PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Association between the Concurrence of Pre-existing Chronic Liver Disease and Worse Prognosis in Patients with an Herb-Polygonum multiflorum Thunb. induced Liver Injury: A Case-control Study from a Specialized Liver Disease Center in China
AUTHORS	Jing, Jing Wang, Rui-lin; Zhao, Xin-yan; Zhu, Yun; Niu, Ming; Wang, Li-fu; Song, Xue-ai; He, Ting-ting; Sun, Yong-qiang; Xu, Wen-tao; Yu, Si-miao; Wang, Li-ping; Guo, Yu-ming; Bai, Zhao-fang; Xiao, Xiao-he; Wang, Jia-bo

VERSION 1 – REVIEW

REVIEWER	Guruprasad P. Aithal Nottingham Digestive Diseases Centre, W/E 1418, E Floor, West Block, Queen's Medical Centre Campus, Derby Road, Nottingham, NG7 2UH
REVIEW RETURNED	03-Jun-2018

GENERAL COMMENTS	Authors describe a case-control study with liver injury related to one particular herbal medicine with or without underlying chronic liver disease and those with chronic liver disease as controls. The number of cases are small to break them into even smaller subgroups and the data appears over analysed. Comments: 1) Study design is explained inaccurately as a retrospective cohort study, but, matched controls are not a part of the cohort as it appears in the Figure 1. So, this is a case control study. 2) Methods: This starts with describing the aim of the study which is unnecessary here. 3) There is no description of ethical approval. 4) There is a long winded description of the obvious which includes already published American College of Gastroenterology Guidelines, R value and RUCAM, but, very little clarity in the text on on how the cases were identified. So, it is unclear how matching of controls was done. 5) in such a large database why did authors chose only 200 controls to match? If they had matched 1: 8 case control ratio as they stated, power of the study would have been enhanced significantly. 6) Some of the subgroups are too small to draw any conclusions. Comparing 8 cases of HILI with preexisting NAFLD and 64 matched NAFLD wouldn't allow robust conclusions. 7) The p value should be corrected for multiple testing. 8) Discussion should be structured along these lines- state salient findings, strengths and limitations, compare and contrast with the literature and clinical implications. As it stands it is difficult to follow the points and in places the discussion is too speculative.

	8) Article summary should be rewritten as some of it doesn't make sense.

REVIEWER	Tamara Milovanovic School of Medicine, University of Belgrade Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia
REVIEW RETURNED	09-Jul-2018

GENERAL COMMENTS	This manuscript is well written. It describes association between the concurrence of pre-existing chronic liver Diseases and malignant prognosis in patients with herb-induced Liver Injury and highlight a very important clinical topic. The methodology of the article is good, results adequately presented and discussed. Based on presented, conclusions are clear and future directions for investigations are given. I recommend to accept the manuscript.

REVIEWER	Cyriac Abby Philips The Liver Unit, Cochin Gastroenterology Group, Philip Augustine Associates, Ernakulam Medical Center, Cochin, India
REVIEW RETURNED	05-Aug-2018

GENERAL COMMENTS	Association between the Concurrence of Pre-existing Chronic Liver Diseases and Malignant Prognosis in Patients with Herb-induced Liver Injury: A Retrospective Observational Cohort Study by Jing et al. Very important work, but needs major revision to be considered. A great deal of unnecessary information has to be trimmed out and the descriptions made crisp, clear and short. Comments to the authors: Throughout the manuscript kindly make changes to 'patients with chronic liver diseases (CLDs)' to 'patients with chronic liver disease (CLD)'. Throughout the manuscript and in the title of the manuscript, kindly modify or restructure the word 'malignant prognosis' to a better description such as 'worse prognosis' or 'higher mortality' or 'higher morbidity' etc…. Please remove the word 'malignant' as it may erroneously portray hepatocellular carcinoma in the context of chronic liver disease. In the abstract section, please remove "using data from the electronic medical records; Adjusted analysis using logistic regression." under the 'Design' heading. In the abstract section, under - Primary outcome measures: Non-recoverable outcomes, including chronicity and fatality, in HILI patients with or without pre-existing CLDs, and patients with matched CLDs. – do not have to mention non-recoverable outcomes – only specify – mortality or morbidity (define this aspect as liver related deaths, non-liver related deaths etc…). There is some confusion regarding the comparisons in the abstract, methods and table sections – CLD+HILI versus CLD without HILI is one group and is there another group? The following comparisons are not easy to understand fully. Abbreviate your objectives to only the needful groups and then minimize descriptions in the Table. In the following descriptions in the Table, * and **** sounds similar. Clearly define your groups and comparisons and only show case HILI in CLD without CLD. I am not sure how looking into etiology within CLD then matching it

	against another control and then against HILI patients would serve the purpose of improved understanding. This is too confusing to the reader. You may also discuss patients with HILI with and without ACLF for a better understanding. ///*The comparisons were analyzed among the 3 groups, including the DILI group, DILI with pre-existing ALD group and matched ALD group. ** The comparisons were analyzed between the DILI group and the DILI with pre-existing ALD group. *** The comparisons were analyzed between the DILI group and the matched ALD group. **** The comparisons were analyzed between the DILI with pre-existing ALD group and the matched ALD group./// Since the authors have assessed HILI with regards to only one herbal agent - Polygonum multiflorum Thunb, the title and subsequent discussions must only include this particular agent and not generalize to all herbal preparations. The authors mention “and we tested whether the concurrence of pre-existing CLDs was an independent risk factor for malignant prognosis in HILI.” – this is confusing – are the authors looking at poor outcomes in CLD patients with HILI or poor outcomes due to HILI in CLD patients? These two are very different aspects and must be homogenized in the manuscript from abstract to conclusions. Please mention and describe HILI as possible, probable and highly probable/definite (as per scoring RUCAM) within the text also and not only the Table. The authors have mentioned diagnosis of HILI in patients with underlying liver disease condition such as alcoholic liver disease and viral hepatitis. This is quite difficult to appreciate and more emphasis on the scoring system that enables us to understand the actual effect of HILI in the patients studied. In the methods section, how was the matching done? Please describe it in detail. Was is propensity score matching or another statistical tool that was utilized? Describe how many patients with pre-existing CLD developed acute on chronic liver failure and provide details on outcomes in this group separately. ACLF patients have a different outcome profile when compared to patients with CLD who develop liver dysfunction not amounting to ACLF. Continuing alcohol use within the previous 3 months is important and not one month. Please recheck and revise accordingly. Patients can develop severe alcoholic hepatitis within the 3 months of excessive alcohol use. If such was associated with a higher risk of developing HILI, this must be showcased. Improve the RESULTS section by providing subheadings to ease flow of reading. Currently the results are showcased very haphazardly and needs to be streamlined. For example: discuss the study groups with regards to demographics first, then discuss clinical features between groups, there after associated conditions between groups and then biochemical and blood parameters followed by liver biopsy findings. After these broad descriptions, then come to each of these subheadings with regards to outcomes studied – univariate and multivariate. Such a flow in description is very important for ease of readability and understanding. Was liver injury pattern and outcomes looked into? Add this aspect also. Quote and discuss the recently published study (and the first) on complementary medicine use and liver injury patterns, toxicology analysis and outcomes. This study also showed the importance of ACLF in HILI patients and described injury parameters that

	predicted death in affected patients (mostly Ayurveda and herbal medicines) - https://www.ncbi.nlm.nih.gov/pubmed/29476406 The authors may remove the discussions and pertinent references on immunopathogenesis of HILI in CLD as this was not specifically studied in this population of patients. Add a paragraph on the limitations of the study. It is a single centre and retrospective study which itself is a limitation. Discuss further limitations and describe need for future prospects.

VERSION 1 – AUTHOR RESPONSE

Point-by-point responds to Reviewers' comments:

Reviewer 1
Reviewer Name: Guruprasad P. Aithal
Comments: Authors describe a case-control study with liver injury related to one particular herbal medicine with or without underlying chronic liver disease and those with chronic liver disease as controls. The number of cases are small to break them into even smaller subgroups and the data appears over analysed.
Response: Thank you very much for Professor Guruprasad P. Aithal's constructive comments and suggestions on our manuscript (ID bmjopen-2018-023567). We have studied comments carefully and have made revision by using the track changes mode in MS Word.

Comment 1: Study design is explained inaccurately as a retrospective cohort study, but, matched controls are not a part of the cohort as it appears in the Figure 1. So, this is a case control study.
Response: We have revised the study design and homogenized related descriptions in our paper according to Reviewer's comment.

Comment 2: Methods: This starts with describing the aim of the study which is unnecessary here.
Response: We have revised the descriptions in the first paragraph of the 'Methods' section at Page 6, Line 11-12, as Reviewer suggested.

Comment 3: There is no description of ethical approval.
Response: The ethical approval had been described under the 'Patient and Public involvement' subheading in the 'Methods' section of submitted original manuscript. At present, we put it under the 'Study design' subheading in the 'Methods' section at Page 7, Line 3-4, according to Reviewer and Editors' comments.

Comment 4: There is a long winded description of the obvious which includes already published American College of Gastroenterology Guidelines, R value and RUCAM, but, very little clarity in the text on how the cases were identified. So, it is unclear how matching of controls was done.
Response: The identification of cases and the method of matching for controls were revised under the 'Study design' subheading in the 'Methods' section at Page 6, Line 17-22, as Reviewer suggested. Additionally, the definitions of different CLDs were added under the 'Diagnostic criteria' subheading in the 'Methods' section at Page 7-8.

Comment 5: In such a large database why did authors chose only 200 controls to match? If they had matched 1: 8 case control ratio as they stated, power of the study would have been enhanced significantly.
Response: As different drugs might have differential effects on prognosis in patients with DILI or HILI, the present study focused on the same herb- Polygonum multiflorum Thunb. associated with HILI in

order to avoid the confounding effects of different drugs. Although the sample size of clinical database (n=193714) in liver disease center was large, the number of patients with PMT-related HILI was limited. In contrast, if we didn't have had a such large database, we wouldn't have enrolled so many patients with one herb-PMT related HILI (n=145). Considering the sample size of cases, the number of matched controls could be affected. However, the present study could provide a useful design for the further large samples study of all-causes DILI in patients with CLD.

Comment 6: Some of the subgroups are too small to draw any conclusions. Comparing 8 cases of HILI with preexisting NAFLD and 64 matched NAFLD wouldn't allow robust conclusions.
Response: We also think some of the subgroup (For instance, HILI with chronic viral hepatitis group and HILI with autoimmune liver disease group) are too small to draw any conclusion. In the paper, we had compared 8 patients of HILI with preexisting NAFLD and 64 matched NAFLD. However, we just have described the presentation of those in the 'Results' section rather than drew conclusions in the 'Discussion' section.

Comment 7: The p value should be corrected for multiple testing.
Response: In the present study, the p value has been corrected for multiple testing by the Bonferroni's correction as Reviewer suggested. The description was displayed under the 'Statistics' subheading in the 'Methods' section at Page 10, Line 4-6.

Comment 8: Discussion should be structured along these lines- state salient findings, strengths and limitations, compare and contrast with the literature and clinical implications. As it stands it is difficult to follow the points and in places the discussion is too speculative.
Response: We have revised the structure and descriptions in the 'Discussion' section at Page 14-18 according to the Reviewer's suggestion.

Comment 9: Article summary should be rewritten as some of it doesn't make sense.
Response: We have rewritten the article summary in the 'Strengths and limitations of this study' section at Page 4 as Reviewer suggested.

Special thanks again to Professor Guruprasad P. Aithal for your constructive comments and suggestions. These comments are very useful for improving our paper.

Reviewer 2
Reviewer Name: Tamara Milovanovic
Comments: This manuscript is well written. It describes association between the concurrence of pre-existing chronic liver Diseases and malignant prognosis in patients with herb-induced Liver Injury and highlight a very important clinical topic. The methodology of the article is good, results adequately presented and discussed. Based on presented, conclusions are clear and future directions for investigations are given. I recommend to accept the manuscript.
Response: Thanks very much to Professor Tamara Milovanovic for your positive comments on our manuscript (ID bmjopen-2018-023567). We have no additional response.

Reviewer 3
Reviewer Name: Cyriac Abby Philips
Comments: Association between the Concurrence of Pre-existing Chronic Liver Diseases and Malignant Prognosis in Patients with Herb-induced Liver Injury: A Retrospective Observational Cohort Study by Jing et al.

Very important work, but needs major revision to be considered. A great deal of unnecessary information has to be trimmed out and the descriptions made crisp, clear and short.
Response: Thanks very much to Professor Cyriac Abby Philips for your helpful comments and suggestions concerning our manuscript (ID: bmjopen-2018-023567). We have made corrections as Reviewer suggested.

Comment 1: Throughout the manuscript kindly make changes to 'patients with chronic liver diseases (CLDs)' to 'patients with chronic liver disease (CLD)'.
Response: The descriptions- 'patients with chronic liver diseases (CLDs)' were corrected as 'patients with chronic liver disease (CLD)' throughout the manuscript according to Reviewer's comments.

Comment 2: Throughout the manuscript and in the title of the manuscript, kindly modify or restructure the word 'malignant prognosis' to a better description such as 'worse prognosis' or 'higher mortality' or 'higher morbidity' etc…. Please remove the word 'malignant' as it may erroneously portray hepatocellular carcinoma in the context of chronic liver disease.
Response: The descriptions- 'malignant prognosis' were corrected as 'worse prognosis' throughout the manuscript as Reviewer suggested.

Comment 3: In the abstract section, please remove 'using data from the electronic medical records; Adjusted analysis using logistic regression.' under the 'Design' heading.
Response: We have removed it under the 'Design' heading in the 'Abstract' section at Page 2 as Reviewer suggested.

Comment 4: In the abstract section, under - Primary outcome measures: Non-recoverable outcomes, including chronicity and fatality, in HILI patients with or without pre-existing CLDs, and patients with matched CLDs. – do not have to mention non-recoverable outcomes – only specify – mortality or morbidity (define this aspect as liver related deaths, non-liver related deaths etc…).
Response: We have revised it under the 'Primary outcome measures' heading at Page 2, Line 11-12, as Reviewer suggested.

Comment 5: There is some confusion regarding the comparisons in the abstract, methods and table sections – CLD+HILI versus CLD without HILI is one group and is there another group? The following comparisons are not easy to understand fully. Abbreviate your objectives to only the needful groups and then minimize descriptions in the Table. In the following descriptions in the Table, * and **** sounds similar. Clearly define your groups and comparisons and only show case HILI in CLD without CLD. I am not sure how looking into etiology within CLD then matching it against another control and then against HILI patients would serve the purpose of improved understanding. This is too confusing to the reader. You may also discuss patients with HILI with and without ACLF for a better understanding.
///*The comparisons were analyzed among the 3 groups, including the DILI group, DILI with pre-existing ALD group and matched ALD group.
** The comparisons were analyzed between the DILI group and the DILI with pre-existing ALD group.
*** The comparisons were analyzed between the DILI group and the matched ALD group.
**** The comparisons were analyzed between the DILI with pre-existing ALD group and the matched ALD group.///
Response: In the abstract, methods and table sections, CLD+HILI versus CLD without HILI is one group (Figure 1). To investigate the impacts of different pre-existing CLDs on HILI, we selected two major types of CLDs (ALD and NAFLD) in CLD+HILI patients and compared ALD or NAFLD patients with HILI patients and HILI+ALD or HILI+NAFLD patients.
The unclear descriptions were revised in the Table section at Page 29-38 as Reviewer suggested. Furthermore, we have clearly defined these groups and comparisons in the 'Methods' and 'Results' sections at Page 6-12. In Table 2 and Table 3, comparisons among the three group (HILI without CLD

group, HILI+CLD group and CLD without HILI group) and the pairwise comparisons (HILI without CLD group vs HILI+CLD group, HILI without CLD group vs CLD without HILI group, HILI+CLD group vs CLD without HILI group) were all needed for distinguishing between HILI and CLD interactions. Additionally, discussing HILI patients with and without ACLF was added in the 'Results' and 'Discussion' sections at Page 13 and Page 17.

Comment 6: Since the authors have assessed HILI with regards to only one herbal agent - Polygonum multiflorum Thunb, the title and subsequent discussions must only include this particular agent and not generalize to all herbal preparations.
Response: The title and subsequent discussions have revised in our paper according to Reviewer's comments.

Comment 7: The authors mention "and we tested whether the concurrence of pre-existing CLDs was an independent risk factor for malignant prognosis in HILI." – this is confusing – are the authors looking at poor outcomes in CLD patients with HILI or poor outcomes due to HILI in CLD patients? These two are very different aspects and must be homogenized in the manuscript from abstract to conclusions.
Response: We were looking at poor outcomes in HILI patients with CLD. The pertinent descriptions have revised and homogenized in the paper from abstract to conclusions. In this study, we were looking into the association between concurrence of CLD and poor outcomes for HILI cases. The multivariable logistic regression was analyzed in patients with PMT-related HILI rather than patients with CLD. Thus, the results suggested that the concurrence of pre-existing CLD was an independent risk factor for worse prognosis in HILI. In contrast, we could not provide the evidence that concurrence of HILI became the independent risk factor for poor outcome in CLD patients.

Comment 8: Please mention and describe HILI as possible, probable and highly probable/definite (as per scoring RUCAM) within the text also and not only the Table. The authors have mentioned diagnosis of HILI in patients with underlying liver disease condition such as alcoholic liver disease and viral hepatitis. This is quite difficult to appreciate and more emphasis on the scoring system that enables us to understand the actual effect of HILI in the patients studied.
Response: The descriptions of the RUCAM scale in HILI patients were added under the 'Clinical characteristics' subheading in the 'Results' section at Page 11, Line 13-17, within the text according to Reviewer's comments.

Comment 9: In the methods section, how was the matching done? Please describe it in detail. Was is propensity score matching or another statistical tool that was utilized?
Response: In this study, we firstly divided enrolled patients with PMT-related HILI into patients with pre-existing CLD and those without CLD. Second, we selected the PMT-related HILI patients with pre-existing CLD defined as the case group, and also identified matched CLD patients without HILI as the control group. For each case, we selected eight controls matched by sex, age (± 4 years old), body mass index (BMI) (± 2 kg/m2), the type of CLD, the daily amount of alcohol intake (± 5 g/d) and the presence or absence of cirrhosis.

Comment 10: Describe how many patients with pre-existing CLD developed acute on chronic liver failure and provide details on outcomes in this group separately. ACLF patients have a different outcome profile when compared to patients with CLD who develop liver dysfunction not amounting to ACLF.
Response: The descriptions and outcomes in patients with pre-existing CLD developed ACLF were added under the 'Outcome' subheading in the 'Results' section at Page 13, Line 6-9, according to Reviewer's suggestions.

Comment 11: Continuing alcohol use within the previous 3 months is important and not one month. Please recheck and revise accordingly. Patients can develop severe alcoholic hepatitis within the 3 months of excessive alcohol use. If such was associated with a higher risk of developing HILI, this must be showcased.
Response: In our paper, histories of excessive alcohol use in HILI patients have been rechecked again. And the unclear descriptions have been revised in the 'Methods' section at Page 7 and in the footnote of 'Table 1' at Page 28 according to Reviewer's comments. In our study, the patients with histories of excessive alcohol use (alcohol intake of ≥40 g/d for men and ≥20 g/d for women) did not drink during the three months prior to the onset of liver injury.

Comment 12: Improve the RESULTS section by providing subheadings to ease flow of reading. Currently the results are showcased very haphazardly and needs to be streamlined. For example: discuss the study groups with regards to demographics first, then discuss clinical features between groups, there after associated conditions between groups and then biochemical and blood parameters followed by liver biopsy findings. After these broad descriptions, then come to each of these subheadings with regards to outcomes studied – univariate and multivariate. Such a flow in description is very important for ease of readability and understanding. Was liver injury pattern and outcomes looked into? Add this aspect also.
Response: The descriptions were revised and partly rewritten in the 'Results' sections at Page 10-14 according to Reviewer's comments. The liver biopsy findings were analyzed and added under the 'Histological findings' subheading in the 'Results' section at Page 12-13 as Reviewer suggested. The liver injury patterns and outcomes were added and described in the 'Results' section at Page 11, Line 11-17, and at Page 13, Line 4-6, as Reviewer suggested.

Comment 13: Quote and discuss the recently published study (and the first) on complementary medicine use and liver injury patterns, toxicology analysis and outcomes. This study also showed the importance of ACLF in HILI patients and described injury parameters that predicted death in affected patients (mostly Ayurveda and herbal medicines) - https://www.ncbi.nlm.nih.gov/pubmed/29476406
Response: The recently published study showed that ACLF could affect the outcomes in patients with HILI. So, we have quoted and discussed it in the 'Discussion' section at Page 17, Line 5-7, as Reviewer suggested.

Comment 14: The authors may remove the discussions and pertinent references on immunopathogenesis of HILI in CLD as this was not specifically studied in this population of patients.
Response: The discussions and pertinent references on immunopathogenesis of HILI in CLD were removed in the 'Discussion' section at Page 17 according to the Reviewer's comment.

Comment 15: Add a paragraph on the limitations of the study. It is a single centre and retrospective study which itself is a limitation. Discuss further limitations and describe need for future prospects.
Response: The paragraph on the limitations of our study were added in the 'Discussion' section at Page 18, Line 1-4, according to the Reviewer's suggestion. In addition, the need for future prospects were described in the 'Discussion' section at Page 18, Line 10-13, as Reviewer suggested.

VERSION 2 – REVIEW

REVIEWER	Cyriac Abby Philips The Liver Unit Cochin Gastroenterology Group, Kochi - Kerala - India
REVIEW RETURNED	09-Oct-2018

GENERAL COMMENTS	The authors have revised the manuscript satisfactorily.