Reviewer 1

Comments for Transmission to the Authors
This is a nice review which complihensive reviewed the litature. Several biomarkers for diagnosis HCC were described in this review.

Question: However, the sensitivity and specificity of some biomarkers were different between Eastern pateints and Western patients. I think the authors should also described such content.

Answer: **The difference in the sensitivity and specificity of biomarkers between ethnicities has now been discussed in depth. We have also used this point to recommend validation studies to take place in the specific population in question in order to maximize sensitivity and specificity.**

Reviewer 2

Comments for Transmission to the Authors
The paper is quite interesting and highlights the unanswered need for a specific and cheap serum or urinary biomarker useful for early diagnosis of HCC especially in not developed countries. Some minor points should be clarified

Question: Write proton nuclear magnetic resonance and mass spectrometry instead of 1H NMR and MS Too many abbreviations are used throughout the paper and this makes the reading difficult. I think that a list of abbreviations should be reported. Furthermore, I do not understand the meaning of some abbreviations such as TSA, TCA, CA, GC-MS, HILIC, and RPLC

Answer: **We have now fully explained the terms at their first appearance and added a list of abbreviations for the readers.**

Question: Introduction: according to the present guidelines percutaneous ablation should be added to the curative treatments of HCC

Answer: Percutaneous ablation is indeed a curative option for HCC, but we specifically identified hepatic resection and orthotopic liver transplantation as the preferred curative treatments in EARLY HCC.

Question: Paragraph The angiogenic switch: dysplastic nodules are often but not always hypoechoic on ultrasound; they can also less frequently appear as hyperchoic or isoechoic.

Answer: **We have updated the section with the extra information.**

Question: Please, correct Paragraph Current surveillance and diagnosis: the Authors should specify that the present guidelines for non invasive diagnosis of HCC apply only to patients with established liver cirrhosis

Answer: **Surveillance recommendation for patients with established liver cirrhosis has been clarified**

Question: I looked for reference 76 in pubmed but I could not find it

Answer: The citation has been updated with a different pubmed referenced review paper.

Question: Discussion: I think that it is too much short. The Authors should add a paragraph highlighting which are at present the more promising candidate metabolites in the blood and urine that could be tested in large series of cirrhotic patients with HCC (especially in the very early and early stage) and without HCC

Answer: **The discussion has been expanded with two new paragraphs that highlight promising candidate metabolites in the blood and urine, and another to explain the importance of identifying population-specific biomarkers for an effective application of the novel investigative tool.**

Reviewer 3

Comments for Transmission to the Authors
Dear Author 1.Serum biomarkers are striking potential tools to screen for and diagnose HCC early thanks to the non-invasive, objective, and reproducible assessments they can potentially enable. α-fetoprotein (AFP) is the biomarker most widely used to test for HCC, but the sensitivity and specificity of AFP vary widely, and total AFP is not always specific, especially when HCC is in its early stages. Whereas the sensitivity of AFP analysis used in conjunction with ultrasound to detect early-stage HCC has ranged from about 40% to 65%, the combination use of newer biomarkers, the Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) and des-gamma-carboxy prothrombin (DCP) (PIVKA-II) plus ultrasound, provides a sensitivity of nearly 85% and a specificity of nearly 95%. I ask some questions.

Question: According to your Table 2,AFP1-3 is much higher in sensitivity and specificity compared to AFP,DCP and AFU. How about the sensitivity and specificity if you use the combination of AFP1-3 and DCP.?

Answer: **The sensitivity and specificity values of the combination of markers, AFP-L3 and DCP, have been added to the table.**

2.Although DCP (PIVKA-II), AFP-L3, and Osteopontin (OPN) exhibit high specificity regarding the diagnosis of HCC, many clinicians use AFP values to follow-up patients with chronic liver diseases due to its higher sensitivity. The results of conventional tumor markers are negative for approximately 30% to 40% of HCC patients; therefore, searching for novel HCC markers must be continued. Squamous cell carcinoma antigen (SCCA) and HSP70 may be considered as key biomarkers for HCC patients when the results for traditional biomarkers are negative.(An Overview of Biomarkers for the Diagnosis of Hepatocellular Carcinoma ,Hepatitis Monthly 2012)

Question: Please tell me how about SCCA and HSP70 for HCC biomarker?

Answer: **Both SCCA and HSP70 have now been included and discussed as potential biomarkers.**

3. HIF-1 plays important roles in many critical aspects of HCC tumorigenesis, progression, and metastasis. It is involved in cellular proliferation, angiogenesis, invasion, and resistance to radiotherapy and chemotherapy. Clinical data also indicate that HIF-1 overexpression is associated with poor prognosis of HCC. More importantly, HIF-1 is identified as a potential target for HCC therapy. (The Role of Hypoxia Inducible Factor-1 in Hepatocellular Carcinoma 2014)

Question: Please comment the biomarker and target therapy of HIF-1 for HCC. Author comment various kinds of biomarker for HCC. Please tell me about some specific biomarker .

Answer: **We have expanded the discussion of HIF 1 to incorporate its potential use as a prognostic marker and as a therapeutic target.**