

from the glycans that are present on the same protein from normal cells. Indeed, numerous studies have observed changes in N-linked glycosylation with the development of cirrhosis and hepatocellular carcinoma (HCC) (*see, e.g.*, Naitoh, A., et al., Highly enhanced fucosylation of serum glycoproteins in patients with hepatocellular carcinoma. *J Gastroenterol Hepatol*, 14: 436-445, 1999; Block, T. M., et al., Use of targeted glycoproteomics to identify serum glycoproteins that correlate with liver cancer in woodchucks and humans. *Proc Natl Acad Sci U S A*, 102: 779-784, 2005). For example, in individuals chronically infected with HCV and having a diagnosis of HCC, the amount of fucosylated N-linked glycan derived from total protein preparations isolated from serum was consistently greater than that of healthy subjects or those with HCV and “inactive” disease (Comunale, M. A., et al., Proteomic analysis of serum associated fucosylated glycoproteins in the development of primary hepatocellular carcinoma. *Journal of Proteome Research*, 6: 308-315, 2006).

[0007] However, it has not yet been possible to identify a single glycoprotein, glycosylation pattern, biomarker suite, or other indicator of HCC that confers a high degree of sensitivity and ability for early detection. There remains an outstanding need for early detectors of HCC.

SUMMARY

[0008] Provided herein are methods for detecting hepatocellular carcinoma in a subject comprising removing IgG and IgM proteins from a biological fluid obtained from the subject; measuring the amount of one or more biomarkers in the biological fluid; determining the age and gender of the subject; and, determining the absence or presence of hepatocellular carcinoma in the subject using an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid.

[0008a] In one aspect, there is provided a method for detecting hepatocellular carcinoma in a subject comprising optionally removing IgG and IgM proteins from a biological fluid obtained from the subject; measuring the amount of one or more biomarkers in the biological fluid obtained from the subject, wherein the biomarkers comprise alpha-fetoprotein, fucosylated kininogen, and alkaline phosphatase; determining the age and gender of the subject; and, determining the absence or presence of hepatocellular carcinoma in the subject using an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid; and wherein the biological fluid is whole blood, serum or plasma.

[0009] The identities of individual biomarkers that are measured in accordance with the present disclosure are both specific and critical to the accuracy of the detection of hepatocellular

carcinoma, but for the sake of brevity, the inventive combinations of biomarkers are omitted from the present summary.

[0010] Also disclosed are assays for detecting hepatocellular carcinoma in a subject comprising measuring the amount of one or more biomarkers in the biological fluid, wherein the assay utilizes reagents for removing IgG and IgM proteins from a biological fluid of the subject prior to the measuring steps; determining the age and gender of the subject; and, determining the absence or presence of hepatocellular carcinoma in the subject using an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid.

[0010a] In another aspect, there is an assay for detecting hepatocellular carcinoma in a subject comprising measuring the amount of one or more biomarkers in the biological fluid, , wherein the biomarkers comprise alpha-fetoprotein, fucosylated kininogen, and alkaline phosphatase, and wherein the assay optionally utilizes a component for removing IgG and IgM proteins from a biological fluid of the subject prior to the measuring steps; determining the age and gender of the subject; and determining the absence or presence of hepatocellular carcinoma in the subject using an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid; wherein the biological fluid is whole blood, serum or plasma.

[0011] The present disclosure also pertains to kits for detecting hepatocellular carcinoma in a subject comprising a reagent for removing IgG protein from a biological fluid, and a reagent for removing IgM protein from a biological fluid from the subject; reagents for respectively measuring one or more biomarkers in the biological fluid; and, instructions for determining the absence or presence of hepatocellular carcinoma in the subject using an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid.

[0011a] In yet another aspect, there is provided a kit for detecting hepatocellular carcinoma in a subject comprising optionally, a component for removing IgG from a biological fluid from the subject; optionally, a component for removing IgM from a biological fluid from the subject; reagents for respectively measuring one or more biomarkers in the biological fluid; , wherein the biomarkers comprise alpha-fetoprotein, fucosylated kininogen, and alkaline phosphatase; and instructions for determining the absence or presence of hepatocellular carcinoma in the subject using an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid; wherein the biological fluid is whole blood, serum or plasma.

[0012] Also provided herein are methods for assigning a subject to a group having a higher or lower probability of hepatocellular carcinoma comprising removing IgG and IgM proteins from a biological fluid from the subject; measuring the amount of one or more biomarkers in the biological fluid; determining the age and gender of the subject; and, assigning the subject to a group having a higher or lower probability of hepatocellular carcinoma based on an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid.

[0012a] In another aspect, there is provided a method for assigning a subject to a group having a higher or lower probability of hepatocellular carcinoma comprising optionally removing IgG and IgM proteins from a biological fluid from the subject; measuring the amount of one or more biomarkers in the biological fluid obtained from the subject , wherein the biomarkers comprise alpha-fetoprotein, fucosylated kininogen, and alkaline phosphatase; determining the age and gender of the subject; and assigning the subject to a group having a higher or lower probability of hepatocellular carcinoma based on an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid; wherein the biological fluid is whole blood, serum or plasma.

[0013] The present disclosure also provides methods for managing treatment of a subject suspected of having hepatocellular carcinoma comprising removing IgG and IgM

proteins from a biological fluid from the subject; measuring the amount of one or more biomarkers in the biological fluid; determining the age and gender of the subject; and, treating the subject based on an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid, wherein the subject is treated for hepatocellular carcinoma when the subject is determined to have that disease based on the output of the function.

[0013a] In a further aspect there is provided a method for managing treatment of a subject suspected of having hepatocellular carcinoma comprising optionally removing IgG and IgM proteins from a biological fluid obtained from the subject; measuring the amount of one or more biomarkers in the biological fluid obtained from the subject, wherein the biomarkers comprise alpha-fetoprotein, fucosylated kininogen, and alkaline phosphatase; determining the age and gender of the subject; and, determining a treatment for the subject based on an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid, wherein the treatment is appropriate for hepatocellular carcinoma when the subject is determined to have that disease based on the output of the function; wherein the biological fluid is whole blood, serum or plasma.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIGS. 1A-1C depict the results of an evaluation of a panel consisting of fucosylated low molecular weight kininogen, alpha-fetoprotein, aspartate aminotransferase (AST), alkaline phosphatase (ALK), age, and gender. FIG. 1A summarizes the AUROC data for the discrimination of all patients with HCC (N=115) from all patients with cirrhosis (N=93); with early-stage HCC (N=69) from cirrhosis (N=93); with AFP negative (AFP⁻, having a level of AFP within the range that is observed among normal subjects, *e.g.*, without HCC) HCC (N=39) from AFP negative cirrhosis (N=84); and with both early-stage and AFP negative HCC (N=29) from AFP negative cirrhosis (N=84). FIG. 1B summarizes the sensitivity values at 90% specificity cutoffs for discrimination of the same respective patient groups in the same study, and

What is Claimed:

1. A method for detecting hepatocellular carcinoma in a subject comprising:
 - optionally removing IgG and IgM proteins from a biological fluid obtained from the subject;
 - measuring the amount of one or more biomarkers in the biological fluid obtained from the subject, wherein the biomarkers comprise alpha-fetoprotein, fucosylated kininogen, and alkaline phosphatase;
 - determining the age and gender of the subject;
 - determining the absence or presence of hepatocellular carcinoma in the subject using an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid; and
 - wherein the biological fluid is whole blood, serum or plasma.
2. An assay for detecting hepatocellular carcinoma in a subject comprising:
 - measuring the amount of one or more biomarkers in the biological fluid, , wherein the biomarkers comprise alpha-fetoprotein, fucosylated kininogen, and alkaline phosphatase, and wherein the assay optionally utilizes a component for removing IgG and IgM proteins from a biological fluid of the subject prior to the measuring steps;
 - determining the age and gender of the subject; and
 - determining the absence or presence of hepatocellular carcinoma in the subject using an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid;
 - wherein the biological fluid is whole blood, serum or plasma.
3. A kit for detecting hepatocellular carcinoma in a subject comprising:
 - optionally, a component for removing IgG from a biological fluid from the subject;
 - optionally, a component for removing IgM from a biological fluid from the subject;
 - reagents for respectively measuring one or more biomarkers in the biological fluid; , wherein the biomarkers comprise alpha-fetoprotein, fucosylated kininogen, and alkaline phosphatase; and,

instructions for determining the absence or presence of hepatocellular carcinoma in the subject using an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid;

wherein the biological fluid is whole blood, serum or plasma.

4. A method for assigning a subject to a group having a higher or lower probability of hepatocellular carcinoma comprising:

optionally removing IgG and IgM proteins from a biological fluid from the subject;

measuring the amount of one or more biomarkers in the biological fluid obtained from the subject, wherein the biomarkers comprise alpha-fetoprotein, fucosylated kininogen, and alkaline phosphatase;

determining the age and gender of the subject; and,

assigning the subject to a group having a higher or lower probability of hepatocellular carcinoma based on an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid;

wherein the biological fluid is whole blood, serum or plasma.

5. A method for managing treatment of a subject suspected of having hepatocellular carcinoma comprising:

optionally removing IgG and IgM proteins from a biological fluid obtained from the subject;

measuring the amount of one or more biomarkers in the biological fluid obtained from the subject, wherein the biomarkers comprise alpha-fetoprotein, fucosylated kininogen, and alkaline phosphatase;

determining the age and gender of the subject; and,

determining a treatment for the subject based on an optimized output of a function of the determined age and gender, and the measured biomarkers in the biological fluid, wherein the treatment is appropriate for hepatocellular carcinoma when the subject is determined to have that disease based on the output of the function;

wherein the biological fluid is whole blood, serum or plasma.

6. The method according to any one of claims 1, 4, and 5, wherein the function comprises respective optimized weighting coefficients for the determined age and gender, and for the measured amounts of the one or more biomarkers.

7. The method according to any one of claims 1, 4, and 5, wherein the biomarkers further comprise one or more of, fucosylated fetuin-A, fucosylated alpha-1-antitrypsin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

8. The method according to any one of claims 1, 4, and 5, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, and aspartate aminotransferase;

alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated alpha-1-antitrypsin;

alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated fetuin-A; or

alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, fucosylated fetuin-A, and fucosylated alpha-1-antitrypsin;

9. The method according to any one of claims 1, 4, and 5, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, and aspartate aminotransferase.
10. The method according to any one of claims 1, 4, and 5, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated alpha-1-antitrypsin.
11. The method according to any one of claims 1, 4, and 5, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated fetuin-A.
12. The method according to any one of claims 1, 4, and 5, wherein the IgG is removed by incubating the biological fluid with Protein A/G, and the IgM is removed by passing the biological fluid through a molecular weight-based filter.
13. The method according to any one of claims 1, 4, and 5, wherein the IgG and the IgM are removed by incubating the biological fluid with polyethylene glycol.
14. The assay according to claim 2, wherein the function comprises respective optimized weighting coefficients for the determined age and gender, and for the measured amounts of the one or more biomarkers.
15. The assay according to claim 2 or claim 14, wherein the biomarkers further comprise one or more of fucosylated fetuin-A, fucosylated hemopexin, fucosylated alpha-1-antitrypsin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT).
16. The assay according to any one of claims 2, 14, or 15, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, and aspartate

aminotransferase;

alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated alpha-1-antitrypsin;

alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated fetuin-A; or

alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, fucosylated fetuin-A, and fucosylated alpha-1-antitrypsin.

17. The assay according to claim 2 or claim 14, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, and aspartate aminotransferase.

18. The assay according to claim 2 or claim 14, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated alpha-1-antitrypsin.

19. The assay according to claim 2 or claim 14, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated fetuin-A.

20. The assay according to any one of claims 2 or 14-19, wherein the component for removing the IgG is Protein A/G, and the component for removing IgM is a molecular weight-based filter.

21. The assay according to any one of claims 2 or 14-19, wherein the IgG and the IgM are removed by incubating the biological fluid with polyethylene glycol.

22. The kit according to claim 3, wherein the function comprises respective optimized weighting coefficients for the determined age and gender, and for the measured amounts of the one or more biomarkers.

23. The kit according to claim 3 or claim 15, wherein the biomarkers further comprise one or more of fucosylated fetuin-A, fucosylated hemopexin, fucosylated alpha-1-antitrypsin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT).
24. The kit according to any one of claims 3, 21, or 23, wherein the biomarkers are
alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, and aspartate aminotransferase;
alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated alpha-1-antitrypsin;
alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated fetuin-A; or
alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, fucosylated fetuin-A, and fucosylated alpha-1-antitrypsin.
25. The kit according to claim 3 or claim 21, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, and aspartate aminotransferase.
26. The kit according to claim 3 or claim 21, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated alpha-1-antitrypsin.
27. The kit according to claim 3 or claim 21, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated fetuin-A.

28. The kit according to any one of claims 3 or 21-27, wherein the component for removing the IgG is Protein A/G, and the component for removing IgM is a molecular weight-based filter.
29. The method according to any one of claims 1, 4, or 5, wherein the hepatocellular carcinoma is early stage hepatocellular carcinoma, alpha fetoprotein negative hepatocellular carcinoma, or early stage hepatocellular carcinoma that is also alpha fetoprotein negative hepatocellular carcinoma.
30. The assay according to claim 2, wherein the hepatocellular carcinoma is early stage hepatocellular carcinoma, alpha fetoprotein negative hepatocellular carcinoma, or early stage hepatocellular carcinoma that is also alpha fetoprotein negative hepatocellular carcinoma.
31. The kit according to claim 3, wherein the hepatocellular carcinoma is early stage hepatocellular carcinoma, alpha fetoprotein negative hepatocellular carcinoma, or early stage hepatocellular carcinoma that is also alpha fetoprotein negative hepatocellular carcinoma.
32. The method according to any one of claims 1, 4, or 5, wherein the biomarkers further comprise one or more of aspartate aminotransferase, fucosylated alpha-1-antitrypsin, alanine aminotransferase, and fucosylated fetuin A.
33. The method according to claim 32, wherein the biomarkers further comprise aspartate aminotransferase or alanine aminotransferase.
34. The method according to claim 33, wherein the biomarkers further comprise fucosylated fetuin A, fucosylated alpha-1-antitrypsin, or both.
35. The assay of claim 2, wherein the biomarkers further comprise one or more of aspartate aminotransferase, fucosylated alpha-1-antitrypsin, alanine aminotransferase, and fucosylated fetuin A.
36. The assay according to claim 35, wherein the biomarkers further comprise aspartate aminotransferase or alanine aminotransferase.
37. The assay according to claim 36, wherein the biomarkers further comprise fucosylated fetuin A, fucosylated alpha-1-antitrypsin, or both.

38. The kit according to claim 3, wherein the biomarkers further comprise one or more of aspartate aminotransferase, fucosylated alpha-1-antitrypsin, alanine aminotransferase, and fucosylated fetuin A.

39. The kit according to claim 38, wherein the biomarkers further comprise aspartate aminotransferase or alanine aminotransferase.

40. The kit according to claim 39, wherein the biomarkers further comprise fucosylated fetuin A, fucosylated alpha-1-antitrypsin, or both.