August 1, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: **3989-Manuscript&Tables**.doc).

Title: Prognostic Factors in Non-Malignant and Non-Cirrhotic Patients with Portal Cavernoma: An 8-Year Retrospective Single-Center Study

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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) Reviewer 1's comment 1: Please complete risk factors for EHPVO/PVT of patients included as for example: local: pancreatitis, surgery, trauma ? systemic: additional prothrombotic disorders, drug induced ?

Answer: we have added these data regarding risk factors for EHPVO/PVT of patients in the Results section in Page 6 as follows.

Thrombotic risk factors of EHPVO were detected in 33 patients. Among them, 11 had positive JAK2 V617F mutation, none had both CD55 and CD59 deficiencies, two had weakly positive anti-cardiolipin IgG antibodies, and none had positive factor V Leiden or prothrombin gene G20210A mutation. Previous history of infection before our admission

included colitis (n=1), pelvic infection (n=1), appendicitis (n=1), intra-abdominal infection secondary to duodenal ulcer perforation (n=1), umbilical cord infection (n=2), megacolon disease of new born (n=1), bacterial dysentery (n=1), and pancreatitis (n=4). Previous history of abdominal surgery before our admission included splenectomy and devascularization for variceal bleeding (n=11), splenectomy for hypersplenism and/or splenomegaly (n=8), splenectomy for traumatic spleen rupture (n=1), partial splenic artery embolization for hypersplenism (n=1), cholecystectomy (n=4), surgical repair of peptic ulcer perforation (n=1), total hysterectomy for hysteromyoma (n=1), and cesarean delivery (n=1). Notably, 7 and 13 patients underwent splenectomy before and after the diagnosis of portal cavernoma, respectively.

(2) Reviewer 1's comment 2: Please extend the statistical analysis by investigating potential differences between study subjects with different risk factors / causes for EHPVO/PVT including overall survival rates of these subgroups.

Answer: as described in the Results section, we had to acknowledge that "given that the thrombotic risk factors of EHPVO were detected in only half of patients, they were not included in the prognostic analysis". This limitation of our study was also emphasized in Discussion section as follows.

The prevalence and prognostic significance of underlying etiological factors in patients with portal cavernoma are discussed elsewhere (Qi X, et al. J Gastroenterol Hepatol. 2013;28:432-42. Qi X, et al. J Gastroenterol Hepatol. 2013;28:148-52. Qi X, et al. Aliment Pharmacol Ther. 2011;33:1087-103. Qi X, et al. J Gastroenterol Hepatol. 2012;27:1036-43.), but not in this study. Therefore, we can not demonstrate the association between survival and prothrombotic factors, including acquired and inherited factors. Further work is warranted to explore the effect of etiological factors on survival.

(3) Reviewer 1's comment 3: Please include - as possible - cross-sectional analysis of clinical risk factors for EHPVO/PVT in cirrhotic patients vs. non-cirrhotic patients as a internal control group.

Answer: the comparison of risk factors for EHPVO/PVT between cirrhotic patients versus non-cirrhotic patients appears to be beyond the task of the present study. Additionally,

thrombotic risk factors were not often detected in cirrhotic patients. Indeed, the thrombotic risk factors were not routinely detected in cirrhotic patients at our center. This is primarily because the important risk factor of development of PVT in liver cirrhosis is the decreased portal blood velocity.

(4) Reviewer 1's comment 4: Please verify the imaging methods used for exclusion of liver cirrhosis: ultrasound ? , CT scan ? MRT ? histology ?

Answer: we have added the imaging methods in Methods section as follows. Liver cirrhosis and hepatocellular carcinoma were excluded on the basis of a history of chronic liver disease, clinical presentation, liver function, alpha-fetoprotein and positive findings on imaging (i.e., ultrasound and CT scans). A liver biopsy was obtained, if a diagnosis of cirrhosis was inconclusive or if hepatocellular carcinoma was suspected. Other abdominal malignancy was excluded by imaging.

In our study, all included patients underwent abdominal color Doppler ultrasound and computed tomography. But only 3 patients underwent liver biopsy.

(5) Reviewer 1's comment 5: Although variceal bleeding with an occurance rate of nearly 80% is the most important complication of EHPVO/PVT, only 50% of study subjects have had episodes of variceal bleeding. How can the authors explain these different findings in comparison to even published data?

Answer: Indeed, the occurrence rate of variceal bleeding is various among the different studies. In the study by Spaander et al. (Spaander MCW, et al. Aliment Pharmacol Ther 2010;32:529-534), variceal bleeding was observed in 22% (23/103) of non-cirrhotic and non-malignant patients with extrahepatic portal vein thrombosis. In the study by Orr et al. (Orr DW, et al. Clin Gastroenterol Hepatol 2007;5:80–86), varcieal bleeding was observed in 53% (32/60) patients with chronic portal and mesenteric venous thrombosis. In the study by Janssen et al. (Janssen HLA, et al. Gut 2001;49:720–724), variceal bleeding was observed in 30% (52/172) of patients with extrahepatic portal vein thrombosis. Therefore, the prevalence of variceal bleeding in our study is consistent with the data from other studies.

(6) Reviewer 1's comment 6: Please specify treatment duration of oral anticoagulation in patients with EHPVO/PVT.

Answer: the use of anticoagulation is described in Methods section as follows. Initially, heparin was regularly administered intravenously at a starting dose of 1,000-1,400 U/hour for five days. Subsequently, oral warfarin was prescribed at the dosage of 2.5-5 mg/day for at least six months and was adjusted to maintain the internationalized normalized ratio (INR) at a target of 2.5 (range 2.0-3.0). A three-day overlap between intravenous and oral anticoagulation was required. Life-long oral anticoagulants were prescribed to patients with thrombophilia.

Additionally, anticoagulation therapy was approved in patients with acute portal vein thromobosis. However, to date, the benefit of anticoagulation therapy in patient with portal cavernoma has not been established. In our study, the use of anticoagulation therapy was just considered in the patients with an acute thrombotic episode or those with prothrombotic risk factors and without high-risk varcieal bleeding. Indeed, only 10 patients with an acute thrombotic episode were given anticoagulation therapy. However, the efficacy of anticoagulation therapy is slight. Among them, only one patient had partial recanalization of portal vein. Additionally, anticoagulation therapy was given for only 2-5 days in five patients, due to increased abdominal pain and anticoagulants-related complications. These patients were converted to thrombolytics. More importantly, oral anticoagulation is often interrupted or discontinued due to the patients' subjectivity. Therefore, it is difficult to precisely evaluate the duration of anticoagulation therapy.

(7) Reviewer 1's comment 7: Completion of diagnostic features of patients with ascites is recommended. Please include laboratory analysis of ascitic fluids for exclusion of spontane bacterial peritonitis, especially in case of elevated WBC.

Answer: the presence of ascites was diagnosed by physical examination, ultrasound and CT scans. The grade of ascites was based on the definitions of the International Ascites Club (grade I: mild ascites only detectable by ultrasound; grade II: moderate symmetrical abdominal distension; grade III: marked abdominal distension). Paracentesis was just performed in patients with ascites unresponsive to diuretics. Thus, not all patients

underwent the paracentesis and laboratory analysis of ascitic fluids. Additionally, we did not observe spontaneous bacterial peritonitis in any patients.

In addition, we rechecked the data regarding the laboratory analysis of ascetic fluids. In our study, three patients presented with grade III ascites, one patient presented with grade II ascites, and 16 patients presented with grade I ascites. Unfortunately, no report regarding laboratory analysis of ascetic fluids was found. Certainly, we clearly recognized the clinical significance of your comment. Therefore, we added this limitation into our study as follows. "The laboratory analysis of ascitic fluids was not performed. We could not exclude the possibility of spontaneous bacterial peritonitis, especially in cases with an elevated WBC."

(8) Reviewer 2's comment 1: Acute thrombotic episode is defined. But I doubt if these patients have cavernoma too. Are t patients with cavernoma with an adding thrombotic episode?

Answer: Yes, the patients with portal cavernoma had a concomitant acute thrombotic episode. Indeed, all included patients were diagnosed with portal cavernoma. Among them, a proportion of patients had an acute thrombotic episode.

(9) Reviewer 2's comment 2. How many patients finally were their liver biopsed? Sometimes it is not easy a diagnosis of cirrhosis vs. secondary changes due to chronic portal vein thombosis.

Answer: we checked the data from the patients included in our study again. Unfortunately, only 3 patients underwent liver biopsy. Two patients underwent liver biopsy during the operation of splenectomy. One patient with HbsAg (+), HbeAb (+) and HbcAb (+) underwent liver biopsy due to the suspected liver cirrhosis secondary to HBV. Results from liver biopsies were negative in the three patients.

However, we would like to emphasize that all patients underwent HBV and HCV detection and were inquired about the history of alcohol use to exclude the possibility of other chronic liver disease. Additionally, all patients underwent abdominal Doppler ultrasound and contrast-enhanced CT scans to exclude the possibility of liver cirrhosis.

Generally, all included patients did not have the CT features of liver cirrhosis, except for portal hypertension and splenomegaly.

(10) Reviewer 2's comment 3. Was etiology study in a protocolized manner? This protocol should be explained in methods.

Answer: Thrombotic risk factors of EHPVO, including JAK2 V617F mutation, CD55 and CD59 deficiencies, anti-cardiolipin IgG antibodies, and factor V Leiden or prothrombin gene G20210A mutation, were detected at our department after September 2009 (Qi X, et al. J Gastroenterol Hepatol. 2013; 28: 148-52. Qi X, et al. J Gastroenterol Hepatol. 2012; 27: 1036-43.). But it should be noted that not all patients underwent the detection. Additionally, not all thrombotic risk factors were detected in these patients.

(11) Reviewer 2's comment 4. Was anticoagulaton a prognosis factor? And how many patients were finally anticoagulated. It is not shown in tables.

Answer: anticoagulation therapy was approved in patients with acute portal vein thromobosis. However, to date, the benefit of anticoagulation therapy in patient with portal cavernoma has not been established. In our study, use of anticoagulation therapy was just considered in the patients with an acute thrombotic episode or those with prothrombotic risk factors and without high-risk varcieal bleeding. Indeed, only 10 patients with an acute thrombotic episode were given anticoagulation therapy. However, the efficacy of anticoagulation therapy is slight. Among them, only one patient had partial recanalization of portal vein. Additionally, anticoagulation therapy was given for only 2-5 days in five patients, due to increased abdominal pain and anticoagulants-related complications. These patients were converted to thrombolytics. Additionally, oral anticoagulation is often interrupted or discontinued due to the patients' subjectivity. Therefore, it was difficult to evaluate the effect of anticoagulation therapy on prognosis.

(12) Reviewer 2's comment 5. I do not understand why leukocyte count is a bad prognostic factor. Did these patients have a myeloprolipherative disorder?

Answer: we would like to add the discussion about the WBC count as the predictor of survival in the discussion as follows. "We also found that an elevated WBC count was the

independent predictor of survival. This might be explained by the fact that the comorbidities, such as acute leukemia (n=1) and multiple liver abscesses (n=1) could be more common in patients with an elevated WBC count. However, it should be noted that the effect size was very small (hazard ratio was very close to 1). Therefore, the significance of WBC count on patients' survival might be clinically slight."

(13) Reviewer 2's comment 6. In view of the number of deaths (7) I think that a a multivariate regresion cox analysis is not possible. I would eliminate this or I will discuss that the model is overfitted and conlusions of this analysis sholud be taken with caution.

Answer: we agreed with your insightful comment. We would like to discuss the limitation of our study as follows. "Given that the number of death was only 7, the multivariate Cox regression analysis might be inappropriate. Indeed, the number of variables included in the multivariate analysis might introduce the risk of overfitting the data, thereby leading to a high risk of false positive results. Therefore, the conclusions of this analysis should be taken with caution and further confirmed in larger studies."

3 References and typesetting were corrected.

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

Sincerely yours,

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