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.

[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.

What is Claimed:

1. A method 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.

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 assay utilizes a reagent 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.

3. A kit for detecting hepatocellular carcinoma in a subject comprising:

a reagent for removing IgG from a biological fluid from the subject;

a reagent for removing IgM 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.

4. A method 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,