Dear Editors,

On behalf of all the authors of the manuscript NO **56825** entitled "**Acute liver failure and death predictors in patients with dengue-induced severe hepatitis**", I would like to express our sincere appreciation for the impressive reviews provided by all the editors and reviewers. We have revised our manuscript in light of their useful suggestions and comments. English language in our revised manuscript has been re-edited by AJE. We attach the English re-editing certificate to this letter. We would like to thank you once again for giving us the opportunity to resubmit this manuscript.

Best regards,

Tongluk Teerasarntipan, MD, MSc

Dear Editors,

This document provides specific responses to the reviewers. A comprehensive revision of the manuscript was performed, and the changes are highlighted in yellow. Our responses are as follows.

Science Editor:

Scientific quality: The manuscript describes a retrospective study of the acute liver failure and death predictors. The topic is within the scope of the WJG.

(1) Classification: Grade B and Grade D

(2) Summary of the Peer-Review Report: This is a very interesting manuscript. The number of patients who enrolled in the study is relatively large, therefore, the statistical power of the analysis would be robust. However, this report seems to contain fundamental problems in the way of multivariate analysis. In the methods needs clarification regarding the acute liver failure. The questions raised by the reviewers should be answered

Response: Thank you for your comment. We have answered all questions raised by the reviewers.

(3) Format: There are 5 tables and 3 figures. A total of 38 references are cited, including 14 references published in the last 3 years. There are 2 self-citations. 2 Language evaluation: Classification: Grade A and Grade B. A language editing certificate issued by AJE was provided. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement, and the Institutional Review Board Approval Form. Written informed consent was waived. No academic misconduct was found in the CrossCheck detection and Bing search. 4 Supplementary comments: This is an unsolicited manuscript. The study was supported by Fatty Liver Unit, Foundation of the Faculty of Medicine, Chulalongkorn University. The topic has not previously been published in the WJG. The corresponding author has not published articles in the BPG.

5 Issues raised:

(1) I found the authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s)

Response: We apologize for the incomplete document submission. We attached a copy of the grant approval document to this letter.

(2) I found the authors did not provide the original figures. 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 apologize for the incomplete submission. We have prepared and arranged the figures using PowerPoint as recommended and have attached the original figures to this letter.

(3) I found the authors did not write the "article highlight" section. Please write the "article highlights" section at the end of the main text.

Response: We apologize for the incomplete submission. We write the "article highlights" as follows:

"Article Highlights

Research Background

Liver injury from dengue infection is frequently observed, but it is usually mild and transient. Approximately 10% of dengue patients have dengue-induced severe hepatitis (DISH), which is defined by a more than 10-fold elevation in transaminase. Acute liver failure (ALF) from dengue infection is extremely rare but results in a high mortality rate.

Research Motivations

The prognosis of patients with DISH has not been well studied. Moreover, information regarding the risk of transitioning from DISH to ALF and death is limited. Evidence regarding effective therapeutic options for patients with ALF from

dengue is still lacking, and therefore, early identification of patients who are at risk of ALF and prompt treatment are keys to improving clinical outcomes.

Research Objectives

We aimed to identify the predictive factors of ALF and death in hospitalized dengue patients, regardless of dengue severity, who had severe hepatitis at presentation. We also aimed to analyze the clinical characteristics of all patients who transitioned from dengue-induced severe hepatitis to ALF.

Research Methods

We retrospectively reviewed 2,311 serologically confirmed adolescent and adult dengue patients who were hospitalized during a 12-year study period. Patients with DISH [n = 134] and DISH with subsequent ALF [n = 17] were included. Predictors of ALF and in-hospital death were identified using logistic regression analysis.

Research Results

The mortality rate was low in DISH patients (0.8%) but was remarkably high if ALF developed (58.8%). In univariate analysis, age, sex, hematocrit, white blood count, atypical lymphocyte count, platelet count, INR, bilirubin, SGOT, SGPT, ALP, albumin, creatinine, MELD score, presence of liver comorbidity and presence of capillary leakage syndrome (CLS) were identified as potential prognostic parameters for ALF or death. In multivariate analysis, the MELD score remained the only predictor of ALF, with an adjusted odds ratio (aOR) of 1.3 (95% confidence interval (CI) 1.1–1.5; $P < 0.001$). An initial MELD score >15 was associated with ALF from DISH with an AUROC of 0.91, sensitivity of 88.2% and specificity of 87.3%. An independent factor associated with death was baseline INR (aOR 10.4, 95% CI 2.6–40.5, $P = 0.001$). INR >1.5 predicted death from DISH with an AUROC of 0.83 (sensitivity of 81.8% and specificity of 86.8%).

Research Conclusions

The MELD score was the best predictor of ALF in DISH patients, a complication from dengue that is associated with high mortality. The presence of ALF and the baseline INR level were independent predictors of death among DISH patients.

Research Perspectives

ALF developing from DISH is rare, but it has a very poor prognosis. Early detection of patients who are at risk of ALF and death is essential. The MELD score and INR level are basic parameters that showed good predictive values for ALF and death among DISH patients.

Conclusions

Both the MELD score and INR level were excellent prognostic predictors for dengue patients with severe hepatitis. The MELD score was the best predictor of ALF, while the baseline INR level and the presence of ALF were the best predictors of mortality. A deterioration of liver function to ALF was associated with high mortality, as it indicated a state of an overwhelming systemic inflammatory response to dengue infection."

We have added "article highlights" in the "discussion" section at the end of the main text from line 3 on page 15 to line 2, on page 17.

6 Re-Review: Required.

7 Recommendation: Conditionally accepted.

Reviewer #1 comments:

This research itself is worth reporting to show the importance of DISH and ALF subsequent to DISH because the physicians in temperate countries would not be familiar with dengue fever and subsequent liver damage.

The number of patient who enrolled in the study is relatively large, therefore, the statistical power of the analysis would be robust. However, this report seems to contain fundamental problems in the way of multivariate analysis.

It is strongly recommended to authors to exclude PT-INR, and creatinine, to eliminate or reduce the risk of multicollinearity with the MELD score. Major concerns In Table 2 and associated discussion, there is a fundamental problem in the way of analysis. As mentioned above in the comments, MELD score is well-established predictive indicator for the prognosis of liver failure. And this score is calculated from T-bil, creatinine, PT-INR, and the status whether the patient is receiving hemodialysis or not. Therefore, there must be strong correlation between MELD score and PT-INR or creatinine. The presence of multicollinearity between MELD and these clinical parameters would affect the results of the regression analysis. It is not appropriate to conclude that the MELD score is the only significant factor that predicts ALF from DISH. We strongly recommend the authors to re-analyze the regression analysis of MELD score and other clinical variables excluding PT-INR and creatinine if the authors really want to insist on the importance of MELD score for prediction of progression to ALF from DISH.

Response:

Thank you very much for your kind comment and valuable suggestion. Because the MELD score is a well-established prognostic tool to predict liver injury with possible multicollinearity issues among the MELD score, INR and creatinine, we performed a multivariable regression analysis using the MELD score without INR and creatinine. The results of the revised analysis are shown in Table 1 on the next page and confirmed the clinical importance of the MELD score in predicting ALF from DISH patients.

Table 1 Re-analysis with additional variables and association with acute liver failure

Parameters	**Univariate analysis**	**Multivariate analysis**

	Odds ratio	95% CI	*P* Value	Adjusted Odds ratio	95% CI	*P* Value
Baseline demographic information						
- Age	1.0	0.9-1.0	0.044	0.9	0.9-1.0	0.033
- Male gender	0.7	0.3-2.1	0.570	3.9	0.5-30.1	0.193
Peripheral blood count-related with dengue severity						
- Hematocrit	1.0	0.9-1.0	0.624	-	-	-
- WBC count	1.0	1.0-1.0	0.419	-	-	-
- % of neutrophils	1.1	1.0-1.1	0.001	-	-	-
- % of atypical lymphocyte	0.9	0.9-1.0	0.218	-	-	-
- Platelet count	1.0	1.0-1.0	0.907	-	-	-
Liver status at diagnosis of DISH						
- Total bilirubin	1.3	1.0-1.6	0.026	-	-	-
- SGPT	1.0	1.0-1.0	0.001	-	-	-
- INR	23.7	5.0-111.9	<0.001	-	-	-
- Albumin	0.1	0.04-0.4	<0.001	0.7	0.2-3.2	0.696
- Creatinine	2.2	1.3-3.9	0.006	-	-	-
- MELD score	1.3	1.2-1.4	<0.001	1.3	1.1-1.5	<0.001
- Presence of liver comorbidity	3.8	1.1-13.9	0.042	4.5	0.4-48.6	0.215

We have revised our re-analysis regarding this issue and discussed it accordingly in the manuscript as follows:

- "Age and sex-adjusted multivariate analysis revealed that the MELD score remained statistically significantly associated with ALF (P = <0.001, adjusted OR = 1.3, 95% CI 1.1 - 1.5)." ("Results" section, page 8, lines 22-25)
- "In multivariate analysis, the MELD score remained the only predictor of ALF with an adjusted odds ratio (aOR) of 1.3 (95% confidence interval (CI) 1.1 – 1.5; P = <0.001)." ("Abstract" section, "Results" subsection, page 3, lines 26-27 and page 4, line 1)

- "Our study demonstrated that INR and creatinine had high ALF predictive values. It needs to be noted that both INR and creatinine are components of the MELD score, which also had a good ALF predictive value in our study. Moreover, the MELD score is an established prognostic predictor of liver disease. After adjusting the MELD score by multivariate analysis with other potential variables and excluding INR and creatinine to avoid multicollinearity effects with the MELD score, our study demonstrated that the MELD score was the best clinical parameter at presentation for predicting the development of ALF from DISH." ("discussion" section, page 12, lines 10-18)
- The re-analysis outcome was revised in the manuscript in the "table 2" on page 25.

Reviewer #1 comments:

In Table 3, there is the same problem as mentioned about Table 2. Three variables out of four in this table correlate strongly in principal each other, therefore, there also be the concern about multicollinearity. The authors should review the selection of the variables that enter the regression analysis.

Response:

Thank you for your comment. To eliminate the concern about multicollinearity among the MELD score, INR and creatinine, we reanalyzed INR and creatinine and excluded the MELD score from the multivariate analysis. We think that mortality from dengue infection is more dependent on multiorgan function rather than liver function alone. The MELD score is primarily designed to reflect liver dysfunction. Therefore, a multivariate analysis to predict mortality using creatinine and INR and excluding the MELD score to avoid any multicollinearity effect would be more appropriate. We also added total bilirubin to the multivariate analysis instead of the MELD score as another liver-related variable. The results from the reanalysis confirmed the clinical importance of INR for mortality prediction in patients with DISH. (Table 2 in this letter)

Table 2 Re-analysis with additional variables and association with death from DISH patients

Parameters	**Univariate analysis**			**Multivariate analysis**		
	Odds ratio	95% CI	*P* Value	Adjusted Odds ratio	95% CI	*P* Value
Baseline demographic information						
- Age	1.0	1.0-1.1	0.817	1.0	1.0-1.1	0.142
- Male gender	0.9	0.3-2.9	0.807	-	-	-
Dengue severity						
- Hematocrit	1.0	0.9-1.1	0.960	-	-	-
- WBC count	1.0	1.0-1.0	0.856	-	-	-
- % of neutrophils	1.1	1.0-1.1	0.036	-	-	-
- % of atypical lymphocytes	1.0	0.9-1.1	0.467	-	-	-
- Platelet count	1.0	1.0-1.0	0.673	-	-	-
Liver status at diagnosis of DISH						
- Total bilirubin	1.0	0.9-1.1	0.789	0.8	0.5-1.3	0.347
- SGPT	1.0	1.0-1.0	0.004	-	-	-
- INR	6.5	2.6-16.2	<0.001	10.4	2.6-40.5	0.001
- Albumin	0.2	0.1-0.5	0.003	0.2	0.04-1.3	0.104
- Creatinine	1.6	1.01-2.4	0.046	1.2	0.8-1.9	0.419
- MELD score	1.1	1.1-1.2	0.001	-	-	-
- Presence of liver comorbidity	1.0	0.1-8.2	0.983	-	-	-

We have revised our re-analysis regarding this issue and discussed the results accordingly in the manuscript as follows:

- "To select the variables for multivariate analysis, we postulated that mortality from dengue infection is dependent not only on liver dysfunction but also on multiorgan dysfunction. Since the MELD score primarily evaluates liver dysfunction, we used creatinine and INR without the MELD score in the

multivariate analysis to avoid any multicollinearity effect among the MELD score, INR and creatinine. In addition to ALF, the age and sex-adjusted multivariate analysis showed that INR was another factor that could predict death, with an adjusted OR of 10.4 (95% CI 2.6-40.5, P = 0.001)." ("Results" section, page 9, lines 7-19)

- "Other independent factors associated with death included baseline INR (aOR 10.4, 95% CI 2.6-40.5, P = 0.001).)." ("Abstract" section, "Results" subsection, page 4, lines 5-6)
- "we postulated that the INR level had a superior mortality predictive performance than the MELD score because a change in the INR level reflects both liver injury and systemic inflammation, whereas a change in the MELD score is mainly related to liver dysfunction." ("Discussion" section, page 13, lines 10-13)
- The re-analysis outcome was revised in the manuscript in the "Table 3" on page 26.

Reviewer #1 comments:

In addition, the authors described that the presence of ALF was the dominant predictor of death from DISH prior to the analysis of Table 3, however, it is obvious that the prognosis of ALF is very poor and the analysis of the odds ratio about the presence of ALF would be almost insignificant.

Response:

We greatly appreciate your thoughtful comment. We agree that ALF from DISH is obviously associated with a very poor prognosis, but many ALF patients did not have ALF at presentation. It would be beneficial if we could detect patients with a high mortality risk before developing ALF; therefore, we performed a multivariate analysis using the information available at presentation without the presence of ALF in the multivariate analysis.

We have added sentences regarding this issue in the "Results" section as follows:

- “ALF developing from DISH was obviously associated with a very poor prognosis; however, many ALF patients did not have ALF at presentation. It would be beneficial if we could detect DISH patients who had a high risk of mortality before they developed ALF. Therefore, we performed multivariate analysis using the clinical and investigation information available at presentation, without the presence of ALF. (“Results” section, page 9, lines7-12)

Reviewer #2 comments:

This is a very interesting manuscript entitled "Acute liver failure and death predictors in patients with dengue-induced severe hepatitis", however needs some improvements.

Firstly, maybe in the title and all main manuscript you must needs definition if you would like to demonstrate the severe cases or all cases of dengue (that is very interesting) or specific with liver injury. So, I suggest you to try to focus in the main objective of your study. The impression is that the study starts to found all cases of dengue, then cases with liver injury and then cases with severe acute hepatitis (acute liver failure or fulminant hepatitis).

Response:

Thank you for your kind comment and suggestion. In this study, we would like to demonstrate liver-related complications from all cases of hospitalized dengue infection, which included every degree of dengue severity: dengue fever, dengue hemorrhagic fever and dengue hemorrhagic fever with dengue shock syndrome. From all the dengue infections, we selected patients who initially had severe acute hepatitis, which was defined as an elevation of transaminases > 10-fold.

We have added details of our study objectives in the "Introduction" and "Material and methods" sections as follows:

- "Our primary objective was to identify the predictive factors of ALF in all hospitalized dengue patients, regardless of dengue severity, who had DISH at presentation, and our secondary objectives were to identify mortality predictors from all hospitalized dengue patients with DISH and to study the clinical characteristics of all patients who had their liver status change from DISH to ALF." ("Introduction" section, page 6, line 2-7)
- "We retrospectively screened 2,396 serologically confirmed all cases of dengue patients, regardless of dengue severity, including DF, DHF, and DHF with DSS, who were hospitalized during the 12-year study period (between

2007 and 2019) at King Chulalongkorn Memorial Hospital, Thailand" ("Materials and methods" section, "Patients" subsection, page 6, lines 9-13)

Reviewer #2 comments:

In the introduction may needs to focus in your "problem", as liver injury and acute liver failure. I suggest you to real describe the scientific hypothesis of your study. Than you must need to clarify the aim of your study. Must important clarification regarding all analysis with dengue cases or focus in severe cases.

Response:

Thank you for your comment and suggestion. We have added the scientific hypothesis of our study, as well as references, in the "Introduction" section as follows:

- "The possible mechanisms of dengue-induced liver injury are a combination of direct viral injury to hepatocytes, dysregulation of immune responses to dengue infection and ischemic liver injury from CLS[5]. We postulated that the degree of liver damage from dengue infection varies according to the main pathophysiology. Mild hepatitis might be caused by direct viral injury to hepatocytes, while severe hepatitis or ALF might be caused by severe dysregulation of the immune response or severe liver hypoperfusion." ("Introduction" section, page 5, lines 19-25)
- We added the supporting scientific reference number 5 (**Treeprasertsuk S**, Kittitrakul C. LIVER COMPLICATIONS IN ADULT DENGUE AND CURRENT MANAGEMENT. *Southeast Asian J Trop Med Public Health* 2015;**46 Suppl 1**:99-107 [PMID: 26506735 DOI: 10.1017/S0022463415000272]) for our hypothesis ("Introduction" section, page 5, line 22).

We clarified our objective of the study in the "introduction" section, page 6, lines 2-7, as follows:

- Original: “We aimed to identify predictive factors of ALF and death and to identify clinical characteristics of ALF in patients with DISH.”
- Revision: “Our primary objective was to identify the predictive factors of ALF in all hospitalized dengue patients, regardless of dengue severity, who had DISH at presentation, and our secondary objectives were to identify mortality predictors from all hospitalized dengue patients with DISH and to study the clinical characteristics of all patients who had their liver status change from DISH to ALF”

Reviewer #2 comments:

In the methods needs clarification regarding the acute liver failure. This world-renowned topic needs clarification. Furthermore, the most important risks regarding Fulminant hepatitis and it's classification (that had vast literature in this topic). You need to describe the classification used as Kings College criteria and/or Cliche criteria / classification to Fulminant hepatitis.

Response:

We greatly appreciate this comment.. We used the Clichy criteria to classify patients with acute fulminant hepatitis and acute liver failure.

We have added the definition as well as the corresponding reference for acute fulminant hepatitis in the “Materials and methods” section, “Patients” subsection, page 6, line 29 and page 7, lines 1-2 as follows:

- “To be more time-frame specific, we diagnosed fulminant hepatic failure in our cohort using the Clichy criteria: encephalopathy that developed within 2 weeks of the onset of jaundice, regardless of ALF etiology.[9].”

Reviewer #2 comments:

Results - Regarding this topic of liver injury and acute liver failure. You need to demonstrate how many cases you had that underwent a liver transplantation (meeting the Kings college criteria or Cliche criteria to classification the fulminant

hepatitis). Moreover, in this severe cases with acute liver failure you must clarify the factor V , encephalopathy, acidosis, latic acid, dialysis, others...

Response:

Thank you for your kind comment and valuable suggestion. We have added the patients' numbers to Table 4 (page 28) to allow a better understanding of our explanation. In our study, 5 patients (patient numbers #4, #5, #9, #12, #15) met the King's college criteria (nonacetaminophen-induced ALF) for liver transplant. The detailed complications of dengue, treatment for ALF and final outcome of each patient is shown in Table 4. Information regarding the serum factor V level was not available in this study. The patients' arterial lactate levels ranged from 1.0 to 3.5 mmol/L after fluid resuscitation. All of these patients had grade 4 hepatic encephalopathy and concomitant renal failure and underwent continuous renal replacement therapy. Three of the five patients (patients #4, #12 and #15) received liver dialysis (MARS and SPAD) as a supportive treatment. Unfortunately, none of these patients underwent liver transplantation due to organ shortages. Only two of the five (patient #4 and #9) patients survived.

We have added information regarding this issue in the "Result" section as follows:

-" In our study, 5 patients (patient numbers #4, #5, #9, #12, #15) met the King's college criteria (non-acetaminophen-induced ALF) for liver transplantation. The detailed complications of dengue, treatment for ALF and final outcome of each patient are shown in Table 4. Information regarding the serum factor V level was not available in this study. The arterial lactate levels of these patients ranged from 1.0 to 3.5 mmol/L after fluid resuscitation. All five patients had grade 4 hepatic encephalopathy and concomitant renal failure and underwent continuous renal replacement therapy. Three of the five patients (patients #4, #12 and #15) received liver dialysis (MARS and SPAD) as a supportive treatment. Unfortunately, none of these patients underwent liver transplantation due to organ shortages. Only two of the five (patients #4 and #9) patients survived. ("Results" section, page 10, lines 6-16)

Reviewer #2 comments:

Discussion - you must need to describe the main limitations of your study. Furthermore, you need to show us the real benefit of your study to the clinical practice.

Response:

Thank you for your kind comment and suggestion. The limitation in our study was mainly due to the heterogeneous timing of blood collection at initial presentation, which ranged from an immediate venous sampling at the first visit to 48 hours after admission. In this study, we selected patients based on transaminase levels, thus we might underdetect the presence of DISH because the transaminases were checked too early. Moreover, since serum transaminase levels increase rapidly, the delayed blood sampling might resulted in an extremely high level of transaminases. This limitation was also found in hematological profiles, especially total WBC count and their differential blood count. Due to the high heterogeneity of liver function tests and peripheral blood count, we cannot confidently conclude that these variables have no role in prognostic prediction in dengue patients. For the real benefit of our study, our result demonstrated an additional clinical usefulness of MELD score and INR level which are basic clinical parameters that could predict the development of liver failure and death. They would be helpful for primary care provider to select patients who are at risk of poor prognosis for close monitoring, as well as to triage patients who need close monitoring in the hospital that have limited human resources or shortage of intensive care unit beds.

We have revised and discussed about our study limitation and benefit in the "Discussion" section as follows:

- "There were some limitations to this study. There was a heterogeneous timing of biochemical blood collection, particularly for the liver function tests, which ranged from an immediate venous sampling at the first visit to 48 hours after admission. In this study, we selected patients based on transaminase levels, and thus, we may have underdetected the presence of DISH because the transaminases were checked too early. Moreover, since serum transaminase

levels increase rapidly, the delayed blood sampling may have resulted in an extremely high level of transaminases. This limitation also applies to the hematological profiles, especially the total WBC count and the differential blood count. Due to the high heterogeneity of the liver function tests and peripheral blood count, we cannot confidently conclude that these variables have no role in prognostication for dengue patients. ("Discussion" section, page 14, lines 14-24)

- "Previously, liver injury from dengue infection has generally been recognized as a mild and transient complication and is believed to only have a subtle impact on prognosis. However, this study noted that some basic bedside parameters, the MELD score and INR, should not be overlooked because they can indicate a poor prognosis. These parameters would be helpful for primary care providers to select patients who are at risk of a poor outcome and need close monitoring, as well as to triage high-risk patients who require intensive care in a hospital that has limited human resources or a shortage of intensive care unit beds." ("Discussion" section, page 14, lines 25-30 and page 15, lines 1-2)