Sincerely yours,

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March 8, 2016

Professor Lian-Sheng Ma,
President and Company Editor-in-Chief
Baishideng Publishing Group Inc.

Manuscript No: 24670

Dear Professor Ma,

Thank you for your letter dated February 12, 2016. We sincerely appreciated your positive evaluation of our manuscript, which included potential acceptance for publication in *World Journal of Gastroenterology* following adequate revisions and responses to the reviewers' comments.

Based on the reviewer's feedback, we carefully revised our manuscript. Changes in the new version are indicated in red.

Enclosed also please find our point-by-point responses to the issues raised by the four reviewers. We would like to take this opportunity to express our gratitude to the reviewers for their constructive and useful remarks. Their comments allowed us to identify areas in our manuscript that needed modification and clarification. We also thank you for allowing us to resubmit a revised version.

As the reviewer pointed out, we couldn't completely exclude liver diseases from our study subjects in this study. However, our manuscript might give a novel concept of serum biomarkers for chronic pancreatitis related to liver diseases. In fact, we are developing a novel Mac-2bp measurement system, which recognizes the characteristic glycan structure of Mac-2bp in liver and pancreas diseases respectively. We believe this novel measurement system should be a useful diagnosis method for chronic pancreatitis in near future.

Tomoki Hata and Koichi Kawamoto were added as a co-author because they contributed to critical revision of the manuscript.

I hope that the reviewing process finds the manuscript acceptable for publication in *World Journal of Gastroenterology*.

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Response to the comments of reviewers

We thank the editor and reviewers for the positive assessment of our manuscript and for identifying areas that required corrections and/or modification. The red-colored text in the revised manuscript is the corrected/modified text. All line numbers mentioned in each response to each comment refer to the small-size numbers that appear on the left margin of the text of the revised manuscript.

Reviewers' Comments to Author:

Reviewer: 1

"Serum Mac-2 binding protein is a novel biomarker for chronic pancreatitis" is a well written interesting manuscript. There are three concerns which require to be addressed by the authors.

1- In the Discussion, lines 257-258, "Our results indicate that increased serum Mac-2bp in subjects without liver diseases should be evaluated for subclinical CP." If detection of high serum levels of Mac-2bp is a consequence of and/or requires liver inflammation (Table 2 and Figure 3), why do the authors make emphasis on 'subjects without liver diseases'? How do the authors rule out a liver disease in this study?

Thank you for the reviewer's comments. Previously, we found serum Mac-2bp as a nonalcoholic steatohepatitis (NASH) biomarker and serum Mac-2bp levels were increased in NASH patients compared with in nonalcoholic fatty liver and healthy volunteers [1] [2]. Although precise mechanisms through which serum Mac-2bp levels increased in chronic pancreatitis (CP) patients in this study are still unclear, some findings in our study indicated that increased serum Mac-2bp in CP patients would be produced from liver. The exclusion criteria from this study included a history of hepatic disease, such as chronic hepatitis C, chronic hepatitis B (seropositive for hepatitis B surface antigen), autoimmune hepatitis, Wilson's disease, or hepatic injury caused by substance abuse. We added some descriptions in our revised manuscript (page 8, line 186-9).

2- Figure 3 (Putative mechanism of serum Mac-2bp changes in chronic

pancreatitis and pancreatic ductal adenocarcinoma) shows a decrease in liver inflammation in patients with PDAC. However, according to Table 1 (Clinical and serological characteristics of the subjects in this study), there are no significant differences for markers of liver inflammation (i.e. AST and ALT) between CP and PDAC patients. How can this be conciliated?

We apologize for our description in Figure 3 which tend to induce misreading for the readers. In our previous study[3], we found pancreatic adenocarcinoma (PDAC) patients had chronic pancreatitis in their noncancerous lesions of pancreas. Therefore, PDAC patients in the present study also would have chronic pancreatitis in their noncancerous pancreas tissues, and pancreatic bioactive substance levels in portal vein would be equally increased from PDAC and CP patients. These pancreatic bioactive substances would evoke hepatic inflammatory changes, and would increase hepatic Mac-2bp production. Alcohol over-intake and/or relative over-nutrition induces steatohepatits in CP and further increases hepatic Mac-2bp production. In contrast, the cessation of alcohol over-intake and no over-nutrition in PDAC would decrease hepatic Mac-2bp production and serum Mac-2bp levels would decrease in PDAC. We modified our Figure 3 according to the figure legend in our revised manuscript.

As reviewer pointed out, serum transaminase (AST, ALT) levels were not different between CP and PDAC patients in our study (Table 1). One of the reasons for this finding would be due to the difference in age. Serum transaminase levels positively correlated with age in general, and age in PDAC patients was significantly higher than that in CP patients in our study. If the age in PDAC patients was the same as that in CP patients, serum transaminase levels would be decreased. In addition, liver metastatic cancer often observed in PDAC patients would exist in our PDAC patients, and these metastatic cancers would have any effects on liver injury. These mechanisms should be investigated in our future study.

3- In the present manuscript the authors conclude that serum Mac-2bp may be a novel and useful biomarker for subclinical CP diagnosis, however Mac-2bp is also reported to be increased in breast cancer, lung cancer, colorectal cancer, and prostate cancer. Can the authors further expand on the analytical performance validation process for Mac-2bp by looking into its

sensitivity, specificity, robustness, accuracy and reproducibility?

Thank you for the reviewer's valuable comments. As reviewer pointed out, serum Mac-2bp levels increase in various cancer (breast cancer, lung cancer, colon cancer, and prostate cancer), viral hepatitis, and autoimmune diseases[4, 5]. We found serum Mac-2bp as a nonalcoholic steatohepatitis (NASH) biomarker and serum Mac-2bp levels were increased in NASH patients compared with in nonalcoholic fatty liver and healthy volunteers [1] [2]. In this time, we don't measure serum Mac-2bp levels in these diseases other than NAFLD, we would like to measure serum Mac-2bp levels in various diseases in our future study, if we succeed to develop a novel detection system for measuring aberrant glycosylation of Mac2-bp. In addition, we would like to investigate the mechanisms through which serum Mac-2bp levels increase in various diseases.

References

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Reviewer: 2

Definition of Subclinical CP needs to be mentioned.

Your subjects are divided into HV, CP and PDAC, but your conclusions suggest using this in subclinical CP. Is there a distinction in the subclinical and CP group? How do you identify and define this should be clear in Methods and study design.

Thank you for the reviewer's correct suggestion. We apologize for the wrong descriptions of our manuscript which generated misunderstanding. In this study, we divided our study subjects into HV, CP, and PDAC. Subclinical CP means "latent chronic pancreatitis" which we think an important background of PDAC. We corrected subclinical chronic pancreatitis (CP) to chronic pancreatitis (CP) to avoid misunderstanding our manuscript (page 4, line 83; page 5, line 107, line 112-3; page 13, line 266).

You should try to give more information in Subgroups who had CP and PDAC, whether how many had co-existing NASH using Mac2bp levels. Also how did you define HV? is it by absence of CP/NASH or both.

As the reviewer suggested, some of our study subjects with CP or PDAC would have NASH. However, we didn't perform liver biopsy of our study subjects in this study. Therefore, we don't have information of our study subjects about NASH.

Healthy volunteers were subjects who revealed no abnormal values in their laboratory evaluation without ultrasound-diagnosed fatty liver in health check-ups. We added some descriptions in our revised manuscript (page 8, line 180-2).

Reviewer: 3

This article described the usefulness of the measurement of serum Mac-2bp for subclinical CP diagnosis. My comment is as follows.

1. There is no mention of the study period.

Thank you for the reviewer's comments. According to the reviewer's comments, we added some descriptions about study period of our study in our revised manuscript (page 8, line 190-1).

2. Please describe a sample size to be required for this study.

The mean values and standard deviations (S.D.) of our study subjects are in the table below.

	HV	CP	PDAC
mean	1.33	2.3	1.32
S.D.	0.72	1.75	0.95
number	59	162	94

In our study, the level of significance (α) was 5%. When we set "power" as 0.8, the necessary sample size was 27. When we set "power" as 0.9, the necessary sample size was 35. In our study, study sample size was abundant for the statistical analysis.

3. The etiology of CP patients in this study is unknown. How many alcohol CP patients are there?

We can know their etiologies of CP patients in 110 patients, 65 patients were alcoholic CP, 42 patients were idiopathic CP, 2 patients were lithogenic CP, and 1 patient was Von Recklinghausen disease.

4. You reported the association between Mac-2bp level and NASH patients. Which of alcohol or over-nutrition is the Mac-2bp level of CP patients associated with?

In 65 alcoholic CP patients, serum Mac-2bp level was 2.74 ± 2.25 μg/mL, which was relatively higher than that of CP patients with other etiologies and as high as that of NASH patients.

5. Does the Mac-2bp level have a difference between asymptomatic CP

patients and symptomatic CP patients?

Although we don't have clinical information which demonstrate the symptoms of our study subjects in details, the result in Table 2 demonstrated serum AMY and Mac-2bp had no correlation. Considering this finding, we suppose there would be little difference between asymptomatic CP patients and symptomatic CP patients.

6. Do PDAC patients in this study include patients derived from CP? If included, does the Mac-2bp level have a difference between PDAC patients derived from CP and other PDAC patients?

Thank you for pointing out critical issue. We also have an interest in this issue. However, we don't have clinical information in details whether PDAC patients were derived from CP or not. Our previous report (ref. 6) demonstrated that all the 76 PDAC patients had CP histology in noncancerous pancreas tissue. Therefore, we think that PDAC patients should have sub-clinical CP in the pancreas.

Reviewer: 4

Thank you for the reviewer's valuable comments and suggestions. As the reviewer pointed out, we couldn't completely exclude liver diseases from our study subjects. Therefore, the effects of subclinical liver diseases in pancreatic disease subjects should be included in our results. To exclude the effects of liver diseases, the combination between serum Mac-2bp and some biomarkers should be needed to screen chronic pancreatitis correctly from general population. However, serum pancreatic enzymes (e.g. amylase, lipase) would be inadequate because of the wide change of their serum levels. Especially, their serum levels were decreased in end-stage chronic pancreatitis. Therefore, novel diagnostic biomarker should be needed. We think our manuscript might give a novel concept of serum biomarkers for chronic pancreatitis related to liver diseases. In fact, we are developing a novel Mac-2bp measurement system, which recognizes the characteristic glycan structure of Mac-2bp in liver and pancreas diseases respectively. We believe this novel measurement system should be a useful diagnosis method for chronic pancreatitis in near future.

1. Mac-2bp is produced in liver due to hepatic inflammation of varied etiologies and is not specifically produced in pancreas.

We suppose as the reviewer's comments.

2. In view of wide prevalence of hepatic steatosis and steatohepatitis, it is unlikely to find a patient only with chronic pancreatitis and absolutely normal liver.

As we described in our manuscript (line 294-319), we suppose Mac-2bp would be produced in liver in chronic pancreatitis patients. Increased pancreatic bioactive substances in chronic pancreatitis patients flow into liver through portal vein, and would increase Mac-2bp production.

We apologize for our description in Figure 3 which tend to induce misreading for the readers. In our previous study (ref. 6), we found pancreatic adenocarcinoma (PDAC) patients had chronic pancreatitis in their noncancerous lesions of pancreas. Therefore, PDAC patients in the present study also would have chronic pancreatitis in their noncancerous pancreas tissues, and pancreatic bioactive substance levels in portal vein would be equally increased from PDAC and CP patients. These pancreatic bioactive substances would evoke hepatic inflammatory changes, and would increase hepatic Mac-2bp production. Alcohol over-intake and/or relative over-nutrition induces steatohepatits in CP and further increases hepatic Mac-2bp production. In contrast, the cessation of alcohol over-intake and no over-nutrition in PDAC would decrease hepatic Mac-2bp production and serum Mac-2bp levels would decrease in PDAC. We modified our Figure 3 according to the figure legend in our revised manuscript.

3. Alcohol being the most common etiology of chronic pancreatitis, it is highly unlikely that the liver is normal in such patients.

We can know their etiologies of CP patients in 110 patients, 65 patients were alcoholic CP, 42 patients were idiopathic CP, 2 patients were lithogenic CP, and 1 patient was Von Recklinghausen disease. As the

reviewer pointed out, alcohol was the most common etiology in our chronic pancreatitis subjects. We agree to the reviewer's comments. In this study, we didn't perform liver biopsy in our study subjects. Therefore, we cannot investigate hepatic histology of our subjects. In these alcoholic chronic pancreatitis patients, we also suppose alcoholic liver injury also existed.

4. There is no attempt in the study to exclude any form of liver disease in patients with chronic pancreatitis.

Thank you for the reviewer's important comments. According to the reviewer's comments, we added some descriptions in our revised manuscript (page 8, line 186-9). The exclusion criteria from this study included a history of hepatic disease, such as chronic hepatitis C, chronic hepatitis B (seropositive for hepatitis B surface antigen), autoimmune hepatitis, Wilson's disease, or hepatic injury caused by substance abuse.

5. Most patients with pancreatic adenocarcinoma have underlying chronic pancreatitis, so discrimination based on Mac-2bp is difficult.

We apologize for our description in Figure 3 which tend to induce misreading for the readers. In our previous study (ref. 6), we found pancreatic adenocarcinoma (PDAC) patients had chronic pancreatitis in their noncancerous lesions of pancreas. Therefore, PDAC patients in the present study also would have chronic pancreatitis in their noncancerous pancreas tissues, and pancreatic bioactive substance levels in portal vein would be equally increased from PDAC and CP patients. These pancreatic bioactive substances would evoke hepatic inflammatory changes, and would increase hepatic Mac-2bp production. Alcohol over-intake and/or relative over-nutrition induces steatohepatits in CP and further increases hepatic Mac-2bp production. In contrast, the cessation of alcohol over-intake and no over-nutrition in PDAC would decrease hepatic Mac-2bp production and serum Mac-2bp levels would decrease in PDAC. We modified our Figure 3 according to the figure legend in our revised manuscript.

6. At this stage it is difficult to propose Mac-2bp as a test for screening of chronic pancreatitis because of high incidence of liver disorders in such patients.

As reviewer's pointed out, pancreas disease patients (including chronic pancreatitis and pancreatic cancer) often have co-existing liver diseases. As we demonstrated in Figure 3, pancreatic bioactive substances in these pancreas disease patients would induce liver inflammation range from subclinical to clinically-evident liver diseases. In this manuscript, we would like to demonstrate that serum Mac-2bp levels were increased in chronic pancreatitis patients with or without clinically-evident liver diseases. Therefore, we believe that serum Mac-2bp would be a novel biomarker for the screening of chronic pancreatitis patients in general populations.