Dear Prof. Fang-Fang Ji,

Thank you for the opportunity to submit our revised manuscript entitled: "Toll-like receptor 4 polymorphisms and the risk of bacterial infections in patients with cirrhosis and ascites" (ref. 37128 in WJH and ref. 36083 in WHG) to be considered for publication in the World Journal of Hepatology.
We have done our best to answer the queries raised by the reviewers and we have marked in green the changes made in the new version of the manuscript.
In addition, as you suggested, we have included the postcodes and Article Highlights, we have uploaded the Audio Core Tip and Grant Support, Institutional Review Board, Informed Consent and Conflict-of-Interest Statements, and we have tried to improve the quality of the figures.
We hope you consider now the article suitable for publication in the World Journal of Hepatology.

We are looking forward to hearing from you.

Sincerely yours,

Edilmar Alvarado
German Soriano
Silvia Vidal
Department of Gastroenterology
Hospital de la Santa Creu i Sant Pau

RESPONSE TO THE EDITORS AND REVIEWERS

Thank you for your interesting comments that have undoubtedly improved our manuscript.

REVIEWERS FROM THE WORLD JOURNAL OF HEPATOLOGY

Reviewer 02904354

This paper is well written and organized. However, the language repetition should be revised, especially the Methods section. Several major comments should be addressed. The authors conducted two similar studies. Notably, both of them achieved a positive conclusion (one in WJG, another in APT). By contrast, the present work achieved a negative conclusion. The same team got contradictive conclusions from several different studies. It should be fully explained. Additionally, the authors enrolled a consecutive cohort of patients from 2005 to 2011. By comparison, the previous work published in APT had an overlapping enrollment period (from 2006-2009). This point should be analyzed by the WJH editors to explore the potential duplicate publication. Certainly, I knew that the conclusions are different. In the Table 4, I did not see the variceal bleeding a potential factor in an univariate analysis. Additionally, the previous decompensation include HE, variceal bleeding, ascites. However, all of them were included in the Table 4.

Thank you for your comments.
We have modified the Methods section to try to avoid language repetition.
We comment now in the Discussion the contradictory conclusions between the present study and our previous restrospective study. Moreover, we explain that the 111 patients from our preliminary retrospective study were also included in the prospective follow-up reported in the present study. However, we do not consider that this is a duplicate publication, because the previous study was a preliminary only retrospective evaluation of the first 111 patients, and the present study is prospective for these 111 patients and 147 patients more.
Regarding the absence of variceal bleeding as potential factor in the univariate analysis of mortality, we did not include this factor because it did not reach statistical significance in the univariate analysis, and we only included in Table 4 those factors with statistical significance in the univariate analysis. We consider that it is now more clear in the new version.

Reviewer 03262644

This is interesting study with the main conclusion that TLR4 SNPs D299G and/or T399I are not related to the different risk of infection or the clinical outcomes in patients with decompensated cirrhosis with ascites as the decompensating event. This is interesting because bacterial infections are along with active alcohol abuse the main reason for deterioration of the liver function in cirrhosis leading to acute decompensation, ACLF and death. According to some retrospective data these 2 SNPs of the TLR4 gene might have been connected to the higher risk of infection in cirrhotic patients, but this has not been prospectively evaluated until this study. The scientific merit of this question arises from the fact that many patients with portohypertensive ascites require long-term antibiotic prophylaxis which is not without risks because of

the drug-related toxicity, possible modification of gut microbiota and selection of resistant bacterial species and loss of the antibiotic efficacy. Therefore new discoveries on the risk-factors responsible for the higher rate of infections in a certain patient would be welcome. According to this manuscript TLR4 SNPs D299G and/or T399I are not among them. Generaly speaking, one of the problems with this study is a very low number of patients with these SNPs (only 28), which has been already addressed by the authors as one of the major limitations. The other problem is that I could not clearly dissect from the provided data did the authors included also the patients with SBP, HRL, variceal bleeding along with the presence of ascites, OR only the patients with ascites without these complications? Furtheron, the authors must be much more precise when claiming "…we excluded patients with ……any other condition determining poor short-term prognosis". Which were these conditions? Page 9, Results section: "Seventy-four of the 332 patients were excluded due to human immunodeficiency virus (HIV) infection in 9 patients, a variable common immunodeficiency in one, advanced hepatocellular carcinoma or some other condition determining poor short-term prognosis in 57, and permanent urinary catheter in 7. Finally, 258 patients were included in the study." What did they mean by this "in 9 patients"- mistake? Page 9, Results: I do not fully understand what genotype/haplotype did they take for the risk group: compound heterozygotes for D299G and T3991I SNP, or else? This should more precisely stated.

Thank you for your comments.
Regarding the low number of patients with the polymorphisms, from the 258 patients included over a 6-year period, only 28 patients presented the polymorphisms, but this percentage is similar to the 10% prevalence of these polymorphisms in the general population. This is already commented as an important limitation of the study in the manuscript.
We have now clarified that all the patients presented ascites at inclusion to the study but they could simultaneously present other complications of cirrhosis (see Table 1). All patients were included on the first day of the hospital admission.
We have now clarified the exclusion criteria and the genetic characteristics of patients (all patients were heterozygous, there were not homozygous).

Reviewer 00071640
This study is well designed and manuscript is well written. The results of this study may add some information to the literature.

Thank you for your comments.

Reviewer 03024263
I read with great interest the article by Dr Alvarado-Tapias and co-authors. Indeed, bacterial infection is common and accounts for major morbidity and mortality in cirrhosis. Bacterial translocation is pathogenetically linked to the development of infections, particularly spontaneous bacterial infections, and other serious complications in cirrhosis. It was shown that small intestinal overgrowth, increased intestinal permeability, impaired intestinal motility, lack of bile acids, sympathetic

over-activity, and local innate and adaptive immunological alterations are important factors involved in the pathogenesis of bacterial translocation. Toll-like receptor 4 (TLR4) and its coreceptor MD-2 recognize bacterial lipopolysaccharide and signal the innate immune response. Two single nucleotide polymorphisms of human TLR4, D299G and T399I, have been identified and suggested to be associated with lipopolysaccharide hyporesponsiveness. In a previous retrospective study, these authors concluded that TLR4 D299G polymorphism could influence not only the predisposition to bacterial infections but also the evolution of the disease in cirrhotic patients (C. Guarner-Argente et al., 2010) . In this prospective study, they received opposite results. The results obtained by the authors are in agreement with other publications. For me it is also not a surprise. The authors are absolutely right when they propose that these results are due to poor functional impact of D299G and T399I TLR4 polymorphisms and/or the multifactorial and complex nature of the immune response. Nevertheless, the problem is very interesting and actual, requiring further research.

Thank you for your comments.

Reviewer 02521807

The study from Edilmar Alvarado-Tapias et al. shows TLR4-related SNPs D299G and/or T399I and their association with risk of infection and its outcome among patients with decompensated cirrhosis by ascites. The authors have published previous reports on this field. The present study shows conflicting results with other previously reported and the authors should be emphatic in their discussion to address this conflicting issue. Moreover some results appear overlapped and need to be clearly expressed to differentiate each study and their new original knowledge offered. In order to analyze plausible reasons for such weakness, the authors should also consider serious limitations in the population size under study, particularly in those exhibiting defined SNPs (D299G and/or T399I). Such is a clear imbalance for statistical considerations and may be a pivotal reason for weakness that must be amended to be statistically supported. During the different clinical conditions of patients enrolled in the study, the text is quite confusing regarding the clinical condition beyond ascites. Other conditions are also depicted by it is not clear whether were they included. It is mandatory to be clear. Besides the exclusion criteria should be clearly stated. Tables including clinical conditions of patients under study are also muddled.

Thank you for your comments.
We have now commented in the Discussion the contradictory results between the present study and our previous retrospective study. Moreover, we have clarified that the first 111 patients from the present manuscript were retrospectively analyzed in our previous preliminary publication.
We agree that the number of patients with the polymorphisms is low. From the 258 patients included over a 6-year period, only 28 patients presented the polymorphisms. This percentage, however, is similar to the 10% prevalence of these polymorphisms in the general population. This is already commented as an important limitation of the study.

We have clarified the clinical conditions at admission. All patients presented ascites at inclusion to the study but they could simultaneously present other complications of cirrhosis, as represented in Table 1.
We have clarified the exclusion criteria and Tables.

Reviewer 01588404
This is an interesting prospective study by the authors looking at 2 polymorphims inTLR gene and risk of bacterial infections in cirrhotics with ascites. Although the probability of transplant free survival was lower in Polymorphism group but did not reach significance. This similar to 2 metanalysis in 2012 and 2016 which showed no impact of these two TLR4 polymorphisms in sepsis. Minor points 1. Did the author consider expanding the analysis to other TLR4 polymorphisms which are shown to impact susceptibility to infections in different ethnic groups?

Thank you for your comments.
We agree that it would be interesting to study other polymorphisms and we have included a comment in the Discussion, as suggestion for future research.

REVIEWERS FROM THE WORLD JOURNAL OF GASTROENTEROLOGY

Reviewer 03024263
I read with great interest the article by Dr Alvarado-Tapias and co-authors. Indeed, bacterial infection is common and accounts for major morbidity and mortality in cirrhosis. Bacterial translocation is pathogenetically linked to the development of infections, particularly spontaneous bacterial infections, and other serious complications in cirrhosis. It was shown that small intestinal overgrowth, increased intestinal permeability, impaired intestinal motility, lack of bile acids, sympathetic over-activity, and local innate and adaptive immunological alterations are important factors involved in the pathogenesis of bacterial translocation. Toll-like receptor 4 (TLR4) and its coreceptor MD-2 recognize bacterial lipopolysaccharide and signal the innate immune response. Two single nucleotide polymorphisms of human TLR4, D299G and T399I, have been identified and suggested to be associated with lipopolysaccharide hyporesponsiveness. In a previous retrospective study, these authors concluded that TLR4 D299G polymorphism could influence not only the predisposition to bacterial infections but also the evolution of the disease in cirrhotic patients (C. Guarner-Argente et al., 2010) . In this prospective study, they received opposite results. The results obtained by the authors are in agreement with other publications. For me it is also not a surprise. The authors are absolutely right when they propose that these results are due to poor functional impact of D299G and T399I TLR4 polymorphisms and/or the multifactorial and complex nature of the immune response. Nevertheless, the problem is very interesting and actual, requiring further research.

Thank you for your comments.

Reviewer 00722239

The authors prospectively investigated 258 cirrhotic patients with ascites regarding correlation of genetic polymorphisms of TLR4 and bacterial infections. Design of study and quality of manuscript are excellent. Although the results were negative for their hypothesis, I think this study provides useful knowledge and worth publishing. In my personal opinion, impact of the immune response via TRL4 recognizing LPS from gram-negative bacilli is probably much weaker in cirrhotic patients than healthy individuals. I have no specific comment for revise.

Thank you for your comments.

Reviewer 0030389

The authors prospectively assessed the relationship between the presence of D299G and/or T399I TLR4 polymorphisms and the incidence of bacterial infections in cirrhotic patients with ascites. In a previous retrospective study, they observed a possible association between the presence of the D299G TLR4 polymorphism and the predisposition to develop bacterial infections in patients with cirrhosis. However, in the present study, they concluded that the presence of D299G and/or T399I TLR4 polymorphisms in cirrhotic patients with ascites is not a relevant risk factor for the development of bacterial infections and does not seem to significantly modify the evolution of the disease. The manuscript is interesting, but there are some major concerns. Major comment #1. The results are negative. They previously reported the association between the presence of the D299G TLR4 polymorphism and the predisposition to develop bacterial infections in patients with cirrhosis. In the present study, they should study a larger number of patients and for a longer period.

We already acknowledge in the manuscript the limitations due to the low prevalence of the TLR4 polymorphisms, the sample size and the short follow-up period. However, we prospectively evaluated 258 patients with decompensated cirrhosis during a 6-year period and with a mean overall follow-up of 26.6 ± 31.7 months. Although we failed to show statistically significant differences in the development of infections under these conditions, the effect- if any- of the studied polymorphisms has little clinical relevance.

Major comment #2. P4, lines 23-26. Objective variable of multivariate analysis should be bacterial infection according to the title of the manuscript.

We have added a multivariate analysis of bacterial infection in the Results section of the manuscript.

Major comment #3. D299 and T399 SNP are associated with each other. However each SNP should be assessed separately.

We found 28 patients (10.8%) heterozygous carriers of D229G and/or T399I polymorphisms. From these 28 patients, 25 had both polymorphisms, one patient was carrier of the D299G polymorphism only and 2 were carriers of the T399I polymorphism only. Therefore, we considered that a reliable separate analysis was not possible and we decided to put all of them together in one group named polymorphisms group.

Major comment #4. P13, lines 28-30. "However, although we observed a trend to a higher predisposition to bacterial infections, infections caused by gram-negative bacilli, infections caused by gram-positive cocci in the polymorphism group," This sentence is not correct, since they only observed a trend for a higher incidence of pneumonia in the polymorphism group (17.9% vs 8.7%, P=0.08)

We indicate that the trend was not statistically significant.

Minor comment #1. Naming of "polymorphism group" seems to be inappropriate. It should be "mutant group" or "minor group".

Due to the relatively high frequency of these TLR4 genetic variations in the general population, we prefer to use the word "polymorphisms" instead of "mutations", as it is also considered by most other authors.

Minor comment #2. P8. "Genomic DNA extraction and polymorphism genotyping". Did they use only one primer for genotyping each SNP?

We have clarified this in the Methods section.

Re viewer 00503536

The manuscript written by Alvarado-Tapias et al. analyzed the relationship between the presence of TLR4 polymorphisms and bacterial infections in cirrhotic patients with ascites. Although TLR4 polymorphisms are thought to be important for determination of susceptibility of bacterial infection, they found no relationship between the presence of TLR4 polymorphisms and bacterial infections in cirrhotic patients with ascites. The results were thus negative, but the analysis is well-performed and the data give important information for understanding the bacterial infection in cirrhotic patients.

Thank you for your comments.