Round-1:

POINT-BY-POINT RESPONSE

Manuscript NO: 58470

Name of Journal: *World Journal of Gastrointestinal Surgery*

Manuscript Type: ORIGINAL ARTICLE

Retrospective Cohort Study

Liver Transplant for Larger Hepatocellular Carcinoma in Malatya: The Role of Gamma Glutamyl Transferase and Alpha-fetoprotein, A Retrospective Cohort Study

Ince V *et al.* The Role of GGT and AFP for advanced HCC

Volkan Ince, Brian I Carr, Harika Gozukara Bag, Veysel Ersan, Sertac Usta, Cemalettin Koc, Fatih Gonultas, Baris Kemal Sarici, Serdar Karakas, Koray Kutluturk, Adil Baskiran, Sezai Yilmaz

Reviewer code: 03538879

First decision: 2020.09.09

Science editor: Ya-Juan Ma

To the Editor in Chief,

World Journal of Gastrointestinal Surgery.

Dear Professor Na Ma,

Please find enclosed a point-by-point response of the original submission entitled "Liver Transplant for Larger Hepatocellular Carcinoma in Malatya: The Role of Gamma Glutamyl Transferase and Alpha-fetoprotein, A Retrospective Cohort Study."

1 MANUSCRIPT REVISION DEADLINE

We request that you submit your revision in no more than 14 days. Please note that you have only two chances for revising the manuscript.

Response: We revised our manuscript and submitted before deadline.

2 PLEASE SELECT TO REVISE THIS MANUSCRIPT

Please login to the F6Publishing system at https://www.f6publishing.com by entering your registered E-mail and password. After clicking on the "Author Login" button, please click on "Manuscripts Needing Revision" under the "Revisions" heading to find your manuscript that needs revision. Clicking on the "Handle" button allows you to choose to revise this manuscript or not. If you choose not to revise your manuscript, please click on the "Decline" button, and the manuscript will be WITHDRAWN.

Response: We done as requested.

3SCIENTIFIC QUALITY

Reviewer's comments and suggestions:

Answer to the Reviewer #1

The authors continued previous studies on the relationship between AFP/GGT and prognosis of HCC in liver transplantation, focused on patients having tumors beyond Malatya criteria patients, MTD > 6.0 cm HCCs, found significantly longer overall-survival and disease-free-survival for patients who had lower values of AFP and GGT, compared with higher values. AFP and GGT, both singly and combined together, represent a simple prognostic identifier for patients with large size HCCs after liver transplantation. The results of this study are helpful for clinical practice. is article meets the publication requirements AND can be accepted.

1. The authors found that AFP groups had non-significant changes in GGT values, AND neither tumor differentiation nor microvascular invasion were different between the 2 AFP groups. This result is the likely cause of the cutoff value of AFP is not ideal?

Response: We thank for valuable comments of Reviewer #1.

"In this study, the AFP cut-off was used as 200 ng/mL, depending on our previous studies. However, specific to this study, we obtained the same results when we applied the AFP cutoff of 150 or 100 or 50 separately. Therefore, based on our other studies, we based the AFP cut-off 200." This phrase was added into the Discussion section.

Answer to the Reviewer #2

1. I strongly suggest authors revise the manuscript according to STROBE statement and use sub-sections. Current structures were too disordered.

Response: We revised the manuscript according to the STROBE statement in line with the editor's suggestions. Sub-sections were used.

2. The introduction section should explain the reason and rationale of this study. Authors should modify relevant contents to let readers clearly understand the meaning of this study. In addition, authors mentioned GGT-II in the introduction, while no further work was done on it. So why mention it?

Response: We thank for valuable comments for the Reviewer #2. The introduction section has been revised in a way that clearly explains our purpose and can be understood by readers. We had mentioned the GGT-II as an additional general information, and we removed the sentence that we mentioned GGT-II.

3. Treatments after surgery may also significantly influence the prognosis. I am wondering the reasons why post-surgery treatments were not involved in analysis.

Response: Post-transplant follow up and treatments such as immunosuppression and chemotherapy were added as stated in the Methods. We also added the post-transplant recurrence rate and management of the patients in to the results section as "Post-transplant recurrence rate was 46% (n=23). None of the patients with post-transplant recurrence were suitable for further surgery, and all of these patients received systemic Sorafenib therapy." However, none of the 50 patients received adjuvant HCC therapy, unless their HCCs recurred.

4. In the last paragraph of results, authors divided the cohort into two groups according to the tumor size. How the 10cm was determined?

Response: We first divided the patients three MTD groups as 6>MTD≥ 8cm, 8>MTD≥10 cm and MTD>10 cm. In 6-8 group there were 4 patients with low AFP and low GGT and all of them survived greater than 5 years and Kaplan-Meier analyses couldn't calculate a p value due to the low patient number. In the 8-10cm group there were significant survival outcomes. So we combined the 6-8 and 8-10 MTD groups. Then we presented the patient data of the two MTD groups as 6-10cm and >10cm patients.

This analysis is not stated in the text to avoid oversizing the study. It can be added as a supplement if desired.

5. The decimal points in the figures was all written as ",". Please revise.

Response: All of the decimal points in the figure were revised as ".".

4 LANGUAGE QUALITY

Please resolve all language issues in the manuscript based on the peer review report. Please be sure to have a native-English speaker edit the manuscript for grammar, sentence structure, word usage, spelling, capitalization, punctuation, format, and general readability, so that the manuscript's language will meet our direct publishing needs.

Response: Prof. Dr. Brian I. Carr is a native English speaker. He is one of our co-authors. English editing was done by him.

5 EDITORIAL OFFICE'S COMMENTS

Science Editor's comments and suggestions:

1- The language classification is Grade C. Please visit the following website for the professional English language editing companies we recommend: https://www.wjgnet.com/bpg/gerinfo/240.

Response: Prof. Dr. Brian I. Carr is a native English speaker. He is one of our co-authors. English editing was done by him.

2-The "Author Contributions" section is missing. Please provide the author contributions

Response: We done as requested

3-The authors did not provide original pictures. 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: We done as requested and submitted as a single powerpoint file"58470-Figures.ppt***" on the system.***

4-PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout

Response: We done as requested

5-The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text.

Response: Article Highlights were added both here and to the online submission system.

Research background.

For many years, liver transplantation for hepatocellular carcinoma (HCC) has been done according to the Milan criteria of patient selection, using a maximum single tumor diameter (MTD) of 5cm and resulting in 75% 5-year survival.

Research motivation.

It has recently become apparent, that an expansion of the Milan criteria might be possible, with patients having slightly larger MTD than 5cm being transplanted with similar good survival. However, the extent by which MTD can be increased has not been clearly identified.

Research objectives.

To try to identify subsets of HCC patients with larger sizes of tumors, who can also have longer survival after liver transplant, despite having an MTD >5cm.

Research methods.

We retrospectively evaluated a prospectively accrued database of 50 patients who had HCCs > 5cm MTD and were treated by living donor liver transplantation.

Research results.

We found that patients with MTD 6-10cm HCCs could be stratified according to presence of low or high levels of serum alpha-fetoprotein (AFP) or gamma glutamyl transpeptidase (GGT), or the 2 serum markers in combination. The results showed that patients with a combination of low AFP plus low GGT had excellent long term survival. Patients who had either low AFP alone or low GGT

alone had intermediate survival, but patients with both high AFP plus high GGT had the worst survival.

Research conclusions.

Measurement of serum AFP and GGT levels permits identification of HCC patients with tumors greater than Milan criteria size who can have excellent post liver transplant survival.

Research perspectives.

Future research will need to be directed towards the identification of new HCC biomarkers that will correlate with tumor biology and will help refine the selection of HCC patients with larger tumors who can also benefit by treatment with liver transplantation. This will increase the percent of HCC patients who can then benefit by increased survival from liver transplantation.

Editorial office director's comments and suggestions:

I have checked the comments written by the science editor. The study was without financial support. I have checked the comments written by the science editor. I have changed the manuscript type "retrospective study" to "clinical trials study". The authors need to fill out the CONSORT Checklist form with page numbers. The author should number the references in Arabic numerals according to the citation order in the text. The reference numbers will be superscripted in square brackets at the end of the sentence with the citation content or after the cited author's name, with no spaces.

Response: There is no funding fort his study. This is a retrospective cohort study as Reviewer #2 mentioned before, so we filled out the STROBE statement with page numbers. References were revised as requested.

Company editor-in-chief's comments and suggestions:

Manuscript under further review

I have reviewed the Peer-Review Report, the full text of the manuscript and the relevant ethics documents, all of which have met the basic publishing requirements, and the manuscript is conditionally accepted with major revisions. I have sent the manuscript to

the author(s) for its revision according to the Peer-Review Report and the Criteria for Manuscript Revision by Authors. Before final acceptance, authors need to correct the issues raised by the editor to meet the publishing requirements.

Response: We thank to the editor-in-chief. We done as requested.

We have answered and made corrections according to the Editor's suggestions and each reviewer, which are highlighted in the manuscript. We have carefully answered and included all the suggestions made the best we could. We believe this report to be novel, interesting and appropriate subject matter to the readership of World Journal of Gastrointestinal Surgery.

All the authors have contributed to and agreed on the content of the manuscript. The manuscript has not been published previously in any language, in whole or in part and is not currently under consideration elsewhere.

6 STEPS FOR SUBMITTING REVISED MANUSCRIPT

Step 1: Author information

Response: We done as requested

Step 2: Manuscript information

Response: We done as requested

Step 3: Abstract, Main text, and Acknowledgements

Response: We done as requested

Step 4: References

Response: We done as requested

Step 5: Footnotes and Figure Legends

Response: We done as requested

Step 6: Automatically Generate Full Text Files

Response: We done as requested

Step 7: Upload the Revision Files

Response: We done as requested

1) 58470-Answering Reviewers: DONE
(2) 58470-Audio Core Tip: DONE
(3) 58470-Biostatistics Review Certificate: DONE ALLREADY PREVIOUSLY SUBMITED
(4) 58470-Clinical Trial Registration Statement: DONE ALLREADY PREVIOUSLY SUBMITED
(5) 58470-Conflict-of-Interest Disclosure Form: DONE ALLREADY PREVIOUSLY SUBMITED
(6) 58470-Copyright License Agreement: DONE ALLREADY PREVIOUSLY SUBMITED
(7) 58470-Approved Grant Application Form(s) or Funding Agency Copy of any Approval Document(s): NONE
(8) 58470-Signed Informed Consent Form(s) or Document(s) : DONE ALLREADY PREVIOUSLY SUBMITED
(9) 58470-Institutional Review Board Approval Form or Document: DONE ALLREADY PREVIOUSLY SUBMITED
(10) 58470-Non-Native Speakers of English Editing Certificate: NATIVE SPEAKER

(11) 58470-Video: N/A
(12) 58470-Image File: DONE
(13) 58470-Table File: DONE
(14) 58470-CONSORT 2010 Statement: DONE – STROBE STATEMENT
(15) 58470-Supplementary Material: N/A

Yours Sincerely,

Dr Volkan Ince MD, FEBS, on behalf of all co-authors.

Round-2:

POINT-BY-POINT RESPONSE

Manuscript NO: 58470

Name of Journal: *World Journal of Gastrointestinal Surgery*

Manuscript Type: ORIGINAL ARTICLE

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Ince V *et al.* The Role of GGT and AFP for advanced HCC

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Reviewer code: 03538879

First decision: 2020.09.09

Science editor: Ya-Juan Ma

To the Editor in Chief,

World Journal of Gastrointestinal Surgery.

Dear Professor Na Ma,

Please find enclosed a point-by-point response of the original submission entitled "Liver Transplant for Larger Hepatocellular Carcinoma in Malatya: The Role of Gamma Glutamyl Transferase and Alpha-fetoprotein, A Retrospective Cohort Study."

Reviewer's comments and suggestions:

Answer to the Reviewer #1
Round 2

1. Many clinical parameters have impacts on the prognosis of liver transplantation for liver cancer. I suggest the author add some clinical parameters during operation, such as blood loss, operation time, etc.
2. The author said "We previously found, by multivariate analysis of a large HCC cohort, that an MTD of 6 cm had a high and significant Hazard Ratio for survival", Therefore, tumor diameter has an important impact on the prognosis of liver cancer. From Figure 3 the author found no patients with MTD >10cm who also had low serum AFP plus low serum GGT levels, so I am wondering why there was no statistical difference in MTD of the two groups in table 3?
3. How the MTD Measured？ From CT image or pathological specimen？

Response: We thank for valuable comments of Reviewer #1.

1. We quite agree with reviewer #1, and that is why we excluded the patients whose follow-up period is lower than 90 days. All of these parameters that reviewer #1's mentioned are strongly related with post-transplant early mortality, so we could focus on HCC related outcomes and not on surgical mortality, by excluding the follow up period of lower than 90 days. But we have the data of intraoperative parameters. It can be added as a supplement if desired.

2. We strongly agree with reviewer #1, that MTD has an important impact on survival. The cause of no statistical difference on figure 3 according to MTD>10cm is related the Kaplan-Meier analysis itself. When you compare two groups with Kaplan-Meier, and if there are no patients in one of the groups, Kaplan-Meier cannot perform a comparison, so cannot calculate any p value, because there are no patients other group.

3. All of the tumor parameters like MTD, number of nodules, were recorded according to pathology reports of explanted livers.

Answer to the Reviewer #2

Round 2

Authors have answered my questions and made appropriate revision. I still suggest authors modify the structure of results section using sub-titles. Current presentation is not friendly to read.

Response: We thank reviewer #2 for valuable contributions. We revised the results section using sub-titles as following.

-Descriptive data

-Survivals according to AFP and GGT alone

-Survivals according to AFP and GGT combinations

-Survivals according to MTD size and AFP-GGT combinations

-Definition of new model

As a result of our analysis, we found that there is a group of patients that can be achieved with longer survival by liver transplantation in patients with HCC with MTD greater than 6cm. We have defined a new model for liver transplantation in patients with HCC with large tumors.

- *MTD≤10cm and AFP≤200ng/mL and GGT≤104IU/mL are good prognostic criteria in patients with HCC beyond Milan and Patients who meet this criteria have 76.2 % 5-year OS by liver transplantation (figure 3A).*

Yours Sincerely,

Dr Volkan Ince MD, FEBS, on behalf of all co-authors.