Manuscript NO: 37348

Title: Potential triggering factors of acute liver failure as a first manifestation of autoimmune hepatitis - a single center experience of 52 adult patients

Dear Editor-in-Chief,

Thank you for reviewing our manuscript and the opportunity for resubmission with additional modifications. We provided a point-by-point response to the Reviewers' comments and a copy of the revised manuscript with significant changes underlined.

In particular, more detailed information was provided differentiating between autoimmune hepatitis, virus-induced de-novo autoimmune hepatitis, and finally, drug-induced autoimmune hepatitis (DILI-AIH), as suggested by the Reviewers. We also added more information related to grade of hepatic encephalopathy and immunosuppressive therapy in our cohort.

Moreover, proofreading of the entire manuscript was done by Professor G. Gores, Head of the Department of Gastroenterology and Hepatology, Mayo Clinic, Rochester

We followed the Journal's guidelines for manuscript preparation and manuscript format.

On behalf of all authors, we are looking forward for a positive decision.

Most sincerely,

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RESPONSES TO COMMENTS BY REVIEWER NUMBER 1

We thank the Reviewer for his/her thoughtful and constructive examination of our manuscript "*Potential triggering factors of acute liver failure as a first manifestation of autoimmune hepatitis - a single center experience of 52 adult patients*". We have addressed all the Reviewer's concerns in detail. All significant changes to the manuscript are underlined. Our responses to the Reviewer's comments are as follows:

Reviewer 1: Previously study indicated that approximately 20-30 % of the patients reveal an acute presentation which may be induced by a triggering agent. The potential triggering factors that may lead to ALF as the initial presentation of AIH is not well discussed. In this study, the results suggested that drugs, viral infections, and surgery in general anesthesia may trigger ALF as the initial presentation of AIH. Furthermore, advanced age and high MELD-score may be potential risk factors for lethal outcome. Consequently, the clinician would be well-advised to accurately document these underlying conditions. Increase of age, MELD-score, and creatinine levels may be risk factors for lethal outcome or need for urgent liver transplantation while higher levels of transaminases come along with improved spontaneous recovery.

Comment: The Reviewer is right. Factors leading to ALF as the initial presentation of AIH are not well described in literature. Generally, autoimmune hepatitis manifests as a chronic hepatopathy with increased liver enzymes. Acute liver failure is not common in these patients. We identified potential triggering factors and discussed these conditions in our manuscript.

RESPONSES TO COMMENTS BY REVIEWER NUMBER 2

We thank the Reviewer for his/her thoughtful and constructive examination of our manuscript "*Potential triggering factors of acute liver failure as a first manifestation of autoimmune hepatitis - a single center experience of 52 adult patients*". We have addressed all the Reviewer's concerns in detail. All significant changes to the manuscript are underlined. Our responses to the Reviewer's comments are as follows:

Reviewer 2: The report by Buechter et al. describes patients suffering from ALF due to AIH in a single medical center over the period of twelve years. The authors find that pharmaceutical use, infections and having surgeries requiring anesthesia could trigger ALF in these patients. The strengths of this study lie in the careful selection of patients over this time period, the use of the AASLD criteria for ALF diagnosis, and the soundness of their conclusions due to use of multiple antibodies and parameters. That being said, there are a couple of areas the authors should address to improve this manuscript.

Comment 1: In the discussion the authors state that higher ALT values are associated with improved spontaneous recovery (which the authors show in their data as well). This should be cited and/or explained in greater detail as it is difficult to understand why a greater degree of liver injury is associated with improved spontaneous recovery.

Response: This is an interesting observation. Our center previously demonstrated that high ammonia, low albumin, and low ALT-levels were associated with worse outcome in childhood acute liver failure. From our long-time clinical experience in patients with acute liver failure, we observed that patients with high ALT-values recovered better than their counterparts with low ALT-values indicating that there is still functioning liver parenchyma despite the fact of acute liver injury. This study by Kathemann et al. is cited and further discussed in our manuscript.

Comment 2: Hepatic encephalopathy was part of the inclusion criteria for the patients included in these studies. How did the grade of HE influence outcomes/HLA profile/antibody profile/etc. In addition, did the cause of ALF lead to more overt/severe hepatic encephalopathy? If there is no correlation, the discussion should still include a statement to address these points.

Response: This is an excellent question. Hepatic encephalopathy (HE) was classified using the West Haven criteria. However, we found HE grade I in 46 of our 52 patients, HE grade II in 2 patients, HE grade III in 2 patients, and finally, HE grade IV in 2 further patients, respectively. Unfortunately, patients with HE grade IV had poor prognosis and died of acute liver failure. We found no correlation between grade of HE and antibody or HLA-profile. Moreover, we also did not found a correlation between the triggering factors mentioned in the manuscript with severity of hepatic encephalopathy. These additional findings were all included to the manuscript.

Comment 3: The abbreviation LKM was not defined on first use (in abstract).

Response: Abbreviation for LKM (anti-liver kidney microsomal antibody) is now defined on first use in the abstract.

Comment 4: The formatting for numbers over 1000 should remove the apostrophe.

Response: We removed the apostrophe for numbers over 1000.

RESPONSES TO COMMENTS BY REVIEWER NUMBER 3

We thank the Reviewer for his/her thoughtful and constructive examination of our manuscript "*Potential triggering factors of acute liver failure as a first manifestation of autoimmune hepatitis - a single center experience of 52 adult patients*". We have addressed all the Reviewer's concerns in detail. All significant changes to the manuscript are underlined. Our responses to the Reviewer's comments are as follows:

Reviewer 3: The study is interesting; however, authors should address these points:

Comment 1: How many patients with acute liver failure did authors have during the study period?

Response: During the study period from 2005 till 2017, we included a total of 565 patients with histologically-proven AIH. However, 52 patients presented acute liver failure and were further evaluated. None of the remaining patients of our cohort (n = 513 patients) developed acute liver failure under therapy with corticosteroids and/or immunosuppressive therapy with e.g. azathioprine. This additional finding was included to the manuscript.

Comment 2: How did authors exclude other causes of acute liver failure? Which viral markers were tested?

Response: Each patient was tested for auto-antibodies (ANA, AMA, ANCA, SMA, LKM, and SLA), IgG, IgM, viral markers (anti-HAV IgM, HBs-Ag, anti-HBc IgM, HBeAg, anti-HBe, anti-HBs, HBV-DNA-PCR, anti-HCV, HCV-RNA-PCR, anti-HDV-EIA, anti-HEV IgM, HEV-PCR, HSV-PCR, CMV-PCR, and EBV-PCR), transferrin-saturation, ceruloplasmin, copper in serum, soluble interleukin-II receptor, α_1-antitrypsin, and finally, GLDH. Liver histology was available for each patient demonstrating typical features of autoimmune hepatitis. Moreover, we used the RUCAM instrument and the AIH score ("Diagnostic Scoring System of the International Autoimmune Hepatitis Group" by Czaja AJ et al.) to further differentiate cause of liver failure. These additional data were included to the manuscript.

Comment 3: Did authors check for Wilson disease as it may be a rare cause of ALF too?

Response: The Reviewer is correct. Wilson disease may induce acute liver failure in few cases. Each patient was tested for ceruloplasmin and free copper in serum. In indeterminate cases, additional examinations were performed (Kayser-Fleischer ring, copper in urine, parameters of hemolysis, and also determination of cupper in the liver biopsy). This information was included to the manuscript.

Comment 4: EBV, HEV, and CMV can be causes of ALF themselves. How did authors differentiate them with AIH?

Response: Please see Comment 2 (serological markers). The reviewer is correct in that way that drugs and viral infections are able to cause ALF. Differentiating between drug-

induced ALF and AIH-induced ALF is difficult on the basis of histology alone. In both cases plasma cell rich inflammation with interface hepatitis can be present. In order to differentiate DILI from AIH clinical, historical and laboratory data have to be considered as stated in the manuscript. If EBV, CMV or HEV infection was suspected, viral detection by means of immunohistochemistry, in situ hybridization, and PCR was performed.

Comment 5: How many patients received steroids? And the dosage?

Response: All patients with AIH-induced ALF received a pulse therapy with steroids starting with 1 mg/kg body weight intravenously. Number of patients (30 patients, 57.7 %) receiving steroids in a daily dose of 7.5 mg to maintain remission was added to the manuscript.

Comment 6: Did authors use azathioprine in these patients?

Response: Azathioprine (in 27 patients, 51.9 %) and in some cases calcineurin-inhibitors (in 7 patients, 13.5 %) were also used to maintain remission. Data regarding immunosuppressive therapy were included to the manuscript.

Comment 7: Data have been duplicated in the text and table. This should be revised.

Response: Duplicated data in the text or table were deleted as suggested by the Reviewer.