 to 5, or N is 2 to 5, or N is 3 to 5, or N is 4 to 5.
4. The method of any one of the preceding claims, wherein N is 1, or N is 2, or N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12.
5. The method of any one of the preceding claims, wherein at least one of the N biomarker proteins is selected from PTGR1 and INHBC.
6. The method of any one of the preceding claims, wherein N is at least two, and wherein at least one of the N biomarker proteins is selected from FBP12, RECQ1, BGLR, CNDP1, SOM2, and GRID2.
7. The method of any one of the preceding claims, wherein N is at least two, and wherein at least one of the N biomarker proteins is selected from INSL5, HEXB, and ERN1.
8. The method of any one of the preceding claims, wherein each of the N biomarker proteins is selected from PTGR1, INHBC, BPIB1, FBP12, RECQ1, BGLR, CNDP1, SOM2, GRID2, INSL5, HEXB, and ERN1.
9. The method of any one of the preceding claims, wherein N is at least two, and wherein at least two of the N biomarker proteins are selected from PTGR1, CNDP1, and ERN1.
10. The method of any one of the preceding claims, wherein N is at least two, and wherein at least two of the N biomarker proteins are selected from PTGR1, INSL5, and HEXB.
11. The method of any one of claims 1 to 8, wherein N is at least two, and wherein at least two of the N biomarker proteins are selected from INHBC, HEXB, and CNDP1.
12. The method of any one of claims 1 to 8, wherein N is at least two, and wherein at least two of the N biomarker proteins are selected from BPIB1, CNDP1, INSL5, HEXB, and ERN1.
13. The method of any one of the preceding claims, wherein the subject has hepatic steatosis.
14. The method of claim 13, wherein the hepatic steatosis is mild, moderate, or severe hepatic steatosis.
15. The method of any one of claims 2 to 14, wherein the subject has NASH.
16. The method of claim 15, wherein the NASH is stage 1, 2, 3, or 4 NASH.

17. A method of determining whether a subject has hepatic inflammation, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least one, and wherein at least one of the N biomarker proteins is selected from MAAI, SAA2, RPN1, and PCOC2.
18. A method of determining whether a subject has hepatic inflammation, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least two, and wherein at least one of the N biomarker proteins is selected from MAAI, SAA2, RPN1, PCOC2, CA198, CTCF, and TACD2.
19. The method of claim 17 or 18, comprising determining whether the subject has NASH with hepatic inflammation.
20. The method of any one of claims 17 to 19, wherein N is 1 to 14, or N is 2 to 14, or N is 3 to 14, or N is 4 to 14, or N is 5 to 14, or N is 6 to 14, or N is 7 to 14, or N is 8 to 14, or N is 1 to 8, or N is 2 to 8, or N is 3 to 8, or N is 4 to 8, or N is 5 to 8.
21. The method of any one of claims 17 to 20, wherein N is 1, or N is 2, or N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12, or N is 13, or N is 14.
22. The method of any one of claims 17 to 21, wherein N is at least two, and wherein at least one of the N biomarker proteins is selected from CA198, CTCF, and TACD2.
23. The method of any one of claims 17 to 22, wherein N is at least two, and wherein at least one of the N biomarker proteins is selected from PPAC, ADIPO, PYY, FCG3B, TRXR1, ACY1, and CCL23.
24. The method of any one of claims 17 to 23, wherein each of the N biomarker proteins is selected from MAAI, SAA2, RPN1, PCOC2, CA198, CTCF, TACD2, PPAC, ADIPO, PYY, FCG3B, TRXR1, ACY1, and CCL23.
25. The method of any one of claims 17 to 24, wherein N is at least two, and wherein at least two of the N biomarker proteins are selected from PCOC2, PYY, and TRXR1.
26. The method of any one of claims 18 to 24, wherein N is at least two, and wherein at least two of the N biomarker proteins are selected from TACD2, TRXR1, and ACY1.
27. The method of any one of claims 18 to 24, wherein N is at least two, and wherein at least two of the N biomarker proteins are CA198 and TRXR1.
28. The method of any one of claims 18 to 24, wherein N is at least two, and wherein at least two of the N biomarker proteins are selected from CA198, FCG3B, and ACY1.
29. The method of any one of claims 17 to 24, wherein N is at least two, and wherein at least two of the N biomarker proteins are selected from RPN1, PYY, and ACY1.

30. The method of any one of claims 18 to 24, wherein N is at least two, and wherein at least two of the N biomarker proteins are selected from TACD2, PPAC, and TRXR1.
31. The method of any one of claims 18 to 24, wherein N is at least two, and wherein at least two of the N biomarker proteins are selected from CTCF, ADIPO, and TRXR1.
32. The method of any one of claims 17 to 24, wherein N is at least two, and wherein at least two of the N biomarker proteins are selected from SAA2, PPAC, and ACY1.
33. The method of any one of claims 17 to 32, wherein the subject has hepatic inflammation.
34. The method of claim 33, wherein the hepatic inflammation is mild, moderate, or severe hepatic inflammation.
35. The method of any one of claims 19 to 34, wherein the subject has NASH.
36. The method of claim 35, wherein the NASH is stage 1, 2, 3, or 4 NASH.
37. A method of determining whether a subject has hepatocellular ballooning, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least one, and wherein at least one of the N biomarker proteins is selected from PTGR1, ATL2, and CNN2.
38. The method of claim 37, comprising determining whether the subject has NASH with hepatocellular ballooning.
39. The method of claim 37 or 38, wherein N is 1 to 5, or N is 2 to 5, or N is 3 to 5, or N is 4 to 5, or N is 1 to 2, or N is 1 to 3, or N is 1 to 4.
40. The method of any one of claims 37 to 39, wherein N is 1, or N is 2, or N is 3, or N is 4, or N is 5.
41. The method of any one of claims 37 to 40, wherein N is at least two, and wherein at least one of the N biomarker proteins is selected from AK1BA and CTLA4.
42. The method of any one of claims 37 to 41, wherein each of the N biomarker proteins is selected from PTGR1, ATL2, CNN2, AK1BA and CTLA4.
43. The method of any one of claims 17 to 42, wherein N is at least three, and at least three of the N biomarker proteins are AK1BA, PTGR1, and ATL2.
44. The method of any one of claims 37 to 43, wherein the subject has hepatocellular ballooning.
45. The method of claim 44, wherein the hepatocellular ballooning is mild, moderate, or severe hepatocellular ballooning.
46. The method of any one of claims 38 to 45, wherein the subject has NASH.
47. The method of claim 46, wherein the NASH is stage 1, 2, 3, or 4 NASH.

48. A method of determining whether a subject has hepatic fibrosis, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least one, and wherein at least one of the N biomarker proteins is selected from ATL2, NFASC, and FCRL3.
49. The method of claim 48, comprising determining whether the subject has NASH with hepatic fibrosis.
50. The method of claim 48 or 49, wherein N is 1 to 8, or N is 2 to 8, or N is 3 to 8, or N is 4 to 8, or N is 5 to 8, or N is 6 to 8, or N is 7 to 8, or N is 1 to 2, or N is 1 to 3, or N is 1 to 4, or N is 1 to 5, or N is 1 to 6, or N is 1 to 7.
51. The method of any one of claims 48 to 50, wherein N is 1, or N is 2, or N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8.
52. The method of claims 48 to 51, wherein N is at least two, and wherein at least one of the N biomarker proteins is selected from CO7, COL11, VGFR2, WNT5A, and PLOD3.
53. The method of any one of claims 48 to 41, wherein each of the N biomarker proteins is selected from ATL2, NFASC, FCRL3, CO7, COL11, VGFR2, WNT5A, and PLOD3.
54. The method of any one of claims 48 to 53, wherein N is at least two, and at least two of the N biomarker proteins are ATL2 and VGFR2.
55. The method of any one of claims 48 to 53, wherein N is at least two, and at least two of the N biomarker proteins are selected from ATL2, COL11, and WNT5A.
56. The method of any one of claims 48 to 53, wherein N is at least two, and at least two of the N biomarker proteins are selected from ATL2, CO7, and WNT5A.
57. The method of any one of claims 48 to 56, wherein the subject has hepatic fibrosis.
58. The method of claim 57, wherein the hepatic fibrosis is mild, moderate, or severe hepatic fibrosis.
59. The method of any one of claims 49 to 58, wherein the subject has NASH.
60. The method of claim 59, wherein the NASH is stage 1, 2, 3, or 4 NASH.
61. The method of any one of the preceding claims, wherein the sample is a blood sample, a plasma sample, or a serum sample.
62. The method of any one of the preceding claims, wherein the subject is at risk of liver disease.
63. The method of any one of the preceding claims, wherein the subject is at risk of developing or of having hepatic steatosis, hepatic inflammation, hepatocellular ballooning, and/or hepatic fibrosis.
64. The method of any one of the preceding claims, wherein the subject is obese.

65. The method of any one of the preceding claims, wherein the method comprises contacting the N biomarker proteins of the sample or samples with a set of N biomarker capture reagents, wherein each biomarker capture reagent of the set of biomarker capture reagents specifically binds to a different biomarker protein being detected.
66. The method of claim 65, wherein each biomarker capture reagent is an antibody or an aptamer.
67. The method of claim 66, wherein each biomarker capture reagent is an aptamer.
68. The method of claim 67, wherein at least one aptamer is a slow off-rate aptamer.
69. The method of claim 68, 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.
70. The method of claim 68 or claim 69, 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.
71. The method of any one of the preceding claims, wherein the subject has or likely has hepatic steatosis, hepatic inflammation, hepatocellular ballooning, hepatic fibrosis, and/or NASH.
72. The method of any one of the preceding claims, wherein the determining comprises analyzing the levels of the N biomarker protein levels using a classification model or an elastic net logistic regression model.
73. The method of any one of the preceding claims, wherein the method comprises determining whether the subject has or likely has hepatic steatosis, hepatic inflammation, hepatocellular ballooning, hepatic fibrosis, and/or NASH for the purpose of determining a medical insurance premium or life insurance premium.
74. The method of any one of the preceding claims, wherein the method further comprises determining a medical insurance premium or life insurance premium.
75. The method of any one of the preceding claims, wherein the method further comprises using information resulting from the method to predict and/or manage the utilization of medical resources.
76. A kit comprising N biomarker protein capture reagents, wherein the N biomarker protein capture reagents bind to the N biomarker proteins of any one of claims 1, 3 to 12, 17, 19, 20 to 32, 37, 39 to 43, 48, and 50 to 56.
77. The kit of claim 76, wherein each of the N biomarker protein capture reagents specifically binds to a different biomarker protein.

78. The kit of claim 76 or 77, wherein each of the N biomarker capture reagents is an antibody or an aptamer.
79. The kit of claim 78, wherein each biomarker capture reagent is an aptamer.
80. The kit of claim 79, wherein at least one aptamer is a slow off-rate aptamer.
81. The kit of claim 80, 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.
82. The kit of claim 80 or claim 81, 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.
83. The kit of any one of claims 76 to 82, for use in detecting the N biomarker proteins in a sample from a subject.
84. The kit of claim 83, for use in determining whether the subject has or likely has hepatic steatosis, hepatic inflammation, hepatocellular ballooning, hepatic fibrosis, and/or NASH.
85. The method of any one of claims 1 to 16, comprising determining whether a treatment for NASH should be administered to the subject, wherein the treatment comprises: testing the subject for diabetes and/or cardiovascular disease, administering a drug to the subject, wherein the drug treats the hepatic steatosis and/or NASH, and/or performing a gastric bypass surgery on the subject.
86. The method of any one of claims 1 to 16, wherein the method comprises determining whether the subject responds to treatment for NASH, comprising:
- a) detecting the level of each of the N biomarker proteins in a first sample from the subject, wherein the first sample is obtained from the subject prior to the subject being treated for NASH, thereby determining a pretreatment status of the subject;
 - b) administering a treatment for NASH to the subject, wherein the treatment comprises: testing the subject for diabetes and/or cardiovascular disease, administering a drug to the subject, wherein the drug treats the hepatic steatosis and/or NASH, and/or performing a gastric bypass surgery on the subject;
 - c) detecting the level of each of the same N biomarker proteins detected in part (a) in a second sample from the subject, wherein the second sample is obtained from the subject after the subject is administered the treatment for NASH, thereby determining a treatment status of the subject; and
 - d) comparing the pretreatment status and the treatment status,

wherein, the subject is responding to the NASH treatment if the comparison indicates that the hepatic steatosis has decreased in severity compared to the pretreatment status of the same subject.

87. The method of any one of claims 1 to 16, comprising determining whether a subject is in need of a liver biopsy, wherein the presence of hepatic steatosis indicates that the subject needs a liver biopsy.

88. The method of any one of claims 1 to 16, comprising determining whether the subject has fatty liver disease.

89. The method of any one of claims 17 to 36, comprising determining whether a treatment for NASH should be administered to the subject, wherein the treatment comprises: testing the subject for diabetes and/or cardiovascular disease, administering a drug to the subject, wherein the drug treats the hepatic inflammation and/or NASH, and/or performing a gastric bypass surgery on the subject.

90. The method of any one of claims 17 to 36, wherein the method comprises determining whether the subject responds to treatment for NASH, comprising:

- a) detecting the level of each of the N biomarker proteins in a first sample from the subject, wherein the first sample is obtained from the subject prior to the subject being treated for NASH, thereby determining a pretreatment status of the subject;
- b) administering a treatment for NASH to the subject, wherein the treatment comprises: testing the subject for diabetes and/or cardiovascular disease, administering a drug to the subject, wherein the drug treats the hepatic inflammation and/or NASH, and/or performing a gastric bypass surgery on the subject;
- c) detecting the level of each of the same N biomarker proteins detected in part (a) in a second sample from the subject, wherein the second sample is obtained from the subject after the subject is administered the treatment for NASH, thereby determining a treatment status of the subject; and
- d) comparing the pretreatment status and the treatment status, wherein, the subject is responding to the NASH treatment if the comparison indicates that the hepatic inflammation has decreased in severity compared to the pretreatment status of the same subject.

91. The method of any one of claims 17 to 36, comprising determining whether a subject is in need of a liver biopsy, wherein the presence of hepatic inflammation indicates that the subject needs a liver biopsy.

92. The method of any one of claims 17 to 36, comprising determining whether the subject has fatty liver disease.

93. The method of any one of claims 37 to 47, comprising determining whether a treatment for NASH should be administered to the subject, wherein the treatment comprises: testing the subject for diabetes and/or cardiovascular disease, administering a drug to the subject, wherein the drug treats the hepatocellular ballooning and/or NASH, and/or performing a gastric bypass surgery on the subject.

94. The method of any one of claims 37 to 47, wherein the method comprises determining whether the subject responds to treatment for NASH, comprising:

- a) detecting the level of each of the N biomarker proteins in a first sample from the subject, wherein the first sample is obtained from the subject prior to the subject being treated for NASH, thereby determining a pretreatment status of the subject;
- b) administering a treatment for NASH to the subject, wherein the treatment comprises: testing the subject for diabetes and/or cardiovascular disease, administering a drug to the subject, wherein the drug treats the hepatocellular ballooning and/or NASH, and/or performing a gastric bypass surgery on the subject;
- c) detecting the level of each of the same N biomarker proteins detected in part (a) in a second sample from the subject, wherein the second sample is obtained from the subject after the subject is administered the treatment for NASH, thereby determining a treatment status of the subject; and
- d) comparing the pretreatment status and the treatment status, wherein, the subject is responding to the NASH treatment if the comparison indicates that the hepatocellular ballooning has decreased in severity compared to the pretreatment status of the same subject.

95. The method of any one of claims 37 to 47, comprising determining whether a subject is in need of a liver biopsy, wherein the presence of hepatocellular ballooning indicates that the subject needs a liver biopsy.

96. The method of any one of claims 37 to 47, comprising determining whether the subject has fatty liver disease.

97. The method of any one of claims 48 to 60, comprising determining whether a treatment for NASH should be administered to the subject, wherein the treatment comprises: testing the subject for diabetes and/or cardiovascular disease, administering a drug to the subject, wherein the drug treats the hepatic fibrosis and/or NASH, and/or performing a gastric bypass surgery on the subject.

98. The method of any one of claims 48 to 60, wherein the method comprises determining whether the subject responds to treatment for NASH, comprising:

- a) detecting the level of each of the N biomarker proteins in a first sample from the subject, wherein the first sample is obtained from the subject prior to the subject being treated for NASH, thereby determining a pretreatment status of the subject;
- b) administering a treatment for NASH to the subject, wherein the treatment comprises: testing the subject for diabetes and/or cardiovascular disease, administering a drug to the subject, wherein the drug treats the hepatic fibrosis and/or NASH, and/or performing a gastric bypass surgery on the subject;
- c) detecting the level of each of the same N biomarker proteins detected in part (a) in a second sample from the subject, wherein the second sample is obtained from the subject after the subject is administered the treatment for NASH, thereby determining a treatment status of the subject; and
- d) comparing the pretreatment status and the treatment status, wherein, the subject is responding to the NASH treatment if the comparison indicates that the hepatic fibrosis has decreased in severity compared to the pretreatment status of the same subject.

99. The method of any one of claims 48 to 60, comprising determining whether a subject is in need of a liver biopsy, wherein the presence of hepatic fibrosis indicates that the subject needs a liver biopsy.

100. The method of any one of claims 48 to 60, comprising determining whether the subject has fatty liver disease.

101. The method of any one of claims 1 to 72, further comprising determining whether a treatment for NASH should be administered to the subject.

102. The method of claim 101, wherein the treatment comprises: testing the subject for diabetes and/or cardiovascular disease; administering a drug to the subject, wherein the drug treats the at least one condition selected from hepatic steatosis, hepatic inflammation,

hepatocellular ballooning, and hepatic fibrosis, and/or the drug treats NASH; and/or performing a gastric bypass surgery on the subject.

103. The method of any one of claims 1 to 72, wherein the method comprises determining whether the subject responds to treatment for NASH, comprising:

- a) determining a pretreatment status of the subject, comprising detecting the level of each of the N biomarker proteins in a first sample from the subject, wherein the first sample is obtained from the subject prior to the subject being treated for NASH, thereby determining a pretreatment status of the subject;
- b) administering a treatment for NASH to the subject, wherein the treatment comprises: testing the subject for diabetes and/or cardiovascular disease; administering a drug to the subject, wherein the drug treats at least one condition selected from hepatic steatosis, hepatic inflammation, hepatocellular ballooning, and hepatic fibrosis, and/or the drug treats NASH; and/or performing a gastric bypass surgery on the subject;
- c) determining a treatment status of the subject, comprising detecting the level of each of the same N biomarker proteins detected in part (a) in a second sample from the subject, wherein the second sample is obtained from the subject after the subject is administered the treatment for NASH; and
- d) comparing the pretreatment status and the treatment status, wherein, the subject is responding to the NASH treatment if the comparison indicates that at least one condition selected from hepatic steatosis, hepatic inflammation, hepatocellular ballooning, and hepatic fibrosis has decreased in severity compared to the pretreatment status of the same subject.

104. The method of any one of claims 1 to 72, further comprising determining whether the subject is in need of a liver biopsy, wherein the presence of at least one condition selected from hepatic steatosis, hepatic inflammation, hepatocellular ballooning, and hepatic fibrosis indicates that the subject needs a liver biopsy.

105. The method of any one of claims 101 to 104, comprising determining whether the subject has fatty liver disease.

106. The method of any one of claims 85, 87 to 89, 91 to 93, 95 to 97, 99 to 102, 104, and 105, wherein the sample is a blood sample, a plasma sample, or a serum sample.

107. The method of any one of claims 86, 90, 94, 98, and 103, wherein the first sample and the second sample are blood samples, plasma samples, and serum samples, and wherein the first sample and the second sample are the same type of samples.
108. The method of any one of claims 85 to 107, wherein the subject is at risk of liver disease.
109. The method of any one of claims 85 to 108, wherein the subject is at risk of developing or of having hepatic steatosis, hepatic inflammation, hepatocellular ballooning, and/or hepatic fibrosis.
110. The method of any one of claims 85 to 109, wherein the subject is obese.
111. The method of any one of claims 85 to 110, wherein the method comprises contacting the N biomarker proteins of the sample or samples with a set of N biomarker capture reagents, wherein each biomarker capture reagent of the set of biomarker capture reagents specifically binds to a different biomarker protein being detected.
112. The method of claim 111, wherein each biomarker capture reagent is an antibody or an aptamer.
113. The method of claim 112, wherein each biomarker capture reagent is an aptamer.
114. The method of claim 113, wherein at least one aptamer is a slow off-rate aptamer.
115. The method of claim 114, 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.
116. The method of claim 114 or claim 115, 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.
117. The method of any one of claims 85 to 116, wherein the subject has or likely has hepatic steatosis, hepatic inflammation, hepatocellular ballooning, hepatic fibrosis, and/or NASH.
118. The method of any one of claims 85 to 117, wherein the determining comprises analyzing the levels of the N biomarker protein levels using a classification model or an elastic net logistic regression model.
119. The method of any one of claims 85 to 118, wherein the method comprises determining whether the subject has or likely has hepatic steatosis, hepatic inflammation, hepatocellular ballooning, hepatic fibrosis, and/or NASH for the purpose of determining a medical insurance premium or life insurance premium.
120. The method of any one of claims 85 to 119, wherein the method further comprises determining a medical insurance premium or life insurance premium.

121. The method of any one of claims 85 to 120, wherein the method further comprises using information resulting from the method to predict and/or manage the utilization of medical resources.