

Dear Editors,

We would like to thank the WJG editors and reviewers for the constructive comments and recommendations. This extensively revised version incorporated most of the suggestions and we believed it has substantially improved. We hope that it would now fulfill the publication priority of WJF.

Here is our point-by-point response to the reviewers.

Reviewer #1:

1- The title should be written with prepositions in small letters.

- **Response: Revised accordingly.**

2- In the "Core tip" third line from bottom: "Combination with other novel markers have shown..." should be corrected to "Combination with other novel markers has shown..."

- **Response: corrected**

3- The authors may have also reported the other most recent prospective which includes studies suggesting the combination between lncRNA and AFP measurements.

- **Response: application of lncRNA was added in page 9. Corresponding references 52 and 53 were added**

4- In Page No.4, the authors mentioned the techniques used to measure AFP starting with immunoelectrophoresis and ending with quantitative automated chemiluminescent enzyme immunoassay. They used the word "lately" which gives the reader an impression that chemiluminescent enzyme immunoassay is developed in the 2000s, as they started with the older techniques which appeared between 1970s and 1980s. The two references that they referred to were dated back between 1980s and 1990s!

- **Response: the sentence was revised in page 5.**

5- In Fig. 1 B, the authors did not show how exactly interfering antibodies bind to both capture and detect antibodies to give false positive results and high value.

- **Response: The explanation was elaborated in the figure legend.**

6- In Page No.7, the authors referred to some studies in which AFPL-3 was used as a biomarker for HCC. However, they ignored other studies that showed higher sensitivity and specificity (Ibrahim, A. A. A., et al. Benha Medical Journal, 35(2018) (3), 312 ; Cerban, R., et al. Surg. Gastroenterol, 24(2019) (1), 37-44 ; El-Halawany, F., et al. MAGHREB-MACHREK, 2(2020) (2), 01-12 ; Ibrahim, H. M., et al. SJBS, 28 (2021) 5760–5764).

- **Response: These more recent publications were discussed on pages 7 and 8. Corresponding references 40-42, 47, 54 were added.**

7- In Page No.8, second line from bottom: ".....3000 to 7190 ng/ml have been reported" should be replaced by " .....3000 to 7190 ng/ml has been reported"

- **Response: corrected on page 9.**

8- At the end of Page No.9, the authors wrote" Use this reference here instead: PMID: 32620274"!!!! In fact, it is reference No.55! 9- In the references, the authors put the doi link for reference No.23 which was not the case for the rest of the references!

- **Response: Removed and references were updated.**

Reviewer #2:

Specific Comments to Authors: Hanif H et al. provided a comprehensive review on the applications and limitations of AFP for HCC. It reviewed the detection performance of AFP in HCC, liver diseases and cirrhosis, and conditions in the absence of liver disease. It also reviewed the combined detection by AFP, AFP-L3 and DCP (GALAD score), which was shown to enhance the HCC detection performance. I think the review was well-written and covered most key aspects around AFP in HCC and hepatic disease diagnosis.

- **Response: we thanked the reviewer for the encouraging comments.**

However, most contents in this review have been reviewed in previous papers. I found quite a few published review papers in the following aspects, which compromised the novelty and significance of the present paper.

- **Response: the comments were well received; we have revised the manuscripts incorporated the newer findings in the various sections of the text (see highlighted text) and updated the following references.**

[Reference 29] Sonbol MB, Riaz IB, Naqvi SAA, et al. Systemic Therapy and Sequencing Options in Advanced Hepatocellular Carcinoma: A Systematic Review and Network Meta-analysis. JAMA Oncol. 2020;6(12):e204930. doi:10.1001/jamaoncol.2020.4930

[Reference 28] Luo P, Wu S, Yu Y, et al. Current Status and Perspective Biomarkers in AFP Negative HCC: Towards Screening for and Diagnosing Hepatocellular Carcinoma at an Earlier Stage. Pathol Oncol Res. 2020;26(2):599-603. doi:10.1007/s12253-019-00585-5

[Reference 17] Wang X, Wang Q. Alpha-Fetoprotein and Hepatocellular Carcinoma Immunity. Can J Gastroenterol Hepatol. 2018;2018:9049252. Published 2018 Apr 1. doi:10.1155/2018/9049252

[Reference 16] Sauzay C, Petit A, Bourgeois AM, et al. Alpha-foetoprotein (AFP): A multi-purpose marker in hepatocellular carcinoma. Clin Chim Acta. 2016;463:39-44. doi:10.1016/j.cca.2016.10.006

[Reference 90] Tzartzeva K, Obi J, Rich NE, et al. Surveillance Imaging and Alpha Fetoprotein for Early Detection of Hepatocellular Carcinoma in Patients With Cirrhosis: A Meta-analysis. Gastroenterology. 2018;154(6):1706-1718.e1. doi:10.1053/j.gastro.2018.01.064

[Reference 91] Parikh ND, Singal AG, Hutton DW, Tapper EB. Cost-Effectiveness of Hepatocellular Carcinoma Surveillance: An Assessment of Benefits and Harms. Am J Gastroenterol. 2020;115(10):1642-1649. doi:10.14309/ajg.0000000000000715

[Reference 5] Thompson Coon J, Rogers G, Hewson P, et al. Surveillance of cirrhosis for hepatocellular carcinoma: systematic review and economic analysis. Health Technol Assess. 2007;11(34):1-206. doi:10.3310/hta11340

[Reference 8] Kim DY, Han KH. Epidemiology and surveillance of hepatocellular carcinoma. Liver Cancer. 2012;1(1):2-14. doi:10.1159/000339016

[Reference 68] Peng C, Li Z, Xie Z, et al. The role of circulating microRNAs for the diagnosis of hepatitis B virus-associated hepatocellular carcinoma with low alpha-fetoprotein level: a systematic review and meta-analysis. *BMC Gastroenterol.* 2020;20(1):249. Published 2020 Jul 31. doi:10.1186/s12876-020-01345-5

[Reference 69] Inoue T, Tanaka Y. Novel biomarkers for the management of chronic hepatitis B. *Clin Mol Hepatol.* 2020;26(3):261-279. doi:10.3350/cmh.2020.0032

[Reference 86] Kobeisy MA, Morsy KH, Galal M, Sayed SK, Ashmawy MM, Mohammad FM. Clinical significance of elevated alpha-fetoprotein (AFP) in patients with chronic hepatitis C without hepatocellular carcinoma in upper EGYPT. *Arab J Gastroenterol.* 2012;13(2):49-53. doi:10.1016/j.ajg.2012.06.004

[Reference 23] Gupta S, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. *Ann Intern Med.* 2003;139(1):46-50. doi:10.7326/0003-4819-139-1-200307010-00012

[Reference 80] Singal AG, Lampertico P, Nahon P. Epidemiology and surveillance for hepatocellular carcinoma: New trends. *J Hepatol.* 2020;72(2):250-261. doi:10.1016/j.jhep.2019.08.025

[Reference 79] Pennisi G, Celsa C, Giammanco A, Spatola F, Petta S. The Burden of Hepatocellular Carcinoma in Non-Alcoholic Fatty Liver Disease: Screening Issue and Future Perspectives. *Int J Mol Sci.* 2019;20(22):5613. Published 2019 Nov 9. doi:10.3390/ijms20225613

[Reference 82] Sumida Y, Yoneda M, Seko Y, et al. Surveillance of Hepatocellular Carcinoma in Nonalcoholic Fatty Liver Disease. *Diagnostics (Basel).* 2020;10(8):579. Published 2020 Aug 10. doi:10.3390/diagnostics10080579

[Reference 54] Wong RJ, Ahmed A, Gish RG. Elevated alpha-fetoprotein: differential diagnosis - hepatocellular carcinoma and other disorders. *Clin Liver Dis.* 2015;19(2):309-323. doi:10.1016/j.cld.2015.01.005

[Reference 47] Wang X, Zhang Y, Yang N, et al. Evaluation of the Combined Application of AFP, AFP-L3%, and DCP for Hepatocellular Carcinoma Diagnosis: A Meta-analysis. *Biomed Res Int.* 2020;2020:5087643. Published 2020 Sep 17. doi:10.1155/2020/5087643

[Reference 27] Bertino G, Ardiri A, Malaguarnera M, Malaguarnera G, Bertino N, Calvagno GS. Hepatocellular carcinoma serum markers. *Semin Oncol.* 2012;39(4):410-433. doi:10.1053/j.seminoncol.2012.05.001

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