Dear editors and reviewers,

Thank you very much for giving us an opportunity to revise our manuscript. We appreciate the editors and reviewers very much for their constructive comments and suggestions on our manuscript entitled “Clinical characteristics and 28-day outcomes of bacterial infections in patients with hepatitis B virus-related acute-on-chronic liver failure” (Manuscript NO.: 53207).

We have studied those comments carefully. Those comments are very helpful for revising and improving our paper, as well as the important guiding significance to other research. According to the those detailed suggestions, we have made a careful revision on the original manuscript. All revised portions are marked in red in the revised manuscript which we would like to submit for your kind consideration.

Kind regards.

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Response to reviewers

Reviewer 1 (Reviewer's code: 03729295)

I would like to make important Specific Comments related your work: 1) We noticed in your study: Original findings: - Pneumonia was the most common site of BIs in patients with ACLF-2 and ACLF-3, and SBP was the most common site of BIs in patients with AD and ACLF-1. - Gram-negative bacteria accounted for the majority of cultured bacteria, and MDROs were common. - The 28-day transplant-free survival rates of patients was very low and decreased with increasing ACLF grade; independent predictors of the 28-day outcomes of the study patients were COSSH-ACLF scores, AKI, BSI, PTA, and invasive catheter. 2) We noticed also: Clinical importance: independent predictors of the 28-day outcomes of first Bacterial infections are enough well documented in patients with hepatitis B virus (HBV)-ACLF as defined by the Chinese Group on the Study of Severe Hepatitis B. Conclusion: summarize appropriately the study data Key problems: - Retrospective cohort study limit (e.g. follow up evaluation) - Small sample size (Power lack) However you have noticed some limitations regarding your work. 3) Future direction: A prospective investigation involving more patients and appropriate design is needed to further elucidate the predictors as you have noticed in the discussion section: "additional prospective randomized studies should be conducted in the future".

Dear authors,

Your work could have been related to a retrospective cohort study focused on clinical characteristics and 28-day outcomes of first Bacterial infections at admission or during hospitalization in patients with hepatitis B virus (HBV)-ACLF as defined by the Chinese Group on the Study of Severe Hepatitis B (COSSH). Your manuscript looks like STROBE Checklist. Please, see my comments as follow:

1) Study motivation/context statement is enough well documented as well as the study methodology.

2) The study limits are enough well described in the discussion section.

3) Observation related manuscript writing and/or presentation:

3.1. Observations or mistakes to take into account:

- Introduction section (line 11): you reported "total bilirubin (TBIL) ≥ 12 mg/dl" as part of new criteria for HBV-ACLF, however in "Method section" you report high bilirubin level (**non-hemolytic**) as exclusion criteria. Why?

Answer: I think this is a very important problem. In definition of ACLF by COSSH, patients with total bilirubin ≥ 12 mg/dl and international normalized ratio ≥ 1.5 are included in the new criteria for HBV-ACLF. In "Method section" we report high bilirubin level (non-hemolytic) as exclusion criteria. Our definition of "non-hemolytic" is congenital non-hemolytic jaundice, which includes Gilbert syndrome, Dubin-Johnson syndrome, Crigler-Najjar syndrome, Rotor syndrome, etc. These diseases are congenital metabolic diseases and need to be excluded by this study. To avoid ambiguity, we changed "non-hemolytic" to "congenital non-hemolytic" in the revised manuscript.

- Method section: Line 3: you talk about "first bacterial infection (BIs)" without give a definition or any reference about this term. I would have wished you give any reference here or define the term in the "definitions section".

Answer: Based on your comments, we have defined the first bacterial infections in the revised manuscript. First BIs were referred to as the first bacterial infections that occurred either at admission or during hospitalization in patients.

Lines 6-8: "bilirubin elevation....": idem the above comment in the "introduction section; line 11". We even noticed in the "method section" different paragraph of exclusion criteria. Why? I think you should organize the statement related "exclusion criteria" to help readers to better understand.

Answer: Patients with HBV-ACLF and acute decompensation of HBV-related chronic liver disease combined with first bacterial infections who were hospitalized in

our Hospital from October 2014 to March 2016 were selected from the electronic database for retrospective analysis. Patients with diseases resulting in bilirubin elevation, such as hemolytic, congenital non-hemolytic, and obstructive jaundice, malignant tumor, and extrahepatic diseases that seriously influence life were excluded in the electronic database. This electronic database contains the etiology of viral hepatitis, alcoholic hepatitis, autoimmune liver diseases, drug-induced liver injury, Wilson's Disease, hemochromatosis, and schistosomiasis. This electronic database is the basis for this retrospective analysis and other studies of our research group. In the first step, We introduce the origin of this database in the part of "Patient enrollment and study design" in this paper. In the second step, we give the exclusion criteria for this study. The exclusion criteria were viral infections other than HBV and hepatic lesions because of other factors, such as alcoholic hepatitis, autoimmune liver diseases, drug-induced liver injury, Wilson's Disease, hemochromatosis, and schistosomiasis.

In the "subsection definitions": About the statement "HBV-ACLF is a complicated syndrome with a high short-term mortality rate that develops in patients with HBV-CLD regardless of the presence of cirrhosis and is characterized by acute deterioration of liver function and hepatic and/or extrahepatic organ failure": I think this paragraph is not necessary, because enough well described across in the abstract and introduction section.

Answer: In the revised manuscript, we removed this paragraph of the definition of HBV-ACLF based on your comments.

- Results section: sub-section "Bacterial detection in patients": Line 18: "....proportion of Gram-negative and -positive *bacteria* ***in*** *was* found between the...": you should delete the word "in" or replace it by the word "infection".

Answer: In the revised manuscript, we delete the word "in" based on your comments.

- Tables: **Table 5**: This table should be better rearranged *a fortiori* for the column related regression analysis to display adequately the results HR (95% CI) and P value.

Answer: We have corrected this error based on your comments. The new Table 5 can display adequately the results HR (95% CI) and P value after adjusting the format.

Table 5 Independent predictors for the 28-day outcomes in hepatitis B virus-related acute-on-chronic liver failure patients combined with bacterial infections

Variables	Survivor	Nonsurvivor	Univariate Cox regression		Multivariate Cox regression	
	(n = 58)	(n = 99)	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (years)	45.5 ± 9.7	49.3 ± 10.7	1.023 (1.005-1.042)	0.013		
Male (%)	53 (91.4)	82 (82.8)	0.693 (0.410-1.170)	0.170		
Cirrhosis (%)	50 (86.2)	85 (85.9)	0.956 (0.543-1.684)	0.877		
SIRS (%)	17 (29.3)	58 (58.6)	2.214 (1.480-3.310)	< 0.001		
Temperature (℃)	37.1 (36.6, 38.0)	37.1 (36.6, 38.0)	1.055 (0.861-1.293)	0.604		
MAP (mmHg)	86.9 ± 11.4	88.9 ± 15.5	1.003 (0.987-1.019)	0.696		
Invasive catheter (%)	6 (10.3)	26 (26.3)	2.026 (1.290-3.181)	0.002	2.173 (1.320-3.579)	0.002
The grade of ACLF						
ACLF-1 (%)	44 (75.9)	35 (35.4)	Reference	< 0.001		
ACLF-2 (%)	12 (20.7)	29 (29.3)	1.977 (1.205-3.243)	0.007		
ACLF-3 (%)	2 (3.4)	35 (35.4)	4.648	< 0.001		

			(2.862-7.548)	
HBV DNA (log10)	3.6 (2.3, 5.5)	4.7 (2.8, 6.6)	1.083 (0.997-1.177)	0.060
WBC (×10^9/L)	7.5 (5.6, 11.3)	10.4 (6.9, 15.0)	1.054 (1.022-1.088)	0.001
NEUT (×10^9/L)	5.4 (3.4, 8.6)	8.5 (5.1, 12.7)	1.058 (1.023-1.094)	0.001
HGB (g/L)	105.0 ± 21.6	109.3 ± 26.3	1.003 (0.994-1.011)	0.516
PLT (×10^9/L)	64.0 (46.8, 88.5)	63.0 (40.0, 98.0)	1.000 (0.995-1.005)	0.984
ALB (g/L)	28.0 (25.0, 31.0)	28.0 (23.0, 31.0)	0.984 (0.945-1.024)	0.435
TBIL (μmol/L)	353.6 ± 130.9	356.4 ± 163.7	1.000 (0.999-1.001)	0.836
DBIL (μmol/L)	257.4 ± 88.3	236.8 ± 113.7	0.998 (0.996-1.000)	0.091
ALT (IU/L)	76.5 (35.3, 127.8)	103.0 (49.0, 352.0)	1.001 (1.000-1.001)	0.003
AST (IU/L)	118.0 (71.0, 165.0)	136.0 (85.0, 367.0)	1.001 (1.000-1.001)	< 0.001
ALP (IU/L)	158.5 (125.8, 211.3)	137.0 (99.0, 179.0)	1.000 (0.998-1.001)	0.721
GGT (IU/L)	49.0 (35.8, 78.8)	56.0 (33.0, 78.0)	0.999 (0.995-1.004)	0.821
Cr (umol/L)	98.5 (79.5, 126.3)	122.0 (87.0, 196.0)	1.002 (1.001-1.003)	0.001
Na (mmol/L)	133.0 (130.0, 136.0)	132.0 (128.0, 136.0)	0.996 (0.962-1.031)	0.830

INR	1.9 (1.7, 2.3)	2.6 (2.1, 3.3)	1.762 (1.487-2.089)	< 0.001		
PTA (%)	33.2 ± 11.1	23.3 ± 9.9	0.937 (0.917-0.957)	< 0.001	0.967 (0.941-0.993)	0.015
CRP (mg/L)	20.5 (13.2, 43.4)	16.2 (8.9, 39.6)	0.998 (0.991-1.005)	0.530		
PCT (ng/ml)	1.2 (0.8, 1.8)	1.2 (0.6, 2.9)	1.017 (1.002-1.033)	0.025		
Ascites (%)	57 (98.3)	96 (97.0)	0.578 (0.183-1.828)	0.351		
AKI (%)	24 (41.4)	61 (61.6)	2.777 (1.734-4.449)	< 0.001	2.187 (1.259-3.799)	0.005
HE (%)	23 (39.7)	76 (76.8)	1.635 (1.089-2.454)	0.018		
AVB (%)	8 (13.8)	26 (26.3)	1.476 (0.942-2.312)	0.089		
Pneumonia (%)	20 (34.5)	59 (59.6)	1.831 (1.222-2.744)	0.003		
SBP (%)	34 (58.6)	33 (33.3)	0.553 (0.364-0.842)	0.006		
BSI (%)	6 (10.3)	20 (20.2)	1.661 (1.016-2.715)	0.043	2.339 (1.384-3.952)	0.002
Other BIs (%)	4 (6.9)	16 (16.2)	1.488 (0.870-2.544)	0.146		
Multiple sites of BIs (%)	6 (10.3)	24 (24.2)	1.797 (1.132-2.852)	0.013		
MDROs (%)	4 (6.9)	11 (11.1)	1.401 (0.728-2.696)	0.313		
CA BIs (%)	4 (6.9)	13 (13.1)	1.537	0.149		

			(0.857-2.757)			
HCA BIs (%)	24 (41.4)	47 (47.5)	1.046 (0.705-1.553)	0.821		
Nosocomial BIs (%)	30 (51.7)	39 (39.4)	0.804 (0.537-1.204)	0.290		
COSSH-ACLF Scores	6.1 (5.8, 6.7)	7.4 (6.6, 8.7)	1.704 (1.498, 1.937)	< 0.001	1.371 (1.127-1.666)	0.002

- Discussion section: Line 31: the word "pneumoni[a]"[26] ; you should write it as "pneumonia"[26]

Answer: We have corrected this error according to your suggestions.

3.2. References: Some reference citation in references section need to be explicit and/or complete: issue number miss often like ref 7 (issue number 10), ref 22 (issue number 31).... I would have wished you consider these observations.

Answer: According to the guidelines for manuscript preparation and submission of this journal, issue number does not need to be provided. Example of the format of references in this guidelines is as follows: *Ma L, Chua MS, Andrisani O, So S. Epigenetics in hepatocellular carcinoma: An update and future therapy perspectives. World J Gastroenterol 2014; 20: 333-345 [PMID: 24574704 PMCID: PMC3923010 DOI: 10.3748/wjg.v20.i2.333]*.

Reviewer 2 (**Reviewer's code:** 03475479)

This article was well-written. Although similar articles about bacterial infection in ACLF were present, this article was interesting and informative regarding with HBV-related ACLF. I have several concerns.

1. How was conditon of peroral nutrition intake. Authors should define how to nutritional support.

Answer: I think this is a very important problem. Nutritional support treatment is very important for patients with HBV-ACLF and may affect the efficacy and prognosis of those patients. Patients with liver failure who were treated in our hospital will be assessed for nutritional status by nutritionist. Energy intake target for patients with liver failure is 30-35 kcal/kg×d. We encourage patients to add meals at night and supplement them with vitamins and trace elements. We gradually increase energy and protein intake to their target amount. Enteral or parenteral nutritional support treatment is given to patients who cannot take oral nutrition. During the course of nutritional support treatment, we monitor liver function, kidney function, blood glucose, blood lipid, blood ammonia, lactic acid, and coagulation of those patients. We have added the changes to the part of "***Patient enrollment and study design***" in the revised manuscript.

2. Use of rifaximine, nucleoside analogues, PPIs should be clarified.

Answer: I think this is a very important problem. (1) The time span of this retrospective study we conducted was from October 2014 to March 2016. Rifaximin was not available in our hospital at this time, so all patients had not been treated with this drug during their hospital stay. (2) All patients with HBV-ACLF received antiviral therapy with nucleoside analogues (enticavir, tenofovir, lamivudine or adefovir based on the condition of patient) after admission. As this is a retrospective study, the details of antiviral treatment for patients who are transferred from other hospitals are not very clear. We will improve this important problem in our future prospective studies. (3) PPIs may have some impact on the occurrence of bacterial infections in patients with end-stage liver disease. Patients with gastrointestinal ulcers, upper gastrointestinal bleeding, or upset stomach after admission will be treated with

PPIs. As this is a retrospective study, the details of PPIs treatment for patients who are transferred from other hospitals are not very clear. We will improve this important problem in our future prospective studies. We have added some supplements in the part of "***Patient enrollment and study design***" and "***limitations in DISCUSSION***" in the revised manuscript.

Reviewer 3 (**Reviewer's code:** 00503536)

The manuscript written by Li et al. describes the importance of bacterial infection in the prognosis of the patients with HBV-related acute-on-chronic liver failure. Bacterial infections are known to be frequently accompanied by liver failure, but there are few reports on the details of the conditions. Therefore, the manuscript is important for the management of those patients. However, there are some concerns that need to be addressed.

Major points,

1. What were the mechanisms of HBV-related acute-on-chronic liver failure in the patients? Was the reactivation of HBV observed in all of those patients? Did the bacterial infections directly contribute the liver failure? The authors should add a comment on that point.

Answer: HBV-related acute-on-chronic liver failure (ACLF) is a complicated syndrome with a high short-term mortality rate that develops in patients with HBV related chronic liver disease (CLD) and is characterized by acute deterioration of liver function and hepatic and/or extrahepatic organ failure.

Hepatic encephalopathy, acute variceal bleed, acute kidney injury, bacterial infections, and reactivation of HBV can all as acute insult to trigger ACLF and play pivotal roles in deterioration of clinical course. Because this is a retrospective study and many patients are transferred from other lower-level hospitals, the reactivation of HBV is not very clear and was not observed in all of those patients. In this study, the level of HBVDNA of the majority of patients (137/159, 86.2%) with HBV-ACLF were positive on admission, and all patients received antiviral therapy during their hospital stay. We have added some supplements in the part of "***limitations in DISCUSSION***" in the revised manuscript and will improve this important problem in our future prospective studies.

Bacterial infections (BIs) trigger ACLF and play pivotal roles in deterioration of clinical course. About 32.6% of patients with ACLF are triggered by BIs in the Chronic Liver Failure Consortium ACLF in Cirrhosis study (***Moreau R***, *Jalan R, Gines P, et al; CANONIC Study Investigators of the EASL–CLIF Consortium. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with*

acute decompensation of cirrhosis. Gastroenterology 2013; ***144****: 1426-1437, 1437.e1-1437.e9*). In our study 46.4% were healthcare-associated BIs and 40.2% belonged to nosocomial BIs, and some of these patients were transferred from other hospitals. We found that some patients did not have the ACLF before BIs, and the ACLF occurred after BIs. In my opinion, BIs directly contribute the ACLF of those patients. But this is a retrospective study and many patients are transferred from other lower-level hospitals, the situation of these patients in other hospitals and the directly contribute the ACLF of BIs are not very clear and was not observed in all of those patients. We have added some supplements in the part of "***limitations in DISCUSSION***" in the revised manuscript and will improve this important problem in our future prospective studies.

2. Are there any differences in the frequency of bacterial infections or the bacterial types between acute HBV-related liver failure and acute-on-chronic HBV-related liver failure? How about the patients with liver failure of other etiologies, such as acute-on-chronic alcoholic liver failure?

Answer: I think acute HBV-related liver failure combined with bacterial infections is a very worthwhile area to study. Compared with acute-on-chronic HBV-related liver failure (ACLF), the incidence of acute HBV-related liver failure is very low, and the frequency of bacterial infections or the bacterial infections types of those patients is unclear. We will improve this important problem in the future studies. Patients with HBV-ACLF and acute decompensation (AD) of HBV-related CLD combined with first BIs were selected for this retrospective analysis. Our research shows that there is no difference of the bacterial types between the ACLF and the AD patients. SBP was the most common site of BIs in the AD and ACLF-1 groups. Pneumonia was the most common site of BIs in the ACLF-2 and ACLF-3 groups. The trend test displayed that as the ACLF grade increased, the incidence of SBP showed a downward trend. No significant difference in the proportion of Gram-negative and -positive bacteria was found between the ACLF and the AD patients.

Alcoholic liver disease is the major etiology in patients with ACLF from Europe and

North America, acute-on-chronic alcoholic liver failure combined with bacterial infections is a very worthwhile area to study. A total of 159 patients with HBV-ACLF and 40 patients with acute decompensation of HBV-related chronic liver disease combined with first BIs were selected for our retrospective analysis. There are 123 patients with acute-on-chronic alcoholic liver failure in our database. In the next step, we will carry out in-depth research on these patients.

3. What were the causes of death in those patients? Liver failure or bacterial infections?

Answer: In my opinion, this is a very important problem needs to be studied. Because this is a retrospective study, information on prognosis of those patients are verified through medical records and telephone contact. Some patients choose to be discharged or transferred to a local hospital due to financial reason, so the specific cause of death in these patients is not completely clear. Some patients who was observed 28-day outcomes in our hospital eventually died cause of liver failure or liver failure related complications (bacterial infections, hepatorenal syndrome, hepatic encephalopathy, acute variceal bleed). We need to conduct additional prospective studies to determine the cause of death of patients with ACLF combined with bacterial infections in the future. We have added some supplements in the part of "***limitations in DISCUSSION***" in the revised manuscript.