

RE: Manuscript ID. 83839

Manuscript TITLE: **Major Complications After Ultrasound-guided Liver Biopsy:
an Annual Audit of a Chinese Tertiary-care Teaching Hospital**

Response to Decision Letter

We highly appreciate Prof. Ma' dedicated work to our manuscript and the two Reviewer's valuable suggestions which are very helpful for improving our paper. To satisfy the requirements of the Reviewers, we held several discussions in our research group to improve our data analyses, result presentation and related discussion in the revision during the past one week.

All changes made to the manuscript are highlighted in red font with clear indication of the locations. The English in revised manuscript was polished, so that some words, sentences were significantly changed which were not totally presented in this document.

Thanks again for your help and all the constructive suggestions.

Point-to-point responses to the reviewers' comments

Reviewer #1

Comments

Chai W. L; and colleagues reported the major complications after ultrasound-guided liver biopsy: an annual audit of a Chinese tertiary-care teaching hospital. I have the following comments related with this manuscript.

-In the abstract section is necessary to add the background. In addition, in methods it should indicate statistical tests.

Response: Thanks for reviewer's valuable suggestion. According to the Reviewers' suggestions, we added the background and indicated statistical tests in the abstract. Revision made in the "**Abstract**" section on Page 3;

"BACKGROUND

As ultrasound-guided percutaneous liver biopsy (PLB) has become a standard and important method in the management of liver disease in our country, a periodical audit on the major complication is needed.

AIM

The aim of this study is to determine the annual incidence of major complications following ultrasound-guided percutaneous liver biopsy and to identify variables that are significantly associated with an increased risk of major complications.

METHODS

A total of the 1857 consecutive cases of PLB were included in our hospital from January 2021 to December 2021. The major complication rate and all-cause 30-day mortality rate were determined. ~~Statistically significant risk factors associated with the occurrence of major complications after ultrasound-guided PLB were investigated.~~ Multivariate analyses were performed by logistic regression to investigate the risk factors associated with major complications and all-cause 30-day mortality following ultrasound-guided PLB."

-In the material and methods section, the groups of patients analyzed in this study should be indicated.

Response: Thanks for reviewer's suggestion. We indicated the groups of patients analyzed in our study in the section of materials and methods.

Revision made in the “**Materials and Methods-Definition**” section on Page 8, paragraph 2, line 15-20;

“All the included factors of both major complication group and no major complication group were compared, and the independent predictors for the occurrence of major complication after PLB were identified. Clinical, radiological and technical variables were investigated statistically for their association with ~~major complications~~ and all-cause 30-day mortality following PLB. ”

Revision made in the “**Materials and Methods-Data analysis**” section on Page 9, paragraph 1, line 1-9;

“ The difference in categorical variables between major complication group and no major complication group are presented as percentages and were compared by using the chi-square or Fisher's exact test, as appropriate. Normally distributed variables are expressed as the mean \pm standard deviation (SD) and compared by the Student's t test. Non-normally distributed variables are expressed as median (Q1, Q3), and were compared by nonparametric test (Mann-Whitney U test). Multivariate analyses were performed by a forward step (likelihood ratio) multivariable logistic-regression model to investigate the risk factors associated with major complications and all-cause 30-day mortality following ultrasound-guided PLB. ”

-Please define the following acronym: PACS.

Response: Thanks for reviewer's suggestion. We revised accordingly.

Revision made in the “**Materials and Methods-Patients and design**” section on Page 6, paragraph 1, line 14;

“picture archiving and communication system (PACS)”

-Should the authors clarify when the laboratory tests were performed? They were performed before or after liver biopsy.

Response: Thanks to the reviewer for these helpful comments. We clarified the laboratory tests in the section of materials and methods.

Revision made in the “**Materials and Methods-Patients and design**” section on Page 6, paragraph 1, line 8-11;

“The haematological tests which included the blood routine examination and coagulation test within 7 days before PLB were needed and on the following day after PLB, the level of hemoglobin was routinely examined.”

-Please indicate which statistical tests were performed for the nonparametric test.

Response: Thanks for your suggestion. We used Mann-Whitney U for nonparametric test in the data analysis.

Revision made in the “**Materials and Methods-Data analysis**” section on Page 9, paragraph 1, line 1-9;

“ The difference in categorical variables between major complication group and no major complication group are presented as percentages and were compared by using the chi-square or Fisher’s exact test, as appropriate. Normally distributed variables are expressed as the mean \pm standard deviation (SD) and compared by the Student’s t test. Non-normally distributed variables are expressed as median (Q1, Q3), and were compared by nonparametric test (Mann-Whitney U test). Multivariate analyses were performed by a forward step (likelihood ratio) multivariable logistic-regression model to investigate the risk factors associated with major complications and all-cause 30-day mortality following ultrasound-guided PLB. ”

-In the table 1 and 2, the percentage data should be reviewed because some of them are incorrect. Moreover, the n of each group should be indicated.

Response: Thanks for your careful examination. The revision was made accordingly. Revision made in the “**Tables**” section on Page 17-19;

Table 1. Demographic, clinical, procedural and pathological characteristics of patients in the major complication and no major complication groups.

Variables	Major complication (n=10)	No major complication (n=1847)	p value
Sex/ Male	8, 80.0%	1100, 59.6%	0.189
Female	2, 20.0%	747, 40.4%	
Age, median (Q1, Q3)	65.0 (54.5, 76.3)	58.0 (47.0, 67.0)	0.156
Comorbidity			
Cardiovascular and cerebrovascular diseases	2, 20.0%	238, 12.9%	0.504
Extensive ascites	0, 0%	35, 1.9%	0.660
Obstructed jaundice	3, 30.0%	137, 7.4%	0.007
Laboratory test			
Platelet count <50×10 ⁹ /L	0, 0%	15, 0.8%	0.922
Prebiopsy hemoglobin level, median (Q1, Q3)	112.5 (76.5, 126.3)	125.0 (112, 139)	0.027
Fibrinogen<2 g/L	5, 50.0%	228, 12.3%	<0.001
PT prolonged 5 s	1, 10.0%	63, 3.4%	0.255
Objectives of PLB			
Focal liver lesions	8, 80.0%	1346, 72.9%	0.336
Diffuse liver disease	2, 20.0%	501, 27.1%	
Prebiopsy application of anticoagulants/ antiplatelet medication/Y	2, 20.0%	79, 4.5%	0.02

Postbiopsy	9, 90.0%	462, 26.6%	<0.001
application of hemostatic medication/Y			
Operator/10-year experience	6, 60.0%	665, 36.0%	0.115
Biopsy technique			
Bare introduction	6, 60.0%	922, 49.9%	0.525
Tru-cut (18G)			
Coaxial introduction	4, 40.0%	925, 50.1%	
Tru-cut (18G)			
Location of targets			
Right	6, 75.0%	1046, 73.0%	0.659
Left	1, 12.5%	301, 21.0%	
Hilar	1, 12.5%	86, 6.0%	
The maximum diameter of targets, median (Q1, Q3)	2.8 (1.7, 6.3)	3.3 (2.1, 5.9)	0.514
Multilesion/Y	4, 44.0%	848, 60.2%	0.336
Postbiopsy hemoglobin level, median (Q1, Q3)	81.0 (65.3, 107.8)	114.7 (109.0, 126.0)	0.001
Repeat biopsy/Y	0, 0%	30, 1.6%	0.685
Num. of specimens, median (Q1, Q3)	2 (2, 2.3)	2 (2, 2)	0.553
Histological analysis			
HCC	0, 0%	278, 15.1%	0.094
ICC	3, 30.0%	222, 12.0%	

Secondary hepatic tumor	2, 20.0%	516, 27.9%
Liver abscess	1, 10.0%	90, 4.9%
Chronic liver disease	0, 0%	393, 21.3%
Others	4, 40.0%	348, 18.8%

PLB: percutaneous liver biopsy; Y: Yes; n: number; HCC: hepatocellular carcinoma; ICC: intrahepatic cholangiocarcinoma;

-In the following phrase: “The results of this annual audit of 1857 liver biopsies in Chinese tertiary-care teaching hospital confirm that..... with published data from other parts of the world. Please add the appropriate references.

Response: Thank you for your suggestion. We added the related references to this sentence.

Revision made in the “**Discussion**” section on Page 11, paragraph 2, line 8;

“The results of this annual audit of 1857 liver biopsies in Chinese tertiary-care teaching hospital confirm that the incidence of major complications (0.53%) following ultrasound-guided PLB is low and in line with published data from other parts of the world [2-8]”

-With respect to the next phrase: “The rate of serious adverse events was 1.1%, and the bleeding rate was 0.58% [4].” The authors should check if the value 0.58% is correct.

Response: Thank you for your question. We checked the reference 4 and confirmed that the bleeding rate was 0.58%, which presented in Table 3. However, in the abstract section, it was suggested that “the most common was bleeding (16 cases, 0.6%)” as shown in the following screenshots.

Abstract

Background & Aims—Although percutaneous liver biopsy is a standard diagnostic procedure, it has drawbacks, including risk of serious complications. It is not known whether persons with advanced chronic liver disease have a greater risk of complications from liver biopsy than patients with more mild, chronic liver disease. The safety and complications of liver biopsy were examined in patients with hepatitis C-related bridging fibrosis or cirrhosis that were enrolled in the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) Trial.

Methods—Standard case report forms from 2,740 liver biopsies performed at 10 study sites between 2000 and 2006 were reviewed for serious adverse events, along with information from questionnaires completed by investigators about details of biopsy techniques used at each hospital.

Results—There were 29 serious adverse events (1.1%); the most common was bleeding (16 cases, 0.6%). There were no biopsy-related deaths. The bleeding rate was higher among patients with platelet counts $\leq 60,000/\text{mm}^3$ and among those with an international normalized ratio (INR) ≥ 1.3 , although none of the patients with an INR > 1.5 bled. Excluding subjects with a platelet count $\leq 60,000/\text{mm}^3$ would have reduced the bleeding rate by 25% (4/16), eliminating only 2.8% (77/2740) of biopsies. Operator experience, the type of needle used, or the performance of the biopsy under ultrasound guidance did not influence the frequencies of adverse events.

Conclusions—Approximately 0.5% of persons with hepatitis C and advanced fibrosis experienced potentially serious bleeding after liver biopsy; risk increased significantly in patients with platelet counts $\leq 60,000/\text{mm}^3$ (K2).

Table 3
Description of Complications Recorded as a Serious Adverse Event Among 2740 Liver Biopsies Performed

Description	No. Serious Adverse Events	Percentages/Liver Biopsy	Percentages/Serious Adverse Event
Bleeding *	16	0.58%	55.2%
Severe pain	7	0.26%	24.1%
Punctured gall bladder	2	0.07%	6.9%
Marked hypotension	1	0.04%	3.4%
Pneumothorax	1	0.04%	3.4%
Syncope	1	0.04%	3.4%
Dehydration	1	0.04%	3.4%
Total	29	1.06%	100.0%

* Hemoperitoneum, 8 cases; subcapsular hematoma, 4 cases; hemobilia, 3 cases, hemothorax, 1 case

Reviewer #2

Comments

In the article by Chai WL et al (WJG-83839-2023), early and late complications after ultrasound-assisted liver biopsy are reviewed. The article analyzes the complications observed in 1857 consecutive cases of liver biopsy performed at a single University Hospital in Eastern China between January and December 2021. The article is not original. However, it is well written and gives useful information about the factors influencing the risk of biopsy in different categories of patients. My comments follow.

A. General Comments:

1. Some typing and grammatical errors need correction. 2. Discussion too long. Please shorten it to no more than 800-900 words.

Response: Thank you for your suggestion. The English in revised manuscript was polished and “Discussion” was shortened, so that some words, sentences were significantly changed which were not totally presented in this document.

B. Major Comments:

1. (Page 7, Line 4,5): Please give more information concerning the nature and reasons of performing a liver biopsy for “non-histological assessment”.

Response: Thank you for your suggestion. We highly agree with your point. Based on the guidelines of the British Society of Gastroenterology, the Royal College of Radiologists and the Royal College of Pathology^[reference 3], the non-histological assessment included the microbiological, biochemical assessment and other assessment for scientific research. We revised the indications for PLB accordingly.

3. **Neuberger J, Patel J, Caldwell H, et al.** Guidelines on the use of liver biopsy in clinical practice from the British Society of Gastroenterology, the Royal College of Radiologists and the Royal College of Pathology. Gut. 2020; 69:1382-1403.

Revision made in the “**Materials and Methods**” section on Page 6, paragraph 3, line 3-4;

“(4) obtain liver tissue for non-histological assessment (microbiology, biochemical, other).”

2. (Page 8, Line 9): How many operators were involved? What was their post-training experience in years?

Response: Thanks for reviewer’s question. A total of 18 operators in our center performed ultrasound-guided PLB and 5 of them had more than 10-year experience on abdominal intervention. We added the information of operators in the section of materials and methods.

Revision made in the “**Materials and Methods**” section on Page 7, paragraph 3, line 17-18;

“A total of 18 operators in our center performed ultrasound-guided PLB and 5 of them had more than 10-year experience on abdominal intervention. ”

3. (Page 9, Line 12): Please mention the method used in the logistic regression and the mode the nonparametric variables were handled.

Response: Thanks for reviewer's suggestion. We revised the section of statistical analysis according to your suggestion.

Revision made in the "**Materials and Methods-Data analysis**" section on Page 9, paragraph 1, line 1-9;

“ The difference in categorical variables between major complication group and no major complication group are presented as percentages and were compared by using the chi-square or Fisher's exact test, as appropriate. Normally distributed variables are expressed as the mean \pm standard deviation (SD) and compared by the Student's t test. Non-normally distributed variables are expressed as median (Q1, Q3), and were compared by nonparametric test (Mann-Whitney U test). Multivariate analyses were performed by a forward step (likelihood ratio) multivariable logistic-regression model to investigate the risk factors associated with major complications and all-cause 30-day mortality following ultrasound-guided PLB. ”

4. (Table 1 & 2): Tables 1 and 2 must be presented in a completely different way. Under their title, one expects to see the actual numbers of the variables and not the corresponding major/minor complications. The complications per variable must be the content of a separate table.

Response: Thanks for reviewer's suggestion. For Table 1 and Table 2 presented the difference of all the included variables between major complication group and no major complication group, we mixed them into one table.

5. (Table 1, Sex/males): Please verify that the comparison is between males vs. females for major vs. non-major complications. If not, please explain.

Response: Thanks for reviewer's suggestion. We compared the percentage of males and females in major and no major complication groups by chi-square test and we added the percentage of females in the next line.

6. (Table 1, interquartile range): The IQR implies that you give the 25% and the 75% of the variance; not their difference. Please correct.

Response: Thanks for reviewer's suggestion. We revised the presentation accordingly.

Revision made in the “**Tables**” section on Page 17-19;

Table 1. Demographic, clinical, procedural and pathological characteristics of patients in the major complication and no major complication groups.

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Objectives of PLB			

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Biopsy technique			
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Hilar	1, 12.5%	86, 6.0%	
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Postbiopsy	81.0 (65.3, 107.8)	114.7 (109.0, 126.0)	0.001

hemoglobin level,			
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Num. of specimens,	2 (2, 2.3)	2 (2, 2)	0.553
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ICC	3, 30.0%	222, 12.0%	
Secondary hepatic tumor	2, 20.0%	516, 27.9%	
Liver abscess	1, 10.0%	90, 4.9%	
Chronic liver disease	0, 0%	393, 21.3%	
Others	4, 40.0%	348, 18.8%	

PLB: percutaneous liver biopsy; Y: Yes; n: number; HCC: hepatocellular carcinoma; ICC: intrahepatic cholangiocarcinoma;

7. (Table 2, Operator): Were other operators involved in PLB's with more or less experience? If not, you must omit this variable.

Response: Thanks for your question. A total of 18 operators in our center performed ultrasound-guided PLB. Five of them had more than 10-year experience on abdominal intervention, 13 of them had less than 10-year experience on abdominal intervention. We added the information of operators in the section of materials and methods. Revision made in the “**Materials and Methods**” section on Page 7, paragraph 3, line 17-18;

“A total of 18 operators in our center performed ultrasound-guided PLB and 5 of them had more than 10-year experience on abdominal intervention. ”

8. (Table 3): In the multivariable results, a unit of change must accompany all the significant risk factors.

Response: Thank you for your suggestion. We added beta value and adjusted the table accordingly.

Revision made in the “**Tables**” section on Page 20, Table 2;

Table 2. Risk factors related to increased risk of major complications and all-cause 30-day mortality.

	β	OR (95%CI)	P value
Risk factors for major complication			
Obstructed jaundice	1.902	6.698 (1.133-39.596)	0.036
Fibrinogen<2 g/L	2.846	17.226 (2.647-112.102)	0.003
Prebiopsy application of anticoagulants/antiplatelet medications	3.181	24.078 (1.678-345.495)	0.019
Postbiopsy hemoglobin level	-0.037	0.963 (0.942-0.985)	0.001
Age	0.091	1.096 (1.012-1.187)	0.025
Risk factors for all-cause 30-day mortality			
Prebiopsy hemoglobin	-0.038	0.963 (0.928-0.999)	0.042
Postbiopsy hemoglobin	-0.043	0.958 (0.930-0.987)	0.005

C. Minor Comments:

1. (Page 8, Line 4): Do you mean “16 μ g (or mg?) of lyophilized powder”? Please clarify.

Response: Thanks to the Reviewer’s question. According to the instructions for Sonazoid, the content of perfluorobutane microspheres was 16 μ l per bottle.

Revision made in the “**Materials and Methods**” section on Page 7, paragraph 3, line 13;

“The Sonazoid was supplied as 16 μ l **perfluorobutane microspheres** and reconstituted with 2 mL of distilled water to make a homogeneous microbubble suspension; ”

2. (Page 8, Line 10): It is better to express it as the “number of passes to obtain adequate tissue specimens”

Response: Thanks to the Reviewer’s valuable suggestion. We revised accordingly.

Revision made in the “**Materials and Methods**” section on Page 8, paragraph 1, line 4;

“The following pertinent variables were investigated and collected: platelet count, PT, fibrinogen, prebiopsy hemoglobin level, comorbidity, application of anticoagulant/antiplatelet medication, operators’ experience, biopsy technique, objective of biopsy, **number of passes to obtain adequate tissue specimens**, postbiopsy application of hemostatic medication, location of target, multilesion, maximum diameter of target, postbiopsy hemoglobin level, repeat biopsy and histological diagnosis.”

3. (Page 11, Line 16,17): Please explain what do you actually mean in the phrase: “to hepatic occupations, especially in the realm of precision medicine”.

Response: Thank you for your suggestion. We adjusted the related sentences.

Revision made in the “**Discussion**” section on Page 11, paragraph 2, line 1-3;

“**Ultrasound-guided PLB plays an increasingly important role in the management of liver disease or abnormal liver function tests^[1-3], as well as in patients with a diagnostic dilemma ^[9].**”

4. (Page 13, Line 5): Please explain what do you mean with the phrase “adjacent to the hepatic Glisson system”. Glisson is not a system. It is known as the liver capse.

Response: Thank you for your suggestion. We did related revision to this sentence.

Revision made in the “**Discussion**” section on Page 12, paragraph 3, line 7;

“This may be ascribed to the nature and location of the biopsy targets; they were more likely to be tumors of biliary origin and developed adjacent to the hepatic **vessels**.”