December 27, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5651-review.doc).

Title: Prognostic Value of M30/M65 for Outcome in HBV Related Acute-on-Chronic Liver Failure

Author: Su-Jun Zheng, Shuang Liu, Mei Liu, M.A. McCrae, Jun-Feng Li, Yuan-Ping Han, Chun-Hui Xu, Feng Ren, Yu Chen, Zhong-Ping Duan

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) Page 8. For ACLF patients, female (13/81) 16.05% and male (68/81) 83.95%. Male patients developed HBV related ACLF more frequently than female patients in Chinese population. Please explain why male is dominant for ACLF.

Reply:

Many studies showed that Hepatitis B virus (HBV) is an important pathogen that chronically infects more men than women [1-3]. And moreover, chronic hepatitis B (CHB) appears to progress more rapidly in males than in females, which may explain why male is dominant in acute-chronic liver failure (ACLF). The molecular mechanism of this gender disparity is partially due to the androgen which could enhance the HBV

replication through response elements (AREs) in HBV genome[4]. Castrated male A/JCr mice infected with Helicobacter hepaticus and those receiving the competitive androgen receptor antagonist flutamide had significantly less severe hepatitis than intact controls, which indicated to some degree male sex hormone have some influence on infectious hepatitis[5]. Meanwhile, some other studies demonstrated that the altered pattern of liver apolipoprotein A-I isoforms[6], the EGF rs4444903 A>G functional polymorphism[7], as well the Toll like receptor 4 D299G[8] is implicated in male chronic hepatitis B progression, may results in a worse outcome of chronic HBV infection.

(2) Page 13. Hepatocyte apoptosis predominates at earlier stages of disease; while as disease staging progresses there is a gradual switch to necrosis, and eventually necrosis predominates in the late stage of liver disease. The ratio of apoptosis vs, necrosis found in control group samples was 54% vs.46%. However, in CHB this ratio changed to 46% vs.54%. It finally reached 33% vs.67% in ACLF. Please explain the estimate of parameter of histological apoptosis and necrosis.

Reply:

Our study demonstrated that the serum M30/M65 ratio decreased gradually from health control to CHB, and further to ACLF, which showed that hepatic cell necrosis play stronger part than apoptosis in the disease progression of chronic HBV infection. In present study, part of CHB patients underwent needle liver biopsy and some ACLF received liver transplantation. The liver tissue slices stained with hematoxylin-eosin were examined by light microscope. The morphological feature of apoptosis and necrosis was easily identified. CHB manifested varying degrees of hepatocellular damage (spotty necrosis, confluent necrosis and bridging necrosis), whereas ACLF manifested massive necrosis or multilobular necrosis whose scope reached over 2/3 of parenchyma area, which clearly indicated that the necrosis was more severe in ACLF than in CHB. Therefore, measurement of serum M65 and M30 levels could reflect the histopathological changes.

(3) Authors conclude that M30/M65 ratio is most suitable prognostic factor compare to MELD and Child-Pugh. Please explain why M30/M65 ratio has prognostic value for

predicting ACLF patients compared to conventional parameter of MELD and Child-Pugh.

Reply:

Considerable studies demonstrated that hepatocyte death plays a fundamental role in liver failure; therefore, identification of new biomarkers reflecting basic hepatocyte necrosis or apoptosis could provide useful insights for disease development and clinical outcomes. M30-antigen, the caspase-cleaved cytokeratin-18, is used to detect apoptosis. Uncleaved cytokeratin-18, detectable as M65-antigen, is also released from dying cells (due to both apoptosis and necrosis). Previous studies and our study results repeated demonstrated that serum M30 and M65 could identify different stage of liver disease. Therefore, we further investigated whether M30/M65 has prognostic value for ACLF patients compared with MELD and Child-Pugh. As we expected, compared with them, M30/M65 ratio has some degree prognostic value, though the AUC was only 0.66, but with the highest specificity 92.6%. I believe that M30/M65 might a complementary measure for MELD or Child-Pugh scores.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Su-jun Zheng, MD, PhD

Artificial Liver Center,

Beijing YouAn Hospital,

Capital Medical University,

Beijing 100069, China;

Fax: +86-10-63295285

Tel: 086 10 63291007

E-mail: zhengsujun003@126.com