Reviewer: 02952159

First of all, we are really grateful for your time and for your efforts. We thank you for providing so many suggestions. We have incorporated all of them in our final manuscript. We now believe that our manuscript is more valuable and this is because your help.

Abstract: 1. which guideline did author mention here?

Response: According to the current **European Association for the study of Liver diseases (EASL) hepatocellular** guidelines

Introduction: 1) The authors used both "ultrasound" and "ultrasonography" in the paper. Please use unified words. I would be interested to know, what is the reason for better wording?

Response: Both terms are equal and represent the same thing. However, as you kindly suggested we unified the terminology. We decided to use ultrasound instead of ultrasonography because ultrasound is most used in several papers.

2) "For recent progress in ultrasonography". Authors should clarify the application of CEUS in differentiation of HCC in their discussion.

Response: Moreover, the application of contrast agents has gained more and more attention. Compared to other imaging modalities, contrast enhanced ultrasound (CEUS) can be performed immediately after conventional US, being a simple, easy to perform and immediately available dynamic imaging tool [1]. The use of CEUS might therefore, shorten the diagnostic and therapeutic work-up of HCC patients.

3) For the diagnosis of small nodular in liver cirrhosis patients, please also discuss the value of the current CEUS LI-RADS classification and cite the CEUS LI-RADS publication

Response: The large applicability of CEUS for the diagnosis of HCC in cirrhosis was questioned because of the risk of a false positive diagnosis in case of cholangiocarcinoma. This has determined the American College of Radiology to release a diagnostic scheme for the characterization of focal liver lesions in patients at risk for HCC named CEUS LI-RADS ® [2]. In a multicenter Italian study, the use of CEUS LI-RADS in small HCC showed that the LR-5 category was 98.5 predictive of

HCC with no risk for misdiagnosis for pure cholangiocarcinoma [3].

Techniques, performance, complications

1) "The smear cytology technique will decrease the number of required passes and of inadequate fragments" Which kind of smear cytology technique?

Response: The smear cytology technique using Papanicolaou`s method will decrease the number of required passes and of inadequate fragments.

2) CEUS used to guide "the poorly visible or invisible nodules on conventional ultrasound, which become clearly visible after contrast injection. Please be more precise if the biopsy was guided by CEUS? During arterial phase or portal venous/late phase?

Response: Using **real time** contrast-enhanced harmonic **ultrasound** (SonoVue) to guide the biopsy will increase its diagnostic sensitivity by targeting: a) the enhanced, vascular areas of the tumor **in the arterial phase**, in case of large tumors which often display central necrosis [16]; b) the poorly visible or invisible nodules on conventional ultrasound which become clearly visible after contrast injection **in both arterial or late phase**[16, 17].

Current indications of LB in the diagnosis of HCC

1) "Nodules measuring between 1 and 2 cm are difficult to characterize using non-invasive methods" please cite some references for this

Response: We are sorry for this mistake. We thank you for your observation. The references were added.

2) "It follows that 50-70% of patients will require a biopsy in order to receive an exact diagnosis" In some clinical circumstances, liver cirrhosis patients with nodules measuring between 1 and 2 cm will still choose resection rather than biopsy. Please clarify.

Response: US guided LB may not be justified in patients with decompensated cirrhosis in whom whatever the nature the nodule, liver transplantation might be

considered. In contrast, in patients with a small nodule and compensated cirrhosis US guided LB should be performed before surgical resection which carries morbidity and mortality higher than those of biopsy itself

3) "Vascular **micro-invasion** is difficult to ascertain by liver biopsy, and its risk can at best be estimated" Since liver biopsy still could not diagnose vascular micro-invasion, there might be some new technologies, such as radiomics methods.

Response: In situations where vascular micro-invasion cannot be estimated the use of imagistic methods might be of real importance. Diffusion-weighted imaging (DWI) an emerging technique in hepatic magnetic resonance imaging (MRI) provided o sensitivity of 93.5% and a specificity of 72.2 % for the prediction of micro-vascular invasion during the preoperative evaluation of HCC [5].

Liver biopsy in the context of personalized medicine

1. is there any guideline or recommendation for the 'histological subtypes' or 'molecular distinct features' of HCC? Please cite references. 2. Authors describe a lot of contents about 'molecular distinct features' of HCC, please make some comment on the relationship between liver biopsy and HCC molecular features. 3. Also please be clearer about the relationship between 'HCC molecular features', 'liver biopsy' and 'personalized medicine'

Response: A new paragraph has been added as a response to these valuable comments.

The discovery of different histological subtypes each with distinct molecular features is still in its infancy and until further evidence, no recommendations can be made on how to treat best different subtypes. For the time being HCC should rather be considered as one disease. On contrary, in the future once all the signaling pathways for each HCC subtype have been described liver biopsy will indeed be necessary for the correct identification of such signaling pathways. Moreover, the identification of distinct signaling pathways for different subtypes of HCC will allow for the development of new treatments. In this ideal but not far from now scenario, liver biopsy will allow for

the correct diagnosis of HCC subtype, the corresponding up-regulated signaling pathways, and the proper choice of specific molecule and ultimately will open the path for a personalized medicine.

Liquid biopsy: The future of liver biopsy

1. Please be specific in what does 'liquid biopsy' refer to? How to perform it?

Response: The following paragraph has been added:

Compared to liver biopsy, liquid biopsy is a non-invasive method used for the identification of circulating tumor cells (CTCs) circulating tumor associated microparticles (MPs) or circulating miRNA/DNA in the blood of patients with HCC. Moreover, it is well accepted by the patients since only 1 ml of blood is enough for the proper identification using either flow cytometry or cell search system. Similar to conventional biopsies CTSs or MPs can be stained for various surface markers specific for HCC.

2. What is the possible disadvantage of 'liquid biopsy'?

Response: A short paragraph containing the limits of liquid biopsy has been added

The term liquid biopsy has been only recently introduced and the technology for cancer by-products identification is still in its infancy. Until more and more data becomes available liquid biopsy cannot be performed in daily practice and should rather be used for research intents. Time will decide the limits of liquid biopsies and whether it can replace or not conventional biopsies. The reported sensitivity and specificity of liquid biopsy in HCC is rather modest than high.

Reviewer: 03475479

Thank you very much for your suggestions. We highly appreciate them and are well taken. Based on your recommendations we have included several paragraphs with the role of new imaging techniques in the management of HCC.

Recently the effectiveness of new imaging techniques representing not only vascularity but also function such as EOB-MRI is known in the management of HCC. Liver biopsy could not reflect the whole signature of tumor as authors discussed in the manuscript. Authors should discuss

the effectiveness of liver biopsy as compared with such techniques (e.g. EOB-MRI, contrast-US, RI,,).

Response:

Dear reviewer we greatly appreciate you effort and the highly valuable comments. As you suggested we have incorporated the effectiveness of EOB-MRI in the management of HCC. We discussed the value of EOB-MRI as opposed to LB in the differential diagnoses between early HCC and high grade dysplastic nodules. And also, highlighted the superiority of EOB-MRI in discriminating between those two entities. Moreover we also highlighted the role of EOB-MRI in predicting microvascular invasion in HCC. The following paragraphs have been added:

Compared to LB, new imaging techniques such as Gd-EOB-DTPA MRI might be more accurate in the differential diagnosis between early HCC and dysplastic nodules. Hyper-intensity at diffusion-weighted imaging (DWI) was shown to be a useful feature for differentiating hypovascular early HCC from dysplastic nodules which appear as hypointense nodules at Gd-EOB-DTPA MRI[6]. A more recent study, reported a sensitivity of 94.7% and specificity of 99.3% in classifying high grade dysplastic nodules which appear hypointense in the hepatobiliary (HB) phase without arterial phase hyperintensity and without DWI restriction [7]. More importantly, the benign nodules appeared hyperintense in the HB phase, and HCC rarely develops from hyperintense hepatic nodules in the HB phase suggesting that this type of nodules require neither treatment nor more intensive follow-up [8].

In situations where vascular micro-invasion cannot be estimated the use of imagistic methods might be of real importance. Diffusion-weighted imaging (DWI) an emerging technique in hepatic magnetic resonance imaging (MRI) provided o sensitivity of 93.5% and a specificity of 72.2 % for the prediction of micro-vascular invasion during the preoperative evaluation of HCC [5].

The articles that we included are:

1. Suh YJ, Kim MJ, Choi JY, Park MS, Kim KW. Preoperative prediction of the microvascular invasion of hepatocellular carcinoma with diffusion - weighted imaging. Liver Transplantation. 2012 Oct 1;18(10):1171-8.
2. Hwang J, Kim YK, Jeong WK, Choi D, Rhim H, Lee WJ. Nonhypervascular Hypointense Nodules at Gadoxetic Acid–enhanced MR Imaging in Chronic Liver

Disease: Diffusion-weighted Imaging for Characterization. Radiology. 2015 Feb 27;276(1):137-46.
3. Renzulli M, Biselli M, Brocchi S, Granito A, Vasuri F, Tovoli F, Sessagesimi E, Piscaglia F, D'Errico A, Bolondi L, Golfieri R. New hallmark of hepatocellular carcinoma, early hepatocellular carcinoma and high-grade dysplastic nodules on Gd-EOB-DTPA MRI in patients with cirrhosis: a new diagnostic algorithm. Gut. 2018 Feb 3:gutjnl-2017.
4. Sano K, Ichikawa T, Motosugi U, Ichikawa S, Morisaka H, Enomoto N, Matsuda M, Fujii H. Outcome of hypovascular hepatic nodules with positive uptake of gadoxetic acid in patients with cirrhosis. European radiology. 2017 Feb 1;27(2):518-25.

Reviewer: 03269732

This paper has made a systematic review on the liver biopsy's role in HCC. Although invasive, liver biopsy play keys roles in the diagnosis of HCC, personalized treatment decision made and prognosis judgement. Considering the advances in highthroughput molecular technologies, liver biopsy may be helpful to the new classification of HCC with therapeutic and prognostic impact. Liquid biopsy was another endeavor direction, with invasive and repeatable merits. In brief, this paper has given some new sights and help for the clinical work. It deserved to be published. Minor revision 1. Some spelling mistakes have been found in this paper. Please revise them. 2. The format of Table 1 dose not accord with the general international standards of paper publication. Please revise it.

1) Dear reviewer we greatly appreciate the kind words that you have provided. We also thank you for your time and your efforts in revising this manuscript. Indeed we did find some minor spelling mistakes and we revised them. Moreover, a native English speaker has revised the spelling mistakes once again.
2) Sorry for this. We did not paid attention to the format of Table one. We read the general international standards and we revised the Table 1 accordingly.