April 08, 2019

Dear Editor,

Please find the revised manuscript in Word format.

Title: Biomarkers versus imaging in the early detection of hepatocellular carcinoma and prognosis

Author: Lavinia Alice Balaceanu

Name of Journal: *World Journal of Clinical Cases*

Manuscript NO: 46690

The manuscript has been improved according to the specific comments and suggestions of the Reviewers. I have also provided the audio version of core tip, the English editing certificate, the copyright license agreement and the conflict of interest disclosure form.

Reviewer's code: 00051081

SPECIFIC COMMENTS TO AUTHORS

The author has reviewed the recent changes of surrogate markers used for early diagnosis and screening of HCC. Although the findings are interesting the style of manuscript presentation requires a major revision so that a potential reader should read the message in a systematic manner. The author has written the findings that someone may find it as a PubMed summary of recent studies missing the aim of being a journal article. Therefore, I recommend author to revise the manuscript thoroughly into a newer version which is easy to read and findings presented in a logical-systematic pathway.

Author's reply

The author thanks to reviewer for careful review of the manuscript. I also thank you for the specific comments and suggestion to revise the style for improving the quality of the manuscript, easy reading and understanding the message. As the reviewer commented, I tried to organize a lot of old and new serum biomarkers in a systematic pathway in an attempt to find arguments for and against the utility of serum biomarkers in clinical practice, under the current guidelines.

Reviewer's code: 03656572

SPECIFIC COMMENTS TO AUTHORS

In this review, the author provided an overview of the current evidence-based information on the clinical utility of serum biomarkers in the early diagnosis of HCC and the prognosis of the disease, including AFP, MicroRNAs, LncRNA and PIVKA-II. The author concluded that although there are important advances in the role of biomarkers in certain stages of the disease, especially in combinations, large studies are needed on certain population groups to introduce biomarkers into clinical practice on a large scale. It was difficult to find a unique combination of biomarkers in the diagnosis of HCC. Imaging techniques still play a leading role in both surveillance and HCC diagnosis. This review is described in detail, which, as valuable information, could help the readers that have better understand the first-hand knowledge of this topic to start novel studies.

Author's reply

The author thanks to reviewer for careful review of the manuscript. As the reviewer commented, there are important advances regarding the role of serum biomarkers in early diagnosis and prognosis of HCC, especially in combination. The different predominant aetiologies of HCC in various geographical areas (i.e. HBV, HCV, alcoholic and non-alcoholic fatty liver disease, cryptogenic disease) make it difficult to find a unique combination of biomarkers for the diagnosis of HC. Large trials involving certain populations groups are needed before biomarkers can be introduced into clinical practice on a large scale. Nonetheless, imaging techniques still play a leading role in both HCC surveillance and diagnosis, as the current guidelines present.

Thanks again for considering my paper in *World Journal of Clinical Cases*.

Best Regards,

Alice Balaceanu

Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Reviewer's code: 03699961

COMMENTS TO AUTHORS

1) General Comments

In this review, the author collected various information with respect to biomarkers and imaging modalities in aid of HCC diagnosis. The number of references is sufficient and well summarized. However, there is no conceptual context. A simple listing of many references does not informative.

Author's reply

The author thanks to reviewer for careful review of the manuscript. I also thank you for the specific comments and suggestions for improving the quality of the manuscript.

The following are concerns that the authors may wish to consider:

2) Specific comments

Major concerns:

1. I believe that the authors think that ethnicity, genetic polymorphism, the predominant etiologies specific in each geographical area, and so on are critical factors in the characterization of HCC. If it is true, the review should categorize data from the point of these critical factors then summarize to give us the insight for a role of biomarkers in HCC diagnosis.

Author's reply

The different aetiologies of HCC in various geographic areas (HBV, HCV, alcoholic and non-alcoholic fatty liver disease, cryptogenic) and possibly the genetic polymorphism makes it difficult to find a unique combination of biomarkers in the diagnosis of HCC. In a systematic review, Klingenberg et al [77] concluded that non-coding RNAs (miRNA and long non-coding (lnc)RNA) can be used for early diagnosis in HCC, due to high sensitivity and specificity; however, most of the studies analysed had included cases with only one or two HCC aetiologies. If an HBV-related HCC panel of miRNAs

(including miR-122 and miR-21) was to be studied for its diagnostic biomarker potential, the miRNAs should also be investigated for their potential in diagnosis of HCC associated with non-alcoholic fatty liver disease, alcohol or HCV infection in large trials with the specific group patients, as demonstrated by Schütte et al [78]. Zhang et al [79] considered the multiple origins of miRNAs, the lack of standardized protocols for pro-analytical manipulation of samples in research, the physiologic processing that would occur after the point of analysis, the unknown miRNAs binding proteins, and the lack of existing large studies on patients and control populations to support any single or combinations of miRNAs in a panel for clinical application for the detection and prognosis of patients with HCC.

2. The author committed to clarify the significance of and to propose a way utilizing serum biomarkers in the early diagnosis and the prognostic prediction of HCC. The purpose, title, and conclusion are not matched in this context.

Author's reply

In Canadian guidelines, serum biomarkers, such as AFP, AFP-L3 (fucosylated component of AFP or lens culinaris agglutinin-reactive fraction of AFP) and des-gamma-carboxy prothrombin (DCP) are more useful in the late-stage or aggressive HCC than in the early stage of small HCC, mainly because the biomarkers are not highly sensitive [4].

In the Japan guidelines, the three serum biomarkers, AFP, AFP-L3 and DCP are used for definitive diagnosis of HCC or for the subsequent surveillance exams [11]. These biomarkers are also used to estimate the efficacy of treatment in HCC patients who presented elevated levels before treatment [11].

Although there have been important advances in our understanding of the roles of various biomarkers in certain stages of the disease, especially in combinations, large studies involving certain population groups are needed before biomarkers can be introduced into clinical practice on a large scale.

3. The author presented a wide variety of microRNAs and long non-coding RNAs. A simple listing of those candidates cannot be informative to many readers. The author should categorize them in a reasonable way such as a functional annotation.

Author's reply

I organized miRNAs and lncRNAs both in text and in tables so that the potential reader can benefit from information in a systematized way.

4. In terms of imaging modalities, CT/MRI and US/CEUS are completely different in subjectivity/objectivity and evaluable regions. If look at the detectability in total, CT/MRI and CEUS may show the similar capability. However, a specific region of the liver may not be evaluated in practice. These facts are critical in clinic. The author should mention the pros and cons of CT/MRI and US/CEUS.

Author's reply

The International guidelines for CEUS recommendations cites dynamic CEUS as capable of evaluating the enhancement patterns of a liver nodule during arterial, portal venous and late phases, with the appearance being similar as contrast-enhanced CT and contrast-enhanced MRI [13]. CEUS has advantages over dynamic CT or MRI according to its features of providing a real-time evaluation of the arterial phase, applicability to renal failure patients, and its ability to diagnose malignant or non-malignant portal vein thrombosis, to select of one or more nodules for biopsy from multiple nodules with different patterns, to localize small HCC for percutaneous ablation and to assess the recurrence [4,13]. Dependence on the operator's experience, and a lower visibility of the sub-diaphragmatic segment of the liver, especially in liver steatosis are the main disadvantages of CEUS [13].

Although dynamic CEUS has an important role in the diagnosis and characterization of small liver tumours, the ultrasonographic differential diagnosis between HCC and intrahepatic cholangiocarcinoma is difficult, sometimes having the same

hypervascularization and washout pattern, as shown by Van Beers et al [109]. This does not happen with contrast-enhanced MRI or CT performed with small-molecular-weight agents, for both intravascular and extravascular extracellular space distribution [108].

5. Hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI and Kupffer cell images of CEUS lead to a new era in imaging of HCC. The author should mention the benefits of these contrast agents and specific images.

Author's reply

The post-vascular phase (also known as the Kupffer phase) can be evaluated with a specific ultrasonographic contrast agent, perfluorobutane having a hydrogenated egg phospatidyl serine shell [13]. Enhancement defect can better characterize the HCC nodule [13].

According to the European Society of Gastrointestinal and Abdominal Radiology (commonly known as ESGAR) consensus, Neri et al [113] revealed that MRI with Gd-EOB- DTPA as the contrast agent is the best technique for characterization of focal lesions with a diameter equal to or greater than 10 mm in a cirrhotic liver [113]. The dual, renal and hepatocyte elimination of Gd-EOB-DTPA makes it useful as a contrast agent for both perfusion imaging in the early phase and for the hepatocyte imaging, in the late phase [113]. Through dynamic contrast-enhanced MRI with Gd-EOB-DTPA, morphological and functional data can be obtained [113]. These features are particularly useful for HCC in cirrhotic liver, in late hepatic arterial phase (hepatic artery and portal vein enhancement) and hepatobiliary phase (delayed by reduced hepatic function) [113]. If MRI combines Gd-EOB-DTPA as a contrast agent with diffuse

weighted imaging technique, additional qualitative and quantitative data can be obtained on the degree of HCC differentiation, microvascular invasion or response to treatment [112].

6. For indeterminate liver nodule, the biopsy with cellular characteristics and stains for glypican-3, glutamine synthetase, heat shock protein 70 and clathrin heavy chain should be helpful but should not be necessary.

Author's reply

For indeterminate liver nodule, the Canadian guidelines recommend the biopsy showing cellular characteristics and positive staining for glypican-3, glutamine synthetase, heat shock protein 70 and clathrin heavy chain as necessary [4].

For HCC diagnosis, the Australian guidelines recommend the four-phase contrast-enhanced CT, contrast enhanced-MRI, in selected case CEUS, and finally PET and liver biopsy [8].

In non-cirrhotic cases, histological and immunohistological tests confirm the HCC diagnosis, as EASL (European Association for the Study of the Liver) guidelines recommend [9].

I agree with the reviewer comment that the biopsy should be helpful but should not be necessary.

7. The author said that HCC diagnosis is done based on four-phase contrast-enhanced CT, contrast enhanced-MRI, in selected case by CEUS, and finally with PET and liver biopsy. I believe that PET may detect HCC in the liver with relatively high background uptake but may not helpful to diagnose a nodule as HCC.

Author's reply

Cho et al [118] revealed the utility of fluorine-18 fluorodeoxyglucose (^{18}F-FDG) PET-CT in early or intermediate HCC, in management of the disease (hepatic resection or liver transplant), but it was found not be useful in very early-stage HCC, without extrahepatic metastases. Of note, accumulation of the ^{18}F-FDG radiotracer in inflammatory liver lesions is one of the limitations of this method for its use in the diagnosis of a hepatic nodule as HCC [118].

Minor concerns:

1. In several parts, English is poor. Please provide a certification of English editing.

Author's reply

I provide the English editing certificate.

2. In several parts, the descriptions from a guide line and from the literature search are mixed and cannot be distinguished. These two types of descriptions should be clearly separated.

Author's reply

I separated the guidelines from the literature search.

3. Do not abbreviate words from the first appearance.

Author's reply

There are no more abbreviated words in the first appearance.