Dear Editors,

Thank you for your review of our manuscript (Manuscript NO. 58780, Case Control Study). Your careful review and constructive comments are greatly appreciated. We have revised the manuscript accordingly, and hope to have an opportunity to publish this paper in *World Journal of Gastroenterology*.
Please find the revised manuscript for your approval. We have responded to the comments of the reviewers as follows.

Responses to the reviewers' comments:

Reviewer #1:

1) As the CHC is a rare primary liver cancer, background literature describing the significance with regard to prevalence and therapeutic consequences is missing.

Response: Thank you very much for your suggestion. We have incorporated the background literature of CHC in the "Introduction" section as follows

"Combined hepatocellular-cholangiocarcinoma (CHC) is increasingly recognized in cirrhotic liver, with a reported prevalence of 0.4-14.2% of all primary liver carcinomas. CHC is the second most common primary liver cancer in cirrhotic liver, followed by intrahepatic cholangiocarcinoma (ICC), excluding perihilar cholangiocarcinoma."

"CHC can have various imaging features overlapping with hepatocellular carcinoma (HCC), ICC and liver metastasis for complex histopathological components. However, the prognosis and treatment of CHC differ from HCC or ICC, therefore, the accurate diagnosis of this tumour type is of great importance for appropriate patient management."

2) The clinical impact of the trial is not stated, neither in the introduction nor in the conclusion.

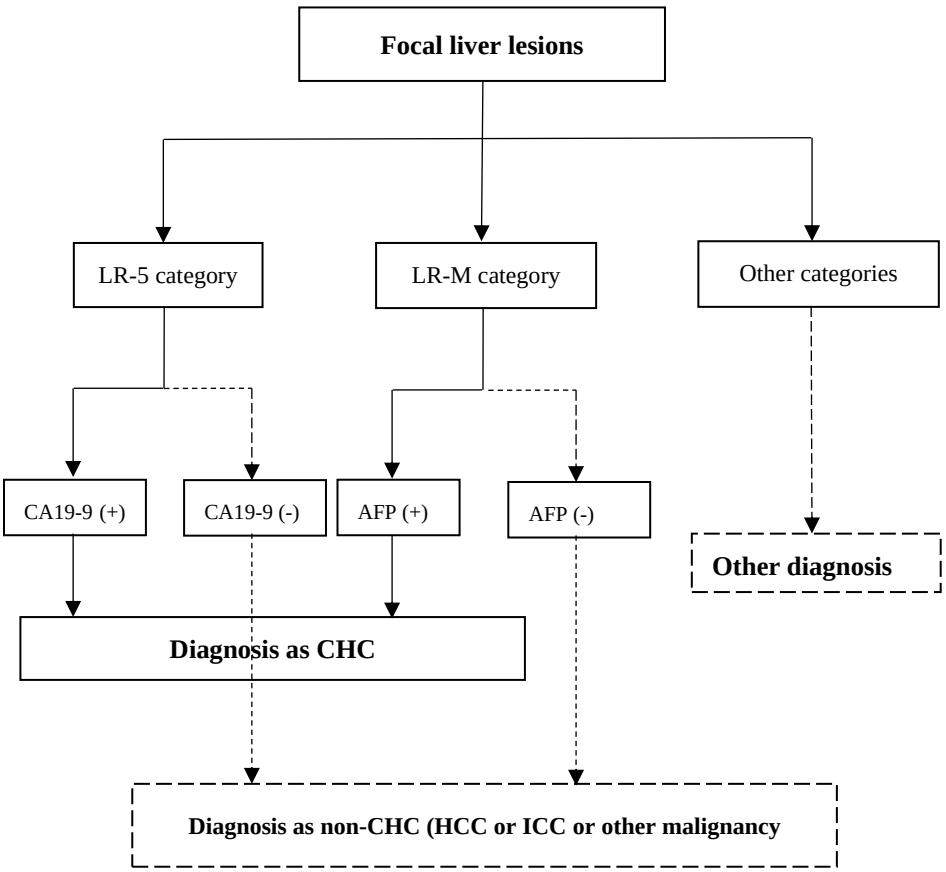
Response: Thank you very much for your suggestion. The impact of this trial has been rewritten in the "Conclusion" section as follows.

"In conclusion, CHC could be accurately diagnosed as malignant by CEUS LI-RADS, with the majority of the lesions in the LR-M category. The combination of CEUS LI-RADS classification with serum tumor markers shows high specificity but low sensitivity for the diagnosis of CHC. These findings could help radiologists and clinical investigators confidently exclude CHC lesions in the clinical setting."

3) One has difficulties to imagine, how this diagnostic tool will be implemented into the clinical routine. A scheme, representing the diagnostic pathway in order to differentiate HCC from CHC for example might help to implement these findings into clinical routine.

Response: Thank you very much for your suggestion. We have added a scheme (Appendix figure 1) to differentiate CHC from HCC or ICC, which might help to implement these findings into clinical routine.

Appendix figure 1: the scheme to differentiate CHC from HCC or ICC



4) Statistical outcomes such as sensitivity, specificity as well as the positive and negative predictive value are summarized for the three diagnostic pathways. The application of these three constellations consisting of a liver nodule classified as LR-5 or LR-M and serum biomarkers AFP and/or CA19-9 seems fairly complicated for the daily clinical application. In order to raise the awareness, these constellations should be examined separately.

Response: Thank you very much for your suggestion. First, the diagnostic criteria of this trial were parallel diagnostic suggestions, indicating that if one of the three criteria were met, the lesion would be suggested as CHC. In addition, we have also investigated the three diagnostic pathways separately. As we can see, all of them show lower sensitivity than the parallel test (Appendix table 1). Therefore, we recommend our more comprehensive diagnostic test. We have created a scheme to increase awareness of this test and for easy implementation into the clinical routine (Appendix figure 1).

Appendix table 1. Diagnostic test results of different diagnostic criteria.

Diagnostic criteria	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
The criteria of this study	0.649	40.0	89.9	58.3	80.9	76.9
Criteria 1[#]	0.533	8.6	98.0	60.0	75.2	74.6
Criteria 2[▲]	0.617	31.4	91.9	NA	NA	76.1
Criteria 3[*]	0.500	0.0	100.0	NA	NA	73.9

[#] represents the diagnostic criteria of CHC as LR-5 or LR-M lesion with simultaneously elevated AFP and CA19-9 (AFP > 20 ng/mL and CA19-9 > 100 U/mL) levels

[▲] represents the diagnostic criteria of CHC as LR-M lesion with elevated AFP level and normal CA19-9 level

[*] represents the diagnostic criteria of CHC as LR-5 lesion with elevated CA19-9 level and normal AFP level.

5 In the clinical routine, nodules at risk of an ICC or CHC might be subjected to a biopsy. How many of the patients did receive a biopsy and what was the histological diagnosis?

Response: Thank you very much for your question. All the subjects enrolled in this study were patients who underwent surgical liver resection and were confirmed to have CHC, ICC, or HCC by pathology.

4) Moreover, these patients might have received a CT scan or MRI scan. Studies by Sagrini et. al. (2019) suggested that CEUS misdiagnosed a higher number as HCC in comparison to CT scans or MRI scan. How do the authors comment on this finding?

Response: Thank you very much for your question. Sagrini et. al. (2019) reported that CEUS examinations observed more malignant liver nodules, in contrast to CT scans and MRI scans (CEUS vs CT vs MRI: 21 vs 7 vs 5 nodules). However, most tumors were assessed as LR-5 rather than LR-M, based on CEUS LI-RADS categorization. Meanwhile, Sagrini et. also highlighted the need to evaluate the characteristics of wash-out and not just its occurrence. As we know, CEUS has the best temporal resolution among the cross-sectional contrast enhanced imaging modalities due to its real time nature. Therefore, CEUS is the best tool that can observe the characteristics of early washout. In addition, some lesions would show partial early washout inpatients with CHC and would thus be classified as LR-M. However, detailed description of this imaging characteristic had not been described in the study by Sagrini et. al. (2019).

Moreover, presence of cirrhosis may contribute to the imaging manifestation of liver nodules[1]. The study by Sagrini et. al. (2019) only evaluated the imaging features of CHC in cirrhotic patients (100%), in contrast to 65.7 % (23/35) cirrhotic patients in our study. Of note, the nonconformity of tumour size between the two studies (median diameter: 2.5 cm vs 4.0 cm) may also contribute to the discrepancy.

Because, the sizes of the liver nodule can affect the imaging features and further change the CEUS LI-RADS categorization[2, 3].

5) In order to fully understand the data in detail, the p-values for the separate echogenic degrees are needed (hypo- vs. hyperenhancement)

Response: Thank you very much for your suggestion. We provided the p-value for the separate sub-groups in the appendix table 2.

In addition, we added the pertinent data in the results according in the "CEUS imaging characteristics" in "Results" as follows.

"In the arterial phase, peripheral irregular rim-like and non-rim-like hyperenhancement were demonstrated in 17.1% (6/35), 82.9% (29/35) of CHC lesions, respectively."

Appendix table 2. Imaging characteristics of the study patients with CHC and those with ICC and HCC.

	CHC	**Non-CHC**		**P1**	**P2**
	(n=35)	**ICC (n=29)**	**HCC(n=70)**		
Echogenicity degree				0.692	0.099
Hypo-	32 (91.4%)	25 (86.2%)	55 (78.6%)		
Hyper-	3 (8.6%)	4 (13.8%)	15 (21.4%)		
APHE pattern				0.757	0.001
Rim-like	6 (17.1%)	6 (20.7%)	0 (0.0)		
Non-rim-like	29 (82.9%)	23 (79.3%)	70 (100%)		

CHC: Combined hepatocellular-cholangiocarcinoma, ICC: Intrahepatic cholangiocarcinoma, HCC: Hepatocellular carcinoma, LI-RADS: Liver Imaging Reporting and Data System, APHE: Arterial phase hyperenhancement

Note: Data are numbers of lesions, with percentages in parentheses

P1 significant difference between CHC and ICC,

P2 significant difference between CHC and HCC,

6) The unit used in table 4 for the serum biomarkers remains unclear.

Response: Thank you very much for pointing out this detail. We have revised the units of AFP and CA 19-9 accordingly to (ng/mL) and (U/mL), respectively.

Reviewer #2:

Response: Thank you for your review of our manuscript. Your careful review and constructive comments are greatly appreciated.

Science editor:

(1) I found the title was more than 18 words. The title should be no more than 18 words;

Response: Thank you very much for your suggestion. The title has been revised within 18 words as “Contrast-enhanced ultrasound in association with serum biomarkers for differentiating combined hepatocellular-cholangiocarcinoma from hepatocellular carcinoma and intrahepatic cholangiocarcinoma”

(2) I found the authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

Response: We have uploaded the approved grant application form(s) accordingly.

(3) I found the authors did not provide the original figures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

Response: Thank you very much for pointing this out. We have uploaded the original figure documents using PowerPoint.

(4) I found the authors did not write the “article highlight” section. Please write the “article highlights” section at the end of the main text.

Response: Thank you very much for pointing this out. We provided an the “article highlights” section at the end of the main text.

Article highlights:

Research background

Combined hepatocellular-cholangiocarcinoma (CHC) is a rare type of primary liver cancer. Due to its complex histopathological characteristics, the imaging features of CHC may overlap with those of hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC).

Research motivation

The contrasted-enhanced ultrasound (CEUS) Liver Imaging Reporting and Data System (LI-RADS) released by the American College of Radiology (ACR) has been reported to be effective for the diagnosis of HCC. However, CHC lesions meeting the criteria for LR-5 classification may compromise the high specificity of LR-5 for the diagnosis of HCC if we only take the imaging features into consideration. Serum biomarkers, especially alpha-fetoprotein (AFP) and carbohydrate antigen 19-9 (CA19-9), have been shown to be helpful in the diagnosis of CHC. However, whether combining CEUS LI-RADS with serum biomarkers is helpful for differentiating CHC from HCC and ICC in at-risk patients has not been fully evaluated.

Research objective

The purpose of this study was to investigate whether the combination of CEUS LI-RADS and serum biomarkers is helpful for differentiating CHC from HCC and ICC in patients with chronic liver disease.

Research methods

Patients with histologically confirmed CHC, ICC and HCC with chronic liver disease between January 2016 and December 2019 were enrolled in this retrospective case control study. HCC patients were finally enrolled after one-to-two (CHC: HCC=1:2) propensity score matching by tumor size, age, and sex. Differences in quantitative variables were tested by the independent sample t-test. The rates of imaging characteristics were compared by using the $\chi 2$ test or Fisher's exact test. Receiver operating characteristic curve analysis was used to investigate the potential of CEUS LI-RADS and serum tumor markers for differentiating CHC from HCC and ICC.

Research results

After propensity score matching, 134 patients (mean age of 51.4±9.4 years, 108 men) were enrolled, including 35 CHC, 29 ICC and 70 HCC patients. Based on the CEUS LI-RADS classification, 74.3% (26/35) and 25.7% (9/35) of CHC lesions were assessed as LR-M and LR-5, respectively. The rates of elevated AFP and CA19-9 levels in CHC patients were 51.4% and 11.4%, respectively. Simultaneous elevation of AFP and CA19-9 was found in 8.6% (3/35) of CHC patients. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and AUC of the aforementioned diagnostic criteria for discriminating CHC from HCC and ICC were 40.0%, 89.9%, 58.3%, 80.9%, 76.9% and 0.649, respectively. When the reported prevalence rate of CHC (0.4–14.2%) was taken into account, the PPV and NPV were revised to 1.6%-39.6% and 90.1%-99.7%, respectively.

Research conclusions

CHCs are more likely to be classified as LR-M than LR-5 by CEUS LI-RADS. The combination of the CEUS LI-RADS classification with serum tumor markers shows high specificity but low sensitivity for the diagnosis of CHC. Moreover, CHC could be confidently excluded with a high NPV.

Research perspectives

The imaging features of CHC are complicated due to its complex histopathological characteristics. In addition, biopsy may misguide the correct diagnosis of CHC due to sampling error or tissue insufficiency. This study investigated the diagnostic value of

the CEUS LI-RADS classification combined with serological tumor markers in differentiating CHC from HCC and ICC. The results showed that the combined diagnostic criteria had high specificity and negative predictive value but low sensitivity for the diagnosis of CHC. These findings could help radiologists and clinical investigators confidently exclude CHC lesions in the clinical setting.

(5) The authors need to fill out the STROBE checklist with page numbers.
Response: We have re-uploaded the STROBE checklist with page numbers.

(6) The highest single-source similarity index in the CrossCheck report showed to be 5%. Please rephrase these repeated sentences.
Response: We have rewritten these repeated sentences.

Special notes for editor science office: In response to “the corresponding author has not published articles in the BPG.”, we would like to clarify that our team have published several articles in World Journal of Gastroenterology (article title: Can contrast enhanced ultrasound differentiate intrahepatic cholangiocarcinoma from hepatocellular carcinoma? by Dr. Qiang Lu from West China Hospital). Using different e-mail address of the corresponding author may lead to the misunderstanding. By the way, ORCID number is more commonly used. I suggest that BPG may also connect the publications through the ORCID, which may give you a more comprehensive profile of the corresponding author.
Best regards,

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2 **Kono Y,** Lyshchik A, Cosgrove D, Dietrich CF, Jang HJ, Kim TK, Piscaglia F, Willmann JK, Wilson SR, Santillan C, Kambadakone A, Mitchell D, Vezeridis A, Sirlin CB. Contrast Enhanced Ultrasound (CEUS) Liver Imaging Reporting and Data System (LI-RADS®): the official version by the American College of Radiology (ACR). *Ultraschall Med* 2017; **38**(1): 85-86 [PMID: 28249328 DOI: 10.1055/s-0042-124369]

3 **Forner A,** Vilana R, Bianchi L, Rodriguez-Lope C, Reig M, Garcia-Criado MA, Rimola J, Sole M, Ayuso C, Bru C, Bruix J. Lack of arterial hypervascularity at contrast-enhanced ultrasound should not define the priority for diagnostic work-up of nodules <2 cm. *J Hepatol* 2015; **62**(1): 150-155 [PMID: 25173969 DOI: 10.1016/j.jhep.2014.08.028]