

results of routine blood tests. In NAFLD, liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are often high.

[0007] The current gold standard to confirm NASH is a histological evaluation of liver biopsy, which is expensive, invasive, and can cause pain, hemorrhage, or even death.

[0008] A simple blood test that would identify and distinguish the various stages of liver disease, including NASH (and thereby reduce the need for liver biopsy), would be highly desirable.

SUMMARY

[0009] In some examples, methods of determining whether a subject has liver disease are provided. In some examples, methods of identifying subjects with hepatic steatosis, hepatic inflammation, hepatic fibrosis, or hepatocellular ballooning are provided. In various examples, the liver disease includes nonalcoholic steatohepatitis (NASH).

[0010] In some examples, methods of determining whether a subject has nonalcoholic steatohepatitis (NASH) are provided. In some examples, methods of identifying subjects with NASH are provided. In some examples, methods of distinguishing subjects with NASH from subjects with hepatic steatosis, hepatic inflammation, hepatic fibrosis, or hepatocellular ballooning are provided. In some examples, methods of determining the severity of NASH are provided.

[0011] In one aspect, the present disclosure refers to 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 PTGR1, and wherein the sample is a blood sample. In some examples, 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. In some examples, 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. In some examples, the method comprises determining whether the subject has NASH.

[0012] In some examples, a method of determining whether a subject has hepatic steatosis is provided, 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, wherein at least one of the N biomarker proteins is selected from PTGR1, INHBC, and BPIB1. In some examples, at least one of the N biomarker proteins is selected from PTGR1 and INHBC. In some examples, N is at least two, at least one of the N biomarker proteins is selected from PTGR1, INHBC, and BPIB1, and at least one of the N biomarker proteins is selected from FBP12, RECQ1, BGLR, CNDP1, SOM2, and GRID2. In

some examples, N is at least two, at least one of the N biomarker proteins is selected from PTGR1, INHBC, and BPIB1, and at least one of the N biomarker proteins is selected from INSL5, HEXB, and ERN1. In some such examples, each of the N biomarker proteins is selected from PTGR1, INHBC, BPIB1, FBP12, RECQ1, BGLR, CNDP1, SOM2, GRID2, INSL5, HEXB, and ERN1.

[0013] In some examples, N is at least two, and at least two of the N biomarker proteins are selected from PTGR1, CNDP1, and ERN1; PTGR1, INSL5, and HEXB; INHBC, HEXB, and CNDP1; or BPIB1, CNDP1, INSL5, HEXB, and ERN1.

[0014] In one example, the present disclosure refers to a method for 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. In some examples, N is at least two, and at least one of the N biomarker proteins is selected from MAAI, SAA2, RPN1, PCOC2, CA198, CTCF, and TACD2. In some examples, 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. In some examples, 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. In some examples, the method comprises determining whether the subject has NASH.

[0014a] In one example, the present disclosure refers to 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.

[0015] In some examples, a method of determining whether a subject has hepatic inflammation is provided, 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, wherein at least one of the N biomarker proteins is selected from CA198, CTCF, and TACD2; or PPAC, ADIPO, PYY, FCG3B, TRXR1, ACY1, and CCL23. In some such examples, each of the N biomarker proteins is selected from MAAI, SAA2, RPN1, PCOC2, CA198, CTCF, TACD2, PPAC, ADIPO, PYY, FCG3B, TRXR1, ACY1, and CCL23.

[0016] In some examples, N is at least two, and at least two of the N biomarker proteins are selected from PCOC2, PYY, and TRXR1; TACD2, TRXR1, and ACY1; CA198 and TRXR1; CA198, FCG3B, and ACY1; RPN1, PYY, and ACY1; TACD2, PPAC, and TRXR1; CTCF, ADIPO, and TRXR1; or SAA2, PPAC, and ACY1.

[0017] In one example, the present disclosure refers to a method for 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. In some examples, 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. In some examples, N is 1, or N is 2, or N is 3, or N is 4, or N is 5. In some examples, the method comprises determining whether the subject has NASH.

[0018] In some examples, a method of determining whether a subject has hepatocellular ballooning is provided, 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, wherein at least one of the N biomarker proteins is selected from AK1BA and CTLA4. In some such examples, each of the N biomarker proteins is selected from PTGR1, ATL2, CNN2, AK1BA and CTLA4.

[0019] In some examples, N is at least three, and at least three of the N biomarker proteins are selected from AK1BA, PTGR1, and ATL2.

[0020] In one example, the present disclosure refers to a method for 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. In some examples, 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. In some examples, 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. In some examples, the method comprises determining whether the subject has NASH.

[0021] In some examples, a method of determining whether a subject has hepatic fibrosis is provided, 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, wherein at least one of the N biomarker proteins is selected from CO7, COL11, VGFR2, WNT5A, and PLOD3. In some such examples, each of the N biomarker proteins is selected from ATL2, NFASC, FCRL3, CO7, COL11, VGFR2, WNT5A, and PLOD3.

[0022] In some examples, N is at least two, and at least two of the N biomarker proteins are ATL2 and VGFR2; or are selected from ATL2, COL11, and WNT5A; or ATL2, CO7, and WNT5A.

[0023] In any of the examples described herein, the subject is at risk of developing hepatic steatosis, hepatic inflammation, hepatocellular ballooning and/or hepatic fibrosis.

[0024] In any of the examples described herein, the subject may be at risk of developing NASH. In any of the examples described herein, the subject may have a NASH

comorbidity selected from obesity, abdominal obesity, metabolic syndrome, cardiovascular disease, and diabetes. In any of the examples described herein, the subject may be obese.

[0025] In any of the examples described herein, a method comprises contacting protein biomarkers of the sample from the subject with a set of biomarker capture reagents, wherein each biomarker capture reagent of the set of biomarker capture reagents specifically binds to a different biomarker being detected. In some examples, each biomarker capture reagent is an antibody or an aptamer. In some examples, each biomarker capture reagent is an aptamer. In some examples, at least one aptamer is a slow off-rate aptamer. In some examples, 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. In some examples, 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.

[0026] In any of the examples described herein, the sample may be a blood sample. In any of the examples described herein, the sample may be selected from a serum sample and a plasma sample.

[0027] In any of the examples described herein, if the subject has hepatic steatosis, hepatic inflammation, hepatocellular ballooning, hepatic fibrosis, and/or NASH, the subject may be recommended a regimen selected from weight loss, blood sugar control, alcohol avoidance, testing the subject for diabetes and/or cardiovascular disease, performing a gastric bypass surgery on the subject, and administering a drug to the subject.

[0028] In some examples, a method described herein is for the purpose of determining a medical insurance premium or life insurance premium. In some examples, a method further comprises determining a medical insurance premium or life insurance premium. In some examples, a method described herein further comprises using information resulting from the method to predict and/or manage the utilization of medical resources.

[0029] In some examples, kits are provided. In some examples, a kit comprises N biomarker protein capture reagents that bind to N biomarker proteins selected from Table 1, 3, 5, or 7, wherein N is at least 1. In one aspect, the present disclosure refers to a kit for determining whether a subject has hepatic steatosis, the kit comprising N biomarker protein capture reagents, wherein each of the N biomarker protein capture reagents bind to a biomarker protein, wherein one of the biomarker proteins is PTGR1. In some examples, each of the N biomarker capture reagents specifically binds to a different biomarker protein. In some examples, each capture reagent is an antibody or an aptamer.

[0030] In any of the examples described herein, at least one aptamer may be a slow off-rate aptamer. In any of the examples described herein, each aptamer may be a slow off-rate aptamer. In some examples, at least one slow off-rate aptamer comprises at least one, at

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 PTGR1, and wherein the sample is a blood sample.
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 N is at least two, and wherein at least one of the N biomarker proteins is chosen from INHBC, BPIB1, FBP12, RECQ1, BGLR, CNDP1, SOM2, and GRID2.
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 chosen from INSL5, HEXB, and ERN1.
7. The method of any one of the preceding claims, wherein each of the N biomarker proteins is chosen from PTGR1, INHBC, BPIB1, FBP12, RECQ1, BGLR, CNDP1, SOM2, GRID2, INSL5, HEXB, and ERN1.
8. 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 (a) PTGR1 and (b) CNDP1 or 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 (a) PTGR1 and (b) INSL5 or HEXB.
10. The method of any one of claims 1 to 7, wherein N is at least three, and wherein at least two of the N biomarker proteins are chosen from INHBC, HEXB, and CNDP1.
11. The method of any one of claims 1 to 7, wherein N is at least three, and wherein at least two of the N biomarker proteins are chosen from BPIB1, CNDP1, INSL5, HEXB, and ERN1.
12. The method of any one of the preceding claims, wherein the subject has hepatic steatosis.
13. The method of claim 12, wherein the hepatic steatosis is mild, moderate, or severe hepatic steatosis.
14. The method of any one of claims 2 to 13, wherein the subject has NASH.
15. The method of claim 14, wherein the NASH is stage 1, 2, 3, or 4 NASH.
16. The method of any one of the preceding claims, wherein the blood sample is a plasma sample or a serum sample.

17. The method of any one of the preceding claims, wherein the subject is at risk of liver disease.
18. 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.
19. The method of any one of the preceding claims, wherein the subject is obese.
20. 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.
21. The method of claim 20, wherein each biomarker capture reagent is an antibody or an aptamer.
22. The method of claim 21, wherein each biomarker capture reagent is an aptamer.
23. The method of claim 22, wherein at least one aptamer is a slow off-rate aptamer.
24. The method of claim 23, 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.
25. The method of claim 23 or claim 24, 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.
26. 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.
27. 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.
28. 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.
29. The method of any one of the preceding claims, wherein the method further comprises determining a medical insurance premium or life insurance premium.
30. 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.

31. A kit for determining whether a subject has hepatic steatosis, the kit comprising N biomarker protein capture reagents, wherein each of the N biomarker protein capture reagents bind to a biomarker protein, wherein one of the biomarker proteins is PTGR1.
32. The kit of claim 31, wherein N is at least two, and wherein at least one of the N biomarker protein capture reagents binds to INHBC, BPIB1, FBP12, RECQ1, BGLR, CNDP1, SOM2, and GRID2.
33. The kit of claim 31, wherein N is at least two, and wherein at least one of the N biomarker protein capture reagents binds to a biomarker protein chosen from INSL5, HEXB, and ERN1.
34. The kit of any one of claims 31 to 33, wherein each of the N biomarker protein capture reagents binds to a biomarker protein chosen from PTGR1, INHBC, BPIB1, FBP12, RECQ1, BGLR, CNDP1, SOM2, GRID2, INSL5, HEXB, and ERN1.
35. The kit of any one of claims 31 to 34, wherein N is at least two, and wherein at least two of the N biomarker protein capture reagents binds to (a) PTGR1 and (b) CNDP1 or ERN1.
36. The kit of any one of claims 31 to 34, wherein N is at least two, and wherein at least two of the N biomarker protein capture reagents bind to (a) PTGR1 and (b) INSL5 or HEXB.
37. The kit of any one of claims 31 to 36, wherein N is at least three, and wherein at least two of the N biomarker protein capture reagents binds to a biomarker protein chosen from INHBC, HEXB, and CNDP1.
38. The kit of any one of claims 31 to 36, wherein N is at least three, and wherein at least two of the N biomarker protein capture reagents binds to a biomarker protein chosen from BPIB1, CNDP1, INSL5, HEXB, and ERN1.
39. The kit of any one of claims 31 to 38, wherein each of the N biomarker protein capture reagents specifically binds to a different biomarker protein.
40. The kit of any one of claims 31 to 39, wherein each of the N biomarker capture reagents is an antibody or an aptamer.
41. The kit of claim 40, wherein each biomarker capture reagent is an aptamer.
42. The kit of claim 41, wherein at least one aptamer is a slow off-rate aptamer.
43. The kit of claim 42, 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.
44. The kit of claim 42 or claim 43, 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.

45. The kit of any one of claims 31 to 44, for use in detecting the N biomarker proteins in a sample from a subject.
46. The kit of claim 45, for use in determining whether the subject has or likely has hepatic steatosis, hepatic inflammation, hepatocellular ballooning, hepatic fibrosis, and/or NASH.
47. The method of any one of claims 1 to 15, 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.
48. The method of any one of claims 1 to 15, comprising determining whether the subject has fatty liver disease.
49. The method of any one of claims 1 to 48, further comprising determining whether a treatment for NASH should be administered to the subject.
50. The method of any one of claims 1 to 48, further comprising determining whether the subject is in need of a liver biopsy, wherein the presence of at least one condition chosen from hepatic steatosis, hepatic inflammation, hepatocellular ballooning, and hepatic fibrosis indicates that the subject needs a liver biopsy.
51. The method of claim 49 or 50, comprising determining whether the subject has fatty liver disease.
52. The method of any one of claims 47-51, wherein the blood sample is a plasma sample or a serum sample.
53. The method of any one of claims 47 to 52, wherein the subject is at risk of liver disease.
54. The method of any one of claims 47 to 53, wherein the subject is at risk of developing or of having hepatic steatosis, hepatic inflammation, hepatocellular ballooning, and/or hepatic fibrosis.
55. The method of any one of claims 47 to 54, wherein the subject is obese.
56. The method of any one of claims 47 to 55, 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.
57. The method of claim 56, wherein each biomarker capture reagent is an antibody or an aptamer.
58. The method of claim 57, wherein each biomarker capture reagent is an aptamer.
59. The method of claim 58, wherein at least one aptamer is a slow off-rate aptamer.
60. The method of claim 59, 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.

61. The method of claim 59 or claim 60, 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.
62. The method of any one of claims 47 to 61, wherein the subject has or likely has hepatic steatosis, hepatic inflammation, hepatocellular ballooning, hepatic fibrosis, and/or NASH.
63. The method of any one of claims 47 to 62, wherein the determining comprises analyzing the levels of the N biomarker protein levels using a classification model or an elastic net logistic regression model.
64. The method of any one of claims 47 to 63, 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.
65. The method of any one of claims 47 to 64, wherein the method further comprises determining a medical insurance premium or life insurance premium.
66. The method of any one of claims 47 to 65, wherein the method further comprises using information resulting from the method to predict and/or manage the utilization of medical resources.