Dear editors and reviewers,

The authors are grateful to the editors and reviewers for their efforts in reviewing the Manuscript （NO: 74031）. The manuscript has been revised thoroughly to address all the comments and suggestions of all the editors and reviewers. The details of the responses to the reviewers' comments are presented in the following pages.

Reviewer #1:

This is a case report highlighted the role of CEUS in liver tumor.

Response:

The authors are grateful to the positive comments of the reviewer.

1. Abstract: not clear about the accuracy of CEUS, is it better than CECT? please clarify. AFP and CA19-9 increased? it was not state in the main text.

Response:

We appreciate the suggestion of the reviewer. CEUS displays exquisite vascularity and tissue perfusion in real time with good spatial and temporal resolution and may more accurately reflect tumor washin and washout times than contrast-enhanced CT. In this case we think that it is better than CECT (Line 32-34). We have presented that "After her hospitalization, laboratory tests showed elevated AFP and CA19-9 level." in Abstract (Line 38-39).

2. Introduction part: authors mentioned that "the treatment schemes and outcomes of the two diseases are different" so please clarify. Apart from surgical resection, which treatment is different?

Response:

We appreciate the suggestion of the reviewer. Following the suggestions, we had revised the part of the Introduction part. When CHC and HCC occur synchronously, due to the unique fiber components of intrahepatic cholangiocarcinoma (ICC) contained in CHC, treatments typically used for multiple HCC, e.g. transcatheterial arterial chemoembolization (TACE) and chemotherapy, provide limit benefits.

The changes in the revised manuscript are marked in red (Line 68-71).

3. Physical examination: please descript PE finding such as BMI, chronic liver stigmata, liver and spleen size.

Response:

We appreciate the suggestion of the reviewer. Following the suggestions, we had revised the part of "Physical examination". The patient's height and weight were 155 cm and 59 kg, respectively, with a body mass index of 24.6 kg/m^2. No swollen lymph nodes were found. The abdomen was soft without rebound tenderness, and the liver and spleen were not palpable under the ribs. There was no percussion pain in the liver area. Mobility dullness was negative, and bowel sounds were normal.

The changes in the revised manuscript are marked in red (Line 84-88).

4. Laboratory tests: please add Hb level, did patient have splenomegaly? why she had low platelet count? Please add TB/DB/ALP/ albumin/globulin. Please add the normal value of PIVKA-II, AFP

and CA19-9. Hepatitis B viral load should be investigated in this patient.

Response:

We appreciate the suggestion of the reviewer. CT showed a slightly enlarged spleen. We added more results in the "Laboratory tests" part following the suggestions: Platelet count, $93x10^9$ /L; white blood cell count, 2.65×10^9 /L; red blood cell count, 3.99×10^{12} /L; hemoglobin, 124 g/L; albumin, 42.2 g/L (normal range: 40.0-55.0 g/L); globulin,23.1 g/L (normal range: 20.0-40.0 g/L); total bilirubin, 10.3 μmol/L (normal range: 5.0-28.0 μmol/L); direct bilirubin, 3.4 μmol/L (normal range: <8.8 μmol/L); alanine aminotransferase (ALT): 50 IU/L (normal range: <40.0 IU/L); aspartate aminotransferase (AST): 61 IU/L (normal range: <35.0 IU/L); Alkaline Phosphatase (ALP) 126 IU/L (normal range: 50.0-135.0 IU/L); hepatitis B surface antigen (+); hepatitis B e antibody (+); hepatitis B core antibody (+); hepatitis C antibody (-);HBV DNA level, 6.32 × 105 IU/Ml (normal range <1.0 × 102 IU/mL); protein induced by vitamin K absence/antagonist-II (PIVKA-II): 54.00 mAU/ml (normal range 6.0-32.5 mAU/ml); alpha-fetoprotein (AFP): 219.00 ng/ml (normal range: <7 ng/ml); serum carbohydrate antigen 19-9 (CA19-9): 38.40 U/ml (normal range: <30 U/ml); normal ranges of carcinoembryonic antigen (CEA) and serum carbohydrate antigen 125 (CA-125).

The changes in the revised manuscript are marked in red (Line 89-101).

5. Imaging examination: Why patient have to investigate both CEUS, CECT? What is the diff between these 2 investigations. As authors mentioned only the diagnosis was considered cirrhosis with a malignant tumor from both investigations.

Response:

We appreciate the suggestion of the reviewer. As CT only considered cirrhosis malignant tumor, and no specific types were identified, surgeons hoped to obtain more information from CEUS. CEUS can assess the microvascular perfusion in real time, as we can see in this case, CEUS considered cirrhosis, and the imaging classification of two tumors was given according to CEUS LI-RADS (The Liver Imaging Reporting and Data System), and one of the masses was identified as HCC.

The changes in the revised manuscript are marked in red (Line 32-34).

6. Final diagnosis: the diagnosis come from imaging result or histopathology? If from histopathology, this should be probably diagnosis or Diff dx.

Response:

We appreciate the suggestion of the reviewer. The diagnosis comes from histopathology. We gave the diagnostic evidence in Fig. 2, and made a relevant description in the note of Fig.2.

The changes in the revised manuscript are marked in red.

7. Discussion: I think that the management that could cure this patient should be liver transplantation. Please disscuss this management in discussion part and pre-operative diagnosis could change the management? How? please discuss as well.

We appreciate the suggestion of the reviewer. Previous studies showed that the survival rate of patients with CHC, who underwent liver transplantation showed inferior survival in comparison to those with HCC alone, and the role and indications of liver transplantation in combined tumor have yet to be defined[1-3]. Therefore, liver resection is currently considered the best treatment for

such patients,and careful preoperative diagnostic evaluation is required to minimize the risk of misdiagnosing HCC[3]. The cited literatures are listed in the end of this letter.

We had discussed it in the discussion part. The added discussion in the revised manuscript are marked in red (Line 148-150).

Reviewer #2:

In this case the authors describe the simultaneously rare occurrence of two different epatic tumors (hepatocellular-cholangiocarcinoma [CHC] and hepatocellular carcinoma [HCC]) and most importantly he showed how a non-invasive refined ultrasound technique (contrast enhanced) was able to distinguish these 2 liver masses mainly on the basis of the arterial and wash-out phases of the enhancement. The case is interesting and quite well written.

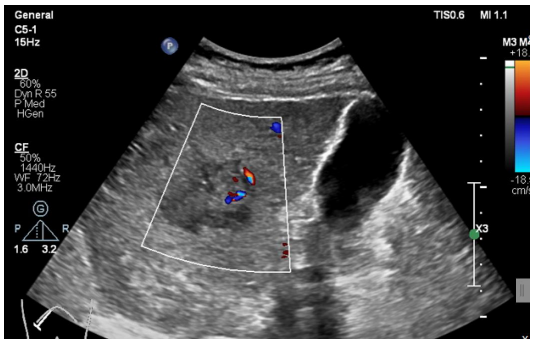
Response:

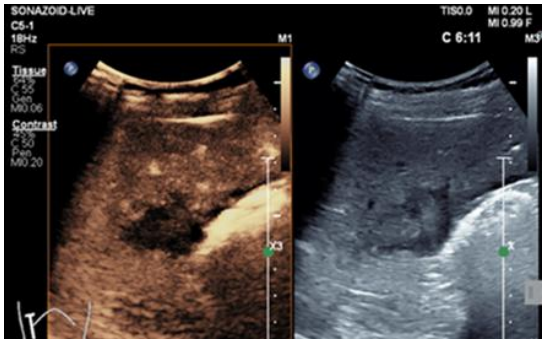
The authors are grateful to the positive comments of the reviewer.

1. First of all in order to replicate such an approach more technicality specifications need to be accurately reported with the adding of some comments: the type of ultrasound equipment; the transducer, the setting of the machine both for B-mode and color: frame rate, PRF, nyquist limits, frame rate etc; is there a preset by the manufacturer? One major point in my view is that not all machines are interchangeable especially as far as the Doppler module is concerned. Moreover the type of contrast should be specified underscoring its availability on the market.

Response:

We appreciate the suggestion of the reviewer. As the parameters were too much to describe in the manuscript, so we only showed all the instrument setup parameters in the pictures. We have added the type of ultrasound equipment and contrast agent to the manuscript (Sonazoid 0.6mL bolus injection, Philips EPIQ7, and C5-1 convex array probe).





The changes in the revised manuscript are marked in red (Line 117).

2. They mentioned too succinctly the pathophysiology underlying the different behavior of contrast in the two neoplastic masses.

Response:

Response:

We appreciate the suggestion of the reviewer. We have added relevant discussion as follows: On CEUS images, rapid arterial phase hyperenhancement (APHE) resulted from the formation of neoangiogenesis, and washout during postarterial phases due to reduced or absent of normal structure of portal triads, are identified as characterics of HCC[4]. Rim-like

hyperenhancement, early (< 60 s) washout and marked washout within 120 s are specific enhancement patterns of ICC on CEUS. Arterial rim hyperenhancement pattern of ICC is associated with a high degree of malignant cell proliferation in the periphery while necrosis or fibrosis in the center of the tumor on pathology[5-7]. The ultrasound manifestations of CHC are related to the proportion of HCC and ICC components in the mass and the nodule size. The amount of the HCC component may be the main determinant of radiologic LI-RADS categories of hepatocellular-cholangiocarcinoma; tumors of LR-4 or LR-5 categories were associated with a larger proportion of the HCC component and a smaller proportion of the CCA component[8].

The changes in the revised manuscript are marked in red (Line 157-159, Line 162-165, Line 168-171).

3. In addition it would be nice to report in a table side by side the more salient ultrasound differentials between the CHC and HCC.

Response: We appreciate the suggestion of the reviewer. A table was prepared in the revised manuscript.

Table 1 Ultrasound manifestation of the two intrahepatic nodules

Nodules	Location	Size(cm)	Boundary	Arterial phase	Portal phase	Post-vascular phase
HCC	S4	2.1*2.0	Clear	Hyperenhancement	Hyperenhancement	Mild hypoenhancement
CHC	S6	3.0*2.7	Unclear	Rim enhancement	Marked hypoenhancement	Marked hypoenhancement

HCC: hepatocellular carcinoma; CHC: hepatocellular-cholangiocarcinoma.

The changes in the revised manuscript are marked in red.

4. What is the added value of contrast ultrasound with respect to CT scan?

Response:

We appreciate the suggestion of the reviewer. The added value is that contrast-enhanced ultrasound displayed exquisite vascularity and tissue perfusion in real time with good spatial and temporal resolution and more accurately reflect tumor washin and washout times than contrast-enhanced CT in this case.

The changes in the revised manuscript are marked in red (Line 32-34).

5. They reported “The patient had a history of drinking (50 g/d)” but they should specify if wine, liquor etc.

Response:

We appreciate the suggestion of the reviewer. We made the revision by clarifying that “The patient had a > 20-year history of alcoholism (approximately 50 mL liquor per day)”.

The changes in the revised manuscript are marked in red (Line 81-82)

References

[1] Garancini M, Goffredo P, Pagni F, et al. Combined hepatocellular-cholangiocarcinoma: a population-level analysis of an uncommon primary liver tumor. Liver Transpl. 2014. 20(8): 952-9.

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[4] Fowler KJ, Burgoyne A, Fraum TJ, et al. Pathologic, Molecular, and Prognostic Radiologic Features of Hepatocellular Carcinoma. Radiographics. 2021. 41(6): 1611-1631.

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[7] Li R, Yuan MX, Ma KS, et al. Detailed analysis of temporal features on contrast enhanced ultrasound may help differentiate intrahepatic cholangiocarcinoma from hepatocellular carcinoma in cirrhosis. PLoS One. 2014. 9(5): e98612.

[8] Choi SH, Jeon SK, Lee SS, et al. Radio-pathologic correlation of biphenotypic primary liver cancer (combined hepatocellular cholangiocarcinoma): changes in the 2019 WHO classification and impact on LI-RADS classification at liver MRI. Eur Radiol. 2021. 31(12): 9479-9488.