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Cirrhotics with monocyte chemotactic protein 1 polymorphism are at higher risk for developing spontaneous bacterial peritonitis – A cohort study

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1st Editorial decision
28-Feb-2021

Ref.: Ms. No. JCTRes-D-20-00145
Cirrhotics with Monocyte Chemotactic Protein 1 polymorphism are at higher risk for developing Spontaneous bacterial peritonitis – A cohort study
Journal of Clinical and Translational Research

Dear Dr. Sankaran,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Mar 30, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: The authors present an interesting study on MCP1 polymorphisms and the risk for spontaneous bacterial peritonitis (SBP). While the polymorphism they investigated has been associated with occurrence of SBP before, the finding that this polymorphism affects survival is novel and interesting.
Major remarks:
- The study cohort is very small. A replication cohort, if possible, would support the data enormously.
- The authors must clarify the causes of mortality. This should also included an analysis if causes of mortality differed between patients with the different MCP1 genotypes.
- The authors should present a survival analysis corrected for presence of hepatocellular carcinoma and Child-Pugh-Stage (or MELD score).
- Inclusion and exclusion criteria must be given in more detail. Was paracentesis performed in all patients?
Minor remarks:
- There are some typing errors
- The abstract is somehow confusing and should be rewritten
- Introduction: There seems to be a misunderstanding: positive blood culture bottles are not a diagnostic feature in SBP
- Please indicate the version of GraphPrism you used
- The names of the bacteria in table 4 are not written correctly
- The analysis and description of allele/genotype frequencies and their significance is a bit confusing. It might be easier to focus on the point that G allele frequency was different.
- In the discussion, when talking about MCP1 gene expression, you mean probably rather genotype frequencies than mRNA levels
- I do not think that frequency of this variant has really been shown to be significantly higher in cirrhotics and not to be present in the general population.

Authors' response

Reviewer's comments & responses:

Reviewer #1: The authors present an interesting study on MCP1 polymorphisms and the risk for spontaneous bacterial peritonitis (SBP). While the polymorphism they investigated has been associated with occurrence of SBP before, the finding that this polymorphism affects survival is novel and interesting**.**

Response: We are grateful for your commentary and suggestions, which we have addressed to the fullest extent as indicated below for every one of your comments.

Reviewers' comments:

1. **The study cohort is very small. A replication cohort, if possible, would support the data enormously.**

   Response: As suggested by the reviewer, we wanted to conduct a replica study. However, as the study was done before the COVID 19 pandemic and later due to lock down, access to the patient samples became limited. Hence a replica study could not be done. We request you to kindly consider the situation.

2. **The authors must clarify the causes of mortality. This should also include an analysis if causes of mortality differed between patients with the different MCP1 genotypes.**

   Response: As suggested by the reviewer, the causes of mortality among the different *MCP1* genotypes were evaluated and the data was analysed statistically using Fischer's exact test.

   The following lines were incorporated in the Result section of the manuscript. "In total, 52 patients (48.59%) died. Table 5 summarizes the causes of death for these patients, with acute - on - chronic liver failure representing the major cause."

3. **The authors should present a survival analysis corrected for presence of hepatocellular carcinoma and Child-Pugh-Stage (or MELD score).**

   Response: The survival curve analysis corrections were made as suggested by the reviewer are Figure 2.

   We have reframed the following lines in the result section of the manuscript: "Kaplan – Meier survival curve analysis corrected for hepatocellular carcinoma and Child Pugh class was done (Figure 2)."

   Advanced hepatocellular carcinoma was an exclusion criterion(as it can independently affect mortality).

4. **Inclusion and exclusion criteria must be given in more detail. Was paracentesis performed in all patients?**

   Response: Yes, diagnostic parcentesis was performed in all the individuals. We have reframed the line in methodology section of the manuscript:

   "Diagnostic paracentesis was performed under aseptic precautions in all patients. When minimal ascites was present, ultrasound guided paracentesis was preferred. Aspirated ascitic fluid was analysed for PMN count, albumin, protein and cytology. Simultaneous blood and ascitic fluid cultures were also sent for assessing microbial growth.

   The following lines were included in the methodology section of the manuscript:

   "Cirrhosis was diagnosed by clinical, laboratory, and radiological findings. Criteria for **inclusion** were liver cirrhosis and ascites detected by abdominal ultrasound. Individuals

with malignant ascites, secondary bacterial peritonitis, advanced HCC, severe heart disease and end stage renal disease were **excluded**."

5. **There are some typing errors**
   Response: The typing errors have been rectified as suggested by the reviewer.

6. **The abstract is somehow confusing and should be rewritten**
   Response: The abstract was rewritten as suggested by the reviewer.

7. **Introduction: There seems to be a misunderstanding: positive blood culture bottles are not a diagnostic feature in SBP**
   Response: As suggested by the reviewer, this line has been modified in the Introduction section as "A high peritoneal neutrophil count (>250 cells / mm3) and / or positive culture are important features of this condition."

8. **Please indicate the version of GraphPrism you used**
   Response: The methodology has been modified as follows "All the statistical calculations were done through Graphpad Prism 5.0"

9. **The names of the bacteria in table 4 are not written correctly**
   Response: The name of the bacteria in table has been modified as *Coagulase negative Staphylococci* and *Brevundimonas vesicularis.*

10. **The analysis and description of allele/genotype frequencies and their significance is a bit confusing. It might be easier to focus on the point that G allele frequency was different.**
    Response: As suggested by the reviewer, the the genotype frequencies were removed and the allele frequency was retained in Table 2.

11. **In the discussion, when talking about MCP1 gene expression, you mean probably rather genotype frequencies than mRNA levels**
    Response: Yes, the genotype frequency is mentioned instead of mRNA levels. Therefore, this line has been modified in the discussion as follows "*MCP1* polymorphism has been studied to play role in various infectious and inflammatory conditions [10-14]".

12. **I do not think that frequency of this variant has really been shown to be significantly higher in cirrhotics and not to be present in the general population**
    Response: This line in discussion was modified as follows "The presence of this variant was seen to be predispose the cirrhotic patients to progressive disease course [17]". There are contrasting evidences regarding this variant to be associated with cirrhotics. However, the presence of the G allele predisposes individuals to hepatic inflammation and fibrosis. Since MCP1 is a chemoattractant, the G variant was shown to be associated with inflammatory diseases and not in healthy controls.

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2nd Editorial decision
12-Apr-2021

Ref.: Ms. No. JCTRes-D-20-00145R1
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Journal of Clinical and Translational Research

Dear Dr. Sankaran,

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For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by May 12, 2021.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: The authors answered most of my questions and revised the manuscript accordingly. However, a few issues remain:
- In table 5, the percentage given after 10 in the AA-group is wrong
- It is unclear how the authors corrected the survival curves for HCC and Child-Pugh-stage C. They look identical to the original ones.
- Concerning inclusion / exclusion criteria: how were end stage renal disease, advanced HCC and severe heart disease defined?

Authors' response

Reviewers' comments:

Reviewer #1: The authors answered most of my questions and revised the manuscript accordingly. However, a few issues remain:

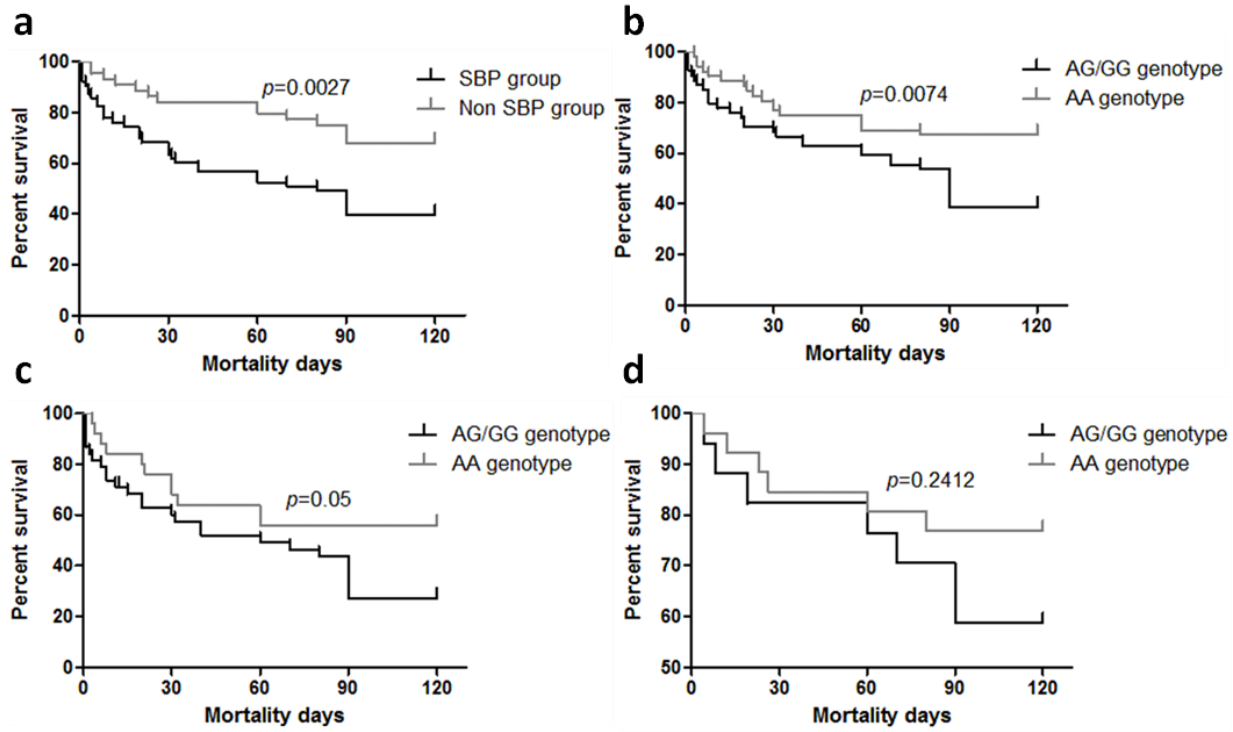
- In table 5, the percentage given after 10 in the AA-group is wrong

Response: As suggested by the reviewer, the percentage given after 10 was changed as 10 (59) in Table 5.

- It is unclear how the authors corrected the survival curves for HCC and Child-Pugh-stage C. They look identical to the original ones.

Response: We apologize for the incorrect figure sent earlier. The survival curves were analysed through Cox proportional hazard model where it was adjusted for HCC and Child-Pugh-class and presented as Figure 2. The survival curve after correction was included in the manuscript.

Survival curve before correction:



Survival curve after correction for HCC and Child-Pugh-class:

The following lines were included in the results section: "Kaplan Meier survival curve analysis was carried out (Figure 2) after making corrections for hepatocellular carcinoma and Child-Pugh class. Overall, Group 1 showed a significantly lower survival than Group 2 with a hazard ratio of 2.007 (95% CI = 1.067 - 3.775, p = 0.030; Figure 2a). Cirrhotic patients with G

allele (AG/GG genotype) had a poor survival than those without G allele (AA genotype), which was statistically significant with a hazard ratio of 1.967 (95% CI = 1.107 - 3.497, p = 0.021; Figure 2b). The presence of G allele resulted in statistically insignificant reduction in overall survival of cirrhotic patients with SBP (hazard ratio of 1.708, 95% CI = 0.8523 to 3.423, p = 0.1312; Figure 2c) and cirrhotic patients without SBP (hazard ratio of 1.787, 95% CI = 0.5902 - 5.409, p = 0.304; Figure 2d) compared with patients having AA genotype."

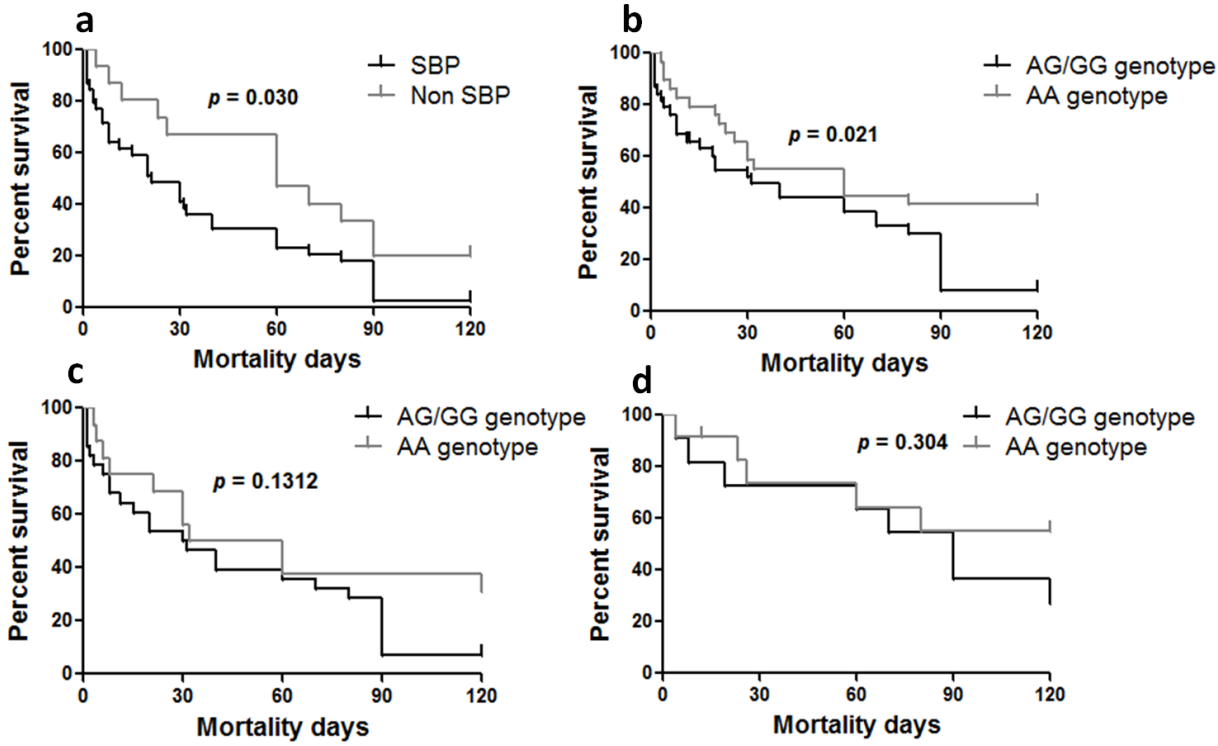


Figure 2. Kaplan-Meier survival curve for overall survival of cirrhotics (a) With and without SBP, (b) With different genotypes of *MCP1*, (c) Among SBP group with AG/GG and AA genotypes of *MCP1* and (d) Among non-SBP group with AG/GG and AA genotypes of *MCP1*. For both (a) & (b), the curves indicate a statistically significant reduction in overall survival due the presence of SBP (p = 0.030) and presence of AG/GG genotype (p = 0.021). (c) Among cirrhotics with SBP, AG/GG variants showed a statistically insignificant reduction in

survival than AA genotype (p = 0.1312) & (d) Among cirrhotics without SBP, AG/GG variants showed a reduction in survival than AA genotype which was not significant (p = 0.304). Log-rank test (Mantel-Cox) test was utilized for comparisons of survival curves. The survival curves corrected for the presence of hepatocellular carcinoma and Child-Pugh class were represented.

- Concerning inclusion / exclusion criteria: how were end stage renal disease, advanced HCC and severe heart disease defined?

Response:

As suggested by the reviewer, we have reframed the following lines in manuscript under methods section defining the end stage renal disease, advanced HCC and severe heart disease :

"Criteria for exclusion were pre-existing chronic renal failure requiring hemodialysis, pre-existing heart failure (New York Heart Association stage III/IV), advanced hepatocellular carcinoma (Barcelona clinic liver cancer-stage C or greater), malignant ascites and secondary bacterial peritonitis."

3rd Editorial decision
28-Apr-2021

Ref.: Ms. No. JCTRes-D-20-00145R2
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Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief

Journal of Clinical and Translational Research

Comments from the editors and reviewers: