Point-by-point replies

First, we would like to thank both of the reviewers and the editor for their invaluable comments, which as we believe, improved the paper substantially. We made requested changes in the manuscript (in red colour) and below please find our point-by point responses:

Reviewer 1 (reviewer's code: 00002232):
Comments to the Author

1)

Comment: In this study, there is a notable lack of comparison between osteopontin and other non invasive markers of portal hypertension. For example, transient elastography, alone or in combination with platelets count+spleen size, has a very good predictive value for clinically significant portal hypertension. In addition, several blood biomarkers have been investigated as noninvasive testing for portal hypertension such as the AST/ALT ratio, BMP7, apelin, vWF, VCAM-1, IL-1beta, among others. The comparison of accuracy for predicting clinically-significant portal hypertension between osteopontin and other non-invasive biomarker is needed to strengthen the study.

Reply: We did not compare osteopontin to other non-invasive markers, as the study was initiated primary to osteopontin evaluation. It is true, that the comparison to other non-invasive markers of portal hypertension is lacking and the implementation of such markers would add important information and strengthen the study. The information about the combination of imaging technique and serum markers for the evaluation of portal hypertension are not frequent in literature. Unfortunately we had not a possibility to examine all the patients with liver stiffness measurement like Cho et al in a recently published study [1]. ***Nevertheless we added comparison with all the parameters, which were available in our patients (platelet count, platelet count/spleen size ratio, AST/ALT ratio) into the revised manuscript, as suggested by reviewer. We calculated the sensitivity, specificity and AUC of above mentioned parameters for the detection of clinically significant portal hypertension in cirrhotic patients (please see the "Results"- page 12, last paragraph). The predictive value of platelet count, platelet/spleen ratio or AST/ALT ratio for HVPG above 10 mm Hg did not reach that of osteopontin in our patients.*** *We agree that also other parameters like BMP7, apelin, vWF, VCAM-1, IL-1 beta markers suggested by reviewer could correlate with the degree of portal hypertension, but in our study we did not have opportunity to measure these parameters, which contrary to common parameters like platelet count, AST activity,*

spleen size or liver stiffness, are not used in routine daily practice. Nevertheless, we also added the lack of some of this data into the limitations of our study (Page 18).

2) Comment: The authors conclude that osteopontin is a non-invasive parameter useful in the stratification of significant portal hypertension. The authors reached this conclusion by performing univariate and correlation statistical test. However, the correlation coefficient between OPN and HVPG was weak (0.25), although significant. The authors should investigate further the robustness of this association. For example, I would recommend performing multivariate linear regression considering HVPG as response variable run for HVPG ≤10 and HVPG>10 mmHg and adjusting the model for possible confounding variables.

Reply: We performed the test with commonly examined laboratory parameters – please see Table 2 in manuscript (Page 27).

3) Comment: Positive and negative predictive values should be also reported together with sensitivity and specificity for osteopontin.

Reply: We calculated positive and negative predictive values for osteopontin in discrimination of clinically significant portal hypertension and we added the values in the text (please see section "Results", page 12, 2nd paragraph).

4) Comment: Transaminase values for cirrhotic patients should be reported in table 1.

Reply: The values of transaminases were added into Table 1 (Page 25).

5) Comment: In the discussion section, the authors state that "the clear relationship between single HVPG measurement and overall survival of patients with cirrhosis is not well documented". This information is not supported by the literature. For example, a reduction in the HVPG to less than 12 mm Hg or a reduction of more than 20% from the baseline value is associated with a decreased risk of variceal hemorrhage and improved survival. Therefore, I

would recommend to modify appropriately this paragraph (Abraldes et al., Hepatology 2003;37:902-8. and D'Amico G, et al. Gastroenterology 2006;131:1611-24).

Reply: We apologize for this misstatement, we had on mind a prognostic value of performing of only a single HVPG measurement. We agree that this was not fully correct, and we changed the text in "discussion" based on reviewer's suggestions and added suggested citation (Page 16, 1st paragraph).

6) Comment: A "study limitations" section should be included in your manuscript. Some limitations to consider are the lack of a validation cohort and the strong regional focus of patients included in this study.

Reply: The section "study limitations" was added to the text; please see the end of "Discussion" (page 18, 2nd paragraph).

7) Comment: Some biochemical parameters do not necessarily follow a normal distribution and this seems to be the case for osteopontin, according to the boxplots shown in figure 1A and B. Therefore, all the parameters that do not follow a normal distribution should be reported as median and interquartile range in the table 1.

Reply: The Table 1 (page 25)was changed and all parameters that do not follow a normal distribution are now reported as median and interquartile range. This information is given also in the legend of the table. The values were also changed in the text.

Reviewer 2 (reviewer's code: 00006258):

1) Comment: it is not clear from Table 1 what the constitution of each group is in terms of etiology (ie how many patients had viral related disease, NASH etc) and this table suggests that some patients did have alcohol-related cirrhosis. The numbers for each etiology should be clearly stated and the authors should comment on validity of grouping small numbers of patients with varying etiology of disease into such analyses.In particular the association of OPN and HCC is well described so discussion of etiologies particularly associated with development of HCC is warranted and more clarity regarding numbers of patients with each disease is important.

Reply: The Table 1 (Page 25) was changed as reviewer suggests – ***the etiologies of cirrhosis are given in numbers*** *(in the previous text this data were given as percentage). It is evident, that most of our patients had cirrhosis due to previous alcohol abuse (about ¾ of all patients). Other etiologies like viral, NASH and others were only in minority. This situation did not allow to do statistically relevant comparison between different groups of patients.* ***Hence the alcoholic etiology against all other etiologies were compared regarding the OPN value and this information was added into the revised manuscript (please see the section "Results", page 12, 1st paragraph).***

As to HCC, we detected carcinoma only in 6 patients (4 had alcoholic etiology, 2 other etiologies). In our opinion, due to very low number of patients with HCC, the information about etiology of cirrhosis is not important for readers. Another issue is the low incidence of HCC in the Czech Republic – but for this fact no clear explanation exists.

2) Comment: The observation that HVPG is higher in cirrhosis is not new, and elevations of circulation OPN in patients with cirrhosis is also well described. The correlation between OPN and survival is clear but predictable based on associations with disease severity (and markers such as platelet count etc as noted by the authors in the discussion) and has been demonstrated in cirrhotic patients with HCC in the past. It would be interesting to see a more detailed breakdown of etiology and survival linked to OPN levels and also cause of death data for the 62 who died (ie not just brief mention of incidence of HCC).

Reply: *We agree with the reviewer, that plasma OPN levels were attributed to liver fibrosis/cirrhosis in patients with various chronic liver diseases by many authors [2-5] (please see the section "Introduction"), but the correlation to HVPG has never been published. The only data regarding the correlation of OPN levels to degree of portal hypertension are based on measurement of spleen vein pressure in 16 patients with schistosomiasis at the time of splenectomy [6]. We added this reference to the manuscript (Please see "Discussion", page 15, 2nd paragraph)*

We also agree, that plasma OPN levels have been described to be significantly elevated in patients with liver cirrhosis and HCC compared to those without HCC [7]. Unfortunately, in our study, no statistically relevant result could be obtained due to low number of patients with HCC

We feel, that the mortality data would be strengthened by detailed description of cause of death, but the data collection was primarily directed to overall mortality, hence we could not give more details. Though we would like to argue that the overall mortality data related to OPN levels provide completely new information with potential clinical impact.

Literature:

1 Cho EJ, Kim MY, Lee JH, Lee IY, Lim YL, Choi DH, Kim YJ, Yoon JH, Baik SK. Diagnostic and Prognostic Values of Noninvasive Predictors of Portal Hypertension in Patients with Alcoholic Cirrhosis. *PloS one* 2015; **10**(7): e0133935 [PMID: 26196942 PMCID: Pmc4511411 DOI: 10.1371/journal.pone.0133935]

2 Syn WK, Agboola KM, Swiderska M, Michelotti GA, Liaskou E, Pang H, Xie G, Philips G, Chan IS, Karaca GF, Pereira Tde A, Chen Y, Mi Z, Kuo PC, Choi SS, Guy CD, Abdelmalek MF, Diehl AM. NKT-associated hedgehog and osteopontin drive fibrogenesis in non-alcoholic fatty liver disease. *Gut* 2012; **61**(9): 1323-1329 [PMID: 22427237 PMCID: Pmc3578424 DOI: 10.1136/gutjnl-2011-301857]

3 Patouraux S, Bonnafous S, Voican CS, Anty R, Saint-Paul MC, Rosenthal-Allieri MA, Agostini H, Njike M, Barri-Ova N, Naveau S, Le Marchand-Brustel Y, Veillon P, Cales P, Perlemuter G, Tran A, Gual P. The osteopontin level in liver, adipose tissue and serum is correlated with fibrosis in patients with alcoholic liver disease. *PloS one* 2012; **7**(4): e35612 [PMID: 22530059 PMCID: Pmc3329460 DOI: 10.1371/journal.pone.0035612]

4 Zhao L, Li T, Wang Y, Pan Y, Ning H, Hui X, Xie H, Wang J, Han Y, Liu Z, Fan D. Elevated plasma osteopontin level is predictive of cirrhosis in patients with hepatitis B infection. *International journal of clinical practice* 2008; **62**(7): 1056-1062 [PMID: 17537188 DOI: 10.1111/j.1742-1241.2007.01368.x]

5 Huang W, Zhu G, Huang M, Lou G, Liu Y, Wang S. Plasma osteopontin concentration correlates with the severity of hepatic fibrosis and inflammation in HCV-infected subjects. *Clinica chimica acta; international journal of clinical chemistry* 2010; **411**(9-10): 675-678 [PMID: 20138033 DOI: 10.1016/j.cca.2010.01.029]

6 Pereira TA, Syn WK, Machado MV, Vidigal PV, Resende V, Voieta I, Xie G, Otoni A, Souza MM, Santos ET, Chan IS, Trindade GV, Choi SS, Witek RP, Pereira FE, Secor WE, Andrade ZA, Lambertucci JR,

Diehl AM. Schistosome-induced cholangiocyte proliferation and osteopontin secretion correlate with fibrosis and portal hypertension in human and murine schistosomiasis mansoni. *Clinical science (London, England : 1979)* 2015; **129**(10): 875-883 [PMID: 26201095 PMCID: Pmc4558314 DOI: 10.1042/cs20150117]

7 Shang S, Plymoth A, Ge S, Feng Z, Rosen HR, Sangrajrang S, Hainaut P, Marrero JA, Beretta L. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. *Hepatology (Baltimore, Md)* 2012; **55**(2): 483-490 [PMID: 21953299 PMCID: Pmc3914762 DOI: 10.1002/hep.24703]