

[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.

[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 measuring the amount of biomarkers comprising alpha-fetoprotein (AFP), fucosylated kininogen, aspartate aminotransferase (AST) and alkaline phosphatase (ALK) in a biological fluid or a separate sample derived from the biological fluid; but prior to measuring the amount of a biomarker that is a fucose-containing glycoform, removing IgG and IgM proteins from a biological fluid from the subject or the separate sample derived from 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 and the separate sample derived from 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 measuring the amount of biomarkers comprising alpha-fetoprotein (AFP), fucosylated kininogen, aspartate aminotransferase (AST) and alkaline phosphatase (ALK) in a biological fluid or a separate sample derived from the biological fluid, but prior to measuring the amount of a biomarker that is a fucose-containing glycoform, removing IgG and IgM proteins from a biological fluid obtained from the subject or the separate sample derived from the biological fluid; 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 or the separate sample derived from 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:
 - measuring the amount of biomarkers comprising alpha-fetoprotein (AFP), fucosylated kininogen, aspartate aminotransferase (AST), and alkaline phosphatase (ALK) in a biological fluid from the subject or in a separate sample derived from the biological fluid;
 - but prior to measuring the amount of a biomarker that is a fucose-containing glycoform, removing IgG and IgM proteins from a biological fluid obtained from the subject or the separate sample derived from 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 or the separate sample derived from the biological fluid;
 - 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 biomarkers comprising alpha-fetoprotein (AFP), fucosylated kininogen, aspartate aminotransferase (AST) and alkaline phosphatase (ALK), and wherein the assay utilizes a component for removing IgG and IgM proteins from a biological fluid of the subject or a separate sample derived from the biological fluid prior to measuring the amount of a biomarker that is a fucose-containing glycoform;
 - 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 or the separate sample derived from the biological fluid;
 - wherein the biological fluid is whole blood, serum or plasma.
3. A method for assigning a subject to a group having a higher or lower probability of hepatocellular carcinoma comprising:
 - measuring the amount of biomarkers comprising alpha-fetoprotein (AFP), fucosylated kininogen, aspartate aminotransferase (AST) and alkaline phosphatase (ALK) in a biological fluid or a separate sample derived from the biological fluid,

but prior to measuring the amount of a biomarker that is a fucose-containing glycoform, removing IgG and IgM proteins from a biological fluid from the subject or the separate sample derived from 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 or the separate sample derived from the biological fluid;

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

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

measuring the amount of biomarkers comprising alpha-fetoprotein (AFP), fucosylated kininogen, aspartate aminotransferase (AST), and alkaline phosphatase (ALK) in a biological fluid from the subject or in a separate sample derived from the biological fluid,

but prior to measuring the amount of a biomarker that is a fucose-containing glycoform removing IgG and IgM proteins from a biological fluid obtained from the subject or the separate sample derived from the biological fluid;

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 or the separate sample derived from 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.

5. The method according to any one of claims 1, 3, and 4, 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.

6. The method according to any one of claims 1, 3, and 4, wherein the biomarkers further comprise one or more of, fucosylated fetuin-A, fucosylated alpha-1-antitrypsin, and alanine aminotransferase (ALT).

7. The method according to any one of claims 1, 3, and 4, 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.

8. The method according to any one of claims 1, 3, and 4, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, and aspartate aminotransferase.

9. The method according to any one of claims 1, 3, and 4, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated alpha-1-antitrypsin.

10. The method according to any one of claims 1, 3, and 4, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated fetuin-A.

11. The method according to any one of claims 1, 3, and 4, wherein the IgG is removed by incubating the biological fluid or the separate sample derived from the biological fluid with Protein A/G, and the IgM is removed by passing the biological fluid or the separate sample derived from the biological fluid through a molecular weight-based filter.

12. The method according to any one of claims 1, 3, and 4, wherein the IgG and the IgM are removed by incubating the biological fluid or the separate sample derived from the biological fluid with polyethylene glycol.

13. 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.

14. The assay according to claim 2 or claim 13, wherein the biomarkers further comprise one or more of fucosylated fetuin-A, fucosylated hemopexin, fucosylated alpha-1-antitrypsin, and alanine aminotransferase (ALT).

15. The assay according to any one of claims 2, 13, or 14, 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.
16. The assay according to claim 2 or claim 13, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, and aspartate aminotransferase.
17. The assay according to claim 2 or claim 13, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated alpha-1-antitrypsin.
18. The assay according to claim 2 or claim 13, wherein the biomarkers are alpha-fetoprotein, fucosylated kininogen, alkaline phosphatase, aspartate aminotransferase, and fucosylated fetuin-A.
19. The assay according to any one of claims 2 or 13-18, wherein the component for removing the IgG is Protein A/G, and the component for removing IgM is a molecular weight-based filter.
20. The assay according to any one of claims 2 or 13-18, wherein the IgG and the IgM are removed by incubating the biological fluid or the separate sample derived from the biological fluid with polyethylene glycol.
21. The method according to any one of claims 1, 3, or 4, 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.
22. 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.

23. The method according to any one of claims 1, 3, or 4, wherein the biomarkers further comprise one or more of fucosylated alpha-1-antitrypsin, alanine aminotransferase, and fucosylated fetuin A.
24. The method according to claim 23, wherein the biomarkers further comprise alanine aminotransferase.
25. The method according to claim 24, wherein the biomarkers further comprise fucosylated fetuin A, fucosylated alpha-1-antitrypsin, or both.
26. The assay of claim 2, wherein the biomarkers further comprise one or more of fucosylated alpha-1-antitrypsin, alanine aminotransferase, and fucosylated fetuin A.
27. The assay according to claim 26, wherein the biomarkers further comprise alanine aminotransferase.
28. The assay according to claim 27, wherein the biomarkers further comprise fucosylated fetuin A, fucosylated alpha-1-antitrypsin, or both.