Response to reviewers' comments

Reviewer #1:

The authors retrospectively analyzed the effect of splenectomy on tumor prognosis after liver transplantation for hepatocellular carcinoma which conducted a retrospective cohort study and suggested that splenectomy should be avoided in patients with hepatitis C complicated with hepatocellular carcinoma.

1. This article has certain clinical value, but some studies on this aspect have been reported (PMID: 34619907),

Response: I have added this study (PMID: 34619907) as one of the references. Thanks for pointing this out.

2. and there have been several studies on the indication of spleen resection for liver transplantation (PMID: 32827564, PMID: 31555908, PMID: 29517676, PMID:33642835).

Response: I originally cited earlier articles as the references. Thanks for these suggestions. I have replaced the earlier references with references from more recent years, as you have suggested.

3. There are also some shortcomings in the study design of this paper, such as mismatching of baseline data and small sample of HCV subgroups.

Response:

- I am very grateful for your comments. I have added the following statement: "Because of surgical indications for simultaneous splenectomy, more HCV patients underwent simultaneous splenectomy. There may be biases in terms of patient selection" in the Discussion Section (Main text: Page 11). Thanks for this advice.
- This is a retrospective study. From 2009 to 2019, a total of 120 HCC patients were included, of which 35 received simultaneous splenectomy and 85 did not. This indicates that the two groups of patients have a similar baseline, except for HCV, platelet count, and AFP level. The Supplementary Table 1 shows the HCV subgroup analysis, similar to Main text Table 1. (Response to reviewers' comments, Page 9-11)
- I have deleted the subgroup analysis of HCV patients from the main text and presented it in Supplementary Table 1 only. This revision will simplify the text and make it more comprehensible.

4. Generally speaking, the innovation of this paper is not recommended, and it can be transferred to other journals of the company.

Response: In addition to several previous studies, the preprint study, "Spleen plays a two-way role in cancer incidence and cancer progression," presented by Yang J, Li Y, Li

Z, and Jiang W, indicates that the impact of splenectomy on cancer development is worth exploring. My study also aims to explore the impact of splenectomy on different groups, such as liver transplant recipients. I sincerely hope that the editors will carefully consider accepting my article. Thanks a lot.

5. A meta analysis has indicated that Splenectomy benefits LT patients in increasing platelet count. However, splenectomy is a morbid procedure as splenectomy increases operation time, intraoperative blood loss, intraoperative RBC transfusion, and postoperative complications. Splenectomy does not improve OS but increase perioperative mortality. Therefore, Splenectomy should be performed only in selective patients (PMID: 29517676).

Response: Thanks for your advice. I have ensured that this meta-analysis is cited in main text (Reference 28). Moreover, I have added the following sentence: "These three studies suggest that splenectomy has a number of short-term risks and should be performed only in carefully selected patients." (Main text: Page 10, Line 23-25)

6. In this paper, the research results for the clinical application value, and is not recommended for patients with port of most scholars directly line resection, splenic artery blood platelets, splenic artery ligation can solve the problems such as (DOI: http://dx.doi.org/10.3748/wjg.v20.i41.15367).

Response: I originally mentioned that "splenic artery ligation is often considered, instead of splenectomy, for achieving the goal of modulation of portal inflow[29]. The effects of splenic artery ligation, compared to splenectomy, were not discussed in this study." in the Discussion Section. I added this reference in my revised article. In the future, I plan to analyze the effect of splenic artery ligation as my next topic. Thanks for your advice.

7. There were many problems in the experimental design of this study.
    I. First of all, of the 120 patients included by the author, only 35 cases were included in the splenectomy group. The sample size was too small, about 1:3 compared with the control group, and the experimental results were not convincing. Secondly, as a clinical study, what is the calculation standard of sample size? How do you calculate a sample of 120? Whether the indications for splenectomy were used as the basis for calculating sample size and were explained evenly, all of these led to the unreliability of the research results of this paper, and there were also the following main problems.

Response:

- This is a retrospective study. To the best of my knowledge, liver transplantation with simultaneous splenectomy is now performed less and less because the indication of simultaneous splenectomy is becoming less frequent.

- I have asked the statistics company to re-run the statistics, all of which were handled in nonparametric statistics. This method may overcome the problem of the small sample size.

i. in splenectomy or not, HBV patients accounted for 66.7%, affecting the results of subgroup data analysis

ii. If baseline data do not match, it is recommended to use the principle of bias matching to correct and re-analyze

iii. Survival analysis should be performed for different subgroups of HCC co-infection

Response:

- I know that one of the limitations of this study is its small patient number. Subgroup analysis makes the problem of the small number of patients more serious. The statistics company suggested using the multivariate Cox regression method instead of subgroup analyses. Hence, I have revised the Conclusion and simply presented the outcome of the cohort, rather than the HCV subgroup.

iv. 5. What are the surgical indications for splenectomy in liver transplantation patients with hepatocellular carcinoma? The author's description is too broad

Response:

- Thank you for pointing this out. I have added the following: “The reasons for simultaneous splenectomy in the 53 recipients were modulation (22/53, 41.5%), thrombocytopenia in recipients with HCV (25/53, 47.2%), and ABO-incompatibility LT (6/53, 11.3%)” (Main text: Page 5, Line 9).

v. 6. Language needs to be partially corrected

Response: I have commissioned an English editing company to revisit the English grammar and fine-tune the text. The certification is attached.

Reviewer #2:

In this paper, the authors present the results of a retrospective case-control study in which the oncologic outcomes were compared between patients with HCC with and without splenectomy. The authors may wish to consider the following comments:

1. Abstract:

I. Passive voice in the background of the abstract makes it unclear whether it is a result of your study or what is known so far.

Response:

I have rewritten this sentence in order to express the meaning more clearly.

“Abstract

Background:

Splenectomy has previously been found to increase the risk of cancer development, including lung, non-melanoma skin cancer, leukemia, lymphoma, Hodgkin's lymphoma, and ovarian cancer. The risk of cancer development in liver transplantation with simultaneous splenectomy remains unclear." (Main text: Page 2, Line 3-6)

II. Conclusions in the abstract refer only to patients with hepatitis C, while sub-analysis by HCV status is only one part of the results.

Response:

The statistics company suggests using the multivariate Cox regression method instead of subgroup analyses. Hence, I have revised the Conclusion and simply presented the outcome of the cohort, rather than the HCV subgroup.

2. Background:

I. There are more suitable recent studies such as meta-analyses on the safety of the splenectomy, to be cited instead of the reference 8: - Yang J, Li Y, Li Z, Jiang W. Spleen plays a two-way role in cancer incidence and cancer progression (still a preprint). - He, Chao; Liu, Xiaojuan; Peng, Wei; Li, Chuan; Wen, Tian-fu. Evaluation of the efficacy and safety of simultaneous splenectomy in liver transplantation patients, Medicine: March 2018 - Volume 97 - Issue 10 - p e0087 doi: 10.1097/MD.0000000000010087.

Response:

- Currently, Yang's study is still a preprint. Although the content can be reviewed online, the online statements specially point out that "preprints are preliminary reports that have not undergone peer review. They should not be considered conclusive, used to inform clinical practice, or referenced by the media as validated information." I cannot cite this article as one of references. However, Yang's study shows that the impact of splenectomy on the cancer development is worth exploring. My study also hopes to explore the impact of splenectomy in different groups, such as liver transplant patients.

II. The second meta-analysis is cited by the authors at the end of the discussion regardless it is an important piece of evidence on this topic. Therefore, the statement "The effects of splenectomy in cancer development after LT has not been discussed in previous literature." should be deleted from the introduction, abstract and discussion, results of this meta-analysis should be reported and a better rationale for this study should be provided.

Response: I originally wanted to emphasize that this was "after liver transplantation".

After careful consideration, I am very grateful for your comments, and following your suggestion, I have deleted the statement, “The effects of splenectomy in cancer development after LT has not been discussed in previous literature.”

3. Methods:
    I. “Between May 2009 and August 2019, 179 patients with HCC underwent LT and received follow-up management.” Were all of them included?

Response:

I excluded 59 patients who had no residual HCCs or who had HCCs without the fitting UCSF criteria on pathologic examinations. I considered the UCSF criteria to be the most important factor, whereas splenectomy was a minor factor. Therefore, I excluded the main factor of the UCSF criteria to explore the importance of minor factors, such as splenectomy.

    II. It is not clear what is the main oncologic outcome in the study, from the text I have the impression that it was overall cancer, from methods that it was only HCC recurrence while the tables report also non-HCC cancers.

Response: I have replaced the term “oncologic outcome” with “HCC recurrence” and “de novo cancer development.” This change makes the meaning clearer.

    III. Statistical analysis: Risk ratios from time-dependent Poisson regression for cohort data with 95%CI would be much more appropriate than p values. Due to the small sample size, even 10% difference in the cancer recurrence between groups was statistically insignificant due to the small power of the study (Table 1). Variables selection in the multivariate regression cannot be based solely on univariate analysis results but also on the clinical significance. P-value is affected by sample size indeed in a small sample, variables may have substantive importance, although they are not significant. Was the multicollinearity and model diagnostics, such as goodness of fit, assessed?

Response: I have asked the statistics company to re-run the statistics, all of which were handled in nonparametric statistics. This method should reduce the problem of small samples.

4. Results:
    I. How “NLR ≥ 3 months after LT” was the main predictor of death since there are patients who died the same day of the surgery (with a survival of 0 days)?

Response: I also encountered this problem; therefore, I didn’t analyze NLR ≥ 3 months after LT as one of the factors in the Cox regression model analyses.

    II. The confidence interval in the association between splenectomy and mortality is too wide, being a consequence of a small sample size.

III. This must be addressed in the limitations as this substantially limits making the inference about splenectomy being a risk factor for mortality.

IV. 95%CI for AFP was 1.096-76.667, in this case, the p-value has no value.

Response: As previously mentioned, I have asked the statistics company to re-run the statistics. This should reduce the problem of small samples.

V. "Because of surgical indications for simultaneous splenectomy, more HCV patients underwent simultaneous splenectomy. There may be biases in patient selection." This is more appropriate and important for the limitations.

Response:

- I am very grateful for your comments. I have added the following statement: "Because of surgical indications for simultaneous splenectomy, more HCV patients underwent simultaneous splenectomy. There may be biases in terms of patient selection" in the limitations paragraph of the Discussion Section (Main text: Page 11).

VI. Is there a result of the splenectomy indications, how many of them were due to surgical indications?

Response:

- I have added the following, "The reasons for simultaneous splenectomy in the 53 recipients were modulation (22/53, 41.5%), thrombocytopenia in recipients with HCV (25/53, 47.2%), and ABO-incompatibility LT (6/53, 11.3%)" (Main text: Page 5, Line 11).

5. Tables:

I. It might be useful to list the 5 non-liver cancers below the table 1 or in the results.

Response: Thank you for this suggestion. I have added the following sentence: "5 of the 85 patients (6.4%) in the non-splenectomy group had de novo cancer development. Of the 5 patients with de novo cancer development, 1 had lung cancer, 1 had urothelial carcinoma, 1 had squamous cell carcinoma of the tongue, 1 had breast cancer, and 1 had adenocarcinoma of the esophagus. In the splenectomy group, no de novo cancer development was found." (Main text: Page 7, Line 24-28)

II. * is redundant, it is clear that for example 0.02 is smaller than 0.05.

Response: The Author Guidelines stated that "Data with statistical significance in a figure or table should be denoted using superscripted alphabetical lettering, such as $^{a}P < 0.05$ and $^{b}P < 0.01$." Hence, I kept the label as "*" to emphasize the statistically significant data.

6. General comments The manuscript needs some reworking/rephrasing to simplify the text and make it more comprehensible.

Response: I have reworked the statistical analysis and rewritten the text simplify it and make it more comprehensible.

7. A native English speaker needs to fine-tune the text.

Response: I have commissioned an English editing company to revisit the English grammar usage, and to fine-tune the text. The certification is attached.

Supplementary Table 1. Patients' characteristics in HCV subgroup

	Non-splenectomy (N=22)	Splenectomy (N=23)	P value
Age (year), median (SD)	62 (10)	59 (9)	0.480
Gender, n (%)			0.208
Male	17 (77.3)	13 (56.5)	
Female	5 (22.7)	10 (43.5)	
BMI, median (SD)	23.5 (2.2)	25.2 (6.6)	0.059
Underlying liver disease, n (%)			
HBV	7 (31.8)	4 (17.4)	0.314
Alcoholism	4 (18.2)	2 (8.7)	0.414
Signs of portal hypertension, n (%)			
Ascites	13 (59.1)	14 (60.9)	1.000
Hepatic encephalopathy	12 (54.5)	9 (39.1)	0.376
Varices bleeding	4 (18.2)	9 (39.1)	0.189

Co-morbidities, n (%)			
Hypertension	6 (27.3)	6 (26.1)	1.000
Diabetes mellitus	9 (40.9)	7 (30.4)	0.542
Pre-operative serum tests, median (SD)			
White blood count (/uL)	4970 (1540)	4560 (3670)	0.143
Platelet count (/uL)	90000 (60000)	56000 (37000)	0.009*
Neutrophil-Lymphocyte ratio	2.33 (3.05)	2.87 (3.35)	0.447
Platelet-Lymphocyte ratio	85.69 (55.58)	65.52 (75.89)	0.540
Total bilirubin (mg/dL)	2.7 (3.2)	1.6 (1.1)	0.241
Creatinine (mg/dL)	0.9 (0.5)	0.8 (0.3)	0.936
Ammonia (ug/dL)	107 (110)	94 (100)	0.242
Albumin (g/dL)	3.0 (1.2)	3.1 (1.2)	0.674
Glucose (mg/dL)	115 (114)	105 (37)	0.503
INR	1.2 (0.4)	1.1 (0.2)	0.555
MELD scores	14 (13)	10 (5)	0.179

AFP (ng/ml)	8.5 (11.5)	17.5 (26.1)	0.014*
Surgical factors			
Surgical type, n (%)			0.622
DDLT	7 (31.8)	5 (21.7)	
LDLT	15 (68.2)	17 (73.9)	
SLT	0	1 (4.3)	
Graft type, n (%)			0.514
Whole graft	7 (31.8)	5 (21.7)	
Partial graft	15 (68.2)	18 (78.3)	
GRWR>0.8, median (SD)	20 (90.9)	20 (87)	1.000
Blood loss (ml), median (SD)	2500 (3100)	1500 (1900)	0.411
Operative time (minutes), median (SD)	552 (170)	616 (151)	0.229
Pathology			
Tumor size (cm), median (SD)	2.4 (1.6)	2.3 (2.3)	0.909

Tumor number, n (%)			0.749
0-1	16 (72.7)	15 (65.2)	
2-3	6 (27.3)	8 (34.8)	
Tumor necrosis, n (%)	12 (54.5)	11 (47.8)	0.768
Lymphovascular invasion, n (%)	2 (9.1)	4 (17.4)	0.665
Outcomes			
Hospital stays (days), median (SD)	21 (11)	18 (11)	0.811
HCC Recurrence, n (%)	0	2 (8.7)	0.489
Secondary cancer, n (%)	1 (5.0)	0	0.465
Mortality, n (%)	2 (9.1)	9 (39.1)	0.035*

The median (interquartile range) is presented for continuous variables, and number (percentage) is presented for categorical variables.

BMI, Body Mass Index; HBV, hepatitis B virus; LT, liver transplantation; INR, international normalized ratio; MELD, the Model for End-stage Liver Disease; AFP, alpha-fetoprotein; DDLT, deceased donor liver transplantation; LDLT, living donor liver transplantation; SLT, split liver transplantation; GRWR, graft-to-recipient weight ratio; HCC,

hepatocellular carcinoma. *P < 0.05.